Short communication

Microbial fuel cell constructed with a micro-organism isolated from sugar industry effluent

D. Prasad, T.K. Sivaram, Sheela Berchmans [*], V. Yegnaraman

Central Electrochemical Research Institute, Karaikudi-630006, Tamilnadu, India

Abstract

Investigations are carried out with *Clostridium* sp. which is isolated from sugar industry effluents, as a biocatalyst for current generation in a microbial fuel cell. Two different configurations of the cell are evaluated. In both cells, the anode compartment consists of suspended cells of *Clostridium* sp. in a nutrient broth in a phosphate buffer. In the first configuration, the cathode compartment consisted of ferricyanide solution (0.05 M). In the second configuration, the cathode compartment consisted of cells of *Thiobacillus ferrooxidans* suspended in a nutrient broth. The second configuration represents a complete microbial cell with the anodic and cathodic reactions driven by micro-organisms. The performance characteristics of the two configurations are evaluated with two different anode materials, graphite and graphite felt.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Microbial fuel cell; Direct electron-transfer; *Clostridium* sp.; *Thiobacillus ferrooxidans*

1. Introduction

A fuel cell is an electrochemical device that continuously converts chemical energy to electrical energy for as long as fuel and oxidants are supplied. Fuel cells theoretically bypass the inefficiencies of internal combustion engine by directly oxidizing and reducing compounds at electrode surfaces. Microbial fuel cells (MFC) seek to use the microbial catalytic abilities of organic compounds, from simple carbohydrates to waste organic matter, for the generation of electricity [1–3]. Abundant energy is stored primarily in the form of carbohydrates and is found in a variety of sources, e.g., waste biomass from the agricultural sector, municipal waste, and energy crops such as corn. The different ways of deriving useful forms of energy from carbohydrates include the production of ethanol and conversion to hydrogen, but these are multi-step approaches and face technical and economic hurdles. An alternative method is the direct conversion of sugars to electrical power. Existing fuel cells catalyzed by transition metals cannot be used to produce electric power from carbohydrates. A viable approach is to use MFCs in which micro-organisms catalyse the oxidation of sugar during their growth processes or in fermentation processes. Bacteria at the anode oxidize organic matter and transfer electrons to a cathode through an external circuit, producing current. Interestingly, MFCs show promise as a method to treat wastewater and produce electricity simultaneously [4,5]. They can be used as biosensors to detect lactate, fructose and BOD [6,7]. MFCs have also been demonstrated for use in remote areas, such as the bottom of the ocean where it is difficult to replace the batteries [8]. The development of intelligent machines that obtain their own operational power by digesting organic matter, called gastrobots has been reported [9].

MFCs are classified into two types depending on how electrons are transferred from the bacteria to the electrode. Mediator MFCs are systems in which electron shuttles, or 'mediators', are added to the system to transfer the electrons between micro-organisms and electrodes. The need for high concentrations of mediators, many of which are toxic chemicals, is believed, however, to make electricity generation impractical on a large scale. On the other hand, mediatorless MFCs show promise for harvesting energy from waste organic matter. These devices rely on the presence of metal-reducing bacteria such as *Shewanella* [10], *Rhodoferax* [11] and *Geobacteraceae* [12], of fermentative

* Corresponding author at: EEC Division, CECRI, Karaikudi-630006, Tamilnadu, India. Tel.: +91 4565 227551; fax: +91 4565 227777.
  E-mail address: sheelaberchmans@yahoo.com (S. Berchmans).

type of bacteria like *Clostridium butyricum* [13]. Metal-reducing bacteria are believed to transfer electrons directly to the anode through the use of electrochemically active redox enzymes in their outer membrane. Power generation from MFCs varies widely as a function of the inoculum, substrate and reactor. Values range from less than 1 to 3600 mW m^{-2} [10,14].

