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ORIGINAL PAPER

Enhanced electricity generation from whey wastewater using combinational cathodic electron acceptor in a two-chamber microbial fuel cell

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Abstract While energy consumption is increasing worldwide due to population growth, the fossil fuels are unstable and exhaustible resources for establishing sustainable life. Using biodegradable compounds present in the wastewater produced in industrial process as a renewable source is an enchanting approach followed by scientists for maintaining a sustainable energy production to vanquish this problem for ulterior generations. In this research, bioelectricity generation with whey degradation was investigated in a two-chamber microbial fuel cell with humic acid as anodic electron mediator and a cathode compartment including combinational electron acceptor. *Escherichia coli* was able to use the carbohydrate originated from whey to generate bioelectricity. The open-circuit potential in absence of mediator was 751.5 mV at room temperature. The voltage was stable for more than 24 h. Humic acid was used as a suitable mediator. In addition, some mixed chemicals were employed as catholyte. Based on polarization curve, the power and current values in the presence of a mixed solution of potassium iodide (KI), ferric chloride [$FeCl_3$ (III)] and manganese chloride tetrahydride ($MnCl_2{\cdot}4H_2O$) with doubling of oxidant (oxygen) concentration using agitation with magnet stirrer in cathode compartment without any buffer solution were boosted to 562.9 μW and 1906.1 μA, respectively, and demonstrated the best result for power generation.

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Introduction

During the past 20 years, much research was done on microbial fuel cells (MFCs), which are electrochemical devices for production of energy from the environment (Li et al. 2009; Allen and Bennetto 1993; Bond and Lovley 2003; Gil et al. 2003; Kim et al. 2002; Liu et al. 2004; Oh et al. 2004; Thygesen et al. 2009) and waste treatment (Venkata Mohan et al. 2008). Several types of substrate have been researched in MFCs for bioelectricity generation (Greenman et al. 2009; Lu et al. 2009; Min and Logan 2004; Min et al. 2005). With growing concerns for exploring other energy sources, waste management, global climate change, and no edible feedstocks, searches for novel technical solutions are in progress. Fuel cells are the alternative energy extractor technology that is studied for a full-scale implementation. These can be grouped into three subcategories: catalysts, enzymes, and microorganisms. Since the turn of the century, research on microbial fuel cells has received a rate increase. MFCs are unique in their ability to exploit microbes, rather than enzymes or inorganic molecules as catalysts to convert chemical energy directly into electricity feedstock (Rismani-Yazdi et al. 2008). MFCs evolve rapidly because of their direct conversion of substrate energy for bioelectricity resulting in high conversion efficiency and operating at room temperature (Lin et al. 2007; Schroeder 2007), preferably not requiring on-gas treatment because the off-gases are composed of carbon dioxide and usually have no employable energy, no need for energy input to cathode (Liu et al. 2004), and finally their potential for tremendous

application in remote locations with no access to a power plant or infrastructure. A group of researchers emphasized the role of carbohydrates in wastes for power generation (Kapdan and Kargi 2006).

The produced bioelectricity varies from different substrates according to the source of substrates (Liu et al. 2005; Morris and Jin 2008; Ren et al. 2007). Cheese manufacturing industry generates large amounts of high strength wastewater characterized by a high biological oxygen demand (BOD) and COD concentrations. (Demirel et al. 2005; Farizoglu et al. 2004; Omil et al. 2003). Whey is a rich by-product of cheese manufacturing process (Ferchichi et al. 2005; Kisaalita et al. 1987). Cheese whey management has attracted more attention, owing to approved limitations (Farizoglu et al. 2004), environmental catastrophic effects, and economic considerations (Yang et al. 2007). Some microbes have been used in mediating microbial fuel cell (Bennetto 1990; Niessen et al. 2004; Reed and Nagodawithana 1991). Besides, one of the most important issues, especially in two-chamber MFCs operating is kinetic efficiency in a cathode compartment. Due to incomplete reduction of oxygen causes low energy conversion efficiency producing reactive intermediates and free radical species, which can be devastating; researching this part of MFC may be essential. A proper approach for improving power output is adding chemical electron acceptors like permanganate in cathode compartment (You et al. 2006). To best of our knowledge, there is no exact information in this special system except for the work done by the current authors (Nasirahmadi and Safekordi 2011). On the other hand, this work is the resumption of the previous one. The aim of this study was to investigate the effect of oxidant concentration on power generation in a two-chamber microbial fuel cell in the presence of humic acid as an anodic mediator, cheese whey as fuel and *E. coli* as biocatalyst in the anode chamber of MFC. The primary goal of this research was to evaluate the MFC for power generation using the treated whey as the substrate with minimum operational manipulation and definitely evaluation of combinational cathodic electron acceptors. The research work was carried out during 2009–2010 in the Department of Chemical Engineering, Noshirvani University of Technology, Babol and Islamic Azad University, Science and Research Branch, Department of Engineering, SEM Laboratory, Tehran, Iran.

