Analytica Chimica Acta 818 (2014) 15–22

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Enhanced response of microbial fuel cell using sulfonated poly ether ether ketone membrane as a biochemical oxygen demand sensor

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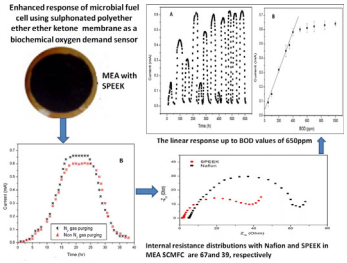
HIGHLIGHTS

- Sulfonated poly ether ether ketone (SPEEK) membrane in SCMFC used to determine the BOD.
- The biosensor produces a good linear relationship with the BOD concentration up to 650 ppm.
- This sensing range was 62.5% higher than that of Nafion®.
- SPEEK exhibited one order lesser oxygen permeability than Nafion®.
- Nafion® shows high anodic internal resistance (67 Ω) than the SPEEK (39 Ω).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form
16 December 2013
Accepted 29 January 2014
Available online 7 February 2014

Keywords:
Biosensor
Nafion®
Microbial fuel cells
Double membrane electrode assembly
Proton exchange membranes

ABSTRACT

The present study is focused on the development of single chamber microbial fuel cell (SCMFC) using sulfonated poly ether ether ketone (SPEEK) membrane to determine the biochemical oxygen demand (BOD) matter present in artificial wastewater (AW). The biosensor produces a good linear relationship with the BOD concentration up to 650 ppm when using artificial wastewater. This sensing range was 62.5% higher than that of Nafion®. The most serious problem in using MFC as a BOD sensor is the oxygen diffusion into the anode compartment, which consumes electrons in the anode compartment, thereby reducing the coulomb yield and reducing the electrical signal from the MFC. SPEEK exhibited one order lesser oxygen permeability than Nafion®, resulting in low internal resistance and substrate loss, thus improving the sensing range of BOD. The system was further improved by making a double membrane electrode assembly (MEA) with an increased electrode surface area which provide high surface area for electrically active bacteria.

1. Introduction

The BOD is an indicator for biodegradable organic load present in wastewater, widely used for the evaluation of wastewater quality [1]. However, the conventional method for determining the BOD is time-consuming (5 days of incubation) and usually requires expertise to achieve reproducible results. Development of alternative methods based on the dissolved oxygen consumption or photometric methods such as luminescence and fluorescence have been undertaken [2–5].

All these biosensors demonstrated a very good relationship with the BOD concentration but presented the limitation of having a very short operational stability and very low substrate versatility and also the measuring range is also very low, generally on the order of 0–110 ppm [4,6].

MFCs have been proposed in the past as biosensors for measuring environmental parameters such as BOD and water toxicity [3,4,7–11]. Varieties of MFC BOD sensors have been developed with the use of an electron-mediator, where the mediators are used to facilitate electron transfer from the microbial cells to

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http://dx.doi.org/10.1016/j.aca.2014.01.059

the electrode. Even though the addition of mediators in these biosensors can enhance the electron transfer, toxicity of mediators results in poor stability of these biosensors. Mediator-less MFCs have been exploited to fabricate novel BOD sensors for continuous and real-time monitoring and showing an operational stability of over 5 years with minimum maintenance [3,4]. In addition, Kim et al. reported that the performance of an MFC as BOD sensor was improved using respiratory inhibitors. The signal from MFCs decreased in the presence of electron acceptors of higher redox potential such as nitrate and oxygen. The addition of azide and cyanide did not change the signal in the absence of the electron acceptors. A huge amount of oxygen diffuses into the anode compartment through the ion-exchange membrane, which separates the anode compartment from the cathode compartment [12]. A novel biomonitoring system using microbial fuel cells has been developed for the purpose of on-line monitoring of the inflow toxic substances into water systems [8]. MFC based sensing was explored to provide for the development of an in situ bioremediation monitoring approach for substrate concentrations and microbial respiration rates [10].

Mirella et al. tested a SCMFC, with an air cathode, as a BOD biosensor for the first time. The aim was to make a more compact and simple system with reduced cost of operation. An air-cathode MFC does not require aeration, recycling chemical regeneration of catholyte. Moreover the range of BOD concentrations detected by the biosensor increased because of a better oxygen supply to the cathode [6]. Upon review of the literature published in the area of fuel cells, it was noted that most of the biosensor related works, both previously as well as currently, utilized Nafion® despite the several problems associated with it.

In previous studies, a linear relationship between substrate concentration and current generated was observed only up to 150 ppm [4]. The SCMFC biosensor output had a linear relationship with the BOD concentration up to 350 mg BOD ppm [6]. The limitation was attributed to system design parameters, like electrode surface area, microbial efficiency, mass transfer, or cathode design [10]. The power production in the MFC among many factors mainly depends on the reactor configuration and the electrode material, performance of proton exchange membrane (PEM), the specific source of substrate and operating conditions such as temperature and pH. Many more improvements in the materials used for MFC construction will be necessary before its practical implementation to make it economically viable with the currently available treatment systems [13].