This study reports the isolation of the microbial species *Clostridium* sp. from an effluent sample collected from a sugar industry. This effluent was chosen on the basis that microorganisms that can assimilate sugar at a greater extent would be present in a sugar industry effluent, because the existing bottleneck for applications of the MFCs is not the conversion efficiency but the conversion rate. Using this microbial species, MFCs were constructed in two different configurations, namely:

- Configuration 1

  Graphite or graphite felt|*Clostridium* sp.

     +deaerated nutrient broth||ferricyanide|graphite.

- Configuration 2

  Graphite or graphite felt|*Clostridium* sp.

     +deaerated nutrient broth||*Thiobacillus ferrooxidans*

     +nutrient broth|graphite.

In configuration 1, the ferricyanide in the cathode compartment becomes depleted with time and in due course the compartment becomes dirty greenish-blue, which indicates the formation of pigments like Prussian blue. It has to be replaced often during the course of the experiments. On the other hand, in the configuration 2, the ferric sulfate present in the nutrient broth of the microbial cathode compartment acts as the electron acceptor and is continuously regenerated by the micro-organisms and hence the solution in the compartment remains clear, unlike the case of ferricyanide. An important objective of the present investigation is to compare the performance of a microbial cathode compartment versus a ferricyanide-containing cathode compartment. The direct electron-transfer capability of the micro-organism and the efficiency of current generation in the absence of a terminal electron acceptor are also investigated. Moreover, the study aims to understand the reactions responsible for current generation. The performance characteristics of both configurations are evaluated with two different anode materials, viz., graphite and graphite felt.

2. Experimental

A molasses sample, collected from a nearby sugar industry, was used as an inoculum for growing bacterial cultures by means of the spread-plating technique. The required quantity of inoculum was determined by the serial dilution method. From the nutrient agar plates, isolated organisms were inoculated into a sterile medium broth, incubated for 24 h, and then used in MFCs. The media for the growth of these micro-organisms were prepared from the following organic nutrients:

- peptone: 10 g;
- beef extract: 10 g;
- sodium acetate: 5 g;
- yeast extract: 1.5 g;
- starch: 1 g;
- glucose: 1 g;
- L-cysteine HCl: 0.5 g.

Immediately before use, sterile solutions of sodium sulfate (0.04%, w/v) and ferric citrate (0.07%, w/v) were added to the medium. The nutrients were dissolved in a phosphate buffer (1000 ml) of composition is: KCl: 1 g; NaH$_2$PO$_4$: 6 g; NaCl: 2.9 g; Na$_2$CO$_3$: 2 g.

The media for the growth of *Thiobacillus ferrooxidans* was:

- Solution A
  - (NH$_4$)$_2$SO$_4$: 3 g;
  - KCl: 0.1 g;
  - K$_2$HPO$_4$: 0.5 g;
  - MgSO$_4$·7H$_2$O: 0.5 g;
  - Ca(NO$_3$)$_2$: 0.01 g;
  - distilled water: 700 ml.
- Solution B
  - FeSO$_4$·7H$_2$O: 44.22 g;
  - H$_2$SO$_4$ (1 M): 10 ml;
  - distilled water: 290 ml.

Solutions A and B were sterilized separately and then mixed asceptically.

A two-compartment cell made of Perspex with a Nafion 961 membrane as the separator was used for the construction of the MFC. Rectangular graphite electrodes (14.4 cm^2) served as the anode and cathode and were suspended from the top cover, which was tightly sealed. Two different configurations of the MFC, as discussed earlier were constructed. In both configurations, the anode compartment consisted of suspended cells of *Clostridium* sp. in a nutrient broth in phosphate buffer. The cathode compartment consisted of 0.05 M ferricyanide solution in the first configuration, while in the second configuration, it was comprised of suspended cells of *T. ferrooxidans* suspended in a nutrient broth. The second configuration thus represents a complete microbial fuel cell. Graphite and graphite felt were evaluated as anode materials, while graphite was used as the cathode in both the configurations. The second configuration was slightly modified to demonstrate direct electron transfer. The addition of sodium sulfate and ferric citrate was deliberately avoided in the nutrient broth solution constituting the anolyte. The volumes of anolyte and catholyte were each around 150 ml.

