CHEMICAL ENGINEERING TRANSACTIONS

VOL. 70, 2018

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Timothy G. Walmsley, Petar S. Varbanov, Rongxin Su, Jiří J. Klemeš

ISBN 978-88-95608-67-9; **ISSN** 2283-9216

DOI: 10.3303/CET1870269

Research and Mathematical Modelling of Direct Bioelectrocatalytic Oxygen Reduction by Laccase

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At this present work, the process of direct bioelectrocatalytic oxygen reduction by laccase was investigated using electrochemical methods and mathematical modelling. The maximum of achieved current density is 640 μA cm^{-2}. The developed mathematical model includes the mass and potential conservation equations and takes into account the porous structure of the electrode active layer. The equations of the mathematical model were solved by numerical methods with own developed software package. The profiles of the components concentration on time and thickness of the active layer of the electrode were obtained. The adequacy of the mathematical model was tested by comparing the experimental and calculated values of the electrodes activity at different loadings of the carbon material. By the golden ratio method, the optimal electrode carbon material loading of 0.92 mg cm^{-2} was found.

1. Introduction

Presently the primary interest of researchers in the field of mathematical modelling of fuel cells (FCs) is related to processes of traditional fuel cells (low-temperature with proton-conductive polymer electrolyte and high-temperature solid oxide), while the papers with mathematical modelling of biofuel cells (BFC) are much less.

This area of research has become especially accessible only in the last decade, and the published to date works on this topic are general, seeking to cover all operational aspects of the BFC.

Mathematical modelling and simulation can play a fundamental role in the further understanding and development of BFC. Adequate mathematical models cooperated with laboratory studies, can be used for studying the reaction medium, engineering of new electrode designs and accelerate the development of practical-useful systems. According to the review published by Rajendran et al. (2017) most mathematical models of biofuel cells are based on the reaction-diffusion differential equations. The primary type of models is based on the principles of formal kinetics. The major part of the models predicts the characteristics of a BFC with separation membrane and with the usage of electron transfer mediators. But practically there is no a mathematical model describing the constructions of membraneless and mediatorless BFC under the direct electron transfer of current generating reactions.

There are some investigations where mathematical modelling and calculations were carried out with the help of commercial package software, e.g. CFD-ACE (Bedekar et al., 2007), COMSOL Multiphysics (Chan et al., 2012), ANSYS FLUENT (Mitrichev et al., 2016), FEM-LAB (Kjeang et al., 2006) or use for calculations the particular software (MATLAB) (Osman et al., 2013). These models operate with the enzyme surface concentration and take it equal to the total amount of enzyme adsorbed at the electrode. Using these models, it is impossible to determine the factors which can affect the final characteristics of the electrodes. In the previous study (Arkadeva et al., 2017) we calculated the laccase distribution within the active layer of the electrode as a result of spontaneous adsorption immobilization. These data have been taken in the present work as initial conditions for determining the electrocatalytic activity of the electrode as a function of the thickness of the active layer.

Please cite this article as: Vasilenko V.A., Arkadeva I.N., Bogdanovskaya V.A., Fokina E.A., Koltsova E.M., 2018, Research and mathematical modelling of direct bioelectrocatalytic oxygen reduction by laccase , Chemical Engineering Transactions, 70, 1609-1614 DOI:10.3303/CET1870269

2. Experimental and model

2.1 Chemicals and methods

In this study, the Laccase Trametes Versicolor - copper-containing enzyme, which can directly reduce (without the use of any mediator) oxygen to water was used. It was produced by the procedure described by Gorshina et al. (2006). Experimental researches were carried out according to Bogdanovskaya et al. (2017). Electrochemical measurements were conducted in a three-electrode cell with separated electrode spaces. The floating electrode was used as a cathode, and it was made in the form of the tablet from hydrophobized carbon black, electrode thickness was equal to 2 mm, the surface area of contact with electrolyte was equal to 1 cm^2. Some amount of multiwalled carbon nanotubes provided by D.I. Mendeleev University of Chemical Technology of Russia (CNT, specific surface area measured by BET method S_{BET}=210 m^2 g^{-1}) was pressed onto the electrode surface. The procedure of CNT fabrication was described by Bogdanovskaya et al. (2016). Then the electrode was placed on the surface of laccase solution, which contained 0,076 mg ml^{-1} of laccase. Adsorptive spontaneous immobilization was carried out for 2 hours. The quantity of adsorbed enzyme was determined spectrophotometrically using a preconstruction calibration curve as the difference of enzyme content in the solution before and after adsorption process.