It is known that PEM influences the power output of the MFC. Nafion® is the most widely used PEM in the MFCs because of its high selective permeability to protons. However, Nafion® 117 being expensive, results in inflation in the production cost of the MFCs. Additionally Nafion® 117 shows a number of problems associated with it such as high cost ($1500 m^{-2}), oxygen leakage from cathode to anode, substrate loss and cation transport and accumulation rather than protons in the long run, Among these, leakage of oxygen into the anode chamber can lower the energy recovery and leads inaccurate measurement of BOD due to the substrate loss from aerobic respiration by facultative bacteria, and they can inhibit the growth of obligate anaerobes. Therefore, it is of great interest to develop a new membrane which can reduce the oxygen diffusion without greatly affecting the internal resistance or power density [14–17]. Among the various polymers, poly ether ether ketone (PEEK) is a promising polymer which is actively being researched by the fuel cell community to replace the Nafion®. PEEK is a low-cost polymer having good thermal stability and mechanical properties. Proton conductivity for this polymer can be achieved by sulfonation (sulfonated PEEK, i.e., SPEEK), and the degree of sulfonation (DS) can be easily controlled by varying the parameters of the sulfonation reaction [18,19]. In our earlier reports, we have described that SPEEK and its performance in MFC was doubled when compared with commercially available Nafion®, which was due to its one order lesser oxygen cross over than that of Nafion®. Further the SPEEK based MFC was used for waste water treatment [16]. Yusof et al. also reported the same that the SPEEK exhibited excellent power generation compared to the Nafion® 117 [20]. In this work we intended to use the SPEEK membrane for biosensor application in SCMFCs and using the electrochemical studies the system efficiency was studied in the aspects of oxygen cross over.

Table 1
Comparison of membrane properties.

Membrane specifications	SPEEK	Nafion®
Proton conductivity (S cm^{-1})	0.5×10^{-2}	1.2×10^{-2}
IEC (meq g^{-1})	1.23	0.952
Water swelling (%)	27	22
Thickness (μm)	170	175
K_0 (cm s^{-1})	2.6×10^{-6}	2.8×10^{-5}
BOD sensing range (ppm)	650	400
Internal resistances (Ω) (anode)	39	65

In the present study, the SPEEK membrane and the Nafion® membrane were evaluated in SCMFC as a biosensor in terms of its BOD detecting range and response time. The effect of the double MEA-MFC was also investigated.

2. Material and methodology

2.1. Electrolyte

Synthesis and characterization of PEM, i.e., SPEEK have been described in earlier reports and its performance in MFC was doubled when compared with a commercially available Nafion® and also its oxygen cross over was one order lesser than that of Nafion® [16]. In this work we plan to increase the degree of sulfonation (DS) to attain equal proton conductivity to Nafion®. In a typical experiment, 10 g of dry PEEK powder (Victrex, England) was dissolved in 150 mL of sulfuric acid under nitrogen atmosphere. To increase DS the reaction mixture was allowed to proceed for 8 h with constant stirring at 50 °C. The reaction was terminated with ice. The synthesized PEM showed good water uptake (27%), ion exchange capacity (1.23 meq g^{-1}) and proton conductivity (0.58×10^{-2} S cm^{-1}) (Table 1). Degree of sulfonation was determined as 70% from FTIR spectrum, based on the method of Khanh Ngan et al. (supplementary data 1) [21]. The only problem with the usage of SPEEK was that at higher degrees of sulfonation it was susceptible to dissolution in water at temperatures (above 60 °C). However, in the case of MFC this problem is not encountered as the MFC operation temperature is usually performed at room temperature which is about 30 °C.

2.2. MFC construction

The fabricated MFC consisted of an acrylic cylindrical chamber 4 cm long and 4 cm in diameter (empty bed volume of 50 mL) separated by a SPEEK or Nafion® proton exchange membrane. The anode electrode used was a carbon cloth which was 30% wet proofed. The cathode was prepared using a carbon cloth having 0.5 mg cm^{-2} platinum coating as a catalyst. The experiment was carried out with a MEA prepared using SPEEK and Nafion® membranes. The MEA was prepared according to the below mentioned brief procedure. The proton conducting membrane was sandwiched between the prepared anode and cathode electrodes and hot pressed at 70 °C under 1 tonne pressures for 2 min. The MEA was further assembled into the cylindrical single-chambered MFC with the cathode-side of the MEA facing the air (single MEA-SCMFC) as our previous designs

[16,17]. The experiment was subsequently carried out with a double MEA prepared using SPEEK membrane. The MEAs were placed on opposite sides of the acrylic cylindrical chamber facing each other (double MEA-SCMFC). Copper wire was used to connect the circuit with an external load. The experiment was conducted in fed batch mode for the calibration of the system; further continuous mode was carried out at room temperature. All tests were done in triplicates and the average value was calculated.

2.3. Culture medium

AW was made with the following constituents: NH_4Cl, 40 mg dm^{-3}; $MgCl_2$, 10 mg dm^{-3}; $CuSO_4$, 0.1 mg dm^{-3}; $CaCl_2$, 5 mg dm^{-3}; $MnSO_4$, 0.1 mg dm^{-3}; $ZnCl_2$, 0.1 mg dm^{-3}; The trace mineral solution consisted 10 mL L^{-1}; phosphate buffer (1.0 Mol dm^{-3} (M), pH7); and distilled water. Trace mineral solution composition contains nitrilotriacetic acid (NTA) 0.0015 mg dm^{-3}; $FeSO_4{\cdot}7H_2O$ 0.0011 mg dm^{-3}; $MnCl_2{\cdot}4H_2O$ 0.0001 mg dm^{-3}; $CoCl_2{\cdot}6H_2O$ 0.00017 mg dm^{-3}; $ZnCl_2$ 0.0001 mg dm^{-3}; $CaCl_2{\cdot}2H_2O$ 0.0001 mg dm^{-3}; $CuCl_2{\cdot}2H_2O$ 0.0001 mg dm^{-3}; H_3BO_3 0.00002 mg dm^{-3}; Na_2MoO_3 0.00001 mg dm^{-3}; $NiSO_4{\cdot}6H_2O$ 0.00026 mg dm^{-3}; NaCl 0.0001 mg dm^{-3}. The BOD concentration of the AW was controlled by adding an appropriate amount of glucose [5,6].