2.1. Preparation of gold substrates immobilized with Clostridium sp. for cyclic voltammetric measurements

Gold electrodes were made from a 2 mm diameter rod of gold fused to a Teflon rod. Electrical contact was made by spot-welding a brass rod to the gold piece. The electrodes were cleaned by means of the standard procedure described elsewhere [15]. The cleaned electrode was immersed in a 1 wt.% cystamine

solution overnight, washed with distilled water, and then treated with 20 µl of 1 wt.% glutaraldehde followed by treatment with *Clostridium* sp. cells suspended in a broth. Next, the electrode was washed with phosphate buffer and used for recording cyclic voltammograms. By this procedure, the micro-organisms were immobilized on to the gold surface. The cyclic voltammograms were recorded in phosphate buffer (pH 7.0) (50 ml of 0.1 M potassium dihydrogen phosphate and 29.1 ml of 0.1 M sodium hydroxide were mixed and made up to 100 ml using distilled water). A Pt foil served as the counter electrode and a calomel electrode was used as the reference. A PARSTAT 2263 system was used to obtain cyclic voltammograms.

2.2. Preparation of specimens for SEM analysis

Graphite felt anodes were removed at the end of the polarization experiments, washed with phosphate buffer and cut into pieces of size of 1 cm × 1 cm for SEM analysis.

2.3. Analysis of fermentation products by HPLC

After studying the performance of the fuel cell by applying a load of 100 Ω, the anolyte solution was removed from the cell and analysed by HPLC to find the end products of fermentation. HPLC (Shimadzu Model LC-8A, Japan) was recorded using the methanol as the mobile phase and silica gel as the stationary phase. A flow rate of 1 ml min^{-1} was maintained. A UV spectrophotometric detector was employed with a wavelength of 254 nm.

3. Results and discussion

3.1. Isolation and Identification of micro-organisms from sugar industry effluents

The micro-organisms were isolated from sugar industry effluents by the spread-plating technique. With the help of biochemical techniques (grams staining, endospore staining) it was found that *Clostridium* sp. is the micro-organism that is primarily grown from the effluent. From the plates, pure cultures were prepared by plating for the second time. These were then used for the studies.

3.1.1. Cell configuration 1

The variation of open-circuit potential (OCP) with time is shown in Fig. 1. In the case of the graphite anode, the OCP values are virtually steady from the start. The OCP varied from 0.48 to 0.51 V over a period of 20 h and continued to remain so with increasing time. In the case of the graphite felt, the OCP values are initially around 0.34 V, increase to 0.67 V over a period of 20 h, and thereafter do not increase much further. After 60 h, the value has reached only 0.71 V.

Polarization curves recorded for the graphite and graphite felt anode materials are shown in Fig. 2. From the curves, it is very clear that a maximum power density of 2.965 and 5.62 W m^{-3} is observed for graphite and graphite felt material, respectively.
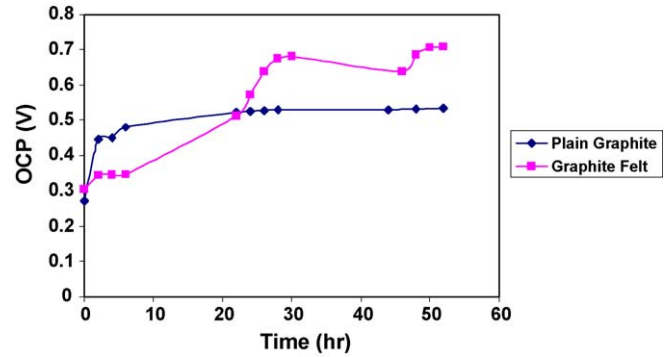

Fig. 1. Open-circuit potential of cell configuration 1.
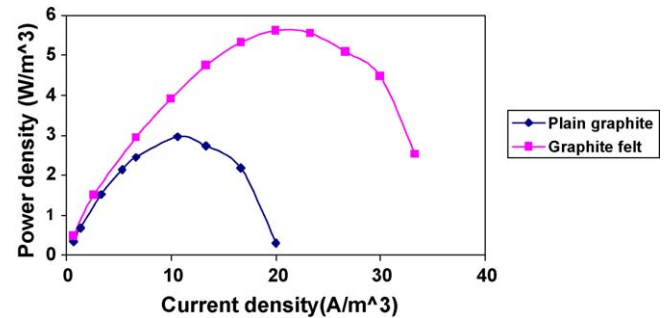

Fig. 2. Polarization curves of cell configuration 1.

