



Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production

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Abstract

Performance of mediator-less and membrane-less microbial fuel cell (ML–MFC) was evaluated to treat synthetic wastewater and actual sewage. The ML–MFC gave COD and BOD removal efficiencies of 88% and 87%, respectively, and TKN removal was around 45–50%. Biomass granulation was observed in the anode compartment of ML–MFC. Effect of distance between the electrodes and total surface area of anode on electricity production was evaluated under variable external resistance. Maximum power density of 10.9 and 10.13 mW/m² was observed at lower spacing between the electrodes (20 cm) and for lesser surface area of the anode, respectively. With variation in the carbon source in the feed, variation in power production was observed.

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Keywords: MFC; Wastewater treatment; Power output; Electrode area; Electrode spacing; Granulation

1. Introduction

High energy requirement of conventional sewage treatment systems are demanding for the alternative treatment technology, which will be cost effective and require less energy for its efficient operation. In addition, due to global environmental concerns and energy insecurity, there is emergent interest to find out sustainable and clean energy source with minimal or zero use of hydrocarbons. Bacteria can be used in fuel cell to catalyze the conversion of organic matter, present in the wastewater, into electricity (Allen and Bennetto, 1993; Kim et al., 2002; Gil et al., 2003; Bond and Lovley, 2003). Microbial fuel cells (MFC), if used for wastewater treatment, can provide clean energy for people, apart from effective treatment of wastewater. The benefits of using MFC for wastewater treatment include: clean, safe, quiet performance, low emissions, high efficiency, and direct electricity recovery.

Traditionally, MFC consists of two chambers, anode and cathode, separated by proton exchange membrane (PEM). Microorganisms oxidize the substrate and produce electrons and protons in the anode chamber. Electrons, collected on the anode, are transported to cathode by external circuit and protons are transferred through the membrane internally. Thus, potential difference is produced between anode chamber and cathode chamber due to dissimilar liquid solutions. Electrons and protons are consumed in the cathode chamber by reducing oxygen, usually from water.

Most of the bacterial species used in fuel cells are known to be inactive for transport of electrons. Hence, for intervention synthetic and natural compounds, called redox mediators, are used, such as, neutral red, methylene blue, thionine, and humic acid (Delaney et al., 1984; Lithgow et al., 1986; Park and Zeikus, 2000). With addition of such mediators, commercial application of MFC for wastewater treatment becomes difficult, because most of these mediators are expensive and toxic in nature. Hence, today it is emphasized to develop mediator-less MFC, by enhancing its power production and reduction of its operational cost, to increase its acceptance as wastewater treatment process.

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Recently, it has been demonstrated that an iron-reducer bacteria *Shewanella putrefaciens*, family *Geobacteraceae* and few other bacteria can directly transfer electrons to electrodes using electrochemically active redox enzymes (Kim et al., 1999a,b; Kaufmann and Lovely, 2001; Magnusson et al., 2000; Bullen et al., 2006).

In the operation of mediator-less MFC several factors are considered as limiting steps for electricity generation, such as, fuel oxidation at the anode, presence of electrochemically active redox enzymes for efficient electrons transfer to the anode, external resistance of the circuit, proton transfer through the membrane to the cathode, and oxygen reduction at the cathode. Proton transfer to the cathode chamber can be a limiting factor when proton permeability of the membrane is poor. Under limited proton transfer conditions, microbial activity and electron transfer to the electrode in anode chamber can be reduced due to change in pH, apart from slow cathode reaction due to limited protons supply (Gil et al., 2003).

Application of MFC in large-scale wastewater treatment, containing suspended solids, might be limited due to high initial cost and fouling of the membrane, requiring replacement. If use of membrane is eliminated, acceptability of MFC for wastewater treatment would increase. A membrane-less microbial fuel cell (ML-MFC) was used by Jang et al. (2004), which converted organic contaminants from artificial wastewater to electricity. Such membrane-less MFC can improve the economic feasibility and acceptability. Hence, the objectives of the present study were: (i) to evaluate effectiveness of membrane-less MFC, inoculated with anaerobic sludge, for chemical oxygen demand (COD), biochemical oxygen demand (BOD) and nitrogen removal from synthetic wastewater and actual sewage; and (ii) to study the effect of surface area and distance between the electrodes on electricity production using graphite electrodes.

