Makara Journal of Science

Volume 22
Issue 1 *March*

Article 7

3-20-2018

Optimization of Laccase Adsorption-Desorption Behaviors on Multi-Walled Carbon Nanotubes for Enzymatic Biocathodes

Alex Lukmanto Suherman
1. Faculty of Medicine and Pharmacy, University of Grenoble Alpes, Grenoble 38400, France. 2. Department of Chemistry, Physical and Theoretical Chemistry Laboratory, Oxford University South Parks Road, Oxford OX1 3QZ, United Kingdom. 3. Laboratoire TIMC-IMAG équipe Systèmes Nanobiotechnologiques, Biomimetiques et Biotechnologies, UMR 5525 CNRS, Grenoble F-38041, France, lex.suherman@chem.ox.ac.uk

Abdelkader Zebda
Laboratoire TIMC-IMAG équipe Systèmes Nanobiotechnologiques, Biomimetiques et Biotechnologies, UMR 5525 CNRS, Grenoble F-38041, France

Donald K. Martin
1. Faculty of Medicine and Pharmacy, University of Grenoble Alpes, Grenoble 38400, France. 3. Laboratoire TIMC-IMAG équipe Systèmes Nanobiotechnologiques, Biomimetiques et Biotechnologies, UMR 5525 CNRS, Grenoble F-38041, France

Recommended Citation

Suherman, Alex Lukmanto; Zebda, Abdelkader; and Martin, Donald K. (2018) "Optimization of Laccase Adsorption-Desorption Behaviors on Multi-Walled Carbon Nanotubes for Enzymatic Biocathodes," *Makara Journal of Science*: Vol. 22 : Iss. 1 , Article 7.
DOI: 10.7454/mss.v22i1.8619
Available at: https://scholarhub.ui.ac.id/science/vol22/iss1/7

Optimization of Laccase Adsorption-Desorption Behaviors on Multi-Walled Carbon Nanotubes for Enzymatic Biocathodes

Cover Page Footnote

This project was conducted under the framework of the Erasmus Mundus Master Biohealth Computing Program 2015 – 2016 under the guidance of the SyNaBi ResearchGroup, TIMC-IMAG Grenoble, France. The corres-ponding author thanks the Indonesia government for it Beasiswa Unggulan 2015 – 2016 grant for partial funding of the project.

Makara Journal of Science, 22/1 (2018), 52-58
doi: 10.7454/mss.v22i1.8619

Optimization of Laccase Adsorption-Desorption Behaviors on Multi-Walled Carbon Nanotubes for Enzymatic Biocathodes

Alex Lukmanto Suherman[1,2,3*], Abdelkader Zebda[3], and Donald K. Martin[1,3]

1. Faculty of Medicine and Pharmacy, University of Grenoble Alpes, Grenoble 38400, France
2. Department of Chemistry, Physical and Theoretical Chemistry Laboratory, Oxford University
South Parks Road, Oxford OX1 3QZ, United Kingdom
3. Laboratoire TIMC-IMAG équipe Systèmes Nanobiotechnologiques, Biomimetiques et Biotechnologies, UMR 5525
CNRS, Grenoble F-38041, France

[*]*E-mail: alex.suherman@chem.ox.ac.uk*

Received November 18, 2017 | Accepted March 23, 2018

Abstract

Laccase adsorption-desorption behaviors on the surface of multi-walled carbon nanotubes (MWCNTs) were investigated using spectrophotometry and voltammetry. The optimum condition for laccase adsorption is 5.0 mg/mL of laccase in 0.01 M phosphate-buffered saline (PBS) at pH 5.0. Laccase adsorption is a reversible phenomenon that is dependent upon the nature of MWCNTs and the concentration of ionic strength in the laccase solution. Chitosan was functionalized as a nanoporous reservoir to minimize laccase desorption. Chitosan was found to protect approximately 97.2% of the adsorbed laccase from MWCNTs during the first six hours of observation. The three-dimensional (3D) biocathode, MWCNTs-laccase-chitosan with a 0.2 cm^2 geometric area, was shown to have a stable open circuit potential (OCP) of 0.55 V, a current density of 0.33 mA cm^{-2} at 0.2 V vs. saturated calomel electrode (SCE), and a stable current for 20 hours of successive measurements. This report provides a new insight into the study of a high-performance laccase-based biocathode via optimization of adsorption and minimization of desorption phenomena.

