Biosensors and Bioelectronics 42 (2013) 156–162

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Co-immobilization of glucose oxidase and xylose dehydrogenase displayed whole cell on multiwalled carbon nanotube nanocomposite films modified electrode for simultaneous voltammetric detection of D-glucose and D-xylose

Liang Li [a,b], Bo Liang [b], Feng Li [a,*], Jianguo Shi [c], Marco Mascini [d], Qiaolin Lang [b], Aihua Liu [b,**]

[a] College of Environment and Safety Engineering, Qingdao University of Science and Technology, 53 Zhengzhou Road, Qingdao 266042, China
[b] Laboratory for Biosensing, Qingdao Institute of Bioenergy & Bioprocess Technology, and Key Laboratory of Bioenergy, Chinese Academy of Sciences, 189 Songling Road, Qingdao 266101, China
[c] Key Laboratory for Biosensors of Shangdong Province, Biology Institute of Shandong Academy of Sciences, 19 Keyuan Road, Jinan 250014, China
[d] Dipartimento di Chimica, Universita degli Studi di Firenze, Via della Lastruccia, 3 50019 Sesto Fiorentino, Italy

ARTICLE INFO

Article history:
Received 16 August 2012
Received in revised form
4 October 2012
Accepted 18 October 2012
Available online 26 October 2012

Keywords:
Voltammetric biosensor
D-glucose
D-xylose
Simultaneous determination
Glucose oxidase
Xylose dehydrogenase-displayed bacteria

ABSTRACT

In this paper, we first report the construction of Nafion/glucose oxidase (GOD)/xylose dehydrogenase displayed bacteria (XDH-bacteria)/multiwalled carbon nanotubes (MWNTs) modified electrode for simultaneous voltammetric determination of D-glucose and D-xylose. The optimal conditions for the immobilized enzymes were established. Both enzymes retained their good stability and activities. In the mixture solution of D-glucose and D-xylose containing coenzyme NAD^+ (the oxidized form of nicotinamide adenine dinucleotide), the Nafion/GOD/XDH-bacteria/MWNTs modified electrode exhibited quasi-reversible oxidation-reduction peak at −0.5 V (vs. saturated calomel electrode, SCE) originating from the catalytic oxidation of D-glucose, and oxidation peak at +0.55 V(vs. SCE) responding to the oxidation of NADH (the reduced form of nicotinamide adenine dinucleotide) by the carbon nanotubes, where NADH is the resultant product of coenzyme NAD^+ involved in the catalysis of D-xylose by XDH-displayed bacteria. For the proposed biosensor, cathodic peak current at −0.5 V was linear with the concentration of D-glucose within the range of 0.25–6 mM with a low detection limit of 0.1 mM D-glucose (S/N=3), and the anodic peak current at +0.55 V was linear with the concentration of D-xylose in the range of 0.25∼4 mM with a low detection limit of 0.1 mM D-xylose (S/N=3). Further, D-xylose and D-glucose did not interfere with each other. 300-fold excess saccharides including D-maltose, D-galactose, D-mannose, D-sucrose, D-fructose, D-cellobiose, and 60-fold excess L-arabinose, and common interfering substances (100-fold excess ascorbic acid, dopamine, uric acid) as well as 300-fold excess D-xylitol did not affect the detection of D-glucose and D-xylose (both 1 mM). Therefore, the proposed biosensor is stable, specific, reproducible, simple, rapid and cost-effective, which holds great potential in real applications.

1. Introduction

D-glucose and D-xylose are the two most important monosaccharides in nature, which are involved in life activities as main carbon resource and energy (Matsushika et al., 2009). Glucose and xylose have been recognized as the ideal sweeteners and nutritional agents. Their related products also have a high functional value, which have been widely applied to the efficacy of food and other health effects of food. On the other hand, xylose and glucose are abundant in plants and agricultural residues, which are important biomass in bioenergy industry (Agblevor et al., 2007), because the fermentation of both xylose and glucose could enhance the utilization efficiency and the yields of fuel ethanol (Elkins et al., 2010). Thus, both fermentability and conversion efficiency of xylose and glucose are important factors in selecting superior engineering strains and increase bioethanol production in bioenergy industry. Therefore, D-glucose and D-xylose are the most parameters during in food industry, medical and biochemical process.

To date, many analytical methods including high performance liquid chromatography (HPLC) (Agblevor et al., 2007; Lloyd and Wyman, 2005; Ohgren et al., 2007), UV–visible spectrophotometry (Cara et al., 2008), capillary electrophoresis (Huber et al., 1994), gas chromatography/mass spectrometry (Kamm et al., 2006) and electrochemical methods (Tatsumi and Katano, 2004) have been developed to analyze monosaccharides. In general, Raman spectra have

* Corresponding author.
** Corresponding author. Tel.: +86 532 8066 2758.
E-mail addresses: lifeng@qust.edu.cn (F. Li), liuah@qibebt.ac.cn (A. Liu).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.10.062

narrower peak widths for aqueous samples and cover a wide spectral range, making it a better technique over UV–visible spectrophotometry for quantitative analysis with complex matrices, for example, the measurement of biomass conversion in a number of species, including poplar (Gierlinger and Schwanninger, 2006), black spruce (Agarwal, 2006), switchgrass (Li et al., 2010), corn stover (Shih and Smith, 2009) and eucalyptus globules (Sun et al., 2011). On the other hand, enzyme-based biosensor such as glucose oxidase (GOD) or glucose dehydrogenase (GDH)-based electrochemical sensor is widely used for glucose analysis (Flexer et al., 2011; Lim et al., 2005; Tsai et al., 2005; Wang et al., 2012; Yu et al., 2011; Zafar et al., 2011). We previously reported that a novel xylose dehydrogenase (XDH) cell-surface-displayed bacteria (XDH-bacteria) using a newly identified ice nucleation protein from *Pseudomonas borealis* DL7 as an anchoring motif (Liang et al., 2012). With coenzyme NAD^+, the XDH-displayed bacteria facilitates the catalysis of the oxidization of xylose and the resultant NADH can be detected (Li et al., 2012; Liang et al., 2012).

