

Electrochemical Evaluation of *Phanerochaete Chrysosporium* Based Carbon Paste Electrode with Potassium Ferricyanide Redox System

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In the present study, a modified carbon paste electrode is reported with fungal biomass of *Phanerochaete chrysosporium* as the modifying agent. Cyclic voltammetric studies with the redox couple of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ are used for electrochemical characterization of modified electrode. These studies revealed that the modified electrode has a greater electrochemical surface area, coverage capacity, and heterogeneous rate constant in comparison to unmodified electrode. Infrared spectra, atomic force and scanning electron microscopic images are used to characterize surface morphology of modified and unmodified electrode. In addition, Nyquist plots showed that charge transfer experienced less resistance on the modified electrode illustrating that *Phanerochaete chrysosporium* has improved the electro catalytic efficiency of the carbon paste.

Keywords: fungal specie; modified carbon paste electrode; cyclic voltammetry; potassium ferricyanide

1. INTRODUCTION

For many years, carbon has been a popular choice as an electrode material as it has wide potential window, chemical inertness and low cost characteristics [1]. Among these carbon electrodes, carbon paste is widely employed as it allows low background current and has composite nature [2]. Moreover, the ease of modification and renewal in these carbon paste electrodes make them a better substitute of macro-electrodes. With the passage of time, these carbon paste electrodes have undergone through a process of modification in order to purposely design the surface of the electrode with the intrinsic properties of the modifier. Modified carbon paste electrode exhibits better and improved response than the unmodified one [3]. The modification procedure plays a crucial role in the pre-

concentration step of stripping analysis as it favors selective and enhanced accumulation of target specie. This augmented accumulation of analyte in turn improves the sensitivity and lowers the detection limit [4]. Moreover, during the detection step it favors the electron transfer kinetics. Modifier used to fabricate the carbon paste electrode can be broadly classified into a chemical or biological agent. The association of modifier with the electrode is carried out by adsorption, covalent binding, coating or even dispersion into a conductive matrix [5]. Among materials of biological origin, fungal biomass can be potent source of modification as it is easy to grow, produce high yield of biomass with unsophisticated fermentation techniques or inexpensive growth media [6]. It is evident from literature that fungal biomass has been exploited as bio sorbent as it has a high proportion of cell wall as compared to other micro-organisms and possess excellent binding capacities owing to carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups [7]. Among fungi, white rot fungi are known by their ability to degrade lignin due to presence of laccase, Mn peroxidase and lignin peroxidase enzymes [8].

In the present study, a white rot fungi *Phanerochaete chrysosporium* is used as a modifier in the preparation of biologically modified carbon paste electrode. *P. chrysosporium* is quite known in literature due to its effectiveness in removal, reduction and detoxification of textile dyes, heavy metals and industrial effluents [9,10]. Cyclic voltammetric studies of this modified carbon paste electrode is carried out with potassium ferricyanide ($K_3[Fe(CN)_6]$) system in order to evaluate its electrochemical surface area, nature of electrode process and electron transfer along with calculation of kinetic parameters (charge transfer coefficient and heterogeneous rate constant). Choice of cyclic voltammetry is made as it is widely used technique in study of redox reactions and provides information about the occurrence of chemical reactions [11]. On the other hand, among a number of benchmark redox systems $K_3[Fe(CN)_6]$ is selected for its surface sensitive electrochemical response especially for carbon material [12].

2. MATERIAL AND METHOD

2.1 Chemicals and reagents

Graphite powder and mineral oil are used as received from Alfa aesar, Germany and MP, Biomedicals, France respectively. Salt of potassium ferricyanide and potassium chloride is obtained from Sigma Aldrich, Germany.

2.2 Equipment

Cyclic voltammetric experiments are performed with a potentiostat/galvanostat (Gamry 600 TM Reference, Germany). This system is equipped with 'EChem Analyst' and 'My Gamry Framework' software for data acquisition and interpretation. This is a three cell electrode system in which modified/unmodified carbon paste electrode is taken as working electrode while saturated calomel electrode (SCE) and platinum wire acted as reference and counter electrode respectively. All

the measurements are performed at ambient temperature ($25^{\circ}\text{C}\pm 2$). pH measurements are performed with a Eutech 2700 pH instrument.

2.3 Culture conditions for *Phanerochaete chrysosporium* biomass

The nutrient medium of *P. chrysosporium* (ATTC 24725) is prepared according to a reported method [13]. The autoclaved nutrient medium is inoculated and left in an orbital shaker for 15 days at 35°C . At the end of 15 days, biomass was harvested, washed with double distilled water and dried in oven at 90°C for 24 hours. The dried biomass was then grinded and sieved through mesh of $250\ \mu\text{m}$.

2.4 Preparation of unmodified and modified carbon paste electrode

Modified carbon paste electrode is prepared as reported previously [14] by mixing graphite powder, mineral oil and *P. chrysosporium* biomass. A homogenized paste of these three components is prepared by mixing them in *w/w* ratio of 65:30:5. This paste is then firmly packed into the electrode body (plastic syringe, i.d 5mm) and an electrical contact is ensured with a copper wire. Unmodified carbon paste electrode is prepared by mixing graphite powder and mineral oil in ratio of 70:30.

