CHEMICAL ENGINEERING TRANSACTIONS
VOL. 63, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Jeng Shiun Lim, Wai Shin Ho, Jiří J. Klemeš

ISBN 978-88-95608-61-7; **ISSN** 2283-9216
DOI: 10.3303/CET1863102

Development of Immobilised Bioanode for Microbial Fuel Cell

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The efficiency of Microbial fuel cell (MFC) performance is based on how well the electron is transferred and finally turned into electrical power in a complete electrical circuit. However, MFC power capacity is still very low compare to similar conceptual fuel cell and one of the major reasons is due to high internal resistance imposed by macro-environment of an MFC. In the present research, the objectives were to develop bio-based anode and its usage in the MFC for power production. The power production was compared using free cells in MFC. The bioanode was developed by mixing cells solution and graphite granules overnight before adding alginate and subjected to homogenisation. The mixture was then immobilised using entrapment method to obtained uniform beads. Initial study was conducted using glucose as fuel and both open circuit voltage (OCV) and closed circuit voltage (CCV) were evaluated using MFC. Results show OCV increased gradually and still increased after 6 h of operation compared to free cells. In CCV profile for free cells show a decrease in voltage generated but then rapidly increased which indicates a 'power-overshoot' phenomenon which was not observe on immobilised based bioanode MFC. The maximum OCV was 2-fold higher for immobilised based bioanode compared to free cells. In conclusion, the immobilised based anode or bio-anode produced was proved viable for MFC application.

1. Introduction

Microbial fuel cells (MFCs) are one of the alternative energy producing technology, producing electric power by utilising microorganisms, to assist redox reactions and by generating electricity from what would otherwise be considered waste. This technology can use bacterium already present in wastewater as catalysts to generating electricity while simultaneously treating wastewater (Gude, 2016). A MFC converts energy, available in a bio-convertible substrate, directly into electricity. This can be achieved when bacteria switch from the natural electron acceptor, such as oxygen or nitrate, to an insoluble acceptor, such as the MFC anode. Fuel cells that use bacteria are classified as two different types: biofuel cells that generate electricity from the addition of artificial electron shuttles (mediators) and MFCs that do not require the addition of mediator.

The electricity generated by MFC is relatively low and often power output is fluctuated due to unstable internal resistance presence in the MFC environment (Wang et al., 2016). Microbial metabolic activity could as well as be hindered by the macro-environment created by the electrolyte and complexity of the substrate used as the source of fuel (Mahmood et al., 2017). Researchers realise that the closer the microbes or electrigens to anode electrode, more electron can be transferred efficiently. In other word, the 'linking species' should be physically closed to the surface of the electrode to promote efficient electron transfer (Schroder, 2007). Immobilisation of microbes to the anodic electrodes has been established previously by several authors such as polymer matrix (Alferov et al., 2014), carbon nanoparticles (Yuan et al., 2011) and on carbon paper using latex as the immobilisation agent (Wagner et al., 2012). Electrodes are first immersed in microbial culture and incubated in this way for several days to ensure microbes are attached to the electrode base for microbial attachment. Such steps are time-consuming as well as risk of non-attachment of microbes on the surface of electron is rather high. The proposed method of immobilisation is to produce controlled form of circular shaped immobilised cell which consists of microbes or electrigens mixed with graphite granules, and bind by alginate. Graphite granules have been reported comparable to other carbon based materials and commonly used for

Please cite this article as: Khairul Aswad Abdul Wahab, Amirul Amin Ahmad Nazri, Ahmad Arttirmeedzee Mohd Azam, Nazlee Faisal Ghazali, Eraricar Md Salleh, Nik Azmi Nik Mahmood, 2018, Development of immobilised bioanode for microbial fuel cell, Chemical Engineering Transactions, 63, 607-612 DOI:10.3303/CET1863102

MFC studies (Wei et al., 2011). Preliminary study on usage of this type of immobilised electrode has been done previously (Mesran et al., 2014) and successfully determined the importance of immobilised based activated carbon as anode. In the present study, immobilised based bioanode was developed and was investigated for its usage in MFC in terms of power performance.

