Abstract:

In the current research exopolysaccharide levan was produced using immobilized cells of *Bacillus subtilis* NRRL B-941. Fourteen immobilization matrices were screened to produce levan. Only sodium alginate showed the characteristics of the best immobilization matrix for the maximum levan yield along with less cell leakage. Immobilization conditions were sodium alginate 3% (w/v), 0.2 M $CaCl_2$, curing time of beads 30 min, 1.5% (w/v) wet weight of *B. subtilis* cells, diameter of beads 3 mm and number of beads 200. Fermentation conditions for the highest levan yield were sucrose as carbon source 200g/L, nitrogen Sources 1.5 g/L, pH 6.5, 42 h incubation time, temperature 37°C, agitation Speed 200 rpm. Immobilization of the cells gave maximum yield of 66±0.28 g/L. Meaningful offshoot of this study was that levan yield lifts from 31±0.36 g/L to 66±0.28 g/L (2.12 folds increase) after the optimization of all the production parameters. The effective and the excellent extraction and precipitation method was ethanol extraction. Viscosity of levan was estimated as 582.55 cP. Thin layer chromatography technique confirms the levan. The Fourier Transform Infrared spectrum (FTIR) of extracted levan highlighted the presence of pyran ring, hydroxyl and carboxyl functional groups and confirmed that the extracted polysaccharide was levan in nature. To assess the thermal stability of levan thermogravimetric analysis, TGA and differential scanning calorimetry, DSC were performed. Glass transition temperature (T_g) of levan was observed to be around 77°C. Onset of thermal decomposition (T_o) of levan took place about at 210°C. The second characteristic peak appeared around 240°C which indicated the melting transition (T_m) of levan. The maximum weight loss (T_p) occurred between the temperature ranges of 500 to 550°C. Scanning electron microscope showed the structural images of immobilized cells in levan and matrices.