Pak. J. Biotechnol. 10 (2) 37 -51 (2013) ISSN 1812- 1837

INVESTIGATION OF DIFFERENT MEDIATORS IN MICROBIAL FUEL CELL WITH CYCLIC VOLTAMMETER

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ABSTRACT
Microbial fuel cells (MFCs) are new bioprocess method for conversion of chemical energy in organic matters to bioelectricity. In some MFCs produced electrons can travels to anode surface directly and need some artificial component to transfer the produced electron to anode surface. This paper tries to highlight different mediators that have been used as electron mediators in the MFCs in recent years. In this research study, *Escherichia coli* ATCC 8739 was used for power production in an anaerobic condition. Methylene blue, thionine, humic acid, potassium fericyanide and neutral red at different concentrations (50 to 500μmoll^{-1}) are selected and analyzed with several concentrations as mediators. Cyclic voltammetry use to study of anodic electrochemistry analysis. All experiment carry out in aerobic an anaerobic condition but at aerobic condition did not obtain significance peak. The obtained results demonstrate that NR has better ability for transfer of electron in this study.

Keywords: Microbial fuel cell; mediators; oxidation and reduction; cyclic voltametry

INTRODUCTION

Fossils fuels are depleting in the world and also this kind of fuels have a lot of disadvantage [1]. Researchers know this phenomena and working to find new alternatives such as renewable energy to overcome energy crisis in future [2, 3]. Microbial fuel cells (MFCs), a new technology utilizing microorganisms as active biocatalyst to mediate direct conversion of chemical energy stored in organic, inorganic matters or bulk biomass into electrical energy has gained considerable interest among researchers in recently years due to energy crisis [4]. Operating on mild conditions and wide variable ranges of biodegradable material as fuel that can be oxidized by the microorganisms are the potential advantages of biological systems over the conventional chemical systems [5]. In MFCs, microorganisms can use different substrate in anaerobic anode chamber and produce electrons and protons [6, 7].

Protons were transferred to cathode chamber through proton exchange membrane or salt bridge system [8-10]. Electron transfer mechanisms divided MFCs in two categories (mediator and mediator less MFC) [11].
Transfer electron directly from anode media to anode electrode surface had shown to happen only at low efficiency [12, 13]. Sometimes you need to add artificial electron transfer in anode compartment to enhance the proficiency of MFCs. A lot of artificial electron mediators are mentioned in the literature such as thionine, methylene blue, neutral red, ferricyanide, methyl viologenor humic acid [12, 14]. Some used mediators in MFCs were summarized in Table 1. Researchers showed that adding artificial electron mediators improve performances of MFCs. But some of MFCs do not need any mediators for production of bioelectricity such as MFCs works with *Rhodoferax ferrireducens* [15], *Geobacter sulfurreducens[15]* and *Clostridium butyricum* [16] do not need any artificial electron mediators for transfer of produced electron to anode electrode.

Table- 1: Mediators was used in several researchers as electron shuttle

Mediators	Substrate	Microorganism	Reference
Neutral Red, Thionine	Glucose	*Actinobacillus succinogenes*	[27, 28]
Fe (III) EDTA	Glucose	*Erwinia dissolven*	[29]
Methylene blue	Glucose	*Saccharomyces cerevisiae*	[30]
HNQ, resazurin, Thionine	Glucose	*Gluconobacter oxydans*	[31]
Methylene blue, Neutral Red, Thionine	Glucose	*Saccharomyces cerevisiae*	[21, 32]
Neutral Red	Lactate, pyruvate, acetate	*Shewanella putrefaciens*	[28]
Neutral Red, 2-Hydroxy-1,4-Naphthoquinone, Methylene blue	Glucose, acetate	*Escherichia coli*	[14, 20, 22, 24, 33]
Thioninee	Glucose, Sucrose	*Proteus vulgaris*	[22]
Methylene blue, Neutral Red	Hydrolyzed Lactose	*Saccharomyces cerevisiae*	[34]
Humic acid	Xylose	*Domestic waste water*	[35]
Anthraquinone-2,6-disulfonate (AQDS)	Lactate	*Shewanella oneidensis*	[36]
Ferric chelate complex	Glucose	*Streptococcus lactis*	[29]

Different kind of voltametry methods have been developed and used for electrochemical reactions. This technique always was used to examine and characterize the electron transfer from microorganisms, biofilms and mediators from aqueous phase in anode chamber to anode surface [17]. In electrochemical technique, different potential is imposed upon to electrodes and resulting current is analyzed with electrochemical instruments. Cyclic voltammetry (CV) is famous method in electro chemical analysis [18].

The main objective of the present study was to investigate the role of different mediators on transfer of electrons in anodic chamber of MFC by using of cyclic voltammetry methods. We also consider the effect of microorganisms' concentration and time incubation period on oxidation and reduction peak in this research.

