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Bioelectricity Generation in Biological Fuel Cell with and Without Mediators

[1]Nader Mokhtarian, [1]Wan Ramli Wan Daud, [2]Mostafa Rahimnejad and [2]Ghasem D. Najafpour

[1]Chemical Engineering Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Selangor Darul Ehsan, Malaysia
[2]Biotechnology Research Lab., Faculty of Chemical Engineering,
Noshirvani University, Babol, Iran

Abstract: As fossil fuel sources are depleted, alternative energy sources are rapidly developing. Renewable energy is much eco-friendly such as biomass which is converted to fuel and energy in many alternative processes. For an alternative energy futuristic, the trends for new alternative renewable energies are gradually increasing. Major efforts were devoted to develop alternative electricity generation processes. Among renewable alternatives, enzymatic fuel cells, microbial fuel cell (MFC) and biological fuel cell (BFC) have created great interests for many researchers due to its possibility of directly harvesting electricity from organic wastes and renewable biomass or other reliable sources. MFC operates under very mild conditions and in wide variable ranges of biodegradable materials used as fuel. The bio-based materials can be oxidized by microorganisms in anode and the biocatalysts have great potential to generate electrons. Biological systems possess number of advantages over the conventional chemical processes. Microbial fuel cell as the newest type of chemical fuel cells is a bioreactor that can generate electricity from what would be considered as organic wastes by means of microorganisms as biocatalysts. In this approach, bioelectricity generation and simultaneous waste treatment may take place in a cell. Therefore, the yield of newly developed system is much higher than any conventional processes. In other hand, direct electron transfers from anaerobic anode chamber to anode surface had shown to take place only at very low efficiency. In MFCs, different substrates and biocatalysts to generate electricity are employed. In this research, *Saccharomyces cerevisiae* (PTCC 5269) was selected for the generation of bioelectricity in a two chamber MFC. Nafion 117 was selected as protons exchange membranes to transfer the generated protons from the anode chamber to the cathode compartment at ambient temperature and pressure. The initial glucose concentration was 30g.l^{-1}. Thionine and neutral red (NR) at several concentrations (50 to 600 μmol.l^{-1}) were utilized as electron shuttle in the anode chamber. Operational performance of the MFC was evaluated with focus on electricity generation. At 200 μmol.l^{-1} neutral red concentration, the maximum obtained voltage, current and power density were 510 mV, 1675 mA.m^{-2} and 186 mW.m^{-2}, respectively. The outer surface of graphite electrode at the end of process was also analyzed by scanning electron microscopy with magnification of 5000. The obtained image demonstrated that microorganisms were uniformly grown on the graphite surface which was accounted for the system high electrical performance.

Key words: Microbial fuel cell · Bioelectricity · Current density · Thionine · Neutral red

INTRODUCTION

The world energy supply nearly 90 percent from fossil fuel is depleted [1]. Consumption of fossil fuel has certainly several disadvantages such as global warming and environment pollution. Knowing energy crisis, researchers are interested to find new alternatives such as renewable energy to overcome energy shortages [2, 3]. The new sources of energy should be renewable and also environmental friendly such as fuel cells [4]. A fuel cell is an electrochemical engine that converts chemical energy of a fuel, such as hydrogen and an oxidant, such as oxygen, directly into electricity [5]. Biological fuel cells (BFCs) are a subset of fuel cells which employ active biocatalysts for production of bioelectricity instead of expensive metal oxide catalysts used in conventional fuel cells. The distinct types of BFCs are defined based on type of biocatalyst used in anode compartment.

Corresponding Authors: Dr. Wan Ramli Wan Daud, Chemical Engineering Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia. E-mail: wramli@eng.ukm.my.

Microbial fuel cells (MFCs) employ living cells to catalyze the oxidation of organic substances, whereas enzymatic fuel cells use active enzymes for the same purpose [6, 7]. MFCs have been considered as an alternatives to conventional batteries for point source electricity generation [8]. The main advantage of MFCs is that they typically have long lifetimes (up to five years) [9, 10] and are completely capable to oxidize simple carbohydrate to carbon dioxide [11].