2.4. Enrichment

The enrichment and adaptation of the electrochemically active bacteria in the SCMFCs was performed in batch mode under a fixed external load of 500 Ω, and was carried out for a period of approximately 30 days. The artificial wastewater was used as fuel, a BOD of 1000 ppm was selected for enrichment and anaerobic sludge (Anna University sewage water treatment plant in Chennai, India) was added to as a bacterial inoculum. Once the current output decreased to the background value (0.015 mA), a new batch was introduced into the reactor without inoculum. Subsequently, the control test was run without substrate that produced current up to 0.015 mA, which is called as background current.

2.5. Oxygen mass transfer coefficients

In MFCs, the oxygen mass transfer coefficient is a measure of the oxygen permeability of the membranes. It is important that the membranes used do not allow oxygen to diffuse from the cathode to the anode compartment. The oxygen mass transfer coefficient, K_0 was determined using a portable dissolved oxygen (DO) probe (*Extech 407510A*, Taiwan) in uninoculated SCMFC reactor. The dissolved oxygen probe was attached to the lid of the anode chamber, and the anolyte was sparged with nitrogen gas to remove DO before carrying out the experiment. The mass transfer coefficient of oxygen in the membrane, K_0, was determined by measuring the DO concentration over time and using Eq. (3)

$$K_0 = -\frac{V}{At}\ln\left[\frac{(C_0 - C)}{C_0}\right] \quad (1)$$

where V is the liquid volume in the anode chamber, A is the membrane cross-sectional area, C_0 is the saturated oxygen concentration in the cathode chamber, and C is the DO concentration in the anode chamber at time t. The diffusion coefficient (D_0, $cm^2\ s^{-1}$) was calculated as [15,16]

$$D_0 = K_0 L_t \quad (2)$$

where L_t denotes the membrane thickness. While the thickness of the commercial Nafion® membrane was 175 μm given by the manufacturer, the thickness of the fabricated SPEEK membranes was 170 μm.

2.6. Internal resistance of MFC measurement

Internal resistance was characterized using electrochemistry impedance spectrometer (EIS) methods. The impedance measurements were taken from 500 kHz to 1 mHz by applying a sine wave (10 mV rms) on top of the bias potentials with a potentiostat ((Bio-Logic VSP, France). The three-electrode mode is used to analyze an individual electrode. To know the Anode internal resistance, Anode and cathode electrodes were used as working and counter electrodes, respectively. The reference electrode was an Ag/AgCl (KCl-sat.) electrode [22]. The internal resistances of reactors were determined using Nyquist plots as previously described [23,24]. The cyclic voltammetry (CV) were performed in the range from −1 to +1 V vs. Ag/AgCl at a scan rate of 10 mV s^{-1} with the same workstation.

2.7. Columbic efficiency (CE)

The columbic efficiency (CE) was calculated using following Eq. (3).

$$CE = \frac{C_p}{C_T} \times 100\% \quad (3)$$

where C_p is the total Coulombs calculated by integrating the current over time. C_T is the theoretical amount of Coulombs that can be produced from glucose and is calculated as previously [16,17].

3. Results and discussion

3.1. Single MEA-SCMFC biosensor response and calibration with different BOD concentration of wastewater

The enrichment and adaptation of the electrochemically active bacteria in the single MEA-SCMFCs (both Nafion® and SPEEK) were performed in batch mode under a fixed external load of 500 Ω, and was carried out for a period of approximately 3 weeks. Initially the system sort was slow and the current output rose after each batch feed, due to reaching a peak before declining due to consumption of the fuel. The MFCs attain the peak current around 3rd or 4th batch (300 or 400 h), beyond which no further increase in the output current was observed which suggested that the anode biofilm was enriched with electrochemically-active bacteria thus rendering it stable.

Once the enrichment of the cells was complete, subsequently the system performance was investigated in a continuous mode. AW was fed into the both MFCs with different BOD concentration ranging from 50 to 1100 mg L^{-1} at an external load of 500 Ω and the flow rate 0.43 $cm^3\ min^{-1}$, the steady-state current generation was monitored. The MFCs were fed at a specific concentration until a stable current was generated and then the feed concentration was changed followed by starvation until a current of approximately 0.014 mA (background current around) was obtained.

Principle of BOD sensing is that the coulomb or current generated from an MFC is proportional to the concentration of fuel used. The MFCs can be used to measure BOD values either by reading the maximum current or calculating the coulomb [4]. Fig. 1A shows the dynamic response of the SPEEK system to different inlet BOD concentrations (i.e., when concentration increased, current increased and vice versa). Very good reproducibility of the system was observed; for a fixed inlet BOD, either in the case of two consecutive batches or when batches with other concentrations were interposed, a stable current was obtained with a coefficient of variation of 2%.

