

**PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANTS FROM
PETROLEUM DEGRADING AQUATIC BACTERIA**

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ABSTRACT

This study was undertaken to produce, isolate and characterize biosurfactants from oil degrading bacteria using various carbon sources and determine the conditions under which specific oil degrading bacteria will produce optimally, biosurfactants required for optimal oil degradation.

Six bacterial isolates, namely *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas putida*, *Pseudomonas oleovorans*, *Sarcinae litoralis* and S₅₈ (an unidentified bacteria) were maintained on nutrient agar slants and subcultured every fortnight. Standardized cultures (1.0 ml) of the bacteria isolates were inoculated into 100 ml capacity conical flasks each containing 50 ml mineral salts medium (MSM) and 0.2 % (v/v) Escravos light crude oil was aseptically added as the sole carbon source. The cultures were agitated in an incubator shaker at 37 °C for a week. The presence and activities of the biosurfactants produced by each bacterial isolate was detected using the swirling beaker method. The growth rates of the bacteria were measured using a spectrophotometer and the best condition of pH, temperature, salinity, concentrations of nitrogen, iron, and magnesium ions which each bacterial species produced optimally were studied. The biosurfactants were isolated and characterized spectrophotometrically between 380 nm and 700 nm and chemically by spraying the TLC chromatograms with specific reagent such as iodine.

The result showed that the maximum absorption wavelength of the iodine-polysaccharide complex was almost the same for each biosurfactant from the various bacteria and ranged from 380 nm to 420 nm, which was characteristic of a polyglucose unit. The reducing sugars from all the samples proved to be glucose units. A long chain fatty acid was obtained from all the samples which exhibited R_f values close to that of Myristic and Stearic

acids. The results further revealed that all the isolates emulsified oil in the swirling beaker test, indicating the presence of biosurfactants. The biosurfactant produced was composed of polyglucose units and fatty acid which is characteristic of glycolipids. During the growth of these bacteria on crude oil-MSM, the oil was degraded best at 28 °C and pH 6.8 and 7.2. Biosurfactant production increased at concentrations of 0.5 % w/v for NaCl and magnesium but decreased as the concentration was increased to 1.0 % w/v and later to 1.5 % w/v. Production of biosurfactant increased at a concentration of 1.0 % w/v for nitrogen. The bacteria produced biosurfactant when diesel was used as the sole source of carbon.

It was concluded that the bacteria employed in this study would be useful in the biodegradation of oil in petroleum contaminated sites because of their ability to produce biosurfactant and emulsify crude oil.