A platinum wire was used as a counter electrode, and a saturated Ag/AgCl electrode was used as a reference electrode. Studies were carried out in 0,2 M phosphate-acetate buffer solution (pH = 4,5). Polarization curves were registered in the oxygen atmosphere using the potentiostat IPC-Pro 3A.

2.2 Mathematical simulation

The developed mathematical model assumes two modelling domains (Figure 1). The first one is the volume of the electrolyte (0<x<δ) and the second one is the active layer, which is the porous material filled with electrolyte (δ<x<h).

The area of the electrolyte volume can be described by the ideal mixing model, i.e., there are no concentration gradients in this region. Since the process proceeds in a buffer solution with a constant supply of oxygen, the concentration values of the protons and dissolved oxygen in this region remain constant. Furthermore, in this region does not occur electrochemical reaction.

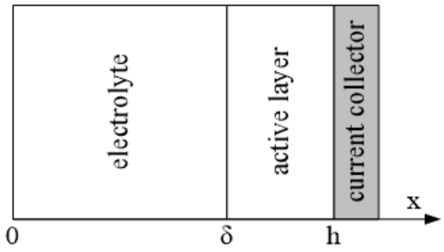

Figure 1: Schematic representation of modelling domains

In the active layer there is the oxygen bioelectroreduction by laccase (L), which schematically can be represented as:

$$L + O_2 \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} LO_2 \tag{1}$$

$$LO_2 + 4\bar{e} + 4H^+ \xrightarrow{k_c} L + 2H_2O \tag{2}$$

It includes the reversible stage of enzyme-substrate complex LO_2 production – reaction (1) and the electron transfer with dissociation of the enzyme-substrate complex to product – reaction (2).

The rates of these electrode reactions can't be described by the Michaelis-Menten equation because only the first stage is enzymatic. The second stage is electrochemical and can described by Buttler-Volmer equation.

The rates of the reactions (1) and (2) respectively (the subscript «c» characterizes the cathodic process):

$$W_1 = k_{1\gamma} c_L c_{O_2} \tag{3}$$

$$W_{-1} = k_{-1\gamma} c_{LO_2} \tag{4}$$

$$W_c = k_{c\gamma} c_{LO_2} exp\left(-\frac{\alpha n F \eta}{RT}\right) \tag{5}$$

where c_j, mol m^{-3} – concentration of the components; α – charge transfer coefficient; n – number of electrons in the electrochemical reaction; η_c , V – the potential drop between the carbon material (CM) of the active layer of the electrode and the electrolyte; $k_{1\gamma}$, $k_{-1\gamma}$, $k_{c\gamma}$ – modified reaction rate constants, related to the corresponding rate constants by: $k_{1\gamma}=k_1^{\gamma}k_{\gamma}$, m^3 mol^{-1} s$^{-\gamma}$; $k_{-1\gamma}=k_{-1}^{\gamma}$, s$^{-\gamma}$; $k_{c\gamma}=k_c^{\gamma}$, s$^{-\gamma}$. The γ subscript characterizes the process, which takes place within the porous CM layer, and the γ superscript corresponds the material's porosity (determines the part of pores, which are opened for electrolyte flow), $0<\gamma<1$ (Koltsova et al., 2000); F, C mol^{-1} – Faraday constant; R, J mol^{-1} K^{-1} – universal gas constant; T, K – temperature.

It was assumed that the enzyme molecules are presented only within the active layer of the electrode, and they are firmly adsorbed on the surface of the CM and can't pass into solution. The amount of the enzyme and enzyme-substrate complex is related to the total amount of the laccase adsorbed on the electrode by the following equation:

$$c_{L^0} = c_{L(t,x)} + c_{LO_2(t,x)} \tag{6}$$

where c_{L^0}, mol m^{-3} – the initial concentration of laccase, distributed within the active layer (obtained in Arkadeva et al., 2017).