Abstrak

Optimasi Perilaku Adsorpsi-Desorpsi Laktase pada Multi-Walled Carbon Nanotubes untuk Biokatoda Berbasis Reaksi Enzimatik. Perilaku adsorpsi-desorpsi *laccase* pada permukaan *multi-walled carbon nanotubes* (*MWCNTs*) telah diselidiki menggunakan spektrofotometri dan voltammetri. Kondisi optimum untuk adsorpsi *laccase* adalah 5,0 mg/mL *laccase* dalam 0,01 M *phosphate-buffered saline* (*PBS*) pada pH 5,0. Adsorpsi *laccase* adalah fenomena reversibel yang bergantung pada sifat MWCNTs dan konsentrasi kekuatan ion dalam larutan *laccase*. *Chitosan* difungsikan sebagai reservoir nanoporous untuk meminimalkan desorpsi *laccase*. *Chitosan* dapat melindungi sekitar 97,2% dari *laccase* teradsorpsi dari MWCNTs selama enam jam pertama pengamatan. Biokatoda tiga dimensi (*3D*), *MWCNTs-laccase-chitosan* dengan area geometrik 0,2 cm^2, menunjukkan potensial rangkaian terbuka (*OCP*) yang stabil pada 0,55 V, kerapatan arus 0,33 mA cm^{-2} pada 0,2 V *vs*. elektroda kalomel jenuh (*SCE*), dan arus stabil selama 20 jam pengukuran berturut-turut. Laporan ini memberikan wawasan baru ke dalam studi tentang biokatoda berbasis *laccase* berperforma tinggi melalui optimalisasi adsorpsi dan minimalisasi fenomena desorpsi.

***Keywords**: adsorption, desorption, laccase, multi-walled carbon nanotubes, spectrophotometry, voltammetry*

Introduction

Enzymatic biofuel cells (EBFCs) have become a promising candidate for powering implantable biomedical devices in living organisms [1-3]. Using specific enzymes, EBFCs have been investigated [1-3] for use in harvesting energy from glucose and oxygen, which are abundantly available in the blood and extracellular fluids of living organisms. For example, an enzyme from the oxidoreductase family, such as laccase (EC 1.10.3.2) [4], can be used in EBFCs. Laccase can be found in many plants, fungi, and microorganisms. In our previous studies, we investigated using laccase to fabricate a three-dimensional (3D) biocathode of EBFCs [5,6].

However, EBFCs may suffer from a sluggish electron transfer rate at the laccase active site of the electrode resulting in low laccase catalytic activity. These outcomes can be indicated by insufficient laccase adsorption and/or desorption (leaching) from the biocathode. Therefore, increasing the amount of adsorbed laccase and enhancing its lifetime by minimizing desorption might improve the performance of EBFCs [6].

Nanomaterials, such as multi-walled carbon nanotubes (MWCNTs), can be utilized as a substrate to increase the amount of adsorbed laccase [7]. MWCNTs have a high surface area for enzyme loading, mass transfer resistance, excellent electrical and mechanical properties, and high conductivity [7,8]. Moreover, the characteristics of the surface of MWCNTs (either pristine or modified), including its charge types, hydrophobicity, surface area, porosity, and mechanical behaviors, are important for the stability and longevity of the adsorbed laccase [5]. However, laccase could possibly be leached out from the MWCNTs surface due to changes in pH and the ionic strength of the system over long periods of time. To overcome this problem, biocompatible polymers, such as chitosan and nafion, can be used to increase the stability and longevity of the adsorbed laccase on MWCNTs.

Chitosan is a natural semi-crystalline polysaccharide derived by partial deacetylation (D) of chitin, which consists of at least 60% D units that are expressed as the degree of deacetylation (DD) from its molar fraction [9,10]. Chitosan with a high DD (>85%) is suitable for preventing the breaking and swelling of biocathodes due to its high stability and biocompatibility [11,12]. Nafion, a sulfonated tetrafluoroethylene-based fluoropolymer-copolymer, is known for its non-permeability to negatively charged enzymes [13,14]. Nafion can also be used as an alternative to chitosan because it offers good biocompatibility and thermal stability in cell cultures and in the human body [15].

The present study investigated the physical adsorption-desorption behaviors of laccase on MWCNTs. Spectrophotometry and voltammetry were used to examine the effects of the MWCNTs surface functional group and determine the presence of chitosan or nafion on the adsorbed laccase. Optimizing the conditions of the adsorption-desorption phenomena can lead to the use of a high-performance 3D laccase-based biocathode in EBFC applications.

Materials and Methods

Laccase (*Trametes versicolor*, EC 1.10.3.2), 2,2’-azino-Bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), NaCl, Na_2HPO_4 (>99%), NaH_2PO_4 (>99%), acetic acid (≥99.8%), chitosan (medium molecular weight), and nafion were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). All aqueous solutions were prepared using nanopure water with a resistivity not less than 18.2 MΩ cm at 25 °C (Purelab Prima, Décines-Charpieu, France). Pristine MWCNTs and Amine ($-NH_2$) functionalized multi-walled carbon nanotubes (MWCNTs-NH_2) (10-15 nm diameter, >95% purity) were purchased from Nanocyl S.A. (Sambreville, Belgium) without further purification.

