



ABSTRACT

The present study deals with the production of tyrosine hydroxylase from *Aspergillus oryzae* IIB-9 under submerged fermentation and its immobilization on ZnO nanocrystals for improved stability and catalytic efficiency towards L-dopa production. The optimized parameters for attaining maximum TH activity were the volume of Vogel's medium (75ml), time of incubation (72 h), initial pH (5.5) and size of inoculum (1.5 ml). Moreover, the TH activity was further enhanced by immobilization on ZnO nanoparticles. The UV-Vis spectrum showed an absorption band for the WG-ZnO-NPs and FP-ZnO-NPs dispersed in isopropanol at 295 and 285nm, respectively. The results of XRD showed the crystalline nature of ZnO-NPs. In FTIR, the observed band from 740/cm to 648.1/cm and 735.8/cm to 650.1/cm showed the stretching vibrations of WG-ZnO-NPs and FP-ZnO-NPs, respectively. The SEM images confirmed the particle size of the ZnO-NPs between 130-170 nm. The optimized TH activity by varying enzyme concentration for the immobilization of TH on WG-ZnO-NPs and FP-ZnO-NPs were 2.52 ± 0.14 IU/ml and 2.41 ± 0.08 IU/ml, respectively. The ZnO-NPs quantity (25-150 mg) and procurement period (5-50 min) were also optimized for the immobilization of TH on ZnO-NPs. Further, the stability of immobilized TH on ZnO-NPs was checked by optimizing the time of incubation (10 min for WG-NPs and 15 min for FP-NPs) and temperature (45°C for WG-NPs and 30°C for FP-NPs). The catalytic efficiency of TH was also investigated by incubating enzymes with different copper, iron, manganese and zinc salts. The maximum catalytic efficiency of the TH enzyme was observed with 50 mM $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ i.e. 3.76 ± 0.06 IU/ml. An 11.05-fold increase in the TH activity was examined by its immobilization on ZnO-NPs. Hence, the immobilization of TH on ZnO-NPs not only increased its activity but also its stability and catalytic efficiency.