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Bioelectricity Generation in Mediator - Less Microbial Fuel Cell: Application of Pure and Mixed Cultures

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Abstract: Microbial fuel cell (MFC) is an emerging biotechnology which converts the organic substrates to electricity by electrogenic bacteria. In this study electricity generation was evaluated in a dual chambered mediator-less microbial fuel cell. Graphite plates were used as electrodes. Glucose with initial concentration of 4 $g.l^{-1}$ was used as an electron donor. Anode chamber of the fabricated MFC was inoculated with two mixed cultures; anaerobic effluent of dairy manufacture (called A) and anaerobic effluent of wastewater treatment plant (called B). *Saccharomyces cerevisiae* PTCC 5269 was used as a pure culture. Power generation using these three inoculums was evaluated in the same condition including room temperature, without agitation and using glucose with initial concentration of 4 $g.l^{-1}$ as substrate. The performance of MFC was analyzed by the measurement of polarization curve. Maximum power and current densities of 30.99 $mW.m^{-2}$ and 391.68 $mA.m^{-2}$; 25.90 $mW.m^{-2}$ and 320.7604 $mA.m^{-2}$ were obtained when culture B and A were used as inoculums, respectively. Power and current densities were 10.0224 $mW.m^{-2}$ and 253.2964 $mA.m^{-2}$ when *S. cerevisiae* was used as a pure culture in MFC. With chemical oxygen demand (COD), removal efficiencies of 54, 71 and 75 % were achieved using *S. cerevisiae,* mix cultures A and B as inoculums, respectively. The investigation was targeted for the capability of mediator-less microbial fuel cell as combined wastewater treatment and electricity production unit.

Key words: *Saccharomyces cerevisiae*; Mediator-less; Microbial fuel cell; Wastewater treatment; Bioelectricity

INTRODUCTION

Energy shortage and environmental pollution have caused global disasters. Thus, active research to find out alternate sources of energy especially renewable sources of energy is a new trend [1]. In recent years, fuel cells have emerged as a renewable energy sources. Among different kinds of fuel cells, microbial fuel cells (MFCs) defined as devices which directly converts microbial metabolism into electricity have attracted researchers attention [2]. MFCs are the major type of bioelectrochemical systems which convert biomass spontaneously into electricity through the metabolic activity of the microorganisms [3]. Typical MFCs are composed of an anode and a cathode chamber separated by a proton exchange membrane (PEM) or salt bridge. Microorganisms in the anode chamber oxidize fuel (electron donor e.g. glucose, acetate, etc.) to produce electrons and protons. Produced electrons are transferred to cathode compartment through the external circuit. Generated protons flow to cathode compartment through the membrane. Electrons and protons are combined in the cathode and reducing electron acceptor (e.g. oxygen) to produce water [4,5]. Principally, the output power depends on the rate of substrate degradation, the rate of electron transfer from the bacteria to the electrode, the circuit resistance and the proton mass transfer in the liquid [6]. Among these parameters, electron transferred

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from microorganism to electrode in anode chamber is important. Since most microbial cells are electrochemically inactive, rapid electron transfer from a microorganism to an electrode needs an electron mediator [7]. Electron transfer from microbial cells to the electrode is facilitated by the help of mediators such as thionine, methyl viologen, humic acid and many mores [8]. However, chemicals that used as a mediator in MFCs are expensive and toxic; therefore, the long-term operation of mediated MFCs cannot be achieved and has not been commercialized [9]. Recently, a number of bacteria such as *Shewanella putrefaciens*, family of *Geobacteraceae* and number of microorganisms stated in the literatures [10-14] found to have ability to transfer produced electrons from oxidized fuel (substrate) to an electrode without using any artificial mediator, making it possible to establish mediator- less MFC [15].