The detection of two or more monosaccharides at the same time through simple, cheap and portable method holds great importance in food and biomedical analysis. Techniques such as ^{13}C-NMR (Rossi et al., 1995), HPLC (Bastianoni et al., 1999) and Raman spectroscopy (Shih et al., 2011) have been applied for simultaneous determination of glucose and xylose; however, these methods are either expensive or difficult in sample pretreatment, which limited their applications in real world analysis. Recently, co-immobilization of two or more enzymes for biosensing has received great attention. For instance, the co-immobilization of three cellulases on the gold nanoparticles was reported for the hydrolytic degradation of cellulose (Cho et al., 2012). The co-immobilization of two or more enzymes was widely reported in electrochemical sequential enzyme biosensors (Cosnier et al., 2006; Eguilaz et al., 2011; Kim et al., 2010; Okuma and Watanabe, 2002) and the detection of maltose using alpha-glucosidase and pyranose oxidase (Odaci et al., 2010). However, in above cases, only one ingredient can be measured. The co-immobilization of GOD and cholesterol oxidase was reported for the amperometric detection of glucose and cholesterol in diabetic mice for the evaluation of the possible diabetes-accelerated atherosclerosis risk, however, the preparation of the modified electrode is too complicated to extend to wide use (Huang et al., 2011). Moreover, the thus-fabricated bienzyme-electrode was actually used separately as glucose sensor and cholesterol sensor because of the amperometric measurement at the same potential of -0.2 V (Huang et al., 2011). Oxidase/sweet-potato-peroxidase bienzyme system-based amperometric biosensors for glucose, ethanol and biogenic amines were also developed, unfortunately, the simultaneous determination of the substrates could not be realized (Castillo et al., 2003). The amperometric detection of glucose and fructose was developed by using GOD, fructose dehydrogenase and tetrathiafulvalene-modified electrode (Campuzano et al., 2004). However, the simultaneous determination of glucose and fructose in the same sample cannot be performed without a HPLC separation step (Campuzano et al., 2004). The probable reason for the common limitation is that amperometric method is not capable of the detection of several substrates simultaneously. Fortunately, voltammetric method may be practical which enables to distinguish peaks well-separated from each other at the same time. On the other hand, it is vital to develop biosensors using two or more enzymes which are specific and sensitive to their respective substrates. Nevertheless, great issues exist such as the difficulty to obtain the necessary enzymes with high activities and stability, especially with higher specificity for real applications.

In present paper, we developed a novel electrochemical biosensor using both GOD and XDH-bacteria co-immobilized on MWNTs-modified electrode. The proposed microbial biosensor is capable of simultaneous determination of glucose and xylose, which is of high specificity, sensitivity, stability, rapidness and low-cost. To the best of our knowledge, this is the first report to address the combination of commercial enzyme and enzyme-displayed whole cell with nanotechnology to construct electrochemical biosensor for simultaneous determination of two important monosaccharides.

2. Materials and methods

2.1. Chemicals and reagents

Nafion (perfluorinated ion-exchange resin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich and used as received. 0.05 wt% Nafion solution was prepared from 5 wt% Nafion. Multiwalled carbon nanotubes (MWNTs) were kindly gifted by Prof. Gebo Pan in Suzhou Institute of Nan-Tech and Nano-Biomics, Chinese Academy of Sciences. Phosphate buffer saline (PBS, 0.1 M, pH 7.4) was used as the supporting electrolyte. Xylose dehydrogenase (XDH) displayed whole cell from *Escherichia coli* BL21 (XDH-bacteria) was developed in our laboratory, whose concentration was estimated to be 1.4×10^8 cfu/mL with XDH activity of 1.89 ± 0.03 U/OD_{600} (Liang et al., 2012). Glucose oxidase (GOD, EC 1.1.3.4 from *Aspergillus niger*, type X-S, lyophilized powder, with activity of 297 units/mg) was purchased from Sigma. The stock GOD solution was prepared in the 0.1 M PBS buffer and stored at 4 °C. D-(+)-glucose was bought from Sinopharm Chemical Reagent Co., Ltd. Glucose stock solutions were mutarotated overnight at room temperature before use. All other chemicals and reagents are of analytical grade and were prepared using ultrapure water.

2.2. Preparation of modified glassy carbon electrodes

The glassy carbon electrode (GCE, diameter of 3 mm) was successively polished to a mirror finish using 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with deionized water. After successive sonication in anhydrous ethanol and ultrapure water, the electrode was rinsed with ultrapure water and allowed to dry at room temperature. In preparation of Nafion/XDH-bacteria/MWNTs/GCE, 10 μL of Nafion-MWNTs suspension (2 mg MWNTs dispersed in 1 mL 0.05 wt% Nafion solution with ultrasonication) was cast onto the inverted GCE surface and dried in air. Next, different volume of XDH-bacteria aqueous dispersion was syringed onto the GCE and dried overnight at 4 °C in refrigerator. At last, 5 μL of Nafion solution (0.05 wt%) was syringed to the electrode surface. The fabrication of Nafion/GOD/MWNTs/GCE is similar to that of Nafion/XDH-bacteria/MWNTs/GCE. For the construction of Nafion/GOD/XDH-bacteria/MWNTs/GCE, 10 μL of Nafion-MWNTs suspension was dropped on the inverted GCE surface and dried in air, followed 5 μL of XDH-bacteria aqueous dispersion and 5 μL of GOD solution mixed together on the inverted GCE and dried overnight at 4 °C in refrigerator. Finally, 5 μL of Nafion solution (0.05 wt%) was syringed to the electrode surface. All the thus-prepared modified electrodes were immersed in PBS buffer to remove any loosely adsorbed enzymes and were stored at 4 °C under dry conditions when not in use.

