Biosensors and Bioelectronics 33 (2012) 100–105

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

A selective and sensitive D-xylose electrochemical biosensor based on xylose dehydrogenase displayed on the surface of bacteria and multi-walled carbon nanotubes modified electrode

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ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form
14 December 2011
Accepted 14 December 2011
Available online 22 December 2011

Keywords:
D-Xylose
Electrochemical biosensor
Xylose dehydrogenase displayed bacteria
Multi-walled carbon nanotubes

ABSTRACT

A novel Nafion/bacteria-displaying xylose dehydrogenase (XDH)/multi-walled carbon nanotubes (MWNTs) composite film-modified electrode was fabricated and applied for the sensitive and selective determination of D-xylose (INS 967), where the XDH-displayed bacteria (XDH-bacteria) was prepared using a newly identified ice nucleation protein from *Pseudomonas borealis* DL7 as an anchoring motif. The XDH-displayed bacteria can be used directly, eliminating further enzyme-extraction and purification, thus greatly improved the stability of the enzyme. The optimal conditions for the construction of biosensor were established: homogeneous Nafion–MWNTs composite dispersion (10 μL) was cast onto the inverted glassy carbon electrode, followed by casting 10-μL of XDH-bacteria aqueous solution to stand overnight to dry, then a 5-μL of Nafion solution (0.05 wt%) is syringed to the electrode surface. The bacteria-displaying XDH could catalyze the oxidization of xylose to xylonolactone with coenzyme NAD^+ in 0.1 M PBS buffer (pH7.4), where NAD^+ (nicotinamide adenine dinucleotide) is reduced to NADH (the reduced form of nicotinamide adenine dinucleotide). The resultant NADH is further electrocatalytically oxidized by MWNTs on the electrode, resulting in an obvious oxidation peak around 0.50 V (vs. Ag/AgCl). In contrast, the bacteria-XDH-only modified electrode showed oxidation peak at higher potential of 0.7 V and less sensitivity. Therefore, the electrode/MWNTs/bacteria-XDH/Nafion exhibited good analytical performance such as long-term stability, a wide dynamic range of 0.6–100 μM and a low detection limit of 0.5 μM D-xylose (S/N = 3). No interference was observed in the presence of 300-fold excess of other saccharides including D-glucose, D-fructose, D-maltose, D-galactose, D-mannose, D-sucrose, and D-cellbiose as well as 60-fold excess of L-arabinose. The proposed microbial biosensor is stable, specific, sensitive, reproducible, simple, rapid and cost-effective, which holds great potential in real applications.

1. Introduction

In nature, carbohydrates can be involved in many important life activities to provide carbon resource and energy (Beckham et al., 2011). D-Xylose (INS 967) has been recognized as the ideal sweetener, nutritional agents and therapeutic agents for diabetics since 1960s (Broekaert et al., 2011). Xylose-related products such as xylan and xylo-oligosaccharides also have a high functional value, which have been widely applied to the efficacy of food and other health effects of food. Additionally, xylose can be used as softener, surfactant, and plasticizer in various industries (Martel et al., 2010). On the other hand, xylose is one of the main hydrolysis products of cellulose; the fermentation of xylose could enhance the utilization efficiency and the yields of fuel ethanol (Elkins et al., 2010). Thus, both fermentability and conversion efficiency of xylose are important factors in identifying superior engineering strains and scaling-up production in bioenergy industry (Lopez-Casado et al., 2008). Therefore, the xylose analysis in food, medicine and biological processes is extremely important. Traditional methods to detect sugars include reduction, chromatography, refractive index, UV and calorimetric method, Raman spectroscopy (Shih et al., 2011) and near Infrared spectroscopy (Morita et al., 2011), although these methods of analysis and detection of various sugars have reached

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doi:10.1016/j.bios.2011.12.027

a high degree of separation and sensitivity level, they still have a wide variety of application constraints. As the conventional method of sugar analysis, reduction method for reducing sugar, which is a feature that can be used Fehling method and 3,5-dinitrosalicylic acid method to determine xylose. However, the Fehling method lost selectivity and was difficult to judge the end-point when the xylose content is less than 0.3%. High-performance liquid chromatography (HPLC) (Cheng et al., 2010; He et al., 2003; Rovio et al., 2008; Sharma et al., 2010; Wan and Yu, 2007) and capillary electrophoresis methods (Grossl et al., 2005; Huang et al., 2005) are ideal for sugar analysis, nevertheless, which have disadvantages such as expensive reagents required in sample preparation, long analysis time and low resolution.

