Controlling methanogenesis and improving power production of microbial fuel cell by lauric acid dosing

P. P. Rajesh, MD. T. Noori and M. M. Ghangrekar

ABSTRACT

Methanogens compete with anodophiles for substrate and thus reduce the power generation and coulombic efficiency (CE) of the microbial fuel cell (MFC). Performance of a baked clayware membrane MFC inoculated with mixed anaerobic sludge pretreated with lauric acid was investigated in order to enhance power recovery by controlling methanogenesis. In the presence of lauric acid pretreated inoculum, MFC produced maximum volumetric power density of 4.8 W/m^3 and the CE increased from 3.6% (for untreated inoculum) to 11.6%. Cyclic voltammetry (CV) and electro-kinetic evaluation indicated a higher bio-catalytic activity at the anode of the MFC inoculated with lauric acid pretreated sludge. With the lauric acid pretreated inoculum a higher catalytic current of 114 mA, exchange current density of 40.78 mA/m^2 and lower charge transfer resistance of 0.00016 Ωm^2 were observed during oxidation at the anode. Addition of lauric acid significantly achieved suppression of methanogenesis and enhanced the sustainable power generation of MFC by 3.9 times as compared with control MFC inoculated with sludge without any pretreatment.

Key words | coulombic efficiency, cyclic voltammetry, lauric acid, microbial fuel cell, suppressing methanogenesis

P. P. Rajesh
PK Sinha Centre for Bioenergy,
Indian Institute of Technology,
Kharagpur – 721302,
India

MD. T. Noori
Department of Agriculture and Food Engineering,
Indian Institute of Technology,
Kharagpur – 721302,
India

M. M. Ghangrekar (corresponding author)
Department of Civil Engineering,
Indian Institute of Technology,
Kharagpur – 721302,
India
E-mail: *ghangrekar@civil.iitkgp.ernet.in*

INTRODUCTION

Biomass is proposed as one of the future energy sources, since it is carbon neutral. Energy present in biomass can be harvested by using various economical and environmentally friendly technologies. Since the energy value of biomass depends on the high-energy electrons found in their constituent carbon molecules, microorganisms can be used to channel these electrons and their energy to form: CH_4 by methanogenesis, H_2 by dark fermentation, and electricity by electrogens (Lee *et al.* 2008). Microbial fuel cells (MFCs) utilize exoelectrogenic bacteria as catalysts, which can promote oxidation of organic matters under anaerobic condition and simultaneously produce an electrical current. Methane production is frequently observed in a bioelectrochemical system (BES) since the growth conditions of exoelectrogens are comparable with those of methanogens (Ferguia *et al.* 2007; Call & Logan 2008). The coulombic efficiency (CE) of MFCs is adversely affected by various parameters among which the coulombic losses caused by the methanogenic consortium are prominent. Enhancing CE by suppressing methanogenesis is an ongoing issue in BES. Inhibition of methanogens was experimentally carried out in different biological systems either through various environmental stresses (Chae *et al.* 2010), through application of chemical inhibitors of methanogens (Liu *et al.* 2011), by applying saturated fatty acids (SFAs) (Zeitz *et al.* 2013) or even by adding marine algae *Chaetoceros* (Božic *et al.* 2009). Methanogenesis can be avoided in MFCs by eliminating the electrochemically inactive bacteria especially methanogens. Multiple studies have already reported on the inhibition of methanogens in MFCs by using chemical inhibitors (Kim *et al.* 2005; Li *et al.* 2012). More & Ghangrekar (2010) studied the effect of ultrasonication and heat pretreatment of inoculum on performance of MFC and found that low frequency ultrasound pretreatment gave better performance.