2.5 Surface characterization of unmodified and modified electrode with FTIR spectra, AFM and SEM images

Surface characterization of unmodified and modified electrode is performed via Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM) and atomic force microscopy (AFM).

FTIR spectra are taken with attenuated total reflectance (ATR) in solid phase in the range of 2000 – 3000 wavenumbers cm^{-1} (Alpha Bruker, Germany). The data obtained from FTIR-ATR spectra is evaluated with OPUS 5.5 software. AFM (Shimadzu, SPM 9500J3) technique is used to characterize unmodified and modified electrode for average roughness. While the SEM images for unmodified and modified electrode are taken at 330X and 400 X magnifications with $50\ \mu\text{m}$ marker respectively for surface morphology (JEOL, JSM-6480).

3. RESULTS AND DISCUSSION

3.1 Surface characterization of unmodified and modified electrode with FTIR spectra, AFM and SEM images

FTIR spectra for both unmodified (UNMCPE) and modified carbon paste electrode (MCPE) are shown in Fig 1a. It can be seen that both the FTIR spectra mainly illustrated peak intensities related to carbon based functional groups that might be due to the greater amount of graphite powder in carbon paste electrodes. In unmodified and modified electrodes, prominent peaks at $2951\ \text{cm}^{-1}$, 2898

cm^{-1} and 2852 cm^{-1} are due to asymmetric and symmetric stretching of methyl and methylene groups. Moreover, a number of peaks appeared between the ranges of $2000\text{-}2200 \text{ cm}^{-1}$ for both types of electrodes. It is reported in literature that mono substituted terminal alkyne group gave peak between $2100\text{-}2140 \text{ cm}^{-1}$ and peak for di-substituted medial alkyne group appeared around $2190\text{-}2200 \text{ cm}^{-1}$.