Electrochemical methods were also reported for the direct detection of xylose: for instance, a pulsed amperometric detection on an electrophoretic microchip (Fanguy and Henry, 2002) and potentiometric biosensor based on field effect transistor utilizing *Gluconobacter oxydan* whole cells (Reshetilov et al., 1996), however, the detection sensitivity is not satisfactory. Due to their high selectivity and sensitivity, electrochemical biosensors are useful for real-time monitoring of the target molecules in a more appropriate device. The biosensor is constructed by immobilization of enzyme onto the electrode surface, which can be realized to detect carbohydrates rapidly, sensitively, and selectively. To date, in combination with HPLC, many enzyme-based biosensing systems, for example, cellobiose dehydrogenase (Elmgren et al., 1992, 1993a,b; Nordling et al., 1993), glucose dehydrogenase (Marko-Varga, 1987), aldose dehydrogenase (Smolander et al., 1993, 1995), hexose oxidase (Maes and Nagels, 1993), pyranose oxidase and oligosaccharide glucose dehydrogenase (Ikeda et al., 1989, 1990; Kiba et al., 1991; Kinoshita et al., 1990; Ruzgas et al., 1996; Tessema et al., 1995) have been developed for the measurement of sugars, nevertheless, these approaches are still time-consuming, expensive, and complex in procedure. In most cases, the enzyme-based sensors were actually used as the electrochemical detectors after HPLC separation. Further, few reports could be found with xylose dehydrogenase (XDH) system for xylose detection. So it is high desire to establish method which is rapid, simple in instrumentation and operation, sensitive, selective and cost-effective.

The rapid development of nanotechnology provides a strong strategy for analytical chemistry, which helps people to understand the nano-bio-interface interaction in depth (Höök et al., 2008; Liu, 2008; Liu et al., 2006b). Nano-structured materials, due to their excellent chemical properties, have been widely used to explore sensitive detection system (Rickerby and Morrison, 2007). For example, carbon nanotubes (CNTs) are ideal materials for the nanoelectronic devices and nano-sensors because of CNTs' unique electronic properties, large surface area, excellent electrochemical performance and good chemical stability (Andrews et al., 2002; Ouyang et al., 2002). Actually, CNTs can facilitate electron transfer reaction (Baughman et al., 1999) and increase the catalytic activity (Banks et al., 2004).

$$\text{Xylose} + \text{NAD}^{+} \xrightarrow{\text{XDH}} \text{xylonolactonc} + \text{NADH} \quad (1)$$

XDH (EC 1.1.1.175) can catalyze D-xylose metabolism, which occurs through a contrasting pathway that differs from the cases for classical D-xylose pathway in most bacteria (Buchert and Viikari, 1988; Suzuki and Onishi, 1973; Yamanaka et al., 1977). XDH could convert D-xylose to D-xylonolactone with NAD^+ (nicotinamide adenine dinucleotide) as its coenzyme, which is reduced to NADH (the reduced form of nicotinamide adenine dinucleotide) as shown in Eq. (1). As an outer membrane protein, ice-nucleation protein has been found in some prokaryote. It is anchored on the surface of bacteria by a glycosylphosphatidylinositol anchor, which benefits the surface display of proteins (Kawahara, 2002; van Bloois et al., 2011). XDH gene had been cloned from the genome of strain *Caulobacter crescentus* NA1000 due to its high selectivity and enzyme activity (Stephens et al., 2007). N terminus gene of ice-nucleation protein had been cloned from the genome of strain *Pseudomonas borealis* DL7 (Wu et al., 2009). Inspired by this idea, a recombinant plasmid pTInaPb-N/Xdh was constructed in the way that XDH gene was fused into N terminus gene of ice-nucleation protein in our laboratory (Liang et al., 2012). The plasmid could be digested into a 1300 bp strip by *Nco* I/*Hind* III double enzyme. Recently, the XDH cell-surface displaying system using a newly identified ice nucleation protein from *P. borealis* DL7 as an anchoring motif was developed in our laboratory (Liang et al., 2012). Considering that the practical applications of xylose detection by intracellular protein could be limited by the cost of purification and the stability of the free enzyme, the strategy of bacteria surface expression of XDH may be an ideal method for sensing application.