2. Methods

2.1. Membrane-less microbial fuel cell

The ML-MFC used in the study was made up of acrylic cylinder having effective height of 60 cm and internal diameter of 15 cm. Anode compartment (depth 26 cm) was placed at bottom and cathode compartment (depth 26 cm) was as at top. Glass wool (4 cm depth) and glass bead (4 cm depth) were placed at the upper portion of the anode compartment, supported by perforated acrylic sheet. Schematic diagram of the ML-MFC used in this study is shown in Fig. 1. Three graphite rods were placed in the anode and cathode chambers, to be used as electrodes. The distances between the respective anode and cathode electrodes were 20 cm, 24 cm, and 28 cm. Total apparent surface area of three identical anode electrodes was 210.64 cm². The fuel was supplied from the bottom of the anode chamber and the effluent was discharged through the cathode chamber at top. The electrodes were connected with copper wire

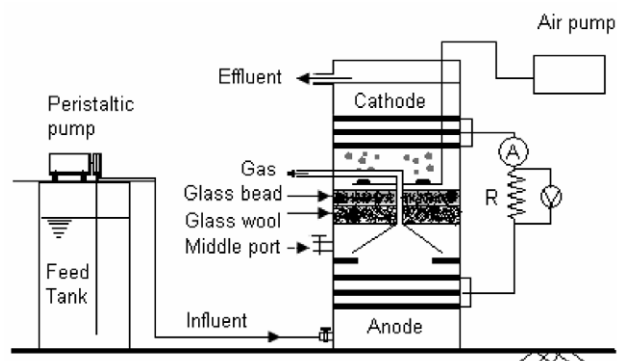


Fig. 1. Schematic diagram of membrane-less microbial fuel cell used in the study.

through the resistance ranging from 10 to 4000 Ω , including the resistance of copper wire and a multimeter.

2.2. Wastewater composition and inoculation of MFC

Wastewater was applied at the rate of 5.011 l/d to the ML-MFC making total hydraulic retention time (HRT) of 49.8 h. The cathode compartment was aerated at rate of 60 ml/min. Synthetic wastewater containing sucrose as a carbon source was used in the study, unless specified, having COD concentration around 325 mg/l. The synthetic wastewater was prepared by adding (mg/l) 312 sucrose, 480 NaHCO₃, 95.5 NH₄Cl, 10.5 K₂HPO₄, 5.25 KH₂PO₄, 63.1 CaCl₂·2H₂O, and 19.2 MgSO₄·7H₂O. Trace metals (Fe, Ni, Mn, Zn, Co, Cu, and Mo) were added as per the composition suggested by Ghangrekar et al. (2005). Influent pH was maintained in the range 7.2–7.6. The ML-MFC was inoculated with anaerobic sludge collected from septic tank bottom. The inoculum sludge was sieved through 1 mm opening sieve and heated at 100 °C for 15 min to suppress the methanogens, cooled at room temperature, and 2.0 l volume of sludge was added to the anode compartment. No microbial addition was carried out in the cathode compartment. The ML-MFC was operated at room temperature ranging from 29 to 33 °C for 75 days.

2.3. Analyses

The potential and current were measured using a digital multimeter and converted to power according to $P = iV$, where P = power (W), i = current (A), and V = voltage (V). All electrical parameters were monitored daily. Under variable external resistance the voltage and current were recorded after allowing the circuit to stabilize for five to ten minutes. Grab samples were collected for determination of influent and effluent characteristics. Parameters, such as, COD, pH, dissolve oxygen (DO), suspended solids (SS), volatile suspended solids (VSS), ammonical nitrogen and total Kjeldahl nitrogen (TKN) were monitored according to APHA et al. (1998). BOD was determined for three days at 27 °C. The nitrate nitrogen was measured using electrode

(Orion make, USA). Determination of pH and DO was carried out daily and COD was determined every alternate day. BOD, SS, VSS, and nitrogen in different forms were determined once in a week, after achieving steady state. Microstructures, of the biofilm developed on the electrodes and granules from anode chamber, were examined using scanning electron microscope (Model JSM-5800, JOEL, Japan). The samples were prepared as per the protocol provided by Fang et al. (1994).