Zeitz *et al.* (2013) successfully inhibited methane production in the rumen fermentation process by supplementing medium- and long-chain SFAs and found out that inhibition depends on SFA chain length and temperature. Medium chain length lauric acid (C_{12}) was found to inhibit the methanogens to a greater extent compared to other fatty acids. SFA inhibits the growth of Gram-positive and

doi: 10.2166/wst.2014.386

methanogenic bacteria via adsorption and disruption of cell membranes (Galbraith & Miller 1973; Soliva *et al.* 2003). Significant decrease in CH_4 formation rate of 90% is reported in specific species of methanogenic archaea by treating with lauric acid (Zeitz *et al.* 2013). To suppress the substrate loss caused by methanogens and to enhance power production of MFC, the objective of this research was to evaluate the performance of MFC inoculated with mixed anaerobic sludge pretreated with lauric acid. Electrochemical analysis like cyclic voltammetry (CV) was also used to study the changes in the redox activity at the anode surface while using lauric acid pretreated inoculum.

MATERIALS AND METHODS

MFC construction and operation

The study was carried out in two dual-chambered aqueous cathode MFCs with an anodic liquid volume of 1.2 L. Baked clayware cylinders served as the anodic chamber of these MFCs and the 8 mm thick wall material of the cylinder worked as a separator (Behera *et al.* 2010). The anode and cathode of the MFC consisted of carbon felt (Panex® 35, Zoltek Corporation) with a projected surface area of 300 and 720 cm^2, respectively. The carbon felt cathode was wrapped over the clayware cylinder and the carbon felt anode was placed as a cylinder of diameter 7 cm inside the anodic chamber (Figure 1). The distance between the nearest face of the anode and cathode was 2 cm. Stainless steel wire was used to connect both the electrodes through an external resistance of 100 Ω.

Anaerobic sludge collected from the bottom of a septic tank was used as the inoculums in the anodic chamber. The control MFC-1 was inoculated with 200 mL of sludge without any pretreatment. To suppress methanogens, the anaerobic sludge was pretreated with a dosage of 1 mg of lauric acid (C_{12}) per mL of sludge and incubated at 65 °C for 30 minutes before inoculating in MFC-2. To see the effect of lauric acid addition on working MFC for enhancing electrogenesis, it was added in MFC-2 once again in the seventh feed at the dose of 1 mg/mL.

Synthetic wastewater containing sodium acetate as a carbon source having chemical oxygen demand (COD) of about 3,000 mg/L was used as the feed as described by Behera *et al.* (2010). These MFCs were operated in batch mode of operation with a feeding frequency of 5 days under controlled temperature varying from 33 to 37 °C.

Analysis and calculations

The performance of MFC was evaluated in terms of voltage and current measured using a data acquisition unit and converted to power according to $P = V^*I$, where P = power (W),

