Bioresource Technology 99 (2008) 8748–8751

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

An electrochemical sensor for detection of laccase activities from *Penicillium simplicissimum* in compost based on carbon nanotubes modified glassy carbon electrode

Jian-Xiao Liu [a,b], Wen-Jing Zhou [b], Ji-Lai Gong [b], Lin Tang [b], Yi Zhang [b]
Hong-Yan Yu [b], Bin Wang [b], Xiang-Min Xu [a,b], Guang-Ming Zeng [a,b,*]

[a] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266003, PR China
[b] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 19 December 2007
Received in revised form 10 April 2008
Accepted 10 April 2008
Available online 3 June 2008

Keywords:
Amperometric detection
Laccase
Penicillium simplicissimum
Compost
Carbon nanotubes

ABSTRACT

An electrochemical sensor for detection of the activity of laccase from *Penicillium simplicissimum* isolated from the composting has been developed. The sensor is based on glassy carbon electrode modified with multi-wall carbon nanotubes (CNTs). The introduction of CNTs into this system can greatly enhance the electrochemical signal in this assay more sensitively, selectively and rapidly than that in conventional spectrophotometric assays. It was found that the optimal pH value of the electrolyte was 5.6. The results showed a good linear correlation between the current and the concentration of laccase activities measured by spectrophotometry, where the current slope was measured by chronoamperometry with a coefficient of 0.9835. Therefore, this electrochemical sensor can be used for rapid detection of laccase activity from *P. simplicissimum*. Furthermore, it may be potentially used for rapid quantification of *P. simplicissimum* according to the relationship between the laccase activities and the biomass.

1. Introduction

Laccase (EC 1.10.3.2) is a well-known multi-copper oxidase with the redox mediators which possess the properties of oxidizing non-phenolic compounds and thus a variety of substrates can be oxidized by this enzyme. It is widely distributed among plants, insects and fungi (Gianfreda et al., 1999). Recently, laccase has also been reported to be widely spread in bacteria (Alexandre and Zhulin, 2000).

Composting is considered as a useful process for the disposal of municipal and agricultural solid waste, in which enzymes play an important role to transform the raw material into fully mineralized organic matter through a variety of biological and biochemical processes (Tiquia, 2002). It was reported that the formation of polyaromatic humic acid structures is associated with phenoloxidase activity in the soil, and laccase is one kind of phenoloxidase (Chefetz et al., 1998).

Laccase activities can be detected with their capability to catalyze one-electron abstraction from phenolic hydroxyl groups. Several methods had been reported to detect the activity of laccase in the literature. Among them, the commonly used method is spectrophotometric assay with different substrates (Bourbonnais et al., 1998). Although the spectrophotometric assay possessed the advantage of high sensitivity, it suffered from the drawback of inability to avoid the interference from turbidity and UV–vis-light-absorbing substances.

As a new generation material of carbon, carbon nanotubes (CNTs) represent an important group of nanomaterials with attractive electronic, chemical and mechanical properties (Baughman et al., 2002). The structural and electronic properties endow them with distinct electrocatalytic activities and capabilities to facilitate direct electrochemical analysis of proteins and enzymes. CNTs were used to synergize the redox mediators to facilitate electron-transfer processes in electrochemical devices such as sensors, biosensors and biological fuel cells and reactors (Shieh and Yang, 2006). In the previous literature, the CNT modified electrode was prepared by casting a CNT/sulfuric acid solution onto a glassy carbon (GC) electrode surface (Zhao et al., 2002). However, the wide applications of CNTs were usually limited by their poor solubility and chemical inertness in aqueous solution. Recently, chitosan (CS) has been reported to disperse CNTs for modification of electrode. Covalent functionalization of CNT with polymers could make the resulting composites more stable and controllable (Liu et al., 2006). Yang used a layer-by-layer self-assembly technique to

* Corresponding author. Address: College of Environmental Science and Engineering, Ocean University of China, Qingdao 266003, PR China. Tel.: +86 731 8822754; fax: +86 731 8823701.
E-mail address: zgming@hnu.cn (G.-M. Zeng).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.04.029

prepare multilayer films of platinum (Pt) nanoparticle-doped and CNTs/CS for electrochemical devices (Yang et al., 2006). Jiang developed a CNTs/CS modified GC electrode to detect nitrite (Jiang et al., 2005).

In our previous work, *Penicillium simplicissimum* has been reported to be inoculated into compost. It was found that the efficiency of lignin degradation was enhanced mainly due to the catalysis of laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) extracellularly released (Yu et al., 2004). Recently, further works have been done to enhance the capabilities of generating laccase and degradation of the lignin for *P. simplicissimum* (Zeng et al., 2006a, 2006b). So it is significantly important to detect the activities of the lignin-degrading peroxidases in composting using a new sensitive and rapid method. Electrochemical sensors were widely used in the area of environmental analysis due to its high sensitivity, selectivity and simplicity. In our previous work, an amperometric sensor has been developed to detect the activities of LiP and MnP extracellular released from *Phanerochaete chrysosporium*, due to the reduction of the enzymatic reaction product on the electrode (Tang et al., 2005).

