Bioelectricity production by mediatorless microbial fuel cell under acidophilic condition using wastewater as substrate: Influence of substrate loading rate

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Microbial fuel cell (MFC) employing low-cost materials (non-coated plain graphite electrodes) without any toxic mediators (aerated cathode and mediatorless anode) was evaluated under acidophilic (anode pH of 5.5) conditions using anaerobic mixed consortia to enumerate the influence of substrate loading rate on bioelectricity generation from anaerobic wastewater treatment at ambient temperature (28 ± 2°C). Experimental data showed the feasibility of power generation from wastewater treatment. However, the overall voltage generation, power yield and substrate degradation was found to depend on the substrate/organic loading rate (OLR). Maximum potential difference of 423 mV (1.66 mA) was recorded at stable operating conditions. Apart from power generation, the fuel cell also demonstrated substrate removal (62.5%). Applied OLR documented marked influence on both power yield and substrate degradation rate. Maximum power yield (274 mW/g COD_R; 50 Ω), was observed at operation OLR of 0.574 kg COD/m^3-day. Voltage and current started decreasing due to substrate exhaustion (COD reduction) in the anode chamber. It is evident from the experimental data that the anodic chamber of MFC mimicked anaerobic suspended growth reactor normally used for wastewater treatment with respect to COD removal. The study documented the advantage of both wastewater treatment and electricity production in a single system.

Keywords: Aerated cathode, bioelectricity, graphite electrode, microbial fuel cell, wastewater.

IT is evident that humankind is increasingly dependent on energy with the advancement of science and technology. The present-day energy scenario in India and around the globe is precarious, thus driving to the search of alternative to fossil fuels. Increasing energy consumption creates unbalanced energy management and requires power sources that are able to sustain for longer periods[1]. Trapping renewable energy from waste organic sources is the present trend of active research[2–7]. In this direction, bioelectricity generation through microbial fuel cells (MFCs) using a variety of substrates, including wastewater is being studied extensively[2,5–17].

It is well known that microorganisms can produce fuels such as ethanol, methane and hydrogen from organic matter. More recently, it has been reported that microorganisms can also convert organic matter into electricity using MFCs[2–7]. MFC is a biochemically catalysed system, which generates electricity by oxidizing biodegradable organic matter in the presence of either fermentative bacteria or enzymes[2–7,18–20]. The biocatalyst present in the anode chamber of MFC generates electrons (e^-) and protons (H^+) through anaerobic respiration of organic substrates. Electron transfer occurs through the electrode (anode) integrated with an external circuit to the cathode. Protons diffuse through the proton exchange membrane (which separates the cathode and anode chamber) into the cathode chamber, where they combine with the electron acceptor. The potential difference between the respiratory system and electron acceptor generates the current and voltage needed to generate electricity[2]. Harvesting electricity from organic wastes through MFC is an attractive source of energy as organic waste is 'carbon-neutral' and oxidation of organic matter only releases recently fixed carbon back into the atmosphere[4]. According to Lovley[4], MFC could fill a niche that is significantly different from that of the better known abiotic hydrogen- and methanol-driven fuel cells. Abiotic fuel cells require high temperatures and expensive catalysts which are toxic, to promote oxidation of the electron donors[21]. Naturally occurring microorganisms catalyse the oxidation of fuels in MFC at room temperature and could potentially be designed to function at any temperature at which microbial life is possible[4,6]. MFC can be considered as a promising alternative for the harnessing of electrical energy from various substrates using different cell configurations, and electron transfer mechanisms[9,17,20,22–25].

Presently, research on MFCs using wastewater as substrate is in the initial stages of laboratory evaluation around the world. The reported work so far is mainly based on using the monoculture at laboratory level[12,20,21,26]. A few studies

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were reported on using wastewater as substrate for production of electricity[2–17,25]. Substantial technical and engineering challenges still remain to achieve sustainable electricity production at full scale. The function and efficiency of MFCs with respect to power generation are generally dependent on factors such as nature of carbon source used[7,20], fuel-cell configuration (single/multiple chamber), dimensions and volume[26,27], nature and type of electrode[15,19], electron acceptors (mediators) present in the cathode chamber[15,28], electrolytes used[22], operating temperature[24], nature of inoculum (biocatalyst) used in the anode chamber[15,28], and nature of the proton exchange membrane[25].