2.3. Apparatus and electrochemical Measurements

Electrochemical measurements were performed using a CHI660D potentiostat (CH Instruments, Chenhua, Shanghai, China). The electrochemical response was measured in a conventional three-electrode system using a bare GCE, or a chemically

modified GCE as working electrode, a Pt wire auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. All potentials were reported in this context with respect to this reference. All measurements were performed at room temperature (~23 °C).

3. Results and discussions

3.1. Voltammetric study of D-xylose and D- glucose based on Nafion/GOD/XDH-bacteria/MWNTs modified GCE

Fig. 1 shows the cyclic voltammograms (CVs) of different modified GC electrodes in 0.1 M PBS (pH 7.4) buffer containing D-glucose or D-xylose, or mixture of both D-glucose and D-xylose. No peak appeared when the Nafion/XDH-bacteria/MWNTs/GCE was in the PBS buffer containing only D-glucose (Fig. 1A, curve b). Clearly, an oxidation peak at 0.55 V in the potential window was observed at Nafion/XDH-bacteria/MWNTs/GCE (Fig. 1A, curve c), which responded to the oxidation of NADH, the resultant product of coenzyme of NAD^+ involved in the oxidation of D-xylose by XDH (Li et al., 2012). On the other hand, at Nafion/GOD/MWNTs/GCE, the direct electrochemistry of GOD was observed (Fig. 1B, curve a) in bare PBS buffer. The CV response of the Nafion/GOD/MWNTs/GCE in the presence of D-xylose and NAD^+(Fig. 1B, curve b) is actually the same as the case in the absence of any substrates (Fig. 1B, curve a). However, the reduction peak at about −0.5 V was decreased when the Nafion/GOD/MWNTs/GCE was measured in 1 mM glucose, which correlated to the glucose's response by the catalytic role of GOD (Kang et al., 2009; Shan et al., 2009). Meanwhile, the Nafion/GOD/XDH-bacteria/MWNTs/GCE showed the same voltammetric response at +0.55 V in 1 mM xylose (Fig. 1C, curve b) and in the mixture of glucose and xylose (each 1 mM) (Fig. 1C, curve c), suggesting that glucose will not affect the voltammetric detection of xylose. Furthermore, the direct electrochemistry of GOD for the Nafion/GOD/XDH-bacteria/MWNTs/GCE was essentially the same as the case in the absence of xylose (Fig. 1C, line a) and in the presence of xylose (Fig. 1C, curve b), suggesting that xylose will not affect the voltammetric detection of glucose. Similarly, the direct electrochemistry of GOD for the Nafion/GOD/XDH-bacteria/MWNTs/GCE was essentially the same as the case in the presence of 1 mM glucose (Fig. 1D, curve b) and in the mixture of glucose and xylose (each 1 mM) (Fig. 1D, curve c), further suggesting that xylose would not affect the voltammetric detection of glucose. The Nafion/GOD/XDH-bacteria/MWNTs/GCE exhibited no voltammetric peak at 0.55 V in bare PBS buffer (Fig. 1D, curve a) and in the presence of glucose (Fig. 1D, curve b). On the other hand, Nafion/GOD/XDH-bacteria/MWNTs/GCE showed no voltammetric response at about 0.55 V in 1 mM glucose (Fig. 1D, curve b); the further addition of 1 mM xylose induced the same peak at about 0.55 V (Fig. 1D, curve c) as that of 1 mM xylose (Fig. 1C, curve b), suggesting that glucose would not affect the voltammetric detection of xylose. Therefore, there is no cross interference between xylose and glucose, which enables to simultaneously detect xylose and glucose in the same solution.

Additionally, the CVs of Nafion/GOD/XDH-bacteria/MWNTs nanocomposite modified GCE at various scan rates were investigated too (Supplementary Information, Fig. S1A). As for the oxidation process