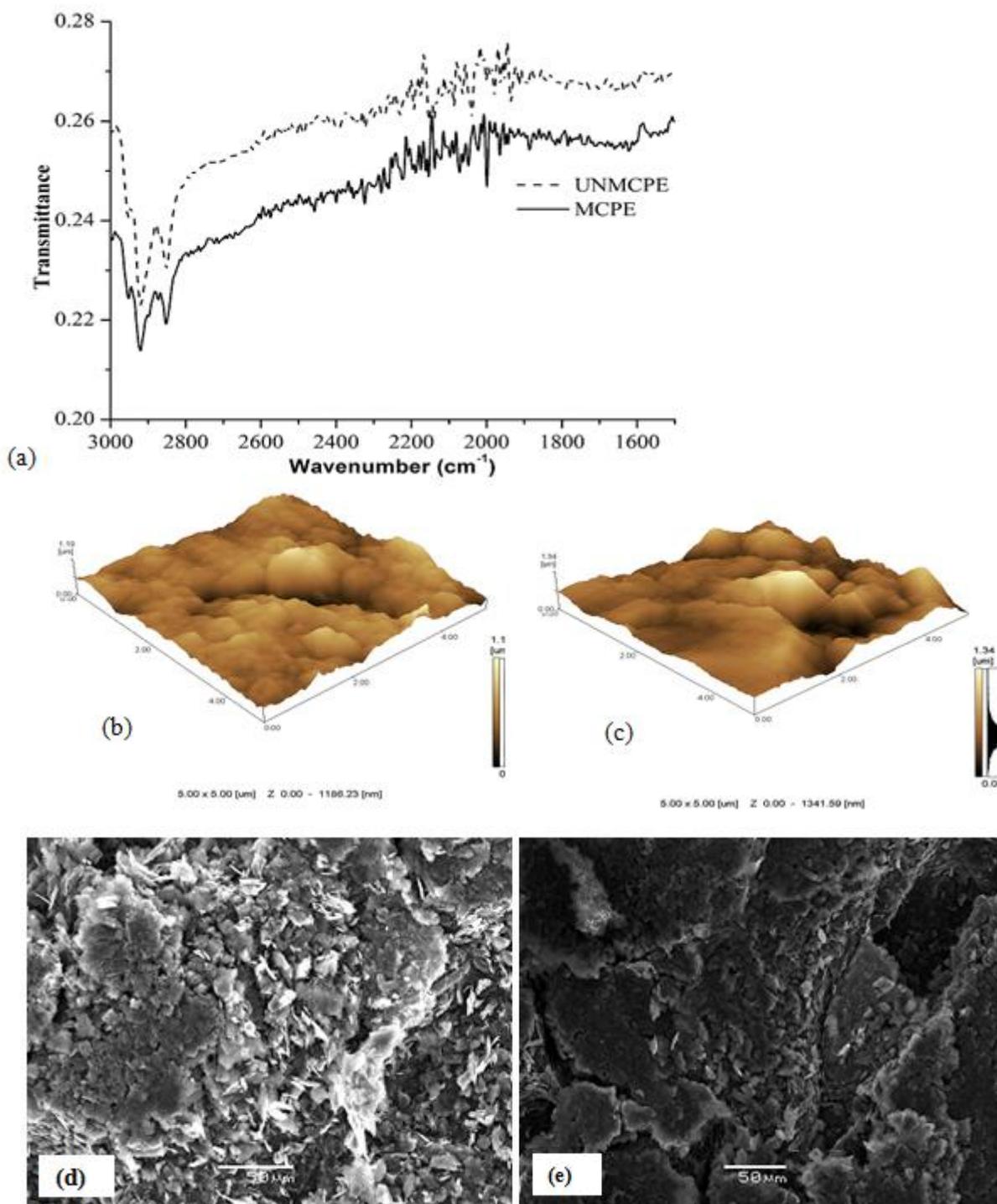


Figure 1. (a) FTIR spectra, (b,c) 3D AFM images and (d,e) SEM images for modified (MCPE) and unmodified (UNMCPE) electrode

In comparison to unmodified electrode a prominent peak at 1620 cm^{-1} is present in modified electrode that is due to NH bending of primary amine groups [15]. Various functional moieties of *P. chrysosporium* are not clearly visible in modified electrode as the carbon material has suppressed them. 3D AFM and SEM images for modified and unmodified electrode are shown in Fig 1b,c and Fig 1d,e respectively.

Modified electrode has a lower average roughness value (92.85 nm) than unmodified one (93.75 nm). This demonstrated that addition of *P. chrysosporium* biomass to the carbon paste has changed the surface morphology of the electrode. It is reported in literature that inclusion of modifier decreased the surface roughness of carbon paste material [16]. The SEM image of unmodified electrode (Fig 1d) showed a homogeneous microstructure and seemed to be covered by a thin film of oil. On the other hand modified electrode depicted irregular surface with fungal biomass appeared to be attached as clusters to the surface of carbon paste (Fig 1e).

3.2 Electrochemical behavior of unmodified and modified electrode

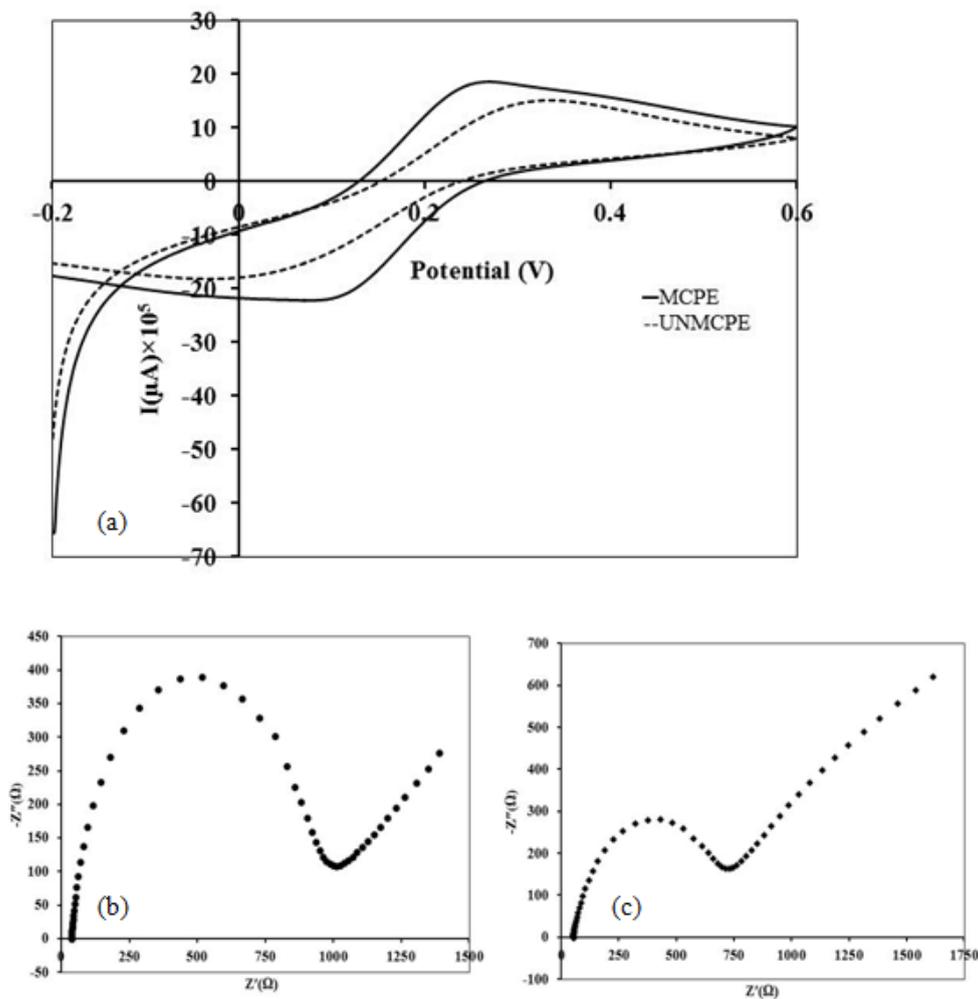


Figure 2. (a) Cyclic voltammogram of modified (MCPE) and unmodified (UMCPE) carbon paste electrode in 5mM $\text{K}_3\text{Fe}(\text{CN})_6/0.1\text{ M KCl}$ system at 100 mV/s (b) Nyquist plots for unmodified (UMCPE) and modified (MCPE) carbon paste electrode