MATERIALS AND METHODS

Escherichia coli ATCC 8739 (*E.coli*) was supplied from Assiut University Mycological Center, Assiut Egypt. The microorganisms were grown at anaerobic condition in an anaerobic jar vessel. 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 gl^{-1}, respectively. The medium was sterilized, autoclaved at 121°C and 15psig for 20 min.

The medium pH was initially adjusted to 7 and the inoculums were inoculated into the media at ambient temperature and pressure. The inoculated cultures were incubated at 30°C. The bacteria were fully grown in a 100 ml flux with agitation 150 rpm for the duration of 24 hours.

All chemicals and reagents used for the experiments were analytical grades and supplied by Dae Jung (Gyonggi-do, Korea). The pH meter, ORION 410 A (Boston, MA0212, USA) model glass-electrode was employed to measure pH values of the aqueous phase. The initial pH of the working solution was adjusted by addition of diluted HCl or 0.1M NaOH solutions. Sodium acetate trihydrate (Junsei Chemical Co., Japan) was used as electron donors in this study. The media for growing of microorganisms and cyclic volatamerty measurement contain Na_2HPO_4, NaH_2PO_4, NH_4Cl, KCl: 4.33, 2.69, 0.31, 0.13 gl^{-1}, respectively. Nutrient mineral buffer (NMB) solution 100 times contained of $MgSO_4$, NTA, NaCl, $MnSO_4.H_2O$, $ZnCl_2$, $CaCl_2$ $2H_2O$, $CoCl_2.6H_2O$, $FeSO_4$.7H2O, $AlK(SO_4)_2.12H_2O$, $CuSO_4$. $5H_2O$, H_3BO_3, $Na_2WO_4.2H_2O$, $NiCl_2.6H_2O$: 3, 1.5, 1, 0.5, 0.13, 0.1, 0.1, 0.1, 0.01, 0.01, 0.01, 0.025, 0.025, 0.024 gl^{-1}, respectively.

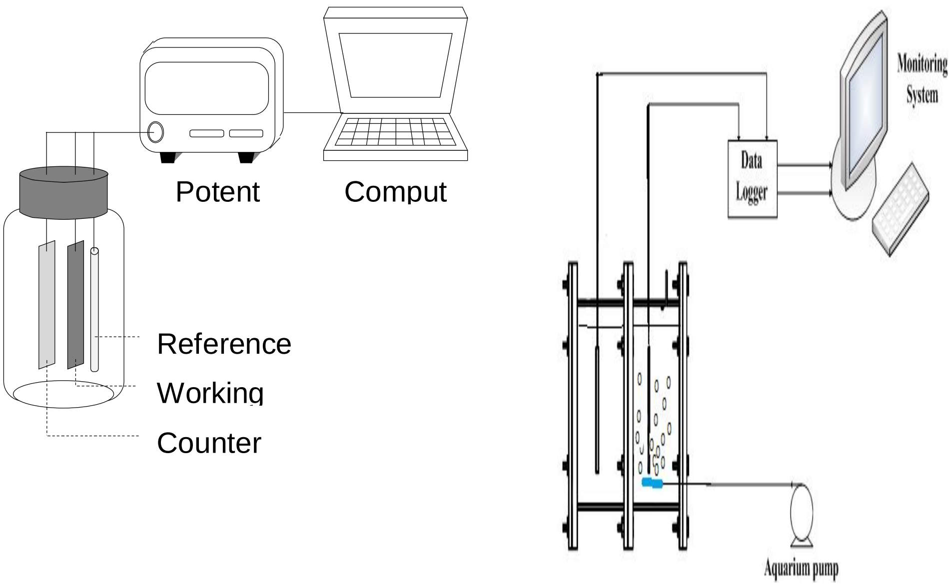
Centrifuge (HANIL MICRO 12, Korea) was used to analyze the effect of active biocatalyst concentration on MFC performances and oxidation and reduction peaks. Vortex mixture (Vortex Genie 2, Scientific Industries, USA) was used to suspension of cell plate after centrifuge.

Pure nitrogen gas was injected into the bottle to remove dissolved oxygen and keep anaerobic condition. Magnetic stirrer was used in the bottle to keep the identical condition used system. Aquarium pump was used for aeration in the bottle with slow flow rate.

Five different famous kinds of mediators was selected and analyzed as electron transfer in anode chamber. Methelene Blue (MB), Neutral Red (NR), humic acid, potassium fericyanide and Thionine was supplied by Merck (Darmstadt, Germany). In

MFC, these chemicals at very low concentrations (50 to 500) µmoll^{-1} were used as mediators.

