BIOELECTRICITY GENERATION FROM SYNTHETIC WASTEWATER TREATMENT IN A MEMBRANE-LESS MICROBIAL FUEL CELL USING METHYLENE BLUE AND/OR NEUTRAL RED AS MEDIATORS

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ABSTRACT

Microbial fuel cells (MFCs) represent a new method for treating wastewaters and simultaneously producing electricity (renewable energy (bioelectricity)) as innovative technologies. Feasibility of using synthetic wastewater as a substrate for electricity generation using anaerobic sludge as a source of microorganisms was investigated after a short acclimatization period of less than 10 days. among two different kind substrate (methanol and acetic acid) Significant reduction in COD of synthetic wastewater by 63% and 75% was observed at initial COD=2743 mg/l and 2560 mg/l respectively in Methanol and Acetic Acid indicated effective wastewater treatment in batch experiments.
The present article deals with the studies of a two chambered salt bridge (membrane-less) MFC anode chamber [(with mediator; plain graphite electrode; acidified by ortho-posphoric acid (pH≈6)]; cathode chamber (50mM potassium ferricyanide [$K_3Fe(CN)_6$] in phosphate buffer; pH ≈7.5; plain graphite electrode) in the presence of mediators. The effect of Methylene Blue (MB) and Neutral Red (NR) as electron mediators and microelements inoculated to anolyte chamber on the power generation in MFCs are reported.
The best performance was obtained in the case of Acetic Acid. Using methylene blue (MB) (0.2mM) as the electron mediator, the maximum power density and current density of 61.718 mW/m^2 and 92.530 mA/m^2 were obtained respectively with CE of 3.94%, which are found to be very promising. The maximum power density and current density of 58.820 mW/m^2 and 89.940 mA/m^2 were obtained respectively with CE of 8.05%.
In the most cases, results show that MB has more effective role than NR. Efforts are being made to improve the performance and reduce the construction and operating costs of MFCs.

KEYWORDS
Microbial Fuel Cell, Bioelectricity, Synthetic wastewater, Salt bridge, Electron mediator.

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1 INTRODUCTION

A lot of efforts has been taken to identify some options to produce energy. A technology using microbial fuel cells (MFCs) that convert the energy stored in chemical bonds in organic compounds to electrical energy achieved through the catalytic reactions by microorganisms has generated considerable interests among academic researchers in recent years.[1] Either single-or double-chamber MFC reactors can be constructed for electricity generation based on microbial oxidation of organic matter. Figure 1A and 1B presents basic configurations of MFC reactors and their electrochemical reactions.[2]

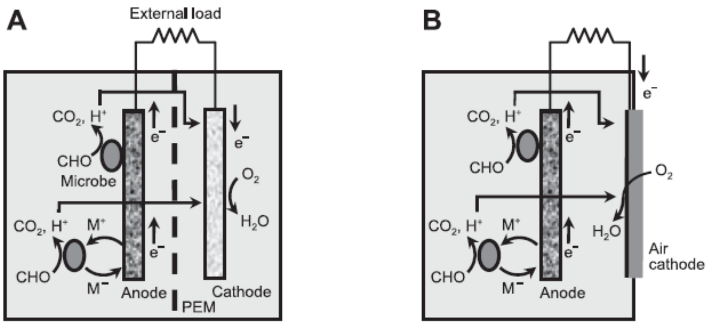

Figure 1. MFC configurations and electrochemical reactions.(A) A double-chamber MFC using oxygen as the cathode electron acceptor.(B)

In microbial cells, electrons released from organics are initially accepted by intercellular electron-shuttling compounds (e.g., nicotinamide adenine dinucleotide [NAD]), and subsequently transferred to electron acceptors via respiratory electron-transport chains. If a mechanism is present by which electrons released from organics can be transferred from any step in the intercellular electron-transfer pathway to an extracellular electrode (i.e., anode), then microbial oxidation of organics can be coupled with electricity generation (i.e., an MFC).[2]

Ferricyanide ($K_3[Fe(CN)_6]$) is very popular as an experimental electron acceptor in microbial fuel cells. The greatest advantage of ferricyanide is the low over potential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential.[3] Equation 1 shows the Ferricyanide oxidation reaction and its redox potential.