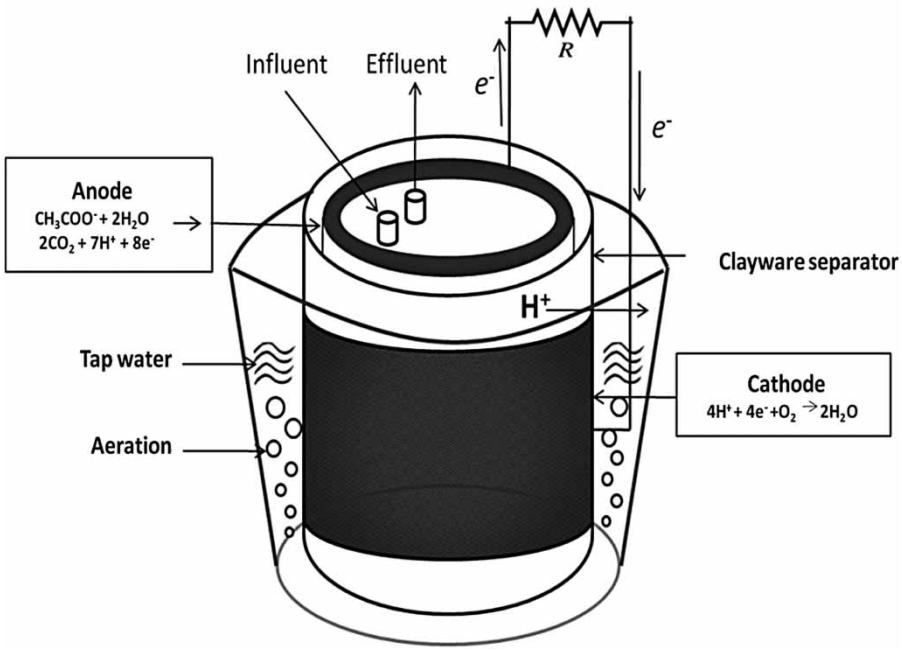

Figure 1 | Schematic diagram of MFC.

I = current (A), and V = voltage (V). Power density (PD) and power per unit volume were calculated by normalizing power to the anode surface area and net liquid volume of the anodic chamber, respectively. Polarization studies were carried out after attaining a stable cell potential by changing the external resistances from 20,000 to 5 Ω using a resistance box (GEC 05 R Decade Resistance Box). Under variable external resistance the voltage and current were recorded after allowing the circuit to stabilize for 10 minutes. Current density (CD) (mA/m^2) was calculated by dividing the current with the surface area (m^2) of the anode. The internal resistance of the MFC was estimated from the slope of line of voltage versus current plot (Picioreanu *et al.* 2007). CV was performed using an Autolab PGSTAT 302N potentiostat (Metrohm, The Netherland) and NOVA 1.9 software.

Influent and effluent COD concentrations were measured by closed reflux colorimetric method (APHA 1998). The pH and conductivity were measured by using a water quality bench meter (Eutech Instruments, Cyber scan, India). Specific methanogenic activity (SMA) of the anaerobic sludge was evaluated in a 250 mL serum flask using the procedure described by Bhunia & Ghangrekar (2007). Sodium acetate was added as 3000 mg COD/L along with other nutrients. Methane production was measured by a liquid displacement system using a flask containing 5% NaOH (w/v) solution. Volatile suspended solids (VSS) of the inoculum were measured according to *Standard Methods* (APHA 1998). The CE of the MFC was calculated by integrating the measured current over time relative to the maximum current possible based on the observed COD removal (Logan *et al.* 2006)

$$\mathrm{CE} = \frac{Ms\ I}{F\ b_{es}\ q\ \Delta\mathrm{COD}} \quad (1)$$

where, Ms is the molecular weight of substrate added, I is the circuit current, b_{es} is the number of electrons exchanged per mole of oxygen (4), q is the flow rate, ΔCOD is the difference in the influent and effluent COD concentrations.

RESULTS AND DISCUSSION

Wastewater treatment and CE

The COD removal efficiency of the MFC-2 was in the range of 65–73%, and it was found to be increasing with each progressive cycle for the first three cycles and thereafter it was more or less stabilized (Figure 2). COD removal of the control MFC-1 was in the range of 85–91%, which was higher as compared to the MFC-2. This indicates utilization of the organic matter from wastewater by acetoclastic methanogens as well as electrogens in the control MFC, hence enhancing COD removal efficiency. The reduction of substrate degradation rate in the MFC-2 suggests the selective inhibition of the methanogenic consortium present in the inoculum. The COD removal efficiency demonstrated by MFC-2 was comparable with the previous methanogen inhibition study using 2-bromoethane sulfonate (Li *et al.* 2012).

SMA of the anaerobic sludge with and without pretreatment was evaluated to quantify the methane-inhibiting potential of lauric acid. SMA was lower (0.152 g CH_4 COD/(g VSS.d)) in anaerobic sludge pretreated with lauric acid whereas a higher methanogenic activity of 0.313 g COD CH_4/(g VSS.d) was observed in sludge without any treatment. These results show that methanogens present in the sludge inoculum are getting significantly suppressed by lauric acid, since the SMA in the anaerobic system is a function of population of methanogens.