Spectrophotometric measurements were performed using an ultraviolet (UV) spectrophotometer (Jenway, Roissy, France). Voltammetry measurements were performed with Potentiostat Bio-Logic VSP-300 (Seyssinet-Pariset, France), using a three-electrode setup consisting of a saturated calomel electrode (SCE) as a reference, a Pt wire counter electrode, and a home-made 3D laccase-based biocathode as a working electrode. The morphology of the biocathode was characterized using Scanning Electron Microscopy (SEM) with an ULTRA 55 FESEM based on the GEMENI FESEM column with a beam booster (Nanotechnology Systems Division, Carl Zeiss NTS GmbH, Oberkochen, Germany) and a tungsten gun.

Immobilization of Laccase. The solutions containing laccase and 0.01 M phosphate buffer solution (PBS), at various laccase concentrations and pHs, were prepared immediately prior to obtaining the measurements. The immobilization of laccase was carried out by incubating 1 mg of MWCNTs and 1 mg of MWCNTs-NH_2 separately in the laccase solutions, followed by medium speed stirring overnight. Subsequently, the substrates were filtered using a vacuum pump, and then they were allowed to dried completely in an ambient temperature environment.

Preparation of the 3D Biocathode. Chitosan powder was dissolved in 0.5% (v/v) acetic acid at 50 °C, resulting in a clear viscoelastic gel after two hours of stirring. Nafion was previously synthesized and characterized in the TIMC-IMAG laboratory. Next, 15.0 mg of MWCNTs-laccase or MWCNTs-NH_2-laccase was mixed gently either with or without a polymer (chitosan or nafion). The resulting homogenous paste was then compressed using a hydraulic press to obtain a pellet with a diameter of *ca.* 5.0 mm and thickness of *ca.* 2.0 mm. A copper wire was connected to one side of the pellet using a conductive adhesive. The perimeter and the covered side of the pellets were isolated using silicone glue, leaving one clean side of the pellet with a geometrical surface area of 0.2 cm^2, which was, subsequently, dried in an ambient temperature environment.

Spectrophotometry and Voltammetry Analysis. An ultraviolet-visible (UV-vis) spectrophotometer was used to study the specific activity of free laccase in the solution phase and to determine the quantity of the adsorbed and desorbed laccase on the MWCNTs or the

MWCNTs-NH_2. These studies were performed using laccase solutions with different pHs and ionic strengths. For the voltammetry studies, open circuit potential (OCP), cyclic voltammetry (CV), and chronoamperometry (CA) methods were used to investigate the electrochemical behaviors of the laccase adsorption-desorption phenomena. The sweeping potential was performed at a voltage ranging from -1.0 V to 1.0 V vs. SCE at a scan rate of 0.2 mV/s. All of the CA measurements were performed with E_i vs. Ref at 200 mV to assess the delivered current density that corresponds to the stability of the 3D laccase-based biocathode.

Results and Discussion

Laccase Activity in the Solution Phase. The specific activity of free laccase in the solution phase, at a concentration of 1 mg/ml in 0.01 M PBS (pH 5.0), was determined using ABTS as a substrate for the enzymatic reduction of dioxygen into water [5]. Because one unit of laccase activity is defined as the amount needed to oxidize 1.0 μmol substrate per minute, the laccase activity in the solution phase (as an initial enzyme activity) was calculated to be approximately 0.72 U/mg (hereafter referred to as laccase activity at day one). The laccase activity in the solution phase was found to decrease as a function of time. As shown in Figure 1, the laccase activity gradually decreased to *ca.* 0.43 U/mg, 0.37 U/mg, 0.33 U/mg, 0.22 U/mg, and 0.20 U/mg, on day two to day six of continuous measurements, respectively. This suggests that laccase activity is unstable over longer periods of time in its solution phase [5]. The decrease in laccase activity is one of the major drawbacks for the practical application of EBFCs. Thus, immobilization of free laccase onto the conducting substrate plays a key role in extending the lifetime of EBFCs.

Kinetic Adsorption of Laccase. The kinetic adsorption of laccase was studied using voltammetry measurements. A 3D-biocathode pellet of pristine MWCNTs was immersed into a solution containing 0.01 M PBS (pH 5.0). The *in-situ* evolution of the OCP, which was observed before and after injection of 1.0 mL laccase solution (Figure 2), demonstrated that laccase adsorption on pristine MWCNTs is a slow phenomenon that occurs over several hours. Injecting laccase solution into the electrolyte solution induces an increase in the OCP value from 0.32 V to 0.54 V. The last value of OCP corresponds to the laccase redox potential at equilibrium. The *in-situ* laccase injection into the electrolyte solution at a cell voltage of 200 mV showed that the anodic current increases after the injection. After laccase adsorption, during successive voltammetry measurement, the current decreased by *ca.* 40.0%. The decrease was attributed to desorption (*ca.* 60.0%) of the adsorbed laccase, as verified by spectrophotometry. This demonstrates that laccase adsorption on MWCNTs is a reversible phenomenon [5]. Furthermore, laccase adsorption on pristine MWCNTs was investigated at different concentrations of laccase in the solution phase. To study the optimum laccase concentration in the adsorption process, 2.0 mg of pristine MWCNTs were incubated in 0.01 M PBS (pH 5.0) containing laccase at different concentrations, ranging from 1.0 mg/ml to 10.0 mg/mL. During the six-hour incubation period, continuous moderate stirring was used. Following this, the supernatants were taken and analyzed using UV-vis spectrophotometry. Consequently (data not shown), laccase was adsorbed optimally (*ca.* 87.2%), into pristine MWCNTs at a concentration of 5.0 mg/mL. This percent adsorption is relatively higher in comparison to laccase derived from other sources at different pHs [7]. For the experiment in which the 3D biocathode was fabricated, 5.0 mg/mL laccase was selected as an optimum condition.

