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Biofuel cell based on horseradish peroxidase immobilized on copper sulfide as anode for decolorization of anthraquinone AV109 dye☆

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ABSTRACT

The potential application of electrochemically formed copper sulfide as horseradish peroxidase mediator in the enzymatic biofuel cell and anthraquinone AV109 dye as a fuel is investigated. The open circuit voltage of 0.52 V and short circuit current of ~3.6 μA/cm^2 are obtained, with the maximum specific power of ~1 μW/cm^2. The influence of internal resistance of the cell is discussed. Decolorization is investigated under open circuit potentials, and under external load of 3.3 kΩ conditions. In both cases, 40% of decolorization is achieved, but are three times faster under external load conditions. Specific energy during decolorization in such cell is estimated to ~5 mWh/m^2. The possible mechanism of the power generation during decolorization of AV 109 dye is discussed.

1. Introduction

With the increasing demands for clean energy [1], biofuel cells could be considered as an environmentally friendly power sources. Enzyme based fuel cells have been reported since the 1960s [2]. Nevertheless, the developments of the enzymatic biofuel cells (EFC) are still in its early days. Compared to conventional fuel cells, research on enzymatic, as well as on microbial, biofuel cells are in the fundamental research stage, because of their low stability and power output [3–5]. Both cells are able to simultaneously produce electric power and removal of different pollutions or oxidation of different biomass related compounds in anode compartment [3,5–7]. Bio-catalytically modified electrodes with enzymes are significant for the performance of biofuel cells. Research in the development of enzyme electrodes for biofuel cell and biosensor applications has been carried out extensively in recent years [3–10]. As a key role in an efficiency of EFC the rate of electron transfer between fuel–enzyme–electrode should be considered. There are two main mechanisms of the electron transfer between enzyme and an electrode [11]. In the direct electron transfer (DET) mechanism, electrons are transferred from or to the electrode on adsorbed, covalently bounded or immobilized enzyme [11,12]. The close contact of the enzyme active sites with the surface of the electrode is crucial for DET. A critical distance between the enzyme active site, which is positioned in the protein shell, and the electrode surface was proposed to be ~2.0 nm [13]. At that distance electron transfer rate is very slow or limited [7]. The second mechanism is mediated electron transfer (MET) where enzyme is attached to a redox mediator. The main role of redox mediator is to increase the rate of electron transfer between the enzyme and the electrode [15].

Peroxidases contain an Fe-porphyrin group as the redox cofactor, and by far, the most applications were described for the commercially available enzyme prepared from horseradish that are in widespread use in biotechnology [12,14]. Horseradish peroxidase (HRP) from *Armoracia rusticana* roots is a versatile, highly specific biocatalyst that have aroused increasing attention due to the possibility of application in various areas such as medicine, diagnostics, chemical synthesis, biosensors and immunoassays [15].

Until now, horseradish peroxidase (HRP) was used in the biofuel cells as an effective cathode material for hydrogen peroxide reduction, produced during glucose oxidation on glucose oxidase anode [16,17].

Many peroxidases such as lignin peroxidase, manganese peroxidase, soybean peroxidase, laccase, and among them horseradish peroxidase, were applied to decolorize and degrade dye in industrial effluents [18–24]. Recently, HRP was used as an effective catalyst for anthraquinone C.I. Acid Blue 225 and C.I. Acid Violet 109 dyes decolorization [25]. It was found that under the optimal conditions, with enzyme concentration 0.15 IU/cm^3, hydrogen

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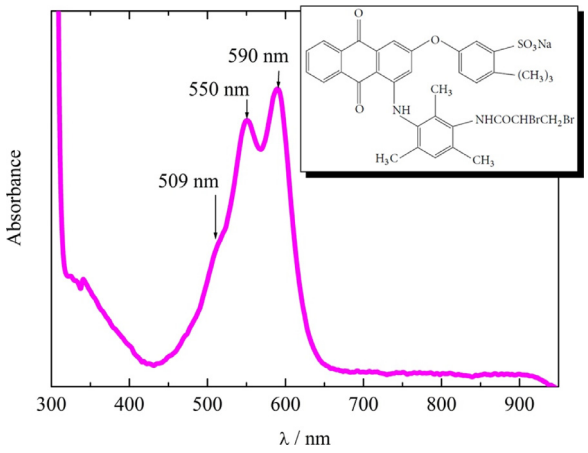

Fig. 1. UV-visible spectra and structure of anthraquinone AV 109 dye.

peroxide concentration 0.4 mmol/L, dye concentration 30 mg/dm^3, at pH 4 and temperature of 24 °C, 85%–95% of C.I. Acid Violet 109 from aqueous solution was decolorized during 15 min. It is interesting to note that using an enzyme concentration of 0.025–0.1 IU/cm^3 decolorization achieved only 35%, which was explained that the AV 109 dye exhibited a rather high inhibitory effect on HRP enzyme.

HRP belongs to the group of oxidoreductases (E. C. 1.11.X) that in the presence of hydrogen peroxide degrades a number of the aromatic compounds [18,26,27]. Horseradish peroxidase catalyzes the oxidation by H_2O_2 of organic molecules that are able to approach the Fe-porphyrin [3]. Because HRP has the critical distance of 1.8 nm [28] a mediator is normally required to achieve electron transfer with an electrode. Among different mediators [3,9,29], metal sulfide was used for some enzyme immobilization on electrode [30–32].