In this work, we aim at exploring the possibility to fabricate a new electrochemical sensor to detect the activity of laccase from *P. simplicissimum* isolated from the composting. This electrochemical sensor is composed of a CNTs/CS modified GC electrode (CNTs-CS/GC). The introduction of CNTs into this system can greatly enhance the electrochemical signal resulting in a more sensitive assay than our previous work. Other parameters such as the optimal culture conditions of *P. simplicissimum* and effect of buffer pH are also investigated.

2. Methods

2.1. Chemicals and microorganism

The microorganism used in this experiment was isolated from a composting process and identified as *P. simplicissimum* by Biolog Microstation System. This strain was one of the prevailing microorganisms in the compost and proliferated rapidly in the screening process (Zeng et al., 2006a).

Multi-walled CNTs were obtained from Shenzhen Nanotech. Port Co., Ltd. (Shenzhen, China). All other chemicals were of analytical grade and used as received. The concentration of 2.0% CS solution was prepared by dissolving CS in 1.0% acetic acid solution with magnetic stirring for about 3 h. All solutions were prepared with double-distilled water. Cyclic voltammetric measurements and amperometric measurements were carried out on CHI660B electrochemistry system (Chenhua Instrument, Shanghai, China).

2.2. Medium and culture conditions

After growing on potato dextrose agar (PDA) for 3 days, the spores of *P. simplicissimum* were scraped from the surface of the agar and blended in the sterile distilled water as inoculum. The basal medium to produce the laccase consisted of maltose 2 g/L, peptone 1.2 g/L, 100 μg/L VB_1 and 0.05% Tween 80. The initial spore concentrations were adjusted between 10^6–10^7 spores/mL through a microscope (CX40RF200, Olympus Optical Co. Ltd., Japan). 1 mL of homogenized spores was inoculated into 100 mL basal medium and incubated for 8 days, at constant temperature of 30 °C and 150 rpm. Then, the contents of each flask were filtered, and the filtrate was the crude enzyme extract. The turbidimetry of the product was characterized by testing the absorption at 420 nm on a 721 visible spectrometer.

2.3. Spectrophotometric measurement of enzymatic activities

Laccase activity in the culture was determined by measuring the oxidation of 500 mM ABTS buffered with 50 mM tartrate buffer (pH 4.0). The change in absorbance was monitored at 420 nm ($\varepsilon_{420} = 36{,}000\ M^{-1}\ cm^{-1}$) on a UV-2250 UV–vis spectrophotometer (Shimadzu, Japan). Enzyme activity was expressed as units (U) which was defined as 1 μM of substrate oxidized per min (Eggert et al., 1996).

2.4. Preparation of CNTs-CS/GC

The CNTs-CS/GC electrode was fabricated according to the previous literature (Liu et al., 2006). Typically, GC electrode (4 mM in diameter) was firstly polished with 0.05 μm alumina slurry, washed ultra-sonically in water and ethanol for 3 min, respectively. Then, the cleaned GC electrode was dried at room temperature (25 °C). For preparation of CNTs-CS/GC electrode, appropriate amount of CNTs was dispersed in 1 mL of 2.0% CS solution under ultrasonication for 15 min. Then 5 μL of the above described CNTs-CS mixture was dropped onto the GC electrode surface with syringe. Finally, the modified electrode was allowed to be dried for 24 h at room temperature.

2.5. Amperometric measurements of enzymatic activities

The laccase activities were determined electrochemically using hydroquinone as substrate. The three-electrode system used in this work consists of a CNTs-CS/GC electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a Pt foil auxiliary electrode. The electrochemical measurement was performed on a VMP2 multichannel potentiostat (Princeton Applied Research, USA). The measurements were conducted in the electrolyte containing 10 mL of 50 mM tartrate buffer and 2.5 mL culture filtrate followed by the addition of different concentrations of hydroquinone. All the measurements were carried out at room temperature unless otherwise mentioned.

3. Results and discussion

Laccase is capable of oxidizing phenols by reducing molecular oxygen by a multi-copper system. The catalytic center consists of three types of copper with different functions (Klis et al., 2006). Hydroquinone is often used as a laccase substrate in enzyme activity assays (Zhang et al., 2007). The electrochemical behavior of this system is well-known. Another advantage of using hydroquinone as substrate consists in its relatively good solubility in water compared to that of other compounds, such as syringaldazyne, used in laccase activity assays (Mayer and Staples, 2002). The molecular reaction models of oxidizing hydroquinone catalyzed with laccase (Yaropolov et al., 1994) can be simplified into the following equation:

$$\text{hydroquinone} + 1/2O_2 \rightarrow p\text{-benzoquinone} + H_2O$$

3.1. Amperometric response of P. simplicissimum culture filtrate

With the properties of good biocompatibility, film-forming ability and insolubility in the aqueous solution, CNTs-CS composite film can be treated as a robust matrix to incorporate enzyme without cross-linking reagents. CS acts as a scaffold for dispersing and incorporating firmly CNTs at the surface of electrode. Especially, the CNTs will lead to more efficient electrical sensing detection, because as electrode materials, it can display good conductivity, and