Effect of the Functional Group of MWCNTs, Ionic Strength and pH Solution. Laccase adsorption on a solid substrate might depend on the surface functional group of carbon nanotubes. MWCNTs-NH_2 can produce a strong covalent interaction between the amine group

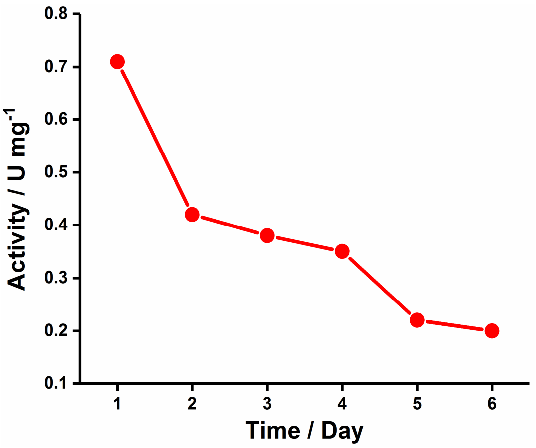

Figure 1. **Linear Relationship between Free Laccase Activity in 0.01 M PBS (pH 5.0) and Time**

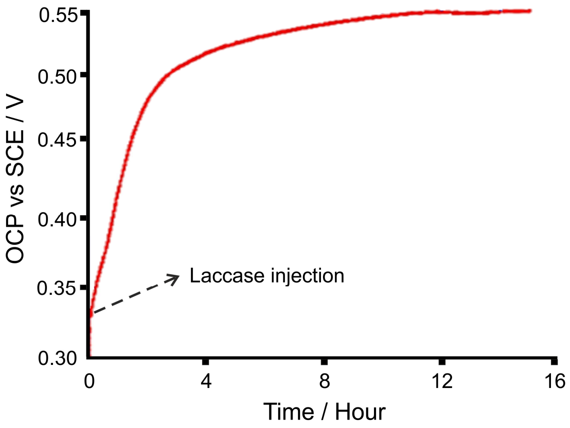

Figure 2. **OCP Evolution of MWCNTs Using Solution Phase Laccase Injection *In Situ* in 0.01 M PBS (pH 5.0).**

of the substrates and the polymeric matrix [16]. According to Brunauer–Emmett–Teller (BET) measurements, pristine MWCNTs (surface area of 280 m^2/g and pore volume of 1.3 cm^3/g) had a surface area that was approximately six-times greater and a pore volume that was five-times larger than MWCNTs-NH_2. Pristine MWCNTs were found to be a better substrate than MWCNTs-NH_2, as shown by the higher laccase adsorption capacity of *ca.* 93.02%. The result suggests that the hydrophobic-hydrophobic interactions between laccase and MWCNTs are more dominant than the ionic interactions in MWCNTs-NH_2 [5,17].

To study the effect of ionic strength during the adsorption of laccase on MWCNTs and MWCNTs-NH_2, laccase was mixed with different concentrations of PBS (pH 5.0). Laccase was found to be less adsorbed both in pristine MWCNTs and MWCNTs-NH_2 at 0.01 M PBS. This indicates that laccase adsorption is also affected by the electrostatic interactions between the charge of the MWCNTs surface groups and laccase. In the case of electrostatic adsorption, increasing the ionic strength decreases the amount of adsorbed laccase because the counterions in the solution compete with laccase in the adsorption on the MWCNTs surface [18,19]. In fact, MWCNTs-NH_2 is more sensitive to the change in ionic strength; this was demonstrated by the fact that less laccase was adsorbed on MWCNTs-NH_2 in comparison to pristine MWCNTs. These results indicate that the contribution of electrostatic adsorption of laccase is more important for MWCNTs-NH_2 than for pristine MWCNTs.