Cyclic voltammograms of unmodified and modified electrodes are shown in Fig 2a. These voltammograms are taken with 5 mM $K_3[Fe(CN)_6]/0.1$ M KCl system. Redox couple $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ gave a well-defined oxidation and reduction peaks during the forward and reverse scan at potential range of -0.2 V to 0.6 V with 100 mV/s scan rate. Modified carbon paste electrode gave more pronounced peaks as compared to unmodified one. In case of modified electrode, an anodic and cathodic peak appeared at 253.9 mV and 108.1 mV respectively. Peak separation value is calculated to be 145.8 mV which indicated a quasi-reversible electrode behavior [17].

The electrochemical impedance spectroscopy (EIS) is a powerful tool to study the interfacial processes on the surface of electrode under the stimulation of a small a.c field. Fig 2b,c demonstrated the Nyquist plot for unmodified and modified electrodes. Nyquist plot for unmodified electrode consisted of a big semicircle arc followed by a smaller Warburg diffusion line that depicted a high resistance is encountered during the electrode reaction. On the other hand, in case of modified electrode, the charge transfer experienced less resistance as the Warburg line predominate indicating a diffusion controlled mechanism [18].

3.3 Electrochemical area of the electrodes

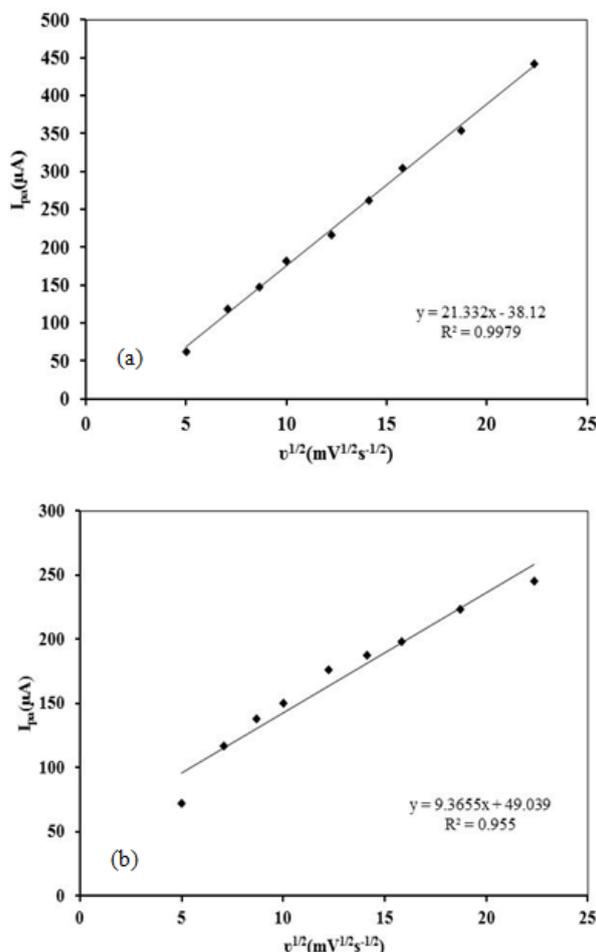


Figure 3. Plot of anodic and cathodic peak current (μA) vs square root of scan rate (mV/s)^{1/2} for modified (a) and unmodified (b) electrode

The relation between redox peak currents and square root of scan rate helped to calculate the electrochemical surface area by using following Randles Sevcik equation [19]:

$$I_p = 0.4463 \times \left(\frac{F^3}{RT} \right)^{1/2} \times n^{3/2} \times A_0 \times D_0^{1/2} \times C v^{1/2} \quad \text{Eq 1}$$

where I_p refers to the peak current, n is the number of electrons transferred, A_0 is the surface area, D_0 is the diffusion coefficient, v is the scan rate and C is the concentration respectively.

