

Anode modification by immobilization of biocatalyst for Microbial Desalination Cell

Umyy Mardiana^{a,b,c}, Christophe Innocent^a, Marc Cretin^a, Buchari^b

Abstract—New strategies are proposed for modification of the anode of a Microbial Fuel Cell (MFC). Immobilization of yeast cells as electrogenic microorganism in MFC was reported using alginate. Yeast cells entrapment within alginate matrices was done through films deposited at the surface of a carbon felt electrode and the resulting anodes were characterized by chronoamperometry. Yeast entrapped within alginate films on carbon felt oxidized glucose and generates a current by direct and mediated electrons transfer from yeast cells to the carbon electrode. The result substantiated that immobilization of yeast for MFC could be a promising method to produce green electricity. The application of energy produce from MFC will be applied in microbial desalination cell (MDC) for sea water desalination.

Index Terms— Bioenergy, MFC, MDC, *Saccharomyces cerevisiae*, Yeast immobilization.

I. INTRODUCTION

Energy, in any form, play the most important role in the modern world. We need energy, especially electrical energy in our daily needs of life. We have been completely dependent on conventional energy sources such as coal and oil for quite a long time. Global warming and climate change refer to an increase in average global temperatures. This is caused primarily by increases in “greenhouse” gases such as carbon dioxide (CO₂). [1][2].

One of the potential sources of renewable energy is the biomass energy. The production of energy from renewable substrates, such as biomass, is important for creating sustainable energy production and reducing global emissions of CO₂. Microbial fuel cell (MFC) technologies represent the newest approach for generating electricity-bioelectricity from biomass using microorganisms. Those later are used as biocatalysts which are able to directly convert resource streams containing biodegradable organic matter (i.e. chemical energy) into electricity [3] [4].

The first observation of electrical current generated by *Saccharomyces cerevisiae* has been reported by Potter [5]. In an MFC, microorganisms oxidize organic matter, producing

electrons captured by anode and then moved through the external circuit to cathode. A membrane generally separates the anode and cathode chambers, and the bacteria grow on anode. The membrane is permeable to protons that are produced at the anode, so that they can migrate to the cathode where they can combine with electrons and oxygen, forming water.

In this study, Microbial Fuel Cell (MFC) based on yeast as biocatalyst will be developed as energy production system and simultaneous applied for desalination seawater using methods based on electro dialysis. As we know that more than 70% of Earth's surface is covered by water, The abundant of this natural resource can then be used for human life. Microbial desalination cells (MDCs) are a newly emerged bio electrochemical technology for simultaneous water treatment and water desalination [6][7].

In this present work a salt-free water will be produced by desalination on an electro dialysis-based cell in which the driving force will be produced by carbohydrates. The integration between MDCs and MFCs will take advantage of bioenergy generated from the oxidation of organic matter to accomplish desalination. Our aim is then to develop a system of water treatment without the need of energy supply, in an attempt to produce energy and to overcome the problem of lack of clean water. A new strategy has been applied to increase the performance of MFC and MDC. The objective were (1) to use of yeast as biocatalyst in MFC, (2) to improve of MFC performance by developing anode modification technics by immobilization of yeast at surface of anode. (3) to investigate the use of anode material in MDC operation.

Studied of immobilization electrode-mediator have been resulted [8][9]. In conventional method, by employing soluble electron-mediators can enhance performance of MFC, but it can cause environmental polluting effect, not considered for continuously industrial process [8][10]. In this study, we propose the anode modification by immobilization by deposit at the surface of a carbon felt of an alginate film containing yeast cells at the surface of carbon felt as anode. Chronoamperometry was used as the main electrochemical method. Scanning electron microscopy was performed for identification of yeast electrode surfaces. neutral red (NR) have been chosen as electron mediator to applied in MDC performances [11]. Further, by employing immobilized mediator and biocatalyst as simultaneously could be contributed to self-sufficient of MFC and MDC which can be used for long term application.

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II. MATERIALS AND METHODS

A. Preparation of electrodes

Carbon felt (CF) (from Alfa Caesar, 1.27 cm thick) and nickel plate were used as anode and cathode material respectively. Materials were previously washed in 1M HCl for 48 hours and then rinsed with ultrapure water to clean from trash material.

