

ARTICLE

Electrochemistry of ABTS at Glassy Carbon Electrodes

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The electrochemical and the mass transport behavior of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) redox couple at glassy carbon electrode (GCE) in phosphate buffer solution (PBS, pH=4.4) is studied in detail by cyclic voltammetry combined with rotating disk electrode system. From the i - E curves recorded at different electrode rotating rate, rate constant, and transfer coefficient for $\text{ABTS}^{2-} \rightleftharpoons \text{ABTS}^{\cdot-} + e$ reaction at GCE electrode and the diffusion coefficient of ABTS^{2-} in PBS are estimated to be 4.6×10^{-3} cm/s, 0.28, and 4.4×10^{-6} cm²/s, respectively. The transfer coefficient with a value of ca. 0.28 differs largely from the value of 0.5 that is always assumed in the literature. The origins for the difference of the rate constant determined and the challenges for estimating the standard rate constant are discussed. The performance for such ABTS^{2-} mediated bio-cathode toward oxygen reduction reaction is discussed according to the over-potential drop as well as current output limit associated with the charge transfer kinetics of $\text{ABTS}^{2-} \rightleftharpoons \text{ABTS}^{\cdot-} + e$ redox reaction and/or the mass transport effect.

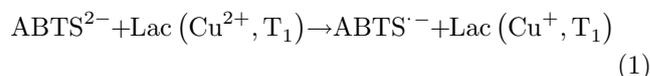
Key words: Diffusional mediator, Enzymatic electrocatalysis, Rate constant, Diffusion coefficient, Electron transfer coefficient

I. INTRODUCTION

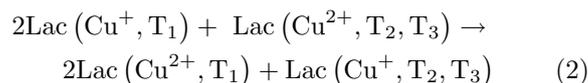
Laccase is an enzyme with multi-copper atoms in oxidases which can effectively reduce oxygen to water [1, 2]. It is one of the most commonly used enzymes in the bio-electrocatalytic cathodes for oxygen reduction reaction (ORR) in biofuel cells [1, 2]. One of the most important challenges in designing such biofuel cells is to effectively shuttle the electrons between the enzyme active sites and the electrode support, since the active centers of enzymes are buried and isolated by its thick peptide backbones. Usually, a mediator is introduced to shuttle the electrons between redox centers of the biocatalyst and the conductive electrode substrate. $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)) redox couple is commonly used as diffusional mediator, because ABTS^{2-} can be fast oxidized to $\text{ABTS}^{\cdot-}$ by the enzyme and $\text{ABTS}^{\cdot-}$ can be relatively easily reduced back to ABTS^{2-} at the electrode at potential (with formal potential of ca. 700 mV *vs.* Ag/AgCl) close to the formal potential of T₁ site in laccase (780 mV *vs.* NHE) [1–8].

In weakly acidic environment (usually pH=4–7), ABTS mediated and laccase catalyzed oxygen reduction reaction composes of several elementary steps [4]. The first step is the reduction of the oxidation state of

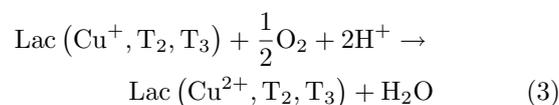
Cu^{2+} at the T₁ site of laccase by ABTS^{2-} :



It is followed by a fast intramolecular charge transfer:



Then after, oxygen is reduced by the reduced state of central Cu ions ($\text{Lac}(\text{Cu}^+, \text{T}_2, \text{T}_3)$) at its T₂ and T₃ sites in laccase molecule:



And the last step in the catalytic cycle is the diffusion of $\text{ABTS}^{\cdot-}$ radical to the electrode surface and $\text{ABTS}^{\cdot-}$ gets reduced there:



Laccase catalyzed ABTS^{2-} oxidation reaction is very fast [1, 2], thus the rate determining step for the net oxygen reduction reaction probably be the 3rd or 4th step or the diffusion of $\text{ABTS}^{\cdot-}$ from the active sites of enzyme to the conductive electrode substrate. Hence, understanding the mass transport and the charge transfer kinetics of the 4th step is of great importance for evaluating or even predicting the performance of such a bio-cathode for ORR.

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Since $\text{ABTS}^{\cdot-}$ is unstable and no such substance can be bought commercially, direct measurement on its electrochemical reduction reaction without involving the consecutive enzyme catalyzed reactions is not possible. While in laccase catalyzed ORR, the kinetics of the reduction of $\text{ABTS}^{\cdot-}$ cannot be easily deduced due to the complications of other chemical reactions involving the transformation of redox states in laccase and ORR.

