Original Article:

Direct electron transfer of laccase enzyme based on RGO/AuNPs/PNR

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ABSTRACT

Biofuel cell has received much attention in recent years due to the global energy demand increase per year. This paper presents a design for *Mytheliophthora thermophile* laccase on the electrode as a biocathode for biofuel cells based on direct electron transfer (DET) between the active site of the enzyme and reduced graphene oxide-gold nanoparticles-poly neutral red (RGO/AuNPs/PNR). The RGO/AuNPs/PNR/laccase biocathode was characterized by cyclic voltammetry (CV) method. The CV experiments demonstrated the activity, direct electron transfer, and stability of immobilized enzyme on the nanocomposite. The results showed that the immobilized enzyme had good stability and performance on the nanocomposite after 10 days. Therefore, the presented method could properly be used in the design of biosensors or biocathode of biofuel cells.

Keywords: Direct electron transfer; Laccase; Reduced graphene oxide (RGO); Gold nanoparticles (AuNPs); Poly neutral red (PNR); Biofuel cell.

INTRODUCTION

Biofuel cells are energy conversion devices that use enzymes, microorganisms or organelle as bio-electro-catalysts [1-3]. Chemical reactions in enzyme based on biofuel cells is be carried on by two different electron transfer methods: direct electron transfer (DET) or mediated electron transfer (MET). In DET, electrons transfer directly between enzyme and electrode surface. In MET, the mediator shuttles the electron between enzyme and electrode surface to reduce the kinetic barrier in the electron transfer. DET is desirable for efficient communication between enzyme and electrode; eliminating the mediator would simplify the construction of biofuel cells [4, 5]. The current and power density in DET systems is traditionally lower than MET systems. Due to advantages of eliminating the mediator, scientists are interested on employing direct electron transfer systems at the anode and cathode of biofuel cells [6, 7]. Laccase is a glycoprotein enzyme which catalyzes the degradation of many phenolic compounds [8, 9]. This enzyme has four copper atoms (Cu^{2+}): copper atom type 1 (T1) is in the enzyme surface. Likewise, a cluster of three coppers contain a copper type 2 (T2) and 2 coppers type 3 (T3) are at depth of the enzyme. Copper T1 captures electron from the substrate and transfers it to the T2/T3 copper cluster. Copper T2 is a resource of electrons and copper T3 reduces oxygen to water [10]. There are different applications of laccases in the enzymatic fuel cells and biosensors. Therefore, optimization of the performance of laccase-based biocathode seems critical [11, 12]. In the present study, laccase enzyme was immobilized on the glassy carbon electrode (GCE) based on RGO/AuNPs/PNR nanocomposite. The RGO/AuNPs/PNR biocathode was evaluated by cyclic voltammetry (CV). Laccase on the RGO/AuNPs/PNR nanocomposite showed an excellent response to reduction of syringaldazine. This new biocathode is a proper choice for practical applications in biosensor and biofuel cell.

MATERIALS AND METHODS

Materials

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) from *Mytheliophthora thermophile* was obtained from Novozyme Company (Portugal). NR, AuNPs and syringaldazine were obtained from Sigma-Aldrich (U.S.A). Na_2HPO_4, NaH_2PO_4, CH_3COOH, CH_3COONa were purchased from Merck (Germany). All electrochemical experiments were repeated at least three times and were performed through deionized water.

Preparation of biocathode

The bare GCE was cleaned by alumina powder and distilled water before modification. The clean electrode was modified by dropping RGO-AuNPs, electropolymerization of NR and immobilization of 4 µL of GOx enzyme (5 mg ml^{-1}) on the electrode surface [13]. The RGO was synthesized from graphite based on Hummers' method [14]. PNR polymer was synthesized by CV in the phosphate buffer containing 100 mM of NR, pH=5.5 and 25 ^oC at a scan rate of 100 mV s^{-1}. The potential was carried out at 100 mV s^{-1} between -1 and +0.6 V for 20 cycles [15].

Cyclic Voltammetry

The electrochemical measurements were performed with Potentiostat Galvanostat (Drop Sens model DRP-STAT 200) at room temperature. A conventional three-electrode cell of volume 5 ml electrolyte, containing 100 mM acetate buffer pH 4.6 (included glassy carbon) was used for the purpose of the experiments. A platinum wire and Ag/AgCl were used as the counter and the reference electrodes respectively. Similarly, electrochemical measurements were obtained by a cyclic potential scan in a potential range from 0.0 to 1.0 V and the scan rate of 100 mV s^{-1}. Before all experiments, acetate buffer solution was bubbled for 30 min, using nitrogen gas.