IVUM soft (Ivium Technology, Netherland) was used to analyze cyclic voltametery. CV was used for oxidation and reduction test. -400 mV to 1000 mV potential range with scan rate of 400mV/s was applied to obtain CV results. Working electrode and sense electrode were jointed together for measurements of all oxidation and reduction peaks. Three electrodes were used to obtained oxidation and reduction peaks. Carbon paper (NARA, Guro-GU, Seoul, Korea) was used as working electrode and Platinum (Platinum, gauze, 100 mesh, 99.9% meta basis, Sigma Aldrich) as counter electrode. Also Ag/AgCl (Ag/AgCl, sat KCl, Sensortechnik Meinsberg, Germany) electrode was utilized as reference electrode. The schematic diagram images and auxiliary equipment of the CV test and MFC cell used in this research are shown in Figure 1.



a **b**

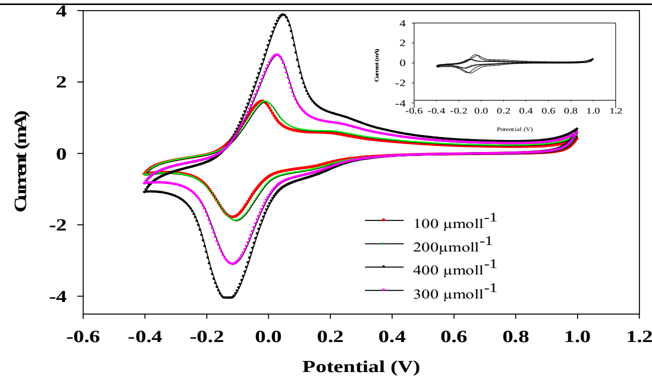
Figure-1: The schematic diagram of equipments, (a) working, Reference and Counter Electrodes for CV test with the auxiliary equipments and (b) Fabricated two chambers MFC

RESULTS AND DISCUSSION

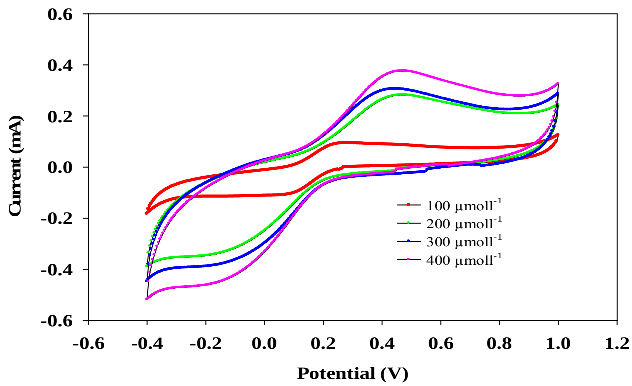
Electron shuttles are normally used in the anode compartment to accelerate transfer of produced electron to anode surface. These components are arti-ficial or any intermediate compounds produced by the microorganism itself. Some microorganisms produce nano-wires to transmit electrons directly without using any mediator but other organisms need to add artificial electron shuttle into the anode chamber. *E. coli* cannot transfer the produced electrons to the anode surface without addition of mediators and electron mediators to transfer produced electrons to anode surface is needed. The produced power and current of the MFC without presence of mediators was very low [19]. Several mediators were used and analyzed as electron mediators in anode compartment.The obtained data are discussed in details as follows.

Effect of mediators: At the first, MB was selected as electron mediator in the anode chamber to transfer of the produced electron to anode surface. Exchange of electrons between electrode surface and bacteria cells was analyzed with CV methods. Upper peak in CV indicate transfer of electron from electrode to bacteria and the lower peak show transfer of electron from microorganisms to electrode. Various concentrations of MB (100 to 400 μmol l^{-1} with an increment of 100 μmol l^{-1}) in aerobic and anaerobic condition were experimented and the results in CV presented in figure 2a. Anaerobic condition investigation was shown inside of figure 2a. The obtained results from CV demonstrate that the oxidation and reduction peak in aerobic condition compare the anaerobic condition was decreased. At the present of oxygen, catholic reaction take place in anode chamber and efficiency of power production in MFC will decrease. Also, several concentrations of potassium ferri-cynide (100, 200,300 and 400μmol l^{-1}), NR (50, 100, 150, 200, 250, 300, 350 μmol l^{-1}) and (d) thionine (100, 200, 300, 400 and 500 μmol l^{-1}) as electron mediators were analyzed in anaerobic and aerobic condition. Current potential curves by scanning the potential from negative to positive potential with 0.1 Vs^{-1} scan rates are shown in Figure 2b, c and d. The maximum current was obtained with NR as mediators at anaerobic condition. The oxidation peak at 350 μmol l^{-1} concentration of NR at deoxy-genated condition was 6 mA and this value obtained at 220 mV imposed potential. Although mediators may well differ in their capabilities to penetrate the bacterial cytoplasmic membrane in their oxidized or reduced form but the most important parameters is their standard redox potential [20]. These obtained results confirm our pervious publication about mediators [21].