The variation in current with BOD concentration shows the linear response (Fig. 1B) up to BOD values of 650 ppm (regression coefficient, $r^2 = 0.97$). The maximum current (0.88 mA) production

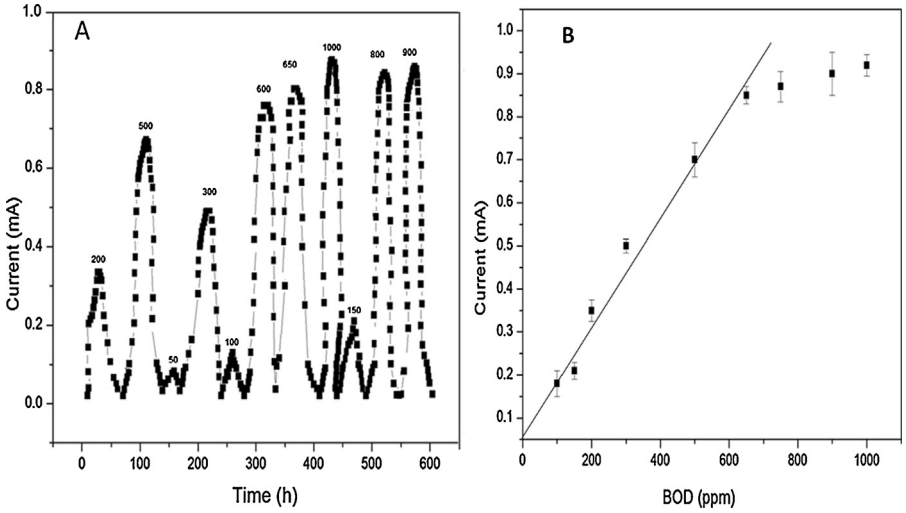

Fig. 1. SPEEK-MEA-SCMFC sensor response to different BOD concentrations with an external load of 500 Ω. Flow rate: 0.43 $cm^3\ min^{-1}$: (A) current generated under different BOD concentrations. (B) Calibration curve for steady-state current in relation to the inlet BOD. Data are the average from two reactors.

was observed for BOD concentration up to 800 ppm and above which the same current values were observed until 1000 ppm of BOD in the influent. The limitation was attributed to system design parameters, such as electrode surface area, microbial efficiency, mass transfer, or cathode design [11]. Fig. 2A and B shows a calibration of current generated vs. BOD in Nafion®. In this case a linear response was obtained up to a BOD concentration of 400 ppm (regression coefficient, $r^2 = 0.98$), the linearity was 62.5% lower than for calibration against current in SPEEK.

3.2. Oxygen permeability

The oxygen flux from cathode to anode through the SPEEK and Nafion® membrane were evaluated using uninoculated MFC reactors by measuring D_0 accumulation in the solution of the anode chamber over time. When SPEEK membrane used as a PEM in uninoculated MFC, the K_0 and D_0 were estimated to be $K_0 = 2.60 \times 10^{-6}\ cm\ s^{-1}$ and $D_0 = 5 \times 10^{-8}\ cm^2\ s^{-1}$, respectively. The MFC with Nafion® showed higher values ($K_0 = 2.80 \times 10^{-5}\ cm\ s^{-1}$, $D_0 = 4 \times 10^{-7}\ cm^2\ s^{-1}$), indicating that the SPEEK can maintain the anaerobic condition of anode by preventing the oxygen from cathode to anode.

3.3. Effect of oxygen on biosensors

The effects of oxygen diffusion into the anode compartment on current generation were evaluated under two different conditions: nitrogen gas sparged and nonsparged. To study the effects of oxygen diffusion on SCMFCs, AW with a BOD of 1000 ppm was used as fuel and external resistance 500 Ω was selected and the experiment was contacted in batch mode. The current output of an SCMFC with Nafion® sparged with nitrogen gas was 0.58 mA and nonsparged was MFC 0.34 mA (Fig. 3A), producing CE values of 30.3% and 12.8%, respectively. The results of this study indicate that a nitrogen gas sparged MFC displayed a much current output than the nonsparged one, due to the continuous removal of diffused oxygen from the cathode. The relatively plentiful existence of facultative or aerobic bacteria at the start might have resulted in the significant difference

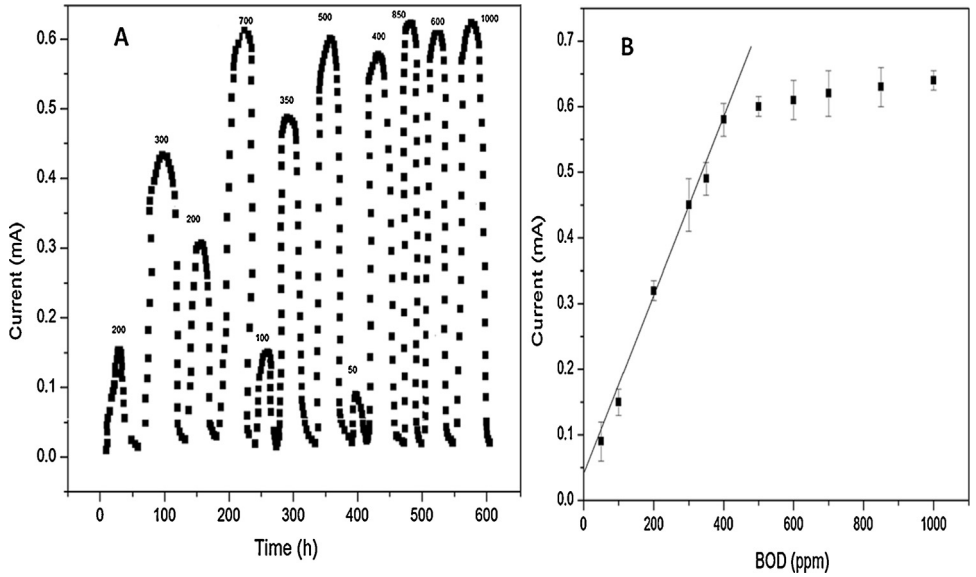

Fig. 2. (A) Nafion®-MEA-SCMFC sensor response to different BOD concentrations with an external load of 500 Ω. Flow rate: 0.43 $cm^3\ min^{-1}$: (A) current generated under different BOD concentrations. (B) Relationship between BOD value and steady-state current.