Materials and methods

The previous materials and methods were applied in this work (Nasirahmadi and Safekordi 2011). Whey was obtained from Gela dairy product Industry (Amol, Iran). Whole whey solution was uniformly acidified by acid solution (HCl, 2N) in an acidic medium to remove excessive proteins. The solution was autoclaved at 15 psig, 121 °C for 15 min, then cooled down to room temperature, centrifuged at 7,000×*g* in sterilized tubes for 15 min to remove aggregated solids. The supernatant (whey supernatant), was refrigerated for 12 h and it was used after adjusting pH to 7 by the concentrated NaOH solution (10 M), as the major constitutive of media for the growth of microorganism. *E. coli* was supplied by laboratory of Rohani Hospital (Babol, Iran). Microorganisms were grown in an anaerobic jar vessel. Humic acid and cathodic electron acceptors were supplied by Merck (Germany). These chemicals with low concentration (200 µmol/L and 1 g/L) were used as mediators in MFC, respectively. The schematic diagram of the fabricated MFC is shown in Fig. 1. The fabricated cells in the laboratory scale were made of glass (Plexy) material.

The volume of each chamber (anode and cathode chambers) was 910 mL with working volume of 800 mL. Sampling access port was provided for the anode, input wire point and the inlet. Selected MFC electrodes were graphite in the size 40 × 90 × 3 mm. Proton Exchange Membrane (PEM, Nafion 117, Sigma-Aldrich) was used to separate the two sections. All chemicals and reagents used in experiments were analytical grade and supplied by Merck (Germany). PH-meter, Hana 211 (Romania) was a model glass electrode used to measure pH levels in the aqueous phase. DNS method was adopted to detect and measure substrate consumption using colorimetric method (Thomas and Chamberlin 1980) and cell growth was also monitored by optical density using spectrophotometer (Unico, USA). In addition, sodium disulfite (1 g/L) was added to anode compartment to minimize oxygen crossover phenomenon during operation which may result in a loss of electron transmission due to aerobic respiration by bacteria like *E. coli*, lowering overall Coulombic efficiency.

The medium and inoculum preparation

The medium prepared for seed culture consisted of glucose, yeast extract, NH_4Cl, peptone: 10, 1, 0.5, and 1 g/L, respectively. The medium was sterilized, autoclaved at 121 °C and 15 psig for 20 min. Treated whey was used as carbon source and the whey's carbohydrate was considered lactose. Medium pH was initially adjusted to 7 and the inoculum was introduced then the culture was incubated at 30 °C. *E. coli* was fully grown for duration of 24 h in 100 ml flux without any agitation. Samples were drawn in interval of 4 h and substrate consumption was analyzed on reduced sugar content by DNS method (Thomas and Chamberlin 1980).

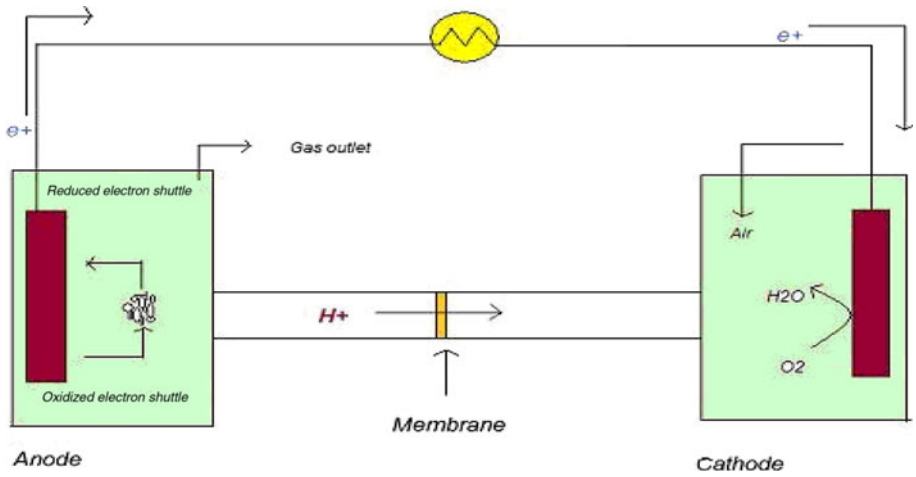

Fig. 1 Schematic diagram of a typical two-chamber MFC applied in the laboratory

Nafion proton exchange membrane and electrodes pretreatment

Nafion was subjected to a course of pretreatment to take off any impurities that has been simmering the film for 1 h in 3 % H_2O_2, 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 microbial fuel cell was not in use to maintain membrane for good conductivity. Electrodes were also exposed to a course of pretreatment, which has been soaking in 100 % ethanol for 45 min and in 1 M HCl for 1 h. After each use, electrodes were washed in 1.0 M HCl followed by 1.0 M NaOH, each for 1 h, to remove any metallic and organic contamination then stored in distilled water before use (Chae et al. 2008).