3. Results and discussion

3.1. COD removal efficiency

The ML–MFC was operated at influent COD concentration in the range of 310–350 mg/l as presented in Table 1. After 17 days of operation, when steady state condition for COD removal reached, the COD and BOD reductions were 88% and 87%, respectively. Volumetric COD loading rate to this ML–MFC was 0.16 kg COD/m³d. After achieving steady state, the average effluent COD was 38 mg/l and BOD₃ at 27°C was 28.6 mg/l. The effluent BOD was determined for unsettled effluent samples containing average SS and VSS of 37.6 and 8.3 mg/l, respectively. Further improvement in the effluent quality is expected by using settling tank after ML–MFC. The observed COD removal efficiency was on the higher side of the maximum reported efficiency in the range of 80–90% (Liu et al., 2004; Jang et al., 2004). Similarly, BOD removal efficiency was higher than 78%, reported by Liu et al. (2004). The minimum unsettled BOD and settled COD values observed in the effluent were 20.9 and 29.14 mg/l, respectively. Further studies are required to explore maximum volumetric loading rate capacity for this ML–MFC.

The COD removal percentage in anode chamber was 46.3% and remaining COD was getting removed in the cathode chamber. For single chamber MFC, operated at HRT of 33 h, COD removal efficiency of 50–70% was reported by Liu et al. (2004). In this study, the ML–MFC was operated at HRT of 24.9 h with respect to anode chamber. Lower COD removal efficiency observed in this case could be attributed to the mixed culture used as inoculum. Further investigation would be necessary to enhance the COD removal in anode compartment and, hence, to increase current production. However, the overall efficiencies observed for COD and BOD removal demonstrated the ability of ML–MFC as an effective wastewater treatment process.

Gas production in anode compartment was measured by providing gas deflector arrangement and inverted cone above the anode chamber in the ML–MFC as shown in Fig. 1. The gas production rate was 29.75 ml/d, which was far lower than the gas production expected in the high rate anaerobic processes. The cathode chamber was aerated by supplying compressed air. As a result, DO in the effluent was in the range of 4–5 mg/l. Even with continuous aeration, lower values of DO in the cathode chamber and in the effluent were due to simultaneous utilization of DO for the cathode reaction, where oxygen is reduced. In addition, some amount of DO might have been utilized for nitrification in the cathode chamber.

3.2. Effect of change of substrate

For evaluating the effect of change of substrate on the performance of ML–MFC, on 19th day of operation lactose and on 20th day dextrose was used as a carbon source instead of sucrose. On the day 35 and 36, settled sewage was used as an influent to the ML–MFC. The performance of ML–MFC with change in substrate, at similar COD concentration, is presented in Fig. 2. When lactose and dextrose were used as substrates, reduction in COD removal up to 80% was observed. However, increase in current production was observed for both of these substrates than sucrose. The current productions were 0.71 and 0.67 mA, when lactose and dextrose were used in the feed, respectively. When sewage was used as an influent (152 mg COD/l), the COD removal efficiency was 75.7%, and the current production was 0.33 mA. The effluent COD concentration

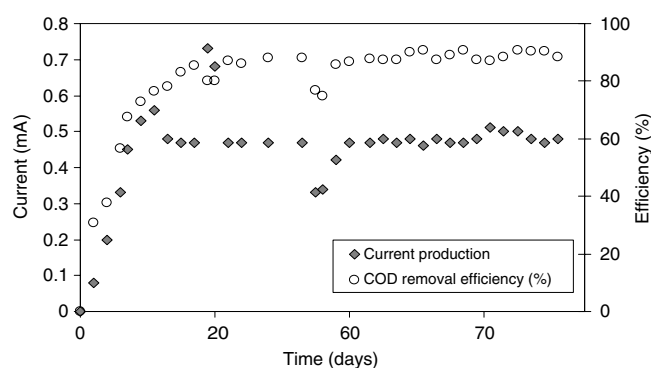


Fig. 2. COD removal and current production observed in membrane-less microbial fuel cell. (On 19th day lactose and on 20th day dextrose was used as a carbon source instead of sucrose in the synthetic wastewater. On 35th and 36th day sewage was used as feed.)