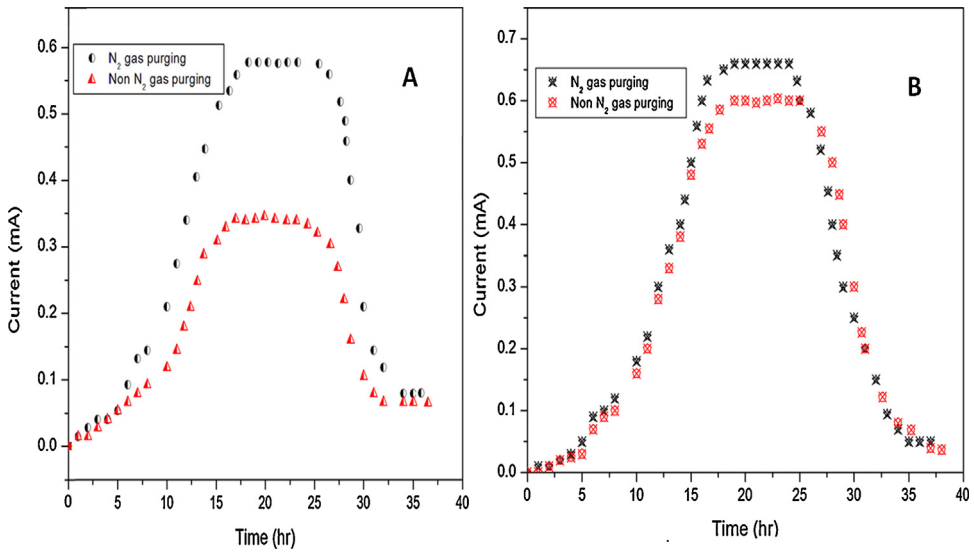

Fig. 3. Comparison of current evolution between a nitrogen gas spared MFC and a nonsparged MFC when BOD of 1000 ppm was used as the substrate at batch mode: (A) Nafion® MEA SCMFC and (B) SPEEK-MEA-SCMFC.

between the two MFCs, due to substrate loss in the nonsparged MFC. Supporting this view, Liu and Logan 2005 reported that up to 28% of the glucose added to an MFC was lost through aerobic bacterial respiration due to the oxygen diffusion via the Nafion® membrane [25]. This study clearly indicates the presence of oxygen causes the substrate loss and affects the sensor application.

In sensor application for accurate measurement of BOD, the signal (electron) produced by substrate should be captured by the anode electrode for the accurate measurement of BOD. The signal from an MFC was reduced in the presence of electron acceptors such as oxygen in the anode and which causes the substrate loss [12] resulting in an inaccurate measurement of BOD. Therefore a high oxygen permeable system was not suitable for accurate measurement of BOD.

However, when nitrogen gas sparged was in the SPEEK MFC, the current generated was 0.66 mA (Fig. 3B) and in nonsparged MFC it produced was 0.60 mA and there was no large differences for both MFCs due to low oxygen permeability from cathode to anode. The CEs of SPEEK with nitrogen sparged and unsparged were 35% and 32% respectively. SPEEK produced high CE than Nafion® because of low substrate losses. The improved dynamic range of the SCMFC with SPEEK MEA studied here, was because of the use of SPEEK as proton exchange membrane. This not only facilitates the proton transport from anode to cathode but also maintains the anaerobic condition in anode by preventing the oxygen diffusion from cathode to anode, and reducing the substrate loss by inhibiting the aerobic bacteria.

3.4. Effect of internal resistance

To study the effect of internal resistance on SCMFCs the AW containing BOD of 1000 ppm was used as fuel and external resistance 500 Ω was selected. In Fig. 4, Nafion® shows high anodic internal resistance (67 Ω) than the SPEEK (39 Ω), again this result implies that the electron transfer rate with SPEEK was higher than the Nafion®, which is due to anaerobic metabolism of bacteria. The same amount of substrate was used in both MFCs but high electron flow was observed in SPEEK. Where the substrate was fully converted to electrons by anaerobic bacteria in case of Nafion® low electron flow was observed due to the aerobic bacteria, which was caused by high oxygen exposure at anode from the cathode.

For a two-chamber MFC, a linear response has been reported up to BOD concentrations of 150 ppm [4]. Mirella et al. reported a linear response up to BOD concentrations of 350 ppm for a SCMFC with Nafion® [6]. They suggest that improving oxygen supply at the cathode improved the dynamic range of the sensor over the two- chamber MFC. Here the increased dynamic response with SPEEK was due to low internal resistance when compared with Nafion®. The low internal resistance was obtained due to low oxygen crossover which attributed high electron flow from bacteria by the anaerobic metabolism.

