Special Issue

http://dx.doi.org/10.3786/sincs2011.20

NANO-MICRO LETTERS ARTICLE

www.nmletters.org

(Proceedings of 2011 Shanghai International Nanotechnology Cooperation Symposium, SINCS 2011, Published online 10 January 2012)

Multilayer Nanopancakes of $LnBO_3$ (Ln=Sm, Gd) as a Novel Support for Immobilization of Laccase

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Abstract: Multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) were obtained without any surfactant or template via a mild Solid-State-Hydrothermal method. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) were used to characterize the as-prepared products. The morphology of as-prepared $LnBO_3$ (Ln=Sm, Gd) was characterized by scanning electron microscopy (SEM), which shows that the nanopancakes have typical diameters of 3∼5 μm while the thickness of the single layer is range of 10∼80 nm. Due to the multilayer structure, the laccase was successfully immobilized on these materials.

Keywords: Multilayer; $LnBO_3$ (Ln=Sm, Gd); Laccase; immobilized

Citation: Xinlin Zhou, Qingsheng Wu and Tiejian Zhu, "Multilayer Nanopancakes of $LnBO_3$ (Ln=Sm, Gd) as a Novel Support for Immobilization of Laccase", Proceedings of Shanghai International Nanotechnology Cooperation Symposium, 80-83 (2011). http://dx.doi.org/10.3786/sincs2011.20

Introduction

Belonging to multicopper proteins, laccases are widely existed in nature especially fungi [1,2]. It is a phenol oxidase that can catalyze oxidation of many organic pollutants in water [3]. So the substrate specificity of laccases was exploited to remove pollutants from the environment without creating the negative effects associated with many other methods [4,5]. Despite laccases having intrinsic appreciable stability, the enzyme is often easily inactivate in practical application due to complex environment conditions, which limits its further industrial application [6,7]. Consequently, immobilized laccases have received much attention from researchers in recent years because of its substantial advantages over free lacases such as continuous reuse, easy separation of the product from reaction media, easy recovery of the enzyme and improvement in enzyme stability, and many different types of methods have been employed in the immobilization of enzymes, such as adsorption, entrapment, cross-link and covalent attachment [8,9].

Recently, it is reported that laccase has been successfully immobilized on many different types of supports, such as activated carbon, magnetic chitosan, porous glass, cellulose-polyamine composite, alginate, kaolinite, polymer beads and membranes polystyrene microspheres, short-range ordered aluminum hydroxide and so on [10,11]. However, leakage, desorption and the loss of enzyme activity was a major problem in laccase immobilization, which was related to many factors involving the enzyme itself, polymer matrix, reaction reagents and process conditions [8]. Therefore, it is a great interest in developing novel technologies on laccase immobilization to improve catalytic activity of laccase and increase its industrial application.

The rare-earth orthoborates have been received numerous studies concerning their crystallographic structures and chemical properties [12]. BO_3^{3-} or BO_4^{5-} of rare-earth borate can formed triangular or tetrahedral,

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while the two groups can interconnected in various ways to form chain, layered, circular, island and shelf-like boron-oxygen anions [13,14]. Rare earth borates are widely used in many fields such as the light source, display imaging, medical due to its high vacuum ultraviolet transparency, wide electronic band gap, exceptional optical damage threshold and physical and chemical stability [15-18]. Up to now, to the best of our knowledge, the studies of laccase immobilized on multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) have not been reported [19].

This paper presents typical multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) as a novel support for immobilization of laccase. It has a potential application in catalysis field, for example, a biosensor for phenol detection.

Experimental Procedures

Materials and method

Materials

All reagents were analytical grade in the synthesis system. H_3BO_3 (>99.0%), $Na_2HPO_4.12H_2O$ (>99.0%), $C_6H_8O_7.H_2O$ (>99.8%) and Ln_2O_3 (Ln= Sm, Gd) (>99.99%) powder were purchased from Shanghai Chemical Reagent Co, Ltd. and used without any purification. Laccase was provided by Shanghai Daidi Industrial Development Co, Ltd. and stored at 4℃ before using.

Method

The laccase@$LnBO_3$ (Ln=Sm, Gd) were prepared by a facile two-step method. The $LnBO_3$ (Ln=Sm, Gd) were prepared by Solid-State-Hydrothermal method. The multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) were employed as the support for the immobilization of laccase, and then the laccase was loaded into $LnBO_3$ (Ln=Sm, Gd) by the physical adsorption method.

Preparation of $LnBO_3$ (Ln=Sm, Gd) nanocrystals

Precursor multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) were synthesized by Solid-State-Hydrothermal method. The detailed reaction procedures are described at elsewhere. In a typical synthesis, 0.6 mmol Ln_2O_3 (Ln=Sm, Gd), 0.72 mmol H_3BO_3, 14 ml deionized water are mixed in 20 ml capacity Teflon-lined autoclave. The autoclave is sealed and maintained at 200℃ constantly for 36 h and then cooled to room temperature naturally. The precipitation is centrifuged and washed with deionized water several times. Finally, as-obtained products are dried under vacuum at 60℃ for 4 h.

