Abstract

The fermentative production of secondary metabolites biosurfactant (rhamnolipid) was carried out by immobilized cells of *Pseudomonas fluorescens*. It gave positive results for all the qualitative tests for biosurfactant production and able to produce sustainable biosurfactant with excessive foaming. Among all the eighteen immobilization matrices, sodium alginate was proved to be the best immobilization carrier for highest rhamnolipid production with least cell leakage. The optimized bioprocess conditions were sodium alginate 3% (w/v), 0.2 M $CaCl_2$, hardening time 30 min, inoculum 1.5% (w/v) wet weight of *P. fluorescens* cells, beads size 3mm and number of beads 200. The maximum rhamnolipid synthesis was attained by consuming 2% (w/v) glucose as a carbon source, 0.1% (w/v) NH_4NO_3 as the nitrogen source and C/N ratio of 20, temperature 30°C, pH 7 and 200 rpm for 24 h. The highest production of RL i.e. 3.49±0.15 g/L was achieved with immobilized cells and 1.71±0.1 g/L with free cells. So, it was determined that immobilized cells offered the 2.04 folds more RL yield as compared to free cells. The positive outcomes of this investigation are, the RL production significantly increases from 1.52±0.08 g/L to 3.49±0.15 g/L (2.29 folds) after the optimization of all the production parameters. The best recovery method was acetone extraction. The biosurfactant was capable to reduce the surface tension of water from 72 to 34 mN/m and formed very stable emulsion with different hydrocarbons and vegetable oils. The emulsification activity was highest against sunflower oil i.e. 70%. The rhamnolipid also exhibited outstanding stability during exposure to high temperature, high NaCl concentration and to huge range of pH. The produced RL displayed maximum antimicrobial activity against *B. subtilis* i.e. 14 mm. The bacterial adherence to hydrocarbons (BATH) assay revealed that the hydrophobicity of cells was effectively reduced from 40 to 16% in the presence of rhamnolipid. Thin layer chromatography (TLC) studies indicated the presence of mono-rhamnolipid. Compositional analysis of RL has shown that the extracted biosurfactant confined lipids (65%) and carbohydrates (35%). The Fourier Transform Infrared spectrum (FTIR) of extracted biosurfactant specify the presence of hydroxyl and carboxyl functional groups and confirmed that the extracted biosurfactant was a rhamnolipid in nature. Moreover, to our knowledge, it is the first report on detailed process optimization from immobilized cells of *P. fluorescens* for maximum rhamnolipid production.