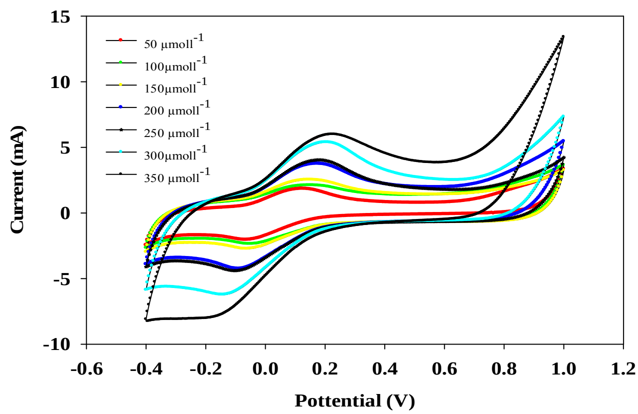
a. MB



b. Fericyanide



c. NR



d. Thionin

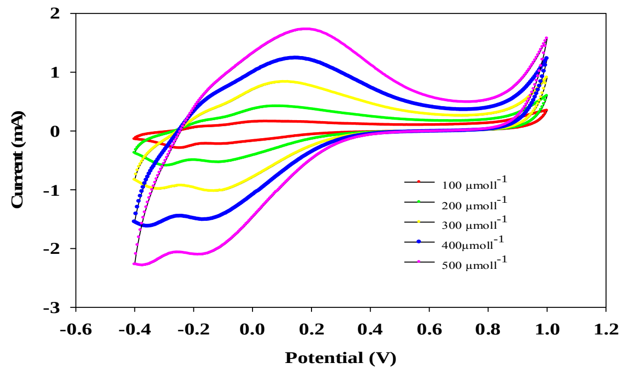


Figure-2: Generated oxidation and reduction peaks from various concentration **o**f MB(100, 200, 300, 400 and 500 µmol l^{-1}) (a) in anaerobic and aerobic condition (inside section a) (b) Potassium Ferricynide (100,200, 300 and 400µmol l^{-1}) (c) NR (50, 100, 150, 200, 250, 300, 350µmol l^{-1}) and (d) Thionine (100,200,300,400 and 500µmol l^{-1}) as electron mediators

And also humic acid with several concentrations in anaerobic and aerobic condition was analyzed as electron mediators but no peaks obtained from CV tests (data not shown here because the obtained peak was too low) for this microorganism.
The MFC performance was evaluated by the polarization curve and bioelectricity production. This technique was used for fabricated MFC. The obtained results indicated that the bioelectricity production without presence of artificial mediators was too low. These result indicated that mediators is necessary for this micro-organisms. The simillar reports was also obtained before [12, 20, 22-24]. All of mentioned mediators at different concentration was analyzed in dual chamber of MFC to obtaine the optimum concentration of each mediator. The maximum produced power, current density and also voltages at the best concentration of each mediator are summarized in Table - 2. The obtained results showed that the mediators were essential when *E. coli* was used as biocatalyst in the MFC. Table - 2 also shows that NR had the best ability for transferring the generated electrons in the anode chamber to the anode surface these results is match with CV test because the maximum oxidation and reduction peak was obtained with NR.
It may be because of the fact that different kinds of electron mediators act to the different parts of a metabolic pathway [25]. The presences of NR in anaerobic anode chamber increased the generated power density was 37.2 times more than the case without mediators in the MFC. Also,

Ieropoulos et al., [20] tested several types of electron mediators for a prototype MFC using *E.coli* but they obtained the maximum power with MB. Also Park and Zeikus [14] studied different mediators for E. coli and obtained maximum MFC performances with NR as electron mediators to anode electrode. Park et al., [12] also modified graphite electrode with NR.

Table-2: Maximum generated power, current and OCV at the optimum concentration of used mediators in MFC

Type of mediators	Optimum oncentration (μ mol.l^{-1})	P_{max} (mW.m^{-2})	I_{max} in P_{max} (mA.m^{-2})	OCV at SS condition (mV)
Without	---	5	35	243
Fericyanide	300	37	346	285
Thionine	400	83	480	432
NR	300	186	852	505
MB	400	94	512	447

Figure- 3 shows the polarization curve and power density of fabricated MFC at the 300µmol l^{-1} of NR as artificial electron mediators. Several concentrations of this mediator were tested in dual chamber of MFC to obtain optimum concentration of this artificial electron shuttle and this mentioned concentration was the best concentration of NR. At the optimum concentration of this mediators (300 µmol l^{-1}), maximum generated power and current were 241 mWm^{-2} and 1483 mAm^{-2}, respectively. Zhang et al., [25] used *E. coli* as active biocatalyst in their research. They used composite anode electrode in the absence of exogenous electron mediators but the produced power was less than 241 mWm^{-2}.

