Bioelectricity power generation from organic substrate in a Microbial fuel cell using *Saccharomyces cerevisiae* as biocatalysts

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Abstract: In recent years, as a novel mode of converting organic matter into bioelectricity, Microbial fuel cells (MFCs) have gained significant attention. Among effective parameters in MFCs, substrate type and concentration play major role on MFC performance. In this study, a dual chamber MFC was used with a wide range of fructose concentrations: 10, 20 30 and 40 g/l. The MFC was inoculated with *Saccharomyces cerevisiae* as biocatalyst. A100µm of neutral red as mediator and also 100µm ferricyanide as oxidizer added to anode and cathode chambers, respectively. The MFC generated an open circuit voltage (OCV) of 690, 768, 548 and 507 mV with concentration of fructose from 10 to 40 $g.l^{-1}$, respectively. Maximum power density of 32.16, 23.7, 18.9 and 10.47 were obtained with substrate concentration of 10 to 40 $g.l^{-1}$, respectively. The maximum value of OCV and power density obtained with 10$g.l^{-1}$ of carbohydrate. To investigate resistance effect on MFC performance, for each substrate concentration data acquisition system was set at optimum value for the resistance which was resulted by the polarization curve. Then maximum power and optimum current density were recorded.

Keywords: *Bioelectricity, External resistance, Fructose, Microbial fuel cell, Saccharomyces cerevisiae*

1. Introduction

As fossil fuel sources are depleted, alternative energy sources are developed. Renewable energy is much eco-friendly such as biomass converted to fuel and energy in many alternative processes.

In the near future, the trends for new alternative renewable energies are gradually increasing.[1-4] Major efforts were devoted to develop alternative electricity generation methods.[5, 6] Among renewable alternatives, microbial fuel cell (MFC) created great interests for many researchers due to its possibility of directly harvesting electricity from organic wastes and renewable biomass.[7] MFC operates under very mild conditions and wide variable ranges of biodegradable materials are used as fuel.[8, 9] The bio base materials are oxidized by the microorganisms in the anode and the biocatalysts have the great potential to generate electrons. Biological systems possess number of advantages over the conventional chemical systems. 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. [10, 11]

A typical MFC consists of anodic and cathodic chambers partitioned by a proton exchange membrane (PEM). Microbes in the anodic chamber oxidize substrates and generate electrons and protons in the process. As an oxidative by-product, carbon dioxide is also produced. However, there is no net carbon emission because of the carbon dioxide originated from renewable biomass incorporated into photosynthetic process. Unlike in a direct combustion process, the electrons are absorbed by the anode and are transported to the cathode through an external circuit. After crossing a PEM or a salt bridge [12], the protons enter the cathodic chamber where they combine with oxygen to form water. Microbes in the anodic chamber generate electrons and protons in the dissimilative process of oxidizing organic substrates.[13, 14] Electric current generation is made possible by keeping microbes separated from oxygen

or any other end terminal acceptor other than the anode and this requires a separate anaerobic anodic chamber. In general, there are two types of microbial fuel cells: mediator and mediator-less microbial fuel cells.[15-18]

Among effective parameters on performance of microbial fuel cell, substrate type and concentrations had a significant effect on cell power.[19-22] The aim of this study was to investigate the effect of fructose, a monosaccharide that could be found in many fruit juices. Substrate concentrations were varied from 10 to 40 $g.l^{-1}$. Also, the influence of external resistance on production of bioelectricity in a dual chamber MFC was evaluated.

2. Methodology

Saccharomyces cerevisiae PTCC 5269 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The microorganisms were grown in an anaerobic jar. The prepared medium for seed culture consists of yeast extract, NH_4Cl, NaH_2PO_4, $MgSO_4$ and $MnSO_4$: 3, 0.2, 0.6, 0.2 and 0.05 $g.l^{-1}$, respectively. Fructose as carbon source was added to the medium with concentration in the range of 10-40 $g.l^{-1}$. 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 for duration of 24 hours in 100 ml flask without any agitation. Substrate consumption was calculated based on determination of reduced sugars in the culture broth. 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 for pH measurements of the samples in aqueous phase. The initial pH of the working solutions was adjusted by addition of dilute HNO_3 or 0.1M NaOH solutions. DNS reagent was employed to detect and measure substrate consumption using colorimetric method and cell growth was also monitored by optical density using spectrophotometer (Unico, USA) at wave length of 620nm.