RESULTS

CV characterization

The catalytic activity of the modified biocathode was investigated by CV in different steps. The bare GCE voltammogram was characterized in 10 mM ferrocyanide at scan rate of 100 mVs^{-1} at room temperature (Fig. 1). The voltammogram of RGO-AuNPs nanocomposite on the GCE in the presence of 100 mM acetate buffer, pH 4.6 and scan rate of 100 mVs^{-1} was studied (Fig. 2). Figure 3 shows the electro-synthesis of PNR on the RGO-AuNPs nanocomposite. The activity of modified biocathode with laccase in buffer and presence of 10 mM syringaldazine as substrate is manifested in Figure 4. The biocathode in buffer was used as a control to show the activity of laccase in presence of syringaldazine. A pair of well-defined redox peaks shows that the RGO/AuNPs/PNR nanocomposite plays an important role in facilitating electron transfer between the laccase and GCE.

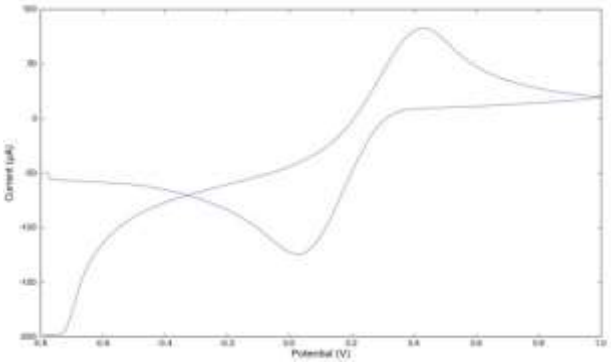

Figure 1. Cyclic voltammogram of the bare GCE in 10 mM potassium ferrocyanide at 25 °C. Scan rate: 100 mVs^{-1}. Scan range: -0.8–1.0 V.

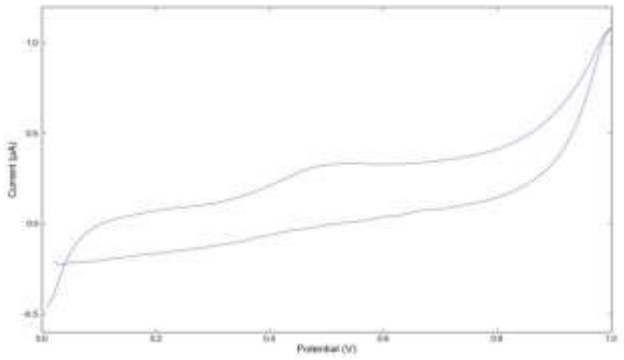

Figure 2. Cyclic voltammogram of the RGO-AuNPs nanocomposite in 100 mM acetate buffer, pH 4.6 at 25 °C. Scan rate: 100 mVs^{-1}. Scan range: 0.0–1.0 V.

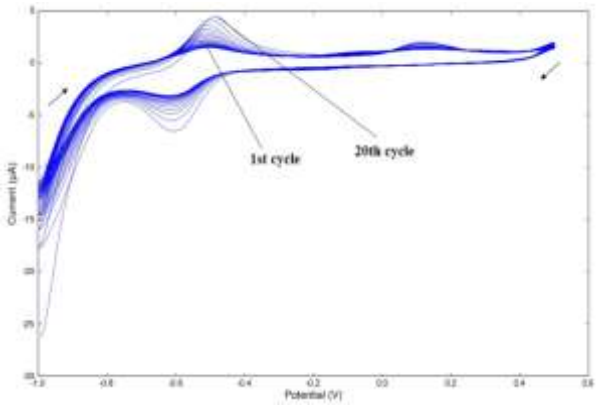

Figure 3. Cyclic voltammogram of the electro-synthesis of PNR on AuNPs/RGO in 100 mM potassium phosphate buffer, pH 5.5 at 25 °C. Scan rate: 100 mVs^{-1}. Scan range: -1.0–0.6 V