Bacteria have recently been introduced as key catalysts in MFCs. Therefore, improvement of biocatalyst on electrode was extensively investigated from the perspective relation with other features; increase electrode surface area by brush carbon electrode [12], activation of electrode surface by chemical treatment (e.g. ammonia) [13]; active selection of electrogenic species [14]. The combined electron transport mechanisms between bacterial cell membrane and electrode surface is believed to be a rate limiting factor which determines the whole MFC system performance. The bacterial electron transfer mechanisms reported so far are; direct electron transfer from outer cell membrane to electrode; electrically conductive nanowire [15, 16]; electron shuttles using externally added or self produced chemicals (e.g. Pyocyanin from *Pseudomonas aeruginosa)* [17, 18].

Mediator Microbial Fuel Cell: Mediators are artificial compounds produced by the microorganism itself. Most of the microbial cells are electrochemically inactive. The electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, neutral red and other electron shuttles. Most of the available mediators are relatively expensive and slightly toxic. Therefore trace amount of mediators may be recommended to enhance the electron transfer from the biocatalysts outer surface to active anode surface area immersed in the microbial culture. The low concentration of mediators may not have inverse effect on biocatalysts activities. The presences of artificial electron mediators are essential to improve the performance of MFCs and BFCs.

Mediator-Less Microbial Fuel Cell: Recently mediators less BFCs are attached more researchers. A mediator-less microbial fuel cell does not require a mediator but uses electrochemically active bacteria to transfer electrons to the electrode (electrons are directly carried from the bacterial respiratory enzyme to the electrode). Some microorganisms produce nanowires to transmit electrons directly without using any mediator but other organisms need to add artificial electron shuttle into anode chamber. Among the electrochemically active bacteria are *Shewanella putrefaciens*, *Aeromonas hydrophila* and many more biocatalysts. Some bacteria, which have pili on their external membrane, are able to transfer their electron production via these pili. Mediator-less microbial fuel cells can run even on wastewater, also derived energy directly from certain aquatic plants. These include reed sweet grass, cord grass, rice, tomatoes, lupines and algae. These microbial fuel cells are called "Plant Microbial Fuel Cells" (Plant-MFC). Given that the power is thus derived from a living plant (in situ-energy production), this variant can provide extra ecological advantages.

Applications of MFCs: Generally MFCs have a wide range of application. Sustainability of MFCs demonstrated that even low voltage but stable power is generated. Long term application of MFC has provided great satisfaction, since small amount of power was generated. Bacteria show low metabolic activities when inhibited by toxic compounds generated from the catabolism of organic compounds. This phenomena may cause a low electron transfer towards electrode. By the aid of MFCs biosensors could be constructed, in which bacteria are immobilized on an electrode and protected behind a membrane. If a toxic component diffuses through the membrane, this can be detected by the changes in potential over the sensor. Such sensors could be extremely useful as indicators of toxicants in rivers, at the entrance of wastewater treatment plants, to detect pollution or illegal dumping of toxic wastes, or to conduct research on polluted sites.

Type of Substrates Used in MFCs: Biological fuel cells directly convert chemical energy present in carbohydrates including sugar and alcohol to electrical energy. During natural microbial catabolism, a substance such as carbohydrate is primarily oxidized in absence of oxygen and electrons are absorbed by active bacteria that in turn produce intermediates. The reaction is as follows:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \; E^* = 0.014v \quad (1)$$

$$\text{(Mediator) Oxid} + e^- \rightarrow \text{(Mediator) Red} \quad (2)$$

In absence of oxygen, electrons are deviated toward the electrode and pass through an external circuit then react with molecular oxygen present in cathodic chamber (Equation 3):

Table 1: Microorganisms, substrates and mediators reported in MFCs implementations for the enhancements of power generation.

Microorganism	Substrate	Mediators	Reference
Actinobacillus succinogenes	Glucose	Neutral Red, Thionin	[30, 31]
Erwinia dissolven	Glucose	Fe (III) EDTA	[32]
Gluconobacter oxydans	Glucose	HNQ, resazurin, Thionine	[33]
Shewanella putrefaciens	Lactate, Pyruvate, Acetate	Neutral Red	[34]
Streptococcus lactis	Glucose	Ferric chelate complex	[35]
Escherichia coli	Glucose, Acetate	Neutral Red, 2-Hydroxy-1,4-Naphthoquinone, Methylene blue	[21, 35-39]
Proteus vulgaris	Glucose, Sucrose	Thionine	[40-42]
Enterococcus faecium	Glucose	Pyocyanin	[43]
Micrococcus luteus	Glucose	Thionin	[23]
Saccharomyces cerevisiae	Hydrolyzed Lactose	Methylene blue, Neutral Red	[27]

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O\ E^* = 1{,}23v \quad (3)$$

Glucose is the most common substrate utilized in MFCs. Conversion of one mole of glucose to carbon dioxide releases 24 electrons:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad (4)$$

Therefore, 2.32×10^6 colon electricity load per mole of glucose enters the electrical cycle. However, it must be considered that the current obtained by a MFC depends on the rate of oxidation and electron transmission efficiency to electrodes.

