Talanta 167 (2017) 208–216

Contents lists available at ScienceDirect

Talanta

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

A double-mediator based whole cell electrochemical biosensor for acute biotoxicity assessment of wastewater

Guanyue Gao[a,b], Deyu Fang[a,b], Yuan Yu[a], Liangzhuan Wu[a], Yu Wang[c], Jinfang Zhi[a,*]

[a] Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Beijing Center for Physical & Chemical Analysis, Beijing 100089, PR China

ARTICLE INFO

Keywords:
Electrochemical biosensor
Acute biotoxicity
Wastewater
Double-mediator
Saccharomyces cerevisiae

ABSTRACT

This work investigates the feasibility and sensitivity of a double-mediator based whole cell electrochemical biosensor to detect the acute biotoxicity of wastewater. The lipophilic mediator menadione was used to mediate the intracellular metabolic activities whereas hydrophilic potassium ferricyanide was employed as extracellular electron acceptor to transport the electron from the menadiol to anode. A chitosan hydrogel polymer film with boron-doped nanocrystalline diamond (BND) particles was electrodeposited onto a glassy carbon (GC) electrode to immobilize *Saccharomyces cerevisiae* cells and the mediators. The feasibility of the as-prepared biosensor was verified by determine the acute biotoxicity of four heavy metal ions(Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}), three phenol pollutants (3,5-dichlorophenol, 4-chlorophenol, phenol) and three real wastewater samples. The IC50 values for Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} are 10.12 mg/L,13.88 mg/L, 17.06 mg/L and 34.56 mg/L. And the IC50 value is 16.48 mg/L, 34.40 mg/L and 44.55 mg/L for 3,5-dichlorophenol, 4-chlorophenol and phenol, respectively. The results of this work indicate that the double-mediator based whole cell electrochemical biosensor could be applied into the acute toxicity assessment of real wastewater samples with excellent performance and highlight their merit as portable and sensitive, which may providing a reasonable and reliable way for wastewater toxicity online detection.

1. Introduction

With the booming of manufacturing industry and popularization of chemical products in the daily life, numerous chemicals and toxic compounds have permeated into the aquatic ecosystems, exerting potential harms to human health, threatening the safety of wild animals and food safety [1]. Besides, due to the powerless regulation and careless management of chemical plants and other manufacturing factories, the leakage accidents and illegal chemical dumping incidents happened frequently, causing not only tremendous harm to the environment but also giving rise to the latent possibility of contamination of downstream water system [2]. However, there are only limited methods for water toxicity and safe evaluation. The water safety assessment techniques are mainly depending on the detection of chemical oxygen demand (COD), biological oxygen demand (BOD) as well as extraction of sewage samples into subsequent laboratory analysis [3–5]. Although these methods can detect the toxicants qualitatively and quantitatively, it is difficult to measure the biotoxicity of every individual toxicant contained in water, since a wide variety of chemical species exist in natural water and a mixture of these may exhibit complex biotoxicity. The usage of above methods is also impeded by their technical limitations such as time-consuming, sophisticated detection procedure, needs of well-trained personnel as well as could not provide the real-time alert of the ambient ecosystem accidents [6].

On the other hand, bioassay tests as an excellent alternative technique in complementary to the above traditional water quality assessment methods, have been used to evaluate the biotoxicity levels of environmental and industrial wastewater. The biotoxicity assay provides a holistic approach that allows to evaluate the toxicity of the total effect of all constituent components, including toxicants and confounding variables in a given complex sample matrix. There have been various bioassays test reported to date for which the method of microbe-based biosensor was used. They can be divided into two main approaches, i.e., optical methods and electrochemical methods. Optical methods are based on the bioluminescence produced naturally by bioluminescent microbes, which using *Vibrio fischeri* [3],*Vibrio qinghaiensis* [5], *Photobacterium phosphoreum* [7] and other genetically engineered luminescent bacteria as signal receptor to determine the biotoxicity of water samples by the change of fluorescence intensity.

* Corresponding author.

http://dx.doi.org/10.1016/j.talanta.2017.01.081
Received 22 October 2016; Received in revised form 23 January 2017; Accepted 29 January 2017
Available online 31 January 2017

Compare to luminescent bacteria methods, the electrochemical biosensor possesses the merits of detect online, prompt response, easy to operate, low-cost and not affected by the turbidity of water samples. Thus, fabricating electrochemical biosensors with high detection sensitivity and stability become an attractive research field in recent years. Many efforts have been made to develop an alternative method for acute biotoxicity estimation and consequently, a whole-cell based electrochemical biosensor assay has been proposed as a rapid and reliable method [8–10]. The sensing scheme of these methods normally rely on monitoring certain physiological changes of microbes under the stimulus of environment pollutants, typically, the respiration chain activity is commonly used to reflect the biotoxicity. However, the main drawback of this kind of microbial electrochemical biosensors is the slow electron transfer between the microbial cell wall and the electrode surface. In order to facilitate the electron transfer rate, many efforts have been devoted to effectively overcome the kinetic barriers. Various artificial electron mediators have been widely used to shuttle the electrons by replacing the oxygen to accept the electrons during the microbial respiration chain between the microbe and electrode, which called mediated electron transfer. The mediators that commonly used can be classified into two categories, i.e., hydrophilic mediators (such as potassium ferricyanide) and lipophilic mediators (such as benzoquinone, menadione, dichloroindophenol, 2,3,5,6-tetramethylphenylenediamine and neutral red, etc.). During the interaction with microbes, a hydrophilic mediator cannot cross the cell membrane and is restricted to reacting with the proteins located on the periplasm, but they possess the advantages of high water solubility and high diffusion coefficient in the aquatic system, which greatly promote their application in biosensor preparation and microbial fuel cells fabrication [11,12]. The lipophilic mediators like menadione, on the contrast, can permeate through cell membrane and interact with the intracellular redox centers in cytoplasm and mitochondria, be reduced by the intracellular enzymes and diffuse or transported out of the cell to transport electrons to the electrode surface. However, using lipophilic mediator as sole mediator in the aquatic system may not be a favorable choice, because their low aqueous solubility can greatly affect their concentration in the detection system and hence impact the magnitude of the current signal. To sort out the aforementioned problem, a double-mediator system comprising a hydrophilic and a lipophilic mediator can make up their drawbacks and enables the intracellular redox systems be accessed, providing a reflection of intracellular cell metabolism activities of target cells and high current signal intensity [13]. The potassium ferricyanide-menadione double-mediator system is commonly used to fundamentally investigate the redox activity of *S. cerevisiae* yeast cells and mammalian cells. For instance, potassium ferricyanide-menadione double-mediator system has been applied to analyze a specific enzyme activity or biochemical process in yeast cell [14,15], single cell imaging using Scanning Electrochemical Microscopy (SECM) [16] and the application in microbial fuel cells [17] etc. The combination of mediators can significantly increase the current magnitude and thus improve the detection sensitivity of the electrochemical biosensor.

