Journal of Scientific & Industrial Research
Vol. 71, September 2012, pp. 621-626

Optimization of power generation in a dual chambered aerated membrane microbial fuel cell with *E. coli* as biocatalyst

Sam A Masih[1], Mercy Devasahayam[1]* and Masochon Zimik[2]

[1]Centre for Transgenic Studies, [2]Department of Cellular and Molecular Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad 211 007, India

Received 17 April 2012; revised 06 August 2012; accepted 07 August 2012

This study presents power generation optimization in a dual chambered aerated membrane microbial fuel cell (MFC) with *E. coli* as biocatalyst at different culture densities (0.5 & 1.0 OD) using sodium acetate as substrate. Optimum parameters were found as follows: voltage, 783 mV; power density, 222.84 mW/cm^2; and coulombic efficiency (CE), 86.56%. CE with sodium acetate as substrate was higher than with fermentable substrate. High CE indicates improved design of dual chambered mediator less aerated membrane MFC for electrical conductance with an application in water remediation.

Keywords: Bioelectricity, Coulombic efficiency, *E. coli*, MFC, Power density

Introduction

Energy in modern society is acquired mainly through burning fossil fuels[1]. Microbial fuel cell (MFC) technology generates electrical energy through oxidation of biodegradable organic matter in presence of biocatalysts (fermentative bacteria or enzyme) under mild reaction conditions[2]. *Escherechia coli* were the first bacteria used in an MFC by Potter in 1911 for power generation[3]. Various other microorganisms (*Geobacter*[4], *Geopsychrobacter*[5], *Pseudomonas*[6] and *Enterobacter*[7]) have been used for electricity generation using MFC technology[4,8]. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are rich sources of these microorganisms[9,10]. An advantage of MFC over hydrogen and methanol fuel cells is diverse range of organic materials used as fuel or substrate[11-14] (acetate, glucose, sucrose, starch etc.). In MFC, substrate and substrate loading rate affect electricity generation[14]. MFC performance decreases rapidly at higher substrate loads[15].

Microorganisms can transfer electrons to anode electrode using exogeneous mediators (potassium ferricyanide, thionine, or neutral red), mediators produced by bacteria or by direct transfer of electrons from respiratory enzymes (cytochromes) to electrode[4]. Several microorganisms (*Enterobacter*[7], *Shewanella putrefaciens*[16], *Geobacteraceaesulferreducen*[4], *Rhodoferax ferrireducens*[17] and *Geobacter metallireducens*[18]) transfer electrons across their membrane directly to anode by forming biofilms on electrode. Several microorganisms (*Actinobacillussuccinogenes, Pseudomonas aeruginosa, Gluconobacteroxydans* and *Lactobacillus plantarum*) use glucose as substrate, *E. coli* utilizes glucose and sucrose, and *Shewanellaputrefaciens* uses lactate, pyruvate, acetate and glucose[16]. Glucose is a commonly used substrate in MFCs. Performance of a MFC containing *Proteus vulgaris* is shown to depend on carbon source in initial medium[19]. MFC with *Enterobacteraerogen* and *Enterobacter cloacae* show high voltage of 990 mV and 1100 mV respectively with sodium acetate substrate[7].

High coulombic efficiency (CE) of 91.4% with *Enterobacterspps* has been obtained using sodium acetate as substrate[7]. Maximum power obtained with *E. cloacae* using sodium acetate is 440 mW/cm^2, which is higher than with sucrose as substrate[7]. With *Enterobacterspps*, high voltage, power density and CE were obtained with non fermentable substrate than fermentable substrate[7]. *E. coli* at various cell densities

*Author for correspondence
E-mail: mercy@shiats.edu.in

with non fermentable substrate gives a maximum voltage[20] (592-779 mV). Other optimum parameters[20] of *E. coli* with fermentable substrate are: power density, 220.66 mW/cm^2; and CE, 85.2%. An application of MFC is in water treatment since high chemical oxidative demand (COD) removal is observed in bacteria pure culture and water sample MFC[20-22].

