Indian Journal of Marine Sciences
Vol. 20, March 1991, pp. 78-79

Emulsification and oil degradation by marine bacteria

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Received 19 March 1990, revised 5 November 1990

Oil emulsifying and solubilizing (ES) factors were isolated from the seawater medium inoculated with different marine bacterial isolates. Oil degradation was slow in first 2 days and on 3rd day 87-94% of oil was degraded by different bacterial isolates. Crude ES factor, which was whitish and gummy in texture, was able to solubilize various hydrocarbons and oil.

Oil degradation in marine environment is an important aspect as the oil pollution due to spill and transportation is increasing. Degradation of oil in the marine environment by natural hydrocarbon degrading bacteria is dependent on the capacity of these bacteria to solubilize and emulsify the oil. Marine microflora plays an important role in the control of oil pollution. Emulsification of the insoluble substrate (oil) is an important step before its degradation by the microorganisms. Few studies on surface active agents have been made with yeast[1,2]. Studies with marine bacteria with respect to oil emulsification are limited and hence the present study.

Isolation of bacteria—Crude oil (0.5 ml, of specific gravity 0.805 g) was added into 250 ml of the seawater medium[3] distributed in 500 ml flasks. The flasks were incubated at 30°C for 15 days. The suspension from flasks was streaked on marine agar media plates and plates were incubated at 30°C again. After 4 d, isolated colonies were picked up and maintained on marine agar containing 1% crude oil.

Isolation of hydrocarbon emulsifying and solubilizing factor—The bacterial isolates 25, 37, 40 and 42 were grown in 1 litre conical flasks each containing 50 ml mineral medium. The composition of the medium was (conc. per litre) 750g natural seawater, 250 ml distilled water, 0.5 g NH_4Cl, 0.5 g K_2HPO_4, 0.5 g NaH_2PO_4 and the *p*H of the medium was 6.8-7.

The natural seawater which was used in the medium was of following composition *p*H 7.8, EC 70.97 m mhos/cuu, TOC 35 $mg.l^{-1}$, total N 2.5 $mg.l^{-1}$, metals Pb 44 ppb, Cd 5 ppb, Cr 47 ppb, Se 350 ppb, Fe 1.7 ppm, Mn 0.1 ppm, Cu 0.04 ppm and Zn 0.28 ppm. To each flask, 8.5 $mg.l^{-1}$ of crude oil was added and kept on shaker at room temperature (30°C). To note the oil degradation, turbidity of the medium was measured at 610 nm using spectrophotometer (Baush and Lomb, model 21) and oil estimation was done gravimetrically. After 5 d, 800 ml of culture broth was centrifuged at 12000 g for 30 min to separate sedimented cells.

Emulsifying and solubilizing (ES) factor separation from aqueous layer—The cell free aqueous broth obtained above was treated with 3 volumes of chilled acetone and allowed to stand for 10 h at room temperature. The precipitate thus obtained was collected by centrifugation. The precipitate was purified again by chilled acetone treatment and vacuum dried at room temperature[4].

Determination of growth and substrate uptake—Growth of the bacterial isolates and substrate uptake were determined by optical density (610 nm) and gravimetric method respectively. At different intervals of time, the flasks were withdrawn and OD was measured at 610 nm by Spectronic 21. The oil in the same flask was extracted with carbon tetrachloride and determined gravimetrically.

Estimation of oil and hydrocarbon emulsifying activity—The crude ES factor was dissolved in distilled water and *pH* adjusted to 5.5 The solution was diluted to 10^{-5}. To 10 ml of the diluted solution, 0.1 ml of crude oil and hydrocarbon to be tested were added. The mixture in a glass stoppered flask shaken vigorously by hand and allowed to stand for 10 min. The mixture was then transferred to calorimetric tube. The turbidity of the stabilized emulsion was measured at 610 nm in a spectrophotometer and emulsifying activity was expressed[5] as D_{610}.

Growth of different bacteria in medium—Bacterial isolates showed slow increase in growth for the first 2d

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