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SIRAWARIS SRIANANT: CRUDE OIL BIODEGRADATION BY PSEUDOMONAS J-45 THROUGH BIOSURFACTANT FORMATION. THESIS ADVISORS : UDOM KOMPAYAK, M.P.H., SHALASAI HUANGPRASERT, M.P.H., PISIT VATANASOMBOON, M.Sc., JIRAPORN SUKUMAVASRI, Ph.D. 157 P. ISBN 974-664-057-7

The objective of this research was to investigate a suitable condition for the biodegradation of crude oil by biosurfactant of *Pseudomonas* J-45 and to study properites of the produced biosurfactant by isolation, purification and identification of biosurfactant.

Pseudomonas J-45 is a bacterium isolated from oil contaminated sites in Thailand. It shows the ability to grow and degrade crude oil under various conditions, i.e., at temperature 25 to 37^{0}C, aeration rates 0.25 to 1.0 v.v.m. and when using of seawater as mineral source. The fermentation of 8 liters of bacterium broth containing 1 % crude oil in 10 liter fermenter showed that the optimum conditions to obtain the highest value of the surface tension reduction were 100 r.p.m. agitation and 1 v.v.m. aeration rate at 30^{0}C. After cultivation, bacterial cells were separated by refrigerated centrifugation at 8,000 r.p.m. at 4^{0}C for 20 min. Cells were dried by lyophilization. The biosurfactant (BS) content both in dry cells and in culture broth was detected. The BS content was found in both phases. It showed that the amount of BS in the broth is 10 times that of the dry cells. Therefore, the BS production for further study was aimed at the BS in culture broth only. The identification for the type of BS in cell and culture broth was attempted. The lipids in cell and broth were separated into 3 groups, neutrallipid, glycolipid and phospholipid by using the chromatographic technique. The BS activity in lipid was investigated using Emulsification Capacity (EC) test. The results revealed that only glycolipid, had EC activity while the other two groups of lipid did not. After that, the types of saccharides bound with lipids were investigated using thin layer chromatography. It showed that the saccharide found in BS is rhamnose. The comparison with the standard saccharides on chromatographic pattern showed that this biosurfactant of *Pseudomonas* J-45 is rhamnolipid type. The biosurfactant was stable over a wide range of pH from 6.0 to 12.0 with optimum activity at pH 8.0 with NaCl concentrations not higher than 5 % and temperature at 55 to 80^{0}C for 3 hours. The critical micelle concentration (CMC) and surface tension value at this point of partially purified biosurfactant were 148 mg/l and 34.5 mN/m, respectively. Preliminary analytical results indicated that biosurfactant was as effective as other commercial synthetic surfactants.