The increase in power density shows the effect of surface area on the performance of the fuel cell.

A current density versus time curve for the MFC (configuration 1, with graphite felt anode) is shown in Fig. 3. The fuel cell was continuously discharged by applying a load of 100 Ω for 4 days. The current density remains constant at about 6.8 A m^{-3} for 3 days and on the fourth day it decreases to 5.26 A m^{-3}.

3.1.2. Cell configuration 2

The variation of OCP with respect to time is given in Fig. 4. With a graphite anode, the OCP is initially around 0.1 V, increases to 0.53 V over a period of 20 h, then to 0.6 V over a period of 45 h, and remains nearly constant afterwards. With a graphite felt anode, the OCP was initially around 0.48 V,
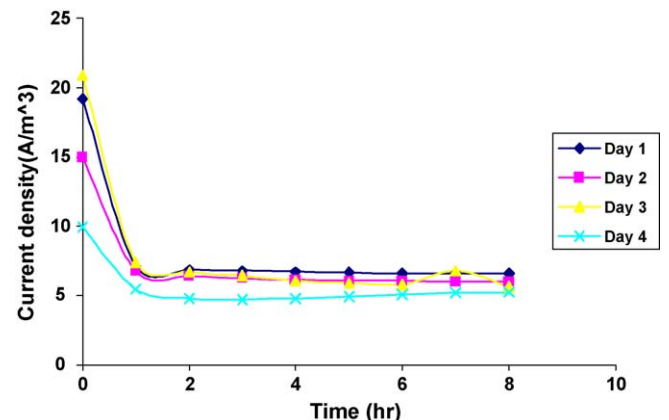

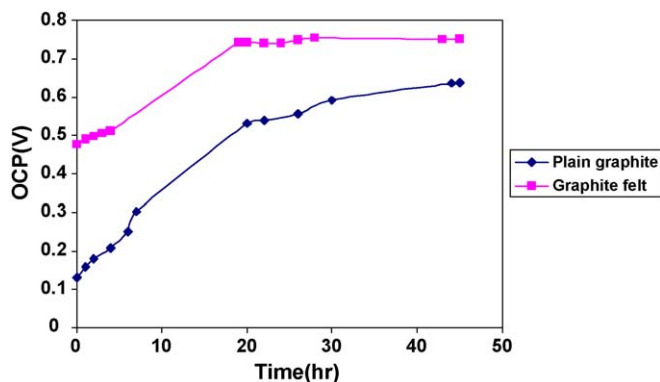Fig. 3. Current density variation of cell configuration 1 under a load of 100 Ω.

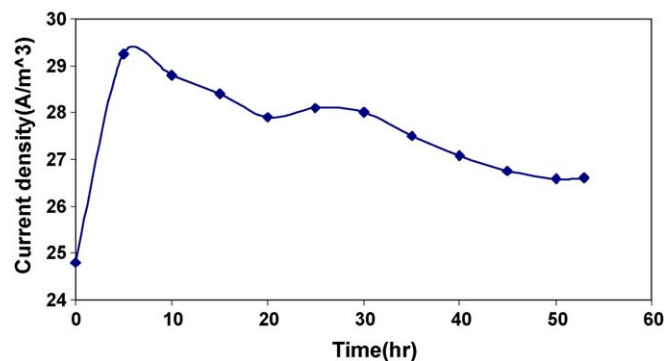Fig. 4. Open-circuit potential variation of cell configuration 2.
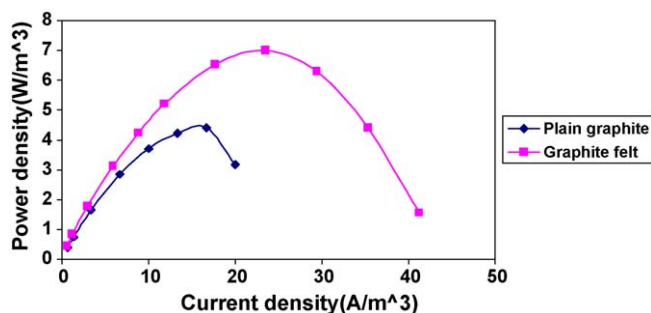