This study presents increased electrical conductance with non-fermentable substrate (sodium acetate) using *E .coli* at varying cell densities (0.5 & 1.0 OD) as biocatalyst.

Experimental Section

Culture and Media

E. coli (MTCC-64) was sub cultured on nutrient agar (peptone, 5; beef extract, 3; NaCl, 5; and agar, 10 g/l) and incubated at 37°C under aerobic conditions. Bacterial cultures were revived according to instructions of IMT, Chandigarh, India. *E. coli* was grown in LB media to an optical density (0.5 or 1 OD) before transfer to anode chamber as reported[20].

Culture Conditions and COD

To determine COD, LB media was inoculated with *E. coli* bacterial culture and grown till an OD_{600} of 0.5. To 50 ml bacterial culture in a conical flask, 0.02 N potassium dichromate (5 ml) was added and kept in a water bath at 100°C for 1 h. After cooling for 10 min, 10% potassium iodide (5 ml) was added followed by 1.1% sulfuric acid (10 ml). Solution was titrated with 0.1 M sodium thiosulphate until a pale yellow color appeared and 1 ml of 1% starch solution till a dark blue color appeared. Solution was again titrated with 0.01 M sodium thiosulphate solution till blue color disappeared (B). COD is determined as, COD = [8 x 100 x (B-A)] / V, where B= volume of titrant used for sample, A= volume of titrant used for distilled water and V= volume of sample to be titrated[20,21].

MFC Construction and Operation

Materials for MFC construction included axenta boxes (AXIVA), tubes (Polytab), copper wires, epoxy material, external resistances (10-500 ohms), graphite electrodes (3 cm x 3 cm x 0.5 cm) (Carbon product, India), strong cation exchange membrane CMI-7000 (Membrane International, NJ, USA) with polystyrene cross linked with divinylbenzene. Membrane was immersed in 1% H_2O_2 for 6 h followed by deionised water for 6 h to increase porosity. Graphite porous electrodes (diam, 0.5 cm) with 5 pores were immersed in deionised water for 24 h to increase porosity as reported[20,21]. Two Axenta chambers (capacity, 300 ml) were interconnected using 2 tubes (9 cm x 3 cm) on either side of cation exchange membrane CMI-7000 (Membrane International, New Jersey)[23,24]. Chambers were autoclaved prior to adding the media in anode chamber and PBS with 100 mM ferricyanide in cathode chamber. A pretreated porous electrode was placed in each chamber using copper wires. Cathode chamber was placed on a magnetic stirrer with a magnetic bar. MFC operation was conducted as reported[20,21] in 2 phases.

Phase 1

Anode chamber contained 300 ml of *E.coli* bacterial culture of 0.5 OD with 1.2 g of substrate (sodium acetate) maintained at pH 5.5 using 88% orthophosphoric acid. PBS (300 ml) containing ferricyanide (100 mM), pH 7.2 was placed in cathode chamber and two chambers connected by copper wires to a multimeter (Orpat, India ODM-200) in external circuit. Voltage was recorded without any resistance (open circuit voltage) for 2-3 days until voltage stabilized[7]. Different resistors were applied in open circuit and current was measured for each resistor. Maximum voltage was found at 100 &! resistor and thereafter this external resistor was used in all subsequent experiments. MFC was operated for 15 days with recordings every 24 h supplemented with fresh substrate at 4 g/l every 3 days[20,21]. After 15 days, circuit was disconnected and COD of anode solution measured.

