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Research paper

Manuscript title: Halloysite nanotubes - An efficient 'nano-support' for the immobilization of α-amylase

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ABSTRACT

Amylases are a class of hydrolytic enzymes which help in the conversion of starch into reduced sugars. Several solid carriers have been utilized for the immobilization of amylase. The current study focused on the utilization of halloysite nanotubes (Hal nanotubes) for the immobilization of α-amylase post their surface functionalization with APTES. The immobilized enzyme was characterized for its ultrastructure and morphology using TEM which revealed the hollow tubular structure of nanotubes. Chemical characterization of Amylase-Hal nanotubes powder was done by FTIR in which the characteristic reflections of pristine Hal nanotubes and amylase were detected in the spectra for Amylase-Hal nanotubes powder. The thermal and crystalline behavior of the immobilized enzyme was studied using DSC and XRD analysis respectively. Further, the effect of time, pH, temperature and metal ions on the enzymatic activity of immobilized enzyme had also been probed into. The optimum pH and temperature for the immobilized amylase was found to be 7.4 and 40 °C respectively. Slight increase in the enzymatic activity was also found for immobilized amylase as compared to free enzyme in the presence of Cu^{2+} and Mn^{2+} ions.

1. Introduction

Enzymes are biocatalysts that are highly competent and specific under diverse environments because of which, they have numerous industrial applications. The enzymes used for such applications are expected to aid in several prospects like efficient consumption of reactants, improvement of the operational time and the maximization of velocity of catalysis (Panzavolta et al., 2005; Kahraman et al., 2007; Tripathi et al., 2007; Sahoo et al., 2011). Limitations associated with the usage of enzymes in industries include low stability, high selectivity towards the process conditions and high cost (Krajewska, 2004; Cao, 2005; Hernandez and Fernandez-Lafuente, 2011). During the last two decades, several advancements have been done in the field of enzyme technology which assist their usage for numerous industrial applications (Veesar et al., 2015). Nelson and Griffin in 1916 discovered that invertase on absorption onto charcoal hydrolyses sucrose (Nelson and Griffin, 1916). Since then immobilization of enzymes on a solid support has gained considerable attention. Immobilization is a technique in which insoluble bio-catalytic derivatives are prepared through coupling of enzymes to organic or inorganic solid supports. Some of the advantages of immobilization of enzymes include enhanced stability towards organic solvents and hostile reaction conditions, repetitive usage, easy separation from the reaction mixture, formulation of product in a controlled manner and high thermal stability (Monsan and Combes, 1988; Mateo and Torres, 2003). Organic support materials lack several properties that the inorganic materials benefit from. Some of the properties include resistibility against microbial attacks and organic solvents, stability and ease of reusability or disposal (Rawtani and Agrawal, 2012a).

Amylases are a class of hydrolytic enzymes which help in the conversion of starch into reduced sugars, syrups and dextrin (Pascoal et al., 2011). They act as an important biocatalyst in food, fermentation, detergent, paper and textile industries (Kirk et al., 2002; Van der Maarel et al., 2002; Gupta et al., 2003; Polaina and MacCabe, 2007). The enzyme due to its many applications in different industries provokes the need to be immobilized in order to favor the improvement of stability and reusability of enzymes like many others (Zhongjie et al., 2013) Several physical and chemical methods are used to immobilize amylase. In chemical method, a formation of covalent or ionic bond between the support and the enzyme occurs, while in physical immobilization techniques, enzymes are either adsorbed or encapsulated on or inside a solid support (Diez et al., 1996; Gancarz et al., 2006; Chen et al., 2009; Sen et al., 2011; Herr et al., 2013). Solid carriers used for the immobilization of enzyme for industrial applications should have porosity, suitable hardness and density (Zhongjie et al., 2013). Glass beads (Kahraman et al., 2007), Zirconium dioxide (Reshmi et al., 2007), metal ceramic powder (Zhongjie et al., 2013), porous molecular sieves (Yiu et al., 2001) and alumina (Reshmi et al., 2006) are some of the solid carriers incorporated

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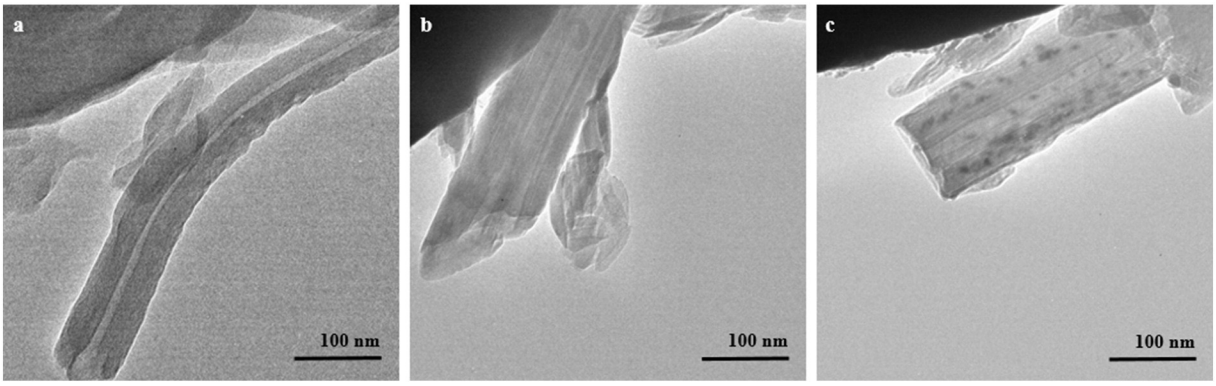

Fig. 1. TEM micrographs of (a) Hal Nanotubes (b) Functionalized Hal Nanotubes and (c) Functionalized Hal Nanotubes with immobilized amylase.

for immobilization of amylase. Clay minerals can also be used as an effective solid support for the immobilization of enzyme (Rahman et al., 2005).