The amount of laccase adsorbed on both MWCNTs and MWCNTs-NH_2 was found to be higher at an acidic pH of 0.01 M PBS. Most types of fungal laccase have an optimum pH in the range of 4.0–5.0 [20,21]. Laccase was found to express higher adsorption in the pristine MWCNTs at pH 4.2 and pH 5.0, with specific activity of 0.63 U/mg (*ca.* 88.0%) and 0.58 U/mg (*ca.* 81.0%), respectively. During the 48-hour follow-up observation period using pristine MWCNTs, the adsorbed laccase activity was found to be most unstable at pH 7.4 because it decreased to *ca.* 30.0%; at pH 4.2 and pH 5.0, the adsorbed laccase activity decreased to *ca*. 6.5% and 3.0%, respectively. Therefore, pH 5.0 was selected as the optimum condition for laccase adsorption.

Effect of Chitosan and Nafion. Biopolymers, such as chitosan and nafion, were investigated to understand their effect on increasing the stability and longevity of the adsorbed laccase on pristine MWCNTs and MWCNTs-NH_2. Laccase desorption behaviors were investigated in the form of 3D-biocathode pellets incorporating the biopolymer, which were incubated in 0.01 M PBS (pH 5.0) for six hours. The results show that laccase desorption was found to be 0.07 U/mg (*ca.* 9.8%) and 0.09 U/mg (*ca.* 12.8%) for pristine MWCNTs and MWCNTs-NH_2, respectively. This demonstrates that laccase is more stable from the leaching out when it is entrapped in the chitosan-MWCNTs 3D matrix. The small amount of desorbed laccase could be attributed to the swelling and/or weakening strength of the polymer matrix due to natural degradation over a longer period of time [22,23]. Therefore, the MWCNTs-laccase-chitosan system was selected as the 3D biocathode for additional experiments.

Electrochemical Characterizations of the 3D Biocathode. Based on the characterizations of the laccase adsorption-desorption behaviors with various parameters described above, a strategy to prevent laccase desorption was constructed (Figure 3). The strategy is based on creating a nanoporous reservoir, in which laccase is confined, near the enzymatic-MWCNTs surface. The advantage of this strategy is that the operational conditions are more likely to be optimal for laccase adsorption. Moreover, the confined laccase in the nanoporous reservoir is in a reduced environment space, which is very favorable for maintaining laccase stability. In contrast to the laccase immobilized on the surface of a solid electrode, laccase and pristine MWCNTs created a new entity that forms the 3D biocathode. In this case, the amount of adsorbed laccase in the 3D biocathode is not limited by the available surface area of the electrode. To assess this strategy, pristine MWCNTs were incubated in 0.01 M PBS (pH 5.0) for 12 hours, then filtered and left to dry completely under ambient temperature. Subsequently, pristine MWCNTs were incubated in a 5.0 mg/ml laccase solution overnight. After vacuum filtration, the MWCNTs-laccase particles were mixed with a 3.0 mg of solid laccase and chitosan gel. The composite was then compressed using a hydraulic press to obtain 3D-biocathode pellets. The nanostructure of the resulting 3D biocathode was examined using SEM. As shown in Figure 4, the highly porous matrix is formed by a dense and homogeneous network of around 10 nm-thick pristine MWCNTs; the spherical structures attached to the nanofibers are attributed to the cross-linked laccase agglomerates. These agglomerates are only visible in the cross-section of the 3D biocathode containing laccase. The agglomerates were found to be distributed all over the MWCNTs framework, suggesting that laccase was homogenously dispersed in the MWCNTs matrix.

The performance of the 3D biocathode, MWCNTs-laccase-chitosan, with the optimized conditions (see above) was then investigated using voltammetry. First, the average OCP was found to be 0.55 V vs .SCE, and the OCP continued to increase up to the optimum redox potential at *ca*. 0.60 V (Figure 5a). Second, CV was performed by sweeping the potential from -1.0 V to 1.0 V and then back to -1.0 V vs. SCE at a scan rate of 0.2 mV/s. This clearly demonstrates a typical electrocatalytic response of laccase incorporated into the pristine

