Title:	Multiple thermostable enzyme hydrolases on magnetic nanoparticles: An immobilized enzyme-mediated approach to saccharification through simultaneous xylanase, cellulase and amylolytic glucanotransferase action
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Keywords:	Immobilization Magnetic nanoparticles (MNPs) Stability Xylanase Cellulase Glucanotransferase
Issue Date:	2018
Publisher:	Elsevier B.V.
Citation:	International Journal of Biological Macromolecules, 120, pp. 1650-1658
Abstract:	Microbe-derived enzymes such as xylanases, cellulases and amylases, are efficient at hydrolyzing plant biomass. Efforts to harness the functionalities of these enzymes towards applications in energy and fuel biosciences, and food and nutrition, continue apace in many laboratories. Given that enzymes derived from mesophile proteomes undergo facile denaturation and/or degradation at ambient temperatures, and require frequent replenishment during bioprocessing, it is desirable that they be replaced by structurally-stable enzymes capable of functioning efficiently and resisting denaturation and degradation, immobilized on solid media to further add to stability and facilitate recovery and reuse. Towards these objectives, we used synthetic magnetic nanoparticles (MNPs) and immobilized upon their surfaces three different structurally-stable hydrolases: a thermostable xylanase (BSX) derived from Bacillus sp. NG-27, a cellulase (RMCel12A) derived from Rhodothermus marinus, and an amylase-cum-glucanotransferase (PfuAmyGT) derived from Pyrococcus furiosus. The MNPs were activated with glutaraldehyde and BSX, RMCel12A, and PfuAmyGT, respectively, were covalently immobilized with efficiencies of ~92%, 45% and 93%. The enzymes and the MNPs were fully characterized before and after immobilization, and the immobilized enzymes were found to be active at 50 °C against synthetic substrates as well as pre-treated biomass derived from corn cob and rice husk. The enzyme-coupled MNPs displayed high stability upon storage properties, high operational stability as well as high reusability (retaining 69, 48, and 50% residual activity after 13 uses for BSX, RMCel12A and PfuAmyGT, respectively). Experiments were also conducted with MNPs loaded simultaneously with all three enzymes. Such immobilized enzyme combinations on MNPs can be used in the saccharification of plant biomass.
Description:	Only IISERM authors are available in the record.
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