Phase 2

Anode chamber contained 300 ml of *E.coli* bacterial culture of 1.0 OD and 1.2 g substrate (sodium acetate) maintained at pH 5.5 using 88% orthophosphoricacid. Ferricyanide (100 mM) in PBS (300 ml) pH 7.2 was placed in cathode chamber. The same procedure was repeated as in the case of phase 1. MFC was monitored using a multimeter (Orpat, ODM-200). Circuit was connected with a fixed load of 100 &! except when different resistors (10-500 &!) were used to determine power generation as a function of load. Current (i) was calculated at a fixed resistance (R) from recorded voltage as i=V/R. Power generated was calculated as P= iV or P= i^2R and normalized by surface area of anode (27.53 cm^2) as reported[20,21]. CE was calculated by estimating substrate removal efficiency from COD[25] as CE = (Cs-Co/Cs) x 100, where Cs is initial COD of 0.5/ 1.0 OD bacterial culture with various substrates, and Co is final COD at the end of 15 day batch cycle[20,21].

Table 1—Comparison of electrical conductance of *E.coli* MFC using various substrates

Parameters	Sucrose[20]		Glucose[20]		Sodium acetate	
E. coli density	0.5 OD	1.0 OD	0.5 OD	1.0 OD	0.5 OD	1.0 OD
Max. voltage, mV	660±0	779±0	592±0	701±0	783±2.6	688±057
Max. current, mA	6.60	7.79	5.92	7.01	7.83	6.88
Current density, mA/cm^2	0.240	0.283	0.215	0.254	0.284	0.249
Power, mW	4356.00	6068.41	3504.64	4914.01	6130.89	4733.50
Power density, mW/cm^2	158.40	220.66	127.43	178.69	222.84	172.01
Initial COD, mg/l	195	220	195	220	187	203
Final COD, mg/l	28.8	67.2	65.6	56.0	25.1	44.0
CE, %	85.20	69.45	66.35	74.54	86.50	78.20

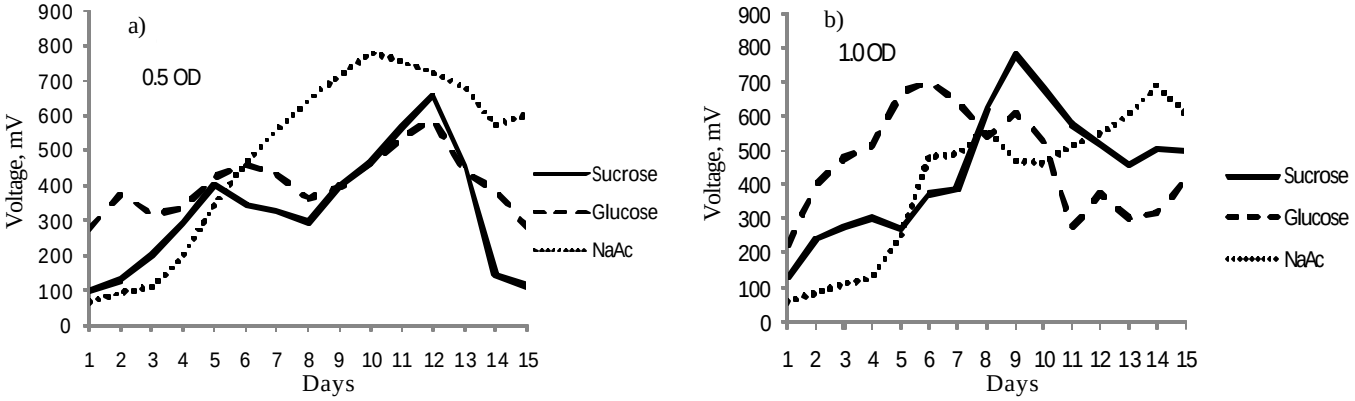

Fig. 1—Comparison of voltage generated during a 15 day batch cycle for *E.coli* at: a) 0.5 OD; and b) 1.0 OD