Table 1

Performance of the ML–MFC for COD and BOD removal

Days	External resistance (Ω)	COD (inlet) (mg/l)	COD (middle) (mg/l)	COD (outlet) (mg/l)	Efficiency (%)	BOD (Inlet) (mg/l)	BOD (middle) (mg/l)	BOD (outlet) (mg/l)	DO (outlet) (mg/l)
0–17	100	310.33–350.46	210.66–249.66	92.33–193.66	37.59–70.91	ND	ND	ND	4.88 (± 0.2)
18–75	100	324.19 (± 9.5)	174.06 (± 11.7)	38.03 (± 5.85)	88.24 (± 1.8)	225.92 (± 8.75)	97.75 (± 20.13)	29.6 (± 6.4)	4.84 (± 0.1)

ND – not determined.

during sewage treatment was 37 mg/l, which was similar to the effluent COD concentration when synthetic feed was used. Variation in the current production under different substrates can be explained based on the half-reaction for these substrates with different proton and electron production. Maximum closed circuit voltage was observed when lactose was used as substrate in synthetic feed; whereas, closed circuit voltage was minimum when sewage was used as feed.

3.3. Nitrogen removal in ML–MFC

The nitrogen was supplemented in the form of ammonical nitrogen to fulfil the nutrient requirement in the synthetic wastewater. During performance evaluation of ML–MFC, the TKN removal of 45.7% was observed while treating synthetic wastewater; and it was 50% when sewage was used as an influent. Different form of nitrogen observed during performance evaluation of ML–MFC is presented in Table 2. Major TKN removal occurred in the cathode chamber. This could be attributed to ammonia stripping due to aeration, and occurrence of simultaneous nitrification and denitrification, in the cathode chamber. Removal of small amount of TKN and $\text{NH}_4\text{-N}$ in the anode chamber, despite of expected anoxic conditions, could be attributed to nitrification, due to diffusion of oxygen from upper cathode chamber in absence of separating membrane, and simultaneous denitrification. Also, small amount of nitrogen removal occurred due to cell synthesis, in both the chambers.

The nitrogen removal capacity of the MFC is not reported in the literature and further investigations are necessary to confirm the mechanism behind the nitrogen removal and its effect on overall power production in the MFC. In presence of nitrate as electron acceptor, decrease in anode potential is expected (Rabaey and Verstraete, 2005). This could be one of the reasons for lower voltage in this ML–MFC. During aerobic nitrification and denitrification by heterotrophic *Bacillus* strains, 33% of nitrogen removal was reported without formation of nitrous oxide (Kim et al., 2005). Nitrogen removal observed in this experiment was slightly higher than this value; indicating that the dominant nitrogen removal mechanism might be due to simultaneous nitrification and denitrification in cathode chamber, because similar *Bacillus* strains are expected to exist in the MFC. Nitrogen assimilation in the biomass is expected to be less, due to low biomass production in the MFCs. Production of sludge in MFC is very less as com-

pared to any other biological treatment process (Jang et al., 2004), because major fraction of the energy produced from oxidation of organic matter is converted to electricity, and the remaining energy is only available for microbial growth.

3.4. Current production

Synthetic wastewater was fed to the ML–MFC in continuous mode after inoculation. Slow increase in current was observed with duration of operation. The ML–MFC took two weeks to produce stable current. At continuous external load of 100 Ω , the current production reached to the maximum value of 0.56 mA on 11th day and dropped to 0.48 mA on 15th day, with nearly constant current production on later days. The open circuit voltage potential of 0.5 V was observed, with closed circuit voltage drop across the resistance as 43 mV. When synthetic feed containing sucrose was used, the power density in ML–MFC, using three electrodes in anode chamber, was observed to be 0.98 mW/m². When, lactose and dextrose were used as a carbon source, the power density was 2.5 and 2.01 mW/m², respectively. However, power density reduced to 0.52 mW/m², when settled sewage was used as feed. The power density observed in this membrane-less MFC, using different substrates, was in general agreement with the values reported in the literature (Jang et al., 2004; Kim et al., 2002; Park and Zeikus, 2003; Bond et al., 2002).

