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Enhancing Activity Of Electrogenic Bacteria In Microbial Fuel Cell By 2-Bromoethanesulphonate Dosing

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Abstract

Methanogens compete for substrate with electrogens in the microbial fuel cell (MFC) and reduces the power output. Performance of three dual chambered earthen pot MFCs, MFC-1 inoculated without 2-Bromoethanesulphonate (BES) addition in the inoculum, MFC-2 and MFC-3 inoculated by adding 0.5mM and 1.0 mM BES in the inoculum sludge, respectively, was compared. MFC-1 showed highest average chemical oxygen demand removal (84 %) as compared to MFC-2 (74 %) and MFC-3 (64 %). An average Coulombic efficiency (CE) of 6.94 ± 1.16 %, 8.99 ± 2.47 % and 11.88 ± 3.97 % was obtained in MFC-1, MFC-2 and MFC-3, respectively. Maximum power density obtained during polarization was 11.31 mW/m^2, 14.14 mW/m^2, and 39.20 mW/m^2 in MFC-1, MFC-2, and MFC-3, respectively. Suppressing methanogens by BES addition, the power produced by MFC-3 was 3.74 times higher than MFC-1. Addition of 1.0 mM BES significantly achieved suppression of methanogenesis and enhanced the power generation.

1. Introduction

Increasing population and industrialization demands higher and higher amount of energy worldwide. This is forcing the research community in search of alternative sustainable energy source to fulfill the ever increasing energy demand. As estimated, the existing reserves of fossil fuels are going to extinct in the next 25-30 years due to its limiting availability. Energy is an essential ingredient of socio-economic development and economic growth. Renewable energy provides a valuable and environmental friendly option to minimize the limited fossil fuel needs. Microbial Fuel Cell (MFC) is one such alternative technology which reclaims the wastewater and generates electricity without causing pollution to the environment.

MFC is a technology which converts chemical energy into electrical energy with the help of bacteria which are used as a catalyst. The first observation of electrical current generated by bacteria is generally credited to Potter [1]. The MFCs are being developed today for power generation as well as treatment of wastewater simultaneously, thus it could offer an alternative source of energy using wastewater as a fuel. The electrogenic bacteria which help in generating electricity can be enriched from sewage and sewage sludge. These bacteria generate electrons and protons from the oxidation of organic matter in the anode chamber. Traditionally MFCs are two chambered device consisting of an anode, where the bacteria grow and generate electrons and protons from oxidation of organic matter, and a cathode where electrons react with oxidant supplied in catholyte. These electrons reduce anode and are transferred towards cathode through external circuit. Protons migrate towards cathode through the separator which separates anode and cathode chamber. On cathode the electrons and protons react with oxygen, generally supplied as an oxidant, to form water.

To enhance the current production, chemical mediators were used in the past to help in carrying the electrons from inside the cell to exogenous electrodes [2]. However, with advancement in knowledge and identification of several bacterial strains having ability to directly reduce the anode, such addition of mediators is considered to be not necessary.

MFC technologies also results in savings in expenses for aeration and excess solids handling as required in conventional sewage treatment plant. The benefits of using MFC for the wastewater treatment include: clean, safe, high efficiency, low emissions, good performance and direct electricity recovery. But this technology is still under research and yet not used for large scale wastewater treatment. The use of polymer proton exchange membrane increases the cost of MFC fabrication and it cannot compete with the existing wastewater treatment technologies. Hence, research is required to find suitable cheaper alternative for this costly membrane. MFC can be constructed at much lower rates using ceramic membrane made from selected clays as a proton exchange membrane [3].

For scaling up of MFCs, advantage of using mixed anaerobic sludge as a source of inoculum over pure culture is that they are easily available in large quantities, more tolerant to environmental conditions, and can accept variety of substrate [4]. Since the growth condition of electrogens and methanogens are similar, therefore methane production in MFC is a common problem observed during operation, particularly when inoculated with mixed anaerobic sludge [5, 6]. Methanogens in the MFCs compete for substrate with electrogenic bacteria and reduce the power output. Therefore, it is necessary to suppress methanogenesis in the MFC, without affecting the activity of electrogenic bacteria in order to enhance the power generation in MFC.

Different treatment methods have been employed to suppress methanogenic activity like exposure to air, heat treatment of the inoculum sludge, acid/alkaline treatment, addition of chemical inhibitors, shock loading, infrared, freezing and ultra-sonication pre-treatment [7-10]. Even a propionic acid with high concentration have significant effect on inhibition of methanogenesis [11]. Bodegaom et al. [12] observed the addition of amorphous $Fe(OH)_3$ at 5 mM concentration inhibited methanogens; however, the sensitivity toward Fe (III) was higher for methanogens culture utilizing H_2/CO_2 as substrate than that with acetate. A slight exposure of anodic inoculum to air also resulted in higher MFC power production [13]. Recently, a microwave pre-treatment shown significant improvement in MFC electricity productivity compared to ozonation pre-treatment [14].

