Enzyme Immobilization on Magnetic Nanoparticles for Enhancing Biocatalysis

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ABSTRACT

Magnetic nanoparticles have a wide range of applications in catalysis, separation, magnetic resonance imaging and drug delivery. This talk focuses on the synthesis, functionalization and application of magnetic nanoparticles for improving biocatalysis. Great progress in synthesizing uniform superaramagnetic nanoparticles with high saturation magnetization has been achieved, and various functional magnetic nanoparticles with specific components have been fabricated for enzyme immobilization in enhancing biocatalysis. Achievements in our research group together with recent progress in the world will be critically reviewed in this talk, and future trends and perspectives in the biocatalysis areas with robust magnetic nanoparticles will be outlined.

Keywords: Biocatalysis, Enzyme, Magnetic nanoparticles

1 INTRODUCTION

Magnetic nanoparticles (MNPs) are well-established nanomaterials that offer controlled size, ability to be manipulated easily[1-3]. Due to their portential applications in medical drug delievery, mangetic resonmance imaging, bioseparation, enzyme immobilization, and so forth, there is still a great interest to develop novel technologies for frabricating various functional MNPs together with new types of magnetic deveices [4-6].

Biocatalysis using the immobilized enzymes has been widely used in food, fine chemical and pharmaceutical industries because they provide many advantages over free enzymes including repeated or continuous reuse, easy separation of the product from reaction media, easy recovery of the enzyme and improvement in enzyme stability. A wide variety of methods have been employed in the immobilization of enzymes, such as adsorption, entrapment, cross-link and covalent attachment. Among these immobilization techniques, magnetic carrier technology has become very attractive for the preparation of immobilized enzymes [7, 8]. The specific magnetic particles can be produced by immobilization of an affinity ligand on the surface of prefabricated magnetic beads, which can be quickly separated from the reaction medium and controlled by applying a magnetic field; then the catalytic efficiencyand stability properties of the enzyme can be greatly improved [9-11].

2 MAGNETIC BIOCATALYST

Novel fabrication methods have been established to obtain uniform magnetic particles, and a series of temperature & pH-responsive, surface-functional, mesoporous smart magnetic nanomaterials were synthesized for enzyme immobiliztion. For example, magnetic mesoporous silica nanoparticles (MMSNPs) with wormhole framework structures were synthesized by using tetraethyl orthosilicate as the silica source and amine-terminated Jeffamine surfactants as template. These nanoparticles exihibited excellent superparamagnetism, and their pore size was regulated by synthesis temperature. When the synthesis temperature increased from 25 ℃ to 80 ℃, the pore size of particles increased from 3.6 nm to 27.1 nm, and the pore volume increased from 0.82 $cm^{3/}g$ to 3.00 cm^3/g, and the BET surface area decreased from 820 m^2/g to 501 m^2/g. Iminodiacerate was attached on these MMSNPs through a silane-coupling agent and chelated with Cu^{2+} (Figure 1).

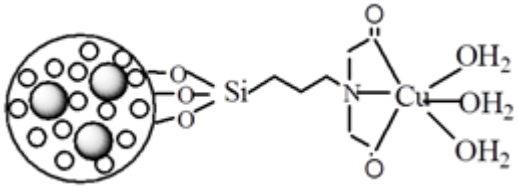

Figure 1 Prepation of MMSNPs-Cu^{2+}

The Cu^{2+}-chelated MMSNPs (MMSNPs-CPTS-IDA-Cu^{2+}) with different pore sizes showed metal affinity adsorption for laccase. The laccase adsorption capacity of MMSNPs-CPTS-IDA-Cu^{2+} increased from 88.4 mg/g to 427.9 mg/g with respect to the pore size increased from 3.6 nm to 27.1 nm. The MMSNPs-CPTS-IDA-Cu^{2+} adsorbed with laccase was used directly as the magnetic laccase catalyst, and the activity recovery of the laccase from the broth was 120.4%. The optimal reaction pH and temperature for the magnetic laccase catalyst was 5.5 (Figure 2) and 60 ^{o}C (Figure 3), respectively. The magnetic laccase catalyst showed high activity within a wide range of pH, and its thermal and storage stability was greatly improved. The magnetic laccase catalyst was used for degradation of various phenolic compounds. The optimal degradation pH was in the range of 5.5-6.0 for various phenolic compounds. The optimal temperature for various phenolic compounds was as follows: 60 ℃ for o-dihydroxybenzene, 25 ℃ for phenol and 45 ℃ for 4-

nitrophenol, 4-methoxyphenol and 2,4-dichlorophenol (Figure 4). The magnetic laccase catalyst remained 87.1% and 82.5 % of its initial activity after 10 batches of in the process of catechol and phenol oxidation, respectively..