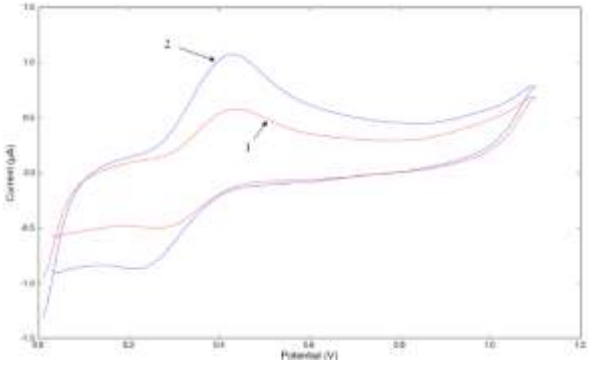

Figure 4. Cyclic voltammograms of the laccase immobilized using the RGO-AuNPs/PNR nanocomposite in buffer (1) and in presence of 10 mM syringaldazine (2) in 100 mM acetate buffer, pH 4.6 at 25 °C. Scan rate: 100 mVs^{-1}. Scan range: 0.0–1.0 V

Direct electrochemistry and eEffect of scan rate

The CV of immobilized laccase on RGO/AuNPs/PNR nanocomposite at different scan rates of 50, 100, 150, 200, 300, 400 and 500 mV s^{-1} was achieved in the potential range from 0.0 to 1.0 V in 100 mM acetate buffer pH 4.6 (Fig. 5A). Figure 5B shows linear behavior of the current changes at different scan rates. The present results demonstrated that the redox process of laccase in the nanocomposite is reversible and fast. Therefore, a direct electron transfer of laccase on RGO/AuNPs/PNR based nanocomposite has been achieved successfully.

Stability

The stability of the presented biocathode was examined by keeping the biocathode at 4 °C for 10 days and measuring its activity. The peak current of the conserved biocathode decreased only 10% of its initial response after being stored for 10 days (Fig. 6). The results showed that the presented biocathode has good stability and biocompatibility for laccase enzyme. Table 1 shows the anodic and cathodic peak cyclic voltammograms of modified biocathode in first day and after 10 days of starvation in 4 °C.

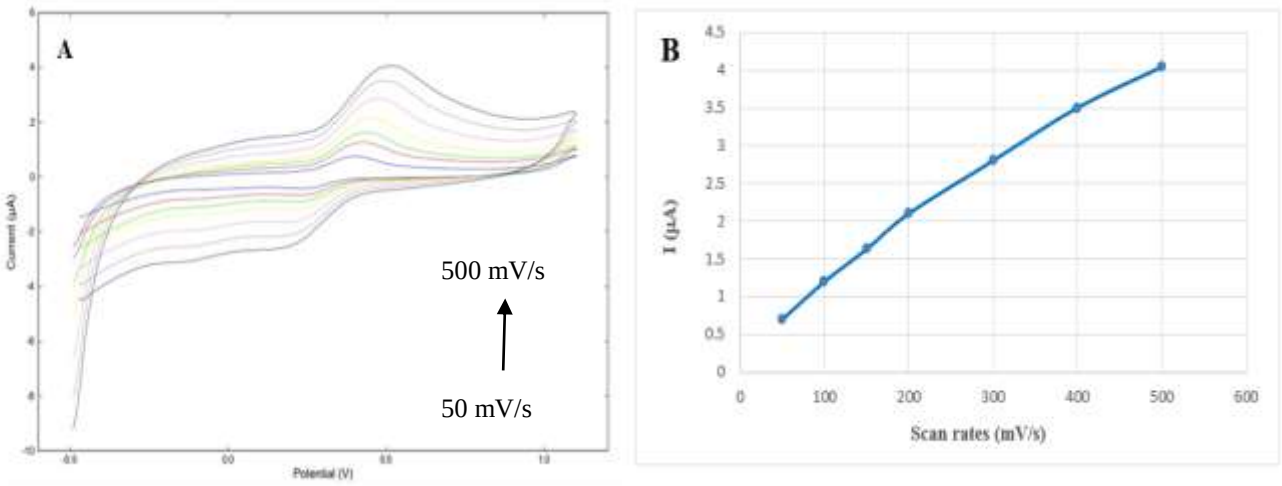

Figure 5. (A) Cyclic voltammograms of the laccase immobilized onto RGO/AuNPs/PNR nanocomposite at a scan rate of 50,100,150,200,300,400 and 500 mVs^{-1} in 100 mM acetate buffer, pH 4.6 at 25 °C. Scan range: 0.0–1.0 V. (B) Relation between the cathodic current peaks and different scan rates of presented biocathode