Fig. 5. Polarization curves of cell configuration 2.

increased to 0.56 V over a period of 10 h, and then remained steady at around 0.74 V.

The polarization behaviour of the cell configuration 2 with either graphite or graphite felt anodes is presented in Fig. 5. The power output is higher in the case of felt. The maximum value is around 4.41 and 6.1 W m^{-3} for graphite and felt, respectively.

The current discharge under a load of 100 Ω for 3 days in the case of the graphite anode is given in Fig. 6. During the first day, the initial value is 8 A m^{-3} and becomes steady at 3.96 A m^{-3}. A steady values of 3.25 and 2.64 A m^{-3} are obtained on the second and third days, respectively. With a graphite felt anode, a steady value of nearly 28 A m^{-3} was obtained (Fig. 7).

Microbial growth on the felt electrode is shown in Fig. 8. Clusters of microbial growth are observed in several places.
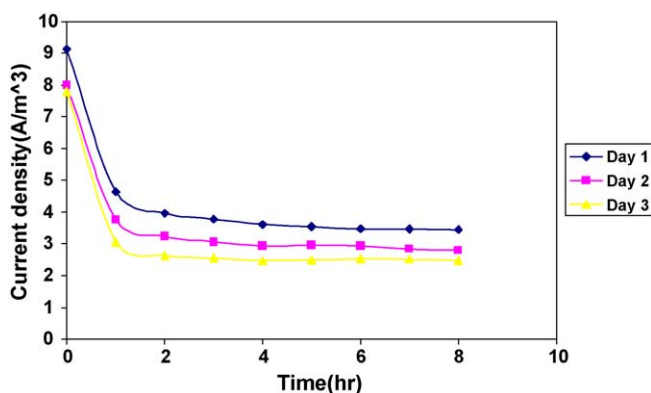

Fig. 6. Current density variation of cell configuration 2 with graphite as the anode under a load of 100 Ω.

Fig. 7. Current density variation of cell configuration 2 with graphite felt as the anode under a load of 100 Ω.

Cyclic voltammograms for *Clostridium* sp. immobilized on a gold electrode in phosphate buffer are given in Fig. 9. At a scan rate of 50 mV s^{-1}, anodic peaks at −0.16 and 0.44 V are observed. The response at −0.16 V looks rather like a plateau. This probably indicates that some limitations, e.g., chemical reactions, affect the electron-transfer kinetics. The origin of such chemical reactions is unknown. On the other hand, the reverse peaks at −0.28 and −0.25 V at 50 mV s^{-1} are well-defined. At a higher scan rate (500 mV s^{-1}), the first set of redox peaks disappear. This also confirms that the electron-transfer kinetics are slow and hence not seen at higher scan rates. Since the redox molecules are easily accessible (they are probably present on the outer membrane), the electroactivity can be recorded by cyclic voltammograms. As it has been already been reported that *Clostridium* sp. have cytochromes on their outer membranes [13], the electrochemical activity is assigned to cytochrome electron transfer.

