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A voltammetric *Rhodotorula mucilaginosa* modified microbial biosensor for Cu(II) determination

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ABSTRACT

It is the first report about the usage of *Rhodotorula mucilaginosa* as a biomaterial to construct a microbial biosensor based on carbon paste for determination of copper. Cu(II) was preconcentrated electrode surface at open circuit and then detected with electrochemical techniques, including Cyclic Voltammetry (CV) and Differential Pulse Stripping Voltammetry (DPSV). Some parameters such as pH of preconcentration solution, preconcentration time, scan rate and effect of interfering heavy metal ions were carried out for optimum responses. The best defined cathodic peak was obtained at pH 5 with 0.05 M $NaNO_3$ and a scan rate of 100 mV/s. The linear range for the developed microbial biosensor was found in the range of 1.0×10^{-7} and 1.0×10^{-5} M (0.0064 and 0.64 mg/L) at the response time of 15 min ($R^2=0.98$). The easy fabrication, sensitivity, low cost and fast response time showed the advantages of the biosensor to conventional techniques.

1. Introduction

Heavy metal determination in environmental control is usually performed by spectroscopic, voltammetric or chromatographic methods which are able to detect single elements or species at low concentrations, but can hardly be used for in-situ analysis or low cost screening procedures. On the contrary, these tasks can be successfully accomplished by biosensors [1]. Using biosensors for quantitative analysis offers an alternative to traditional toxicity tests and classical analytical methods. Biosensors allow a fast, on-site measurement of metal ion concentration by using low cost compact portable equipment offering the same sensitivity and accuracy as the routine techniques do.

Virtually all organisms require copper as a catalytic cofactor for biological processes such as respiration, iron transport, oxidative stress protection, peptide hormone production, pigmentation, blood clotting and normal cell growth and development. However, copper also participates in redox reactions that generate the hydroxyl radical, which causes catastrophic damage to lipids, proteins and DNA. Consequently determination and environmental control of copper should be taken into consideration [2].

In the development of biosensors some biologically active materials such as enzymes, cells, plant and animal tissue have been mostly used. Because of their high specific activities and analyte sensitivities, enzymes are mostly used in the biosensor construction. But most of enzymes used in the microbial biosensor construction are unstable and costly for routine analysis of the specific substances [3]. On the other hand, whole cell microbial sensors have received recent attention; because enzyme purification is unnecessary, whole cell microbial sensors are simple and inexpensive systems to construct biosensors and enzymes are usually more stable in their natural environment in the cell [4]. In addition to these, microbial biosensors are easy to operate and their short responsive time makes them suitable for online and field monitoring [5,6].

The use of biomasses as modifying agents for the preparation of sensors has attracted much interest. The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Cu, Cr, Ni, U and Zn from aqueous solutions. Most of yeast can adsorb a wide range of metal ions or be strictly specific in respect to only one metal ion [7–9]. In addition, there are some advantages to use yeast in the biosensor construction such as speed of growth on a variety of different carbon sources. Yeast is particularly robust with a wide physicochemical tolerance, e.g. pH, temperature and ionic strength and tough cell [3,7].

This study describes a microbial biosensor for selective, not time consuming Cu(II) determination based on carbon paste electrode modified with a lyophilized yeast cell, *Rhodotorula mucilaginosa*. There are a lot of methods related to electrochemical detection of heavy metals using ion exchange voltammetry, but a few studies taking place in literature about the usage of yeasts for determination of heavy metals at biosensor applications. As far as we know, application of biosensor based on carbon paste electrode modified

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with *R. mucilaginosa* for determination of copper from aqueous solutions has not been reported yet.

R. mucilaginosa was selected because of its strong affinity toward copper. Bioaccumulation capacity of *R. mucilaginosa* for Cu(II) has been studied before. The promising results were found for removal of high concentrations of copper ions from the solutions by metal uptake property of *R. mucilaginosa* cells [10]. The present method is reasonably selective and has short responsive time. It is also simple to prepare and does not require excess preliminary treatment.

2. Materials and methods

2.1. Chemicals

All solutions were prepared with distilled water. Stock solutions of Cu(II), Ni(II), Zn(II) and Pb(II) (1.0×10^{-3} M) were prepared from the corresponding analytical grade metal nitrates (Merck, Darmstadt, Germany). Standard solutions of metal ions were prepared freshly by diluting stock solutions. Electrolyte solution was prepared from the analytical grade $NaNO_3$ (Merck, Darmstadt, Germany). Graphite powder (−300 mesh) and paraffin liquid (Merck, Darmstadt, Germany) were used for preparing the carbon paste electrode (CPE). Yeast Peptone Glucose medium (YPG) was used for cultivation of culture. Other chemicals used for cultivation medium were also prepared from analytical grade. The pH of preconcentration solutions was set using 0.1 M KOH or 0.1 M HNO_3 (Merck, Darmstadt, Germany).