B. Procedure for the immobilization of yeast cells

Immobilization within alginate was done from a procedure previously designed [12], CaCl₂ (Sigma) was used as gelation agent. A solution at 3% (w/w) of yeast, *Saccharomyces cereviceae* was prepared in a 0.85% (w/w) NaCl (Sigma) solution. Neutral Red (NR) at 0.5% (w/w) as a mediator was prepared in buffer phosphate (PB) pH7. 2% (w/w) Na-alginate powder (Sigma) was dissolved in ultrapure water at 75-80°C for 30 min. Na-alginate and yeast cells solutions were mixed in a ratio 1:1 then NR was added in this solution. Films formation have been gained through the deeping of a carbon felt in a yeast-alginate solution Neutral Red. Carbon felt was immersed for 1 hour, followed in CaCl₂ then left for 12 hours for complete gel formation in desicator. The product were washed with ultrapure water to remove excess calcium ion and untrapped cells, and then stored at 4°C prior used and simply activated at 30°C for 30 minute before applied.

C. MFC and MDC design and experimental procedure

The MFC dual chamber (2*80 mL) is constituted of an anodic and a cathodic chamber separated by Nafion® 117 (DuPont, USA) as proton exchange membrane. As anode, Carbon felt modified by immobilization yeast alginate have been used, meanwhile nickel plate (5 cm x 1.5 cm) have been applied as cathode electrodes respectively. A schematic of the MFC and MDC system are shown in Fig 1. All chamber used have been closed by cover compartment cell

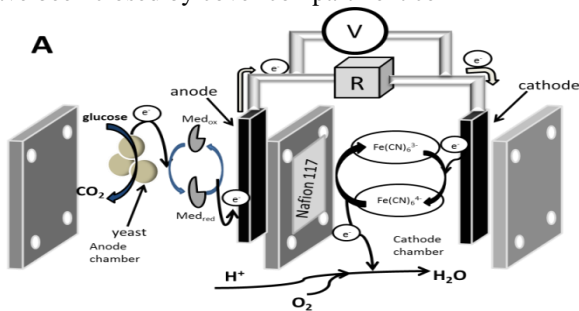


Fig.1. Design compartment of A). Microbial fuel cell (MFC)

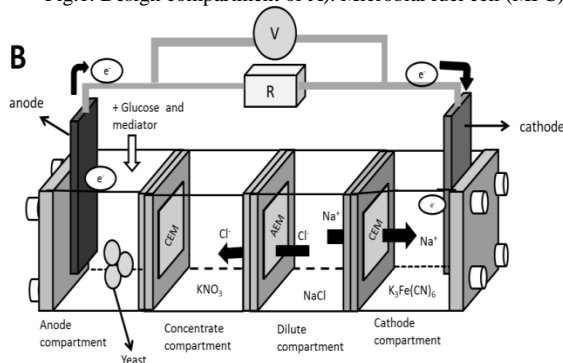


Fig.1. Design compartment of B). Microbial desalination cell (MDC)

Anodic chamber is constituted of a solution of glucose

monohydrate 0.1M (Sigma Aldrich) in buffer pH 7. Yeast *Saccharomyces cerevisiae* as biocatalyst immobilization in film deposited at the surface of anode was packed into anodic chamber. The experiments were conducted with Neutral Red (NR) entrapped in the alginate yeast solution as molecule facilitating electron transfer (i.e. mediator). The cathodic compartment consists of potassium ferricyanide solution (Sigma Aldrich) at 0.02 M in phosphate buffer pH 7. In MDC operation, the composition of anode and cathode are the same with MFC. The addition chamber was provide to MDC operation, they are dilute and concentrate chamber. The dilute and concentrate chambers consist of NaCl 1 M (Merck, France) and KNO₃ (Sigma Aldrich, France) 1M, respectively, as observed in previous study [13][14].

The external resistance R of the device (Fig. 1b) was fixed at 1k Ω , using a resistance box. All desalination process was operated at ambient temperature (25±1°C). CMX (CMI-7000) and AMX-SB (AMI-7001) (from Tokuyama Soda- Japan.) have been used as cation exchange membrane (CEM) and anion exchange membrane (AEM). The voltage (E) in desalination process was recorded using a digital multimeter Voltcraft model VC 850 and the current (I) generated was determined from the equation $I = E/R$. 2 mL of dilute and concentrate solution have been taken for the measurement of salt concentration by ionic chromatography (DIONEX ICS 900 for cation and DIONEX ICS 1000 for anion).

III. RESULTS AND DISCUSSION

A. Yeast-alginate film on carbon felt as a product of yeast immobilization

As shown, the thickness of the alginate-yeast film is around 15 μ m with a high roughness because of the presence of yeast cells (Fig 2b). Yeast cells are well identified as spherical shape is around 4 μ m (Fig.2c). Yeast cells are shrouded within matrix of alginate with a homogeneous deployment on its surface favorable for electrocatalysis.