3.5. Cyclic voltammetry

In cyclic voltammetry (CV), the MFC with SPEEK showed the strongest activity (the highest redox peak), whereas the MFC with Nafion® exhibited lower electrochemical activities as evident from

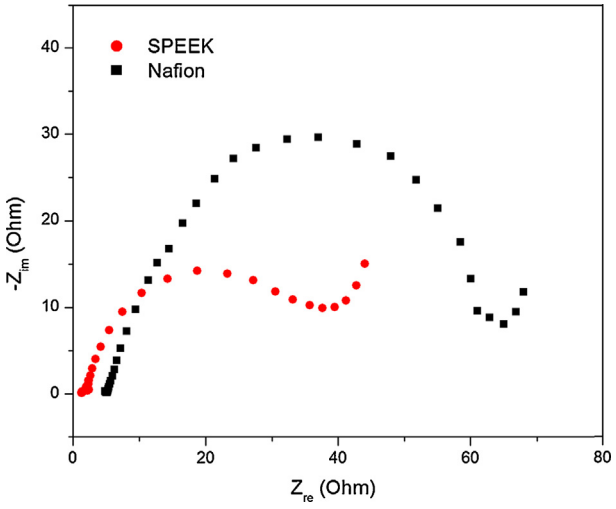

Fig. 4. Comparison of internal resistance distributions in Nafion® based MEA SCMFC and SPEEK based MEA SCMFC using 1000 ppm of BOD as the substrate and 1 M phosphate buffer at batch mode. A conventional three-electrode system was employed to measure impedance with the anode as the working electrode, the cathode as the counter electrode, and the frequency range taken from 500 kHz to 1 mHz with a sinusoidal perturbation of 10 mV amplitude.

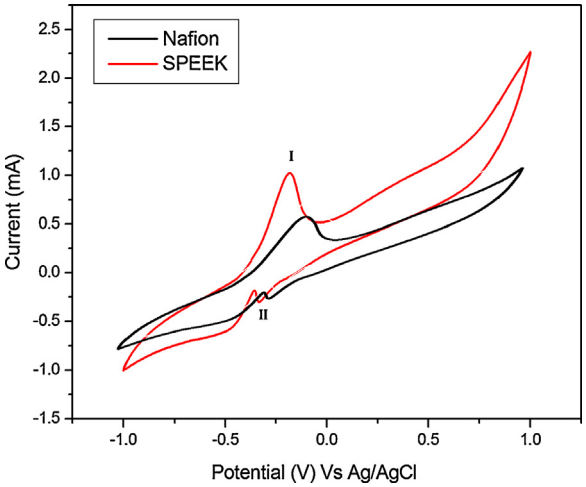

Fig. 5. Cyclic voltammetry of MFCs based Nafion® and SPEEK in 1000 ppm of BOD as the substrate and 1 M phosphate buffer at batch mode. A three-electrode system was employed with the anode as the working electrode, the cathode as the counter electrode, and an Ag/AgCl reference electrode. The potentials were shifted from −1 to 1 mV with a scan rate of 10 mV s^{-1}.

their lower peaks (oxidation and reduction peak I and II, respectively). Each anodic peak indicated the presence of some redox species in the microorganisms (Fig. 5). In general, the primary electron transfer process (cytochromes, other redox proteins, electron shuttles and so on) usually occur in the range of −0.45 to −0.1 V (vs. Ag/AgCl) [26,27]. The redox potential range of the glucose oxidation is from −0.4 to −0.2 V [28]. In the present study, the peaks observed between −0.3 to −0.2 V (vs. Ag/AgCl), indicated the feasibility of converting glucose into electrical energy by making use of the micro-organisms. Further, it was considered as proof for the presence of extracellular redox species in microorganisms grown on anode. The results of the cyclic voltammogram indicated that the enriched anode media possessed electroactive microorganisms and could directly communicate with the electrode surface. This was because the redox active molecules were found to be accessible by the cyclic voltammetric technique and were probably present in the outer membrane of microorganisms.

This redox reaction has been quasi reversible since the oxidation peak current I was higher than the reduction peak current II in all cases (Fig. 5 SPEEK and Nafion®) [29]. This correlation designated redox reaction to the diffusion of a redox compound rather than the compound or cellular component confined to the electrode surface. This refuted the possibility of the activity produced by the adsorption (accumulation) of an electron shuttle, or by redox sensitive cellular component attached to the anode and confirmed the enhancement of current output in MFCs. From Fig. 4, it was clearly seen that the SPEEK membrane showed higher anodic peak than a Nafion® which implied that more redox compounds were generated in SPEEK containing MFC. It can thus be stated that higher number of redox compounds were observed with SPEEK based MFCs since SPEEK provided a better anaerobic environment than Nafion®. The observation of higher performance of SPEEK based MFC is supported by the works of Kim et al. [30] who demonstrated the higher oxidation peak being associated with *Shewanella putrfaciens* grown in an anaerobic environment as against the ones grown in aerobic conditions. They attributed this higher electrochemical activity to the cytochromes on the cell surface, with the orientation of the electrochemically active heme group towards the cell surface. Further, they reported that electrochemical activity was not observed in the suspensions of *S. putrefaciens* strains grown under aerobic condition.

3.6. Response of double MEA-SCMFC with SPEEK biosensor with different BOD concentration of wastewater

The effect of the double MEA on the system response was investigated by the active area of 25 cm^2. The double MEA-SCMFCs were fed with AW containing different concentrations of BOD, until a steady current was generated. The cells were then starved until a current of 0.015 mA (background current) was obtained subsequent to which, MFC sensors were fed with fresh feed containing a different concentration of BOD. Fig. 6A shows the variation in current output of the double MEA-SCMFC with time when fed with different concentrations of BOD. A good linear correlation was obtained between the current and BOD concentrations up to 750 ppm (Fig. 6B). The high response of the double MEA-SCMFCs towards the high BOD concentration was attributed to its design configuration such as the large surface area of electrode which provided a high surface area for electrochemically active bacteria along with a large membrane area for the proton transport [23,31,32].