In this paper, we constructed a novel D-xylose electrochemical biosensor using XDH-displayed bacteria (XDH-bacteria) combination of multi-walled carbon nanotubes. The proposed microbial biosensor is capable of high specificity, high sensitivity, improved stability, rapidness and low-cost. To the best of authors' knowledge, this is the first report to address combination of bacteria-surface-expression-of-enzyme with nanotechnology for sensitive and selective electrochemical biosensor application.

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. Multi-walled carbon nanotubes (MWNTs) were kindly gifted from Prof. Dr. Gebo Pan in Suzhou Institute of Nan-Tech and Nano-Biomics, Chinese Academy of Sciences. All other reagents were of the highest grade available and used without further purification. All solutions were prepared with Milli-Q water.

2.2. Bacterial strains and plasmids, growth of bacteria-displayed XDH

The details for the construction of plasmid pTInaPbN/Xdh and the growth of bacteria-displayed XDH can be found elsewhere (Liang et al., 2012). Briefly, *Escherichia coli* BL21 (DE3) expressing XDH on the cell surface was used. Plasmid pTInaPbN/Xdh was used to express InaPb-N/XDH on the cell surface. Strains harboring the expressing vectors pTInaPb-N/Xdh were grown at 37 °C in Luria–Bertani (LB) medium supplemented with kanamycin to a final concentration of 30 μg/mL with stirring at 200 rpm. When OD_{600} value of bacterial was up to 0.5, they were induced with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.5 mM) at 25 °C. After 24 h of growth, the harvested cells were washed and diluted to unit cell density (OD_{600} = 1.0) with 50 mM PBS buffer (pH 8.0) and stored at 4 °C. The concentration of the XDH-displayed *E. coli* is assayed to be 1.4×10^8 cfu/mL. The XDH activity of the whole cell reached to 1.89 ± 0.03 U/OD_{600} after the enzyme expression induced by IPTG (one unit of enzyme activity is 1 μmol NADH generated per min per OD_{600} whole cells.), suggesting that XDH was successfully displayed on the surface of ice-nucleation protein bacteria.

2.3. Preparation of Nafion-MWNTs and Nafion/XDH-displayed bacteria/MWNTs composite films modified glassy carbon electrodes

The bare glassy carbon electrode (GCE, 3 mm in diameter) was polished carefully with 1.0-, 0.3- and 0.05-μm alumina slurry, and sonicated in anhydrous ethanol and water, respectively. Finally, the GCE was thoroughly rinsed with distilled deionized water.

Nafion–MWNTs composite was prepared by mixing 1 mL Nafion (0.05 wt%) and 2 mg MWNTs and sonicated for 10 min until a homogeneous aqueous dispersion resulted. Then, 10 μL of the mixture was dropped on the inverted electrode, and dried under room temperature to acquire Nafion–MWNTs composite film modified GC electrode. The Nafion/XDH-bacteria/MWNTs composite film modified GC electrode was prepared in this way: 10 μL Nafion–MWNTs composite was first dropped on the GCE. Next, 10 μL of XDH-bacteria aqueous dispersion was added to the XDH solution and mixed together on the inverted GCE, and dried overnight under room temperature. Finally, 5-μL of Nafion solution (0.05 wt%) is syringed to the electrode surface. Before use, the modified electrodes were washed repeatedly with Milli-Q water to remove the loosely combined modifiers. The Nafion/XDH-bacteria/GCE was prepared for comparison.

2.4. Apparatus and methods

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 an Ag/AgCl (3.3 M KCl) electrode 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 discussion

