



PAPER

 α -amylase immobilized on magnetic nanoparticles: reusable robust nano-biocatalyst for starch hydrolysisRECEIVED
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4 July 2018R P Dhavale¹, S B Parit², Subasa C Sahoo³, P Kollu⁴, P S Patil⁵, P B Patil⁶ and A D Chougale⁶¹ School of Nanoscience and Technology, Shivaji University, Kolhapur, Maharashtra—416004, India² Department of Chemistry, The New College, Shivaji University, Kolhapur, Maharashtra—416012, India³ Department of Physics, Central University of Kerala, Kasaragod, Kerala—671314, India⁴ CASEST, School of Physics, University of Hyderabad, Gachibowli, Hyderabad, Telangana—500046, India⁵ Thin Film Magnetism group, Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge CB3 0HE, United Kingdom⁶ Department of Physics, The New College, Shivaji University, Kolhapur, Maharashtra—416012, IndiaE-mail: ashokdchougale@gmail.com**Abstract**

Immobilization of enzymes on magnetic nanoparticles (MNPs) could offer reusability along with increased stability and robust operation at different physicochemical conditions. In this work, magnetically recoverable nano-biocatalyst was prepared by immobilizing industrially important α -amylase on chitosan coated Fe₃O₄ MNPs (AMNPs) and evaluated for its activity and stability. MNPs were characterized by x-ray diffraction, field emission scanning electron microscope and vibrating sample magnetometer. Functional groups on MNPs and chitosan coated MNPs along with immobilization of α -amylase on chitosan coated MNPs were analyzed by Fourier transform infrared spectroscopy. Amount of immobilized α -amylase was optimized by varying enzyme concentration and incubation time during immobilization process. The activity and stability of the AMNPs and free α -amylase were investigated. It was found that AMNPs have better starch digestion capacity at different physicochemical conditions than that of free α -amylase. The immobilized enzyme retained 66% activity after 20 days compared to 18% activity of the free enzyme. AMNPs were reused for 20 times, without significant loss of activity, by magnetically separating the nano-biocatalyst from the reaction mixture after each starch hydrolysis cycle.

Introduction

Use of biocatalysts (enzymes) for various chemical processes allows to limit the use of hazardous chemicals [1–4]. One of the noteworthy properties of enzymes like excellent selectivity and high reactivity permit them to perform the most complex chemical processes in a simple way. The limitation of the use of enzymes is that they work under the most benign experimental and environmental conditions [5, 6]. Therefore, the engineering of enzymes from biological entities, to make them compatible in industrial reactors, is a very exciting goal. Different techniques are employed to improve the enzyme features which allow them to be used under industrially relevant conditions. Techniques such as immobilization, genetic engineering, microbiology, protein engineering, proteins chemistry etc have contributed to impressive developments in this field [7–9]. Immobilization of the enzymes improves storage and operational stability [10–12]. Enzymes are immobilized on a solid support by a covalent bond via various chemical bonding methods, such as cross-linking, multifunctional reagents, or surface reactive functional groups [13–17]. Several nanoparticle-based supports such as Au, Cu, silica etc have been utilized for the immobilization of different enzymes [18–20]. Along with above-discussed benefits, immobilization of the enzyme on magnetic nanoparticles (MNPs) offers the additional benefits of easy magnetic separation and reuse [21].

The hydrolysis of starch to low molecular weight sugars, which have more economic values, is one of the most important industrial process [22–25]. Use of α -amylase for hydrolysis of starch completely diminishes the practice of chemical hydrolysis and hence it shares about 25% of universal trade of enzymes [22]. α -amylase