CHAPTER I

INTRODUCTION

The rapidly increasing of population in the world has resulted in an increase in the area of polluted soil, air and water. As the number of people continues increasing day by day it also brings with it a growing pressure on air, water and land resources. In order to cater to the demands of the people, the rapid expansion of industries, food, health care, vehicles, etc. is necessary. But it is very difficult to maintain the good quality of life with all these new developments, which are unfavorable to the environment in which we live, if proper management is not applied (Sasikumar et al., 2003).

Hazardous wastes are the wastes which, by reason of their chemical activity or toxic, explosive, corrosive, or other characteristics, cause danger or likely will cause danger to health or the environment, whether alone or when coming into contact with other wastes (Lagrega et al., 2001). In the year 2004, 40,821,000 tons of hazardous wastes, regulated under the Resource Conservation and Recovery Act (RCRA) of 1976, were generated in the United States (US. EPA., 2004). Among these large amount of hazardous wastes, used oil and petroleum wastes were the most serious concern.

Used oil, petroleum and petroleum-by product wastes contain a number of chemicals, potentially harmful to human health and the environment, especially polycyclic aromatic hydrocarbons (PAHs). PAHs are hydrocarbon compounds with multiple benzene rings. PAH molecules are made up of two or more fused benzene rings. They were classified by the United States Environmental Protection Agency as priority toxic pollutants because of their toxicity. PAHs can cause adverse effects to human health and the environment. Some PAHs have been identified as having the potential to cause cancers or genetic mutations (Wilson and Jones, 1993; International Association of Research Chemists, 1983). PAHs are generally occurs as a complex mixtures and the burning of organic materials such as fossil fuel and petroleum products is mainly responsible for their ubiquitous distribution in the environment (Volkering et al., 1992; Fernandez et al., 1992). Because of the widespread use of materials containing PAHs, accidental spillage and improper disposal during the

processing, transportation, and use of these materials have resulted in a number of contaminated sites presenting serious health and ecological risks. Bioremediation technologies, the use of living organisms to degrade environmental contaminants, have increasingly been proposed to decontaminate those sites. These technologies offer several advantages over other remediation techniques. For example, it can be done on-site, is less expensive, transforms waste permanently, has greater public acceptance and site disruption is minimal (Boopathy, 2000).

Acenaphthylene is one of the common PAHs found as a contaminant in petroleum refining and coal tar distillation facilities. It is also found in soil, sediment and water in petroleum contaminated sites and as one of the major components of PAHs in urban air of Thailand (Wise et al., 1988; Panther et al., 1996). Isolation and characterization of a wide variety of bacteria from the environmental samples possess ability to metabolize acenaphthylene and PAHs makes bioremediation of acenaphthylene contaminated sites and resources possible (Muller et al., 1991). Their successful applications, however, require a profound understanding of the biochemical pathway by which acenaphthylene is degraded and the enzymatic reactions involved in the degradation of acenaphthylene by these strains. A clear understanding of the fate of acenaphthylene in the environment is dependent on an understanding of the metabolic pathways. Moreover, strategies to improve remediation technologies for PAHs-contaminated sites require a broader understanding of not only the optimum environmental conditions for remediation but also the biochemical pathways involved in degradation and in the eventual formation of oxidized products (Vila et al., 2001). Because of these reasons, successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry. The pathways for the biodegradation of some PAHs such as naphthalene, phenanthrene, fluorene and pyrene are well documented. However, only a very few numbers of bacteria capable of utilizing acenaphthylene and the catabolic pathway involving acenaphthylene degradation have been reported.

A *Rhizobium* sp. CU-A1, isolated from petroleum contaminated soil in Thailand, capable of utilizing acenaphthylene as a sole source of carbon and energy has been reported from our laboratory (Paengthai, 2000). To elucidate the acenaphthylene degradation pathway in this organism, blocked mutants incapable of utilizing this compound as a sole carbon and energy source have been constructed by

using transposon mutagenesis via transposon Tn*5* (Kriangkripipat, 2001). These mutants were useful for isolation and characterization of acenaphthylene-degrading intermediates.

Objectives

The objectives of this work were isolation, purification and identification of acenaphthylene degrading intermediates from *Rhizobium* sp. CU-A1-transposon mutants to propose the complete acenaphthylene catabolic pathway in *Rhizobium* sp. CU-A1.

Isolation, purification and characterization of some enzymes involving acenaphthylene degradation from *Rhizobium* sp. CU-A1 were included.

Hypothesis

Oxidation of acenaphthylene by bacteria has rarely been reported. Moreover, a complete pathway for acenaphthylene degradation has not yet been proposed.

Rhizobium sp. CU-A1 was capable of utilizing acenaphthylene as a sole source of carbon and energy, therefore, complete catabolic degradation should be occurred during its growth on this compound. Catabolic pathway could be elucidated by construction of blocked mutants defective in acenaphthylene degradation. The blocked mutants accumulated various acenaphthylene degrading metabolites. Purification and identification of these intermediates lead to catabolic pathway elucidation.

Scope of the Study

Accumulated intermediates of acenaphthylene degradation formed from *Rhizobium* sp. strain CU-A1-mutants defective in acenaphthylene degradation were purified and identified. The complete acenaphthylene catabolic pathway was proposed. In addition, enzymes involved in acenaphthylene oxidation was purified

and characterized. Oxygen atoms incorporating into acenaphthylene molecule catalyzed by the purified enzyme was studied by using $^{18}O_2$. These results could be used as a basic information for the remediation of acenaphthylene contaminated site and for the improving of the bioremediation technologies.