The flow of dissolved oxygen and protons in the electrolyte arises from their migration in the electric field (for protons) and diffusion due to the concentration gradient, and it can be described by the Nernst-Planck equation. Since the active layer is CM, which, as expected, due to its roughness, porosity and other irregularities can be described using a mathematical apparatus developed for fractal structures. Thus, the equation of material balance for an element of a small volume of the active layer, as in Koltsova et al. (2000), has the form:

$$\frac{\partial^{\gamma} c_j}{\partial t^{\gamma}} = z_j D_{j\gamma} \frac{F}{RT} \frac{\partial c_j}{\partial x} \frac{\partial \Phi_l}{\partial x} + D_{j\gamma} \frac{\partial^2 c_j}{\partial x^2} + S_j \tag{7}$$

where z_j – particle's charge; Φ_l , V – the potential of the liquid (ion-conducting) phase; S_j – j-particles source term (the multiplication of the stoichiometric coefficient of the particle by the reaction rate with the corresponding sign – «-» for reagents, «+» for products); t, s –time; $D_{j\gamma}$, m^2 s$^{-\gamma}$– the component's diffusion coefficient within the porous active layer, related to general diffusion coefficient by $D_{j\gamma}=D_j^{\gamma}S^{(1-\gamma)}$; S, m – channel cross-sectional area available for the electrolyte flow; x, m – electrode thickness coordinate; D_j, m^2 s^{-1} – particle's diffusion coefficient.

According to the Eq(7), the equations for the components concentration change within the active layer of the cathode are:

$$\frac{\partial^{\gamma} c_{O_2}}{\partial t^{\gamma}} = D_{\gamma}^{O_2} \frac{\partial^2 c_{O_2}}{\partial x^2} - W_1 + W_{-1} \tag{8}$$

$$\frac{\partial^{\gamma} c_{H^+}}{\partial t^{\gamma}} = z_{H^+} D_{\gamma}^{H^+} \frac{F}{RT} \frac{\partial c_{H^+}}{\partial x} \frac{\partial \Phi_l}{\partial x} + D_{H^+} \frac{\partial^2 c_{H^+}}{\partial x^2} - 4W_c \tag{9}$$

$$\frac{\partial^{\gamma} c_L}{\partial t^{\gamma}} = -W_1 + W_{-1} + W_c \tag{10}$$

The potential drop was determined as:

$$\eta = \Phi_s - \Phi_l - V_{oc} \tag{11}$$

where the V_{oc} – the open circuit voltage.

The connection between the potential and the current density was expressed by the Poisson equation and for the potential of the solid phase Φ_s (CM, electron-conducting) and of the liquid phase Φ_l as in Do et al. (2014) taking into account the Bruggeman correlation for the difference in the conductivity of the ion and electronic phases:

$$\frac{\partial}{\partial x}\left[(1-\varepsilon)^{1.5} \kappa_S \frac{\partial \Phi_S}{\partial x}\right] = -i \tag{12}$$

For the potential of the ionic (liquid) phase:

$$\frac{\partial}{\partial x}\left[\varepsilon^{1.5} \kappa_l \frac{\partial \Phi_l}{\partial x}\right] = i \tag{13}$$

where ε – volume fraction of the liquid phase in the active layer of the electrode; κ_s, κ_l, A $(V{\cdot}m)^{-1}$ – the conductivity of the electron (s) and the ion (l) conducting phases; i, A m^{-3}– local current density.

The local current density is related to the rate of the electrode reaction by the Faraday equation:

$$i = -nFW_c \tag{14}$$

The value of the total current density was obtained by spatial integration of the local current densities along the thickness of the active layer of the electrode:

$$I_t = \int_0^{\delta} i(t,x)dx \tag{15}$$

where δ, m – active layer thickness.