3.5. Effect of spacing between anode and cathode on power production

The power production at different distance between the anode and cathode electrodes was evaluated. Single electrode from anode and cathode compartments was used with spacing between the electrodes as 20 cm, 24 cm and 28 cm. Fig. 3 shows the power production observed at variable external resistance and under different spacing between the anode and cathode electrodes. Under variable external resistance, the power density increased with decrease in distance between the electrodes. Maximum power density of 10.9, 8.6, and 7.4 mW/m² was observed at electrode spacing 20, 24 and 28 cm, respectively. The maximum power density was observed at external resistance between 900 Ω and 1200 Ω . Decrease in power density was observed with increase in resistance beyond 1200 Ω , indicating importance of external load for controlling power production. These results suggest that, at higher external resistance the electron transfer through the external circuit to the cathode

Table 2
Different form of nitrogen removal observed in ML–MFC

Carbon source	TKN (inlet) (mg/l)	TKN (middle) (mg/l)	TKN (outlet) (mg/l)	$\text{NH}_4\text{-N}$ (Inlet) (mg/l)	$\text{NH}_4\text{-N}$ (middle) (mg/l)	$\text{NH}_4\text{-N}$ (outlet) (mg/l)	NO_3 (inlet) (mg/l)	NO_3 (middle) (mg/l)	NO_3 (outlet) (mg/l)
Synthetic wastewater	49.41 (± 3.47)	39.40 (± 1.45)	26.84 (± 4.87)	44.23 (± 3.46)	32.91 (± 2.08)	12.88 (± 2.39)	1.46 (± 0.23)	1.65 (± 0.12)	2.65 (± 0.38)
Sewage	20.16	ND	10.08	8.4	ND	0.5	1.28	ND	7.83

Results expressed for sewage are average values of two samples.

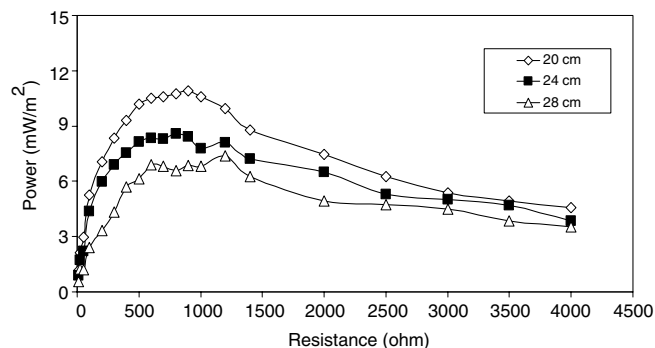


Fig. 3. Effect of spacing between anode and cathode on power production. The power density observed under variable resistance is indicated at different distances between the anode and cathode as \diamond – 20 cm, \blacksquare – 24 cm, and \triangle – 28 cm.

might be the limiting factor. In addition, this suggests that, ML–MFC should be constructed by placing electrodes as close as possible to increase power output. The power density observed using single electrode in the membrane-less MFC agree with the range of 0.6–15 mW/m², reported in the literature for MFC using membrane (Kim et al., 2002; Park and Zeikus, 2003; Bond et al., 2002; Bond and Lovley, 2003; Chaudhuri and Lovley, 2003). The observed power generation reveals that, the membrane-less MFC can also be effective in producing power while treating wastewater.

3.6. Effect of electrode surface area on power production

Out of the three electrodes provided in the anode chamber single, double and triple electrodes (area 70.21 cm², 140.43 cm² and 210.64 cm², respectively) were connected to the circuit to evaluate effect of the anode surface area on power production. Fig. 4 illustrates that, as area of anode increases power density decreases. Thus it indicates that, in a comparatively bigger reactor, area of anode is utilized less efficiently than those of smaller reactors, used as BOD sensors (Chang et al., 2004). In this ML–MFC, when areas of anode electrodes were 70.21 cm², 140.43 cm² and 210.64 cm²

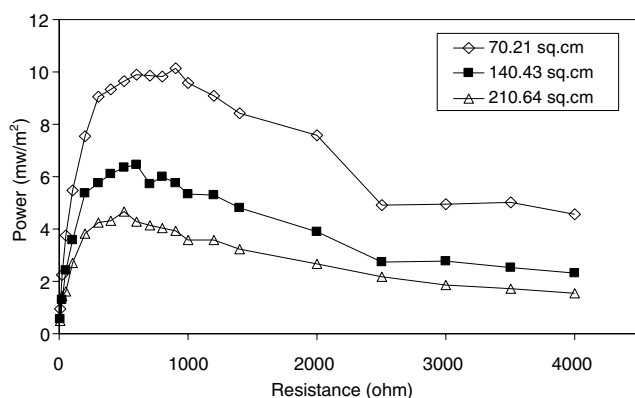


Fig. 4. Effect of surface area of anode on power density. The apparent surface area of the anode used was \diamond – 70.21 cm², \blacksquare – 140.43 cm², and \triangle – 210.64 cm².

maximum power densities observed were 10.13 mW/m², 6.45 mW/m² and 4.66 mW/m², respectively. The use of third electrode had very little effect on enhancement of power production as compared to two electrodes, and it improved net power production (mW) only by 8.9%. However, increase in net power production was observed by 28.6% when two electrodes were used as anode instead of single electrode. Although, as the area of anode was made twice and thrice, the increase in net power production (mW) was 28.6% and 40%, respectively; indicating that power generation was limited by some other factor, apart from anode surface area. The probable limitation could be electron transfer from microbial cell to the anode surface, in absence of addition of external mediator.