3.1. Electrocatalytic oxidation of xylose with the XDH-displayed bacteria in the presence of NAD^+ in solution

As shown in Eq. (1), D-xylose could be catalytically oxidized to D-xylonolactone in the presence of XDH with NAD^+ as its cofactor, and the NAD^+ is reduced to NADH, which is expected to be oxidized on the electrode surface. Initially, we examined whether the XDH-displayed bacteria (XDH-bacteria) prepared in our laboratory exhibited any electrocatalyticity to the oxidation of xylose. Using bare GCE as working electrode, we measured cyclic voltammogram (CV) in 0.1 M PBS buffer solution (pH 7.4) containing XDH-bacteria (10 μL), NAD^+ (4 mM) and xylose (1 mM). Surprisingly, a very strong oxidation peak around 0.6 V (vs. Ag/AgCl) was observed (Fig. 1, line e). Neither xylose, nor XDH-bacteria nor NAD^+ nor mixture of xylose and *E. coli*-displaying XDH in the same buffer solution can be directly oxidized at the electrode in the studied potential window (Fig. 1, lines a–d). Therefore, it is supposed that XDH-displayed on the bacteria could catalyze the oxidization of xylose to xylonolactone with coenzyme NAD^+, in which NAD^+ is reduced to NADH. The resultant NADH is irreversibly oxidized at the electrode, from which an obviously oxidation peak around 0.6 V appeared, which is in agreement with the direct oxidation of NADH at bare glassy carbon electrode (Liu et al., 2006a). The proposed mechanism is schematically shown in Fig. S1. The peak current is linear with the D-xylose concentration in the range of 1 mM to 10 mM, which further support the catalytic role of the XDH-bacteria. However, no signal was observed when D-xylose concentration was less than 0.1 mM. Thus, although the XDH-bacteria in the supporting electrolyte can be used for catalyze the reaction of xylose, which can be used for determination of xylose. Nevertheless, the sensitivity is very low. Further, the real application may be limited because the XDH-bacteria solution must be added for every measurement, which will consume more XDH-bacteria biocatalyst.

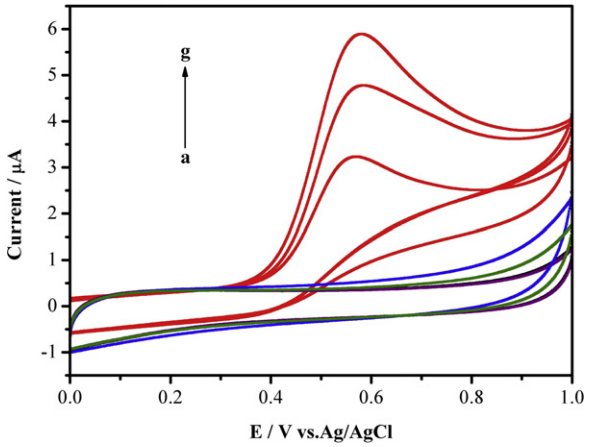

Fig. 1. CVs of 10 mM xylose (a), 10-μL XDH-displayed *E. coli* (b), 4 mM NAD^+ (c), 10 mM xylose + 10 μL XDH-displayed *E. coli* (d) and 10-μL XDH-displayed *E. coli* + 4 mM NAD^+ with varying xylose concentration (red lines (e), 1 mM (f), 4 mM, and (g), 10 mM) in 0.1 M PBS buffer solution (pH7.4). Scan rate, 50 mV s^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.2. Optimization of the conditions for the fabrication of the bioelectrode

The XDH-bacteria is immobilized on the electrode surface to construct electrochemical biosensor may solve problem addressed in Section 3.1. The XDH-bacteria loading in the cast film on the electrode surface may influence the sensitivity of the microbial biosensor. So it is necessary to optimize the XDH-bacteria loading. Biosensors were prepared by varying volume of the bacteria in the cast solution. CVs of a Nafion/XDH-bacteria/GCE were recorded in 0.1 M PBS buffer solution. There is no peak in the absence of XDH-bacteria (Fig. 2A, black line), while a broad oxidation peak at Nafion/XDH-bacteria/GCE was observed when 5 μL of XDH-bacteria was loaded (Fig. 2A, purple line). Further, the anodic current increased with the increasing XDH-bacteria loading amount in the sequence of 5 μL, 7.5 μL, 10 μL (Fig. 2A, purple, blue, and red lines, respectively). That is, the oxidation current increases with the XDH-bacteria amount in the cast film on the electrode. The further increase in the XDH-bacteria loading made the current decrease. Interestingly, no peak appeared when the XDH-bacteria loading volume was increased to 15 μL (Fig. 2A, green line). The voltammetric behavior of the Nafion/XDH-bacteria/GCE in D-xylose solution was further studied by differential pulse voltammetry (DPV), because DPV has a better resolution and signal-to-noise ratio (Liu et al., 2005). The differential pulse voltammograms of Nafion/XDH-bacteria/GCE varying the XDH-bacteria loading on the electrode are shown in Fig. 2B. Apparently, no peak current was observed for the case of 15 μL XDH-bacteria loading, which was in consistence with the CV data shown in Fig. 2A. The exact reason for that is unclear for the moment. It suggests that the velum thickness increasing with loading of the XDH-bacteria solution. When the XDH-bacteria loading amount reached 15 μL, the velum may be so thick that probably blocks electron transfer between buffer solution and electrode surface. The peak current is a function of the XDH-bacteria loading (Fig. 2C). Obviously, the highest peak current response was obtained when a 10 μL of XDH-bacteria was applied.