Preparation of laccase@$LnBO_3$ (Ln=Sm, Gd) nanocrystals

The multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) was employed as carriers for the immobilization of laccsse, and the laccase was immobilized on these materials by the physical adsorption method. In a typical procedure, 100 mg of $LnBO_3$ (Ln=Sm, Gd) support was suspended in 10 mL of phosphate buffer (pH = 7.0) containing a certain amount of laccase (about 20 mg). The mixture of the supports and laccase solution was slowly stirred at room temperature for 12 h. Subsequently, the laccase immobilized on $LnBO_3$ (Ln=Sm, Gd) was separated by a centrifuge. Then the nanopancakes were washed with 10 mL of buffer solution by shaking for 5 min and separated quickly using a centrifuge. The washing procedure was repeated several times until no protein was detected in the supernatant. Finally, the immobilized nanopancakes were stored at 4℃ before using.

Characterization

The morphology and structure of the samples were inspected by using a field emission scanning electron microscope (FE-SEM, Hitachi S4800, Japan) at an accelerating voltage of 5 KV. The phase purity and crystallinity of the samples were characterized by X-ray powder diffraction (XRD) performed on a D8 Focus diffractometer (Bruker) with Cu Ka radiation (λ = 0.154056 nm), employing a scanning rate of 0.02° s^{-1}, in the 2θ ranges from 10° to 70° Infrared spectra (4000-400 cm^{-1}) are recorded by Nicolet 5DX FTIR Spectrometer equipped with a TGS/PE detector and a silicon beam splitter with 1 cm^{-1} resolution.

Results and discussion

The SEM analysis of $LnBO_3$ (Ln=Sm, Gd) obtained at 200℃ for 36 h

The morphology of as-prepared $LnBO_3$ (Ln=Sm, Gd) was characterized by scanning electron microscopy (SEM) and shown in Fig. 1, and the micrographs showed that the nanopancakes have typical diameters of 3∼5 μm while the thickness of the single layer is range of 10∼80 nm, and these microparticles are nonaggregated with narrow size distribution. The pseudovaterite $LnBO_3$ (Ln=Sm, Gd) multilayer self-assembled nanopancakes exhibit advantages in high ratio surface area and analogy-graphite layer structure, which are favorable for potential application in enzyme immobilization.

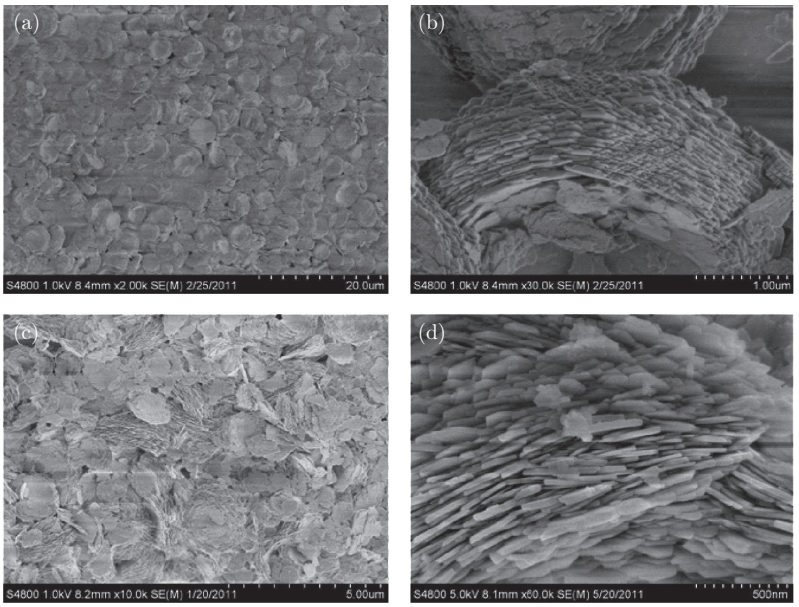

Fig. 1 Typical SEM images of as-prepared $SmBO_3$ (a) and $GdBO_3$ (C) via additive-free S-S-H method at 200℃ for 36 h. The corresponding high magnified images are labeled as b and d.

The XRD patterns analysis of $LnBO_3$ (Ln=Sm, Gd) obtained at 200℃ for 36 h

The XRD patterns of samples were investigated and shown in Fig. 2, and the results showed that the diffraction patterns have great similarity to the JCPDS file for $SmBO_3$ (No. 13-0479) and $GdBO_3$ (No. 13-0483) respectively. All of them have the primitive-lattice hexagonal phase structure. The XRD patterns of $LnBO_3$ (Ln=Sm, Gd) shown in Figure 3 indicated that these two compounds crystallize in the same structure.