Maximum CE of 9.8% was obtained in MFC-2, which was approximately three times higher than the CE of MFC-1 (Figure 2). The CE obtained in this study was found to be more than that in the previously mentioned the methanogen inhibition study with 2-bromoethane sulfonate addition (Li *et al.* 2012). These results indicate increase in the maximum coulombs recovered when sludge was pretreated with lauric acid, which is likely due to the decrement in substrate consumption by methanogens. The low CE obtained in the control MFC-1 indicates relatively more

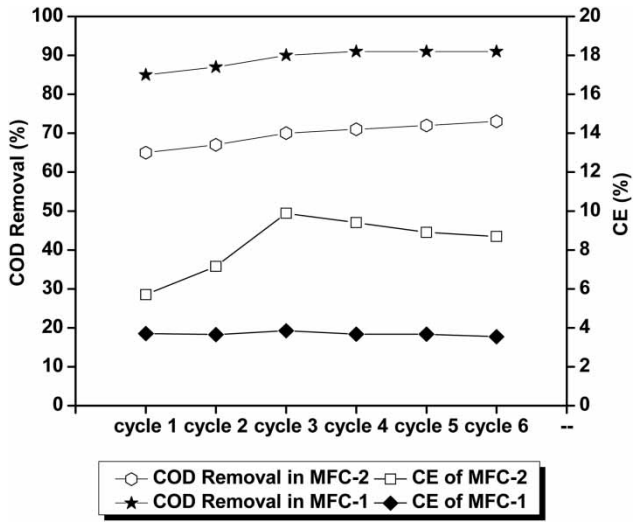

Figure 2 | Comparison of COD removal efficiency and CE in control MFC-1 and MFC-2.

substrate was utilized by the methanogenic consortium, which acted as the major competitor for substrate oxidation. However, gradual reduction of CE was observed from cycle-3 to cycle-6 in MFC-2, which can be explained by the re-acclimatization of the methanogenic consortium for the consecutive feed cycles. This drop in CE could be recovered by intermittent dosing of the lauric acid in the anodic chamber. When lauric acid was added further after six cycles of operation, the CE increased to 11.6% in MFC-2, demonstrating utility of this pretreatment for restoring CE.

Electricity generation

During the initial period of operation, MFC-2 generated a maximum operating voltage of 380 mV and it increased up to 580 mV in cycle 6 at an external resistance of 100 Ω. The control MFC-1, inoculated with untreated anaerobic sludge, produced a lower operating voltage in the range of 280–290 mV (Figure 3(a)). A maximum open circuit voltage of 820 and 710 mV was obtained for MFC-2 and MFC-1, respectively.

The anode potential of MFC is an important parameter which determines the electron-transfer kinetics of exoelectrogens (Marcus *et al.* 2007). A lower anode potential of −430 mV was obtained for MFC-2 as compared to control MFC-1 (−390 mV). A sustainable volumetric PD of 3.36 W/m^3 was produced by MFC-2 at an external resistance of 100 Ω, which was 3.9 times higher than the control MFC-1. MFC-2 generated an average current of 5.4 mA, while the control MFC-1 delivered only 2.8 mA. Nearly doubling of the current produced by MFC-2, inoculated with lauric acid pretreated anaerobic sludge, indicates the effectiveness of lauric acid pretreatment for the selective control of acetoclastic methanogens.

After the completion of six cycles, lauric acid solution was once again added (1 mg/mL of sludge volume) in MFC-2 to find any improvement in power generation. A maximum operating voltage of 656 mV and sustainable PD of 4.3 W/m^3 could be obtained after the addition of lauric acid solution. A corresponding decrease in the anode potential (−460 mV) was also observed. It shows enhanced utilization of acetate for electron recovery by anodophiles while controlling methanogenesis by addition of lauric acid.