Results and Discussion

Comparison of Voltage Generation in a Dual Chambered Membrane MFC

Effect of different substrates and bacterial culture density (0.5 & 1.0 OD) under acidophilic conditions in a 15 day cycle supplemented with sodium acetate substrate every 3 days were studied. Using a multimeter, voltage was recorded for individual bacterial culture samples in 3 individual experiments with the mean used for all subsequent calculations. Standard deviation (SD) of triplicate was calculated and was less than 0.05% (Table 1). Using a 0.5 OD culture of *E. coli*, highest recorded voltage using following substrates was: sucrose, 660; glucose, 592; and sodium acetate, 783 mV (Fig. 1a; Table 1). Using 1.0 OD culture of *E. coli*, highest recorded voltage using following substrates was: sucrose, 779; glucose, 701; and sodium acetate, 688 mV (Fig. 1b; Table 1). From earlier studies[7,26], maximum voltage obtained was: *E. coli*, 600; *Enterobacteraerogen*, 990; and *E. cloacae*, 1100 mV. It was observed that voltage decreased after reaching a peak for *E.coli* specific MFC during 15 day batch cycle (Fig. 1). sucrose as substrate (Fig. 1) gave a higher voltage than glucose while sodium acetate gave a higher voltage than both sucrose and glucose (Table 1). High voltage generated using sodium acetate demonstrate significant effect of non fermentable substrate on power generation (Table 1).

Comparison of Current Generation in a Dual Chambered Membrane MFC

Current generated by dual chambered aerated membrane MFC inoculated with pure *E. coli* was calculated from voltage recordings as reported earlier[20] (Table 1). Using a 0.5 OD culture of *E. coli*, highest recorded current using following substrates was: sucrose, 6.60; glucose, 5.92; and sodium acetate, 7.83 mV (Table 1). Using 1.0 OD culture of *E. coli*, highest recorded current using following substrates was: sucrose, 7.79; glucose, 7.01; and sodium acetate, 6.88 mV (Table 1). From earlier studies[27], highest current generated is 5.43 mA from a mixed culture MFC using a 50&! external resistor. Overall comparison of current profiles from *E.coli* MFC indicates that sucrose or sodium acetate as substrate result in a more stable current than glucose[20]. In *E.coli* MFC, it was observed that voltage

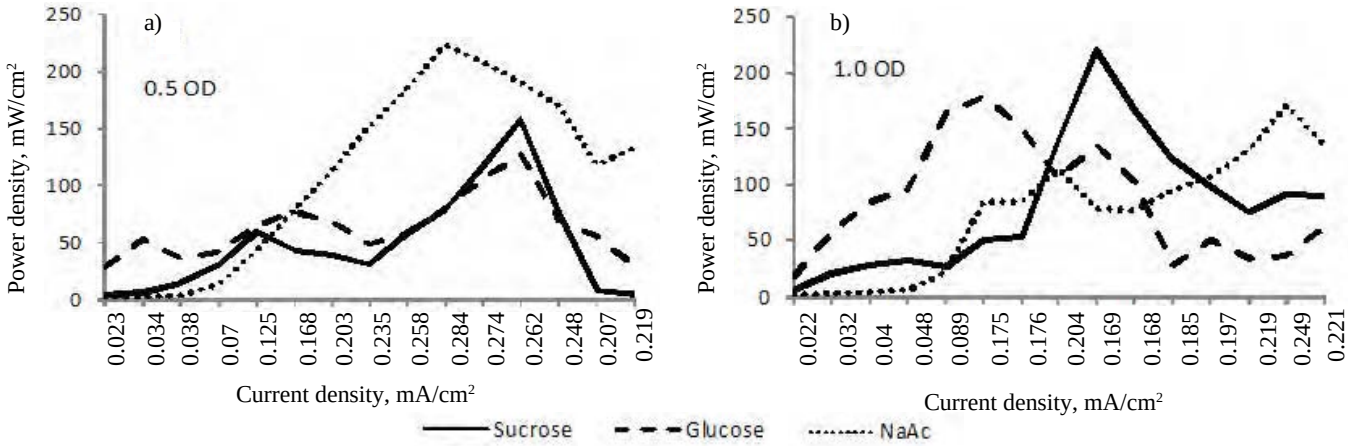

Fig. 2—Polarization curves generated with current density shown for sodium acetate during a 15 day batch cycle for *E. coli* at: a) 0.5 OD; and b) 1.0 OD

