Abstract

The fermentative production of rhamnolipid biosurfactant was carried out by free and immobilized cells of *pseudomonas putida* 922. This bacterium was able to synthesize sustainable biosurfactant (rhamnolipid) with excessive foam-forming properties. The enhanced rhamnolipid production was achieved by using glucose as a carbon source and $NaNO_3$ as the nitrogen source. The optimum rhamnolipid production was carried out at C:N of 20 and the optimized bioprocess condition was temperature 30°C, pH 7 and 200 rpm for 42 h. The results obtained from time course study indicated that the highest biosurfactant production occurred and established in the stationary growth phase. The best production of 1.63 g/L was achieved with free cells and 1.78 g/L with alginate entrapped cells of *P. putida* 922. The biosurfactant was able to reduce the surface tension of water from 72 to 31mN/m and form stable emulsion with a range of hydrocarbons and vegetable oils. The emulsification activity was highest against coconut oil i.e. 83±3.6%. The rhamnolipid also showed remarkable stability during exposure to high temperature (up to 121°C), high NaCl concentration (14%) and to a wide range of pH (2-12).The produced rhamnolipid was recovered by chloroform: methanol extraction and show striking antimicrobial activity against *E. coli, Micrococcus luteus, Candida albicans, Streptococcus epidermidis* and *Klebsiella Pneuminiae.* TLC studies designated the presence of two dominant rhamnolipid types i.e. mono and di-rhamnolipid. Compositional analysis revealed that the extracted biosurfactant contained lipid (68.66%, w/w) and carbohydrate (31.34%, w/w). The Fourier Transform Infrared spectrum of extracted biosurfactant specify the presence of hydroxyl, carboxyl, amine and methoxyl functional groups and inveterate that the extracted biosurfactant was a rhamnolipid.