2. Methodology

2.1 Preparation of cell suspension

Previously, anaerobic sludge from an oleochemical company in Pasir Gudang, Johor was diluted to 100 times dilution using sterile distilled water. 5 mL diluted sample was then added to a 250 mL flask containing Luria-betani medium overnight to obtain starter culture. The starter culture was stored as 5 mL stock in -80 °C prior to use. For MFC operation, cell suspension was prepared by pipetting 5.0 mL of mixed culture (previously cultured from environmental sample) in Luria-Bertani medium and was incubated at 37 °C at 175 rpm for 24 h. Prior to use, cell culture was sampled at absorbance an optical density (OD) value of 2 (as previously determined that it was within log phase of the mixture culture growth at 600 nm of visual wavelength). Cell suspension was centrifuged at 27 °C and 5,000 rpm for 15 minutes and pellet was re-suspend in M9 minimal medium (pH 7.0) containing 6.0 g disodium hydrogen phosphate (Na_2HPO_4), 0.5 g sodium chloride (NaCl), 3.0 g potassium dihydrogen phosphate (KH_2PO_4), 1.0 g ammonium chloride(NH_4Cl) and 20 mM magnesium sulfate ($MgSO_4.7H_2O$) to OD = 2.

2.2 Immobilisation of cell-aginate-graphite (CAG)

Immobilisation of CAG was adapted from Mesran et al. 2016 with slight modification. Cell suspension was mixed with 6.0 % (w/v) of graphite and left to stir overnight to achieve maximum binding between the cell and the graphite. Next, 3.0 % (w/v) of sodium alginate was added little by little while being homogenised at low speed to prevent clumping of the mixture. CAG mixture was then poured slowly in to a 5 mL pipette tip and drop slowly into a beaker containing 3.0 % (w/v) Calcium Chloride ($CaCl_2$) solution (Figure 1). The formed beads was let to harden for 1 h to ensure complete reaction of calcium alginate and subsequently, was washed three times with distilled water prior to use.

Figure 1: Image of immobilised cell-alginate-graphite preparation.

2.3 MFC set-up and operation

A single chambered MFC was configured in such a way that the anodic compartment was half filled (approximately 2.5 cm from the cathode electrode) with immobilised cell-alginate-graphite and making sure that the beads touched the copper wire connected to the external circuit of the MFC. The MFC was connected with an auto-logged multi-meter to obtain voltage values. The anodic compartment was filled with glucose solution, approximately 250 mL of volume before MFC was operated. The MFC was run for 12 to 13 h or otherwise stated in order to obtain a stable open circuit voltage (OCV) before attaching the MFC circuit with external resistant (50 to 50,000 Ω). It was also considered as acclimatisation period for the microorganisms to adapt to their microenvironment (Zhang et al., 2016). The OCV value was recorded via multi-meter every 1.5 h.

2.4 Power analysis

Voltage was measured using a digital multimeter and subsequently calculate the current (I) and power (P) calculated based on Ohm's law which defined power (P) = IV. Power density and current density were then calculated by normalising calculated power with volume of the anodic chamber (0.00025 m^3).

2.5 Glucose concentration determination

To investigate the power produced over time with glucose consumption, glucose analysis was done based on Dintrosalicyclic acid based assay (DNS). The MFC was operated in CCV orientation using 1,000 Ω as the external resistant. DNS was done by mixing 1.0 mL of sample from the MFC anodic liquid with 1.0 mL of dinitrosalicyclic acid solution and incubated at 95 °C for 5 min before diluting it with 3.0 mL distilled. Prior to cool, the samples were measured spectrophotometrically at 540 nm wavelength to estimate the reducing sugar concentration. Glucose was used to build up standard curve.

3. Results and Discussions

3.1 Open circuit voltage (OCV)

Without any external resistance in the MFC electrical circuit, there was no current produced but voltage recordings produced were from the differences of chemical potential presence in the electrolyte. These values were considered as an indication of whether the system can be used for producing power with the created anodic environment. Figure 2 shows an immediate increase in OCV for free cells and no solution was removed from the chamber during this period of operation. With immobilised cells, no increment of potential or voltage within a period of 4.5 h of operation. The differences in OCV state between free cells and immobilised cells in this period of time probably due to the low cell multiplication during substrate metabolization in the immobilization state (Junter and Jouenne, 2004). In different situation, it was reported that in immobilisation state limitations include biofilm surface distribution, cells to substrate ratio, efficient oxygen transfer and volumetric productivity which may affect the fermentation process in an aerated bioreactor (Freeman and Lilly, 1998). In contrast, OCV increased gradually and still increased after 6 h of operation compared to free cells. Large errors was observed which believed to be microbial activities dependent, occurs independently due to the nature of mixed culture with different level of metabolic pathway. Shifts in bacterial diversity were observed during transition through metabolic processes such as methanogenesis, anaerobic or aerobic fermentation (Bretschger et al., 2010). In addition, it was also stated by Hogson et al. (2016) that the metabolic shifts which occurs in an MFC system indicates the segregation of anodic microbial communities which also affecting the power generation. The highest OCV was more than 2-fold after 9 h of operation for immobilised based MFC compared to free cells and further operation shows immobilised based MFC was stable until the COD dropped at 15 h of operation. The curves well-imitated the real microbial growth curves that may reflect the availability of substrates in the anodic chamber.