Power Generation in MFC: Power generation in an MFC is affected by many influential parameters including bioreactor system configuration, type of microorganism, organic substrates known as biomass fuel types, concentration, ionic strength, pH and temperature. MFCs are especially suitable for powering small systems and wireless sensors that have only low power requirements to transmit signals such as temperature to receivers in remote locations. MFCs themselves can serve as distributed power systems for local uses, especially in underdeveloped regions of the world. MFCs can also be readily modified to produce hydrogen instead of electricity in wastewater treatment plant. The objective of this paper was to exhibit the mechanism and effect of electron shuttle in MFCs and BFCs performances for bioelectricity generation.

A significant improvement in cell current has been observed with addition of electrochemical mediators that facilitate the electron transfer between bacteria and electrode [17, 19, 20]. Typically, MFCs performances have been enhanced by addition of electron shuttles with e.g. *Shewanella* and *Pseudomonas*; in particular gram-positive bacteria, *Escherichia coli and Bacillus*, which were otherwise unable to transfer electrons from their internal electron transport chain to outer electron acceptor. Electron mediator which has a redox potential close to that of $NADH/NAD^+$ can facilitate electron shuttling between the reaction center inside the cell and terminal electron acceptor (anode electrode). Several exogeneous electron mediators such as methyl viologen, methylen blue, neutral red and thionin have been used in MFCs (Table 1). Park and Zeikus [21] have shown the interactions between bacterial cultures and electron mediators exist. Incorporation of mediators onto the graphite electrode increased power output by 10-fold; because of the facilitated interaction of these mediators with NAD^+ [22-24]. The soluble redox mediators used in MFCs for the improvement of electron transfer are summarized in Table 1.

In this paper, we demonstrated the yeast, *S. cerevisiae*, which was previously known as inactive exoelectrogenically, can produce electricity in an MFC and power may be enhanced by mediators such as thionin and neutral red. *S. cerevisiae* has not been practiced in MFCs as it's electrogenic activity was considered too low to be implemented in a MFC.

MATERIALS AND METHODS

Saccharomyces cerevisiae PTCC 5269 was supplied by Iranian Research Organization for Science and Technology (Tehran, Iran). The microorganisms were grown at anaerobic condition in an anaerobic jar. The prepared medium for seed culture consisted of glucose, yeast extract, NH_4Cl, NaH_2PO_4, $MgSO_4$ and $MnSO_4$: 10, 3, 0.2, 0.6, 0.2 and 0.05 $g.l^{-1}$, respectively. The medium was sterilized, autoclaved at 121°C and 15psig for 20 min.

The medium pH was initially adjusted to 6.5 and the inoculums were introduced into the media at ambient temperature. The inoculated cultures were incubated at 30°C. The bacteria were fully grown in a 100ml flask

without any agitation for the duration of 24 hours. Substrate consumption was calculated base on determination of remained sugars in the culture.

All chemicals and reagents used for the experiments were analytical grades and supplied by Merck (Darmstadt, Germany). The pH meter, HANA 211(Romania) model glass-electrode was employed to measure pH values of the aqueous phase. The initial pH of the media was adjusted by addition of diluted HNO_3 or 0.1M NaOH solutions.

The gold plated samples were observed with a Scanning Electronic Microscope (SEM) (Phillips XL30, Holland). Finally, images of the samples were taken under SEM at magnifications of 500 and 5000. SEM images were used to demonstrate the physical characteristics of the electrode surface.