2.2. Microorganism and culture conditions

R. mucilaginosa was used in this study as biomaterial to construct microbial biosensor and obtained from Culture Collection of Biotechnology Research Laboratory, Biology Department, Ankara University, Turkey.

Yeast Peptone Glucose (YPG) medium was used for cultivation. Microorganism was incubated for 5 days at 30 °C on a rotary shaker at 100 rpm (New Brunswick Scientific Innova 4230). After incubation period, the cells of *R. mucilaginosa* were harvested by centrifugation at 5000 rpm for 10 min. The supernatant was discarded and washed distilled water. Centrifugation and washing process was repeated three times and then obtained biomass was lyophilized (Edwards, England).

2.3. Preparation of microbial biosensor

The modified CPE was prepared my making a homogenous paste with various amounts of lyophilized cell, graphite powder and paraffin liquid. The paste mixture firmly packed into the hole of electrode body (0.5 cm^2) and pressed with a steal rod. Then electrode surface was smoothed on a paper.

2.4. Apparatus and experimental method

Electrochemical experiments were performed at room temperature with CH Instruments 660B model Electrochemical Analyzer (CH Instrument USA). The instrument was equipped with conventional three electrode cell containing supporting electrolyte solution, 0.05 M $NaNO_3$, deoxygenated by bubbling pure nitrogen before measurements. Working electrodes were carbon paste electrodes modified with *R. mucilaginosa*. The counter electrode was a platinum wire and the reference electrode an Ag/AgCl (saturated KCl). The pH values were measured with a pH meter (Consort P 500).

Experiments were done using the accumulation, medium change and voltammetry scheme. In the accumulation step the modified CPE was immersed in a stirred copper ion solution for a selected time at open circuit. After this step, biosensor was taken out and surface washed carefully with distilled water. Finally, biosensor was placed in the electrochemical cell filling with 15 ml of 0.05 M $NaNO_3$ and voltammetrically measured. Cyclic voltammetry assays were performed with both modified and unmodified CPEs. A 5 mg of lyophilized biomass (5.0% (w/w)) was used for making paste and potential range of −600 to 800 mV was employed and to back (versus the Ag/AgCl reference electrode). Scan rates of 100 mV/s was used in the analysis. For Differential Pulse Stripping Voltammetry assays, modified CPE was prepared with a 10 mg lyophilized biomass (10% (w/w)) and the other technique parameters were identical with CV assays. The background current was also obtained after the accumulation step in the absence of Cu(II) (blank solution) at open circuit.

2.5. SEM measurements

The surface morphology of the microbial biosensor was examined by Scanning Electron Microscopy and elemental analysis was taken out with Energy Dispersive X-ray Spectra (JEOL JSM-7000F Field Emission SEM-EDAX).

3. Results and discussion

3.1. Cyclic voltammograms of microbial biosensor

Cyclic voltammograms of unmodified and modified CPEs were obtained in 0. 05 M $NaNO_3$ for 1.0×10^{-3} M Cu (II). The cyclic behaviors of the biosensors were showed in Fig. 1. The effect of *R. mucilaginosa* modification on the electrode response for the determination of Cu(II) was clearly seen in Fig. 1(a). Well defined cathodic peak which belongs to copper was obtained at about 300 mV by modified CPE.

3.2. SEM characterization and elemental analysis of the developed microbial biosensor surface

The SEM micrograph of the microbial biosensor surface after loading of 1.0×10^{-3} M of Cu(II) were given in Fig. 2. The results of elemental analysis were also given in Table 1. Because of neither N nor Na was not found in the elemental analysis, the data verified that the round shapes which seemed on the SEM micrographs were belong to the oxidized copper ions.

3.3. The effect of scan rate

The effect of different scan rates on the electrode response for the voltammetric determination of 1.0×10^{-3} M Cu(II) with the *R. mucilaginosa* modified microbial biosensor was studied by varying

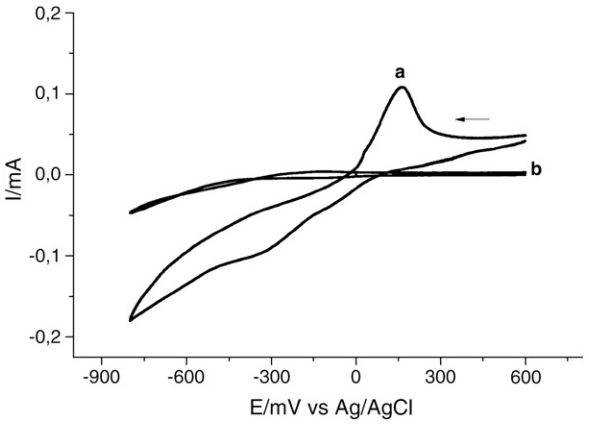

Fig. 1. Cyclic voltammograms of *R. mucilaginosa* modified CPE (a) and unmodified CPE (b). (Concentration of Cu(II): 1.0×10^{-3} M, detection: cyclic voltammetry in 0.05 M $NaNO_3$ with 100 mV/s scan rate, preconcentration time: 15 min).