In the present work, we have firstly characterized the electrochemical behavior of $\text{ABTS}^{2-} \rightleftharpoons \text{ABTS}^{\cdot-} + e$ redox reaction and estimated the kinetic parameters of the oxidation reaction of ABTS^{2-} and the diffusion coefficient of ABTS^{2-} from the measured (i - E) curves. Then we deduced the corresponding kinetic parameters for the electrochemical reduction of $\text{ABTS}^{\cdot-}$ based on the principle of micro-reversibility and the Butler-Volmer equation [9, 10]. Finally we discuss the possible origins for the differences of the kinetic parameters obtained in present work from that of the literature results and discuss how the above results can be used to predict the performance of practical bio-cathode.

II. EXPERIMENTS

ABTS^{2-} (98.5%, Aldrich) was used as received. Both phosphoric acid and potassium dihydrogen phosphate trihydrate of analytical grade (from Sinopharm) were used without further purification. Millipore Milli Q water is used to prepare a buffer solution (0.2 mol/L KH_2PO_4) with a pH=4.4 (denoted PBS here after). 0.5 mmol/L ABTS^{2-} +PBS was used as the electrolyte throughout the present measurements. The viscosity of the above electrolyte was measured by Ubbelohbe viscometer.

The working electrode (WE) was a glassy carbon disk electrode (5-mm diameter, from Tianjin Ai Da limited Corp., denoted as GCE hereafter). Before the measurements, the working electrode was firstly polished (on a cloth) with aqueous alumina slurries with grain size from 3 μm to 0.5 μm (Micropolish II, Buehler), then ultrasonically cleaned in acetone and water for three times each. The counter electrode was made from Pt wire, and a Ag|AgCl (with saturated KCl) electrode was served as reference electrode (Pine Instrument).

A standard three-electrode cell was used for electrochemical measurement and the cell was purged with nitrogen gas (5 N, Nanjing Special gas Corp.) for 10 min prior to each measurement, and electrolyte in the cell was constantly purged with N_2 throughout the measurement. Electrochemical measurements were performed using an electrochemical instrument (CHI400a, Shanghai Chenghua, China). The electrode rotating speed is controlled by a rotating disk electrode system (AFM-SRCE, Pine instruments, USA). All the experiments were carried out at room temperature. All the potentials in this work are given with respect to normal hydrogen electrode (NHE). Calculated diffusion coefficient

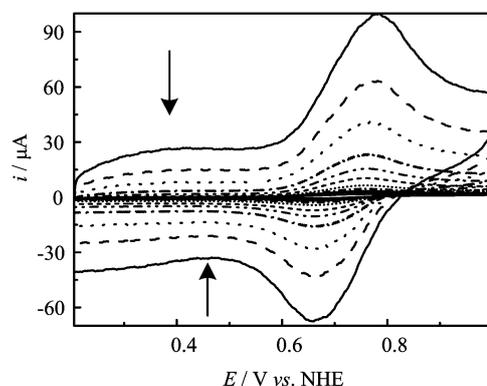


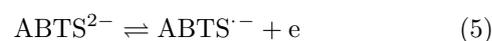
FIG. 1 Cyclic voltammograms of the $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple at a glassy-carbon electrode as a function of potential scan rate. The starting electrolyte contains 0.5 mmol/L ABTS^{2-} and 0.2 mol/L phosphate buffer with pH=4.4, electrode rotating speed is 0 r/min. Arrow directions show the potential scan rates as 2000, 1000, 500, 200, 100, 50, 20, 10, 5, 2, and 1 mV/s.

and reaction rate constant were the average value based on three parallel experiments.

III. RESULTS AND DISCUSSION

A. Cyclic voltammetric characterization of electrochemical behavior of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$

Figure 1 displays representative cyclic voltammograms (CVs) recorded in PBS containing 0.5 mmol/L ABTS^{2-} at a GCE at various potential scan rates. The pair of peaks, which appear at ca. 0.75 V (in the positive-going scan) and 0.66 V (in the negative-going scan) correspond to the oxidation and reduction of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple:



From Fig.1 it is found that: (i) The anodic peak potential increases with the potential scan rate. (ii) There is a good linear relationship between the peak current (both anodic and cathodic currents) and root square of the scan rates (Fig.2). (iii) The difference between potentials of the reduction and oxidation peaks ($E_{\text{pa}} - E_{\text{pc}}$) is ca. 90 mV, this value agrees well with literature results measured at pH=4.0 [3]. It is obviously higher than the theoretical value of 59 mV for an ideal reversible system determined with cyclic voltammetry [9–11] and those measured at pH=7.0 (70 mV) [4]. (iv) For each cyclic voltammogram (Fig.1), the ratios of charges under the respective reduction and oxidation peaks are close to unity. All the above results suggest that, the oxidation and reduction reactions of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple is quasi reversible under present reaction condition.