The glass (Pyrex) material was used for the fabrication of laboratory scale MFC. The volume of each chamber (anode and cathode chambers) was 850 ml with a working volume of 760 ml. The sample port was provided for anode chamber, wire point input and inlet port. The selected electrodes in MFC were graphite plates, size of 40×70×2*mm*.. Table 2 shows a list of components and materials used for the fabrication of stacked MFCs. Proton exchange membrane (PEM; Nafion 117, Sigma-Aldrich) was used to separate two compartments. The Nafion area separated two chambers was 3.79 cm^2. Nafion, proton exchange membrane was subjected to a course of pretreatment to take off any impurities that was boiling the film for 1h in 3% H_2O_2, washed with deionized water, 0.5 M H_2SO_4 and finally washed again with deionized water. In order to maintain membrane for good conductivity, the cell anode and cathode compartments were filled with deionized water when the microbial fuel cell was not in use. Mediators such as MB, NR, Ferric chelate complex and thionine were supplied by Merck (Darmstadt, Germany). These chemicals in MFC at very low concentrations (100 to 500 $\mu mol.l^{-1}$) were used as mediators. The schematic diagram and auxiliary equipments of the fabricated MFC cell is shown in Fig. 1.

The performance of the MFC system was evaluated by polarization curve. Polarization curve was obtained by an external resistance. Power and current were calculated based on following equations:

$$P=I*E \quad (5)$$

$$I=(E/R_{ext}) \quad (6)$$

Where P is the generated power; E is measured cell voltage; R_{ext} is external resistance; I is produced current.

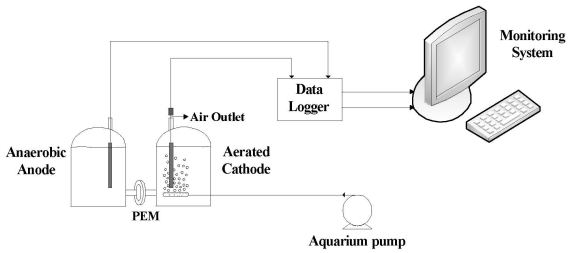


Fig. 1: Schematic diagram of fabricated MFC with the auxiliary equipments.

The produced current and power normalized by the surface area of the anode electrode. Analog digital data acquisition was fabricated to record data point in every 4min. The system had measurements for variable resistances which were imposed to the MFC. The current in MFC was recorded and divided by the obtained voltage using the defined resistance. Then, the system provides power calculation by multiplication of voltage and current. Also the online system demonstrates polarization graphs for power generation and MFC voltage with respect to current. The online system had the ability to operate automatically or manually. While it operates in auto-mode, the assembled relays were able to regulate automatically the resistances. Voltage of MFC was amplified and then data were transmitted to a microcontroller by an accurate analog to digital converter. The microcontroller was also able to send the primary data to a computer by serial connection. In addition, a special function of MATLAB software (7.4, 2007a) was used to store and synchronically display the obtained data.

Columbic efficiency (CE) can be calculated by division of total coulombs obtained from the cell and theoretical amount of coulombs that can be produced from glucose; resulted as Equation 7:

$$CE= (C_p/C_T)\times 100 \quad (7)$$

Total coulombs obtained by integrating the current over time (C_p), where C_T is theoretical amount of coulombs that can be produced from carbon source, calculated as follows:

$$C_T= (FbSV.M^{-1}) \quad (8)$$

In Equation 8, *F* is Faraday's constant, b is the number of moles of electrons produced per mole of substrate (24 moles of electron were produced per mole of

Table 2: Basic component was used for dual chamber MFC.

Item	Materials	Company
Anode electrodes	Graphite plate	ENTEGRIS, INC. FCBLK-508305-00004, USA
Cathode electrodes	Graphite plate	ENTEGRIS, INC. FCBLK-508305-00004, USA
Anode Chambers	Plexiglas	Neonperse, Iran
Cathode chambers	Plexiglas	Neonperse, Iran
Proton exchange Membranes	Nafion 117	Sigma alderich, USA
Connection the cells	Copper wire	Khazar Electric, Iran

Table 3: Produced power density, current density and voltage at various concentration of thionine as electron mediators.

Optimum concentration (μ mol.l^{-1})	Pmax(mW.m^{-2})	I max (mA.m^{-2})	OCV at S.S. condition(mV)
200	30	500	380
300	38	512	419
400	47	610	425
500	60	688	452
600	60.4	689	453

Table 4: Produced power density, current density and voltage at various concentration of NR as electron mediators.

Optimum concentration (μ mol.l^{-1})	Pmax(mW.m^{-2})	I max (mA.m^{-2})	OCV at S.S. condition(mV)
50	53	810	390
100	108	870	452
150	142	1100	460
200	186	1675	510
250	187	1676	511

glucose oxidation in anaerobic anode chamber), S is substrate concentration and M is molecular weight of the used substrate (M= 180.155 g.mol^{-1}) [25, 26].