Data acquisition system

Analog digital data acquisition was used to register data point in every 6 s. The system had measurements for variable resistances, which were imposed on the MFC. The current in MFC was recorded, dividing the obtained voltage by the defined resistance. Then, the system provides power calculation by multiplication of voltage and current. In addition, the online system determines the polarization curves seen for power generation and MFC voltage with respect to current. The online system can operate while it operates in auto-mode; the assembled relays are able to regulate automatically the resistances. Voltage of MFC was amplified and then data was transmitted to a microcontroller by an accurate analog to digital converter. The microcontroller also sends the primary data to a computer by serial connection. In addition, special function of MATLAB software (7.4, 2007a) was used to store and synchronically display the obtained data.

Results and discussion

The schematic diagram of the fabricated MFC cell shown in Fig. 1 was used for power generation using whey as carbon source. The low-cost feed source was used for power production. Figure 2 shows open-circuit voltage (OCV) recorded for the MFC in period over 90 h.

The OCV measured for an MFC is the utmost voltage that can be obtained with the system, with the limitations imposed by the specific bacterial community. For an MFC, as with any power source, the objective is to maximize power output and therefore to obtain the highest current or current density under conditions of the maximum potential. As a general concept, the OCV is exactly achieved under a condition where there is infinite resistance. As that resistance is reduced, the voltage is decreased. Thus, it was expected to have the smallest possible loss in voltage as the current is increased to maximize the power production over the current range of interest. Initially, the voltage was less than 400 mV and then gradually increased. At this stage, carbon sources are utilized and products are formed. After

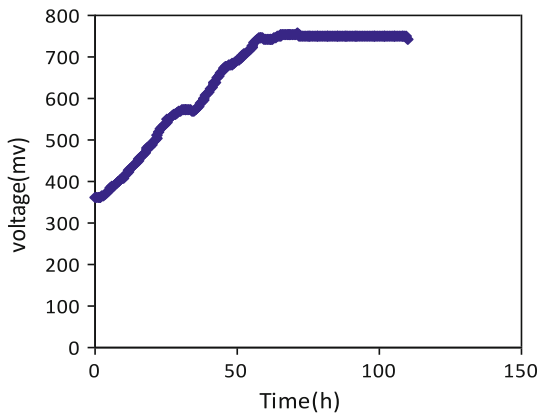

Fig. 2 Open-circuit voltage (OCV) of the MFC

60 h of operation, the OCV reached to a maximum value of 751.5 mV. The OCV was completely stable for more than 24 h. Finally, rapid utilization of substrate and accumulation of products may lead to stationary phase where the cell density and voltage remains constant. After 30 h, cell may start to die as the cell growth rate balances the death rate. It is well known that the biocatalytic activities of the cell gradually decrease as they age. To put it in a nutshell, the instability was a result of lactose consumption by *E. coli* in the anode compartments after 30 h. Figure 3 shows polarization curve presence of humic acid as mediator without using any catholyte.

A polarization curve is used to characterize current as a function of voltage. By changing the circuit external resistance (load), a new voltage will be obtained, and hence a new current at that resistance. Therefore, to obtain a polarization curve it should be used a series of different resistances on the circuit, measuring the voltage at each resistance. Then the current will be calculated as $I = E/R$, or the current density normalizing by an electrode surface area (usually the anode-not adapted here), and plot voltage versus current to obtain the polarization curve. This curve shows us how well the MFC maintains a voltage as a function of the current production. MFC researchers typically use the top point of the power curve to report the "maximum power", which for this case shown in Fig. 7 would be 562.9 μW. When reporting polarization and power densities, it is important to include the OCV and show a complete curve up to the maximum power, and then include a few points to the right of the maximum power to fully establish the peak in the power (density) curve. While generating power is a main goal of MFC operation, it is an important objective to extract as much of the electrons stored in the biomass as possible as current.