The basic aim of the present study is to design MFCs employing low-cost materials without using toxic mediators, which will have the possibility to be implemented in the wastewater treatment plants in the economical perspective. The designed MFC (graphite electrode without any coating) employing aerated cathode and mediatorless anode was evaluated at acidophilic conditions using anaerobic mixed consortia to enumerate the influence of substrate/organic loading rate (OLR) on the performance of the MFC in terms of bioelectricity generation from anaerobic wastewater treatment.

Experimental

Anaerobic mixed inoculum

Anaerobic mixed consortia taken from laboratory-scale UASB reactor was used to inoculate the anode chamber of the MFC. Inoculum after sampling was centrifuged (5000 rpm, 20°C) and washed with saline buffer (three times) and the resultant pellet was enriched in the designed synthetic wastewater (glucose, 3 g/l; NH_4Cl, 0.5 g/l; H_2PO_4, 0.20 g/l; K_2HPO_4, 0.20 g/l; $MgCl_2$, 0.25 g/l; $CoCl_2$, 20 mg/l; $ZnCl_2$, 10 mg/l; $CuCl_2$, 10 mg/l; $CaCl_2$, 4 mg/l; $MnCl_2$, 10 mg/l) keeping the bottle closed with a rubber septum (100 rpm; room temperature) under aseptic anaerobic microenvironment. After harvesting three times under aseptic anaerobic microenvironment, the mixed consortium was inoculated to start up the anode chamber of the MFC.

MFC configuration and operation

The dual-chambered MFC was designed and fabricated in the laboratory using perplex material (Figure 1; Table 1). Total volume of both anode and cathode compartment was the same (0.75 l) and each chamber was provided with sample port, wire point inputs (top), inlet and outlet ports. Proton exchange membrane (PEM; NAFION 117, Sigma–Aldrich) was used to separate the two chambers after boiling sequentially in 30% H_2O_2, deionized water, 0.5 M H_2SO_4 and deionized water each for 1 h. After pretreatment, the PEM was fixed using washers and clamps between the two chambers. Both anode and cathode electrodes were made of graphite plates (5 × 5 cm; 1 cm thickness) and were positioned at a distance of 6 cm on either side of the PEM. The anode was perforated by providing nine uniform-size holes of 0.7 cm diameter to increase the surface area. The electrodes had a surface area of 83.56 sq. cm (anode) and 70 sq. cm (cathode). Prior to use, the electrodes were soaked in deionized water for a period of 24 h. Contact

Table 1. Design criteria of dual chambered mediatorless microbial fuel cell

Reactor configuration	Dual chamber
Anode chamber	Suspended growth
Anode inoculum	Mixed anaerobic consortia
Mediator-anode	Nil
Mediator-cathode	Air
Volume of anode and cathode (l)	0.7
Anode and cathode material	Graphite plate
Surface area of electrodes (sq. cm)	83.56 (anode), 70 (cathode)
Membrane	Proton exchange membrane
Feeding nature (l/cycle)	Batch (0.65 l/feeding cycle)
Organic loading rate (kg COD/m^3-day)	0.517, 0.574, 0.646 and 1.033
Operating temperature (°C)	28 ± 2
Operating pH	5.5 (anode), 7.5 (cathode)