The results indicate the feasibility of converting glucose into electrical energy by making use of the micro-organisms, *Clostridium* sp. isolated from sugar industry effluents. A higher current output is observed with a graphite felt anode and this is due to the increase in surface area. Cell configuration 2 can be considered as a complete microbial fuel cell as micro-organisms are used in the cathode as well as in the anode compartment. The cyclic voltammogram indicates that the *Clostridium* sp. is
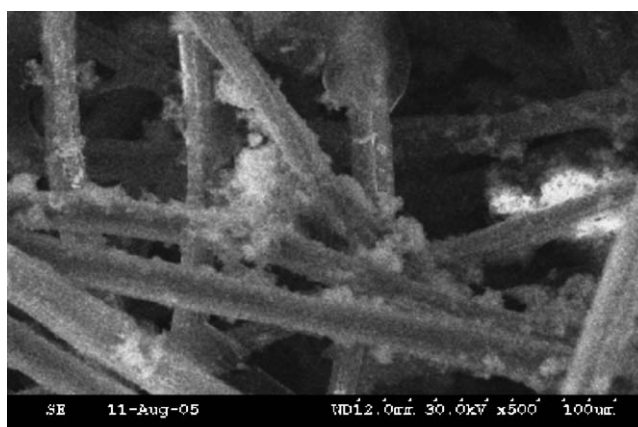

Fig. 8. Scanning electron micrographs of micro-organism growth in graphites felt.

Table 1
Comparison of power density obtained in this study with other reported values

Sl. no.	Mode of operation	Electrode material	Anolyte	Catholyte	Power density (W m^{-3})	Reference
1	Batch	Anode: felt; cathode: graphite	*Clostridium* sp. broth	*Thiobacillus ferrooxidans* broth	8.44	This work
2	Continuous	Anode: graphite felt; cathode: Pt coated graphite felt	Artificial wastewater	Air in wastewater	102	[17]
3	Continuous	Anode: graphite granules; cathode: Woven graphite mat	Synthetic waste water	Potassium ferricyanide	90	[18]
4	Continuous	Anode: perforated graphite felt; cathode: Pt coated graphite felt	Artificial wastewater	Air in wastewater	7.6	[19]

electroactive and can directly communicate with the electrode surface. This is because redox active molecules are found to be accessible by the cyclic voltammetric technique and are probably present in the outer membrane. The existence of cytochromes on the outer membrane of *Clostridium* sp. has already been reported [13]. The anode compartment gradually turns black as the fermentation proceeds. The sulfate is converted into sulfide by the *Clostridium* sp. and the sulfide molecules combine with iron citrate present in the medium to produce a black-coloured sulfide.

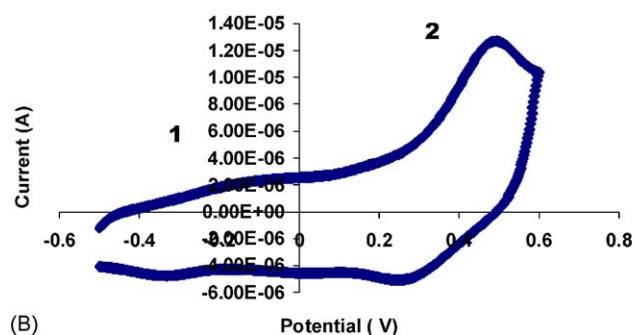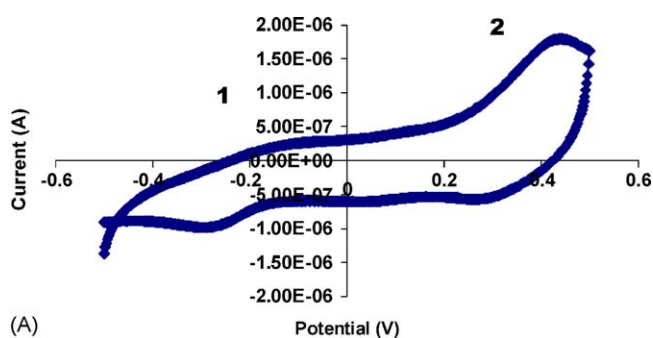There are two sources of current generation in the anode compartment, namely:

$$SO_4^{2-} + 10H^+ + 8e \rightarrow H_2S + 4H_2O \tag{1}$$

$$glucose \rightarrow oxidized\ products + electrons \tag{2}$$

$$cytochrome\ b\ (Fe^{3+}) + e \rightarrow cytochrome\ b\ (Fe^{2+}) \tag{3}$$

$$cytochrome\ c\ (Fe^{3+}) + e \rightarrow cytochrome\ c\ (Fe^{2+}) \tag{4}$$

The micro-organism anaerobicaly metabolises glucose. The glucose is oxidized into simpler molecules. The electrons generated during the process are accepted by sulfate ions (terminal electron acceptor), which produce sulfide ions. The sulfide ion production is indicated by the appearance of black-colour in the broth. The sulfide ion reacts with ferric citrate to produce a black precipitate of ferric sulfide. The ferric ions act as an indicator. The sulfide ions are oxidized at the electrode surface and contribute to the current flow. Another route for current generation is that the electrons obtained during the oxidation of glucose are directly transferred to the electrode through the cytochromes, which act as inbuilt mediators for electron flow. A cell has been constructed to explain direct electron transfer, without sulfate ions and ferric citrate. In this case the micro-organisms communicate directly with the electrode surface through redox proteins present on the outer membrane. The results obtained using such cell are given in Fig. 10. The OCP increases to 0.83 V and the maximum power deliverable is 4.04 W m^{-3}. A higher voltage is found to be produced in this case.

In the cell configuration 1, ferricyanide acts as an electron acceptor in the cathode compartment. In cell configuration 2, the electrons are accepted by ferric sulfate present in the catholyte and it is regenerated by the micro-organisms. Thus, the electron acceptor will not become depleted; there will be continuous regeneration of ferric ions. The cell configuration represents a complete microbial cell in the sense that micro-organisms drive both the anodic and the cathodic reactions.

The fermentation products have been analysed by HPLC and it is observed that products like acetate, butyrate, citrate are
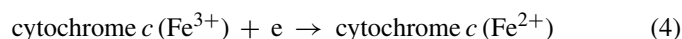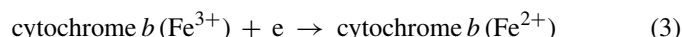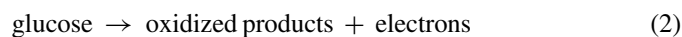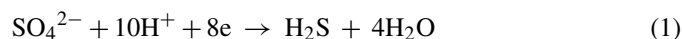

Fig. 9. Cyclic voltammograms showing redox behaviour of micro-organism at (A) 50 mV s^{-1} and (B) 500 mV s^{-1}.
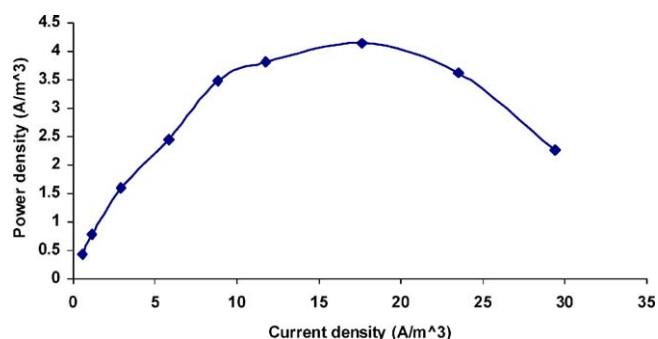

Fig. 10. Polarization curves for cell configuration 2 without ferric citrate and sodium sulfate.

present in the anode compartment. This suggests that glucose on metabolism is oxidized into simpler organic compounds.

A comparison with power density values obtained by other workers is given in Table 1. The result are expressed in $W\,m^{-3}$ in accordance with the present trend [16]. Each biocatalyst will have specific requirements and occupy a certain volume in the reactor thus will decrease both the free space and pore size. Every study refers to a specific combination of reactor volume, membrane separator, catholyte, anode surface and organic loading. Compared with some of the other data, the power density value obtained in this study is one order less. Improvements will be sought in terms of new cell configurations and electrode materials. A better understanding of the mechanism of electrons transfer between micro-organisms and electrodes should also lead to enhanced performance.