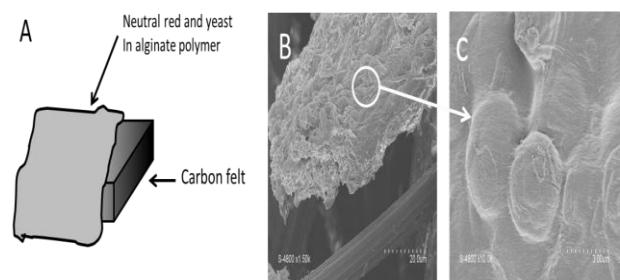


Fig 2. SEM images : A. Yeast film deposited at the surface of anode (CF/Y-Alg-NR). B and C showing yeast cells immobilization within an alginate film deposited on carbon felt

B. MFC performance

According results above, we have been applied immobilized yeast to prepare the anode of the MFC. The aim of this part is to build a MFC and to determine its basic electrical properties through the monitoring of the discharge current (on a 1 k \cdot resistance) vs time during 30 days. Anodic compartment were built from a yeast cells suspension (i.e. without immobilization) (CF/Y-NR in suspension) and from a yeast-alginate film within NR (CF/Y-Alg-NR film). Results

are presented in Fig 3.

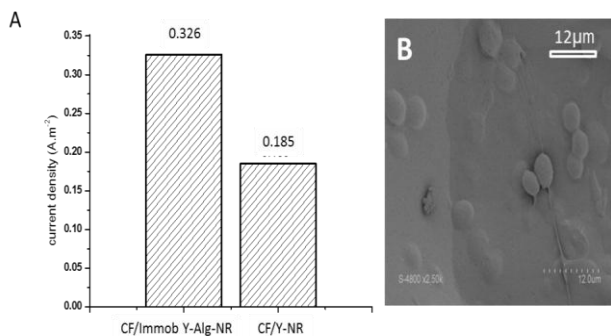


Fig.3. (A) The current density (j) delivered by the MFC working in the anodic compartment with yeast cells and NR in suspension (CF/Y-NR), and CF/Immob Y-Alg-NR film in 0.1 M glucose. (B) SEM image showing the anode of model C after use in MFC.

In CF/Immob Y-NR, it was assumed that, the electrons transfer occurs within alginate polymer and involving neutral red molecule and the electron flux presents a high stability because of the encapsulation of NR and yeast cells close to the electrode surface within the alginate film. The very low current measured from the yeast in suspension cell (CF/Y-NR) experiment can be linked to the fact that the contact of yeast to anode is low. The recycling of NADH to NAD⁺ is important to keep the glycolysis process continuous [15].

C. MDC operation

In this study, first, we have operated on MDC by applying yeast and mediator in solution. CF without modification and nickel plates have been used as anode and cathode respectively. Next focus is the use of electrodes modified by immobilization of yeast. The current density has been recorded and the concentration of ion salt removal has been monitored through the measurement of the concentration of the dilute solution. The second part, MDC by using CF with modification (CF/immob Y-Alg-NR) have been applied as anode in MDC. Results are presented Fig 4.

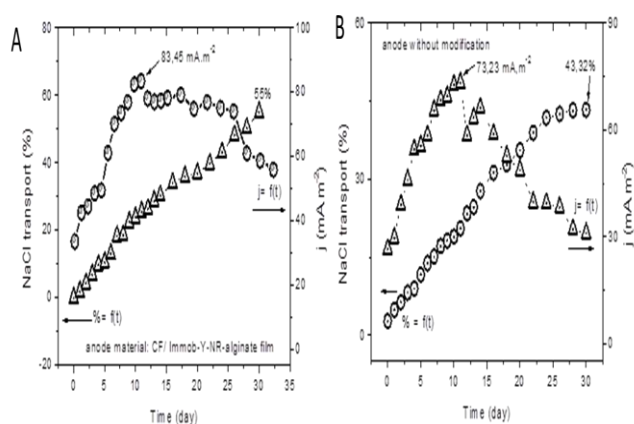


Fig.4 NaCl transport (%) and j (A.m⁻²) obtained from MDC after observed for 30 days of operation equipped (A) carbon felt (CF) without modification as anode material (CF/Y-NR). (B) from anode modification (CF/Immobilized Yeast-Alginate-Nickel Red).