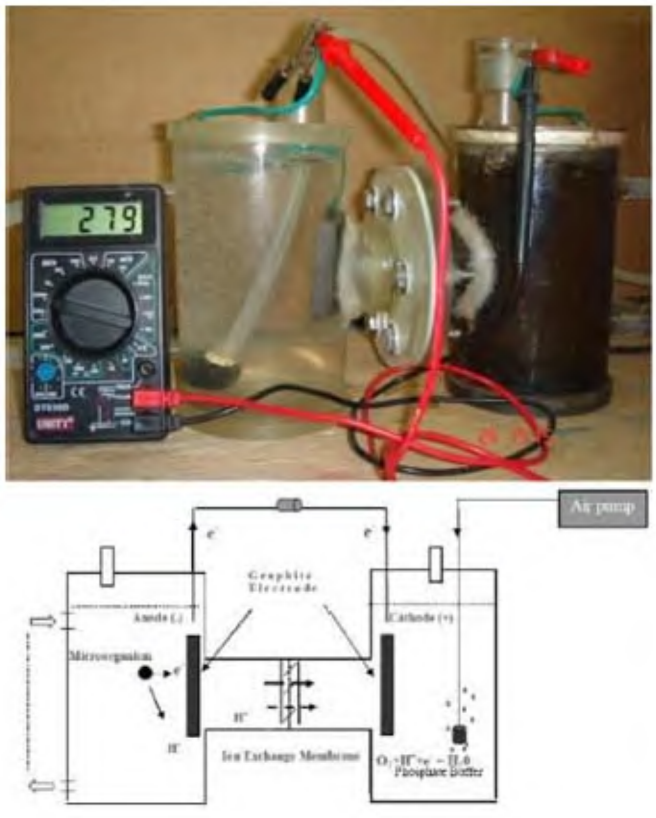

Figure 1. Photograph of dual-chambered aerated microbial fuel cell along with schematic details.

between electrodes and copper wires was sealed with epoxy material.

The cathode chamber of the MFC was filled with aerated phosphate buffer (50 mM K_2HPO_4; pH 7.5) as catholyte. Air was continuously sparged employing an air pump attached with a sparger and dissolved oxygen (DO) concentration was maintained between 4 and 5 mg/l. The anodic chamber prior to startup was inoculated with anaerobic mixed microflora dissolved in 700 ml of designed synthetic feed. No mediator was used in the anodic chamber of the MFC. During operation, the anode chamber was maintained at acidophilic pH condition (5.5) to sustain the growth of acidophilic culture. Anolyte (synthetic feed and mixed microflora) was recirculated continuously at a rate 100 ml/min using a peristaltic pump in order to provide effective contact between the substrate and mixed consortia. Constant removal of chemical oxygen demand (COD) and voltage output were considered as indicators for satisfactory formation of the biofilm and stable operating conditions. Before changing the feed, the inoculum was allowed to settle down (30 min) and exhausted feed (650 ml) was pumped out under anaerobic conditions. The settled mixed inoculum (50 ml by volume) was used for subsequent experiments to sustain microflora. Fresh synthetic feed (650 ml) was loaded once the voltage started decreasing. After every feeding event, the anode chamber was sparged with N_2 for 2 min to maintain anaerobic microenvironment. Feeding, decanting and recirculation operations were carried out using peristaltic pumps (Micllins, India) controlled by electronic timer (ETTS, Germany). Performance of the MFC was evaluated at three substrates/OLRs (0.517 kg COD/m^3-day, 0.574 kg COD/m^3-day, 0.646 kg COD/m^3-day and 1.033 kg COD/m^3-day). MFC was operated in batch mode at constant operating temperature (28 ± 2°C).

Analytical methods

Current (I) and potential (V) measurements were recorded every 3 h using digital multimeter (Metravi 901) by connecting 50 Ω as external circuit. For polarization, current generation was monitored at various external resistances (500 to 50 Ω) connected for a few minutes and readings were noted after stabilization of voltage. Power (W) was calculated using the equation $P = IV$, where I is in amperes and V is in mV. Power density (mW/sq. m) and current density (mA/sq. m) were calculated by dividing the obtained power and current with the surface area (sq. m) of the anode. Power yield (W/kg of COD removed) was obtained by dividing power with the amount of COD removed. Volumetric power (mW/cubic m) and current (mA/cubic m) were calculated based on the anode liquid volume. pH, COD and volatile fatty acids were monitored in the anodic chamber of MFC during operation, according to standard methods[29]. The mixed consortium formed on anode was subjected to scanning electron microscopy (SEM). Prior to SEM imaging samples were washed with saline water followed by 0.05 M phosphate buffer (pH 7.2) and fixed in glutaraldehyde (2.5%; 24 h). Fixed samples were dehydrated in a series of graded alcohol (10–90%) and scanned in SEM (Hitach S-3000N) after drying. The performance of MFC was evaluated by estimating the substrate (COD) removal efficiency (ξ) during the cycle operation using eq. (1). C_{SO} represents the initial COD concentration (mg/l) in the feed and C_S denotes COD concentration (mg/l) in the reactor outlet.

$$\xi = (C_{SO} - C_S)/C_{SO}. \quad (1)$$

OLR (kg COD/m^3-day) was calculated using eq. (2):

$$\text{OLR} = \{[\text{COD}_0 \times \text{feed rate}]/\text{reactor volume}\}. \quad (2)$$

Substrate degradation rate (SDR, kg COD/m^3-day) was calculated to study the rate and pattern of COD removal during the cycle operation according to eq. (3):

$$\text{SDR} = \{[(\text{COD}_0 - \text{COD}_T) \times \text{feed rate}]/\text{reactor volume}\}, \quad (3)$$

where COD_0 and COD_T represent COD (mg/l) at times '0' and 'T' respectively.