$$Fe(CN)_6^{3-} + e^- = Fe(CN)_6^{4-} \qquad (E^\circ = 0.430V) \tag{1}$$

Proton exchange membranes (PEMs) are flouropolymer membranes that ideally transfer protons from a region of lower reduction potential to a region of higher reduction potential. The other membranes used for the purpose are dialysis membranes, salt bridge, carbon papers, liquid Nafion layer, etc.[4] Nafion is the most intensively studied fuel cell membrane as it provides high ionic conductivity but the main limitations of Nafion are its high cost, so it is important to find an alternative. Min et al compared the performance of a PEM and a salt bridge in an MFC inoculated with G. metallireducens. The power output using the salt bridge MFC was 2.2 mW/m^2 that was an order of magnitude lower than that achieved using Nafion but it is cost-effective.[5] On the other hands membranes and Kaolin septum are prone to fouling if the fuel is something like municipal wastewater. Membrane-less MFCs are desired if fouling or cost of the membrane becomes a problem in such applications.[6]

Most of the microbial cells are electrochemically inactive .So in Mediator Microbial Fuel Cell, The electron transfer from microbial cells to the electrode is facilitated by mediators. Unless the species in the anode chamber are anodophiles, the microbes are incapable of transferring electrons directly to the anode. The outer layers of the majority of microbial species are composed of non-conductive lipid membrane, peptididoglycans and lipopolysaccharides that hinder the direct electron transfer to the anode.[1]

Mediators are the compounds with very low redox potential, lower than that of the cellular forward reactions. Hence, they trap and draw the electrons from membrane-bound reactions involving proton and electron transfer and supply those electrons to the anode electrode which is at a lower potential than the mediator itself.[4] This cyclic process accelerates the electron transfer rate and thus increases the power output. Good mediators should possess the following features:

- able to cross the cell membrane easily;
- able to grab electrons from the electron carries of the electron transport chains;
- possessing a high electrode reaction rate;
- having a good solubility in the anolyte;
- nonbiodegradable and non-toxic to microbes;
- low cost.[1]

Typical synthetic exogenousmediators include dyes and metallorganics such as neutral red (NR), methylene blue (MB), thionine, meldola's blue (MelB), 2-hydroxy-1,4-naphthoquinone (HNQ), and Fe(III)EDTA.[7]
Mediators are compounds added to the growth media at specific concentrations that squeeze out electrons from the growing cell and supply to the anode electrode.[4]
If no buffer solution is used in a working MFC, there will be an obvious pH difference between the anode and cathode chambers, though theoretically there will be no pH shift when the reaction rate of protons, electrons and oxygen at the cathode equals the production rate of protons at the anode.[1] With the addition of a phosphate buffer (pH 7.0), pH shifts at the cathode and anode were both less than 0.5 unit and the current output was increased about 1 to 2 folds.[8]
Fuel type, concentration and feed rate are important factors that impact the performance of an MFC. With a given microbe or microbial consortium, power density varies greatly using different fuels. Many systems have shown that electricity generation is dependent on fuel concentration both in batch and continuous-flow mode MFCs. Usually a higher fuel concentration yields a higher power output in a wide concentration range.[1] Moon et al. (2006) investigated the effects of fuel concentration on the performance of an MFC. Their study also showed that the power density was increased with the increase in fuel concentration.[9]

2 MATERIAL AND METHOD

2.1 MFC configuration

A two-chambered MFC used in the study was made up of Plexiglas (the thickness of each sheet was 0.5 mm). The working capacity of each chamber (anode and cathode) was 2L and

connected by a U-shaped glass tube salt bridge (inner diameter=1cm; contact cross section=0.78cm^2). The glass tube salt bridge was filled with 3M KCl and sealed with 12% Agar on the end of that. The contact area was sealed carefully with epoxy material.
Anodic chamber was designed to have sample/feed port, wire point input (top) and outlet port (bottom) to discharge reactor, then it was sealed with washers to ensure anaerobic microenvironment. Figure 2 shows the MFC reactor.

Figure 2. Photograph of two-chambered MFC used in the experiments.

Anode and Cathode electrodes were made of flat graphite, with 99.9% purity and 7.9cm×8.3cm×1.2cm dimensions. Total surface area of each electrode was 65.6cm^2, with no coated. The electrodes were connected with copper wire (length=32cm; diameter=1mm). the current and voltage between the anode and the cathode were measured with a multimeter.

2.2 Culture and medium

Granolas anaerobic sludge obtained from anaerobic digester of Kesht Va Sanate Emam khomeini wastewater treatment plant in Ahvaz then was washed and used as inoculums sludge. using sludge (mixed culture) provided a wide range of microorganisms in contact with feed and occurred an effective treatment. Methanol and/or Acetic Acid were used as the energy source for bacteria.
anolyte was consist of feed (COD~3000mg/l), inoculums sludge (VSS~6000mg/l), mediator, microelements and phosphate buffer to keep fix the pH. In anode chamber, acidophilic pH (6.0) was maintained to sustain the activity of acidogenic bacteria and to inhibit the activities of the methanogenic bacteria in order to enhance hydrogen production (H^+), hydrogen ions transfer to catholyte through salt bridge and contribute in electrochemical reactions.