Fig 4a. reported that, NaCl transport could reach 43% and the maximum current density was 73.3 mA.m⁻². The volume of each chamber was 80mL. The maintenance of MDC perform has been carried out by the addition of glucose in

anolyte and replacement of PF in catholyte every 13th day of the process. At the second week of running, the current has declined, then we added 1.4 g of glucose and changed the PF solution at 0.02 M. The improvement of the current value does not occur, it can be assumed that yeast lifetime is reached. Meanwhile, from the CF/Immobilized Yeast-Alginate-Nickel Red, the maximum of current density and NaCl transport have been achieved 83 mA.m⁻² and 55%, respectively. At 11th day of running, the currents have decreased slightly and at 20th day, there has been declined until the end of process. Results shows that by using anode modification, the effectivity of MDC could improve

IV. CONCLUSION

A biological fuel cell operating by glucose oxidation by baker's yeast, *Saccharomyces cereviceae* was performed. The effect of anode modification and without modification have been studied. The immobilization of yeast at the surface of anode could improve the performance of MFC and MDC. After 1 month operation, 55 % of ions moved from the dilute compartment to the concentrate compartment. The maximum current density during the process was 83 mA.m⁻². Enhancing of MDC based yeast fuel cell performance has been improving. Further, the possibility of utilization modified electrode material also gets a good attention from scientist to improve the power generated by yeast fuel cells.

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REFERENCES

- [1] Singh B.R. Singh, O. (2004): Global Trends of Fossil Fuel Reserves and Climate Change in the 21st Century.
- [2] Steinberg, S. (1999): Fossil fuel decarbonization technology for mitigating global warming, *Int. J. Hydrogen Energy.*, 24, 771-777. doi:10.1016/S0360-3199(98)001.
- [3] Verstaete, W. Rabaey, V (2007): *Microbial Fuel Cells: Methodology and Technology*, Environ Sci and technol., 1-12.
- [4] Logan, B.E., Regan, J.M., 2006: Electricity-producing bacterial communities in microbial fuel cells., *Trends Microbiol.*, 14, 512-8. doi:10.1016/j.tim.2006.10.003.
- [5] Potter, A.M.C., B. Character, N. Sep. 2010: *Electrical Effects Accompanying the Decomposition of Organic Compounds* Published by: The Royal Society Electrical Effects accompanying the Decomposition of Organic, Royal Society Publishing, 84, 260-276.
- [6] Mehanna, M., Saito, T. Yan, J. Hickner, M., Cao, X. Huang, X. (2010): Using microbial desalination cells to reduce water salinity prior to reverse osmosis, *Energy Environ. Sci.*, 3 (2010) 1114. doi:10.1039/c002307h.
- [7] Luo, H., Xu, P., Roane, T.M., Jenkins, P.E., Ren, Z., 2012: Microbial desalination cells for improved performance in wastewater treatment, electricity production, and desalination, *Bioresour. Technol.*, 105, 60-66. doi:10.1016/j.biortech.2011.11.098.
- [8] Popov AL, Kim JR, Dinsdale RM, Esteves SR, Guwy AJ, Premier GC. The effect of physico-chemically immobilized methylene blue and neutral red on the anode of microbial fuel cell. *Biotechnology and Bioengineering* 17. (2012) : 361-70.
- [9] Wang K, Liu Y, Chen S. Improved microbial electrocatalysis with neutral red immobilized electrode. *Journal of Power Sources* 196. (2011) : 164-8

- [10] [10] Park DH, Zeikus JG. Electricity generation in microbial fuel cell using neutral red as an electronophore. *Apply. Environ. Microbiol* 66.(2000) : 1292-97.
- [11] Raghavulu SV, Goud RK, Sarma PN, Mohan SV. Bioresource Technology *Saccharomyces cerevisiae* as anodic biocatalyst for power generation in biofuel cell : Influence of redox condition and substrate load. *Bioresource Technology* 102. (2011) : 2751–7.
- [12] Liu R, Li J, Shen F. Refining bioethanol from stalk juice of sweet sorghum by immobilized yeast fermentation. *Renewable Energy* 33. (2008) : 1130–5.
- [13] Mardiana, U, Innocent, Jarrar H, C, Cretin, M, Buchari, B, Gandasasmita, S. Electropolymerized neutral red as redox mediator for yeast fuel cell, *Int J of electrochem. Sci.* 10. (2015) : 8886-8898.
- [14] Mardiana U, Innocent C, Cretin M, Buchari B, Gandasasmita S. Yeast fuel cell: Application for desalination. *IOP Conference Series: Materials Science and Engineering* 107. (2016) : 012049.
- [15] Feldmann, H. *Yeast Molecular Biology - A Short Compendium on Basic Features and Novel Aspects*; Adolf-Butenandt-Institute, University of Munich: Munich, 2005.



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