Figure 4 shows polarization curve presence of KI and $FeCl_3$ (III). Figure 5 shows polarization curve presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$. Figure 6 shows polarization curve presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$ with doubling of oxidant concentration without using any buffer solution or stirring in cathode compartment and finally Fig. 7, shows polarization curve as Fig. 6, but using agitation in cathode compartment. The presented data show power and voltage with respect to current generated in present MFC without using any magnet stirrer or buffer solution except Fig. 7 in cathode compartment. Maximum power generation and current among these taken figures

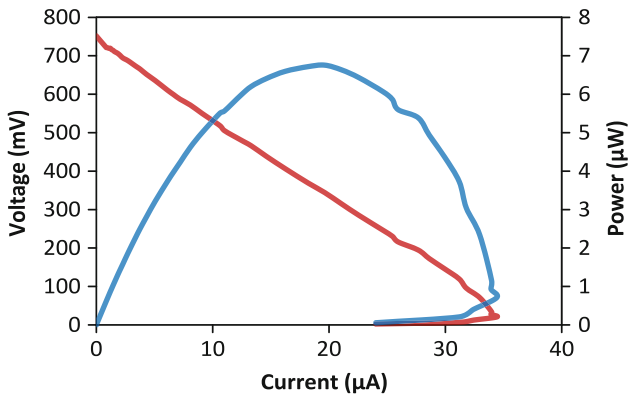

Fig. 3 Polarization curve presence of Humic acid

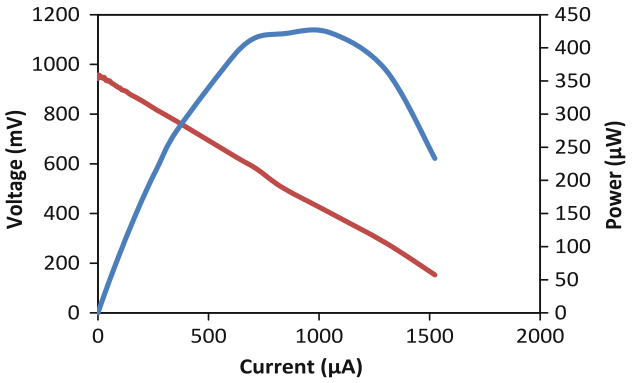

Fig. 4 Polarization curve presence of KI and $FeCl_3$ (III)

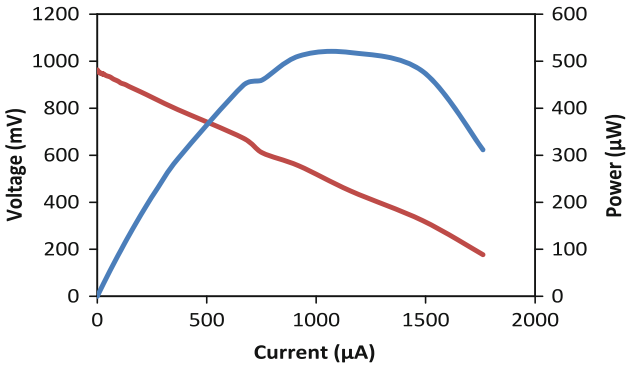

Fig. 5 Polarization curve presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$

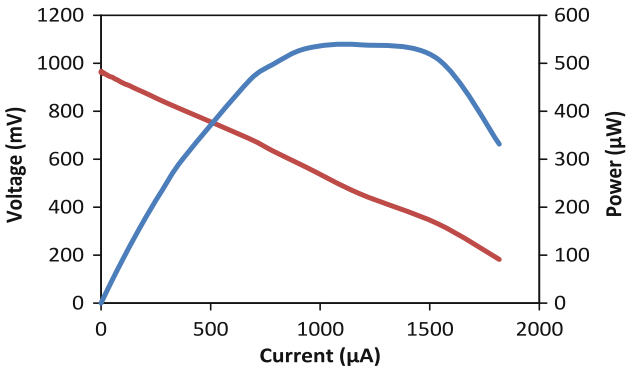

Fig. 6 Polarization curve presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$ with doubling of oxidant concentration without using any buffer solution or stirring in cathode compartment

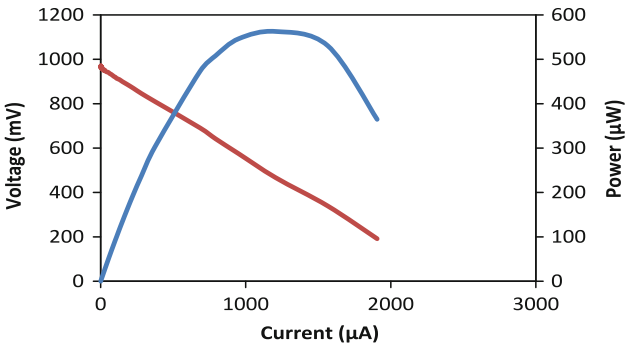

Fig. 7 Polarization curve presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$ with doubling of oxidant concentration using agitation in cathode compartment without any buffer solution

were 562.9 μW and 1906.1 μA, respectively. As it is clear, a remarkable increase in power is observed in comparison with previous work that just was adding mediators without using any combinational mode or changing in other factor effecting on MFCs like aeration or likewise (Nasirahmadi and Safekordi 2011).