With increase in external resistance, power density increased up to certain resistance and then decreased. For maximum power density the favourable external resistances were 900, 600 and 500 Ω for electrode surface areas of 70.21 cm², 140.43 cm² and 210.64 cm², respectively. When resistance was increased by more than the respective value for that electrode area, power density was decreased. Thus, external resistance is also among the factors, to control movement of electrons in the system. In this reactor total effective area of electrodes was not utilized efficiently, hence power production was not proportional to the area of electrode. Logan et al. (2005) have stated that, as area of anode was increased power density was increased from 12 mW/m² to 13.4 mW/m², demonstrating that power density was not doubled when the second electrode was added.

3.7. Voltage at different external resistance

Variation of voltage, observed across the variable external resistance, at different distances between anode and cathode (20 cm, 24 cm, and 28 cm) and at different surface areas of anode (70.21 cm², 140.43 cm² and 210.64 cm²) is presented in Fig. 5. With increase in distance between the electrodes, decrease in voltage was observed. The maximum voltage of 358 mV was observed at 20 cm distance between the electrodes at external resistance of 4000 Ω . At same external resistance the voltage was 310 mV, when distance between the electrodes was 28 cm. However, no significant change in the voltage was observed with respect to change in surface area of anode. Voltage increased up to 360 mV in all the three cases with increase in external resistance.

3.8. Current production at different external resistance

The current production at different distances between the anode and cathode and at different surface areas of anode is shown in Fig. 6. Upon increase in the external load, drop in current was observed under all the situations and the current production was brought to minimum and nearly constant value beyond 3500 Ω . The observed current under variable resistance and at different distances between the anode and cathode showed that, for external resistance greater than 1400 Ω , the current production was almost

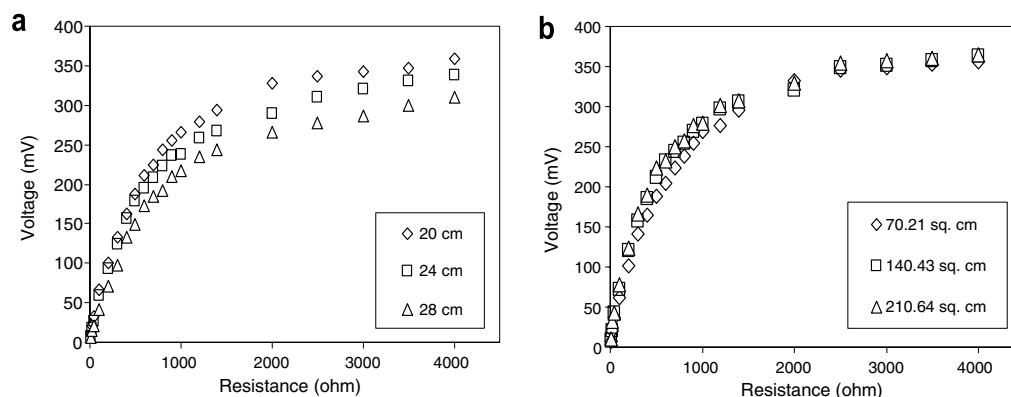


Fig. 5. Variation of voltage observed across variable external resistance. (a) At different distance between the anode and cathode, and (b) at different surface area of anode.

