



# Lipase immobilized on magnetic multi-walled carbon nanotubes

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## ABSTRACT

Magnetic iron oxide nanoparticles were loaded onto the surfaces of multiwalled carbon nanotubes (MWNTs) by the impregnation method. The obtained magnetic nanotubes were characterized with high-resolution transmission electron microscopy (HRTEM), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD). *Yarrowia lipolytica* lipase was covalently immobilized on the magnetic MWNTs (M-MWNTs). M-MWNT-lipase was characterized with XPS spectra and XRD patterns. The structural change of the immobilized lipase was analyzed through circular dichroism spectroscopy. M-MWNT-lipase was utilized for the resolution of (*R*, *S*)-1-phenyl ethanol in the organic solvent of heptane. Compared to the native lipase, the lipase immobilized on M-MWNTs has significantly improved its enzymatic activity for the resolution of (*R*, *S*)-1-phenyl ethanol in heptane. M-MWNT-lipase can be easily recovered after catalysis. In addition, the effect of sonication time on the catalytic activity was also investigated. It is found that, up to 30 min sonication, the catalysis by M-MWNT-lipase is almost not affected. While, the catalytic activity of the native lipase is decreased with sonication time.

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## 1. Introduction

Practical use of enzymes has been realized in various industrial processes, and is being expanded in new fields, such as fine-chemical synthesis, pharmaceuticals, biosensors, and biofuel cells (Kim et al., 2006). Enzymatic catalysis in non-aqueous media has been paid a great deal of attention, because organic compounds as substrates are very sparingly soluble in water and are sometimes unstable in aqueous solutions. The catalytic activity displayed by enzyme in organic solvents is significantly lower than in water. To improve enzyme activity and stability, enzymes have generally been studied with the enzymes immobilized on a solid support (Laurent et al., 2008). Immobilization of enzymes can facilitate enhancing catalytic activity, stability and selectivity as well as recycling the enzymes (Kim et al., 2006; Bai et al., 2006; Dyal et al., 2003). Nanomaterials can serve as ideal supporting materials for enzyme immobilization, because nanoscale materials provide the upper limits in balancing the key factors that determine the efficiency of biocatalysts, including surface area, mass transfer resistance, and effective enzyme loading (Wang et al., 2006, 2010; Jia et al., 2003).

Lipases are produced in high yields and show high chemo-, region- and stereo-selectivity (Hu et al., 2009a,b; Lu et al., 2009;

Kosaka et al., 2007; Lumor and Akoh, 2008), they have been widely used as biocatalysts in organic synthesis. Magnetic nanoparticles have been used for lipase immobilization (Dyal et al., 2003; Kim et al., 2005), such as liquid-modified magnetic nanoparticles (Jiang et al., 2011) and hydrophobic magnetic nanoparticles (Lee et al., 2009) are used to immobilize lipase. The immobilized lipase has a variety of applications. They include resolution of racemic compounds (Bai et al., 2006; Chaubey et al., 2009; Yilmaz et al., 2011; Hu et al., 2009a,b; Andrade et al., 2010), biodiesel production (Dus-san et al., 2011), and ester hydrolysis at oil–water interface (Jiang et al., 2011).

Recently, carbon nanotubes (CNTs) have been used for enzyme immobilization (Gao and Kyratzis, 2008; Feng and Ji, 2011). CNTs consist of graphitic sheets, which have been rolled up into a cylindrical shape. The length of CNT is in the size of micrometers with diameters up to 100 nm (Tasis et al., 2006). CNTs exhibit extraordinary mechanical, electrical and thermal properties as well as bio-compatible property (Shim et al., 2002). Enzyme immobilization is a promising biotechnological application of CNTs (Gao and Kyratzis, 2008; Hansen et al., 2010). For fundamental studies, a broad range of enzymes have been covalently attached onto CNTs (Asuri et al., 2006). The conjugates are stable at high temperatures, providing a unique combination of useful attributes such as low mass transfer resistance, high activity and stability, and reusability. High enzyme-loadings on multiwalled carbon nanotubes can be achieved (Zhang et al., 2009; Ji et al., 2010).

In this work, magnetic multi-walled carbon nanotubes (M-MWNTs) is prepared and used to immobilize lipase. The conjugate

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