Coenzyme M (CoM) is a cofactor which is normally present in all methanogens but not in other bacteria or archaea [15]. It is actively involved in the terminal step of methane biosynthesis. The methanogenic inhibitors involved in this group include 2-bromoethanesulfonate (BES), 2-chloroethanesulfonate (CES), 2-mercaptoethanesulfonate (MES), and lumazine. These chemicals have ability to competitively inhibit the methyl transfer reaction during methane formation [16].

It was reported that an injection of BES, even at very low dose (0.1–0.27 mM), effectively inhibited the bioactivity of methanogens and lowered methane production from 66% to 25% [17]. BES addition in a microbial electrolysis cell (MEC) not only suppresses the methanogens but also helped in improvement of hydrogen production. Almost complete inhibition was achieved at 572 µM of BES dose with hydrogen production increment [18]. BES with chloroform not only inhibits methanogenesis but also H_2 dependent homoacetogenesis responsible for acetate formation [19]. It was reported that the BES addition increased the CE from 40% to 70% using a ferric iron medium. In the absence of the inhibitor, methane accumulation in the headspace of the reactors was observed [6].

Lot of research work has been conducted using earthen pot microbial fuel cell with different operating condition, in order to lower down the cost of MFC and increase power generation while achieving wastewater treatment [20-22]. However, these studies could not address the suppression of methanogens using chemical inhibitors. This study aimed at evaluating performance of earthen pot MFCs inoculated with chemically pretreated anaerobic sludge at different dosage of BES. The performance of these MFCs is compared with performance of MFC inoculated without BES pretreatment to the inoculum sludge.

2. Materials and methods

2.1 Experimental setup

The study was carried out using three double-chambered microbial fuel cells (MFC-1, MFC-2 and MFC-3) which were made up of earthen pot of 0.42 liter capacity anodic volume and plastic containers having a working volume of 4.0 L acting as cathode chamber. Earthen pot made from locally available red soil (major compound present: Na_2O - 3.95%, MgO - 0.654%, Al_2O_3 - 26.3%, SiO_2 - 57.5%, P_2O_5 - 1.13%, K_2O - 1.78%, SO_3 - 0.258%, CaO - 0.791%, Fe_2O_3 - 4.75%, Ti - 0.658%) was used in this study. Stainless steel mesh having total surface area of 166 cm^2 was used as anode electrode and graphite plates, having total surface area of 68.25 cm^2, was used as cathode electrode in all the MFCs. The graphite plate cathode is placed at the nearest distance of 1.0 cm at the centre of the plate and maximum distance of 2.5 cm at the edge of the plate from the earthen pot. Tap water was used as cathodic electrolyte which was continuously aerated with an aquarium air pump. The anode and cathode electrodes were connected externally with concealed copper wire through external load resistance of 100 Ω resistance.

2.2 Inoculation and MFC operation

Anaerobic mixed sludge collected from septic tank bottom was used as the inoculum in the anodic chamber of the MFC-1 without any pretreatment. Chemical treatment was given to sludge inoculum used in MFC-2 and MFC-3 with 0.5 mM and 1 mM of 2-bromoethanesulphonate, respectively. Synthetic wastewater containing acetate as a carbon source having chemical oxygen demand (COD) of about 3000 $mg \cdot L^{-1}$ was used in this study. The other ingredients of the synthetic wastewater were similar to as described by Jadhav and Ghangrekar [23]. These MFCs were operated in the temperature range of 33 to 35°C, maintained by using aqueous heater. MFCs were operated in fed batch mode with a feed cycle of five days for total duration of 30 days. After reaching to a stable power generation and COD removal, polarization study was conducted by varying the external load resistance.

2.3 Analysis and calculations

The chemical composition of red soil, used for fabrication of earthen pot, was determined by X-ray fluorescence analysis (model-AXIOS, Panalytical, Netherland). The performance of MFCs was evaluated in terms of voltage (*V*) and current (*I*) measured using a digital multimeter with data acquisition unit (Agilent Technologies, Malaysia) and converted to power according to

$P = I * V$ (1)

Where, P = power (W), I = current (A), and V = voltage (V). Power density and power per unit volume were calculated by normalizing power to anode surface area and net liquid volume of anode chamber, respectively. The current density i_d was calculated using

$i_d = V/(R*A_d)$ (2)

Where, R the external resistance (Ω) and A_d (m^2) is the surface area of the anode. The Coulombic efficiency (CE) is defined as the ratio of total Coulombs actually transferred to the anode from the substrate, to maximum possible Coulombs if all substrate removal produced current. The CE of the MFC operated under fed batch mode over a period of time t, was calculated as [24].

$$CE = \frac{M\int_0^t I dt}{Fbv\Delta COD} \quad (3)$$

Where, v is the volume of the anode chamber of MFC. M = 32, molecular weight of oxygen; F, Faraday's constant = 96485 C/mol; b = 4, the number of electrons exchanged per mole of oxygen; ΔCOD is the difference in the influent and effluent COD.