Clay is formed through the combination of one or more clay minerals with traces of metal oxides and organic matter. Clay minerals such as kaolinite and montmorillonite are generally formed through the gradual chemical weathering of rocks over a long period of time. Clay mineral consists of layers that are negatively charged, with hydrated cations placed in the interlayer spaces balancing the negative charge (Murray, 1991, 2000; Joseph et al., 2013). Hal is one such clay mineral that is chemically an aluminosilicate and is similar to kaolin which has the same chemical formula as $Al_2Si_2O_5(OH)_4$ (Levis and Deasy, 2002). Hal nanotubes are ultra-small hollow tubes whose diameters are generally lesser than 100 nm. The lengths normally range from about 500 nm to over 1.2 μm. Aluminum, silicon and hydrogen elements are the key constituents of these nanotubes (Rawtani and Agrawal, 2012a, 2012b). The chemical properties of the outer surface of Hal nanotubes are similar to that of silica (SiO_2), and bears a negative charge at higher pH (zeta potential) while alumina (Al_2O_3) constitutes the inner core and provides a positive charge at lower pH (Tari et al., 1999). Due to their large specific surface area, high biocompatibility and nanotubular structure, they are used as support materials. They have been widely used for the loading and controlled release study of various substances which range from low molecular mass organic molecules to complex biochemical molecules (Yuan et al., 2015). Some of the low molecular mass compounds loaded on Hal nanotubes include benzotriazole (Abdullayev and Lvov, 2010), glycerol (Suh et al., 2011), ibuprofen (Tan et al., 2013, 2014), resveratrol (Vergaro et al., 2012) and 5-fluorouracil (Rao et al., 2014). Biochemical substances like lipase (Vaidya et al., 2008), metalloporphyrins (Machado et al., 2008), urease (Zhai et al., 2010), antisense oligonucleotides (Shi et al., 2011), and insulin (Abdullayev and Lvov, 2011) have also been loaded in the Hal nanotubes. Enzymes like

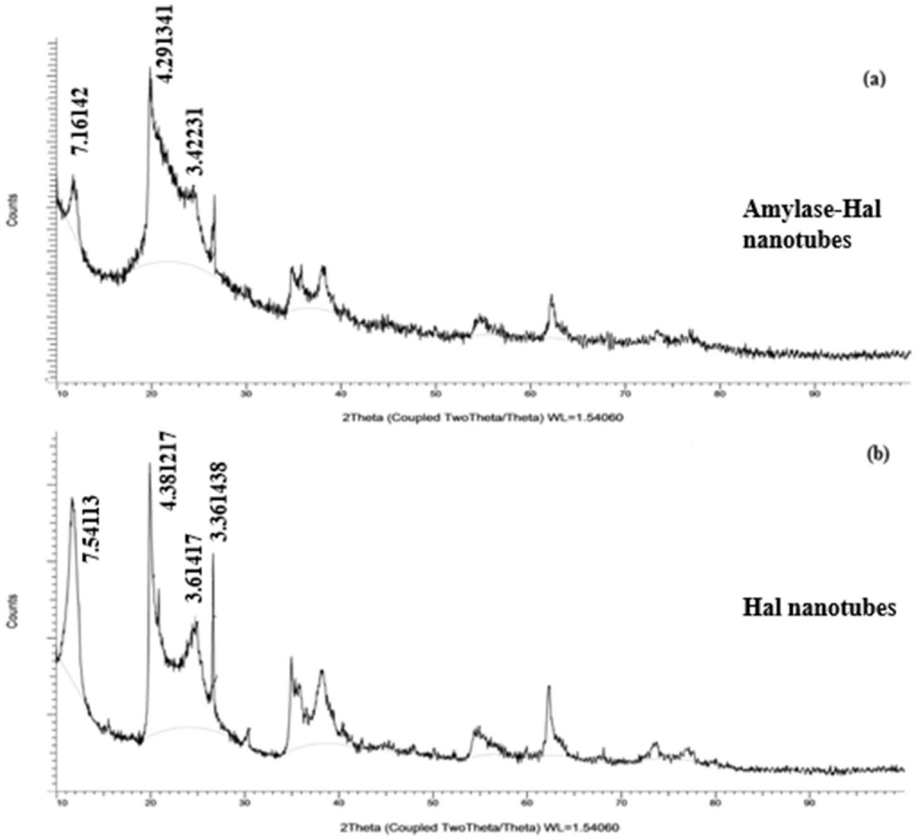

Fig. 2. XRD Patterns of Amylase-Hal nanotubes and Hal nanotubes.