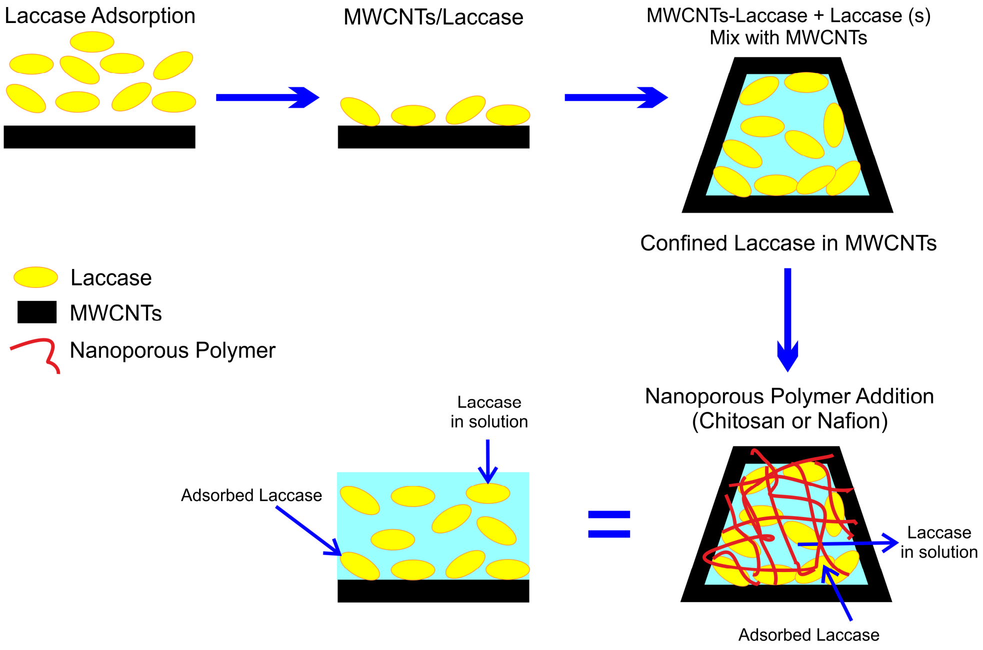

Figure 3. The Biocathode Modification Strategy used to Prevent Laccase Desorption

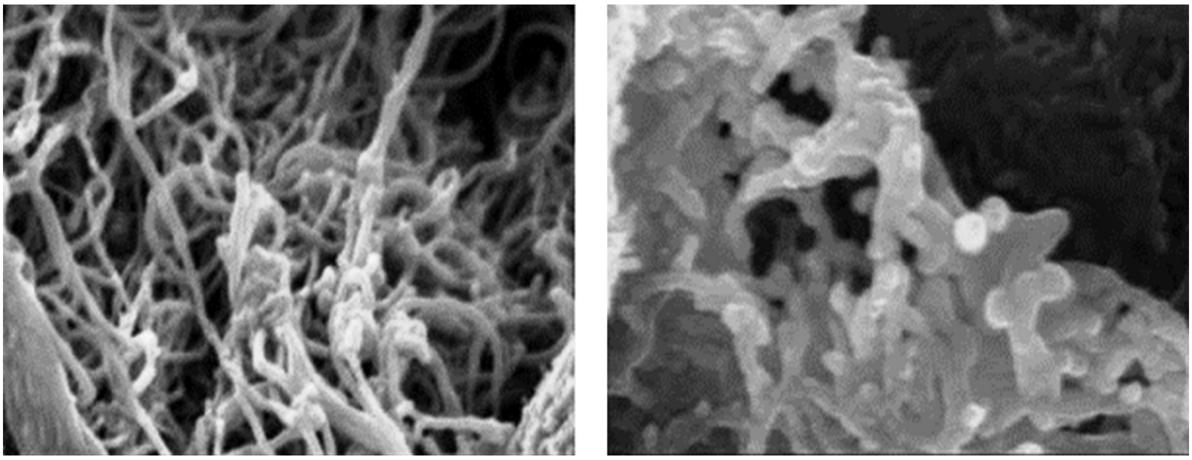

Figure 4. SEM Micrographs of the Cross-section of (a) MWCNTs-laccase and (b) MWCNTs-laccase-chitosan.

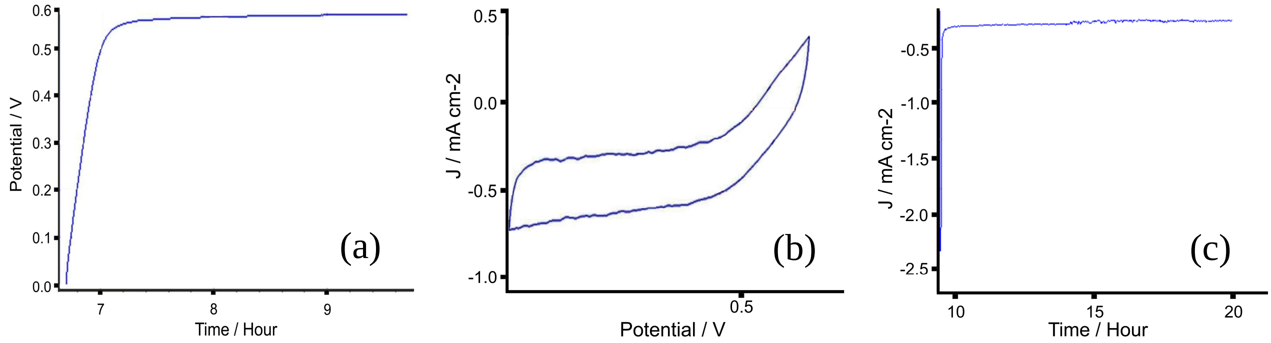

Figure 5. Electrochemical Characterizations of the 3D Biocathode MWCNTs-laccase-chitosan in 0.01 M PBS (pH 5.0). (a) OCP Evolution, (b) CV at a Sweeping Potential Ranging from -1.0 V to 1.0 V vs. SCE at a Scan Rate of 0.2 mV/s, (c) CA at 200 mV vs. SCE

MWCNTs. This was indicated by a cathodic wave starting at 0.55 V vs. SCE (Figure 5b). In addition, there was no oxidative peak current from the sweeping potential window of 0.50 V to 0.0 V vs. SCE. This also indicates that laccase was incorporated into the pristine MWCNTs framework, and it maintained its electrocatalytic activity via oxygen reduction. Finally, the CA measurement at ambient temperature and without O_2 saturation revealed a stable current density of 0.33 mA/cm^2 at 200 mV vs. SCE for at least 20 hours of observations (Figure 5c). This stable current density value is sufficient for operating EBFCs in living organisms [24-26].