The fabricated cells in laboratory scale were made of Plexiglas material. The volume of each chamber (anode and cathode chambers) was 800 ml with working volume of 600 ml (75% of total volume). The sample port was provided for anode chamber, wire point inputs and inlet port. The selected electrodes in MFC were graphite felt in size of 50×35×2 mm. Proton exchange membrane (PEM; NAFION 112, Sigma–Aldrich) was used to separate two compartments. The Nafion area separated the chambers was 3.79 cm^2. Nafion as a proton exchange membrane was subjected to a course of pretreatment to take off any impurities. The membrane pretreatment started with boiling the film in 3% H_2O_2 for 1h, washed with deionized water, 0.5 M H_2SO_4, and then washed with deionized water. The anode and cathode compartments were filled by deionized water when the biological fuel cell was not in use to maintain and preserve the membrane for good conductivity. Natural Red and Ferricyanide were supplied by Merck (Germany). These chemicals with the concentrations of 100 $\mu mol.l^{-1}$ & 100 $\mu mol.l^{-1}$ were used as mediators in anode and cathode of MFC, respectively.

In the microbial fuel cell, *S. cerevisiae* was used as a biocatalyst for production of bioelectricity from carbohydrate source. This microorganism was grown under anaerobic condition in the biofuel cell. Fixed incubation time and enriched medium was used. The obtained data showed that *S. cerevisiae* had good ability to consume substrate under anaerobic

condition. The Fabricated cell for the experimental set up with auxiliary equipments is shown in Fig. 1.

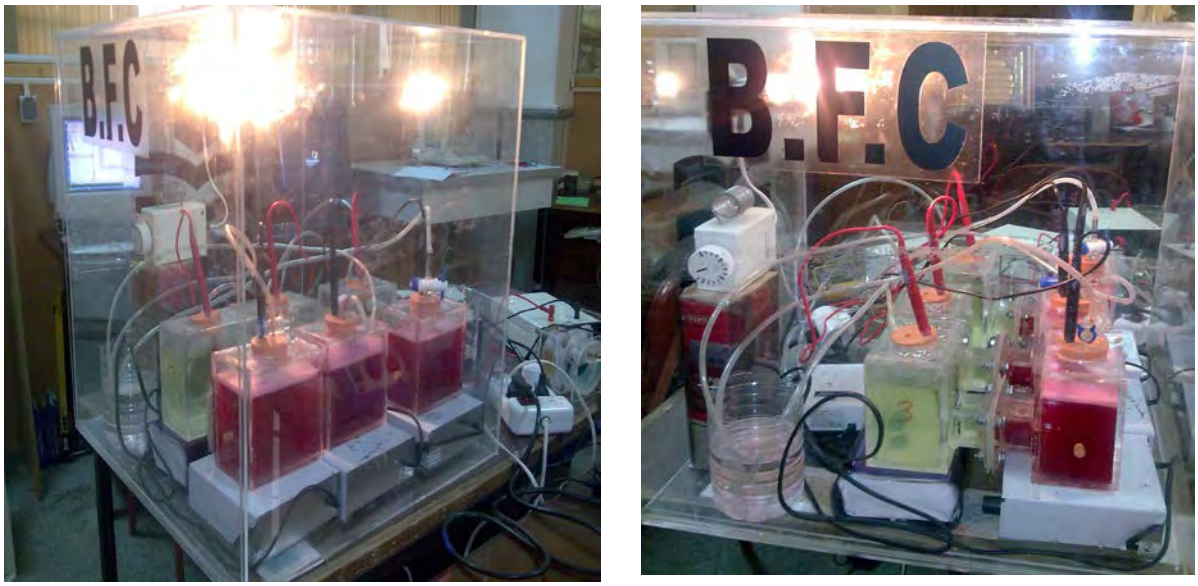

Fig. 1. The laboratory-scale MFCs with thermal controller in enclosed space

3. Result and Discussion

To start up the process, *S. cerevisiae* was inoculated into anode chamber. Fructose fed to microbial fuel cell with concentrations ranged from 10 to 40 $g.l^{-1}$. The output result in the form of open circuit voltage (OCV) was recorded by the data acquisition system. Biochemical activity of the microorganisms gradually increased electricity generation. At incubation time 64, 68, 59 and 57 hours after inoculation, the output OCV remained constant while the cell growth proceeded to stationary phase. The recorded voltages were 690, 768, 548 and 507 mV for 10, 20, 30 and 40 $g.l^{-1}$ of fructose, respectively. Due to stability of process operation after incubation time, the polarization curves were also recorded by data acquisition system in order to evaluate the performance of the MFCs. Fig. 2 shows the substrate concentration was increased (10 to 40 $g.l^{-1}$) the power and current density were decreased. With substrate concentration of 10 $g.l^{-1}$, maximum power and current density generated were 32.16 $mW.m^{-2}$ and 96.59 $mA.m^{-2}$, respectively.