Polarization and internal resistance

Polarization behavior of MFC was studied to obtain potential and PD against CD for both these MFCs. A maximum PD of 148 and 285 mW/m^2 was obtained for MFC-1 and MFC-2, respectively, showing a 92% increase of power generation with lauric acid pretreatment of inoculum (Figure 3(b)). MFC-2 could produce more power as compared to the 2-bromoethane sulfonate treatment (Li *et al.* 2012) and external resistance variation study (Chae *et al.* 2010), which shows the effectiveness of lauric acid for inhibition of methanogens over the other environmental stress method applied earlier. A maximum volumetric PD of 4.28 W/m^3 could be obtained in MFC-2. With the enrichment and maturation of biofilm on the anode, an increased microbial activity and hence a decrease in internal resistance can be observed (Lu *et al.* 2009). The internal resistance of MFC-2 (37 Ω) was found to be lower than the control MFC-1 (55 Ω).

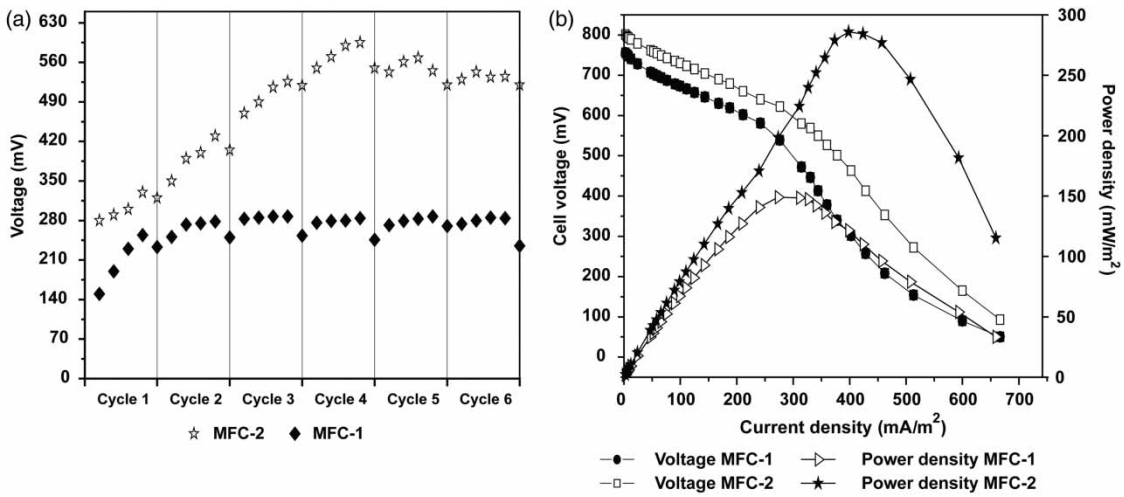

Figure 3 | (a) Comparison of cell voltage generation and (b) polarization curves for the MFC-1 inoculated with untreated anaerobic sludge and MFC-2 inoculated using lauric acid pretreated anaerobic sludge.

Working potential of the electrodes was measured as a function of current by varying the circuit load. For both the MFCs there was a gradual increase in the anode and decrease in the cathode potential with increase in current. The difference between the relative drop of cathode potential (E_c) with decreasing resistance for the MFCs was insignificant but the relative increase of anode potential (E_a) for the control MFC-1 was found to be more significant. Anode potential of the control MFC-1 was found to be −390 mV as compared to −430 mV for MFC-2 at 20 KΩ resistance (Figure 4(a)). These results show that there is a better anodic oxidation in MFC-2 as compared to the control MFC-1. Anode potential of MFC-1 dropped to −200 mV at a CD of 314 mA/m^2; whereas that of MFC-2 was −290 mV at the same CD. MFC-2 could produce a voltage of −268 mV up to a CD of 501 mA/m^2.

Bio-electrochemical evaluation

CV was performed for the bio-electrochemical evaluation of both these MFCs immediately after the fresh substrate was replaced. It helps to elucidate the electrochemical reaction occurring at the electrode surface by the direct electrochemical detection of the redox signals (Raghavulu *et al.* 2012). A slow scan rate of 1 mV/s was used from −1.0 (E_{min}) to +1.0 V (E_{max}). A current response of the electrode was recorded with continuously time varying potential (Figure 4(b)). CV was performed for the anodic half-cell with carbon felt as a working electrode, Ag/AgCl as a reference electrode (+197 vs. standard hydrogen electrode) and graphite rod as a counter electrode.