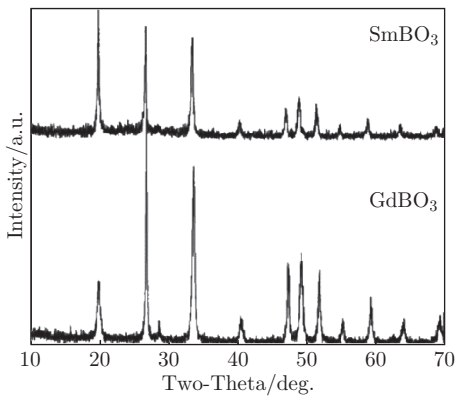

Fig. 2 XRD patterns of as-prepared $LnBO_3$ (Ln=Sm, Gd) via additive-free S-S-H method at 200℃ for 36 h.

The FTIR spectra analysis of $LnBO_3$ (Ln=Sm, Gd) , laccase and laccase@$LnBO_3$

Figure 4 shows that the two compounds have the similar IR spectrum. The absorbance peaks are assigned to the vibration mode of the ring anion $B_3O_9^{9-}$. A

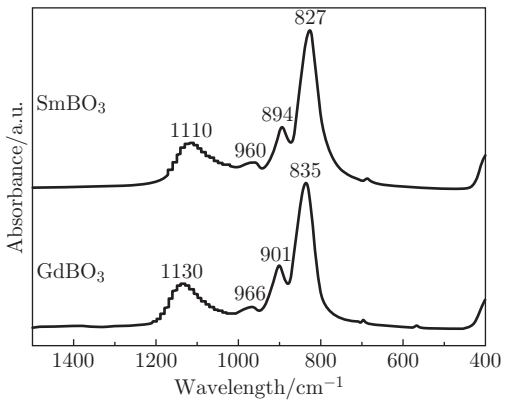

Fig. 3 FTIR spectra of various $LnBO_3$ samples prepared via additive-free S-S-H method at 200℃ for 36 h.

feature of this model is that the $B_3O_9^{9-}$ group is involving a planar ring with D_3 symmetry. The assignment model is proposed in hexagonal $LnBO_3$ as follows: Due to the stretching vibrations of the ring sketch of the cyclic trimeric ion and the terminal B-O and bending vibrations of them, the absorption bands in the region of 800-1200 cm^{-1} and below 500 cm^{-1}, respectively [12-15]. To investigate the binding between the laccase and the multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd), FTIR spectra for the multilayer nanopancakes, lacasse and the multilayer nanopancakes with immobilized laccase were measured. Figure 4 shows the FTIT spectra of $SmBO_3$, laccase and lacasse@$SmBO_3$. Comparing to the typical absorption peaks of lacasse at 3401, 2923, 1649 cm^{-1} and the main absorption peaks of $SmBO_3$ at 1110, 960, 894, 827 cm^{-1}, the absorption of Lacasse@$SmBO_3$ include all of above peaks. So it is evident that the laccase was successfully immobilized

on $SmBO_3$. As far as $GdBO_3$ and laccase@$GdBO_3$ are concerned, the same result can be obtained from Fig. 5.

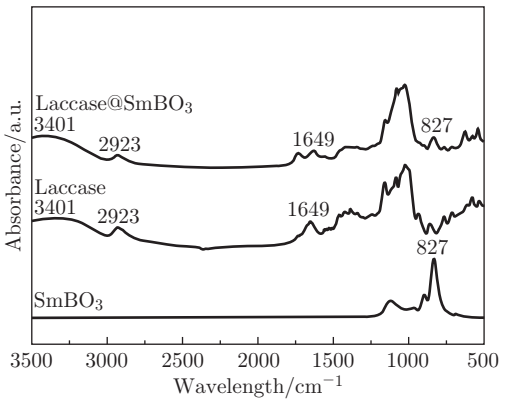

Fig. 4 FTIR spectra of $SmBO_3$, Laccase and Lacasse@ $SmBO_3$.

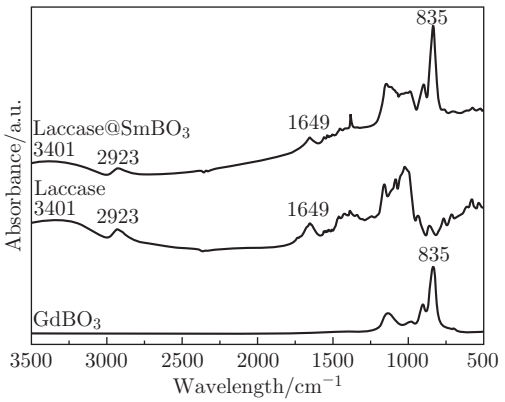

Fig. 5 FTIR spectra of $GdBO_3$, Laccase and Lacasse@ $GdBO_3$.

Conclusion

In this study, a novel multilayer nanopancakes of $LnBO_3$ (Ln=Sm, Gd) have been successfully prepared without any surfactant or template via a mild Solid-State-Hydrothermal method, while laccase was successfully immobilized on these multilayer nanopancakes through physical adsorption method. Present study has enlarged the family of support for laccase immobilization and may provide a basis for the development of suitable biocatalysts for various phenols removal and detection at large scale.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (No. 51072134), the Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials (No. 2012MCIMKF03) and Chang jiang Delta Union Project (No. 10140702017).

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