RESULTS AND DISCUSSION

The performance of the microbial fuel cell was evaluated by the polarization curve and power density. The main goal of research was to operate MFC and enhance output power and receive maximum generated current under optimum potential condition. Fig. 2a shows polarization curve and power density for a mediator less MFC.

Mediators are normally used to enhance the performance of MFCs [27]. Mediators are artificial compounds or produced by microorganism itself. Some microorganisms produce nanowires to transmit electrons directly without using any mediator but other organisms need to add artificial electron shuttle into anode chamber [28]. Yeasts are not able to transfer the produced electrons to the anode surface without addition of mediators. At first, thionine with 200 μmol.l^{-1} concentration was selected as mediator to enhance the power generation. Power and current were enhanced to 16 mW.m^{-2} and 250 mA.m^{-2}, respectively (Fig. 2b).

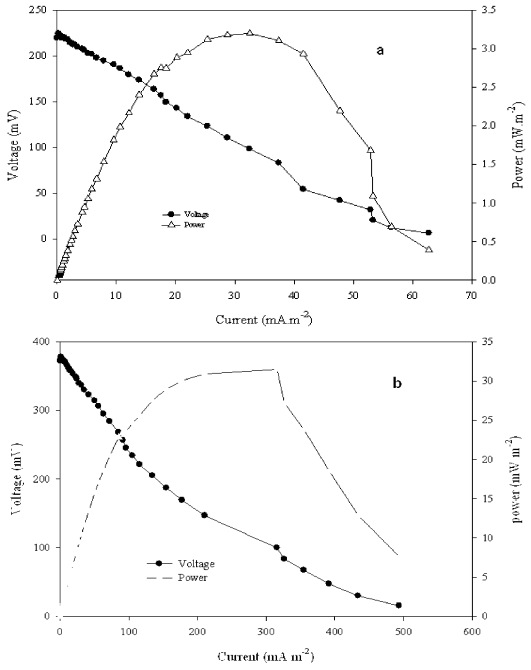


Fig. 2: Effect of mediators on polarization curve. (a) without mediators,(b) 200 μmol.l^{-1} thionine as mediators in anode chamber.

Thionine with 200 μmol.l^{-1} concentration enhanced generated power in MFC (Fig. 2b). In order to obtain the best concentration of thionine as mediators in anode chamber, when the other factors were constant in the MFC; the concentration of thionine was stepwise increased. Four different concentrations of thionine

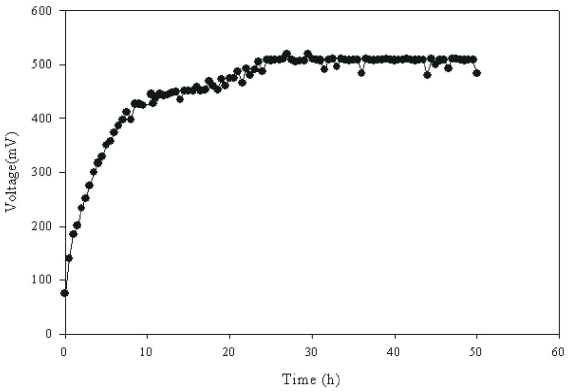


Fig. 3: Open circuit voltage produced in a dual chamber MFC using S. cerevisiae as the active biocatalyst and 200µmol.l-1 NR as mediators.