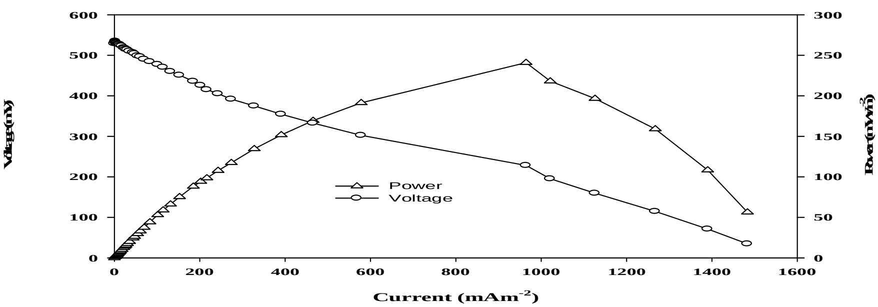


Figure-3: Polarization curve and power density production with optimum concentration of NR as mediators in dual chamber of MFC.

Effect of substrate concentration: After investigation different mediators, the obtained result indicated that NR was the best electron shuttle for this study. Effect of electron donor's concentration in anode compartment was investigated. As concentration of NR was kept constant (300 µmol l^{-1}), the effect of acetate concentration in anaerobic and aerobic condition was studied. The obtained data are presented in figure - 4. By increasing the substrate concentration changes the oxidation and reduction peak and also the place of maximum current changed in the current-potential curve (figure - 4).

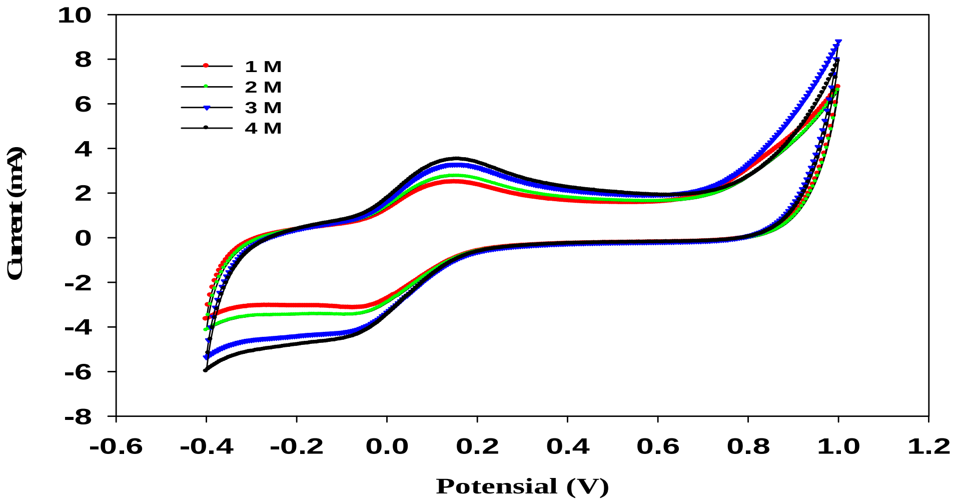


Figure-4: Effect of substrate concentration on CV in anaerobic (a) and aerobic (b) condition, NR with 200 µmol l^{-1} as electron mediator.

Effect of incubation time: Figure - 5 shows the effect of incubation time on CV results. After incubation the produced peak was very short but it increased after time. The system was analyzed after incubation, 3, 6, 9, 16, 24, 30, 36 and 42 hours. Time only changed the oxidation and reduction peaks on CV test but no change in the place of peak. Also the obtained result indicated that after 36hours incubation time additional time has no effect on oxidation peak in CV analysis. It is related to cell growth with incubation time at the present of NR (data not shown). Maximum power and current was obtained after 36 hours of incubation time and it was 190mWm^{-2} and 1532mAm^{-2} respectively.