Here in the present study, a linear correlation can be seen (Fig 3a,b) between these two parameters in case of unmodified and modified electrodes (Eq 2, Eq 3)

$$I_{pa} (\mu A) = 9.36v^{1/2} (mV^{1/2}s^{-1/2}) + 49.039, r^2 = 0.955 \quad \text{Eq 2}$$

$$I_{pa} (\mu A) = 21.33v^{1/2} (mV^{1/2}s^{-1/2}) - 38.12, r^2 = 0.9979 \quad \text{Eq 3}$$

According to Randles Sevcik equation, such behavior corresponded to an electrochemical process that is dependent on electrode potential and diffusion [20]. This linear relationship between anodic peak current and square root of scan rate indicated a reversible reaction where the electroactive specie follow the diffusion process to reach the surface of the electrode and such reaction is limited by mass transport [21]. In short, procedure of electron transfer is mass transport while the whole process is diffusion based. The electrochemical surface area for unmodified and modified electrodes is found to be 0.1248 cm^2 and 0.2844 cm^2 for 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl electrolyte by taking these values of parameters: $T = 298 \text{ K}$, $R = 8.314 \text{ J/Kmol}$, $F = 96480 \text{ C/mol}$, $n = 1$ and $D_0 = 7.6 \times 10^{-6} \text{ cm}^2/\text{s}$.

3.4. Effect of scan rate

The effect of scan rate is studied within the range of 25-500 mV/s for both type of electrodes. This parameter helped to evaluate various aspects of electrochemical procedure.

As discussed before, a linear correlation is observed between anodic peak current and square root of scan rate. Moreover, oxidation peak current value for the redox couple $\text{Fe}(\text{CN})_6^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ increased with increasing scan rate due to heterogeneous kinetics.

Based on the plot of logarithm of peak current and scan rate (Fig 4a,b), a straight line is obtained for both electrodes fulfilling the following equations

$$\log I_{pa} (\mu A) = 0.3823 \log v (V/s) + 2.534, r^2 = 0.9592 \quad \text{Eq 4}$$

$$\log I_{pa} (\mu A) = 0.627 \log v (V/s) + 2.854, r^2 = 0.9877 \quad \text{Eq 5}$$

According to above plot, a slope value of 0.5 and 1 is obtained for diffusion or adsorption controlled redox process. Thus this plot gave idea whether the redox process on the surface of electrode involved diffusion or adsorption as the rate determining step. A slope of 0.3823 is obtained in case of unmodified electrode (Eq 4) that showed the electron transfer on the surface of electrode is purely controlled by the diffusion process. On the other hand, a slope value of 0.627 for modified electrode (Eq 5) is also close to theoretical value (0.5) of diffusion controlled process [22].

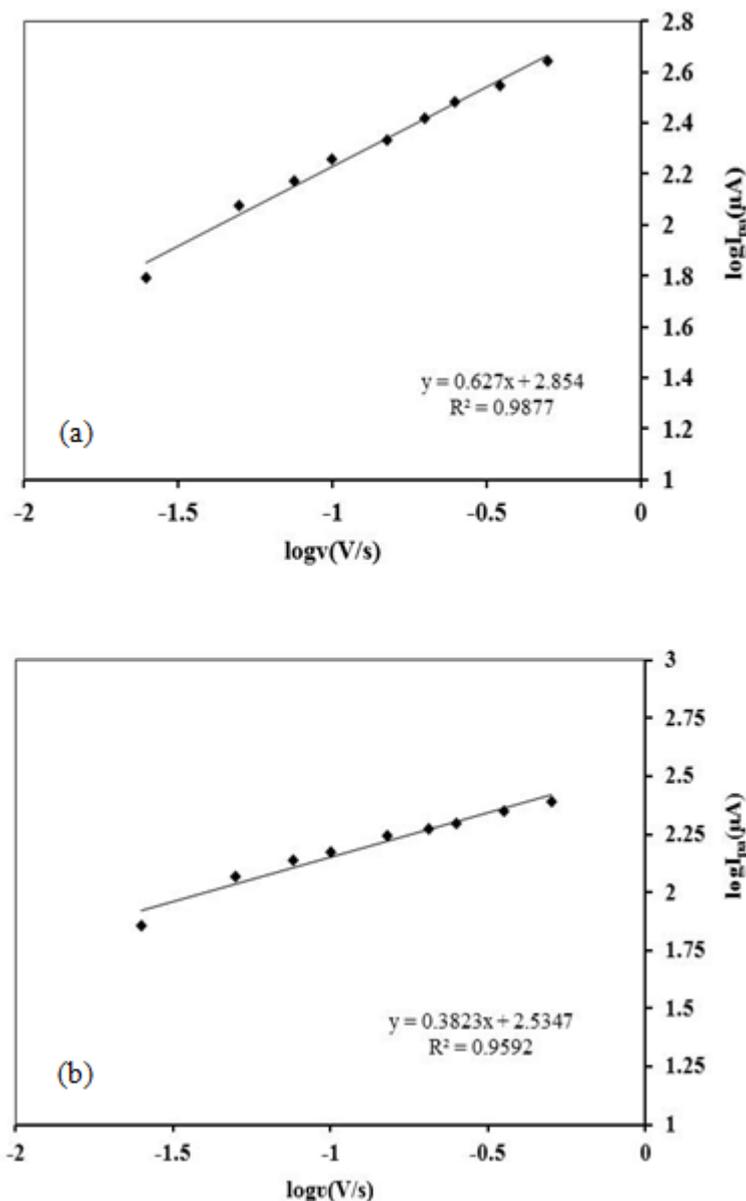


Figure 4. Plot between logarithm of peak current (μA) and logarithm of scan rate (V/s) for modified (a) and unmodified carbon paste electrode (b)