Eq(8) and Eq(9) are the differential equations of parabolic type, solved by the sweep method. Eq(10) is an ordinary differential equation of the first order, solved by the implicit Euler method. Eq(12) and Eq(13) are equations of elliptic type, solved using the establishment method, adding the time derivative.

In contrast to works, where model equations were solved using the commercial software, in this work the model equations were solved by the finite-difference method, the program module was implemented in the C++ programming language.

3. Results and discussion

3.1 Results of electrochemical experiments

Figure 2 shows the polarization curves for the oxygen electroreduction by laccase for electrodes with different amounts of CM applied to the electrode. It was found the limiting current of the electroreduction of oxygen by laccase increases with the increasing of the amount of CM, and the greatest activity of the electrode was observed with 0.94 mg of CM.

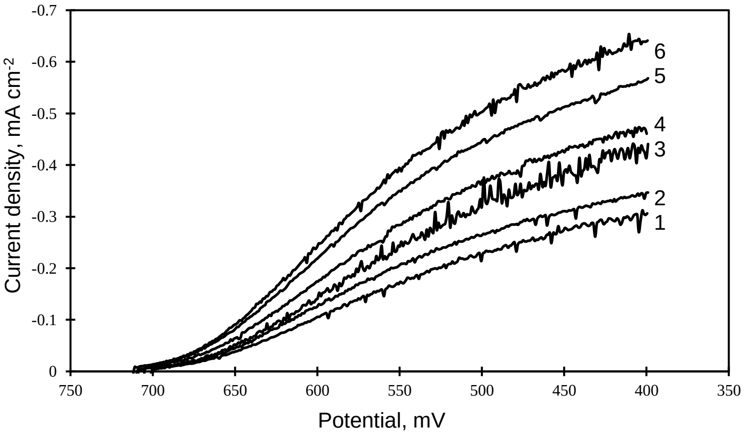

Figure 2: Polarization curves for the oxygen electroreduction by the electrode with laccase, adsorbed on CM CNT, which was pressed into the electrode with an amount: 1 – 0,37 mg; 2 – 0,46 mg; 3 – 0,48 mg; 4 – 0,73 mg; 5 - 0,86 mg; 6 – 0,94 mg. Potential sweep rate 1 mV s^{-1}

In Arkadeva et al., 2017 it was shown that further increase of the CM amount leads to decreasing the activity of the laccase-based electrode. The efficiency of the enzyme adsorption also decreases. In Bogdanovskaya et al. (2017) it was assumed that usage of this type of CM allows laccase to adsorb not only on the surface of the material, but penetrate into its mesopores and also other voids. If the optimal CM loading for a given electrode is overloaded, the proportion of voids decreases, and accordingly the size of the potentially suitable space for laccase adsorption.

The obtained data were applyed to develop a mathematical model of the bioelectrocatalytic oxygen reduction by laccase.

3.2 Results of mathematical modeling

The solution of the mathematical equations was carried out for experiments with the loading of CM 0,6 mg. The reaction constants and the conductivity coefficients were found by the scanning method. The simulation parameters are listed in the Table 1. The literature data were taken as the initial approximations.

Table 1: Simulation parameters

Parameter	Value	Reference	Parameter	Value	Reference
T, K	298	experimental	k_c, $m^3\ mol^{-1}\ s^{-\gamma}$	$1{,}43{\cdot}10^{-13}$	fitted
$c_L{}^0{}_{(t=0,\ x)}$ mol m^{-3}	$1{,}375{\cdot}10^{-3}$	Arkadeva et al. (2017)	κ_l, A $m^{-1}\ V^{-1}$	0,055	fitted, initial Chan et. al. (2012)
$c_{O2}{}^{bulk}$, mol m^{-3}	1,38	experimental	κ_s, A $m^{-1}\ V^{-1}$	0,046	fitted, initial Chan et. al. (2012)
$c_{H+}{}^{bulk}$, mol m^{-3}	$31{,}6{\cdot}10^{-3}$	experimental	ε	0,8	fitted, initial Osman et al. (2013, b)
D_{O2}, $m^2\ s^{-1}$	$2{\cdot}10^{-9}$	Osman et al. (2013, a)	α	0,5	experimental
D_{H+}, $m^2\ s^{-1}$	$1{\cdot}10^{-9}$	Chan et al. (2012)	n	4	
k_1, $m^3\ mol^{-1}\ s^{-\gamma}$	1000	fitted, initial Do et al. (2014)	V_{oc}, V	0,18	experimental
k_{-1}, $s^{-\gamma}$	646	fitted, initial Do et al. (2014)	γ	0,94	Arkadeva et al. (2017)