Pure and mixed cultures of organisms are used as inoclua in MFCs but due to high costs, pure microorganisms may not be suitable for the practical operation such as treatment of industrial effluents. Mixed cultures (i.e. soil, wastewater) containing significant amounts of electrogenic bacteria can be used as the cost-effective inocula for MFCs. However, the non-electrogenic bacteria (i.e. methanogenic bacteria, denitrifying bacteria) in mixed cultures consume organic substrates without generating electricity [16, 17].

The main aim of the present study was to design a MFC incapable to treat wastewater and generate power simultaneously while employing low-cost materials without using toxic mediators. The electrical performance of the fabricated MFC (graphite electrode without any coating) employed in an aerated cathode and mediator-less anode. The system was evaluated at ambient condition using anaerobic mixed consortia and glucose as the sole source of electron.

Experimental

Chemicals, Anaerobic Pure and Mixed Cultures: Mixed cultures were collected from anaerobic effluent of dairy manufacture (Gela-Amol) (culture A) and anaerobic effluent of wastewater treatment plant (Babol) (culture B). *Saccharomyces cerevisiae* PTCC 5269 was supplied by Iranian Research Organization for Science and Technology, Tehran, Iran used as inoculums. Two mixed consortium and pure cultures were inoculated to a seed culture containing (glucose, 4 $g.l^{-1}$; NH_4Cl, 0.5 $g.l^{-1}$; yeast extract, 3 $g.l^{-1}$; KH_2PO_4, 0.7 $g.l^{-1}$; K_2HPO_4, 1 $g.l^{-1}$; peptone, 1 $g.l^{-1}$) separately. The pH of media was adjusted to 6.8. 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.

MFC Configuration and Operation: The dual-chambered MFC was designed and fabricated in Biotechnology Research laboratory (BNUT, Iran) using perplex material. Figures 1a and 1b show photograph and schematic diagram of the designed and fabricated MFC and Table 1 contains its design parameters. Total volume of both anode and cathode compartment was the same (0.08 l) and each chamber was provided with sampleport, wire point inputs (top). Proton exchange membrane (PEM; NAFION 117, Sigma–Aldrich) was used to separate the two chambers. Proton exchange membrane, Nafion, was subjected to a course of pretreatment to take off any impurities through boiling for 1 h in 3% H_2O_2, washed with deionized water, 0.5 M H_2SO_4 and finally washed with deionized water. After pretreatment, the PEM was fixed using washers and clamps between two chambers. In order to maintain membrane for good conductivity, the anode and cathode compartments were filled with

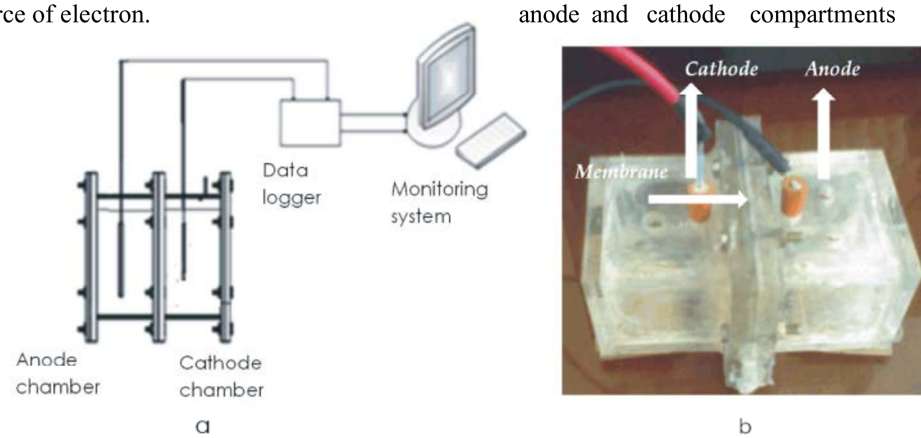

Fig. 1: a) Photograph of dual-chambered microbial fuel cell and b) schematic details

Table 1: Design criteria of dual chamber mediator-less microbial fuel cell

Reactor configuration	Duel chamber
Anode chamber	Suspended growth
Anode inoculums	Mixed anaerobic consortia or pure bacteria
Mediator-anode	Mediator less
Working valium of anode and cathode (l)	0.05
Anode and cathode material	Graphite felt
Surface area of electrodes (cm^2)	6.25
Membrane	Proton exchange membrane (Nafion 117)
Operating temperature	Room temperature (22 ± 2°C)
Operating pH	6.8

deionized water when the microbial fuel cell was not in use. Both anode and cathode electrodes were made of graphite plates (2.5 ×2.5 cm, without any coating). The electrodes were positioned at a distance of 1 cm on either side of the PEM. Copper wire has been connected electrodes through resistance and a data logger.

