

ABSTRACT

A cloned esterase gene from thermophilic bacterium *Thermotoga naphthophila* XD57-0650 was expressed in *E.coli* to produce recombinant esterase enzyme and immobilize *T. naphthophila* esterase onto silica coated iron oxide ($\text{Fe}_3\text{O}_4@\text{SiO}_2$) magnetic nanoparticles via covalent binding. 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) was employed for coupling of the amino functional group on the nanoparticle surface and the amino group $-\text{NH}_2$ of the recombinant esterase enzyme. Fourier transform infrared (FTIR) spectroscopy showing particular bond shift confirmed the binding of esterase to the particles. Enzymatic activity assays were performed for both forms of enzymes through colorimetric method by monitoring the dissociation rate of its specific substrate, ethyl acetate; and titration method using olive oil as natural substrate. Maximal activities were observed at 77°C for free and immobilized enzyme solution; however concerning thermal stability, the immobilized enzyme retained more than 70% of its residual activity, after enzyme incubation beyond 80°C for 15 minutes, compared to less than 45% for the free enzyme. Enhanced reusability of esterase in continuous processes was observed as it kept 28% of its initial activity even after nine sequential cycles, suggesting favorable operational stability of the enzyme. Immobilized enzyme was applied to enhance the hydrolytic capacity of xylanase enzyme for sugarcane bagasse to produce fermentable sugars in a saccharification experiment and it was found to augment the sugar yields by 1.36 folds even at a modest loading (20% v/v of reaction volume) showing a considerable potential in industrial applications.