Current in the voltammograms is a direct indication of the release of electrons from the bacterial cell by the oxidation of substrate (Li *et al.* 2012). MFC-2 had higher catalytic currents (oxidative current, 114 mA; reductive current, 31 mA) as compared to control MFC-1 (oxidative current, 8.1 mA; reductive current, 7.4 mA) in the anodic and cathodic limits of the potential scan, respectively. Higher oxidative current as compared to the reductive current observed in MFC-2 indicates that there are limitations of the cathodic reaction due to the associated internal losses (Velvizhi & Venkata Mohan 2012). Despite similar operating conditions in the anodic chamber, higher oxidative current in MFC-2 indicates a higher activity of electrocatalytic anodic biofilm as compared to MFC-1. The CV of MFC-1 indicates the existence of lower oxidation rate as the current was low. The sigmoidal-shape curve of the voltamperogram of MFC-1 represents an electrocatalytic curve at increasing bio-electrocatalytic activity (Fricke *et al.* 2008). A similar curve with less hysteresis was observed during forward and reverse scan of MFC-1 across the same potential range.

Electro-kinetic evaluation

Tafel analysis is used for evaluating performance of MFCs in terms of proton and electron transfer (Srikanth & Venkata Mohan 2012). A Tafel plot was constructed for these MFCs during polarization in order to calculate the electrode's kinetic parameters. The linear portion of the Tafel slope can be used to analyze the number of electrons transferred in the process along with determination of exchange CD (i_0).

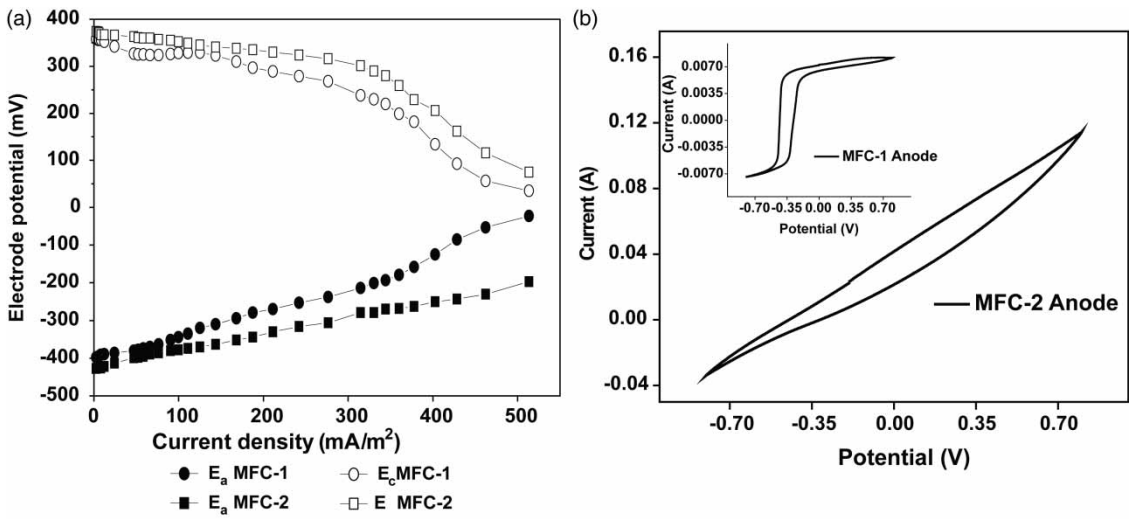

Figure 4 | (a) Electrode potential of the MFCs and (b) comparison of anodic half-cell voltammograms of the control MFC-1 and MFC-2.