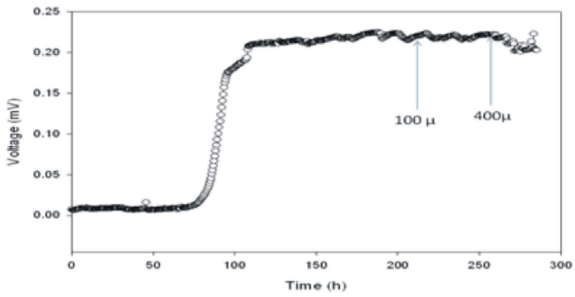


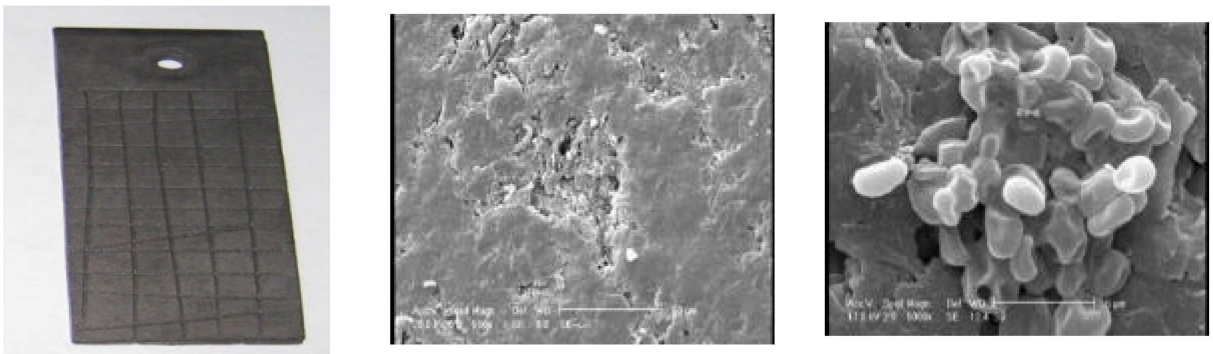
Fig. 4: Effect of mediators in mixed culture of microorganisms on MFC. Close circuit potential of MFC (Rext= 1000 Ω). Swime waste water was used as inoculums NR added to anode chamber after steady sate condition.

(300, 400, 500 and 600 µmol.l^{-1}) were examined and data are presented in Table 3. Maximum power and current obtained at thionine concentration of 600 µmo.l^{-1}.

NR was also chosen as mediators. Several concentrations of NR as mediator were experimented in the MFC to obtain optimum concentration of the artificial electron shuttle. Table 4 shows the effect of electron mediators (NR concentrations) on power and open circuit voltage (OCV). At the optimum concentration of the mediator (200 µmol.l^{-1} NR), maximum obtained power and current were 186 mW.m^{-2} and 1675 mA.m^{-2}, respectively. These achieved power and current were less than of the obtained power and current for NR as mediators at optimum concentration.

Data for the open circuit voltage at the optimum concentration of mediators were recorded by online data acquisition system computer aided and interfaced with the MFC. The recorded data for the duration of 50 hours of operation time are illustrated in Fig. 3. At the starting point for the experimental run, the voltage was less than 100mV and then the voltage gradually increased. After 22 hours of operation, the OCV reached to a maximum value and the stable value was about 500mV. The obtained OCV was stable for the entire operation time. Then 24 moles of electrons and protons are produced by the consumption of one mole of glucose in an anodic chamber at anaerobic condition. For determination of CE, set 500Ω resistance at external circuit for 25 hours and the produced current was measured. The average value for the obtained current was 218.3 mA.m^{-2}. In this study, the CE was calculated based on Equations 7 and 8. The value of CE at optimum concentration of NR as mediator was 24.5 %.

Another step of the experiment, anaerobic activated sludge was selected as mixed culture of active biocatalyst in dual chamber of MFC. Two different concentration of NR was added at different operation time in the anode compartment. The generated power by the MFC did not increase by addition of mediators (Fig. 4).



(A) (B) (C)

Fig. 5: Attached microorganisms on anode surface at the end of period. Scanning Electron Microscopy of the biofilm observed on the surface of anode electrode with magnification of 500 and 5000.

That was probably due to nature of existing mixed culture microorganisms; as the consortium of living organisms was able to produce exogenous mediators or nanowire to transfer of produced electrons to anode surfaces [27, 29]. Maximum produced power and current without any electron shuttle in the anode chamber was 4 $mW.m^{-2}$ and 72 $mA.m^{-2}$, respectively. At concentration of 200 $\mu mol.l^{-1}$neutral red, the obtained voltage, current and power densities were 510 mV, 1,600 $mA.m^{-2}$ and 190 $mW.m^{-2}$, respectively.

Graphite was used as electrode in the MFC fabricated cells. The normal photographic image of the used electrode before employing in the MFC as anode compartment is shown in Fig. 5a. Scanning electronic microscopy technique has been applied to provide surface criteria and morphological information of the anode surface. The surface images of the graphite plate electrode were successfully obtained by SEM. The image from the surface of graphite electrode before and after experimental run was taken. The sample specimen size was 1×1cm for SEM analysis. Figs. 5b and 5c show the outer surface of the graphite electrode prior and after use in the MFC, respectively. These obtained images demonstrated that microorganisms were grown on the graphite surface as attached biofilm. Some clusters of microorganism growth were observed in several places on the anode surface.

