Immobilization of recombinant β-glucosidase on magnetic nanoparticles and its potential in production of ginsenoside F_2 and Compound K

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In the present study, *BbBgl* gene was cloned and expressed in *E. coli* BL21. The β-glucosidase was purified by Ni-NTA magnetic beads to obtain an enzyme with specific activity of 37 U/mg protein using *p*NP-Glc as substrate. The enzyme activity was optimized at pH 5.0, 35°C, 2 or 6 U/ml. The recombinant β-glucosidase was immobilized to magnetic Fe_3O_4 nanoparticles. Furthermore, the optimal conditions for the immobilization and some characteristics of immobilized enzyme were studied. The optimal immobilization conditions observed were enzyme 0.15 mg (1 mg/mL, 0.15 mL), magnetic Fe_3O_4 nanoparticles 3 mg, pH 5.0, immobilization time 4 h. The results showed that the optimal reaction temperature of immobilized enzyme kept in a high activity from 35°C to 40°C. Thermal stability of the immobilized enzyme also had an improvement, the residual activity retained 62% and 57% after 180 h at 35°C and 40°C, respectively, while free enzymes only showed 55% and 51% remnant activity at the same condition. The immobilized enzyme also exhibited good operational stability. In terms of the thermal and operational stability, the immobilized enzyme could be better used in many more applications than the free enzymes. Moreover, the enzyme exhibits strong tolerance against high substrate concentration (up to 40 g/L ginsenoside Rd) with a molar biotransformation rate of 81% within 12 h. The magnetic nanoparticle immobilized β-glucosidase from *B. breve* can biotransform major ginsenoside Rd selectively to F2 and CK, indicating a great potential in industrial application for efficient production of rare ginsenoside F2 and CK.

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