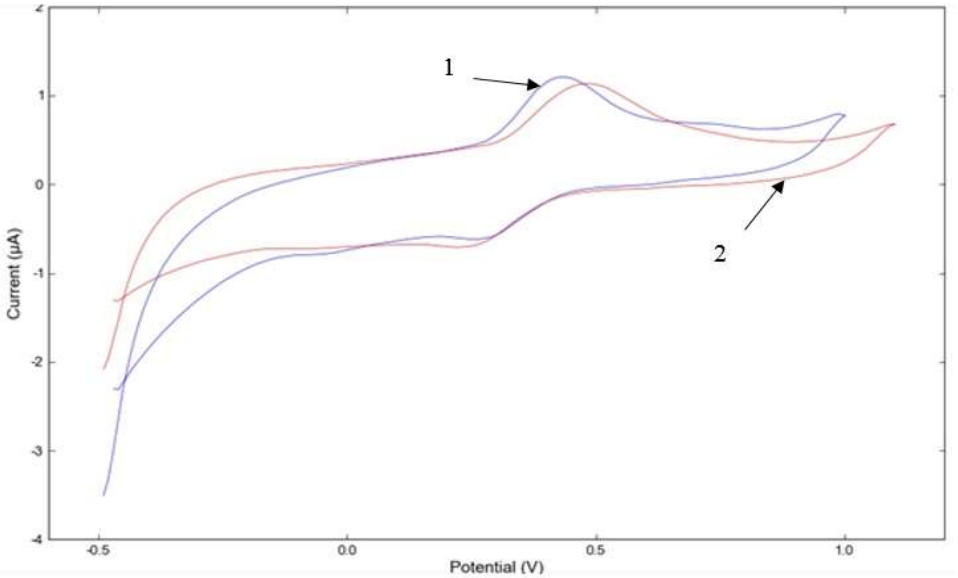

Figure. 6 Cyclic voltammograms of RGO-AuNPs/PNR/laccase modified electrode after 1 day and 10 days of starvation in the 100 mM acetate buffer at pH 4.6 and 25 °C. Scan rate: 100 mVs^{-1}. Scan range: -0.5–1.0 V.

Table 1. Stability of presented biocathode after 10 days of enzyme immobilization

Time	Anodic peak current (µA)	Cathodic peak current (µA)	Cathodic potential (V)
First Day	1.22	-0.59	0.25
After 10 Days	1.14	-0.70	0.23

DISCUSSION

The most important point in constructing a biofuel cell is immobilization of enzyme onto electrode surface. RGO-AuNPs/PNR on electrode surface was a good nanocomposite for laccase immobilization. The immobilized laccase on the RGO-AuNPs/PNR keep its biologic activity and showed good response to 10 mM syringaldazine. Because, RGO and AuNPs have nanostructures that increase the surface of electrode and enhance the electron transferring between electrode and laccase. PNR is an organic dye by phenazine structure that contains electron-donating pendant amine groups and at least one ortho or para free position [16, 17]. Therefore, RGO-AuNPs/PNR nanocomposite has good electric conductivity and a high rate of electron transfer without any electron transfer mediator for new enzyme immobilization. The direct electrochemistry of enzyme using nanomaterials and omitting the toxic mediators for electron transferring in the fields of biofuel cells and biosensors is very important issue [18, 19]. This process is controlled by the redox monolayer species adsorbed on the electrode surface [20, 21]. For redox monolayer modified electrode in voltammetric studies, the peak potentials and different parameters that calculated by Laviron method confirmed the direct and fast electron transfer of electron from laccase to electrode surface [22].

CONCLUSION

This paper we was immobilized the laccase enzyme on GCE modified by RGO-AuNPs/PNR. The electrochemical results of biocathode showed a pair of well-defined redox peak, corresponding to direct electron transfer of immobilized laccase on the RGO-AuNPs/PNR in the presence of syringaldazine as a substrate. Furthermore, the results manifest enhanced DET and high activity and stability of modified biocathode after 10 days. As a result, the method presented here could be properly used in laccase-based biosensors or as a bioelectrode in enzymatic biofuel cells.

"The authors declare no conflict of interest"

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