The cathode chamber of the MFC was filled with aerated phosphate buffer (pH=6.8) as catholyte. The anodic chamber prior to start up in three isolated experiments was inoculated with two anaerobic mixed microflora; A and B and pure culture of microorganism (*S. cerevisiae* PTCC 5269) dissolved in seed culture feed and no mediator was used in the anodic chamber.

Analysis: All chemicals and reagents used for the experiments were analytical grades and supplied by Merck (Germany). The pH meter, HANA 211 (Romania) model glass-electrode was employed to measure pH values of the aqueous phase. Chemical oxygen demand (COD chromate) of the wastewater was measured by the closed reflux colorimetric method [18].

Analytical Method: Polarity technique was adopted to analyze experimental data in terms of voltage and current density. Polarization curves were obtained using an adjustable external resistance. Power and current were calculated based on following equations:

$$P = I \times E \qquad (1)$$

$$I = \left(\frac{E}{R_{ext}}\right) \qquad (2)$$

where, P is generated power and E measured cell voltage, R_{ext} denotes external resistance and I indicate produced current. The online recorded produced current and power were normalized by the surface area of the used anode. Analog digital data acquisition was fabricated to record data point in every 4 min. Measurements were carried out at variable resistances which were imposed to the MFC. The current in the MFC was automatically calculated and recorded dividing the obtained voltage by the specified resistance. Then, the system provides power calculation by multiplication of voltage and current. The provisions were provided for online observation of polarization curve showing the variation of power density 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 display synchronically the obtained data. The power, current and voltage were automatically recorded by the computer connected to the system [19].

RESULT AND DISCUSSION

Power generation in mediator less microbial fuel cell was investigated in three separate experiments using A, B and pure cultures as inoculums. In all experiments, MFCs operation condition was the same.

As shown in Figure 2, the MFC inoculated with mixed culture B produced the highest power density. This might be due to existing electrogenic bacteria that transfer electron to electrode without interfering mediator in anode chamber. Although, the non electrogenic bacteria (i.e. methanogenic bacteria, denitrifying bacteria) in mixed cultures consume organic substrates without generating electricity, but electrogenic bacteria existing in mixed cultures have more electrochemically activity than used pure culture of the microorganism (*S. cerevisiae*) lead to more power generation in MFCs. Data are shown in Table 2. In this study, MFCs can simultaneously achieve the COD removal and the electricity generation.