Results and discussion

After the anode chamber of the MFC was inoculated with adopted anaerobic mixed consortia, the fuel cell was operated with designed synthetic wastewater as feed to support the formation of biomass and subsequent adaptation to the new microenvironment. Constant substrate (COD) removal efficiency and voltage output were considered as indicators to assess the stable performance of the MFC. Fuel cell was operated continuously for 35 days. Experimental data showed the feasibility of electricity generation from wastewater treatment (Figures 2–3; Table 2). However, the performance and stabilization tendency with respect to power generation was found to be dependent on the substrate loading rate. The performance of MFC with respect to voltage and current output during operation is depicted in Figure 2. During the initial stage after inoculation, a gradual rise in the voltage was observed, which approached a maximum of 423 mV on the 33rd day (Figure 2). During first feeding cycle, a drop in potential difference was observed after the fifth day of the operation, which could be due to the exhaustion of carbon in the feed. Fresh feed was supplemented after a drop in potential difference was observed. A steady increase in potential difference was observed with every additional feed and this might be attributed to the adaptation, phenomenon and development of the biofilm on the graphite surface of the anode. The fuel cell showed a retention time ranging be-

tween 6 and 9 days with respect to potential drop. Maximum power output of 1.66 mA was observed during the fourth cycle on the 33rd day after feeding, when measured at 50 Ω (Figure 2). Current generation also showed a gradual increase with each additional feed cycle.

During operation, the anodic chamber was continuously monitored for substrate (COD) removal efficiency (Figure 3; Table 2). It is evident from the experimental data that the substrate loading rate (OLR) documented its marked influence on power yield and substrate degradation. However, it was observed that power yield and substrate degradation did not show any compatibility. At operated lower OLR (0.517 kg COD/m^3-day), the fuel cell registered a SDR of 0.323 kg COD/m^3-day along with a power yield of 168 mW/g COD_R (50 Ω). Maximum substrate (COD)

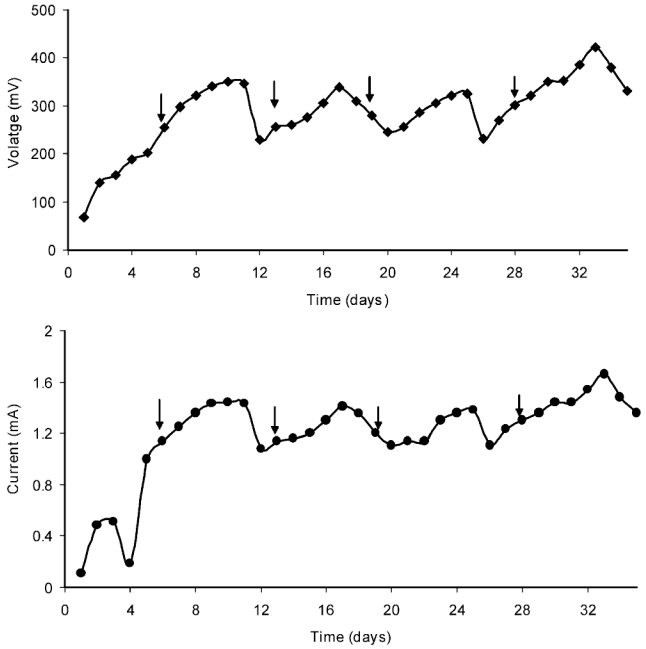

Figure 2. Variation in voltage and current generation during operation of MFC (arrow indicates shift in feed).

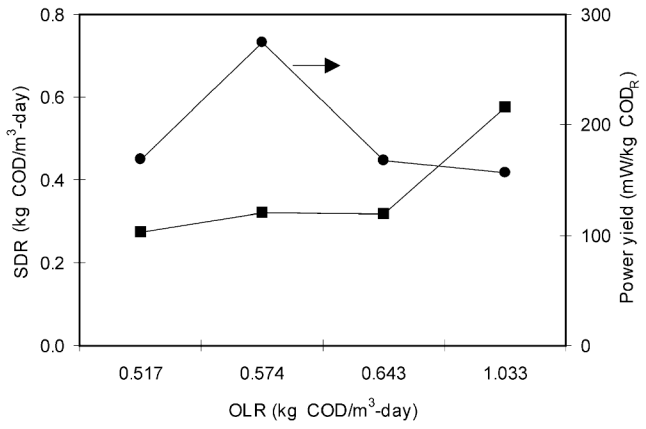

Figure 3. Variation of substrate degradation rate and power yield as a function of organic loading rate.