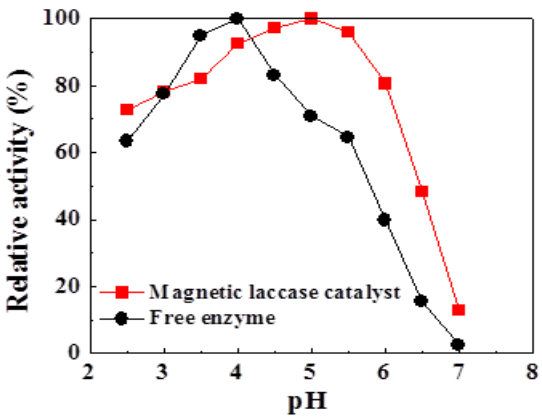

Figure 2 Reaction pH for magnetic laccase catalyst

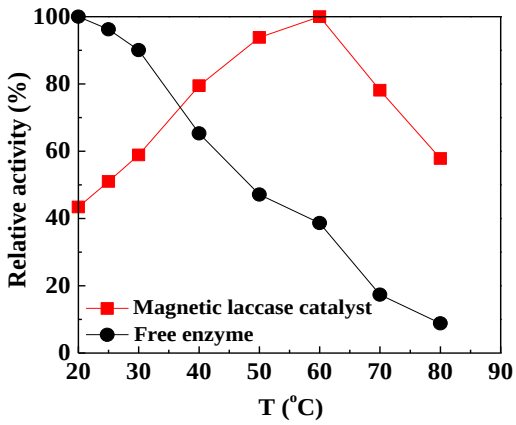

Figure 3 Temperature for magnetic laccase catalyst

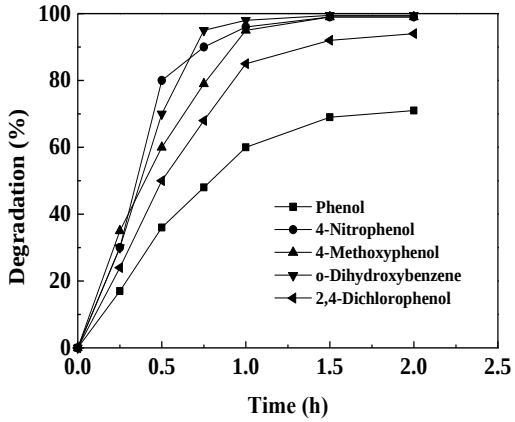

Figure 4 Degradation of various phenolic compounds

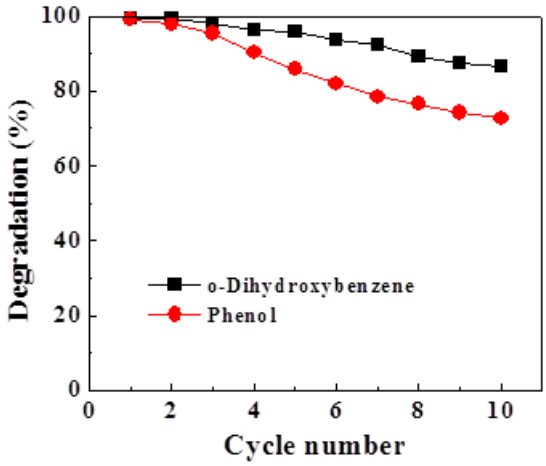

Figure 4 Reuse of magnetic laccase catalyst

3 DEVICE

A magnetically fluidized bed (MSFB) was built for continous degradation of phenolic compounds (Figure 5). Because of good mixing and mass transfer in MSFB, the degradation rate of phenol using the magnetic laccase catalyst maintained more than 99% at a flow rate of less than 450 mL/h and decreased slowly to 91.5% after 40 h of the continuous operation. The present work indicated that the immobilized laccase on magnetic mesoporous supports together with the MSFB provided a promising avenue for the continuous enzymatic degradation of phenolic compounds in industrial wastewater.

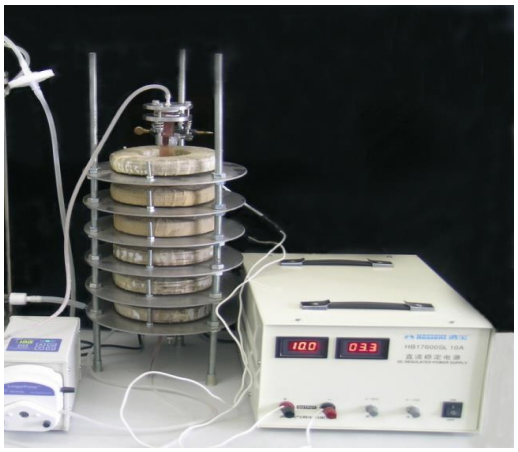

Figure 5 Magnetically fluidized bed

Alternative magnetic field reactor (AFFR) was generated for intensifying the reaction rate of the magnetic laccase catalyst (Figure 6). The reaction rate was enhanced as the magnetic field frequency and strength were increased (Figure 7).