Conclusion

Laccase adsorption on MWCNTs is a reversible phenomenon that is dependent upon the nature of the surface of the MWCNTs; however, it is a very slow process that needs several hours to achieve. In both the pristine and modified MWCNTs, the amount of adsorbed laccase was dependent upon the concentration of the ionic strength in the laccase solution. This finding suggests that laccase adsorption is derived from the electrostatic attraction between the negative charge of laccase and the positive charge of the MWCNTs surface. Laccase adsorption in MWCNTs-NH_2 is more sensitive to the change in ionic strength. Pristine MWCNTs were found to be a better substrate than MWCNTs-NH_2, as shown by their higher laccase adsorption capacity of *ca.*93.02%. Chitosan demonstrates better protection to minimize the desorption levels of laccase in the 3D biocathode; thus, chitosan should be considered as a polymer to enhance the biocompatibility and stability of the 3D biocathode. This report presents another perspective for studies of high-performance laccase-based biocathodes via optimization of adsorption and minimization of desorption phenomena.

Acknowledgement

This project was conducted under the framework of the Erasmus Mundus Master Biohealth Computing Program 2015–2016 under the guidance of the SyNaBi Research Group, TIMC-IMAG Grenoble, France. The corresponding author thanks the Indonesia government for it Beasiswa Unggulan 2015–2016 grant for partial funding of the project.

References

[1] M. Rasmussen, S. Abdellaoui, S.D. Minteer. 2016. Enzymatic biofuel cells: 30 years of critical advancements. Biosens. Bioelectron. 76: 91-102, doi:10.1016/j.bios.2015.06.029.

[2] S. El Ichi, A. Zebda, J.P. Alcaraz, F. Boucher, J. Boutonnat, P. Cinquin, D.K. Martin. 2015. Biocompatible implantable biofuel cell, in: IECBES 2014, Conf. Proc. - 2014 IEEE Conf. Biomed. Eng. Sci. “Miri, Where Eng. Med. Biol. Humanit. Meet.” 51-55. doi:10.1109/IECBES.2014.7047553.

[3] M.A. Hannan, S. Mutashar, A. Samad and Hussain A. 2014. Energy harvesting for the implantable biomedical devices: issues and challenges. Bio-Medical Engineering OnLine. 13: 79-101, doi: 10.1186/1475-925X-13-79.

[4] D.M. Mate, M. Alcalde. 2017. Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microb. Biotechnol. 10: 1457-1467, doi:10.1111/1751-7915.12422.

[5] S. El Ichi-Ribault, A. Zebda, S. Tingry, M. Petit, A.L. Suherman, A. Boualam, P. Cinquin, D.K. Martin. 2017. Performance and stability of chitosan-MWCNTs-laccase biocathode: Effect of MWCNTs surface charges and ionic strength. J. Electroanal. Chem. 799: 26-33, doi:10.1016/j.jelechem.2017.05.018.

[6] S. El Ichi-Ribault, A. Zebda, A. Laaroussi, N. Reverdy-Bruas, D. Chaussy, M.N. Belgacem, A.L. Suherman, P. Cinquin, D.K. Martin. 2016. Laccase-based biocathodes: Comparison of chitosan and Nafion. Anal. Chim. Acta. 937: 43-52, doi:10.1016/j.aca.2016.07.029.

[7] A.P.M. Tavares, C.G. Silva, A.M.T. Silva, J.M. Loureiro, J.L. Faria. 2015. Laccase immobilization over multi-walled carbon nanotubes: Kinetic, thermodynamic and stability studies, J. Coll. Interface Science. 454: 52-60, doi:10.1016/j.jcis.2015.04.054.

[8] J.H. Park, H. Xue, J.S. Jung, K. Ryu. 2012. Immobilization of laccase on carbon nanomaterials. Korean. J. Chem. Eng. 29: 1409-1412, doi:10.1007/s11814-012-0024-1.

[9] J. Ma, Y. Sahai. 2013. Chitosan biopolymer for fuel cell applications. Carbohydr. Polym. 92: 955-975, doi:10.1016/j.carbpol.2012.10.015.

[10] A. Muxika, A. Etxabide, J. Uranga, P. Guerrero, K. de la Caba. 2017. Chitosan as a bioactive polymer: Processing, properties and applications. Int. J. Biol. Macromol. 105: 1358-1368, doi:10.1016/j.ijbiomac.2017.07.087.