removal efficiency (62.5%) was observed during this phase of MFC operation. With increase in substrate loading (0.574 kg COD/m^3-day), increased yield in both power (274 mW/g COD_R; 50 Ω) and substrate degradation (0.366 kg COD/m^3-day) was observed. Subsequent increase in OLR (0.646 kg COD/m^3-day) documented an increase in SDR (0.384 kg COD/m^3-day), but a decrease in power yield (167 mW/g COD_R; 50 Ω). Subsequent increase in OLR also registered higher degradation rate of substrate with stabilized power yield. Decrease in COD concentration indicated effective functioning of the selectively enriched mixed microflora in metabolizing the carbon source present in wastewater as electron donors. It is evident from the experimental data that the anodic chamber of MFC acted as anaerobic suspended growth reactor normally used for wastewater treatment with respect to COD removal efficiency along with power generation. It could be presumed that the MFC studied with the specified operating conditions could be operated with higher power yield, i.e. up to OLR of 0.574 kg COD/m^3-day. Reduced power yield even at higher SDR observed in this study might be attributed to the effective mobility of electrons generated during metabolism towards the electrode because of using plain graphite electrode as anode material.

Figure 4 shows the polarization curve as a function of current density, potential and power density measured at variable resistances (50–500 Ω) and at operating OLR of 0.646 kg COD/m^3-day. Current generation in different resistors was observed once the maximum voltage was attained. The curve depicted a maximum power density of 66.21 mW/sq. m (216.01 mA/sq. m) at lower resistances (50 Ω) studied. Current generation showed decreasing trend with increase in resistance and is consistent with the reported literature[10,15], which indicated a typical fuel cell behaviour. At higher resistance used (500 Ω), relatively less power density of 19.74 mW/sq. m (46.67 mA/sq. m) was observed. Relatively less drop in voltage was observed at lower resistance indicating less potential drop. Voltage stabilization was comparatively rapid at higher resistances studied. Effective electron discharge observed at lower resistances might be the probable reason for further potential drop and slow stabilization of the voltage at lower resistances. Oxidation of substrates by microbes was observed to be more at lower resistance than at higher resistance, where microbes donated electrons to the anode as the electrons were discharged in a closed circuit[8,11]. Rozendal and co-workers[30] reported power density of 563 mA/sq. m (10 Ω) using MFC fabricated with platinum-coated titanium graphite felt anode and aerated cathode. Relatively lower power (216.01 mA/sq. m; 50 Ω) observed in this study might be because of using graphite electrode without any coating.

Each of the microbial groups involved in anaerobic degradation had a specific pH optimum and could grow in a specific pH range. The optimum range for all methanogenic bacteria (MB) was between 6.0 and 8 with an opti-

Table 2. Details of MFC operation with respect to output parameters

OLR (kg COD/m^3-day)	pH variation	VFA buildup (mg/l)	Voltage (mV)	Current (mA)	COD reduction (%)	SDR (kg COD/m^3-day)
0.517	5.5–4.6	+546	350	1.44	62.5	0.323
0.574	5.5–6.8	–10	423	1.66	53.3	0.306
0.646	5.5–6.8	+106	339	1.41	59.5	0.384
1.033	5.5–6.8	–70	332	1.38	60.0	0.419

OLR, Organic loading rate; VFA, Total volatile fatty acids (+ indicates VFA generation; – indicates VFA consumption); SDR, Substrate degradation rate.

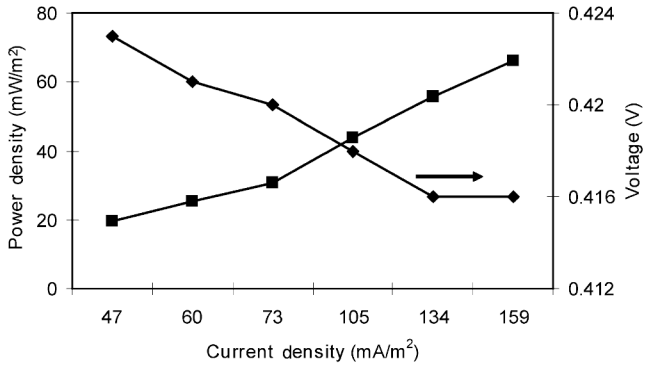

Figure 4. Polarization curve generated during stable performance at operating OLR of 0.646 kg COD/m^3-day.