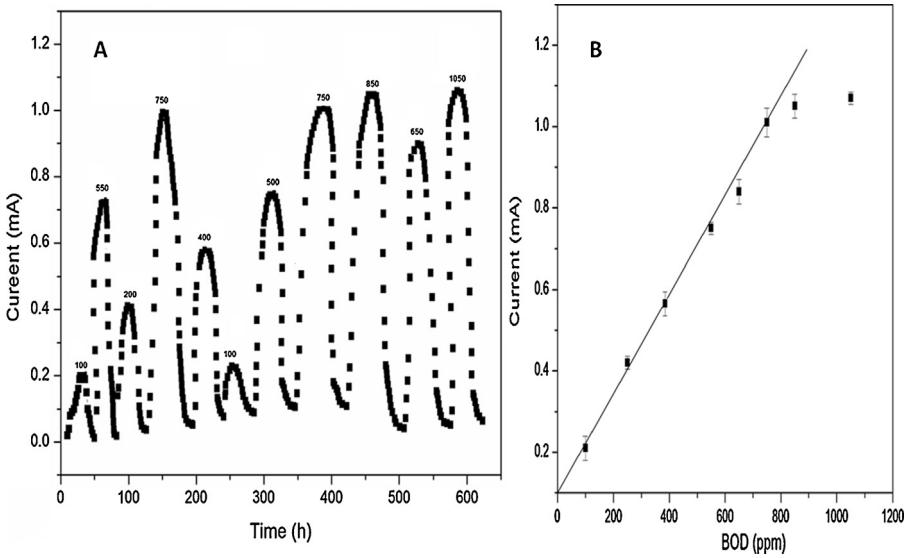

Fig. 6. (A) Variation in current in Double MEA-SCMFC biosensor for response to different BOD concentrations: (B) calibration curve for steady-state current and BOD concentration. Data are the average from two reactors.

Table 2
Change in steady state current, current response time and coulombic efficiency on increasing the flow rate of AW in both single and double MEA-SMFCs.

Flow rate ($cm^3\ min^{-1}$)	Steady-state current (mA)		Response time (min)		HRT (min)	Coulombic efficiency (%)	
Single MEA and double MEA	Single MEA	Double MEA	Single MEA	Double MEA	Single MEA and double MEA	Single MEA	Double MEA
0.25	0.26	0.33	420	240	192.3	30	37
0.38	0.35	0.45	300	180	131.6	35	51
0.46	0.42	0.60	240	120	108.7	42	60
0.54	0.56	0.72	220	100	92.5	50	72
0.65	0.57	0.88	200	80	76.9	60	80
0.73	0.58	0.90	190	79	68.4	55	70

3.7. *Effect of flow rate on SCMFCs*

To study the effect of flow rate on SCMFCs, the AW was used as fuel with a BOD of 500 ppm and external resistance set to 500 Ω. The solution was fed into the cell at a specific feeding rate until a stable current was generated following which, the flow rate was further increased in a step-wise manner. The response time was defined arbitrarily as the time taken to reach 95% of the new steady-state current. The average data calculated from the data obtained from three different reactors is given in Table 2.

Table 1 shows the change in steady state current and current response time upon increasing the flow rate of AW from 0.26 to 0.73 $cm^3\ min^{-1}$ in the two MFCs. In both single MEA as well as double MEA, upon increasing the flow rate of AW, the steady-state current was observed to increase with each step in flow rate, varying from 0.26 to 0.58 mA and 0.33 to 0.90 mA for single MEA and double MEA respectively. In fact, this increase was observed up to a flow rate of 0.54 $cm^3\ min^{-1}$ and 0.65 $cm^3\ min^{-1}$ for single MEA and double MEA respectively. However, when the cells were fed at higher flow rates (i.e., 0.65 $cm^3\ min^{-1}$ in single MEA and 0. 073 $cm^3\ min^{-1}$ in double MEA) only a small increase (about 2% in single MEA and 3% in double MEA) in the current produced was observed.

The increase in current with flow rates indicated an enhancement in the rate of mass transport by electrochemical activity of bacteria which were present in the anode electrode. Double MEA system facilitated the mass transport up to a flow rate of 0.65 $cm^3\ min^{-1}$ beyond which, there was no significant increase in current. This suggested that the electrically active bacteria which were present in more abundance in the double MEA, consumed the fuel faster from the AW due to the larger surface area of the electrode. The double MEA was associated with a considerably faster response of the system for 0.65 mL min^{-1} requiring only 80 min to reach a stable current. Whereas, in the case of single MEA, a longer time of 3.5 h was required to reach a stable current. Table 1 describes the response of the MFC-based sensors to different flow rates in terms of the CE observed for each feeding rate and compares it with those obtained for single MEA biosensor. As stated earlier, the higher electrode surface area of double MEA-SCMFC biosensor resulted in a better performance with a CE of 80% in contrast to 60% obtained with the single MEA.