Generally, it is found that the cell with a felt anode yields the higher current/power and this can be attributed to the enhanced surface area of the anode material. On the other hand, it is noticed that the initial voltage of the cell using graphite felt anode starts at a lower value than that with graphite but reaches a higher steady value. Is the porous nature of felt, requiring possibly a longer duration for flooding of the pores, responsible for this? This will be investigated into in our future research.

4. Conclusions

In this study, microbial fuel cells have been fabricated using two configurations. The performance of the cell is evaluated in terms of two electrode materials, graphite and graphite felt. The increase in surface area results in higher current output. The observed current output arises due to two components, namely, current arising due to electrochemical oxidation of sulfide generated during metabolism and direct electron transfer of electrons produced during the metabolic oxidation of glucose. The direct electron-transfer component is delineated and the current characteristics arising due to direct electron-transfer alone is studied.

This work demonstrates the construction of a complete microbial cell, wherein both anodic and cathodic reactions are controlled by micro-organisms and also demonstrates how the current originating from direct electron transfer alone can be investigated separately.

References

[1] S. Suzuki, I. Karube, T. Matsunaga, Biotechnol. Bioeng. Symp. 8 (1978) 501–511.
[2] L.B. Wingard Jr., C.H. Shaw, J.F. Castner, Enzyme Microb. Technol. 4 (1982) 137–142.
[3] D.R. Bond, D.E. Holmes, L.M. Tender, D.R. Lovely, Science 295 (2002) 483–485.
[4] H. Liu, R. Ramnarayanan, B.E. Logan, Environ. Sci. Technol. 38 (2003) 2281–2285.
[5] J.K. Jang, T.H. Pham, I.S. Chang, K.H. Kang, H. Moon, K.S. Cho, B.H. Kim, Process Biochem. 39 (2004) 1007–1012.
[6] I.S. Chang, J.K. Jang, G.C. Gil, M. Kim, H.J. Kim, B.W. Cho, B.H. Kim, Biosens. Bioelectron. 19 (2004) 607–613.
[7] H.J. Kim, M.S. Hyun, I.S. Chang, B.H. Kim, J. Microbiol. Biotechnol. 9 (1999) 365–367.
[8] A. Shantaram, H. Beyenal, R. Raajan, A. Veluchamy, Z. Lewandowski, Environ. Sci. Technol. 39 (2005) 5037–5042.
[9] S. Wilkinson, Auton Robots 9 (2000) 99–111.
[10] H.J. Kim, H.S. Park, M.S. Hyun, I.S. Chang, M. Kim, B.H. Kim, Enzyme Microb. Technol. 30 (2002) 145–152.
[11] S.K. Chaudhuri, D.R. Lovely, Nat. Biotechnol. 21 (2003) 1229–1232.
[12] D.R. Bond, D.R. Lovely, Appl. Environ. Microb. 69 (2003) 1548–1555.
[13] H.S. Park, B.H. Kim, H.S. Kim, H.J. Kim, G.T. Kim, M. Kim, I.S. Chang, Y.K. Park, H.J. Chang, Anaerobe 7 (2001) 297–306.
[14] K. Rabaey, G. Lissens, S.D. Siciliano, W. Verstraete, Biotechnol. Lett. 25 (2003) 1531–1535.
[15] T.M. Vergheese, S. Berchmans, J. Electroanalchem. 570 (2004) 35–46.
[16] K. Rabaey, W. Verstraete, Trends Biotechnol. 23 (2005) 291–298.
[17] K. Rabaey, P. Clauwaert, P. Aelterman, W. Verstraete, Environ. Sci. Technol. 39 (2005) 8077–8082.
[18] H. Moon, I.S. Chang, J.K. Jang, B.H. Kim, Biochem. Eng. J. 27 (2005) 59–65.
[19] Z. He, S.D. Minteer, L.T. Angenent, Environ. Sci. Technol. 39 (2005) 5262–5267.