Table 1 | Tafel analysis for the control MFC-1 and MFC-2

MFC	Electrode	Exchange CD (i_0), mA/m^2	Charge transfer coefficient × 10^4	Charge transfer resistance (R_{ct}), Ω m^2
Control MFC-1	Anode	24.87	0.66	0.00025
	Cathode	19.88	0.76	0.00032
MFC-2	Anode	40.78	0.66	0.00016
	Cathode	22.91	1.23	0.00028

The i_0 gives an idea about the intrinsic rate of the redox reaction of a chemical species at an electrode at equilibrium, and as the reaction rate increases, i_0 value increases. Tafel analysis showed a higher exchange CD at the anode in MFC-2 as compared to control MFC-1 (Table 1). This indicates efficient electron transfer from the biocatalyst to the anode during oxidation in MFC-2, due to enhanced electrogenesis. The lower exchange CD of control MFC-1 might be due to more utilization of substrate by methanogens. The difference between i_0 at the cathode of these MFCs during reduction was found to be less, indicating similar electron acceptor conditions prevailed in the cathodic chamber (Srikanth & Venkata Mohan 2012). The resistance offered by the electrode material for the transfer of charges at the electrode–electrolyte interface is expressed as charge transfer resistance (R_{ct}) (Jadhav *et al.* 2014). The anodic charge transfer resistance of MFC-2 was smaller as compared to the control MFC-1, which facilitated better electron acceptance and discharge at the anode. Higher R_{ct} was incurred in the cathode of MFC-2 as compared to the anode, which resulted in the increased barrier of electrons to discharge into the catholyte, possibly due to use of low conductivity tap water as catholyte.

CONCLUSION

The performance of MFC is adversely affected by competition between acetoclastic methanogens and electrogens for the substrate utilization. Pretreatment of anaerobic sludge to be used as an inoculum with lauric acid is an efficient approach for the selective inhibition of methanogens. The behavior of MFC in terms of polarization, anode potential and electrokinetic evaluation signified the higher performance of the MFC inoculated with anaerobic sludge pretreated with lauric acid. Considerable reduction in SMA was observed in lauric acid pretreated anaerobic sludge. Significant improvement in power generation and CE can be achieved by using this pretreatment method. Higher activity of biocatalyst on the anode surface after lauric acid pretreatment is evident by the higher oxidative current, higher exchange CD and lower charge transfer resistance. Sustainability in power generation can be achieved by providing the lauric acid dosing intermittently.

ACKNOWLEDGEMENT

The grant received from the Department of Science and Technology, Government of India (File No. DST/TSG/NTS/2010/61), to undertake this work is duly acknowledged.

REFERENCES

APHA 1998 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Behera, M., Jana, P. S. & Ghangrekar, M. M. 2010 Performance evaluation of low cost microbial fuel cell fabricated using earthen pot with biotic and abiotic cathode. *Bioresource Technology* **101** (4), 1183–1189.

Bhunia, P. & Ghangrekar, M. M. 2007 Required minimum granule size in UASB reactor and characteristics variation with size. *Bioresource Technology* **98** (5), 994–999.

Božic, A. K., Anderson, R. C., Carstens, G. E., Ricke, S. C., Callaway, T. R., Yokoyama, M. T., Wang, J. K. & Nisbet, D. J. 2009 Effects of the methane-inhibitors nitrate, nitroethane, lauric acid, Lauricidin® and the Hawaiian marine algae *Chaetoceros* on ruminal fermentation *in vitro*. *Bioresource Technology* **100** (17), 4017–4025.

Call, D. & Logan, B. E. 2008 Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. *Environmental Science & Technology* **42** (9), 3401–3406.

Chae, K. J., Choi, M. J., Kim, K. Y., Ajayi, F. F., Park, W., Kim, C. W. & Kim, I. S. 2010 Methanogenesis control by employing various environmental stress conditions in two-chambered microbial fuel cells. *Bioresource Technology* **101** (14), 5350–5357.

Freguia, S., Rabaey, K., Yuan, Z. & Keller, J. 2007 Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. *Environmental Science and Technology* **41** (8), 2915–2921.