From Fig.1 the formal (midpeak) potentials for the $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple is estimated to be

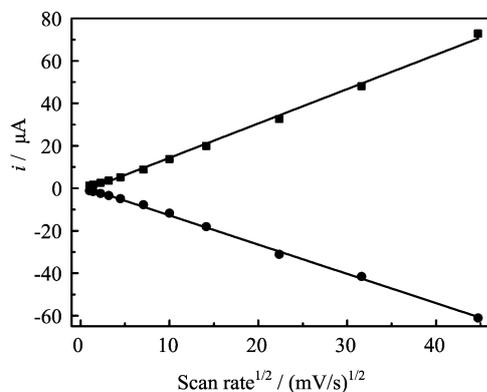


FIG. 2 Cathodic (circle) and anodic (square) peak currents of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple at a glassy-carbon electrode (data from Fig.1) as a function of the square root of the scan rates, the lines are linearly fitted from the data points.

0.71 V. From the anodic and cathodic peak currents the concentration of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple within the diffusion layer is estimated to be roughly the same, thus the midpeak potential can be taken as the equilibrium potential (and standard electrode potential) for this specific system [2, 12]. Obviously, the equilibrium potential for $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple is only ca. 70 mV negative to the formal potential for ORR at the $\text{Cu}(\text{T}_2, \text{T}_3)$ sites of laccase (ca. 0.78 V) [13]. However, taking that the standard potential for ORR at pH=4.4 is 0.97 V when using $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple mediated laccase as cathode for ORR, an overpotential of at least 0.3 V is necessary or the energy loss will be up to 25%.

B. Estimation of kinetic parameters of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox reactions

The general expression for the faradaic current of reaction (5) is

$$i = i_+ - i_- \\ = nFA (k_+ \cdot c_{\text{ABTS}^{\cdot-}}^s - k_- \cdot c_{\text{ABTS}^{2-}}^s) \quad (6)$$

where A is the geometric area of the GCE, F is Faraday constant, c^s is the surface concentration of the reacting species. It can also be expressed by the mass transport of the reacting species through the diffusion layer:

$$i_+ = nFA \cdot D_{\text{ABTS}^{2-}} \cdot \frac{c_{\text{ABTS}^{2-}}^b - c_{\text{ABTS}^{2-}}^s}{\delta} \quad (7)$$

$$i_- = nFA \cdot D_{\text{ABTS}^{\cdot-}} \cdot \frac{c_{\text{ABTS}^{\cdot-}}^b - c_{\text{ABTS}^{\cdot-}}^s}{\delta} \quad (8)$$

where D is the diffusion coefficient and c^b is bulk concentration of the reacting species, and δ is the diffusion layer thickness. For disk electrode with rotating speed

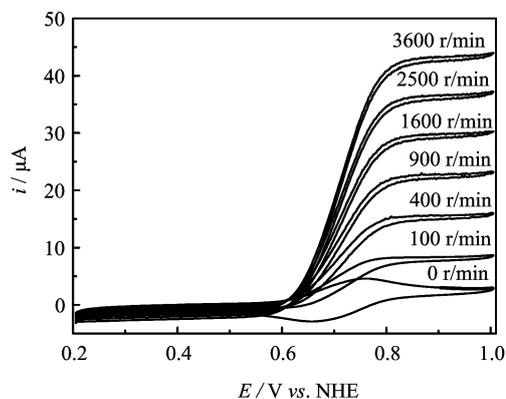


FIG. 3 i - E curves of ABTS^{2-} oxidation in solutions containing 0.5 mmol/L ABTS^{2-} in 0.2 mol/L phosphorate buffer solution (pH=4.4) as a function of electrode rotating speed as indicated in the figure, potential scan rate of 10 mV/s.