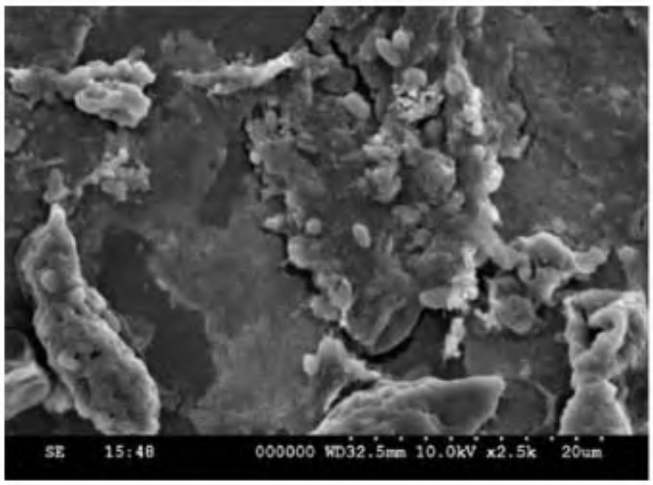

Figure 5. SEM images of bioelectricity-producing mixed consortia (× 2.5 K).

mum near pH 7.0, while acetogenic bacteria (AB) had lower pH optimum around 6.0. AB are notably less sensitive to pH variations[30]. The designed synthetic feed prior to feeding was adjusted to pH 5.5, which facilitated growth of AB and at the same time inhibit the functioning of MB. This susceptible environment created facilitates the generation of molecular H_2 as a metabolic intermediate in the anaerobic environment. During fuel cell operation, VFA was also analysed in the outlet of the anode compartment after the completion of every cycle (Table 2). Higher concentration and accumulation of VFA (+546 mg/l) was observed at the end of first cycle, which was reduced to +106 mg/l after the completion of the second cycle. After the second cycle, a gradual reduction in VFA was observed throughout the run, which might be due to low acetate and propionate concentrations. Accumulation of high concentration of VFA lowers the voltage generation. Increased potential difference was observed at lower VFA concentrations which could be visualized in the fourth cycle (–10 mg VFA/l; 1.66 mA). However, VFA concentration alone may not influence the potential difference created in the given system, as voltage generation depends on a number of other factors such as mass transfer, temperature and internal resistance. The decrease and increase in pH was consistent with the corresponding VFA data. VFA and pH were integral expressions of the acid-base conditions of anaerobic process, as well as intrinsic index of the balance between microbial groups in the anaerobic system[11,31,32]. VFA formation and persistent acidogenic metabolism encountered in the anode chamber presumably indicated a micro-environment congenial to the proliferation of H_2-producing AB and inhibitory for MB. Due to usage of acidophilic conditions, AB outbeats MB resulting in VFA accumulation in the system, thus leading to a drop in system pH. Also due to adopted batch mode operation of the MFC, VFA accumulation in the system was not observed after replacement of feed. VFA composition revealed the presence of formic acid, butyric acid and acetic acid along with propionic acid. However, formic acid concentration was comparatively on the higher side and susceptibility to formic acid in the anaerobic process suggested that mixed acid fermentation condition persisted in the anodic chamber. Propionate concentration was comparatively low, and negligible ethanol and lactic acid production was detected. This phenomenon suggests the survival of H_2-producing species, rather than H_2-consuming species.

Figure 5 shows SEM (× 2.5 K magnification) image of adopted anaerobic mixed consortia formed on the anode surface. The image shows morphologically similar bacterial groups with short-chain rods (~20 µm approximate length) present in clusters. Throughout the experiments, analyte pH was maintained at acidophilic conditions (5.5) to sustain the AB growth and to suppress methanogenic activity, which facilitated congenial environment for H_2

production through anaerobic fermentation. This is an essential intermediate for electricity generation in MFC operation. Experimental data revealed the effectiveness of acidophilic conditions maintained for power generation within a short period of adaptation time. Moreover, using mixed microbial culture could be considered as a practical, cost-effective and promising approach to achieve bioelectricity generation at large scale. Typically, the mixed consortia employed do not require soluble mediators, but can donate electrons directly to the electrode surface by attachment[6,33,34]. The mixed acidogenic inoculum not only produced power in association with COD reduction, but also facilitated transfer of electrons to the anode without an exogenous mediator. Phenazines produced by bacteria were recently shown to enhance electron transfer in MFCs[7,32]. Utilizing mixed anaerobic cultures for production of electricity is a more useful phenomenon and has practical advantages.