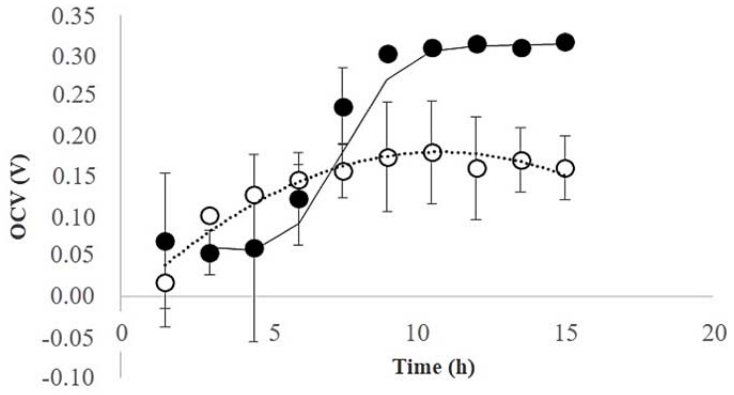

Figure 2: OCV profiles. Both free cells (○) and immobilised bio-anode (●) was compared in terms of OCV using synthetic wastewater as fuel

3.2 Closed-circuit voltage

A direct effect of the immobilisation system can be easily extracted from the closed circuit orientation where external resistant was applied to the MFC full electrical circuit. Voltage and power curves were established based on the results obtained as shown in Figure 3 and 4. The profile for free cells show a decrease in voltage generated but then rapidly increased which indicates a 'power-overshoot' phenomenon after converted to power density as shown in Figure 4. The 'power-overshoot' phenomenon was not observed using immobilised bioanode based MFC which indicates a stable MFC system.

Power-overshoot was reported in many scenarios. One scenario was observed mainly due to low anodic potential during acclimatisation or biolfilm formation at the anode, which in most cases occurs in free cell based MFC (Zhu et al., 2013). At the lowest potential region will cause decreasing of electron transfer activity. The present results show that at power was linearly increased with the increased of voltage produced, which is not applicable to the reasoning given. In another scenario, if the electron production in the anodic compartment exceeded electron reduction at the cathode, a power overshoot is not generated (Kim et al., 2017). In contrast, since the air-cathode was exposed to uncontrollable air supply which could cause high cathodic potential and lead to power overshoot.

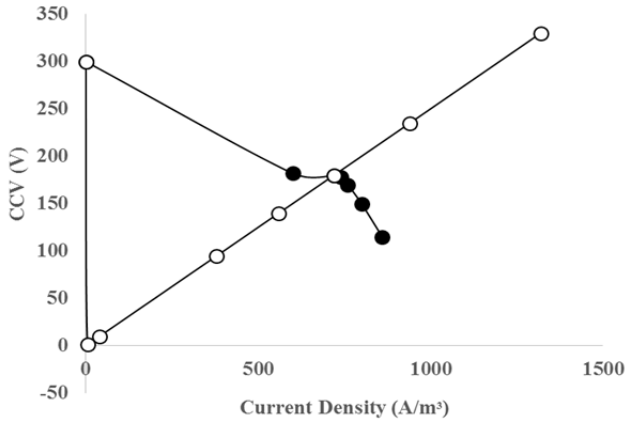

Figure 3: CCV profile. Both free cells (○) and immobilised bio-anode (●) was compared in terms of CCV using different external resistance (50 to 50,000 Ω) using 10 % glucose

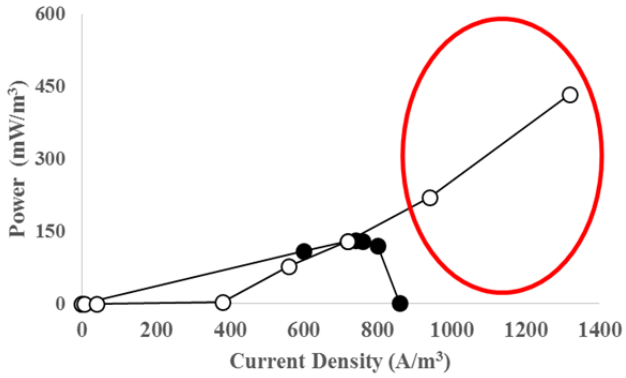

Figure 4: Power curve. Both free cells (○) and immobilised bio-anode (●) was compared in terms of CCV using different external resistance (50 to 50,000 Ω) using 10 % glucose. The red circle indicates power-overshoot.