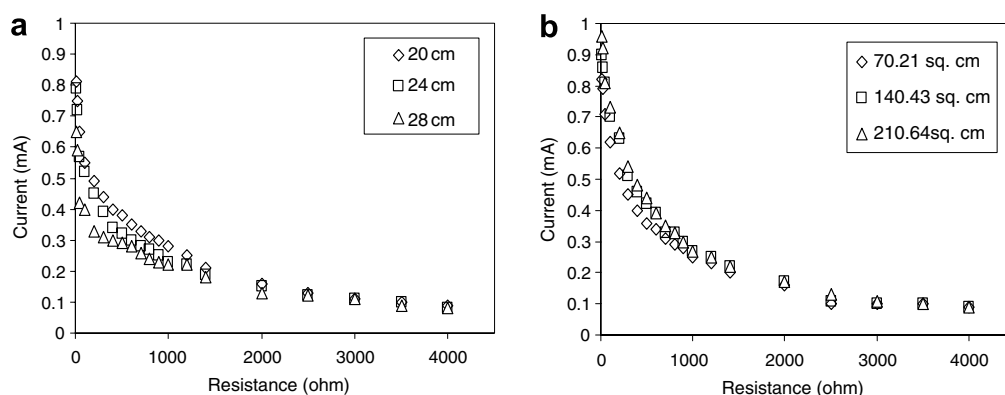


Fig. 6. Variation of current observed under variable external resistance. (a) At different distance between the anode and cathode, and (b) at different surface area of anode.

independent of the distance between the electrodes and surface area of the anode. Even below 1400Ω external resistance, the effect of distance between the electrodes was of little significance. Slightly higher current production was observed when the electrodes were kept close. However, variation in the surface area of anode showed no significant effect on the current production. This suggests that, transport of electron from the bulk liquid to the electrode surface is the limiting parameter for electricity production in this ML–MFC.

3.9. Granulation in biomass in ML–MFC

In the ML–MFC after 35 days of operation granules formation was visible in the anode chamber. Sludge sample was collected on 50th day for microscopic examination from anode chamber. The sludge was granulated partially having 1 to 2 mm size of the granules. The colour of typical granules was dark gray. The SEM images of a typical granule showed that the granule had multiple cracks on the surface, with highly porous inner structure (image not shown). Previous studies have shown that methanogenic granules degrading carbohydrates were tightly packed with a smooth dense surface comprising variety of fermentative acidogenic microorganisms (Fang et al., 1994). The porous

structure of the granules with multiple cracks on the surface, observed in this study, was likely to facilitate the passage of nutrients and substrate as well as the release of hydrogen, which had a very limited solubility of 1.58 mg/l in water (Fang et al., 2002). These granules, unlike those of methanogenic USAB granules, did not exhibit a layered structure because of the simplicity of the acidification process. They resembled to hydrogen production acidogenic sludge agglutinate into granules, in a well-mixed reactor treating synthetic wastewater containing sucrose, as reported by Fang et al. (2002).

3.10. Biofilm growth on electrode surface

Small portion of the biofilm was scratched from the electrode surface on 70th day of operation after emptying the ML–MFC. The biofilm formation on anode was black in colour and on the cathode it was brownish-gray in colour like aerobic activated sludge. SEM image revealed typical bacterial growth on the surface of anode electrode (image not shown). A close examination revealed that there were different predominant bacterial morphologies on the electrodes. On the anode small rod shaped bacteria (3–5 μm in length and 1–2 μm in width) were dominating, resembling either to Fusiform Bacilli as reported by Fang et al. (2002) during

hydrogen production from sucrose containing wastewater or to family of *Geobacteraceae* (Bond et al., 2002; Chaudhuri and Lovely, 2003). On the cathode surface, long rod shaped bacteria (greater than 50 µm in length and 5–7 µm in width) were dominating along with other coccal bacterial morphotypes. These were similar to bacteria found in aerobic granular sludge cultivated on glucose based synthetic wastewater (Wang et al., 2004).

4. Conclusions

The membrane-less microbial fuel cell, inoculated with mixed anaerobic sludge demonstrated its effectiveness as a wastewater treatment process along with electricity production, without incorporating any costly component, such as mediator and membrane. The COD, BOD and TKN removal were achieved at varying levels. Granulation of biomass was observed in the anode compartment of the ML–MFC. Maximum power density was observed at 20 cm spacing between the electrodes. Further studies would be necessary to optimize the electricity production from this ML–MFC. Efforts should be made to maximize the transport of electrons from the bulk to the electrode surface by identifying procedure for enrichment of anodophilic bacteria from the mixed anaerobic sludge to optimize electricity production. With further improvements and optimization, it could be possible to increase power generation. Thus, the combination of wastewater treatment along with electricity production might help in compensating the cost of wastewater treatment.

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