During MFC operation, relatively lower concentration of H_2 (0.1 mmol) and negligible amount of methane was observed in the anode chamber. This observation confirms the capability of mixed consortia for generation of H_2 as intermediate. Reduced concentration further enumerates its role in the power generation. During the metabolic process[6], reduced energy substrates ($NADH/FADH_2$) produced in the microbes reoxidize to release H^+. The protons flow back into the cell through the ATPase enzyme creating one ATP molecule from one ADP molecule for every 3–4 protons. Finally, the electrons are released to a soluble terminal electron acceptor such as nitrite or sulphate or oxygen. When the electron exits the respiratory chain at a reduction potential less than that of the terminal electron acceptor, the bacteria obtain less ATP and a potential relative to the remaining oxygen is used for electricity generation in the MFC. For each electron that is produced, a proton must be conducted to the cathode through the electrolyte to sustain current generation and the electrons and protons react with oxygen at the cathode.

Conclusion

This study documented the feasibility of bioelectricity generation from anaerobic wastewater treatment using a MFC fabricated with low-cost anode materials (non-coated plain graphite electrodes), without any toxic mediators (aerated cathode and mediatorless anode). Acidophilic (anode pH of 5.5) conditions maintained during the experiments using anaerobic mixed consortia also helped in bioelectricity generation along with effective substrate removal. The substrate loading rate showed significant influence on the overall performance of MFC with respect to power generation and substrate removal. COD removal efficiency observed in the anode chamber enumerated the functioning of MFC as alternative wastewater treatment unit in addition to renewable energy generation. The procedure was cost-effective and environmentally sound and sustainable due to utilization of wastewater as substrate. Further, power was generated *in situ* along with wastewater treatment, utilizing low-cost and non-coated electrodes and mediatorless anode. This process could be effectively integrated to wastewater treatment plant, wherein renewable energy could be generated from wastewater in addition to treatment. The dual activity could significantly reduce the cost associated with the current wastewater treatment methods.

1. Katz, E., Shipway, A. N. and Willner, I., *Biochemical Fuel Cells*, John Wiley, 2003, vol. 1, pp. 1–9.
2. Logan, B. E., Feature article: Biologically extracting energy from wastewater – Biohydrogen production and microbial fuel cells. *Environ. Sci. Technol.*, 2004, **38**, 160A–167A.
3. Liu, H., Ramanarayanan, R. and Logan, B. E., Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.*, 2004, **38**, 2281–2285.
4. Lovely, D. R., Bug juice: Harvesting electricity with microorganisms. *Nature Rev. Microbiol.*, 2006, **4**, 497–508.
5. Shukla, A. K., Suresh, P., Berchmans, S. and Rajendran, A., Biological fuel cells and their applications. *Curr. Sci.*, 2004, **87**, 455–468.
6. Logan, B. E. and Regan, J. M., Microbial challenges and fuel cell applications. *Environ. Sci. Technol.*, 2006, **40**, 172–180.
7. Rabaey, K. and Verstraete, W., Microbial fuel cells: Novel biotechnology for energy generation. *Trends Biotechnol.*, 2005, **23**, 291–298.
8. Mano, N., Mao, F. and Heller, A., Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant. *J. Am. Chem. Soc.*, 2003, **125**, 6588–6594.
9. Jang, J. K., Pham, T. H., Chang, I. S., Kang, K. H., Monon, H., Cho, K. S. and Kim, B. H., Construction and operation of a novel mediator and membrane-less microbial fuel cell. *Proc. Biochem.*, 2004, **39**, 1007–1012.
10. Min, B. and Logan, B. E., Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environ. Sci. Technol.*, 2004, **38**, 5809–5814.
11. Liu, H., Cheng, S. and Logan, B. E., Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.*, 2005, **39**, 658–662.
12. Rabaey, K., Lissens, G., Siciliano, S. D. and Verstraete, W., A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.*, 2003, **25**, 1531–1535.
13. Min, B., Kim, J. R., Oh, S. E., Regan, J. M. and Logan, B. E., Electricity generation from swine wastewater using microbial fuel cells. *Water Res.*, 2005, **39**, 4961–4968.
14. Ghangrekar, M. M. and Shinde, V. B., Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. *Bioresour. Technol.*, 2007, **98**, 2879–2885.
15. Venkata Mohan, S., Saravanan, R., Veer Raghavulu, S., Mohanakrishna, G. and Sarma, P. N., Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: Effect of catholyte. *Bioresour. Technol.*, doi: 10-1016/j.biotech2006.12.026.
16. Prasad, D., Sivaram, T. K., Sheela Berchmans and Yegnaraman, V., Microbial fuel cell constructed with a micro-organism isolated from sugar industry effluent. *J. Power Sources*, 2006, **160**, 991–996.
17. Oh, S. E. and Logan, B. E., Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Res.*, 2005, **39**, 4673–4682.