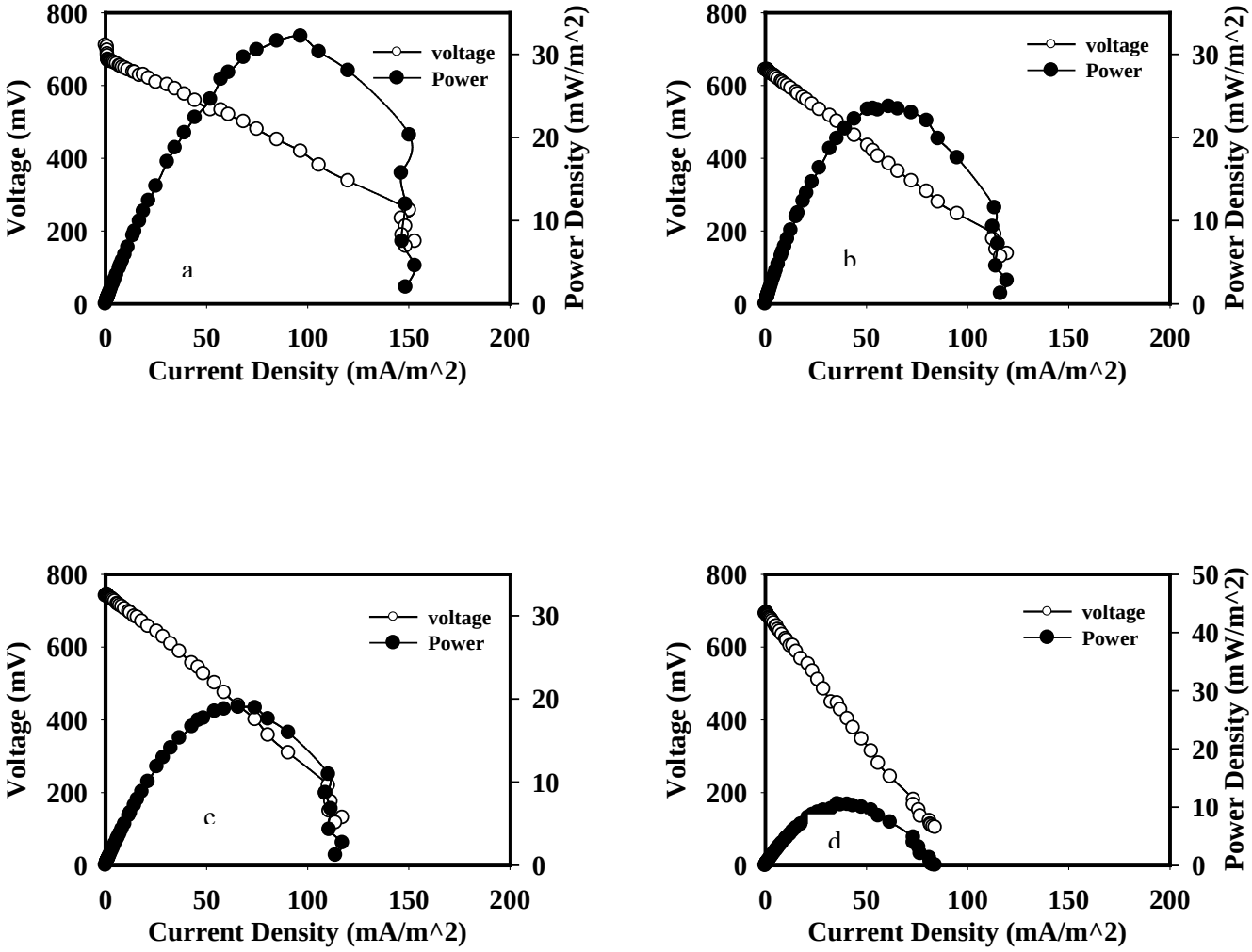

Fig. 2. Effect of different concentrations of fructose on polarization curves
a) 10 g.l^{-1}, b) 20 g.l^{-1}, c) 30 g.l^{-1}, d) 40 g.l^{-1}

As the electrical resistance applied to plot polarization curve varied in the range of 65535 to 0.1 kΩ, the pick point of the graph occurred at 3.88kΩ. Pick point demonstrated maximum power density and the optimum current density were proportional to applied resistance. The MFC performance is illustrated in Fig. 3. The cathode and anode of MFC were connected together through a circuit of 3.88kΩ as an external resistance. Due to presence of resistance, the power and voltage were considered as operational electricity (see Fig. 3).