Electron transfer in anode compartment was promoted by humic acid (brown) in the present MFC. Humic acid was selected as a mediator in MFC with concentration of 200 μmol/L. Humic acid is one of the major components of humic substances, which are dark brown and major constituents of soil organic matter humus that contributes to soil chemical and physical quality and are also precursors of some fossil fuels. They can also be found in peat, coal, many upland streams, dystrophic lakes, and ocean water. In fact, it is various complex organic acids obtained from humus; insoluble in acids and organic solvents. Humic acid had augmented the power production and cell current in MFC with diluted cheese whey as substrate. Maximum power generation was 6.75 μW while the current was boosted to the highest value of 34.4 μA. Moreover, the effect of mixed cathodic electron acceptors and doubling of oxidant concentration was investigated. The results of present polarization curves in Figs. 4, 5, 6 and 7 showed that KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$ collectively with doubling of oxidant concentration using agitation and lack of buffer solution in cathode compartment was a better choice for power generation in MFC with values of 562.9 μW and 1906.1 μA, respectively. Figure 8a, b and c show SEM images. These images demonstrated impurities accumulated on graphite surface. It is recommended to retreat electrode with that mentioned method in this paper to surmount efficiency. In addition, the images of the surface characteristic of graphite plate electrode with magnification of 4,410, 5,000 and 20,000 was obtained successfully by SEM. Graphite electrode was removed at the end of experiment and cut into pieces of about 1 × 1 cm for SEM analysis. Figures 8a, b and c show the outer surface of the graphite electrode with mentioned magnification, respectively. SEM images demonstrated microorganism has grown and impurities accumulated on the graphite surface.

Conclusion

These results are promising to fund on MFC technology using treated whey and combinational cathodic electron acceptors for power generation. Bioelectricity generation was successfully achieved in the MFC. Whey was used as carbon source for production of bioelectricity from *E. coli*. The MFC performance was enhanced using combinational chemical mediators. The power and current production in the presence of KI, $FeCl_3$ (III) and $MnCl_2{\cdot}4H_2O$ with doubling of oxidant concentration using agitation in cathode compartment without adding any buffer solution had increased to 562.9 μW and 1906 1μA, respectively. The two-chambered MFC with dairy industry wastewater, *E. coli* and combinational cathodic electron acceptor demonstrated their potential for bioelectricity generation. Combinational cathodic electron acceptors and oxygen concentration addition identified as effective factors for bioenergy production in dual chambered MFC. The maximum voltage was 0.971 V. According to SEM images, pretreatment of electrodes are needed for the next operation.

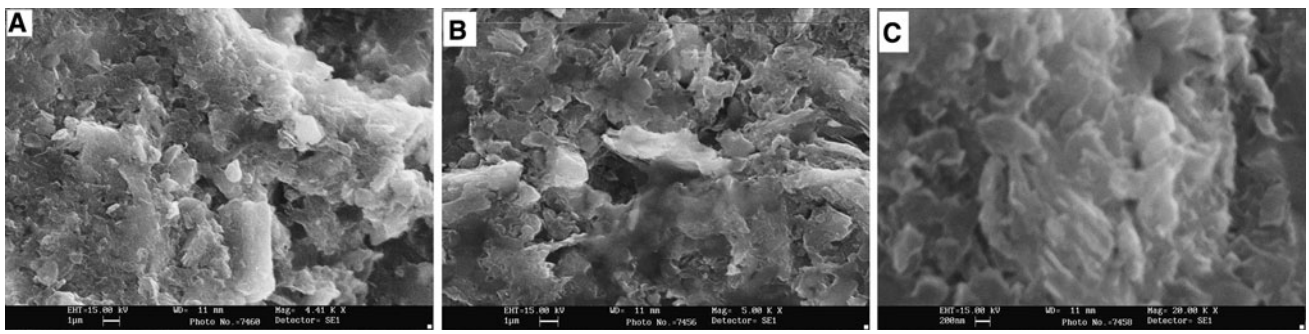

Fig. 8 Graphite electrode with different magnification

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