Fricke, K., Harnisch, F. & Schröder, U. 2008 On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. *Energy & Environmental Science* **1** (1), 144–147.

Galbraith, H. & Miller, T. B. 1973 Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. *Journal of Applied Bacteriology* **36** (4), 647–658.

Jadhav, D. A., Ghadge, A. N., Mondal, D. & Ghangrekar, M. M. 2014 Comparison of oxygen and hypochlorite as cathodic electron

acceptor in microbial fuel cells. *Bioresource Technology* **154**, 330–335.

Kim, J. R., Min, B. & Logan, B. E. 2005 Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Applied Microbiology and Biotechnology* **68** (1), 23–30.

Lee, H. S., Parameswaran, P., Kato-Marcus, A., Torres, C. I. & Rittmann, B. E. 2008 Evaluation of energy-conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. *Water Research* **42** (6–7), 1501–1510.

Li, Z., Qing, C., Shungui, Z., Yong, Y. & Haoran, Y. 2012 Methanogenesis control using 2-bromoethanesulfonate for enhanced power recovery from sewage sludge in air-cathode microbial fuel cells. *International Journal of Electrochemical Science* **7**, 6512–6523.

Liu, H., Wang, J., Wang, A. & Chen, J. 2011 Chemical inhibitors of methanogenesis and putative applications. *Applied Microbiology and Biotechnology* **89** (5), 1333–1340.

Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. & Rabaey, K. 2006 Microbial fuel cells: Methodology and technology. *Environmental Science and Technology* **40** (17), 5181–5192.

Lu, N., Zhou, S. G., Zhuang, L., Zhang, J. T. & Ni, J. R. 2009 Electricity generation from starch processing wastewater using microbial fuel cell technology. *Biochemical Engineering Journal* **43** (3), 246–251.

Marcus, A. K., Torres, C. I. & Rittmann, B. E. 2007 Conduction based modelling of the biofilm anode of a microbial fuel cell. *Biotechnology and Bioengineering* **98** (6), 1171–1182.

More, T. T. & Ghangrekar, M. M. 2010 Improving performance of microbial fuel cell with ultrasonication pre-treatment of mixed anaerobic inoculum sludge. *Bioresource Technology* **101** (2), 562–567.

Picioreanu, C., Head, I. M., Katuri, K. P., van Loosdrecht, M. C. M. & Scott, K. 2007 A computational model for biofilm-based microbial fuel cells. *Water Research* **41** (13), 2921–2940.

Raghavulu, S. V., Babu, P. S., Goud, R. K., Subhash, G. V., Srikanth, S. & Mohan, S. V. 2012 Bioaugmentation of an electrochemically active strain to enhance the electron discharge of mixed culture: process evaluation through electro-kinetic analysis. *RSC Advances* **2** (2), 677–688.

Soliva, C. R., Hindrichsen, I. K., Meile, L., Kreuzer, M. & Machmüller, A. 2003 Effects of mixtures of lauric and myristic acid on rumen methanogens and methanogenesis *in vitro*. *Letters in Applied Microbiology* **37** (1), 35–39.

Srikanth, S. & Venkata Mohan, S. 2012 Change in electrogenic activity of the microbial fuel cell (MFC) with the function of biocathode microenvironment as terminal electron accepting condition: influence on overpotentials and bio-electro kinetics. *Bioresource Technology* **119**, 241–251.

Velvizhi, G. & Venkata Mohan, S. 2012 Electrogenic activity and electron losses under increasing organic load of recalcitrant pharmaceutical wastewater. *International Journal of Hydrogen Energy* **37** (7), 5969–5978.

Zeitz, J. O., Bucher, S., Zhou, X., Meile, L., Kreuzer, M. & Soliva, C. R. 2013 Inhibitory effects of saturated fatty acids on methane production by methanogenic Archaea. *Journal of Animal and Feed Sciences* **22** (1), 44–49.

First received 14 May 2014; accepted in revised form 26 August 2014. Available online 6 September 2014