or current was generated without a lag phase for all substrates at both bacterial density (0.5 & 1.0 OD) and it increased by 48 h (Fig. 1), indicating that anodic electron transfer was facilitated by *in situ* oxidation of hydrogen synthesised by biocatalyst and not through a biofilm formation on anode[20,28]. It has been observed that there is a direct association between presence of hydrogen formation and electrical conductance in an MFC[28]. Voltage and subsequently current generated in *E. coli* specific MFC decreased (Fig. 1) during 15 day batch cycle, indicating absence of a biofilm on anode[20]. This was unlike that observed with *Enterobacterspps* specific MFC where eventual formation of a biofilm was indicated[7].

Comparison of Power Density in Dual Chambered Membrane MFC

Power generation was calculated from mean voltage readings as P = V x I/A where A is surface area of electrode (27.53 cm^2) (Table 1). With 0.5 OD *E. coli*, highest recorded power density using following substrates was: sucrose, 158.40; glucose, 127.43; and sodium acetate, 222.60 mV (Fig. 2a). With 1.0 OD *E. coli*, highest recorded power density using following substrates was: sucrose, 220.66; glucose, 178.69; and sodium acetate, 172.01 mV (Fig. 2b; Table 1). This lower electrical conductance with sodium acetate at biocatalyst concentration of 1.0 OD compared to 0.5 OD was also observed with *Enterobacterspps*, indicating an optimal bacterial concentration for maximum power output[7,20]. Highest power generated using *E. coli* in present study with sodium acetate as substrate was 6130 mW with a power density of 222.6 mW/cm^2 (Table 1). Highest power recorded using *E.coli* as biocatalyst and with

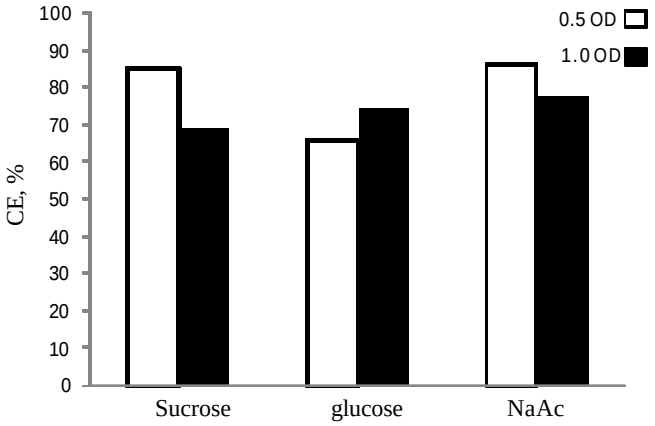

Fig. 3—Comparison of coulombic efficiency

electron mediators is reported as 728 mW[26,29]. While maximum power density obtained with *P. vulgaris* was 452 mW/m^2 using composite graphite electrodes and electron mediators[30]. *E. cloacae* gave a power density of 440 mW/cm^2, the highest reported till date[7].

Coulombic Efficiency (CE) of E. coli Dual Chambered Aerated Membrane MFC

E.coli specific MFC with sucrose as substrate demonstrate higher electrical conductance than glucose as substrate[20]. However, sodium acetate gave higher electrical conductance than both sucrose and glucose (Table 1). Voltage and current generated and power generated for each individual batch when using *E.coli* as biocatalyst during 15 day batch cycle showed high CE (Table 1). In *E. coli* specific MFC, highest CE using following substrates was: sucrose, 85.2; glucose, 74.54; and sodium acetate, 86.56% (Fig 3, Table 1). Similarly, with *Enterobacterspps* high CE (83-92%) was obtained when using sodium acetate as substrate[7]. From earlier

reports[31] in a single chambered MFC, CE obtained using following substrates was: acetate, 72.3; butyrate, 43; propionate, 36; and glucose, 15%. With *Proteus vulgaris* and glucose as substrate a CE of 50% has been reported[19]. High CE obtained in present study demonstrates improved design of MFC and requirements of specific substrate and optimal biocatalyst concentration.