3.4.1. Calculation of surface coverage

Using the slope value from the plot of scan rate and peak current (Fig 5a,b) and with the help of following formula the surface coverage capacity for unmodified and modified electrodes (Eq 6, Eq 7) is calculated.

$$I_p = \frac{n^2 F^2 \Gamma A v}{4RT} \tag{Eq 6}$$

where Γ is the surface coverage of adsorbed molecule (in mol/cm^2) and others are commonly known parameters in their values, as discussed above. The surface coverage capacity for unmodified and modified electrodes is $2.73 \times 10^{-6} \text{ mol/cm}^2$ and $2.83 \times 10^{-6} \text{ mol/cm}^2$ respectively. In case of both

electrodes, correlation coefficient values of 0.85 and 0.96 illustrated that peaks current values are proportional to scan rate. This proportionality demonstrated a thin layer phenomenon [23].

$$I_p(\mu A) = 0.321v(mV/s) + 107.03, r^2 = 0.8556 \tag{Eq 7}$$

$$I_p(\mu A) = 0.7584v(mV/s) + 88.83, r^2 = 0.9616 \tag{Eq 8}$$

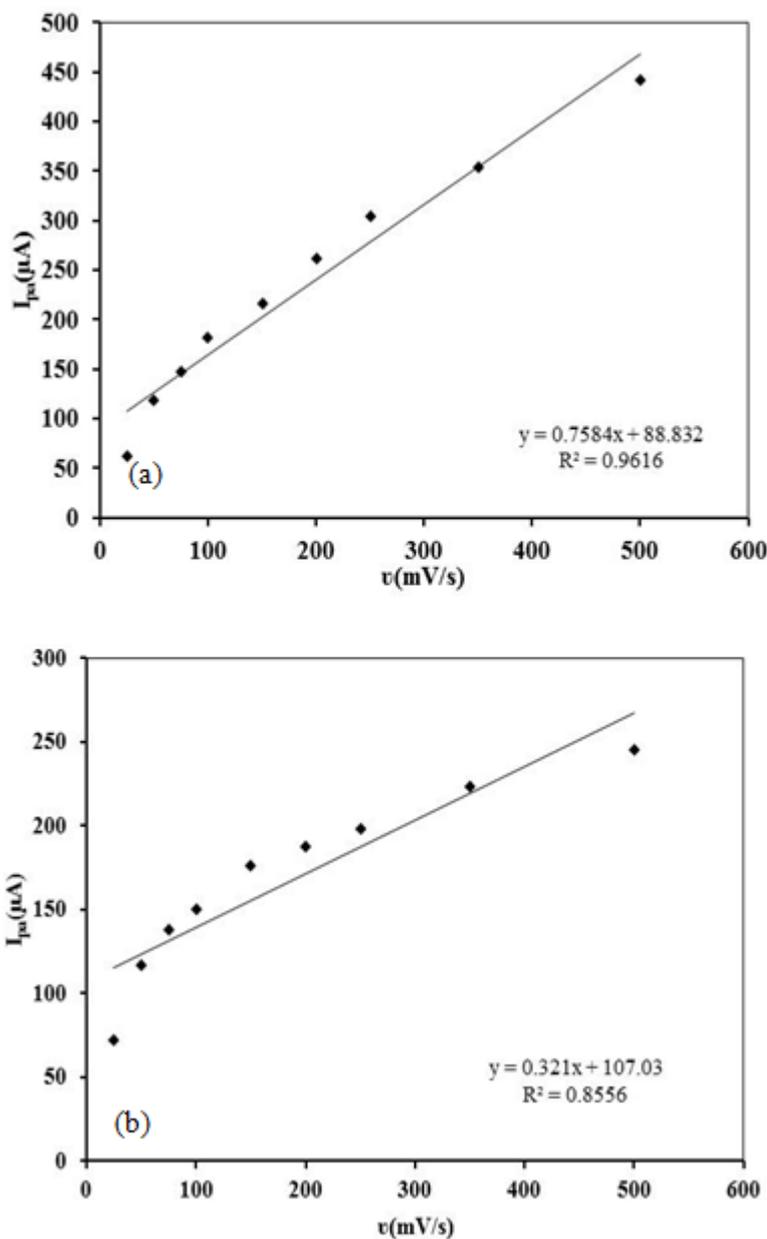


Figure 5. Plot between peak current (μA) and scan rate (mV/s) for modified (a) and unmodified carbon paste electrode (b)

3.4.2. Mechanism for the electrode reaction

Normalized peak current values are obtained by dividing the peak current intensity to the square root of sweep rate. These normalized peak current values are then plotted against scan rate in

order to evaluate mechanism of electrode reaction. In case of modified electrode, it showed EC mechanism (Fig 6a). In EC mechanism an electron transfer reaction is followed by a chemical reaction and the product of chemical reaction is electrochemically inactive [24]. Here normalized peak current values are increased with increasing scan rate

As far as unmodified electrode is concerned (Fig6b), the plot showed the typical shape of ECE mechanism. The nomenclature 'E' stands for the electron transfer reaction while 'C' denotes a chemical reaction. Here an electron transfer reaction is followed by chemical reaction.