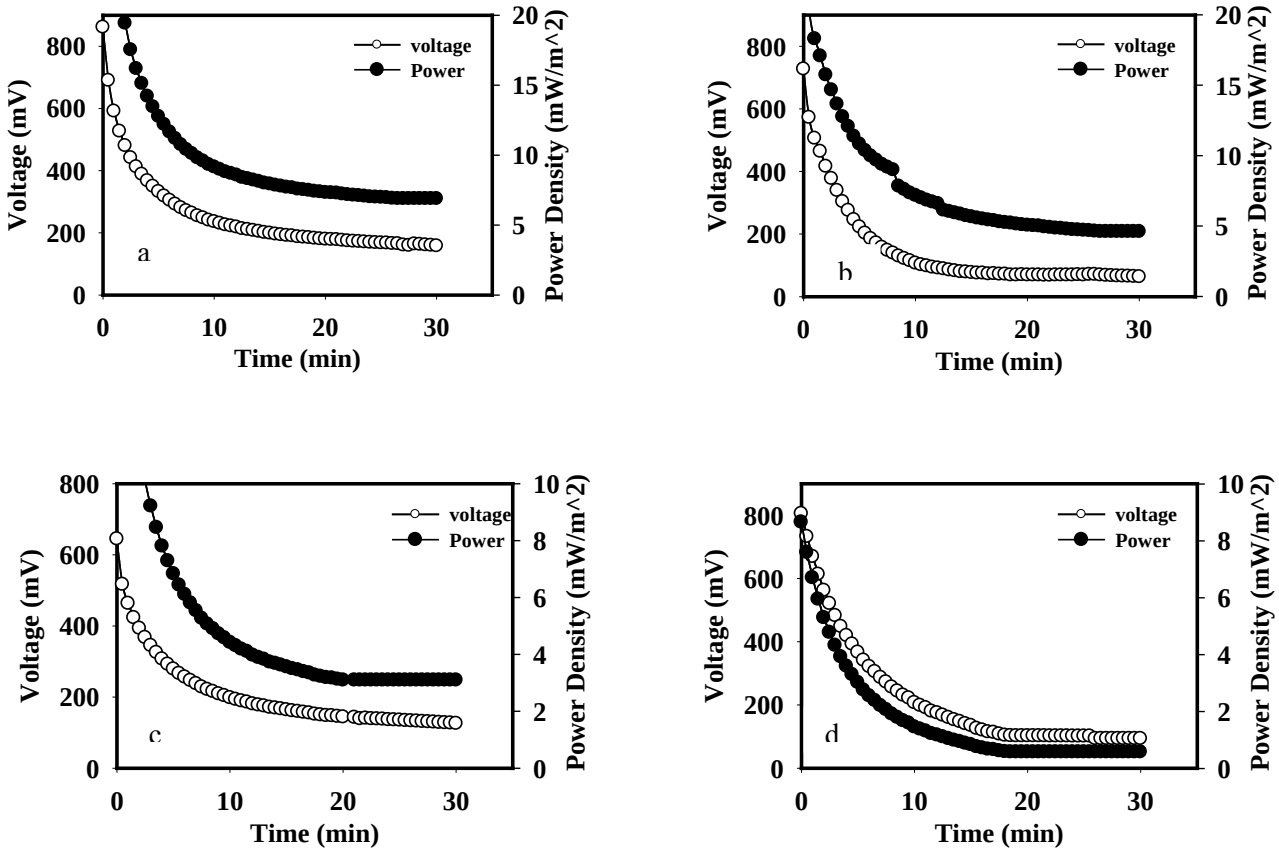

Fig. 3. Effect of external resistance on power density and voltage of MFC fed with a) 10 g.l^{-1}, b) 20 g.l^{-1}, c) 30 g.l^{-1} and d) 40 g.l^{-1} of fructose solution

The performance of MFC with respect to time was monitored. The voltage and power density in experimental runs for the course 30 minutes were obtained. In addition, current density was also recorded but due to complexity of presented plots the data are not shown. However the recorded data were averaged and summarized in Table 1.

Table1. Mean power and current density and mean voltage in presence of 3.88 kΩ as an external resistance

Fructose Concentration g.l^{-1}	Mean Power Density mW/m^{-2}	Mean Current Density mA/m^{-2}	Mean Voltage mV
10	11.26	56.25	196.02
20	9.8	39.17	248.2
30	5.86	36.88	165.7
40	2.3	15.86	145.9

4. Conclusions

The effect of substrate concentrations was investigated in a dual microbial fuel cell. Fructose was chosen as the simple carbon source with concentrations ranged from 1 to 40 $g.l^{-1}$. *S. cerevisiae* as the biocatalyst successfully oxidized the soluble substrate. The biocatalysts in the media with concentration of 10 $g.l^{-1}$ of substrate demonstrated the maximum power and optimum current density of 32.16 mW/m^{-2} and 96.59 mA/m^{-2}, respectively. The proportional resistance to the pick point of the polarization curve at 3.88 kΩ, was applied to the circuit as an external resistance in the operating system. The obtained averaged power and current density were 11.26 mW/m^{-2} and 56.26 mA/m^{-2}, respectively. The presented data were allocated to fructose with total sugar concentration of 10 $g.l^{-1}$.

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