Effect of Biocatalyst Density On Power Output

In an MFC containing *P. vulgaris*, power output has been observed to increase with increasing bacterial density till an optimal bacterial density, after which power output decreases[30]. Similarly, MFC containing a 1:20 dilution of a log phase *E.cloacae* culture inoculum as biocatalyst gives a maximum current of 0.38 mA[32]. In present study, at *E.coli* bacterial density greater than 100 fold to a bacterial inoculums, maximum current of 7.79 mA was obtained. Also, high electrical conductance observed was facilitated by high inoculum density of *E.coli* bacterial culture[7,20]. Present study demonstrates high bacterial density as biocatalyst is required for maximum power output and high coulombic efficiency.

Conclusions

For a dual chambered aerated membrane MFC with *E. coli* as biocatalyst, non fermentable substrate sodium acetate gave higher power density of 222.6 mW/cm^2 than fermentable substrate. Biocatalyst optimization is essential for maximum power generation since a low bacterial culture concentration (0.5 OD) showed higher electrical conductance than 1.0 OD with sodium acetate. Thus for maximum power generation by MFC, optimization of substrate and biocatalyst concentration is essential.

Acknowledgements

Authors thank Prof R B Lal, Vice Chancellor and Prof A K A Lawrence, Registrar, Sam Higginbottom Institute of Agriculture, Technology and Sciences for encouragement and support.

References

1 Runnalls O J C & Mackay D, Energy growth, in *Env Sci Eng*, edited by J A Henary & G W Heinke, (Prentice Hall, USA) 1989, 46-85.

2 Rabaey K & Verstraete W, Microbial fuel cells: novel biotechnology for energy generation, *Trends Biotechnol*, **23** (2005) 291-298.

3 Potter M C, Electrical effects accompanying decompostion of organic compounds, *Proc Roy Soc Lond*, **84** (1911) 260-276.

4 Bond D R & Lovley D R, Electricity production by *Geobactersulfurreducens* attached to electrodes, *Appl Environ Microbiol*, **69** (2003) 1548-1555.

5 Holmes D E, Nicoll J S, Bond D R & Lovley D R, Potential role of a novel psychrotolerant member of the family *Geobacteraceae*, *Geopsychrobacterelectrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell, *Appl Environ Microbiol*, **70** (2004) 6023-6030.

6 Rabaey K, Boon N, Siciliano S D, Verhaege M & Verstraete W, Biofuel cells select for microbial consortia that self-mediate electron transfer, *Appl Environ Microbiol*, **70** (2004) 5373-5382.

7 Masih S A, Devasahayam M, Srivastava R & Gupta S, *Enterobacter* species specific microbial fuel cells show increased power generation with high coulombic efficiency, *Trends Biosci*, **5** (2012) 114-118.

8 Park D H & Zeikus J G, Improved fuel cell and electrode designs for producing electricity from microbial degradation, *Biotechnol Bioeng*, **81** (2003) 348-355.

9 Niessen J, Harnisch F, Rosenbaum M, Schroder U & Scholz F, Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation, *Electrochem Commun*, **8** (2006) 869-876.

10 Zhang E, Xu W, Diao G & Shuang C, Electricity generation from acetate and glucose by sedimentary bacterium attached to electrode in microbial-anode fuel cells, *J Power Sources*, **161** (2006) 820-825.

11 Catal T, Li K, Bermek H & Liu H, Electricity production from twelve monosaccharides using microbial fuel cells, *J Power Sources*, **175** (2008) 196-200.