CONCLUSION

A MFC was designed, fabricated and operated successfully for bioelectricity production. The system used pure glucose as substrate at concentration of $30g.l^{-1}$. The gram positive microorganism, *S. cerevisiae* and anaerobic activated sludge were incorporated in MFC as biocatalyst. The maximum generated power density without addition of any mediators in anode chamber was very low (3.2 $mW.m^{-2}$). In order to achieve the maximum power density in MFC, thionin and NR as electrons mediators with several concentrations were examined in anaerobic anode compartment. The obtained results indicated that NR with concentration of 200 $\mu mol.l^{-1}$ maximum produced power up to 186 $mW.m^{-2}$.

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REFERENCES

1. Logan, B., B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey, 2006. Microbial Fuel Cells: Methodology and Technology†. Enviromental Scence and Technology. 40(17): 5181-5192.
2. Lovley. D., 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Current Opinion in Biotechnology. 17(3): 327-332.
3. Strik,D., H. Terlouw, H. Hamelers and C. Buisman, 2008. Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC). Applied Microbiology and Biotechnology. 81(4): 659-668.
4. Wen, Q., Y. Wu, D. Cao, L. Zhao and Q. Sun, 2009. Electricity generation and modeling of microbial fuel cell from continuous beer brewery wastewater. Bioresource Technology. 100(18): 4171-4175.
5. Steele B.C.H. and A. Heinzel, 2001. Materials for fuel-cell technologies. Nature. 414(6861): 345-352.
6. Minteer, S.D., B.Y. Liaw and M.J. Cooney, 2007. Enzyme-based biofuel cells. Current Opinion in Biotechnology. 18(3): 228-234.
7. Rahimnejad, M., N. Mokhtarian, G. Najafpour, W. Daud and A. Ghoreyshi, 2009. Low voltage power generation in a biofuel cell using anaerobic cultures. World Applied Sciences Journal. 6(11): 1585-1588.
8. Lyon, D.Y., F. Buret,T.M. Vogel and J.M. Monier, Is resistance futile? Changing external resistance does not improve microbial fuel cell performance. Bioelectrochemistry. 78(1): 2-7.
9. Moon, H., I. Chang and B. Kim, 2006. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresource Technology. 97(4): 621-627.
10. Kim, B.H., I.S. Chang, G. Cheol Gil, H.S. Park and H.J. Kim, 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. Biotechnology Letters. 25(7): 541-545.
11. Bond, D.R. and D.R. Lovley, 2005. Evidence for involvement of an electron shuttle in electricity generation by Geothrix fermentans. Applied and Environmental Microbiology. 71(4): 2186.
12. Logan, B., S. Cheng, V. Watson and G. Estadt, 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environmental Science and Technology. 41(9): 3341-3346.