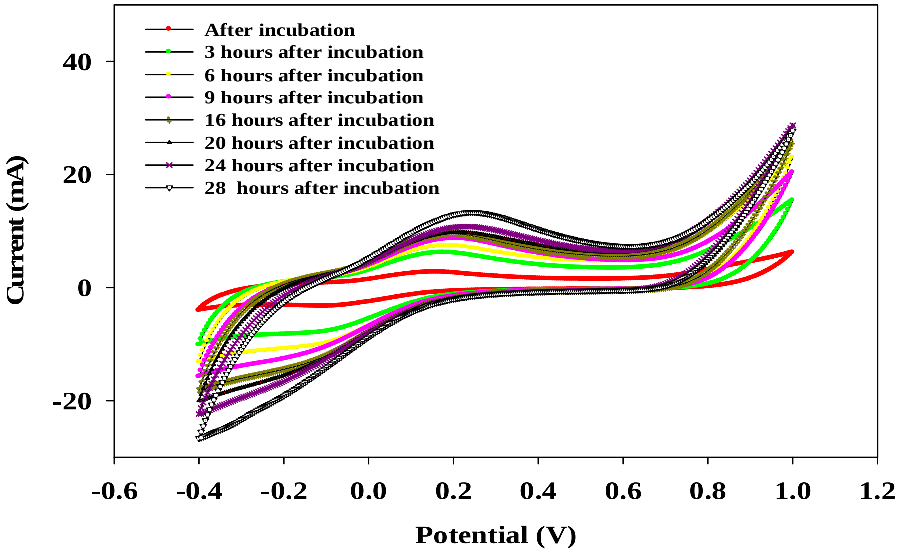
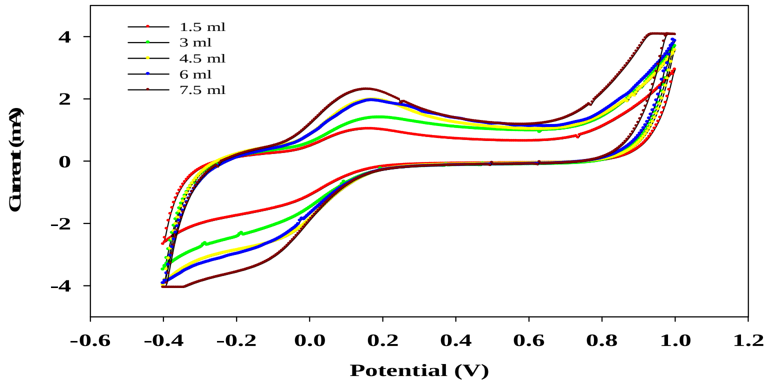


Figure -5: Effect of incubation time on CV. 1ml biocatalyst, 1ml acetate 1M as substrate and 200µmol l^{-1} NR was used as mediators. After incubation (1), 3 hours (2), 6 hours (3), 9 hours (4), 16 hours (5), 24 hours (6),30 hours (7) , 36 hours (8) and 42 hours .

Effect of E. coli concentration: MFCs are hybrid bio-electrochemical system which directly converts chemical energy to power via electro-chemical reactions involving bio-chemical pathways. Microorganisms in these systems serve as active biocatalysts and converting the stored energy in chemical bonds of bio-convertible substrate into bioelec-tricity then microorganisms plays an important role on it. The effects of microorganisms concentration on oxidation and reduction peak are presented in figure - 6. In each step 1.5ml of *E. coli* culture was centrifuge at 10,000 rpm for 8 min at room temperature. Cell plate resuspended by vortex mixture for approximately 15 seconds and these concentrated microorganisms were used as bio-catalysts and added solution in each time and investigated at anaerobic and aerobic condition (Figure 6).

a



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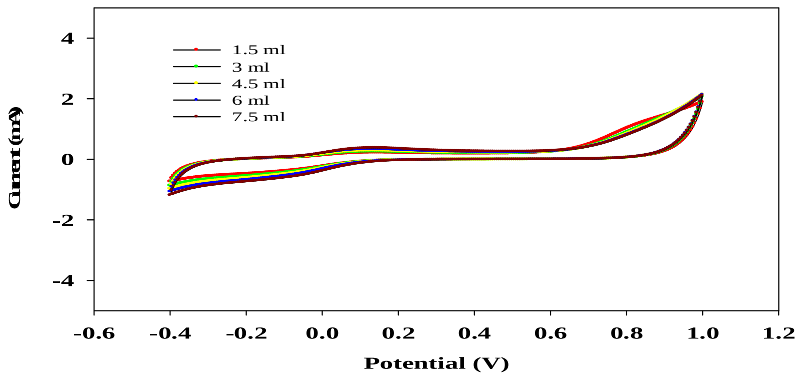

Figure-6: Effect of microorganisms concentration on CV. 1ml acetate 1M as substrate and 200 µmol l^{-1}NR was used as mediators

Effect of attached bacteria on anode surface: In the next stage, anode electrode with attached micro-organisms was analyzed with CV in the present and absent of mediator. The system was analyzed in anaerobic anode chamber. Before formation of any active biofilm on anode surface, no oxidation and reduction potential peak were record in CV test (Figure 7a). Current potential curves by scanning the potential from negative to positive potential after formation of active biofilm are shown in figure 7b. Two oxidation and one reduction peak was obtained with CV test. One peak was obtained in forward scan from -400 to 1000 mV and the other one for oxidation and reduction peak was obtained in reverse scan rate from

1000 to -400 mV. Similar results by alcohol as electron donors in anode chamber was reported [26]. Current potential curves by scanning the potential from negative to positive potential in present of several concentration of NR (100, 200 and 300 µmol l^{-1}) are shown in figure - 7c. Figure - 7 demonstrated that active biocatalyst on anode surface increased oxidation and reduction peak.