The equations of the mathematical model were calculated using the parameters from the Table 1 and the values of the current density as a function of the cell voltage were obtained. The model was tested for different CM loading: the Figure 3 shows the experimental and calculated data for the values of current density depending on the mass of the CM. The results of the calculation are consistent with the experimental data. By the usage of golden ratio method it was found that the optimal electrode carbon material loading is 0.92 mg cm^{-2}.

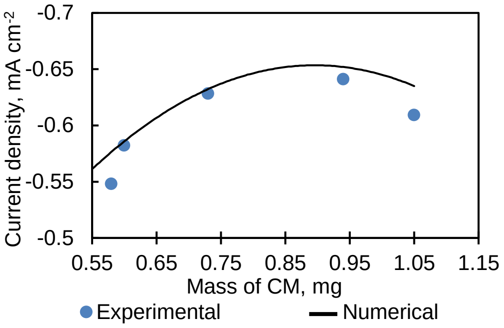

Figure 3: The experimental and the calculated volumes of the electrode activity on the loading of CM

The calculated distributions of the components over the thickness of the electrode active layer in 100 s after the start of the bioelectrocatalytic oxygen reduction are shown on Figure 4.

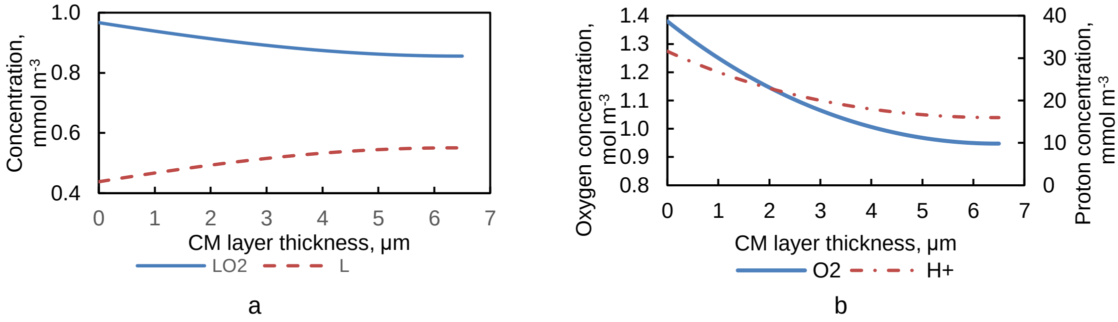

Figure 4: Calculated concentration distributions in the active layer: a) laccase and enzyme-substrate complex; b) oxygen and protons

It was revealed that at the first steps of the calculation the components concentrations varied significantly, and then remained nearly unchanged. Probably such character of the dependence is due to the high values of the reactions rate constants.

In the future, the obtained data will be used to optimize the design of the enzymatic FC cathode.

4. Conclusions

In this paper, a detailed dynamic mathematical model was developed on the basis of the experimental studies of the activity of the electrode based on laccase in the bioelectroreduction of oxygen to water. As a result of the simulation, the distribution profiles of the concentrations of the components along the thickness of the active layer of the cathode were obtained. The dependences of the current density on the cell voltage for different loads of the carbon material (CNT) were calculated. The calculated data are consistent with the experimental data, which indicates the adequacy of the model. The optimal loading of the electrode by the carbon material was also found.

Acknowledgments

Authors gratefully acknowledge Doctor of chemical sciences, Prof. Gorshina N.N. and Doctor of chemical sciences, Prof. Yaropolov A.I. for providing the laccase and research assistance.

This study is partially supported by the state contract № 13.10932.2018/10.11.

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