CHAPTER VI

CONCLUSIONS

The majority of bacteria isolated from engine oil and petroleum contaminated sites were gram negative rods. However, five of the one hundred and thirty isolates identified as biosurfactant-producers using the drop-collapse test and emulsification index (E_{24}) as preliminary screening method.

Five isolates of biosurfactant-producing bacteria, designated A102, A103, B202, P2 and P3 were isolated from engine oil contaminated soil from Burirum province and fuel oil contaminated soil in Bangkok. The identified by using biochemical characterization and by using 16s rDNA sequence alignment. The results showed their species were *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Enterobacter* sp. and *Burkholderia cepacia*, respectively.

However, only two promising isolates P2 and P3, namely *Enterobacter* sp. and *Burkholderia cepacia* had greater the degree of emulsion formed than those of all five isolates. Therefore, *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 were selected for further studies when cultivated on mineral salt medium at 37°C for 72 hours. Subsequently, the types of biosurfactant and biosurfactant production were studied. Moreover, this study determined physicochemical properties of the biosurfactant.

Pseudomonas aeruginosa and *Pseudomonas* sp. (A102, A103 and B202) have been previously reported to involve in biosurfactant-producing bacteria. This present found in A102, A103 and B202 that formed complete E_{24} as 74.08 ± 0.101%, 67.86 ± 0.087% and 70.59 ± 0.074%, respectively and was able to reduce the surface tension to below 40 $mN.m^{-1}$. The values of the surface activity were *P. aeruginosa* A102,

37.0 ± 0.02 $mN.m^{-1}$, *P. aeruginosa* A103, 30.0 ± 0.05 $mN.m^{-1}$, *Pseudomonas* sp. B202, 32.0 ± 0.02 $mN.m^{-1}$. It was well known that the genus *Pseudomonas* accumulates a variety of biosurfactant. Subsequently, this report was interesting in *Enterobacter* sp. P2 and *Burkholderia cepacia* P3.

Enterobacter sp. P2 and *Burkholderia cepacia* P3 were isolated from fuel oil contaminated soil in Bangkok. They formed complete E_{24} as 85.88 ± 0.071% and 86.19 ± 0.060%, respectively following able to lower the surface tension of the culture to 26.0 ± 0.52 $mN.m^{-1}$ and 25.0 ± 0.52 $mN.m^{-1}$. Afterward, the biosurfactants were excreted by *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 following partial purified of biosurfactants. Subsequent, the partially purified biosurfactants were studied for instance structure, characterize and properties of biosurfactant. Mass spectrometry indicated the compound possesses mass estimated 550 m/z were approach with molecular mass of glycolipids. Moreover, the glycolipids were produced by *Enterobacter* sp. and *Burkholderia cepacia* P3 had a critical micelle concentration of about 3.3 $mg.l^{-1}$ and 1,995 $mg.l^{-1}$. In part of stability of biosurfactant at maintained at a range temperature of 30°C-75°C that temperature had effect the stability of emulsification index however noteworthy to mention that the biosurfactant retained approximately 56.26% and 62.91%, respectively of its original activity at 75°C. In addition to, it was able of producing glycolipid effectively from various carbon and nitrogen sources was successfully isolated. Among the carbon and nitrogen substrates examined, 44.4 mM glucose + 75 mM $NaNO_3$ was the most efficient one for glycolipid production as 18.72 ± 0.071 g $glucose.l^{-1}$ and 40.67 ± 0.992 g $glucose.l^{-1}$ when compare on mineral salt medium containing 11.1 mM glucose as carbon source (17.49 ± 0.338 g $glucose.l^{-1}$ and 37.01 ± 0.118 g $glucose.l^{-1}$). The results demonstrated that could to improve biosurfactant production in

Enterobacter sp. P2 and *B. cepacia* P3 by 9.99% and 7.07%, respectively. Whatever in *Enterobacter* sp. P2 when add 2% vv^{-1} supplemented carbon source found that had no effect on biosurfactant production. Nevertheless, the adding 2% vv^{-1} supplemented carbon source found that olive oil and sunflower oil improved production in *B. cepacia* by 20.75% and 48.80%, respectively. However, the glycolipid production by *Enterobacter* sp. and *B. cepacia* were optimal in batch cultures when cultivated with the temperature and agitation rate at 37°C, 250 rpm for 72 hours. Environmental factors such as pH, salinity and temperature also affected biosurfactant activity. This statement found *Enterobacter* sp. and *B. cepacia* that no produced when the mineral salt medium contained NaCl in range 0.1-2.0% wv^{-1}. Moreover, no biosurfactant were produced and growth when the medium contained 2.0% wv^{-1} NaCl.