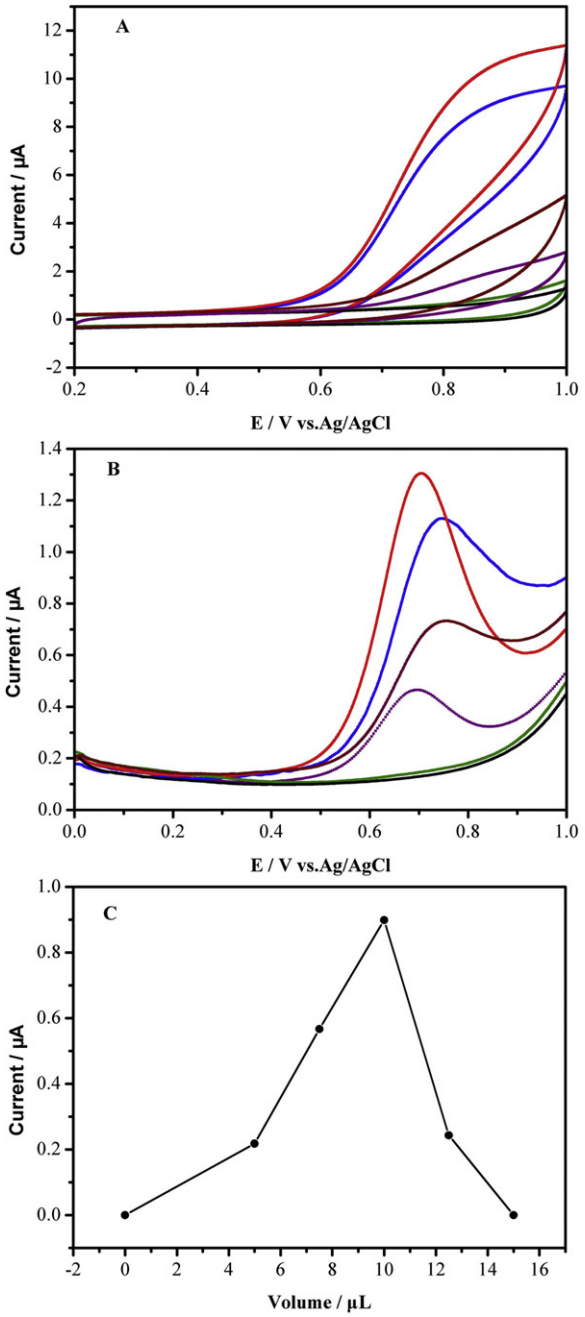

Fig. 2. (A) CVs of Nafion/XDH-bacteria/GCE with different amount of XDH-bacteria loading in 0.1 M PBS buffer solution (pH 7.4) containing 4 mM xylose and 2 mM NAD^+. Scan rate, 50 mV s^{-1}. (B) Differential pulse voltammograms of Nafion/XDH-bacteria/GCE with different amount of XDH-bacteria loading. XDH-bacteria loading: black line, 0 μL; purple line, 5 μL; blue line, 7.5 μL; red line, 10 μL; brown line, 12.5 μL and green line, 15 μL. The differential pulse voltammetry conditions: amplitude, 0.05 V; pulse width, 0.2 s; sampling width, 0.0167; pulse period, 0.5 s. (C) The dependence of oxidation peak current as a function of strain loading amount. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Therefore, a 10 μL of XDH-bacteria was used for subsequent experiments.

