CHAPTER I

INTRODUCTION

1.1 Statement of problem

With the rapid development of industry and agriculture, there are considerable amounts of contaminated sites around the world. These sites are a result of leaking of pipelines and underground petroleum storage tanks and spilling of manufacturing oil or gas manufacturing production. Furthermore, due to high growth rate of the world populations, developments of various industries have been increased. The major organic chemical waste categories include organic aqueous waste (pesticides), organic liquids (solvents from dry cleaning), oils (lubricating oils, automotive oils, hydraulic oils, fuel oils) and organic sludges/solids (painting operations, tars from dyestuffs intermediates). Consequently, hazardous chemical contamination of the environment has been increasing in the last few decades (Evans *et al.*, 2004).

Various methods have been used to clean up the hydrocarbon contaminated sites. Physical and chemical treatments have been used for removing contamination from petroleum polluted soil or waters and for washing petroleum storage tanks (Desai and Banat, 1997). Chemical surfactants were widely used in industrial applications. Most of these compounds were synthesized chemical origin. Most were also toxic to the environmental, not easily biodegradable and their manufacturing processes and byproducts could be environmentally hazardous.

Nowadays, increasing environmental awareness and emphasis on a sustainable society in harmony with the global environment, natural surfactants have been becoming much more important (Holmberg, 2001). Among natural surfactants, ones of microbial origin are especially classified into "biosurfactants" (Desai and Banat, 1997; Banat *et al.*, 2000). Biosurfactants were first discovered as extracellular amphiphilic compounds in research into hydrocarbon fermentation, which started in the late 1960s. At the beginning when biosurfactants were discovered, they attracted attention as "alternative surfactants" due to their high biodegradability and safety. During the last decade, unique properties of biosurfactants, like biological activities, which are not observed at all in conventional chemical surfactants, have one after another been found. Therefore, biosurfactants have been increasingly attracting attention in various fields as multifunctional materials for the new century.

Alternatively, biosurfactant was one of the useful treatments to either reduced or removed the contaminated oils and fats from the environment. Biosurfactants were a group of surface-active molecules produced mainly by hydrocarbon degrading microorganisms. Some of the advantages of biosurfactants over synthetic ones included lower toxicity, biodegradability, selectivity, specific activity at extreme temperatures, pH and salinity, the possibility of their production through fermentation, their potential applications in environmental protection and management, crude oil recovery, as antimicrobial agents in health care and food processing industries (Desai and Banat, 1997).

Numerous researchers showed that the addition of biosurfactant could enhance the solubilization and bioavailability of hydrocarbons in batch scale studies. Van Dyke *et al.* (1993) reported that rhamnolipids from *Pseudomonas aeruginosa* increased 40 to 80% and 30 to 70% recovery of hydrocarbons from contaminated sandy-loam and silt-loam soil, respectively. Moreover, Scheibenbogen *et al.* (1994) demonstrated that 56% of the aliphatic and 73% of the aromatic hydrocarbons were recovered from hydrocarbon contaminated sandy-loam soil by treatment with rhamnolipids. Some researchers also reported that biosurfactants could form complexes with heavy metals. Herman *et al.* (1995) and Miller (1995) noted that in a soil experiment, the monorhamnolipid biosurfactant produced by *P. aeruginosa* ATCC 9027 could remove 1696, 43% and 48% of the sorbed Cd^{2+}, Pb^{2+} and Zn^{2+} from sandy soil (68%sand, 4.7%silt, 8.8%clay).

To summarize, biosurfactants had similar properties to the synthetic surfactant with added advantage of being biodegradable. Furthermore, the advantages had prompted a very high interest in the isolation of new microorganisms able to produce biosurfactants. Some of the organisms reported previously as biosurfactant producers included among others *P. aeruginosa* (Robert *et al.*, 1989), *Bacillus licheniformis* (Javaheri *et al.*, 1985), *Arthrobacter* sp. (Rosenberg *et al.*, 1979), *Staphylococcus* sp. (Nweke and Okpokwasili, 2003) and *Flavobacterium* sp. MTN11 (Bodour *et al.*, 2004).

1.2 Objectives

According to determine whether bacteria isolates, screened and isolated from contaminated soils in Thailand, were able to produce biosurfactants. The biosurfactant productions by these bacteria were studied in this research. There are three objectives of this research:

1.2.1 To screen, isolate and identified biosurfactant-producing bacteria

1.2.2 To characterize of partial purified biosurfactant

1.2.3 To determine parameters affecting biosurfactant production such as specific substrate and nitrogen sources

1.3 Hypothesis

1.3.1 Bacteria screened and isolated from various soils in Thailand which has been exposed to hydrocarbon and organic wastes might have the ability to produce biosurfactants.

1.3.2 Carbon and nitrogen sources affecting biosurfactant production will be beneficial for further to raise biosurfactant production.

1.4 Scope of study

1.4.1 Screening, isolation and identification of biosurfactant-producing bacteria

The biosurfactant-producing bacteria, which produce biosurfactant were screened and isolated from various soils in Thailand by drop collapse method and emulsification index (E_{24}). The biosurfactant-producing bacteria were identified by biochemical characterization and comparison of 16s rDNA gene sequence.

1.4.2 Identification of the biosurfactant type

The analytical characterization of the biosurfactants produced by biosurfactant-producing bacteria was examined. Therefore, partially purified biosurfactant was subjected to analysis with thin layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and mass spectrometry which identified of biosurfactant type.

1.4.3 Characterization of biosurfactant for biosurfactant-producing bacteria

1.4.3.1 Partial purification of biosurfactant production

1.4.3.2 Physiochemical properties and activity of biosurfactant

a) Critical micelle concentration (CMC)

The CMC was determined by interpreting a plot of surface tension compared log surfactant concentration (Rosen, 1989). As the concentration increases, the surface tension decreases until the CMC was reached. The CMC was a significant parameter in solubilization, and mobility of contaminants could be expected to be highest at or above the CMC of the aqueous surfactant solution (Deshpande *et al.*, 1999).

b) Stability of biosurfactant at various of temperatures and pH

Stability a constant temperature several of 30–75°C and pH (2.0–12.0) studies were done by emulsification index (E_{24}).

1.4.4 Factors involving biosurfactant-producing bacteria growth and its biosurfactant production

Types and concentrations of an alternative carbon sources (glucose, maltose and sucrose), nitrogen sources ($NaNO_3$, $(NH_4)_2SO_4$ and CH_4N_2O) towards growth and biosurfactant-production were investigated. Moreover, various types oil were supplemented during growth to determine if addition of oil improve biosurfactant production. The ability of the biosurfactant to emulsify some liquid hydrocarbons, such as olive oil, sunflower oil, soybean oil and diesel oil was determined. Besides were determined to effect of biosurfactant-producing bacteria in Luria Bertani broth (LB) and nutrient broth (NB) which compared mineral salt medium (MSM).

1.4.5 Determination of the effect of NaCl, temperature and pH on the activity of the biosurfactant

Growth studies growth of isolation and biosurfactant production at various temperatures in the range 30-45°C and various NaCl concentrations and pH.

1.5 Expected result

Screening and/or isolation of bacteria were able to produce biosurfactants from contaminated soil and to determine the effect of carbon source, nitrogen source, temperature, pH and NaCl concentration on the production of biosurfactant further information of in order to rise to biosurfactant production.

1.6 Thesis Organization

This thesis is comprised of seven different chapters. First, this chapter provides the introduction part of this research. Second, the theoretical background and literature review is described. Third, the research methodology is explained. After that, the results and discussions are demonstrated; then, these results are being concluded. Finally, the suggestion and future works is orderly described.