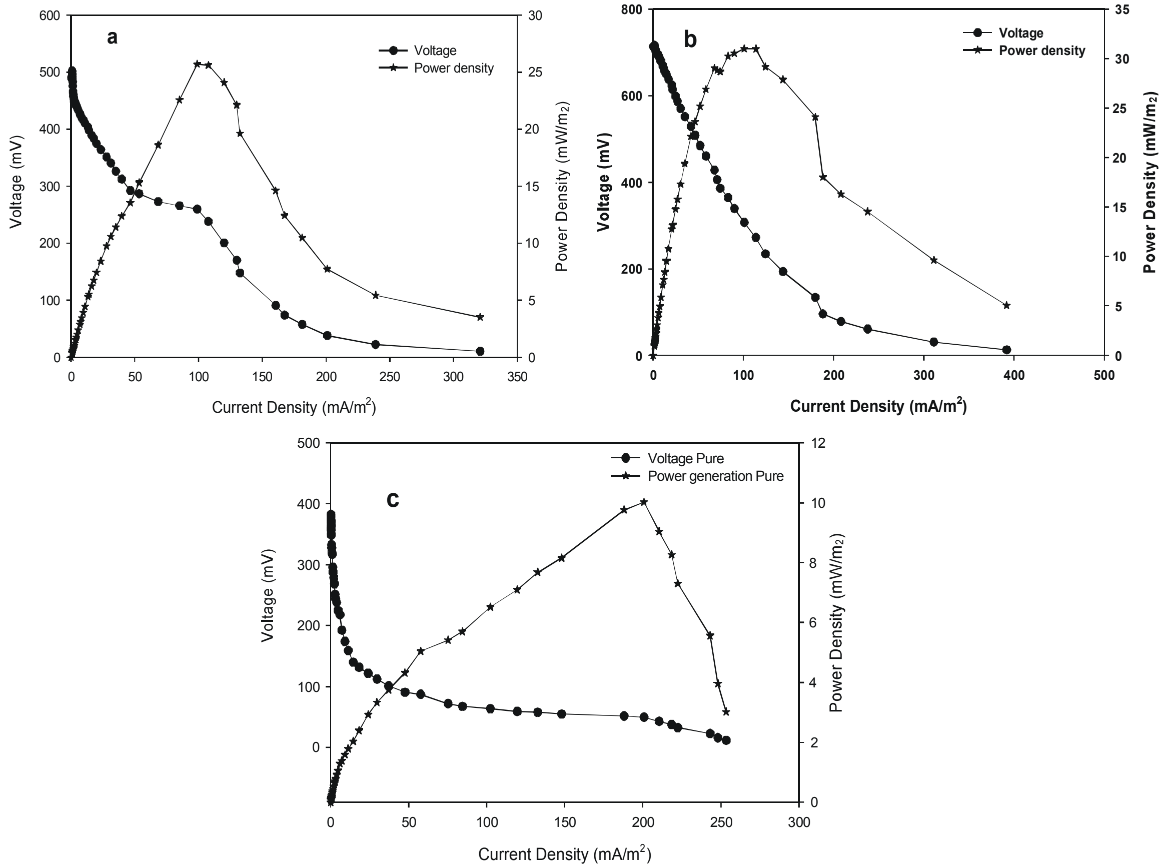

Fig. 2: Power generetion in MFCs with a) culture A, b) culture B and c) pure culture as inoculums

Table 2: Comparison of MFCs inoculated with several inoculums in power generation and COD removal

Inoculums	COD removal (%)	OCV (mV)	Power Density (mw/m^2)
S. cerevisiae	54.43	290	10.0224
A	71.25	500	25.6989
B	75.02	730	30.9924

CONCLUSION

The results revealed the feasibility of bioelectricity generation using a MFC fabricated with low-cost anode materials (non-coated plain graphite electrodes), without any toxic mediators. Performance of MFCs with respect to power generation was found to be dependent on application of various inoculums. In this study, maximum power generation was achieved by anaerobic wastewater as inoculums. Maximum power and current densities for anaerobic effluent of wastewater treatment plant and anaerobic effluent of dairy manufacture were 30.99 mW.m^{-2} and 391.68 mA.m^{-2}; 25.90 mW.m^{-2} and 320.7604 mA.m^{-2}, respectively. Power and current densities were 10.0224 mW.m^{-2} and 253.2964 mA.m^{-2} when *S. cerevisiae* was used as pure culture. Utilizing of wastewater for the production of bioelectricity from an anaerobic treatment is a feasible, economical and sustainable alternative. Most important advantages of energy produced from wastewater were the absence of environmental emissions, simultaneous recovery of energy and wastewater treatment. Although, the non electrogenic bacteria (i.e. methanogenic bacteria, denitrifying bacteria) in mixed cultures consume organic substrates without generating electricity, but electrogenic bacteria existing in mixed cultures that have more electrochemically activity than used pure culture of the microorganism (*S. cerevisiae*) lead to more power generation in MFCs.

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