3.3. Enhanced electrochemical response of Nafion/XDH-bacteria/MWNTs composite-modified electrode

As shown before, with coenzyme NAD^+, the XDH-displaying bacteria facilitates the catalysis of the oxidization of xylose and the resultant NADH was oxidized at the electrode. We previously reported that a poly(acrylic acid)–wrapped–MWNTs composite coated electrode exhibited good electrocatalyticity in the oxidation of NADH. Thus, it is supposed that the combination of XDH-bacteria and MWNTs could enhance the detection sensitivity. The cyclic voltammetric responses of the Nafion/XDH-bacteria/GCE and Nafion/XDH-bacteria/MWNTs/GCE in the presence of 4 mM D-xylose and 2 mM NAD^+ in 0.1 M PBS (pH 7.4) was performed. The NADH oxidation process emerges at a high positive potential of ~0.70 V at the Nafion/XDH-bacteria/GCE, however, the potential of NADH oxidation is lowered to ~0.50 V at Nafion/XDH-bacteria/MWNTs/GCE (Fig. 3A). That is, the overpotential for the oxidation of NADH is reduced (~0.2 V) at Nafion/XDH-bacteria/MWNTs/GCE. Such a decrease in the overpotential for the oxidation of NADH at a Nafion/MWNTs based electrode could be ascribed to the high local density of electronic states in MWNTs, which is attributed to their helicity and possible topological defects (Nugent et al., 2001). Especially, at the Nafion/XDH-bacteria/MWNTs nano-composite modified electrode (Fig. 3A, line b), the peak current was significantly higher than the value at the Nafion/XDH-bacteria/GCE (Fig. 3A, line a), suggesting the electrocatalytic oxidization o f NADH by MWNTs, which accords well with our previous result (Liu et al., 2006a). Then, the current–time curves for Nafion/XDH-bacteria/GCE and Nafion/XDH-bacteria/MWNTs/GCE were performed separately under the optimum conditions established above, for which the responses of the biosensors to D-xylose were investigated under

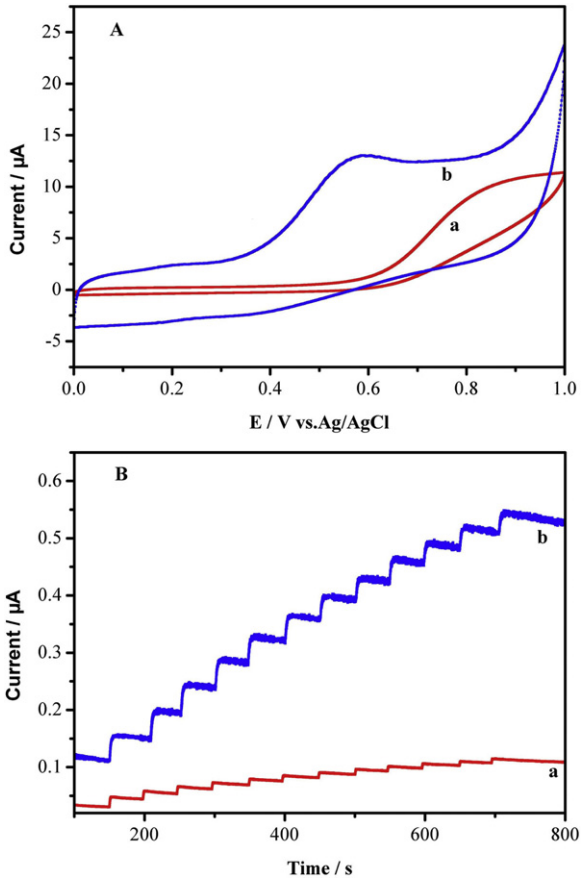

Fig. 3. (A) CVs of the Nafion/XDH-bacteria/GCE (a) and Nafion/XDH-bacteria/MWNTs/GCE (b) in the presence of 4 mM D-xylose and 2 mM NAD^+ in 0.1 M PBS (pH 7.4). (B) Current–time curves. (a) Nafion/bacteria-XHD/GCE at +0.7 V with increasing concentration of D-xylose in 10 μM steps; (b) Nafion/XDH-bacteria/MWNTs/GCE at +0.5 V with increasing concentration of D-xylose in 10 μM steps.

stirring in 0.1 M PBS (pH 7.4). It showed that the current response of Nafion/XDH-bacteria/MWNTs/GCE (Fig. 3B, line b) is much higher than that for Nafion/XDH-bacteria/GCE (Fig. 3B, line a), suggesting the electrocatalytic role of MWNTs. Moreover, the catalytic current increases with the increasing concentration of D-xylose in the solution, which is in accordance with the CVs observation as shown in Fig. 3A.