of ω , $\delta = 1.61D^{1/3}\nu^{1/6}\omega^{-1/2}$, ν is the kinematic viscosity of the electrolyte solution. From Eqs.(6) and (7) one can deduce that

$$\frac{1}{i} = \frac{1 + \frac{x+y}{\sqrt{\omega}}}{FA (k_+ c_{\text{ABTS}^{2-}}^b - k_- c_{\text{ABTS}^{\cdot-}}^b)} \\ x = \frac{k_+}{1.61D_{\text{ABTS}^{2-}}^{-2/3}\nu^{1/6}} \\ y = \frac{k_-}{1.61D_{\text{ABTS}^{\cdot-}}^{-2/3}\nu^{1/6}} \quad (9)$$

If one can measure the Faradaic current of reaction given in Eq.(5) as a function of electrode rotation speed at constant potential and by plotting of $1/i$ vs. $1/\sqrt{\omega}$, from the intercept and slope of the curve one can easily deduce the rate constant and the diffusion coefficient.

For such purpose, further measurements were carried out using cyclic voltammetry with rotating disk electrode under various electrode rotating speed. Figure 3 presents i - E curves of ABTS^{2-} oxidation in solutions containing 0.5 mmol/L ABTS^{2-} + 0.2 mol/L PBS as a function of electrode rotating rates. From Fig.3 it is seen that in the potential region from 0.8 V to 0.6 V, the current is mass transport limited to a great extent. With the further potential negative shift from 0.6 V to 0.4 V, the reaction is under both mass transport and kinetic control. With the further potential negative shift, the current does not change with the electrode potential, but within this potential region currents increase very slightly with the electrode rotation speed. Anyway, at all electrode rotation speed the diffusion limited cathodic current is less than 5% of the diffusion limited current for ABTS^{2-} oxidation. This suggests that under the present condition, the bulk concentration of $\text{ABTS}^{\cdot-}$ is less than 5% of that of ABTS^{2-} . On the other hand, with the help of convective force provided by the electrode rotation $\text{ABTS}^{\cdot-}$ produced

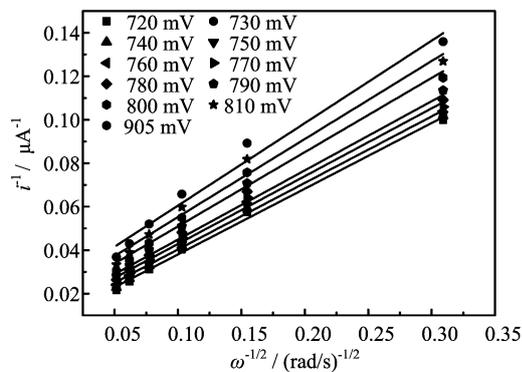


FIG. 4 The inverse of Faradic current $1/i$ as a function of the inverse of the root square of the electrode rotating speed $\omega^{-1/2}$. Data taken from Fig.3 at various constant potentials.

at higher potentials has been diffused away from the electrode before the electrode potential becomes lower enough for $\text{ABTS}^{\cdot-}$ radical to be reduced, thus we think the reduction of $\text{ABTS}^{\cdot-}$ is negligible under the present condition. Hence, within the mixed controlled potential region the faradaic current I can be expressed (*i.e.*, Eq.(9) can be simplified) as:

$$\frac{1}{i} = \frac{1}{i_k} + \frac{1}{i_{l,a}}$$

$$= \frac{1}{nFAk_+ + c_{\text{ABTS}^{2-}}^b} + \frac{1.61\nu^{1/6}\sqrt{\omega}}{nFAC_{\text{ABTS}^{2-}}^b D_{\text{ABTS}^{2-}}^{2/3}} \quad (10)$$

where i_k is the purely kinetic controlled current, $i_{l,a}$ is the anodic diffusion limited current. Figure 4 displays the plots for $1/i$ vs. $\omega^{-1/2}$ at various constant potentials in the region from 0.72 V to 0.81 V, the Faradic currents i are taken from Fig.3. Note the double layer charging current must be corrected when taking the values for the Faradic currents, since at a low electrode rotation rate the double layer charging current, *e.g.*, ca. $3.5 \mu\text{A}$ at 10 mV/s, is close to that of faradaic current at lower over-potentials.

