Abstract

On mapping the present global scenario, it is discernible that the demand for microbial extracellular polymeric substances (EPSs) will continue to increase as promising alternatives to petroleum-based synthetic polymers, due to their biodegradable and non-toxic properties. Microbial EPSs exhibit a wide range of distinct physicochemical characteristics, due to which they can find potential applications in the areas of healthcare, biomedical, food, cosmetics and environment. Despite these advantages, the viability and sustainability of microbial EPS production at a large scale are questioned, primarily due to very low product yields. The present thesis thus focused on designing, developing and optimizing a bioprocess for the production, purification and partial characterization of an EPS from a thermophilic bacterium, *Bacillus licheniformis,* followed by its performance evaluation as a reducing and stabilizing agent in nanoparticle synthesis. Initially, a suitable production medium was formulated through systematic screening and optimization of essential nutrients. This was achieved by using methodologies like the Plackett–Burman (PB) design followed by the application of the Support Vector Machine–Teaching Learning Based Optimization (SVM-TLBO) technique. Medium optimization resulted in about five-fold increase in EPS production. Subsequently, optimization of the critical process parameters was accomplished in a 3.7 L bench-top fermenter by employing the Response Surface Methodology–Teaching Learning Based Optimization (RSM-TLBO) method. An optimal combination of agitation (222 rpm), aeration (1.2 vvm) and temperature (49°C) enhanced EPS production further by about 20%. This optimization study showed that temperature had a more pronounced effect on EPS production, followed by agitation and aeration. A multi-step downstream processing strategy was developed for the purification of the EPS product. The strategy involved extracting the EPS from the cell-free supernatant by acetone precipitation, followed by deproteination and dialysis. The crude EPS obtained was then purified by ion exchange and gel filtration chromatography. The average molecular weight of the purified EPS was determined to be 1510 kDa. Thereafter, the chemical composition and physical properties of the purified EPS were determined by employing sophisticated analytical techniques including FTIR, GC-MS, SEM, XRD etc. The findings revealed that the purified molecule was a heteropolysaccharide, mainly composed of glucose, fructose and galactose. The molecule exhibited considerable thermal stability with a degradation temperature of 257°C. Since the quantity of the final purified product was limited, the partially purified product was evaluated for its performance as a reducing and stabilizing agent in silver nanoparticle synthesis. The silver nanoparticles had a narrow size distribution (8-32 nm) with an average particle diameter of 16 nm. These nanoparticles were found to be stable in the solution for up to three months. In a global scenario, where the commercialization of EPS is limited due to low product yields, this study holds the promise and potential to make a noteworthy contribution towards realizing feasible EPS production from a thermophilic bacterium.

Keywords: Microbial exopolysaccharide; Fermentative production; Medium optimization; SVM-TLBO method; Process optimization; RSM-TLBO technique; Chromatographic purification; Partial Characterization; Nanoparticle synthesis & stabilization