

The foremost task undertaken in the present study deals with the screening of different microbial strains capable of producing maximum biosurfactant. Out of 78 oil field bacterial isolates three *Pseudomonas* were selected for their ability to produce biosurfactants. Besides this, bacterial strain procured from Microbial Type Culture Collection (MTCC) were also tested for their surface active properties. The appearance of such surface active compounds in the culture broth grown on water miscible/immiscible substrate were easily monitored by measuring surface tension of the cell free broth. The reduction in surface tension of the medium provided a rapid method for assay of maximum biosurfactant formation prior to their actual isolation. A convenient way for rapid identification of biosurfactant producing bacterial strains was developed by assaying cell surface hydrophobicity. Evidence exhibiting direct correlation between biosurfactant production by *P. putida*, *P. aeruginosa*, *P. dimunita*, *S. marcescens*, *A. protophormiae* and their cell surface hydrophobic properties were obtained. The simplicity of these techniques (HIC, SAT, BATH, RP) suggests its implementation in screening of biosurfactant producing bacterial strains from the national sources. Moreover this inexpensive technique provides a step further for assaying biosurfactant formation prior to its actual isolation using organic compounds.

Investigation done on psychrophilic strain of *Arthrobacter protophormiae* during its growth on water immiscible carbon source (*n*-hexadecane) showed surface tension reduction from 68.0 mN/m to 30.6 mN/m. This Antarctic strain could grow and produce biosurfactant in the presence of high NaCl concentration (10.0 to 100 g/l). Although the biosurfactant was isolated by growing the organism under psychrophilic conditions (10°C) it exhibited stable activity over a wide range of temperature (30°C-100°C) and pH (2 to 12). The biosurfactant was effective in recovering upto 90% of residual oil from an oil saturated sandpack column.

Scanning electron micrograph of *n*-hexadecane grown *Pseudomonas* showed that cells were elongated and lot of extracellular material was secreted in the vicinity of cell surface as compare to nutrient broth grown cells. Another interesting characteristic

feature seen in case of hydrocarbon grown bacterial cells were the presence of blebs/vesicles.

Gas chromatographic analysis of residual hydrocarbon done to study the uptake mechanism of water immiscible substrate showed that *P. putida* could utilize 96% of the hydrocarbon for biomass and biosurfactant formation. Thus, showing an exceptionally high capacity to metabolize hydrocarbons.

A biosurfactant exhibiting excellent emulsification activity and surface properties was isolated during growth of *Serratia marcescens* on 2% (w/v) sucrose. Reduction in surface tension values and increase in the yield of biosurfactant during the late log phase of growth indicates that the biosurfactant is a secondary microbial metabolite. The biosurfactant formed stable emulsions with a wide variety of hydrocarbon (Kerosene oil - 100%, Decane - 98%, Pristane - 100%, Mobile oil - 94%, Crude oil - 85%). Interesting by, the emulsion formed was stabilized by the surfactant and does not revert to separate oil and water phase even after 90 days. The isolated biosurfactant was found to be composed mainly of carbohydrate and lipid (30% and 40% respectively) showed that it is stable at high temperature and over a wide range of pH.

Nutritional requirements for optimum production of biosurfactant by *S. marcescens* showed 2% sucrose as a suitable carbon source for the maximum growth and biosurfactant production. Sodium nitrate was the preferred nitrogen source for the optimal biosurfactant production. Effect of different concentration of individual metal cations Fe^{3+} (12 ppm); K^+ (750 ppm); Ca^{2+} (15 ppm). Trace elements (1ml) are optimal for biosurfactant production by *S. marcescens*. Increase in agitation speed resulted in increase in biosurfactant production with 200 rpm being optimum, fermentation studies were done in 6.5L fermenter. Biosurfactant yield was shown to increase by 2.5 g/l than that by shake flask.

To exploit the potential commercial ability of the precipitated biosurfactant in

MEOR a sandpack column was prepared. The precipitated *Serratia* biosurfactant (0.1% aqueous solution) was effective in recovery of 90%, 85%, 82%, 80% and 80% of kerosene oil, n-paraffin, motor oil, tank bottom sludge and Assam crude oil respectively from their hydrocarbon saturated columns. Moreover, the same biosurfactant solution could remove sticky crude oil from the walls of the containers. The isolated biosurfactant also exhibited antimicrobial activity specially against gram positive microorganisms. The biosurfactant passed the toxicity test on mice thereby giving an impact that it could open up new arenas from the pharmaceutical and drug manufacturing point of view.