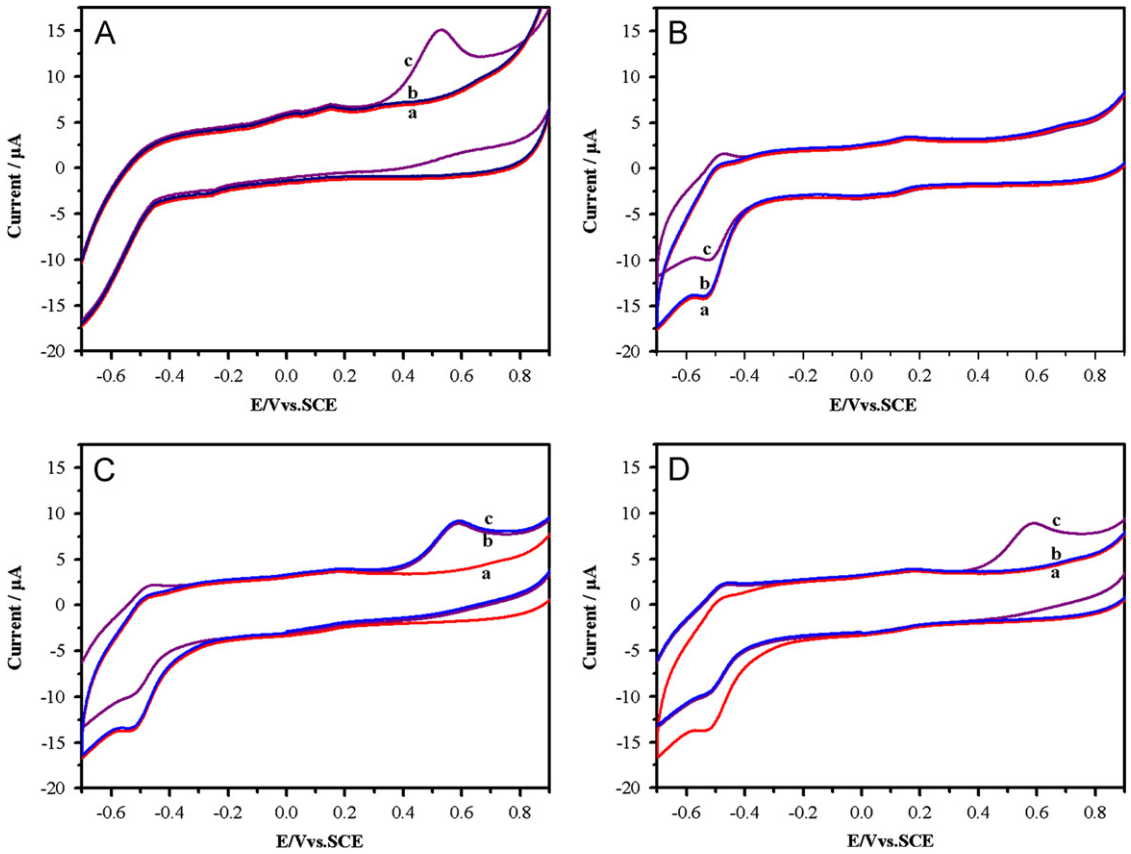

Fig. 1. (A) CVs of Nafion/XDH-bacteria/MWNTs/GCE in ambient-air 0.1 M PBS (pH 7.4) in the absence (a) and presence of 1 mM D-glucose (b) and presence of 1 mM D-xylose+1 mM NAD^+(c); (B) CVs of Nafion/GOD/MWNTs/GCE in ambient-air 0.1 M PBS (pH 7.4) in the absence (a) and presence of 1 mM D-xylose+1 mM NAD^+ (b) and presence of 1 mM D-glucose(c); (C) CVs of Nafion/ GOD/XDH-bacteria/MWNTs/GCE in the absence (a) and presence of 1 mM D-xylose+1 mM NAD^+(b) and presence of 1 mM D-xylose+1 mM NAD^+ +1 mM D-glucose(c); (D) CVs of Nafion/GOD/XDH-bacteria/MWNTs/GCE in the absence (a) and presence of 1 mM D-glucose (b) and presence of 1 mM D-glucose+1 mM D-xylose+1 mM NAD^+ (c). Scan rate, 50 mV s^{-1}.

of NADH in this composite film, the peak current increased with the increase of the square root of the scan rate (Supporting Information, Fig. S1B), suggesting a diffusion-confined electrochemical process (Eguílaz and Villalonga, 2011). To the contrary, the peak current was linear with the scan rate from 20 mV/s to 300 mV/s (Supporting Information, Fig. S1C) indicated that the redox process of GOD in this composite film was a quasi-reversible and surface-confined electrochemical process (Zhao et al., 2008).

3.2. Optimization of GOD and XDH-bacteria loading on the Nafion/GOD/XDH-bacteria/MWNTs nanocomposite modified GCE

The enzyme loading on the electrode could affect the performance of the as-prepared biosensor. On the other hand, the enzyme utilization rate is very important to reduce the cost of enzyme-based biosensors for practical applications. In the present research, the voltammetric response of the Nafion/GOD/XDH-bacteria/MWNTs nanocomposite modified GCE was investigated by varying GOD (in μg) and XDH-bacteria(in cfu) loading in the film, and their CV responses of the prepared biosensors toward the oxidation of either 1 mM D-glucose or 1 mM xylose (in the presence of NAD^+) were recorded. Three separate electrodes were prepared and measured for each enzyme loading. First, effect of the XDH-bacteria loading in the cast film on the electrode on the sensitivity of the microbial biosensor was studied. By doing so, different Nafion/GOD/XDH-bacteria/MWNTs/GCE electrodes were prepared by varying loading amount of the XDH-bacteria (in cfu) and a fixed GOD (10 μg) loading in the cast film on the electrode, and their responses toward the oxidation of 1 mM D-xylose + 1 mM NAD^+ and 1 mM D-glucose were recorded. The voltammetric response of the modified electrode as a function of XDH-bacteria loading is depicted in Fig. 2A. Obviously, there was no peak at about +0.55 V in the absence of XDH-bacteria on the electrode. A current of 1.47 μA at Nafion/GOD/XDH-bacteria/MWNTs/GCE was observed when 3.5×10^5 cfu of XDH-bacteria was loaded on the electrode. Further, the anodic current increased with the increasing XDH-bacteria loading amount up to 7.0×10^5 cfu (Fig. 2A). That is, the oxidation current response increased with the XDH-bacteria amount in the cast film on the electrode from 3.5×10^5 cfu to 7.0×10^5 cfu. Thereafter, the further increase in the XDH-bacteria loading made the current decrease. For instance, the peak current at +0.55 V was 3.33 μA when the XDH-bacteria loading amount was increased to 1.05×10^6 cfu, which was lower than that value for 7.0×10^5 cfu XDH-bacteria, but higher than that value for 3.5×10^5 cfu XDH-bacteria. There was only 0.87 μA when the XDH-bacteria loading increased to 1.4×10^6 cfu. The velum thickness is proposed to increase with loading of the XDH-bacteria solution (Li et al., 2012). When the XDH-bacteria loading amount was higher than 1.05×10^5 cfu, the velum may be thick enough to block electron transfer between buffer solution and electrode surface. The highest peak current was obtained when a 7.0×10^5 cfu of XDH-bacteria solution was used to modify the electrode. The voltammetric response of the prepared biosensor toward the oxidation of 1 mM D-glucose was 2.2 μA at about −0.5 V (data not shown), which is essentially unchanged with the loading amount of XDH-bacteria in the cast film. So a 7.0×10^5 cfu of XDH-bacteria solution was loaded on the electrode for co-immobilization in the subsequent experiments.

