TITLE: Covalent immobilization of a glucose-stimulated β-glucosidase from *Humicola insolens* on functionalized ferromagnetic nanoparticles and its biotechnological application

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ABSTRACT: Magnetic nanoparticles can be very efficient support for enzyme immobilization due to their ideal characteristics to balance the main factors that determine the efficiency of biocatalysts, including specific surface area, resistance to mass transfer, effective enzymatic loading and easy recovery using an external magnetic field. In this work, the recombinant β-glucosidase from *Humicola insolens* (Bglhi) was covalently immobilized on synthetized Fe3O4 nanoparticles derivatized with chitosan and glutaraldehyde to produce Bglhi-MNPs. The immobilization efficiency and yield of Bglhi-MNPs were 145% and 8.3%, respectively. Bglhi-MNPs retained about 50% of the initial activity even in the tenth reuse cycles. The optimum temperature and pH of catalysis were 50 °C and pH of 6.0, respectively. The immobilized enzyme was less thermostable ($t_{1/2}$ 50°C < 10 min) and less stimulated by glucose (about 1.4-fold) when compared to the free enzyme ($t_{1/2}$ 50°C = 60 min; stimulation by glucose of 1.7-fold). Bglhi-MNPs hydrolyzed *p*-nitrophenyl-β-D-glucopyranoside with maximal velocity and an apparent affinity constant of 71.2 ± 3.1 U mg^{-1} and 0.34 ± 0.02 mmol L^{-1}, respectively, resulting in a catalytic efficiency of 209.4 U mg^{-1}/mmol L^{-1}. The immobilized enzyme showed higher synergistic effect (3.0-fold) with Celluclast 1.5 L for the hydrolysis of Whatman no. 1 filter paper as compared with the estimated effect for free enzyme (2.3-fold). Futhermore, Bglhi-MNPs was able to act on pretreated fraction sugarcane bagasse releasing about 26.8% more glucose than the unsupplemented commercial cocktail. These results demonstrate that covalent immobilization was a good strategy for Bglhi immobilization and indicated the excellent application potential of Bglhi-MNPs in the hydrolysis of lignocellulosic biomass.

Keywords: β-glucosidase, Covalent immobilization, ferromagnetic nanoparticles, hydrolysis of lignocellulosic biomass.

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