The copper sulfide is an inexpensive, environmentally friendly material, and thus in this paper, we investigated the possibilities of simultaneous oxidation of Acid Violet 109 dye as a fuel and electric power generation, using HRP covalently immobilized on the copper sulfide electrode in the biofuel cell.

2. Experimental

2.1. Chemicals

Copper sulfide was synthesized galvanostatically (j = 1 mA/cm^2, 600 s) onto both sides of the pure copper sheet (99.99%, Alfa-Aesar) 2 cm × 4 cm, from solution contained 0.1 M Na_2S (prepared from Na_2S × $9H_2O$, p.a. Carlo Erba) and 0.1 M Na_2SO_4 as supporting electrolyte. Prior to use, copper was degreased in acetone in an ultrasonic bath, etched in 5 M HNO_3 for 10 s and washed with distilled water. Horseradish peroxidase (EC 1.11.1.7; donor: hydrogen peroxide oxidoreductase), with a specific activity 200–250 IU/g solid and dithiothreitol were obtained commercially from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide was purchased from Merck (Darmstadt), and its concentration was determined several times using its molar absorption coefficient (ε = 43.6 /M·cm^{-1}) at λ = 240 nm by dilution of the supplied H_2O_2 (30%) solution.

Anthraquinone dye used in this paper, C. I. Acid Violet 109 (AV 109), was obtained from Lanaset (Lanaset Violet B). The chemical structure and typical UV-visible spectra of the AV 109 spectra was shown in Fig. 1. The decolorization was followed at 590 nm, using LLG uniSPEC-2 UV-visible spectrophotometer.

2.2. Immobilization of HRP on the copper sulfide electrode

Prior to the immobilization, HRP was reduced, to obtain surface thiol groups which can improve covalent bonding onto copper sulfide, according to the method described in the literature [33]. The copper sulfide electrode was immersed in 12 cm^3 of HRP solution (1 mg/cm^3) prepared in 0.1 mmol/L phosphate buffer solution (PBS) for 24 h at 4 °C. After the immobilization was finished, subsequently electrode was rinsed with the immobilization buffer and 1 M $CaCl_2$ to remove loosely bonded enzyme. Immobilization efficiency was calculated on the basis of difference between offered and recovered HRP amount in the supernatant and washings. The amount of HRP in the supernatant and washings was determined using a modified method of Lowry with bovine serum albumin as reference [34]. The adsorbed mass of the protein on the electrode was determined to be 0.4 mg or ~0.08–0.1 IU. The obtained HRP-CuS electrode was stored refrigerated (4 °C) until used.

2.3. Biofuel cell and methods

Biofuel cell was consisted of two 150 cm^3 Pyrex beakers connected with U-shaped glass pipe, as ionic conductor, with the diameter of 0.8 cm filed with the electrolyte. As electrolyte 100 cm^3 0.1 M Na_2SO_4 buffered with 0.05 potassium biphtalate, pH = 4.1, was used. In one beaker with copper sulfide-HRP anode, magnetic stirring was applied, while in the other pure electrolyte and Pt-mesh cathode (1 cm × 10 cm rolled into a cylinder) with the air bubbling, ~10 mL/s, was used. As a reference, saturated calomel electrode was used.

Experiments were performed using Gamry PC3 potentiostat/galvanostat, and current and/or voltage was measured using PeakTech 4390 USB DMM digital multimeter and *R*-substitution box MA 2200, 1 MΩ–33 Ω, (Iskra-Kranj, Slovenia). Optical micrograph was obtained with optical microscope Olympus CX41 connected to PC.

The chemical composition of the copper sulfide sample was analyzed using an Energy Dispersive Spectrometer (EDS) Isis 3.2, with a SiLi X-ray detector (Oxford Instruments, UK) and a computer multi-channel analyzer. For the EDS analysis to avoid influence of underlay metallic copper, copper sulfide was prepared by prolonged oxidation, 60 min, during which CuS particles fell down from the electrode. Particles were filtered and properly washed with distilled water using Büchner funnel.

3. Results and discussion

3.1. Synthesis of the copper sulfide

Copper sulfide was formed by anodic oxidation of the pure copper in 0.1 M Na_2SO_4 and 0.1 M Na_2S. Anodic polarization curve, shown in Fig. 2, was characterized by the absence of any reaction at potentials more negative than –1.1 V. Some small peak positioned at –1.05 V, and a huge peak at –0.95 V was observed during the anodic scan to the potentials of –0.75 V. The reversible standard potential for the reaction [35]:

$$Cu_2S + 2e = 2Cu + S^{2-} \quad (1)$$

is –1.133 V (SCE), therefore at potential of ~ –0.9 V, oxidation of the metallic copper to Cu_2S phase is thermodynamically possible. Increasing anodic potentials, few different peaks connected with the oxidation reactions were observed in Fig. 2(a) and could be connected to the Cu_2S phase transformation and higher potentials with S^{2-} oxidation. Different $Cu_{2-x}S$ phases (x = 0 – 1) tend to coexist, resulting in many possible phases. Some of the most common phases of the copper sulfide at room temperature are