

Production of Biosurfactant to Combine Two-stage Culture with Immobilization of Resting Cells in *Bacillus subtilis*

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ABSTRACT

Biosurfactants is a term that mainly refers to surface active molecules that are produced by a variety of microorganisms. As with most surface active materials, they have a hydrophilic and a hydrophobic portion. In general the advantages of these biomolecules over synthetic surfactants are their low-toxicity, high-biodegradability and the easy to use cheap, renewable substrate for commercial production. The increasing interest in the potential application of microbial surface active compounds is based on the ecological compatibility concern in biotechnology development. This study employs a semi-continuous culture system which combines a two-stage process with the immobilization of resting cells for lipopeptide biosurfactant formation in *Bacillus subtilis*(ATCC 21332). Results appeared a decrease of the surface tension to 30 dyne/cm in the culture broth at 8hr under two-stage culture, which led to shorter process and higher productivity. A chitosan prepared by deacetylation of chitin was chosen as the carrier for cell immobilization. Various types of chitosan beads were prepared through cross-linkage and functional groups modification chemically to obtain better resistant and absorbent capacity. The ultrastructure and the pore size distribution of the chitosan beads from different preparation were observed under SEM from different preparation. High loading of 8.7×10^7 cells/ml-bead was achieved in carboxyl modification of CCB. The maximum production of surfactin from broth quantified by HPLC to reach 750mg/L in two-stage culture of *B. subtilis*. This paper deals with the application of a novel semi-continuous cultural system combined with cell immobilization also to simplify separation process by foam recovery. A very active biosurfactant which can reduce the surface tension to 23 dyne/cm from foam collection liquid, the lowest value have ever been appeared in literature, it exhibits the excellent technical creativity and composite applications regarding industrial considerations. Key word: *Bacillus subtilis*, biosurfactant, surfactin, cell immobilization, semi-continuous cultural system, SEM, HPLC.

Keywords : surfactin ; biosurfactant ; immobilization ; resting-cell ; *Bacillus subtilis*

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