18. Ikeda, T. and Kano, K., Bioelectrocatalysis-based application of quinoproteins and quinoprotein-containing bacterial cells in biosensors and biofuel cells. *Biochim. Biophys. Acta*, 2003, **1647**, 121–126.
19. Niessen, J., Schroder, U. and Scholz, F., Exploiting complex carbohydrates for microbial electricity generation – A bacterial fuel cell operating on starch. *Electrochem. Commun.*, 2004, **6**, 955–958.
20. Niessen, J., Schroder, U., Rosenbaum, M. and Scholz, F., Fluorinated polyanilines as superior materials for electrocatalytic anodes in bacterial fuel cells. *Electrochem. Commun.*, 2004, **6**, 571–575.
21. Larminie, J. and Dicks, A., *Fuel Cell Systems Explained*, John Wiley, UK, 2003.
22. Oh, S. E., Min, B. and Logan, B. E., Cathode performance as a factor in electricity generation in microbial fuel cells. *Environ. Sci. Technol.*, 2004, **38**, 4900–4904.
23. Liu, H., Grot, S. and Logan, B. E., Electrochemically assisted microbial production of hydrogen from acetate. *Environ. Sci. Technol.*, 2005, **39**, 4317–4320.
24. Moon, H., Chang, I. S. and Kim, B. H., Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. *Bioresour. Technol.*, 2006, **97**, 621–627.
25. Scott, K. and Shukla, A. K., Polymer electrolyte membrane fuel cells: Principles and advances. *Rev. Environ. Sci. Biotechnol.*, 2004, **3**, 273–280.
26. Park, D. H. and Zeikus, G., Improved fuel cell and electrode designs for producing electricity from microbial degradation. *Biotechnol. Bioeng.*, 2003, **81**, 348–355.
27. Liu, H., Ramanarayanan, R. and Logan, B. E., Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.*, 2004, **38**, 2281–2285.
28. Kim, N., Choi, Y., Jung, S. and Kim, S., Development of microbial fuel cells using *Proteus vulgaris*. *Bull. Kor. Chem. Soc.*, 2000, **21**, 44–48.
29. APHA, Standard methods for the examination of water and wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA, 1998, 20th edn.
30. Rozendal, A. R., Hamelers, M. V. H. and Buisman, N. C., Effects of membrane cation transport on pH and microbial fuel cell performance. *Environ. Sci. Technol.*, 2006, **40**, 5206–5211.
31. Bailey, J. E. and Ollis, D. F., *Biochemical Engineering Fundamentals*, McGraw-Hill, New York, 1986.
32. Brenner, D. J., Introduction to the family Enterobacteriaceae. In *The Prokaryotes* (eds Starr, M. P. *et al.*), Springer-Verlag, Berlin, 1981, pp. 1107–1127.
33. Bond, D. R. and Lovley, D. R., Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.*, 2003, **69**, 1548–1555.
34. Kim, B. H., Park, H. S., Kim, H. J., Kim, G. Y., Chand, L. S., Lee, J. and Phung, N. T., Enrichment of microbial community generating electricity using a fuel cell type electrochemical cell. *Appl. Microbial. Biotechnol.*, 2004, **63**, 672–681.

ACKNOWLEDGEMENT. S.V.R. thanks the Indian Council of Medical Research, New Delhi for providing Junior Research Fellowship to carry out this work.

Received 6 November 2006; revised accepted 28 February 2007