On the other hand, the Nafion/GOD/XDH-bacteria/MWNTs/GCE based biosensor was fabricated with 7.0×10^5 cfu of XDH-bacteria loading and varying GOD amount, and the responses of the prepared biosensors toward the oxidation of both 1 mM D-glucose and 1 mM D-xylose (1 mM NAD^+) were recorded separately. Observed from Fig. 2B, at a very low level of GOD loading (2.5–10 μg), the increase in the enzyme loading led to an enhancement in the response signal for the oxidation of D-glucose and then reached a plateau at higher loadings of GOD (10–20 μg), but there was an unobvious decrease of response current was found at loading of higher than 20 μg, which is in accordance with the literature (Si et al., 2011). The exact reason for the plateau is unclear for the moment. It is well known that the redox center, flavin adenine dinucleotide (FAD) is buried inside the GOD. When the GOD loading amount was increased on the electrode, the FAD electron transfer for additional GOD molecules is probably spatially hindered gradually, thereafter, no more increase in electron transfer with further increasing GOD loading. The oxidation current response was 4.3 μA at +0.55 V for the prepared biosensor toward the oxidation of 1 mM D-xylose (in the presence of 1 mM NAD^+) (data not shown), which was actually the same value with the varying of GOD loading. In order to retain the performance of biosensor for glucose and reduce GOD amount, a quantity of 10 μg of GOD was therefore used for co-immobilization in the later experiment. Taken together, a loading amount of 7.0×10^5 cfu of XDH-bacteria and 10 μg of GOD was applied for the preparation of the Nafion/GOD/XDH-bacteria/MWNTs/GCE.

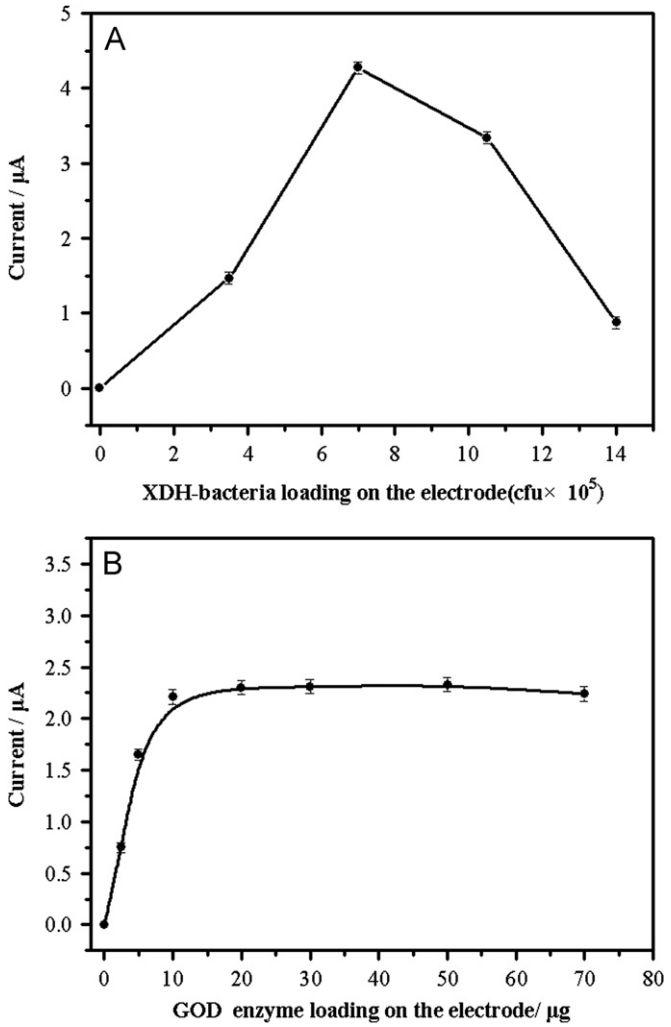

Fig. 2. Effect of enzyme loading for the Nafion/GOD/XDH-bacteria/MWNTs/GCE on the oxidation of both 1 mM xylose (in the presence of 1 mM NAD^+) and 1 mM glucose. (A) The dependence of oxidation current at +0.55V on the loading amount of XDH-bacteria, and (B) the dependence of cathodic current at −0.5V on the loading amount of GOD at the modified electrode. Scan rate, 50 mV s^{-1}.

3.3. Simultaneous voltammetric detection of glucose and xylose based on Nafion/GOD/XDH-bacteria/MWNTs modified GCE

The CVs of the Nafion/GOD/XDH-bacteria/MWNTs/GCE electrode in 0.1 M PBS (pH 7.4) containing different concentrations of D-glucose and D-xylose were performed under the ambient-air condition (Fig. 3A). The oxidation peak current at 0.55 V linearly increased with the increasing concentration of D-xylose ranged from 0.25 to 4 mM (Fig. 3B). The linear regression equation is $y=1.2436+2.0509x$ with a correlation coefficient (R) of 0.999. The limit of detection (LOD) is 0.1 mM xylose (S/N=3). Clearly, the reduction peak around −0.5 V decreased with the increase of glucose concentration due to the oxygen consumption (Fig. 3A). On the other hand, it was found that the decrease in cathodic current was linearly increased with the increase in glucose concentration ranging from 0.25 mM to 6 mM (Fig. 3C). The linear regression equation is $y=4.7692-0.1721x$ with R of 0.999. The detection limit of the biosensor was estimated to be 0.1 mM for glucose at a signal-to-noise ratio of 3. The summary of the analytical features of the biosensor for D-xylose and D-glucose is shown in Table S1. Our LOD value for D-xylose is lower than those obtained from field effect transistor-based sensor utilizing *G. oxydan* whole cells (LOD, 0.5 mM) (Reshetilov et al., 1996), and HPLC with electrochemical detection using a thin-layer cell housing a custom manufactured copper working electrode (LOD, 0.19 mM) (Wring et al., 1998). Although the LOD of 0.1 mM for glucose rather high than those values reported by other electrochemical methods, it is still favorably good for food and biomass analysis, usually the concentration of glucose in these samples are higher than 0.1 mM. Till date, other techniques such as ^{13}C –NMR (0–25 mM glucose; 12–30 mM xylose) (Rossi et al., 1995), HPLC(0–27.8 mM glucose, 5.33–32 mM xylose) (Bastianoni et al., 1999), Raman spectroscopy (177.78 mM glucose;46.67 mM xylose) (Shih et al., 2011) have been applied for simultaneous determination of glucose and xylose, which showed wider linear range. However, these methods are either expensive or difficult in sample pretreatment, which will limit their applications in real world analysis. Therefore, this Nafion/GOD/XDH-bacteria/MWNTs nanocomposite modified electrode can be served as a biosensor for glucose and xylose.