Two different mediators such an Methylene Blue (MB) and Neutral Red (NR) with the same concentrations (0.2 mM) were used for carrying out the experiments. the chemical formula of mediators and their redox potential are presented in table 3.

Table 1. Identification of two mediators that are used in experiments.

mediators	Methylene Blue	Neutral Red
Chemical formula	$C_{16}H_{18}ClN_3F.XH_2O$ X=2-3	$C_{15}H_{17}ClN_4$
Redox potential	-0.145 mM	-0.320 mM

Microelements provide nutrients to microorganisms growth and increase the ionic strength of the media in the MFC, so they effects on the performance of the MFC. Addition of the salt probably increases the conductivity of the medium by decreasing the internal resistance; thus, higher current generation was observed when salt was added in the medium. Mohan et al. investigated on the effects of salt in performance of MFCs, they found that no current was detected in the absence of salt in the medium.[4] so the synthetic wastewater was enriched by adding 0.5 g/l NH_4Cl, 0.25 g/l KH_2PO_4, 0.3 g/l $MgCl_2$ and 0.005 g/l $CaCl_2$.

in this study, were used of ferricyanid in catholyte because of its greatest advantage as a mediator and the pH maintained at 7.0 by adding 200 mM phosphate buffer. table 2 described the catholyte components.

Table 2. Catholyte components and their values

Catholyte components	Chemical formula	values
ferricyanid	$K_3Fe(CN)_6$	32.88 g/l (200 mM)
Phosphate buffer	K_2HPO_4	20 g/l (230 mM)
Ortho-phosphoric acid 86%	H_3PO_4	By pH=7.0

2.3 Analyses

Power (W) was calculated using $P = IV$, where I is in amperes and V is the voltage in mV. Current density (mA/m^2) is calculated by dividing the obtained current with the surface area (m^2) of anode. Power density (mW/m^2) is calculated by dividing the obtained power with the surface area (m^2) of anode.
The columbic efficiency was calculated by equation 2.

$$\eta c(\%) = \frac{C_P}{C_T} \times 100 \quad (2)$$

Where C_p is the harvested coulombs calculated by integrating the current over operation time (corrected for the contribution of coulombs generated by anaerobic sludge decay); And C_T is the theoretical value of coulombs from substrate that was added at the beginning of operation of the MFC. C_T is calculated by equation 3.

$$C_T = \frac{F n S v}{M} \quad (3)$$

Where F is Faraday’s constant (96,487Cmol^{-1} electron), n is the mol number of electrons produced per mol of substrate (in COD units) oxidation, S is the substrate concentration (in g L^{-1} COD), v is the effective volume of the MFC (L), And M is the molecular weight for oxygen (M= 32).

Table 3. Number of electrons produced per mol of different substrates

Substrate	Reaction	Number of electrons produced per mol
Methanol	$CH_3OH + H_2O \rightarrow CO_2 + 6H^+ + 6e^-$	n=6
Acetic Acid	$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$	n=4

2.4 MFC operation

two-chambered MFC was operated in fed batch mode, the duration of each batch mode varied depending on the substrate concentration, but in general required 140h, after each duration, the anode chamber discharged and washed for the next duration. MFC reactor maintained at room temperature (20-25°C). parameters such as current, voltage and pH were monitored during the day and determination of COD was carried out twice a day. MLSS, VSS and EC were determined at the beginning and the end of each batch mode. All the parameters analysis according to Standard Methods for Water and Wastewater Analysis [10]. Operating conditions illustrated in table 4.

Table 4. Operating condition in each run of experiments

No.	Run / parameters	1	2	3	4
1	Substrate	Methanol	Methanol	Acetic Acid	Acetic Acid
2	COD as feed (mg/l)	2743	2871	2560	2808
3	Mediators	MB	NR	MB	NR
4	Mediator concentration (mM)	0.2	0.2	0.2	0.2
5	VSS (mg/l)	6140	5980	5640	5931

3 RESULT AND DISCUSSION

The two-chambered MFC was operated in 4 runs according to table 4. Electricity generation began to increase after started. the best results obtained in third run, when MB and Acetic Acid were chosen as a mediator and feed respectively.

the experiments shows that MB is a more suitable electron mediator than NR for fuel cells. in the case of MB as the mediator, maximum current density and power density, 92.53 mA/m^2 and 61.72 mW/m^2 (144hr) were observed respectively when Acetic Acid used as feed, and in the presence of Methanol, maximum current density and power density, 85.06 mA/m^2 and 53.67 mW/m^2 (144hr) were obtained respectively. Using NR as the electron mediator, maximum current density and power density, 89.94 mA/m^2, 58.82 mW/m^2 (140hr) and 65.6 mA/m^2, 38.28 mW/m^2 (138.5hr) were obtained in the presence of Acetic Acid and Methanol respectively. (see *Figure 3*)