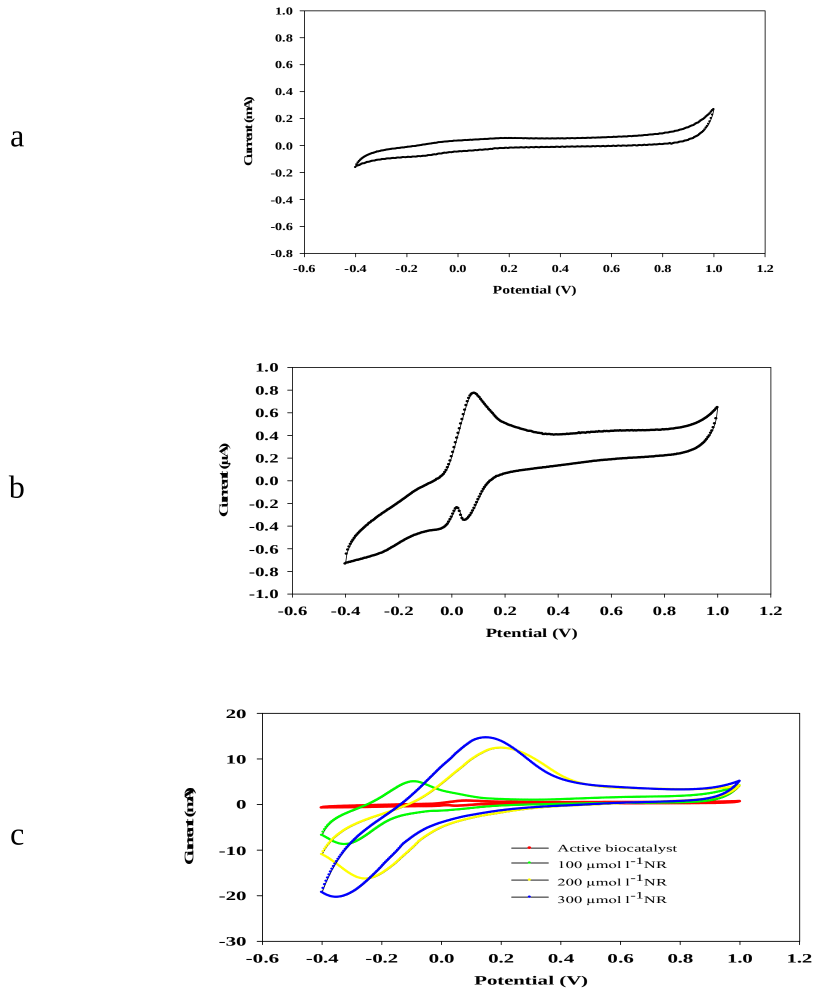

Figure-7: Effect of attached bacteria (active biocatalyst) on oxidation and reduction peak. (a) Absence of biofilm, (b) active biocatalyst without mediators and (c) active biocatalyst with different concentration of NR.

CONCLUSIONS

MFC can produce electrons and protons from sustainable materials. The generated electrical power in MFCs can be improved by adding some electron mediators. These artificial electron shuttles enhance to travel produced electrons to anode surface. Five electrons mediators at different concentrations and conditions were examined and analyzed the oxidation and reduction peaks in CV. The obtained results indicated that NR had the best ability among the used mediators to transfer the produced electrons to anode surface. In aerobic condition in all done experiments, the cyclic voltammograms not generated significant oxidation and reduction peaks. The obtained data demonstrated that anaerobic condition in anode chamber is so essential because in present of oxygen the produced electron reacts with oxygen in anode chamber and the produced power decreased.

ACKNOWLEDGMENTS: The authors wish to acknowledge Biotechnology Research Center, Babol Noshirvani University of Technology, Babol, Iran for the facilities provided to accomplish the present research.