3.4. Calibration curve of the microbial sensor

The current–time curve was obtained with Nafion/XDH-bacteria/MWNTs/GCE by using amperometry at an applied potential of ~0.5 V (Fig. 4). The oxidation current increased after addition of D-xylose and reached at 95% steady-state value within 5 s (Fig. 4A). The calibration curve for D-xylose is shown in Fig. 4B. The current linearly increased with the increasing concentration of D-xylose over the concentration of 0.6–100 μM. The linear regression equation is $y = 0.02966 + 0.02915x$, with a correlation coefficient $R = 0.999$. The limit of detection (LOD) is 0.5 μM xylose (S/N = 3). Recent papers reported the electrochemical methods capable of detection of D-xylose based on a pulsed amperometric detection on an electrophoretic microchip (LOD, 20 μM) (Fanguy and Henry, 2002), capillary zone electrophoresis with amperometric detection (LOD, 1 μM) (Wang et al., 2003), potentiometric biosensor based on field effect transistor 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). Obviously, our LOD value by using the Nafion/XDH-bacteria/MWNTs composite-based biosensor is much better than other electrochemical xylose sensors reported so far.

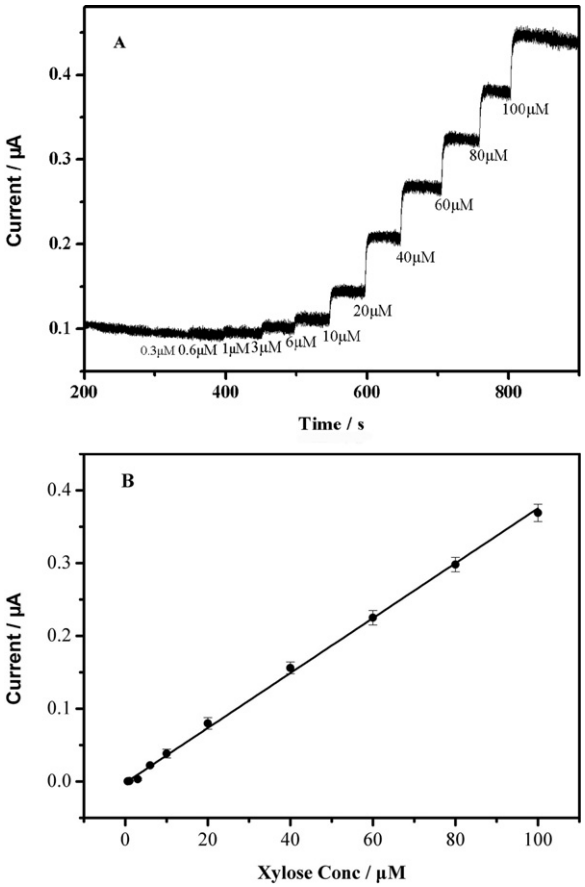

Fig. 4. (A) Current–time curve obtained for the Nafion/XDH-bacteria/MWNTs/GCE on the successive addition of xylose in 0.1 M PBS (pH 7.4) containing 2 mM NAD^+, on which the xylose concentration labeled is the xylose concentration in the PBS solution. Applied potential, +0.5 V vs. Ag/AgCl. (B) Typical calibration graph of the biosensor for xylose.

3.5. Selectivity of the biosensor

As shown above, cell harboring pTInaPb-N/Xdh strain exhibited good activity to D-xylose. Here we further investigated the substrate (saccharides) specificity of the XDH-bacteria. The voltammetric response of this sensor to the successive addition of D-xylose (10 μM) and a series of saccharides including D-maltose (3 mM), D-galactose (3 mM), D-mannose (3 mM), D-glucose (3 mM), D-sucrose (3 mM), D-fructose (3 mM), D-cellbiose (3 mM) and L-arabinose (0.6 mM) as well as D-xylitol (3 mM) were shown in Fig. 5. Compared with the current for 10 μM D-xylose (Fig. 5, arrows a–c), these analogues actually showed background currents (Fig. 5, arrows d–k), suggesting that there is no interference in the presence of 300-fold excess of these substrates. In the case of L-arabinose (3 mM) as a substrate, small oxidation current was observed due to the catalysis of the cell-displaying XDH (data not shown), which means 300-fold excess L-arabinose will affect the detection of D-xylose. However, when the concentration of L-arabinose is 0.6 mM, no current was detectable though the concentration of L-arabinose is 60-fold higher than D-xylose (Fig. 5, arrow l). Usually the concentration of D-xylose is much higher than that of L-arabinose in the actual degradation products of lignocelluloses and food samples (Brown, 1983), which suggests no interference from L-arabinose to the detection of D-xylose in real samples. Therefore, it is clear that this sensor can be used to selectively detect D-xylose without interference.