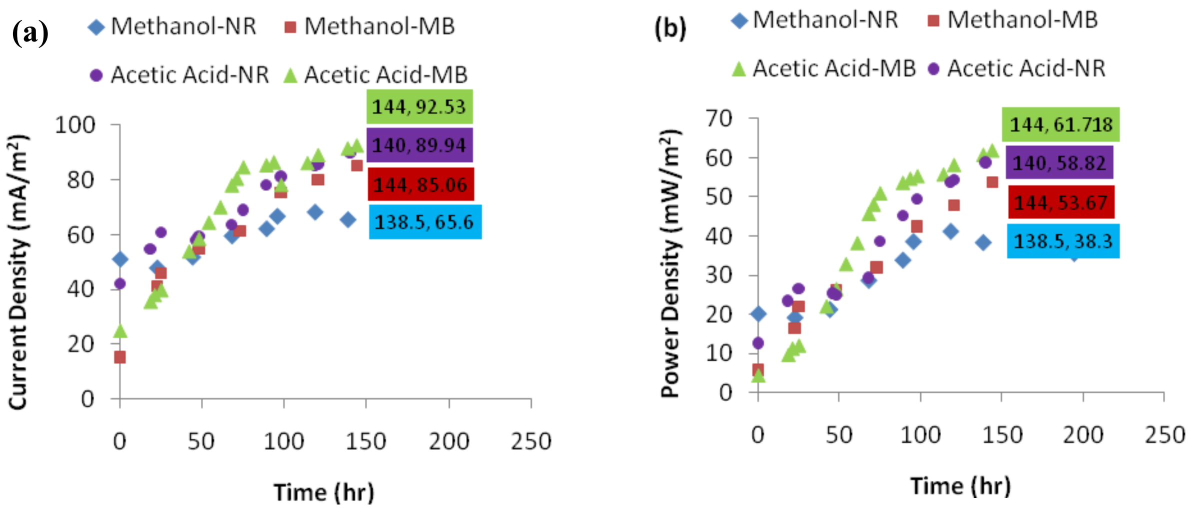

Figure 3. Current density (a) and power density (b) obtained using Methanol or Acetic Acid in presence of MB or NR during the operation time.

According to the redox potential values of electron mediators, NR (-0.320mV) that has a more negative redox potential than MB (-0.145mV) is expected to show higher power density when compared with MB but it has suggested by Mohan et al. that NR is more efficient when competing for the electron at the cell interaction stage but not efficient in transferring the electron to the anode electrode.[7]

Columbic efficiency has a reverse relation with COD removal efficiency. in this study COD removal for Acetic Acid and Methanol in presence of MB, 75% and 68% and in presence of NR, 56% and 52% were obtained respectively, so the columbic efficiency, 3.94%, 12.98%, 8.05% and 12.64% were obtained respectively. (see *Figure 4*)

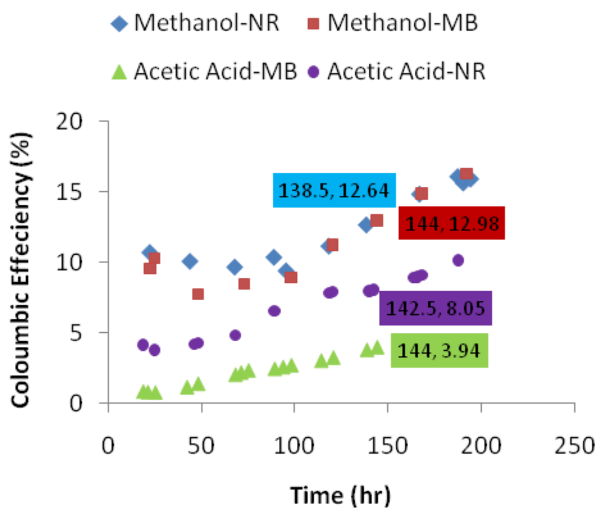

Figure 4. columbic efficiency changes during the operation time.

4 CONCLUSIONS

Salt bridge MFC is the simplest biological fuel cell that needs to be optimized for maximizing the power generation in any type of MFCs. In this study, regardless of the feeds type, MB has an effective role than NR to transfer electrons in all operating runs and cause significant increases in electricity generation.

In Compare with feeds, decomposition of Acetic Acid release more electrons than Methanol in the anode chamber, so the best performance was obtained when has been used of Acetic Acid in presence of MB.

Using mediators improved the power generation and it is found that some factors can effect on MFC 's proceeds such as type of the mediator, mediator concentration and ionic strength. all these factors need to be optimized in different kind of MFC to enhance energy generation.

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