[11] J. Kumirska, M.X. Weinhold, J. Thöming, P. Stepnowski. 2011. Biomedical activity of chitin/chitosan based materials-influence of physicochemical properties apart from molecular weight and degree of N-Acetylation. Polymers (Basel). 3: 1875-1901, doi:10.3390/polym3041875.

[12] C.K.S. Pillai, W. Paul, C.P. Sharma. 2009. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Prog. Polym. Scie. 34: 641-678, doi:10.1016/j.progpolymsci.2009.04.001.

[13] Y. Freijanes, V.M. Barragán, S. Muñoz. 2016. Chronopotentiometric study of a Nafion membrane in presence of glucose. J. Memb. Sci. 510: 79-90, doi:10.1016/j.memsci.2016.02.054.

[14] L. Dehabadi, I.A. Udoetok, L.D. Wilson. 2016. Macromolecular hydration phenomena: An overview of DSC studies on sulfonated tetra-fluoroethylene-based fluoropolymer–copolymer (Nafion) and cellulose biopolymer materials. J. Therm. Anal. Calorim. 126: 1851-1866, doi:10.1007/s10973-016-5673-6.

[15] S. Meredith, S. Xu, M.T. Meredith, S.D. Minteer. 2012. Hydrophobic Salt-modified Nafion for Enzyme Immobilization and Stabilization. J. Vis. Exp. 65: e3949, doi:10.3791/3949.

[16] A. Korani, A. Salimi. 2015. High performance glucose/O2compartment-less biofuel cell using DNA/CNTs as platform for immobilizing bilirubin oxidase as novel biocathode and integrated NH2-CNTs/dendrimer/glucose dehydrogenase/nile blue as bioanode. Electrochim. Acta. 185: 90-100, doi:10.1016/j.electacta.2015.10.090.

[17] T.J. Su, J.R. Lu, R.K. Thomas, Z.F. Cui, J. Penfold. 1998. The adsorption of lysozyme at the silica-water interface: A neutron reflection study. J. Colloid Interface Sci. 203: 419-429, doi:10.1006/jcis.1998.5545.

[18] Y. Sugimoto, Y. Kitazumi, O. Shirai, M. Yamamoto, K. Kano. 2016. Understanding of the effects of ionic strength on the bimolecular rate constant between structurally identified redox enzymes and charged substrates using numerical simulations on the basis of the poisson-boltzmann equation. J. Phys. Chem. B. 120: 3122-3128, doi:10.1021/acs.jpcb.6b00661.

[19] H. Yue, D.H. Waldeck, J. Petrović, R.A. Clark. 2006. The effect of ionic strength on the electron-transfer rate of surface immobilized cytochrome c. J. Phys. Chem. B. 110: 5062-5072, doi:10.1021/jp055768q.

[20] M.M. Atalla, H.K. Zeinab, R.H. Eman, A.Y. Amani, A.A.E.A. Abeer. 2013. Characterization and kinetic properties of the purified trematosphaeria mangrovei laccase enzyme. Saudi J. Biol. Sci. 20: 373-381, doi:10.1016/j.sjbs.2013. 04.001.

[21] I. Stoilova, A. Krastanov, V. Stanchev. 2010. Properties of crude laccase from Trametes versicolor produced by solid-substrate fermentation. Adv. Biosci. Biotechnol. 1: 208-215, doi:10.4236/abb.2010.13029.

[22] E. Szymańska, K. Winnicka. 2015. Stability of chitosan-A challenge for pharmaceutical and biomedical applications. Mar. Drugs. 13: 1819-1846, doi:10.3390/md13041819.

[23] D. Ren, H. Yi, W. Wang, X. Ma. 2005. The enzymatic degradation and swelling properties of chitosan matrices with different degrees of N-acetylation. Carbohydr. Res. 340: 2403-2410, doi:10.1016/j.carres.2005.07.022.

[24] E. Katz, K. MacVittie. 2013. Implanted biofuel cells operating in vivo-methods, applications and perspectives-feature article. Energy Environ. Sci. 6: 2791, doi:10.1039/c3ee42126k.

[25] T. Miyake, K. Haneda, N. Nagai, Y. Yatagawa, H. Onami, S. Yoshino, T. Abe, M. Nishizawa. 2011. Enzymatic biofuel cells designed for direct power generation from biofluids in living organisms. Energy Environ. Sci. 4: 5008, doi:10.1039/c1ee02200h.

[26] A. Zebda, S. Cosnier, J.P. Alcaraz, M. Holzinger, A. Le Goff, C. Gondran, F. Boucher, F. Giroud, K. Gorgy, H. Lamraoui, P. Cinquin. 2013. Single glucose biofuel cells implanted in rats power electronic devices. Sci. Rep. 3: 1516. doi:10.1038/srep01516.