13. Cheng, S. and B.E. Logan, 2007. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochemistry Communications. 9(3): 492-496.
14. Kim, J.R., B. Min and B.E. Logan, 2005. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Applied Microbiology and Biotechnology. 68(1): 23-30.
15. Gorby, Y.A., T.J. Beveridge and W.R. Wiley, 2005. Composition, reactivity and regulation of extracellular metal-reducing structures (nanowires) produced by dissimilatory metal reducing bacteria. Pacific Northwest National Laboratory (PNNL), Richland, WA.
16. Reguera, G., K.D. McCarthy, T. Mehta, J.S. Nicoll, M.T. Tuominen and D.R. Lovley, 2005. Extracellular electron transfer via microbial nanowires. Nature. 435(7045): 1098-101.
17. Rabaey, K., N. Boon, M. Hofte and W. Verstraete, 2005. Microbial phenazine production enhances electron transfer in biofuel cells. Environmental Science & Technology. 39(9): 3401-8.
18. Rabaey, K. N. Boon, S. Siciliano, M. Verhaege and W. Verstraete, 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Applied and Environmental Microbiology. 70 (9): 5373.
19. Allen, R.M. and H.P. Bennetto, 1993. Microbial fuel cells: electricity production from carbohydrates. Applied Biochemistry and Biotechnology. 39: 27-40.
20. Park, D.H. and J.G. Zeikus, 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. Applied and Environmental Microbiology. 66(4): 1292-7.
21. Park, D. and J. Zeikus, 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. Applied and Environmental Microbiology. 66(4): 1292.
22. Park, D.H. and J.G. Zeikus, 2003. Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnology and Bioengineering. 81(3): 348-355.
23. Choi, Y., E. Jung, H. Park, S. Jung and S. Kim, 2007. Effect of initial carbon sources on the performance of a microbial fuel cell containing environmental microorganism micrococcus luteus. Notes. 28(9): 1591.
24. Ringeisen, B.R., E. Henderson, P.K. Wu, J. Pietron, R. Ray, B. Little, J.C. Biffinger and J.M. Jones-Meehan, 2006. High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. Environmental Science & Technology. 40(8): 2629-2634.
25. Park, D. and J. Zeikus, 2002. Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnology and Bioengineering. 81(3): 348-355.
26. Park, D. and J. Zeikus, 1999. Utilization of electrically reduced neutral red by Actinobacillus succinogenes: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. Journal of Bacteriology. 181(8): 2403.
27. Vega, C. and I. Fernández, 1987. Mediating effect of ferric chelate compounds in microbial fuel cells with Lactobacillus plantarum, Streptococcus lactis and Erwinia dissolvens. Bioelectrochemistry and Bioenergetics. 17(2): 217-222.
28. Lee, S., Y. Choi, S. Jung and S. Kim, 2002. Effect of initial carbon sources on the electrochemical detection of glucose by Gluconobacter oxydans. Bioelectrochemistry. 57(2): 173-178.
29. Park, D. and J. Zeikus, 2002. Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens. Applied Microbiology and Biotechnology. 59(1): 58-61.
30. Grzebyk, M. and G. Pozniak, 2005. Microbial fuel cells (MFCs) with interpolymer cation exchange membranes. Separation and Purification Technology. 41(3): 321-328.
31. Bennetto, H., 1990. Electricity generation by microorganisms. Biotechnology. 1(4): 163-168.
32. Park, D., S. Kim, I. Shin and Y. Jeong, 2000. Electricity production in biofuel cell using modified graphite electrode with neutral red. Biotechnology Letters. 22(16): 1301-1304.
33. Schröder, U., J. Nießen and F. Scholz, 2003. A generation of microbial fuel cell with current outputs boosted by more than one order of magnitude (Angew Chem Int Ed 42: 2880-2883). Angew. Chem., 115: 2986-2989.
34. Ieropoulos, I., J. Greenman, C. Melhuish and J. Hart, 2005. Comparative study of three types of microbial fuel cell. Enzyme and Microbial Technology. 37(2): 238-245.

35. Bennetto, H., G. Delaney, J. Mason, S. Roller, J. Stirling and C. Thurston, 1985. The sucrose fuel cell: efficient biomass conversion using a microbial catalyst. Biotechnology Letters. 7(10): 699-704.
36. Thurston, C., H. Bennetto, G. Delaney, J. Mason, S. Roller and J. Stirling, 1985. Glucose metabolism in a microbial fuel cell. Stoichiometry of product formation in a thionine-mediated Proteus vulgaris fuel cell and its relation to coulombic yields. Microbiology. 131(6): 1393-1399.
37. Shin, S., Y. Choi, S. Na, S. Jung and S. Kim, 2006. Development of bipolar plate stack type microbial fuel cells. Bulletin-Korean Chemical Society. 27(2): 281.
38. Rabaey, K., N. Boon, M. Hofte and W. Verstraete, 2005. Microbial phenazine production enhances electron transfer in biofuel cells. Environ. Sci. Technol., 39(9): 3401-3408.
39. Najafpour, G., M. Rahimnejad, N. Mokhtarian, W. Daud and A. Ghoreyshi, Bioconversion of Whey to Electrical Energy in a Biofuel Cell Using *Saccharomyces Cerevisiae*. World Applied Sciences Journal (Special Issue of Biotechnology & Genetic Engineering)., 1-5.
40. Oh, S.E. and B.E. Logan, 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. Applied Microbiology and Biotechnology. 70(2): 162-169.
41. Allen, R. and H. Bennetto, 1993. Microbial fuel-cells. Applied Biochemistry and Biotechnology. 39(1): 27-40.
42. Mathuriya, A. and V. Sharma, 2009. Bioelectricity production from paper industry waste using a microbial fuel cell by Clostridium species. J. Biochem Tech., 1(2): 49-52.
43. Rahimnejad, M., N. Mokhtarian, G. Najafpour, A. Ghoreyshi and W. Dahud, 2009. Effective Parameters on Performance of Microbial Fuel Cell. IEEE. 3(2): 432-438..