3.6. Reproducibility and stability of the biosensor

The reproducibility of successive tests using the same sensor was investigated. Five successive measurements using the same biosensor were carried out at the concentration level of 10 μM D-xylose. The relative standard deviation (RSD) of the current responses is 7%. The repeatability of the sensor construction was evaluated by preparing five sensors and determining their sensitivity separately. The repeatability of the sensors expressed as RSD was below 10%, which indicates a very good repeatability in biosensor construction.

The long-term stability of the Nafion/XDH-bacteria/MWNTs /GCE was examined. The current response of the modified electrode

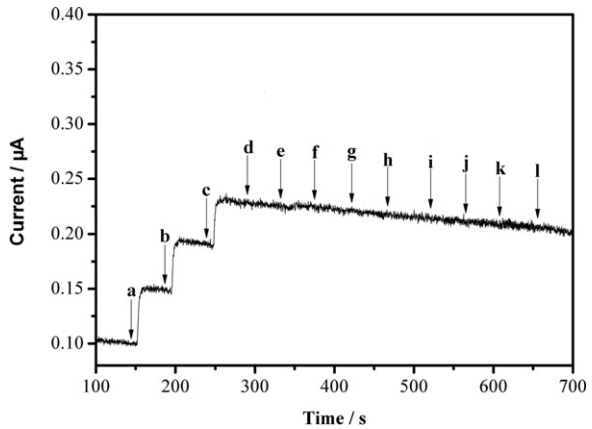

Fig. 5. Current–time curve obtained for the Nafion/XDH-bacteria/MWNTs/GCE on the successive addition of 10 μM D-xylose (a–c), 3 mM D-maltose (d), 3 mM D-galactose (e), 3 mM D-mannose (f), 3 mM D-glucose (g), 3 mM D-sucrose (h), 3 mM D-fructose (i), 3 mM D-xylitol (j), 3 mM D-cellbiose (k), 0.6 mM L-arabinose (l) in 0.1 M PBS buffer (pH 7.4).

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

Sample	Conc (μM)	Found (μM)	Recovery	RSD
1[a]	8.43	17.98	95.5%	6.3%
2[b]	7.02	16.83	98.1%	3.2%
3[c]	10.13	20.42	102.9%	8.9%

[a] The degradation product of straw after physico-chemical processes.
[b] The fermentation liquor of degradation bacteria, which could utilize microcrystalline cellulose and xylan as the carbon source for growth.
[c] Local Tsingtao beer.

was measured daily in a PBS buffer in the presence of 10 μM D-xylose. The biosensor was stored under 4 °C when it was not used. During one-month test, the response of the modified electrode was almost unchanged for the first 20 days' measurement. After that, the current response began to decrease sharply, however, the current value still remained 58% of the initial response after 1 month. Thus the sensor is favorably stable. Actually, the thus-prepared microbial electrochemical biosensor is more stable compared with a conducting-polymer-nanotubule-coated electrode (Valentini et al., 2004) and is also competitive with previously reported tyrosinase-conjugated polysacchride coated electrode (Liu et al., 2005).

3.7. Analysis of real samples

The Nafion/XDH-bacteria/MWNTs/GCE bioelectrode was used to detect D-xylose in real samples such as beer and two products of lignocellulose degradation samples collected from actual degradation process. The samples were accurately diluted with buffer solution to fit into the linear range of the method before measurement. For each sample, three determinations were performed. The concentrations were calculated based on the calibration graph and were shown in Table 1. The RSD values are within 9%, demonstrating that the precision and stability of the calibration graph were suitable for quantifying xylose in real samples. The recoveries of the xylose standards added are 95.5–102.9%, which reveal good accuracy of the proposed method.

4. Conclusions

In conclusion, we developed a novel Nafion/bacteria-displaying XDH/MWNTs nanocomposite electrode. The modified electrode is highly sensitive towards D-xylose and provides a rapid response, a low detection limit (0.5 μM) and a broad linear range (0.6–100 μM). The proposed microbial biosensor exhibited good reproducibility, long-term stability and no interference from saccharides. Due to its excellent performance, this nano-biocomposite electrode is expected to find possible application in anodic biocatalysts, biofuel cells and other bioelectrochemical devices.

Acknowledgments

This work was supported by 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. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.12.027.

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