3.3 Glucose concentration analysis

In addition to the power performance, glucose concentration was monitored by hour basis to link the voltage generation over time. It was observed that glucose concentration decreased after 2 h of MFC operation and stabilised for few hours even the voltage decreased over time (Figure 5). High concentration of glucose probably needed in terms of long last or sustainable power production based on the present results, but higher glucose could be inhibitory to some microorganisms (Borole et al., 2009). The inhibitory effect could well explain the decrease of voltage produced, probably at the same time affecting the electro activity of the microorganisms in the anodic chamber.

The effect of high glucose concentration on the voltage produced was further investigated by adding 5.0 % (w/v) after 24 h of MFC operation. There were no significant changes after adding the glucose solution on the voltage produced as shown in Figure 6 which has probable inhibition effect of excessive glucose on the microbial growth or metabolic activity (Borole, 2009).

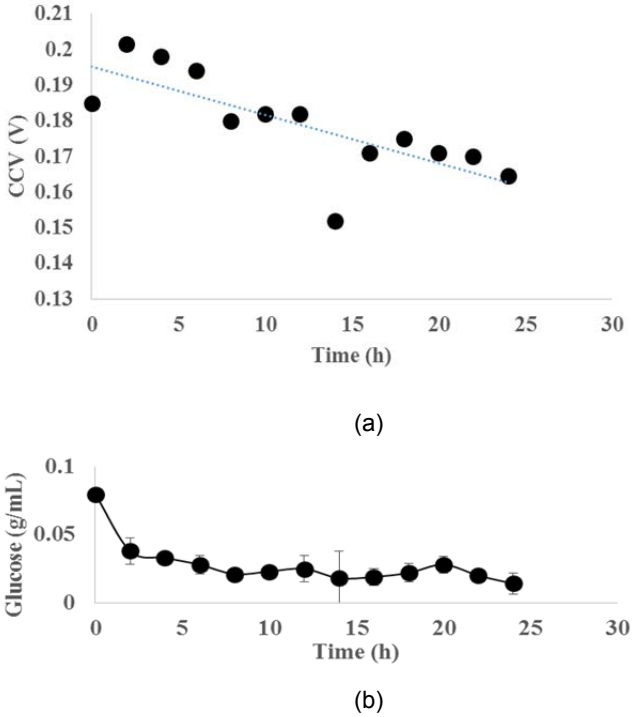

Figure 5: Voltage generation (a) and glucose remained in the anodic compartment (b) during closed circuit less than 1,000 Ω external resistance

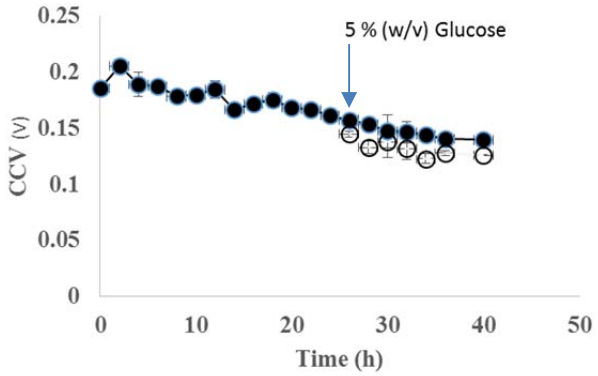

Figure 6: CCV profile with additional 5.0 % (w/v) glucose solution

4. Conclusion

The immobilised CGA developed and shows promising role as a bio-anode for MFC application. Based on the power and voltage curves the usage of immobilised CGA as bioanode could well exited the problem of 'power overshoot' for better MFC performance. Furthermore, immobilised based MFC via glucose concentration effect shows that the voltage produced can be sustained even addition of glucose is added. Further investigations on the inhibitory effect of glucose would be benefit on the basis of sustaining the voltage produced by immobilized CGA.