However, in spite of the improved sensitivity of mediated biosensors, it seems there are still some drawbacks that hinder their practical application. The primary concern of these sensors is most mediators reported were usually added into the aqueous solution directly and their concentrations were kept at a relatively high level, which can be harmful to the cell [18]. Besides, the addition of the mediators may also cause second contamination of aquatic system. Thus, to fully display the merit of electrochemical biosensor, achieve real-time detection, an integrated and portable electrochemical biosensor is needed.

In present work, an integrated and miniaturized whole-cell based electrochemical biosensor was successfully fabricated. A yeast strain (S288C) was employed as the biological recognition element. The yeast cells were immobilized on a chitosan hydrogel polymer film with boron-doped nanocrystalline diamond (BND) particles which has electrodeposited onto a glassy carbon (GC) electrode. The conductivity of the as prepared electrode has greatly improved because of the uniform distribution of BND particles. The biotoxicity of four heavy metal ions, three phenol pollutants and three real wastewater samples were examined by the prepared biosensor. The results indicate that the double-mediator based whole-cell electrochemical biosensor prepared can be applied into the toxicity assessment of wastewater samples and display the advantages of integrated, miniaturized and sensitive, which imply a reasonable way for real wastewater online toxicity detection.

2. Materials and methods

2.1. Chemicals and reagents

$Pb(NO_3)_3$, $CuSO_4{\cdot}5H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2$, 3,5-dichlorophenol, 4-chlorophenol and phenol were provided by Beijing Lanyi Chemical Products Co., Ltd., China. Yeast extract, beef extract and peptone were obtained from Beijing Aoboxing Bio-tech Co., Ltd., China. Potassium ferricyanide was purchased from Arcos USA. Menadione was provided by Adamas Reagent Co., Ltd. Glucose was purchased from Alfa Aesar. All reagents were of analytical grade and used as received without further purification. All solutions were prepared with deionized water (18.0 MΩ cm, Milli-Q Purification System, Millipore) freshly before use.

2.2. Cultivation of microorganism

Saccharomyces cerevisiae (S288C) and *Escherichia coli* (ATCC25922) were inoculated from China General Microbiological Culture Collection Center (CGMCC). A 300 mL flask containing 100 mL autoclaved Yeast Extract Peptone Dextrose medium (YEPD, 0.5% yeast extract, 1% peptone, 1% glucose) was inoculated with a colony of *S. cerevisiae* and grown aerobically at 30 °C for 16 h on a rotary shaker at 200 rpm to allow the *S. cerevisiae* grow into stationary phase. *E. coli* was cultured in 100 mL autoclaved Nutrient Broth solution (10g L^{-1} peptone, 3g L^{-1} beef extract, and 5g L^{-1} NaCl) for 16 h at 37 °C. The microorganism cells were harvested by centrifugation at 6000 rpm for 5 min at room temperature, then washed twice with PBS and resuspended in PBS. The final concentration of *S. cerevisiae* and *E. coli* cell suspension were adjusted by a Secoman Uvikon UV–Vis spectrophotometer at the wavelength of 600 nm (OD_{600}). The cell suspensions with desired concentration were stored at 4 °C until required. The cell suspension was used for the experiments on the day of harvesting.

2.3. Preparation of BND-chitosan hydrogel polymer film

A solid-state diffusion method has been applied to the preparation of boron-doped nanodiamond powder [19]. Detonation nanodiamond was treated in air at 425 °C for 5 h before boron doping to remove the sp^2 carbon. Then the heat-treated nanodiamond and boron powder was wet mixed with ethanol in the ratio of 1:2. The mixture was heated in 900 °C for 24 h under hydrogen gas flow. The boron-doped nanodiamond was finally collected after centrifuging, washing and drying. Chitosan solution was prepared by ultrasonically dissolve 1 wt% chitosan flakes in 1% acetic acid and 0.01 M KCl solution at 40 °C. 0.5 mg/mL boron-doped nanocrystalline diamond (BND) was added into the chitosan solution prepared above and ultrasonic agitate for 3 h until the BND nanoparticles were completely dispersed in the chitosan solution, forming BND-chitosan hydrogel. The 1 cm×1 cm glassy carbon electrodes were applied in present work and the electrodes were polished by 30–50 nm α-alumina powder each time before use to remove the oxidation layer. The polished glassy carbon electrodes were successively ultrasonic washed by nitrate, ethanol and distilled water. A conventional three-electrode system was applied to electrodeposit the BND-chitosan hydrogel onto the glassy carbon electrodes. The polished