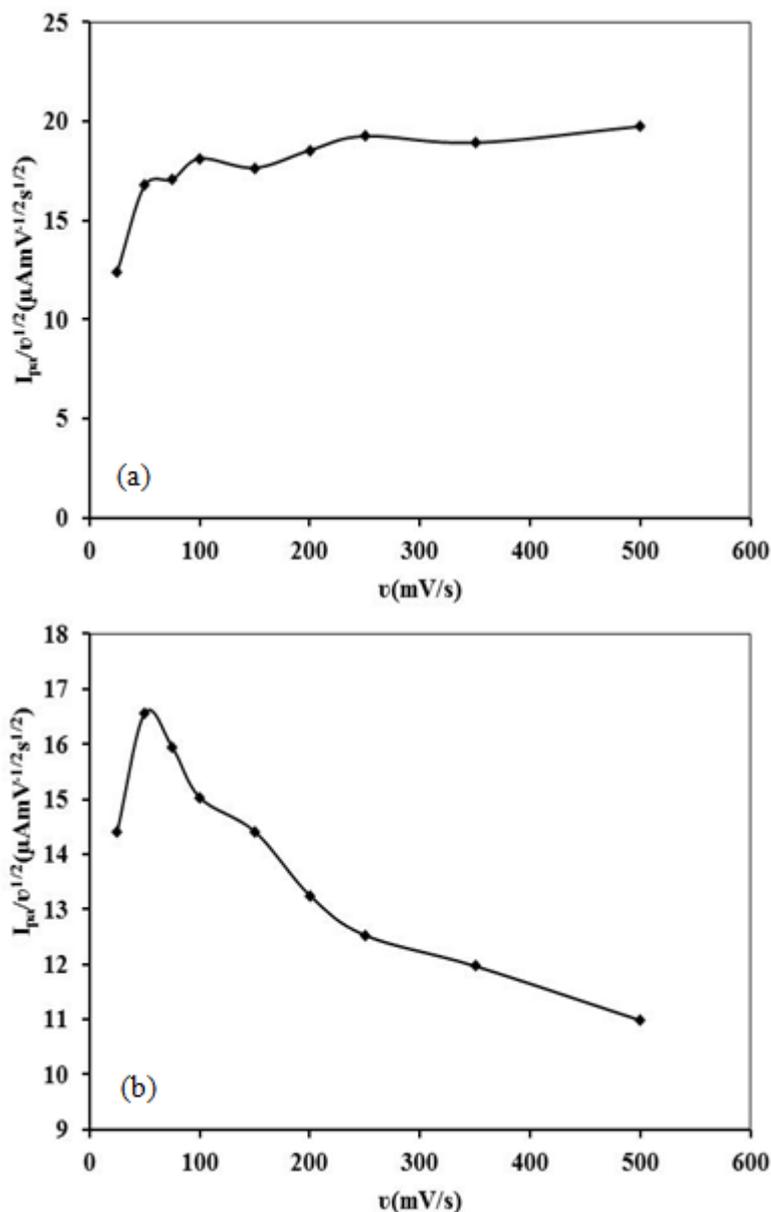


Figure 6. Variation of peak current function ($I_p/v^{1/2}$) ($\mu A m V^{-1/2} s^{1/2}$) with scan rate (mV/s) for modified (a) and unmodified carbon paste electrode (b)

The product of chemical reaction is electroactive and undergoes the electron transfer reaction thus the values of products for both the reactions are maintained under equilibrium control. In case of ECE mechanism, the normalized peak current intensity decreases with an increment in scan rate [25].

3.4.3. Calculation of kinetic parameters

According to the procedure of Laviron [26], the variation of peak potential with logarithm of scan rate is used to calculate charge transfer coefficient and heterogeneous rate constant for a surface confined redox couple reaction.