Polarization studies were carried out after reaching a stable cell potential by changing the external resistances from 10,000 to 10 Ω. The internal resistance of the MFC was measured from the slope of line from the plot of voltage versus current [25]. Open circuit voltage (OCV) and operating voltage (OV) across 100 Ω resistance were measured after reaching to stable value. Influent and effluent COD concentrations, pH and volatile suspended solids of the inoculum were measured according to APHA standard methods [26].

3. Results and discussion

3.1 Organic matter removal

After initial acclimation for the total duration of two weeks, all the MFCs used in the present study delivered stable performance for organic matter removal. The COD removal obtained in MFC-1 was 13.5 % and 31.2 % higher than MFC-2 and MFC-3, respectively (Table 1). This higher COD removal indicates the presence of active methanogens in the sludge inoculum of MFC-1. The decrease in COD removal in MFC-2 and MFC-3 compared to MFC-1 shows BES has effectively suppressed methanogenic activity in the anode chamber of these MFCs. The COD removal efficiency of MFC-2 was 15.6% higher than MFC-3 demonstrating higher dose of BES in MFC-3 is resulting in more suppression of methanogens and hence decreasing the COD removal.

Table 1. COD removal efficiency of the earthen pot MFCs obtained at different feed cycles

Feed Cycle	COD removal efficiency (%)		
	MFC-1	MFC-2	MFC-3
1	68.2	61.7	52.5
2	72.2	64.4	56.8
3	78.9	80.2	66.4
4	86.8	72.7	64.2
5	89.3	69.2	61.6
6	79.8	76.4	68.4

However, the COD removal obtained in the present study with 0.5 and 1.0 mM BES was quite lower than 87 % reported with 0.95 mM BES for treatment of dairy wastewater [27]. Presence of active methanogens has been observed in different MFCs inoculated with anaerobic sludge along with naturally occurring microbial communities [5, 6]. Substrate utilization by these methanogens reduces power output of the MFC, hence to harvest more power from this device suppression of methanogens is utmost necessary.

3.2 Electricity generation

Power density is an important parameter for determining the cost-effectiveness of MFCs, if electricity production is the sole purpose of their application. The current and voltage generation in all the MFCs increased with the feed cycles with simultaneous increase in COD removal efficiency. The maximum voltage generation across 100 Ω external resistance in the MFC-3 was 39 % and 48 % higher than MFC-2 and MFC-1, respectively. The highest sustainable power density obtained in MFC-3 was 39.17 mW/m^2 (1552 mW/m^3); which was 42.90 % and 78.60 % higher than MFC-1 and MFC-2, respectively (Table 2). This power density was quite higher than 30 mW/m^2, reported earlier for iron oxide-coated carbon paper electrode with BES addition [6].

Table 2 Electrical parameters obtained for earthen pot MFCs at different feed cycles

Feed cycle	Param-eter	MFC-1	MFC-2	MFC-3
1	OCV	459 ± 24	565 ± 36	499 ± 24
	OV	39 ± 5	89 ± 13	165 ± 10
	PD	0.91	4.8	16.4
	VPD	36	188	648
2	OCV	545 ± 38	614 ± 13	536 ± 24
	OV	74 ± 14	128 ± 9	167 ± 6
	PD	3.3	9.87	16.8
	VPD	130	390	664

3	OCV	569 ± 18	598 ± 21	547 ± 11
	OV	119 ± 10	155 ± 9	189 ± 14
	PD	8.5	14.5	21.5
	VPD	337	572	850
4	OCV	581 ± 28	607 ± 15	587 ± 19
	OV	132 ± 8	154 ± 7	255 ± 12
	PD	10.49	14.3	39.17
	VPD	415	565	1552
5	OCV	572 ± 23	526 ± 20	603 ± 14
	OV	126 ± 16	148 ± 28	214 ± 13
	PD	9.56	13.2	27.6
	VPD	378	522	1086
6	OCV	589 ± 19	606 ± 21	522 ± 27
	OV	117 ± 16	139 ± 11	206 ± 9
	PD	8.24	11.6	25.56
	VPD	326	460	1010

OCV- Open circuit voltage (mV)
OCV- Open circuit voltage (mV)
OV-Open circuit voltage (mV)
PD- Power density with 100 Ω, (mW.m^{-2})
VPD- Volumetric power density with 100 Ω, (mW.m^{3})

The maximum sustainable power delivered by MFC-2 and MFC-3 was higher than MFC-1 in all feed cycles (Table 2). This indicates that BES addition strongly suppress the methanogenesis in these MFCs, and provided conditions favorable for growth of electrogens to harness more power. However, the power densities obtained in the present study was quite lower than 3700 mW/m^2 reported while treating cellulosic biomass using MFC in the presence of fermentative inhibitors [28], demanding further research to enhance power.