3.4. Selectivity of the biosensor

The Nafion/GOD/XDH-bacteria/MWNTs/GCE exhibited good activity to D-glucose and D-xylose. Here we further investigated the substrate (saccharides) specificity of the biosensor. The CV responses of this sensor to the addition of mixture of D-glucose and D-xylose (each 1 mM) and a series of other saccharides including D-maltose, D-galactose, D-mannose, D-glucose, D-sucrose, D-fructose, D-cellobiose, D-xylitol (each 300 mM) and L-arabinose (60 mM) were measured. The Δi_p is defined as the difference of the peak current from CVs of the Nafion/GOD/XDH-bacteria/MWNTs/GCE in the presence of saccharide and in the presence of bare buffer solution, which are shown (Supplementary Information, Table S2). Compared with the peak current in the presence of D-glucose and D-xylose (each 1 mM), these sugars or analogs exhibited no peak currents, suggesting that there is no interference in the presence of 300-fold excess these substrates. In the case of L-arabinose (300 mM) as a substrate, small oxidation current was observed due to the catalysis of the sensor (data not shown), which meant 300-fold excess L-arabinose would affect the detection of D-xylose (Li et al., 2012). However, when the concentration of L-arabinose was 60 mM, no current was detectable though the concentration of L-arabinose was 60-fold higher than D-xylose. Common interfering substances such as ascorbic acid, dopamine and uric acid (each 0.1 M) exhibited no interference to the measurement of D-glucose and D-xylose (each 1 mM) (Supplementary Information, Table S2). Therefore, this sensor can be used to selectively detect D-glucose and D-xylose without interference from other sugars and common interfering species.

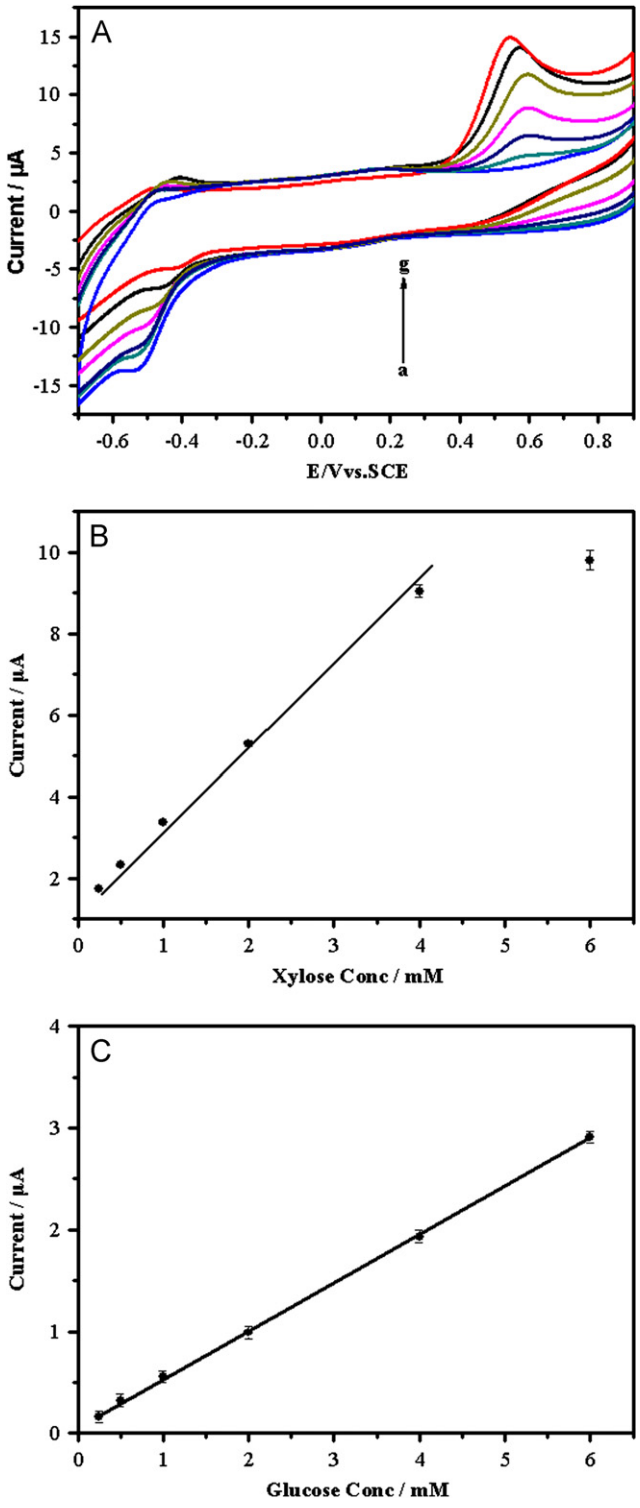

Fig. 3. (A) CVs of Nafion/GOD/XDH-bacteria/MWNTs/GCE in ambient-air 0.1 M PBS (pH 7.4) containing 6 mM NAD^+ in the presence of different concentrations of glucose and xylose. (a) in the absence of glucose and xylose, and (b–g) in the presence of both glucose and xylose: each 0.25 mM (b), each 0.5 mM (c), each 1.0 mM (d), each 2.0 mM (e), each 4.0 mM (f) and each 6 mM (g). (B) Typical calibration graph of the biosensor for xylose, the relative standard deviations of the error bars are within 3.2%. (C) Typical calibration graph of the biosensor for glucose. Scan rate, 50 mV s^{-1}.