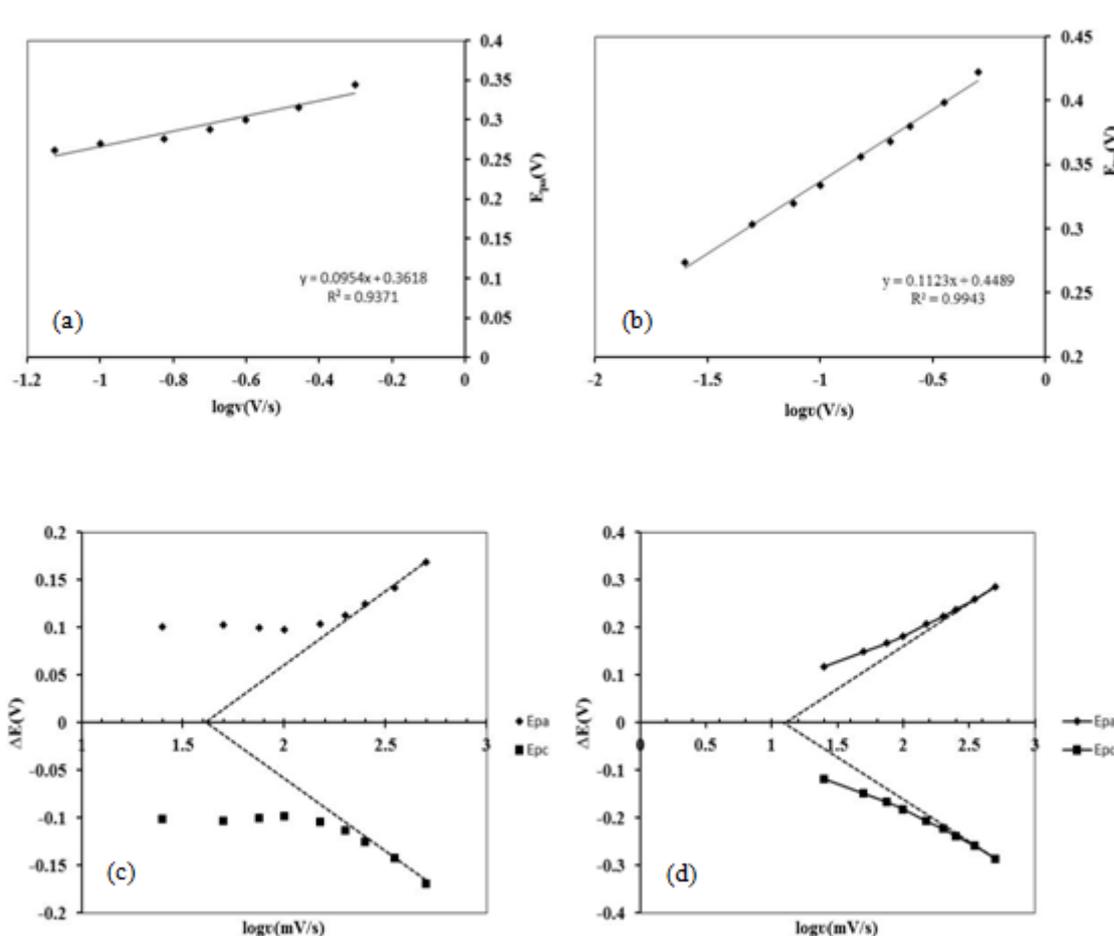


Figure 7. Plot between peak potential (V) and logarithm of scan rate (V/s) for modified (a) and unmodified carbon paste electrode. (b).Plot between peak potential separation (V) and logarithm of scan rate (mV/s) for modified (c) and unmodified carbon paste electrode (d)

From the slope values of graph plotted between anodic peak potential and logarithm of scan rate (Fig 7a,b), values of charge transfer coefficient is found to be 0.525 and 0.619 for unmodified (Eq 9) and modified electrodes (Eq 10).

$$E_p(V) = 0.1123 \log v(V/s) + 0.4498, r^2 = 0.9943 \tag{Eq 9}$$

$$E_p(V) = 0.0954 \log v(V/s) + 0.3618, r^2 = 0.9371 \quad \text{Eq 10}$$

Following equation (Eq 11) is applied to calculate charge transfer coefficient between the redox couple and carbon paste electrode.

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log \frac{RT}{nFv} - \alpha(1-\alpha) \frac{n_\alpha F \Delta E_p}{2.303RT} \quad \text{Eq 11}$$

where k_s is the heterogeneous rate constant, α is the charge transfer coefficient, v is the sweep rate and all other parameters have their conventional meaning.

For the calculation of heterogeneous rate constant, the following equation is used.

$$k_s = \frac{nF\alpha v_a}{RT} = \frac{nF(1-\alpha)v_c}{RT} \quad \text{Eq 12}$$

where v_c and v_a are the potential scan rates at intercept of the straight line fits to anodic and cathodic data respectively. These potential scan rates can be calculated from the graph plotted between ΔE ($E_p - E^0$, where E^0 is the formal electrode potential) and logarithm of scan rate (Fig 7c,d). The calculated rate constant value for unmodified and modified electrode is 22.48 s^{-1} and 38.8 s^{-1} respectively.

4. CONCLUSION

A carbon paste electrode modified with *P. chrysosporium* is reported. Cyclic voltammetric studies are used to highlight its electrocatalytic activity in comparison to an unmodified electrode. Modified electrode has a greater electrochemical surface area and coverage capacity than unmodified one. Transfer of electron mechanism is studied on both type of electrodes and calculation of kinetic parameters illustrated that modified electrode has a faster electron transfer. In short, it can be concluded that redox systems differ dramatically in their sensitivity to the state of carbon electrode's surface.

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