3.3 Polarization and internal resistance

Polarization study was carried out to evaluate the effect of external resistance on power production. During polarization the maximum power density of 11.31 mW/m^2 (Figure 1) was obtained in the MFC-1; whereas, it was 14.14 mW/m^2 in the MFC-2 and 39.20 mW/m^2 in MFC-3, respectively. The power density of MFC-3 was 3.74 times and 2.75 times higher than MFC-1 and MFC-2, respectively. The increase in power output was more pronounced in MFCs treated with BES.

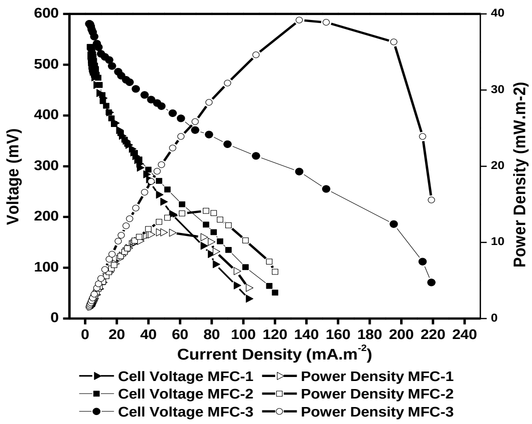

Figure 1. MFC polarization curves and power density for MFC-1, MFC-2 and MFC-3

The internal resistance of the MFC-1, MFC-2 and MFC-3 was 306 Ω, 247 Ω and 182 Ω, respectively. The lower internal resistance of the MFC-2 and MFC-3 compared to MFC-1 indicates addition of BES resulted in suppression of methanogens and favoured development of more enriched biofilm of electrogenic species on the anode surface, which reduced the activation loss. Suppression of methanogens might have also decreased the competition for substrate and reduced the population of alternate bacteria occupying the space on electrode. This enhanced the supply of reduced species toward the electrode and decreased the anode concentration losses.

3.4 Coulombic efficiency

The highest CE obtained by MFC-1, MFC-2 and MFC-3 was 8.22 %, 10.55 % and 15.36 %, respectively (Figure 2). The CE was more in the MFC-3 where the inoculum was treated with higher BES dose as compared to MFC-2 where 50% lower BES dose was given during pretreatment of inoculum. The low CE obtained in MFC-1 demonstrates that a large portion the substrate was utilized by methanogenic microorganisms during the substrate oxidation which could not be made available for electrogens for current generation. Thus, MFC-1 demonstrated better performance for organic matter removal due to presence of methanogens as well as electrogens and gave lower power output than the MFC-2 and MFC-3; wherein the methogens were suppressed in the later. However, the maximum CE observed in MFC-2 and MFC-3 in the present studies were quite lower than 70% as reported earlier for iron oxide-coated carbon paper electrode with BES addition[6]. Hence there is a scope to improve system performance further by modifying the electrodes and system configuration. It is reported that the addition of higher BES dose (50 mM) suppressed methanogenesis and increased Coulombic efficiency by 40 % as compared to without BES [29]. This suggests that the combined effect of better electrode material along with chemical inhibitors can enhance the power generation in MFCs.

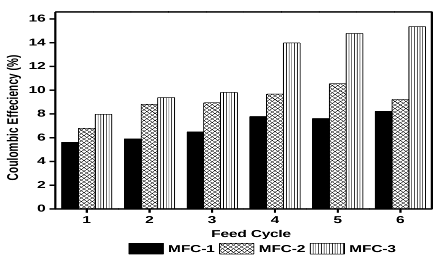

Figure 2. Coulombic Efficiency obtained at different feed cycles

4. Conclusions

The study demonstrated that in MFCs, inoculated with mixed anaerobic sludge, methanogens act as a major alternative to consume substrate and compete with electrogenic bacteria for substrate and hence reduce the power generation and Coulombic efficiency. Injection of 2-bromoethanesulphonate in the anode chamber of MFC proved to be effective method for controlling methanogenesis in anodic biofilms without damaging electrogenic bacteria. MFC with 1 mM BES dose gave 3.74 times higher power density as compared to MFC operated without BES dosing. Further improvement in power output of MFC can be made by modifying the electrode materials and system configurations.

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