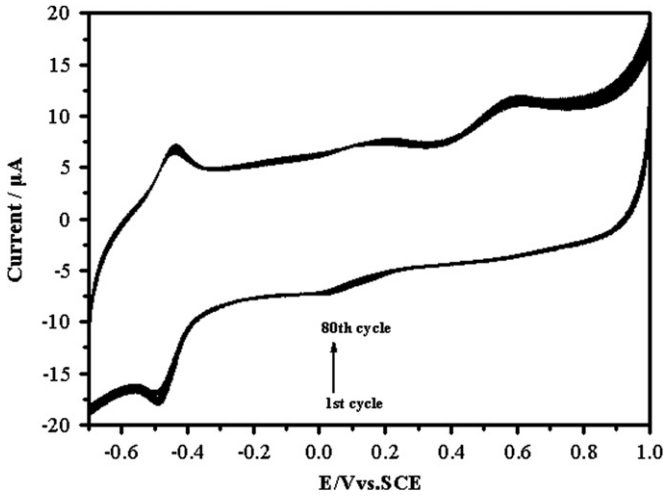

Fig. 4. CVs of the Nafion/GOD/XDH-bacteria/MWNTs/GCE in 0.1 M PBS (pH 7.4) containing 1 mM NAD^+, 1mM glucose and 1 mM xylose for 80 cycles. Scan rate, 100 mV s^{-1}.

3.5. Reproducibility and stability of the biosensor

Three different electrodes were fabricated using the same procedure, and their CV responses were measured in the same glucose and xylose solution. The corresponding currents were compared at the same peak potential. A continuous measurement of CV had been carried out in a 0.1 M PBS (pH 7.4) to evaluate the operational stability of this biosensor. It was found that both peak currents for xylose and glucose retained 95% their initial values and no obvious potential shifts were observed after 80 scans (Fig. 4), which showed that the modified electrode had good operational stability. The long-term stability of the Nafion/GOD/XDH-bacteria/MWNTs/GCE was examined. The current response of the modified electrode was measured daily in a PBS buffer containing 1 mM NAD^+, 1 mM glucose and 1 mM xylose. The biosensor was stored under 4 °C when it was not used. During one-month test, there was no apparent decrease in the current response in the first continuous 5 days' measurements (Supplementary Information, Fig. S2). After that, the current responses gradually decreased. However, the current values still remained 83% initial response for glucose and 61% initial response for xylose after 1 month. To verify the reproducibility of this method, 5 modified electrodes were fabricated with the same procedure. The relative standard deviation (RSD) of the peak currents were 1.9% for 1 mM glucose and 2.5% for 1 mM xylose, which were calculated from the CVs when measuring 5 modified electrodes, indicating that the sensor displayed a better reproducibility. The analytical features of the biosensor are also collected in Table S1.

3.6. Analysis of real samples

The Nafion/GOD/XDH-bacteria/MWNTs/GCE bioelectrode was used to detect D-glucose and D-xylose in real samples such as beer, peach juice and product of lignocellulose degradation samples collected from actual degradation process. The sample solutions were first filtered through a 0.22-μm membrane, and the filtrate was collected. Before measurement, the filtrate was diluted successively with PBS buffer, which was further aliquoted into 5.000 ml containing 5 mM NAD^+. For each sample solution, three repetitive measurements were performed. The concentrations of the real samples were calculated based on the calibration graph multiplying the dilution ratios (Table 1). To estimate the recovery of the method over the spiked standard concentration, 5 μL standard solution containing both D-glucose and D-xylose (each 1.000 M) was spiked into above diluted real sample solution and stirred well before CV measurements were performed again. The RSD values are within 8%, demonstrating that the precision and stability of the calibration graph were suitable for measuring D-glucose and D-xylose in real samples. The recoveries of the D-glucose and D-xylose standards added are 96.6–98.5% and 95.7–101.5%, respectively, which indicate good accuracy of the proposed method.

Table 1
Recovery analysis of samples quantified with the calibration graph using the proposed method.

Sample		Conc. (mM) [a]	Found (mM)[a]	Recovery (%)	RSD (%)
1# [b]	Glucose	0.37 ± 0.01	1.34 ± 0.03	96.6	5.2
	Xylose	3.38 ± 0.03	4.39 ± 0.07	101.5	8.0
2# [c]	Glucose	3.85 ± 0.05	4.84 ± 0.08	98.5	2.9
	Xylose	2.02 ± 0.02	2.93 ± 0.02	95.7	3.3
3# [d]	Glucose	3.15 ± 0.02	4.13 ± 0.06	97.5	6.8
	Xylose	3.37 ± 0.04	4.37 ± 0.07	99.5	4.7

[a] All values were obtained as the average of three repetitive measurements plus-minus standard deviation.
[b] 1# represents local beer.
[c] 2 # represents local peach juice.
[d] 3# represents the fermentation liquor of degradation bacteria, which could utilize microcrystalline cellulose and xylan as the carbon source for growth.

4. Conclusions

In conclusion, we successfully established a novel method for the simultaneous voltammetric detection of D-glucose and D-xylose using the Nafion/GOD/XDH-bacteria/MWNTs modified electrode. The proposed biosensor is stable, specific, reproducible, simple, rapid and cost-effective. Therefore, it is envisioned that this bienzyme based electrochemical biosensor will be found promising applications.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (Nos. 31200598 and 21275152) and the Hundred-Talent-Project (No. KSCX2-YW-BR-7) and the Knowledge Innovation Project in Biotechnology (No. KSCX2-EW-J-10-6), Chinese Academy of Sciences.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.10.062.