By extrapolating the $1/i$ vs. $\omega^{-1/2}$ curves to an infinite electrode rotation speed, the purely kinetic controlled current i_k and the rate constant k_+ at various over-potentials ($\eta = E - E^e = E - 0.71$ V) are derived, which are given in Fig.5. For the case when the reaction only takes place in one direction the potential dependence of k_+ can be written as follows:

$$k_+ = k_0 \cdot \exp\left(\frac{\beta n F \eta}{RT}\right) \quad (11)$$

where β is the electron transfer coefficient, which is found to be 0.28 ± 0.2 under present condition. It is much smaller than the usually assumed value of ca. 0.5 [10]. This indicates that the potential energy curves at the intersection points between the reactants and products are asymmetric. Further investigations are

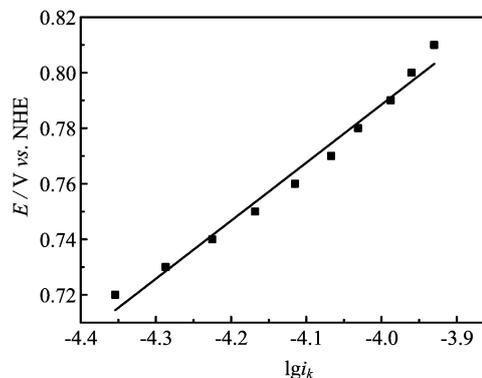


FIG. 5 Plot of electrode potential as a function of the logarithm of the kinetic current of ABTS^{2-} oxidation (i_k), data points are extrapolated from the data in Fig.4 to infinite electrode rotation speed. The line is the linearly fitted plot of the data points.

underway to understand what a transition state may be involved for such discharge process.

From Fig.5 and Eq.(11), the rate constant k_0 at the equilibrium potential is found to be $(4.6 \pm 0.2) \times 10^{-3}$ cm/s. It should be mentioned that the estimation of standard rate constant k_0^0 remains challenging because the bulk concentration of $\text{ABTS}^{\cdot-}$ can be hardly well controlled and measured due to the fact that no commercial $\text{ABTS}^{\cdot-}$ is available. It should be noticed that the standard rate constant for this system has been reported, the value was just rate constant at equilibrium under the specific reaction conditions used in the individual study (*e.g.*, certain ABTS^{2-} initial concentration, which may be orders of magnitude higher than that of $\text{ABTS}^{\cdot-}$ under the conditions where the rate constant is estimated). Furthermore, the double layer charging effect has most probably not being taken into account when deducing such results using cyclic voltammetry with a fast potential scan rate, thus the numbers from different groups can differ up to orders of magnitude [3, 4]. The rate constant estimated in the present work is close to the value of 4.54×10^{-3} cm/s reported in Ref.[3], but is three times smaller than that reported in Ref.[4] (ca. 0.01 cm/s which is estimated using a transfer coefficient of 0.5). The present results indicate that one should be very careful when taking such values for the further evaluation of reaction kinetics in ABTS mediated and laccase catalyzed ORR at the cathode of biofuel cell.

On the other hand, based on the BV equation and with a transfer coefficient of 0.72, the rate constants for the electrochemical reduction of $\text{ABTS}^{\cdot-}$ radical at different potentials can be deduced obviously, the potential dependent rate constant change is ca. two times slower than that for the opposite oxidation reaction. Our present results demonstrated that the redox reaction of the $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ couple at the electrode is not fast enough, which may kinetically limit the ORR

process [4, 11]. Furthermore, whether such redox couple can serve as a good redox mediator in solution phase, it also depends on the diffusion and mass transfer coefficients [14–16].

C. Estimation of the diffusion and mass transfer coefficients of ABTS^{2-} and $\text{ABTS}^{\cdot-}$

The diffusion coefficient of ABTS^{2-} anions can be determined by taking the mass transport limited current recorded with the rotating electrode system at various electrode rotating speed according to the following equation [17]

$$i_{l,a} = 0.62nFAD_{\text{ABTS}^{2-}}^{2/3}\nu^{-1/6}c_{\text{ABTS}^{2-}}^b\omega^{1/2} \quad (12)$$

The kinematic viscosity ν of the electrolyte solution 0.2 mol/L PBS + 0.05 mol/L ABTS^{2-} used in this work is determined to be $1.04 \times 10^{-2} \text{ cm}^2/\text{s}$ by the Ubbelohde viscometer. From the plot of $i_{l,a}$ vs. $\omega^{1/2}$ (Fig. 4), $D_{\text{ABTS}^{2-}}$ is estimated to be $(4.4 \pm 0.2) \times 10^{-6} \text{ cm}^2/\text{s}$. On the other hand, it can also be estimated from the CVs recorded at different scan rates (Figs. 1 and 2) according to the following equation:

