ABSTRACT
The present work deals with the production of tyrosine hydroxylase from Bacillus subtilis SDSC-Env-i6 under submerged fermentation and its immobilization on Al2O3 nanocrystals for improved stability and catalytic efficiency towards L-dopa production. The biochemical reaction was carried out using acetate buffer as an extractant. The maximum L-dopa production (1.376 mg/ml±0.003), L-tyrosine consumption (2.115 mg/ml±0.001) and TH activity (4.9 U/ml±0.04) was achieved with 50 ml harvesting medium, pH 6.5, 48 h of incubation and inoculum size of 1.5 ml. By using broth at 4°C, maximum inhibition was obtained with 10 ppm and 15 ppm concentration and 1 min of time of inhibitor addition at 35°C (0.061±0.01 µmole) and 65°C (0.093±0.05 µmole) for benserazide and carbidopa, respectively. While for supernatant at – 20°C, maximum inhibition by benserazide and carbidopa was with 30 ppm concentration and after 2 (0.129±0.001 µmoles) and 5 (0.341±0.001 µmole) min of their addition. The thermostability and catalytic efficiency of TH was evaluated by incubating it at different temperatures and with different concentrations of copper and calcium salts, respectively. At optimized conditions, TH activity was further enhanced by immobilization on Al2O3 nanoparticles. The maximum activity of immobilized TH by varying enzyme concentration (0.1-0.6 ml), Al2O3 nanoparticle concentration (25-300 mM) and procurement period (10-80 min) was 12.2±0.12 U/ml, 14.4±0.14 U/ml and 15.3±0.05 U/ml, respectively. A 5-fold higher TH activity was recorded after its immobilization on Al2O3 NPs. The UV-VIS spectrum exhibited an absorption band for TH immobilized Al2O3 nanoparticles dispersed in distilled water at 253 nm. In FTIR analysis, band obtained at 520-900/cm was characterized as finger print region of Al2O3 nanoparticles. The XRD results proved the crystalline nature of these nanoparticles. Moreover, SEM images confirmed the particle size of Al2O3 nanoparticles between 124-130 nm. The results are highly significant ($p \leq 0.05$) and indicate potential commercial utility of immobilized TH on Al2O3 nanocrystals.