References

Agarwal, U.P., 2006. Planta 224, 1141–1153.
Agblevor, F.A., Hames, B.R., Schell, D., Chum, H.L., 2007. Applied Biochemistry and Biotechnology 136, 309–326.
Bastianoni, S., Donati, A., Gastaldelli, A., Marchettini, N., Martini, S., Rossi, C., 1999. Chemical Physics Letters 310, 38–42.
Campuzano, S., Loaiza, Ó.A., Pedrero, M.a., de Villena, F.J.M., Pingarrón, J.M., 2004. Bioelectrochemistry 63, 199–206.
Cara, C., Ruiz, E., Oliva, J.M., Saez, F., Castro, E., 2008. Bioresource Technology 99, 1869–1876.
Castillo, J., Gáspár, S., Sakharov, I., Csöregi, E., 2003. Biosensors and Bioelectronics 18, 705–714.
Cho, E.J., Jung, S., Kim, H.J., Lee, Y.G., Nam, K.C., Lee, H.-J., Bae, H.-J., 2012. Chemical Communications 48, 886–888.
Cosnier, S., Mousty, C., Cui, X., Yang, X., Dong, S., 2006. Analytical Chemistry 78, 4985–4989.

Eguílaz, M., Villalonga, R., 2011. Analytical Chemistry 83, 7807–7814.
Eguilaz, M., Villalonga, R., Yanez-Sedeno, P., Pingarron, J.M., 2011. Analytical Chemistry 83, 807–7814.
Elkins, J.G., Raman, B., Keller, M., 2010. Current Opinion in Biotechnology 21, 657–662.
Flexer, V., Durand, F., Tsujimura, S., Mano, N., 2011. Analytical Chemistry 83, 5721–5727.
Gierlinger, N., Schwanninger, M., 2006. Plant Physiology 140, 1246–1254.
Huang, Q., An, Y., Tang, L., Jiang, X., Chen, H., Bi, W., Wang, Z., Zhang, W., 2011. Analytica Chimica Acta 707, 135–141.
Huber, C., Grill, E., Oefner, P., Bobleter, O., 1994. Fresenius' Journal of Analytical Chemistry 348, 825–831.
Kamm, B., Kamm, M., Schmidt, M., Starke, I., Kleinpeter, E., 2006. Chemosphere 62, 97–105.
Kang, X., Wang, J., Wu, H., Aksay, I.A., Liu, J., Lin, Y., 2009. Biosensors and Bioelectronics 25, 901–905.
Kim, D.C., Sohn, J.I., Zhou, D., Duke, T.A.J., Kang, D.J., 2010. ACS Nano 4, 1580–1586.
Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S., 2010. Bioresource Technology 101, 4900–4906.
Li, L., Liang, B., Shi, J., Li, F., Mascini, M., Liu, A., 2012. Biosensors and Bioelectronics 33, 100–105.
Liang, B., Li, L., Mascin, M., Liu, A., 2012. Analytical chemistry 84, 275–282.
Lim, S.H., Wei, J., Lin, J.Y., Li, Q.T., KuaYou, J., 2005. Biosensors and Bioelectronics 20, 2341–2346.
Lloyd, T.A., Wyman, C.E., 2005. Bioresource Technology 96, 1967–1977.
Matsushika, A., Inoue, H., Kodaki, T., Sawayama, S., 2009. Applied Microbiology and biotechnology 84, 37–53.
Odaci, D., Telefoncu, A., Timur, S., 2010. Bioelectrochemistry 79, 108–113.
Ohgren, K., Vehmaanpera, J., Siika-Aho, M., Galbe, M., Viikari, L., Zacchi, G., 2007. Enzyme and Microbial Technology 40, 607–613.
Okuma, H., Watanabe, E., 2002. Biosensors and Bioelectronics 17, 367–372.
Reshetilov, A.N., Doqnova, M.V., Dovbnya, D.V., Boronin, A.M., Leathers, T.D., Greene, R.V., 1996. Biosensors and Bioelectronics 11, 401-408.
Rossi, C., Marchettini, N., Donati, A., Medaglini, D., Valassina, M., Bastianoni, S., Cresta, E., 1995. Biomass and Bioenergy 8, 197-202.
Shan, C., Yang, H., Song, J., Han, D., Ivaska, A., Niu, L., 2009. Analytical Chemistry 81, 2378–2382.
Shih, C.-J., Lupoi, J.S., Smith, E.A., 2011. Bioresource Technology 102, 5169–5176.
Shih, C.J., Smith, E.A., 2009. Analytica Chimica Acta 653, 200–206.
Si, P., Ding, S., Yuan, J., Lou, X.W., Kim, D.-H., 2011. ACS Nano 5, 7617–7626.
Sun, L., Simmons, B.A., Singh, S., 2011. Biotechnology and Bioengineering 108, 286–295.
Tatsumi, H., Katano, H., 2004. Chemistry Letters 33, 692–693.
Tsai, Y.C., Li, S.C., Chen, J.M., 2005. Langmuir 21, 3653–3658.
Wang, Z., Etienne, M., Quilès, F., Kohring, G.-W., Walcarius, A., 2012. Biosensors and Bioelectronics 32, 111–117.
Wring, S.A., Terry, A., Causon, R., Jenner, W.N., 1998. Journal of Pharmaceutical and Biomedical Analysis 16, 1213–1224.
Yu, P., Zhou, H., Cheng, H., Qian, Q., Mao, L., 2011. Analytical Chemistry 83, 5715–5720.
Zafar, M.N., Wang, X., Sygmund, C., Ludwig, R., Leech, D., Gorton, L., 2011. Analytical Chemistry 84, 334–341.
Zhao, X., Mai, Z., Kang, X., Zou, X., 2008. Biosensors and Bioelectronics 23, 1032–1038.