$$i_{p,a} = 2.69 \times 10^5 n^{3/2} c_{\text{ABTS}^{2-}}^b D_{\text{ABTS}^{2-}}^{1/2} v^{1/2} A \quad (13)$$

here v is the potential scan rates. From Eq.(13) and Fig. 2, the $D_{\text{ABTS}^{2-}}$ is deduced to be $(4.5 \pm 0.4) \times 10^{-6} \text{ cm}^2/\text{s}$. The deviation of the diffusion coefficients estimated from these two methods is ca. 2.3%. The slightly higher error bar for that obtained using the latter method may be probably related to the facts that the double layer charging current is not easy to be corrected when using cyclic voltammetry with different scan rates in the stationary cell.

From the diffusion coefficient and the thickness of diffusion layer δ , the mass transfer coefficient $k_m = D/\delta$ can be roughly estimated. For a electrode rotating rate of 2500 r/min, the thickness of the diffusion layer is ca. 8.0 μm , thus the mass transfer coefficient is ca. $5.5 \times 10^{-3} \text{ cm/s}$. For commonly used stationary system only with natural convection, δ can be as large as ca. 100 μm , k_m is ca. one order of magnitude smaller than the former case (ca. $4.4 \times 10^{-4} \text{ cm/s}$). Comparing mass transfer coefficient with the rate constant of the charge transfer reaction ($4.6 \times 10^{-3} \text{ cm/s}$), it is clearly seen that in the stationary cell, mass transport will be the rate determining step for the oxidation of ABTS^{2-} to $\text{ABTS}^{\cdot-}$.

In the case of $\text{ABTS}^{\cdot-}$, since at the beginning of experiments, no such anion radicals exists in the solution, so it is not possible to directly measure its diffusion and mass transport coefficient. Based on the fact that ABTS^{2-} has just one more electron than $\text{ABTS}^{\cdot-}$ and its structure in the electrolyte probably does not change much when losing one electron, we can assume that the diffusion coefficient for $\text{ABTS}^{\cdot-}$ is approximately the same as that for ABTS^{2-} . Considering the fact

that the amount $\text{ABTS}^{\cdot-}$ produced by electrochemical oxidation of ABTS^{2-} in the present study is limited (whose bulk concentration may be 10^6 times smaller than $[\text{ABTS}^{2-}]$), thus we think for present case without the involvement of laccase and ORR, mass transport are always the rate determining step (RDS) for the reaction (5). For biocathode with ABTS mediated and laccase catalyzed ORR, even the chemical reaction may be fast enough, the poor mass transport in aqueous solution may further reduce the cathode performance of ORR reaction mediated with such redox couple. On the other hand, for electrodes immobilized with both enzyme and mediators the diffusion layer thickness may be greatly reduced, however, the diffusion coefficient may also be greatly reduced. The mass transport effect as well as the voltage loss due to the low redox potential for such mediator may probably explain why biofuel cells based on such mediated redox couple can only give very low power output [1–2, 8]. Our results strongly suggest that for developing efficient electron transfer mediated biocathode for ORR, searching for better mediators with higher mass transport rate, the higher redox potential as well as electrode kinetics for the redox reactions is critical. Further investigations are under way on this issue.

IV. CONCLUSION

The electrochemical behavior for the oxidation and reduction reactions of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple and their mass transport behavior are examined systematically using cyclic voltammetry combined with the rotating disk electrode system. Our results demonstrated that reaction $\text{ABTS}^{2-} \rightleftharpoons \text{ABTS}^{\cdot-} + e$ is only quasi reversible in solution with pH=4.4 and 0.48 mmol/L ABTS^{2-} with exchange rate constant and transfer coefficient of ca. $4.6 \times 10^{-3} \text{ cm/s}$ and 0.28, respectively. The diffusion coefficient ABTS^{2-} is estimated to be $4.4 \times 10^{-6} \text{ cm}^2/\text{s}$. And the standard rate constant for the electrochemical reduction of $\text{ABTS}^{\cdot-}$ and the diffusion coefficient are estimated to be of the same order, based on the principle of microreversibility and Butler-Volmer equation. And we found that the potential dependent changes in reaction rate for $\text{ABTS}^{\cdot-}$ reduction are twice slower than that for the opposite reaction. Possible roles for the diffusion and charge transfer processes of $\text{ABTS}^{2-}/\text{ABTS}^{\cdot-}$ redox couple in the ABTS mediated and laccase catalyzed ORR are discussed.

V. ACKNOWLEDGMENTS

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