12 Logan B E, Cheng S, Watson V & Estadt G, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells, *Environ Sci Technol*, **41** (2007) 3341-3346.

13 Behera M & Ghangrekar M M, Performance of microbial fuel cell in response to change in sludge loading rate at different anodic feed pH, *Biores Technol*, **100** (2009) 5114-5121.

14 Lu N, Zhou S G, Zhuang L, Zhnag J T & Ni J R, Electricity generation from starch processing wastewater using microbial fuel cell technology, *Biochem Eng J*, **43** (2009) 246-251.

15 Rabaey K, Lissens G, Siciliano S D & Verstraete W, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency, *Biotechnol Lett*, **25** (2003) 1531-1535.

16 Kim H J, Park H S, Hyun M S, Chang I S, Kim M *et al*, A mediatorless microbial fuel cell using a metal reducing bacterium, *Shewanellaputrefaciens*, *Enz Microbial Tech*, **30** (2002) 145-152.

17 Chaudhari S K & Lovely D R, Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells, *Nature Biotech*, **21** (2003) 1229-1232.

18 Min B, Cheng S & Logan B E, Electricity generation using membrane and salt bridge microbial fuel cells, *Water Res*, **39** (2005) 1675-1686.

19 Allen R M & Bennetto H P, Microbial fuel cells: electricity production from Carbohydrates, *Appl Biochem Biotechnol*, **39** (1993) 27-40.

20 Devasahayam M & Masih S, Microbial fuel cells demonstrate high coulombic efficiency applicable for water remediation, *Indian J Exp Biol*, **50** (2012) 430-438.

21 Masih S, Devasahayam M, Haider J & Mishra S, Remediation of Inland water and waste water samples using microbial fuel cell technology, *Trends Biosci*, **4** (2011) 175-179.

22 Liu H & Logan B E, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ Sci Technol*, **38** (2004) 4040-4046.

23 He Z, Minteer S D & Angenent L, Electricity generation from artificial wastewater using an upflow microbial fuel cell, *Environ Sci Technol*, **39** (2005) 5262-5267.

24 Rabaey K, Clauwaert P, Aelteman P & Verstraete W, Tubular microbial fuel cells for efficient electricity generation, *Environ Sci Technol*, **39** (2005) 8077-8082.

25 Logan B E, *Microbial Fuel cells* (Wiley, New Jersey) 2008.

26 Park D H & Zeikus J G, Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanellaputrefaciens*, *Appl Microbiol Biotechnol*, **59** (2002) 58-61.

27 Mohan S V, Vulu S V R, Srikanth S & Sharma P N, Bioelectricity production by mediatorless microbial fuel cell under acidophilic conditions using wastewater as substrate, influence of substrate loading rate, *Curr Sci*, **92** (2007) 1720-1726.

28 Zhuang K, Vemuri G N & Mahadevan R, Economics of membrane occupancy and respiro-fermentation, *Mol Syst Biol*, **7** (2011) 500-509.

29 Zhang T, Zeng Y, Chen S, Ai X & Yang H, Improved performances of *E. coli* catalyzed microbial fuel cells with composite graphite/PTFE anodes, *Electrochem Commun*, **9** (2007) 349-353.

30 Yuan Y & Kim S, Improved performance of a microbial fuel cell with polypyrrole/carbon black composite coated carbon paper anodes, *Bull Korean Chem Soc*, **29** (2008) 1344-1348.

31 Chae S J, Günes F, Kim K K, Kim E S, Han G H *et al*, Synthesis of large-area graphene layers on poly-nickel substrate by chemical vapor deposition: wrinkle formation, *Adv Mater*, **21** (2009) 2328-2333.

32 Nimje V R, Chen C Y, Chen C C, Tsai J Y, Chen H R *et al*, Microbial fuel cell of *Enterobacter cloacae*: Effect of anodic pH microenvironment on current, power density, internal resistance and electrochemical losses, *IntJ Hydrogen Energy*, **36** (2011) 11093-11101.