The alternating magnetic field facilitates the diffusion of substrate from the liquid to the magnetic laccase catalyst. The movement of the magnetic laccase catalyst with constantly changed direction under alternating magnetic field has better stirring function than the movement with onstant direction in shake flask. (Figure 8).

The magnetic laccase catalyst can be easily separated from the reaction solution and reused.The magnetic laccase catalyst etained 85% of its reaction rate after 10 consecutive operations.

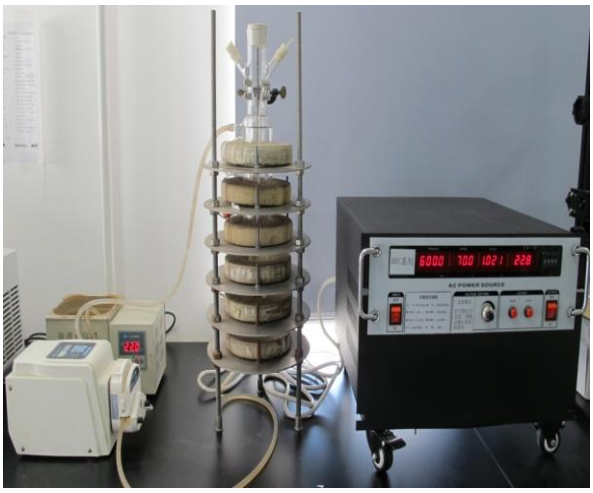

Figure 6 Alternative magnetic field reactor

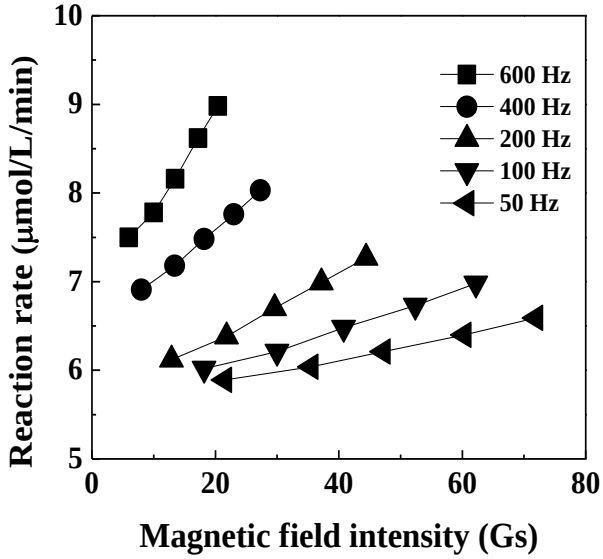

Figure 7 Magnetic field frequency and intensity

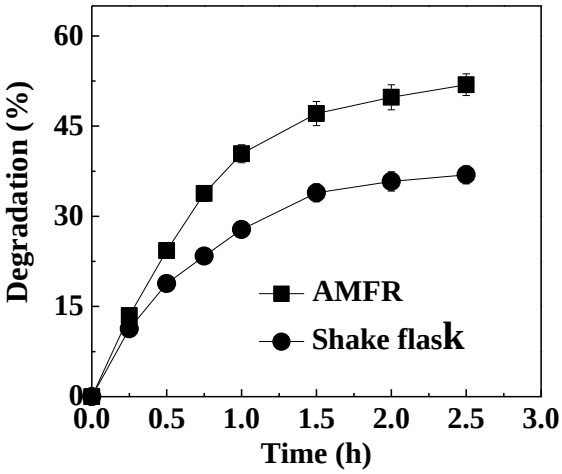

Figure 8 Alternative magnetic field reactor & shake flask

4 CONLUSION

In order to improve the catalytic stability and recovery of enzymes, magnetic composite materials to immobilize enzyme for improving biocatalysis were fabricated successfully through multi-scale design method on chemical engineering materials. Catalytic properties of the magnetic laccase catalyst are regulated at the multi-scale levels of molecule, mesostructure and process engineering. The magnetic laccase catalyst has high catalytic activity, and can be easily recovered by magnetic field for reuse. In presence of an external alternating magnetic field, the apparent rate of the magnetic laccase catalyst increased significantly than that of the control without alternating magnetic field. The phenol degradation was enhanced further in a continuous treatment process by the magnetic laccase catalyst in a magnetically stabilized fluidized bed because of good mixing and mass transfer. These results will not only contribute to establish economic & environmental friendly biotransformation process, but also promote cross-disciplinary of material science, biotechnology and chemical engineering.

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