Acknowledgements

The authors would like to acknowledge the Ministry of Higher Education Malaysia (MOHE) and the Department of Bioprocess and Polymer, Faculty of Chemical and Energy Engineering, Universiti Teknologi

Malaysia (UTM) for the facilities and financial support (University Grant. Q.J130000.2544.10H33 and 2546.14H20).

References

Alferov S.V., Minaicheva P.R., Arlyapov V.A., Asulyan L.D., Alferov V.A., Ponamoreva O.N., Reshetilov A.N., 2014, Bioanode for a microbial fuel cell based on Gluconobacter oxydans immobilised into a polymer matrix, Applied Biochemistry and Microbiology, 50, 637 – 643.

Borole A.P., Mielenz J.R., Vishnivetskaya T.A., Hamilton C. Y., 2009, Controlling accumulation of fermentation inhibitors in biorefinery recycle water using microbial fuel cells, Biotechnology for Biofuels, 2, 7, DOI: 10.1186/1754-6834-2-7.

Bretschger O., Osterstock J.B., Pinchak W.E., Ishii S., Nelson K.E., 2010, Microbial Fuel Cells and Microbial Ecology: Applications in Ruminant Health and Production Research, Microbial Ecology, 59, 415 - 427.

Freeman A., Lilly M.D., 1998, Effect of processing parameters on the feasibility and operational stability of immobilised viable microbial cells, Enzyme and Microbial Technology, 23, 335-345.

Gude V.G., 2016, Wastewater treatment in microbial fuel cells – an overview, Journal of Cleaner Production, 122, 287–307.

Hogson D.M., Smith A., Dahale S., Stratford J.P., Li J.V., Gruning A., Bushell M.E., Marchesi J.R., Rossa C.A., 2016, Segregation of the Anodic Microbial Communities in a Microbial Fuel Cell Cascade, Frontiers in Microbiology, 7, DOI: 10.3389/fmicb.2016.00699.

Junter G.A., Jouenne T., 2004, Immobilised viable microbial cells: from the process to the proteome… or the cart before the horse, Biotechnology Advances, 22, 633-658.

Kim B., An J., Chang I.S., 2017, Elimination of Power Overshoot at Bioanode through Assistance Current in Microbial Fuel Cells, ChemiSusChem: chemistry and sustainability, Energy and Materials, 10, 612 - 617.

Mahmood N.A.N., Ghazali N.F., Ibrahim K.A., Ali M.A., 2017, Anodic pH Evaluation on Performance of Power Generation from Palm Oil Empty Fruit Bunch (EFB) in Dual Chambered Microbial Fuel Cell (MFC), Chemical Engineering Transactions, 56, 1795 – 1800.

Mesran A.H., Mamat S., Pang Y.R., Hong T.Y., Muneera Z., Ghazali N.F., Ali M.A., Mahmood N.A.N., 2014, Preliminary Studies On Immobilised Cells-based Microbial Fuel Cell System On Its Power Generation Performance, Journal of Asian Scientific Research, 4, 428-435.

Schroder U., 2007, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency, Physical Chemistry and Chemical Physics, 9, 2619–2629.

Wagner R.C., Porter-Gill S., Logan B.E., 2012, Immobilisation of anode-attached microbes in a microbial fuel cell, Global Journal of Science Frontier Research, 11, 12–20.

Wang X., Zhang E., Yuc E.H., Scott K., 2016, Low Cost Materials for the Air Cathodes in Single-Chamber Microbial Fuel Cells: A Mini Review, Chemical Engineering Transactions, 51, 37 – 42.

Wei C., Sieng L., Hussaini A., Zulkarnian A., Apun K., Bilung L., Vincent M., 2011, Microorganism-immobilized carbon nanoparticle anode for microbial fuel cells based on direct electron transfer, Applied Microbiology and Biotechnology, 89, 1629 – 1635.

Yuan Y., Zhou S., Xu N., Zhuang L., 2011, Microorganism-immobilised carbon nanoparticle anode for microbial fuel cells based on direct electron transfer, Applied Microbiolology and Biotechnology, 89,1629-35.

Zhang E., Zhai W., Luo Y., Scott K., Wang X., Diao G., 2016, Acclimatisation of microbial consortia to alkaline conditions and enhanced electricity generation, Bioresources Technology, 211, 736 - 742.

Zhu X., Tokash J.C., Hong Y., Logan B.E., 2013, Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials, Bioelectrochemistry, 90, 30 - 35.