REFERENCES

1. Ghasemi,M., W.R. Wan Daud, M. Ismail, M. Rahimnejad, A.F.Ismail, J.X.Leong, M.Miskan and K. Ben Liew, Effect of pretreatment and biofouling of proton exchange membrane on microbial fuel cell performance. Int. J. Hydrogen Energy 38: 5480–5484 (2013).
2. Lovley, D., Microbial fuel cells: novel microbial physiologies and engineering approaches.Curr. Opin. Biotechnol. 17(3):327-332 (2006).
3. Strik,D., H.Terlouw, H. Hamelers and C. Buisman, Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC). Appl. Microbiol. Biotechnol. 81 (4): 659-668 (2008).
4. Ghasemi, M., W.R.W. Daud, S.H. Hassan, S.E. Oh, M. Ismail, M. Rahimnejad and J.M.Jahim, Nano-structured Carbon as Electrode Material in Microbial Fuel Cells: A Comprehensive Review. J. Alloys Compd. 580:245–255 (2013).
5. Picioreanu, C., K. Katuri, M. van Loosdrecht, I. Head and K. Scott, Modelling microbial fuel cells with suspended cells and added electron transfer mediator. J. Appl. Electrochem. 40(1):151-162 (2010).
6. Liu, H., R. Ramnarayanan and B. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ. Sci. Technol. 38(7): 2281-2285 (2004).
7. Huang, L., J. Chen, X. Quan and F. Yang, Enhancement of hexavalent chromium reduction and electricity production from a biocathode microbial fuel cell. Bioprocess Biosyst. Eng. 33(8): 937 - 945 (2010).
8. Liu, Z.D. and H.R. Li, Effects of bio-and abio-factors on electricity production in a mediatorless

microbial fuel cell. Biochem. Eng. J. 36(3): 209-214 (2007).
9. Kim,M.S. and Y.Lee, Optimization of culture conditions and electricity generation using Geobacter sulfur reducens in a dual-chambered microbial fuel cell. Int. J. Hydrogen Energy 35: 13028-13034 (2010).
10. Larrosa, A., L.J. Lozano, K.P. Katuri, I. Head, K. Scott and C. Godinez, On the repeatability and reproducibility of experimental two-chambered microbial fuel cells. Fuel 88(10):1852-1857 (2009).
11. Tang,X., Z. Du and H. Li, Anodic Electron Shuttle Mechanism Based on 1- Hydroxy -4- Aminoanthra quinone in Microbial Fuel Cells. Electrochem. Commun. 12: 1140-1143 (2010).
12. Park,D., S.Kim, I.Shin and Y.Jeong, Electricity production in biofuel cell using modified graphite electrode with neutral red. Biotechnol. Lett. 22(16): 1301-1304 (2000).
13. Lovley, D., Bug juice: harvesting electricity with microorganisms. Nat. Rev. Microbiol. 4(7): 497-508 (2006).
14. Park, D. and J. Zeikus, Electricity generation in microbial fuel cells using neutral red as an electro-nophore. Appl. Environ. Microbiol. 66(4): 1292 (2000).
15. Chaudhuri, S.K. and D.R. Lovley, Electricity generation by direct oxidation of glucose in media-torless microbial fuel cells. Nat. Biotechnol. 21(10): 1229 - 1232 (2003).
16. Park, H.S., B.H. Kim, H.S. Kim, H.J. Kim, G.T. Kim, M. Kim, I.S. Chang, Y.K. Park and H.I. Chang, Novel Electrochemically Active and Fe (III) - reducing Bacterium Phylogenetically Related to Clostridium butyricum Isolated from a Microbial Fuel Cell. Anaerobe 7(6): 297-306 (2001).
17. Fricke, K., F. Harnisch and U. Schröder, On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. Energy Environ. Sci. 1(1): 144-147 (2008).
18. Dietrich, M., J. Heinze, C. Krieger and F.Neugebauer, Electrochemical Oxidation and Structural Changes of 5,6-Dihydrobenzo [c] cinnolines. J. Am. Chem. Soc. 118(21): 5020-5030 (1996).
19. Rahimnejad, M., N. Mokhtarian, G. Najafpour, W. Daud, and A. Ghoreyshi, Low Voltage Power Generation in aBiofuel Cell Using Anaerobic Cultures. World Appli. Sci. J. 6(11): 1585-1588 (2009).
20. Ieropoulos, I., J. Greenman, C. Melhuish and J.Hart, Comparative study of three types of microbial fuel cell. Enzyme Microb. Technol. 37(2): 238-245 (2005).
21. Najafpour, G., M. Rahimnejad and A.Ghoreshi, The Enhancement of a Microbial Fuel Cell for Electrical Output Using Mediators and Oxidizing Agents. Energy Sour. Part A. 33(24): 2239-2248 (2011).
22. Bennetto,H., Electricity generation by microorganisms. Biotechnol. 1(4): 163-168 (1990).

23. Schröder, U., J. Nießen and F. Scholz, 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 (2003).
24. Grzebyk, M. and G. Pozniak, Microbial fuel cells (MFCs) with interpolymer cation exchange membranes. Sep. Purif. Technol. 41(3): 321-328 (2005).
25. Zhang, T., Y.Zeng, S.Chen, X. Ai and H.Yang, Improved performances of E.coli catalyzed microbial fuel cells with composite graphite/ PTFE anodes. Electrochem. Commun. 9(3): 349-353 (2007).
26. Kim, J., S. Jung, J. Regan and B. Logan, Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour. Technol. 98(13): 2568-2577 (2007).