The current generation from an MFC is determined by several physical and biochemical factors, including (1) the microbial activity to oxidize fuel, (2) electron transfer rate from the microbes to the electrode, (3) circuit resistance, (4) proton transfer from the anode compartment to the cathode compartment, (5) oxygen supply and reduction at the cathode and (6) oxygen diffusion into the anode compartment through the membrane. Among these, the most serious problem in an MFC as a BOD sensor is the oxygen diffusion into the anode compartment, which consumes electrons in the anode compartment thereby reducing the coulomb yield [5]. In the present study, many of the problems mentioned above were addressed effectively using the double MEA-SCMFC with SPEEK. The larger surface area of the double MEA facilitated better activity of the electrochemically active bacteria that actively oxidized large amounts of substrate from the AW which in turn, increased the electron mass transfer. With regard to the problems associated with circuit resistance, it is known that, the employment of an MEA reduces the distance between electrodes, which contributes significantly to reduce the internal resistance (circuit resistance) of MFCs [14,20,23]. On comparing with the single MFC, the double MEA-SCMFC with air cathode has an additional advantage of improved oxygen supply at cathodes which further facilitates the reduction reaction at the cathodes. More importantly, the problem of oxygen diffusion into the anode compartment was greatly reduced with the use of SPEEK (having a lower oxygen diffusion coefficient). SPEEK was found to exhibit clear differences in phase separation behavior and morphology from perfluorosulfonic polymers (Nafion®) [16,21]. Hydrophilic and hydrophobic phase separation is less distinctive due to a smaller hydrophobic backbone, fewer acidic functional groups linked to the aromatic ring, and a less-flexible polymer chain.

It is also reported that the water channels developed in hydrated sulfonated poly-arylether ketone membranes are relatively narrow and highly branched to result in dead-end channels [21]. Thus, the oxygen permeation coefficient of the SPEEK polymer has been found to be lower than those of Nafion®. Among the various polymers, poly ether ether ketone (PEEK) is a promising candidate which is actively being researched by the FC community to overcome the drawbacks of Nafion®. It has good thermal, mechanical and chemical resistance due to the presence of aromatic groups in polymer chain. In addition, the preparation of SPEEK membrane allows direct casting from organic solutions and is therefore less expensive than perfluorosulfonic membranes. Importantly, the PEEK can be easily sulfonated directly by concentrated sulfuric acid solution at room temperature. However, the degree of sulfonation is limited for SPEEK based systems because of their degrading nature when over-sulfonated at higher temperature.

The above mentioned effects directly increase the dynamic BOD sensing range of the double MEA SCMFC type biosensor by approximately 114% when compared with the range obtained and reported using the commercial membrane Nafion® in SCMFC [6]. A linear response was in fact observed up to a BOD concentration of 750 ppm, in contrast with SCMFC with Nafion® in which linearity was observed up to a maximum of 350 ppm BOD as observed and reported by Millea et al. [6]. These studies conclusively proved that the utilization of SPEEK based double MEA SCMFC displayed an enhanced sensing range which has potential applications in detecting high BOD of up to 750 ppm.

4. Conclusion

This study demonstrates that a SCMFC, with SPEEK MEA, has the potential to be used as a biosensor for labile BOD. SPEEK makes it suitable for maintaining the anaerobic environment in the anode chamber of the MFC resulting in decreased substrate loss and thus improving the sensing range of BOD. Double MEA

provides a larger surface area for electrochemically active bacteria that actively oxidize larger amount of substrate from the AW, in a way increasing the electron mass transfer and also reduce the response time (80 min) which also reduces the hydraulic retention time (HRT) of the biosensor (i.e., 76.9 min).

Acknowledgements

The authors thank the Department of Science and Technology (DST) India (letter no. DST/TSG/AF/2010/09, dt.01-10-2010) and the UGC-Meritorial Student Fellowship (2010–2013) Department of Chemistry, Anna University Chennai, India for their financial support to carry out this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.01.059.

References

[1] A. Eaton, D.L.S. Clesceri, A.E. Greenberg, Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, 1995.
[2] N. Pasco, K. Baronian, C. Jeffries, J. Hay, Appl. Microbiol. Biotechnol. 53 (2000) 613–618.
[3] 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.
[4] B.H. Kim, Chang, G.C. Gil, H.S. Park, H.J. Kim, Biotechnol. Lett. 25 (2003) 541–545.
[5] B.H. Kim, I.S. Chang, H. Moon, American Scientific Publishers 10 (2006) 1–12.
[6] D.L. Mirella, P.C. Tom, M.H. Ian, S. Keith, Water Res. 43 (2009) 3145–3154.
[7] G.C. Gil, I.S. Chang, B.H. Kim, M. Kim, J.K. Jang, H.S. Park, H.J. Kim, Biosens. Bioelectron. 18 (2003) 327–334.
[8] M. Kim, M.S. Hyun, G.M. Gadd, H.J. Kim, J. Environ. Monit. 9 (2007) 1323–1328.
[9] Y. Lei, W. Chen, A. Mulchandani, Anal. Chim. Acta 568 (2006) 200–210.
[10] J.M. Tron, J.D. Fortner, M. Plotze, J.B. Hughes, A.M. Puzrin, Biotechnol. Lett. 30 (2008) 1385–1390.
[11] J.M. Tron, J.D. Fortner, M. Plotze, J.B. Hughes, A.M. Puzrin, Biosens. Bioelectron. 24 (2008) 586–590.
[12] I.S. Chang, H. Moon, J.K. Jang, B.H. Kim, Biosens. Bioelectron. 20 (2005) 1856–1859.
[13] B. Manaswini, S.J. Partha, T.M. Tanaji, M.M. Ghangrekar, Bioelectrochemistry 79 (2010) 228–233.
[14] K.J. Chae, M. Choi, F.F. Ajayi, W. Park, I.S. Chang, I.S. Kim, Energy and Fuels 22 (2008) 169–176.
[15] O. Lefebvre, Y. Shen, Z. Tan, A. Uzabiaga, I.S. Chang, H.Y. Ng, Bioresource Technol. 102 (2011) 6291–6294.
[16] S. Ayyaru, S. Dharmalingam, Bioresource Technol. 102 (2011) 11167–11171.
[17] S. Ayyaru, P. Letchoumanane, S. Dharmalingam, A.R. Stanislaus, J. Power Sources 217 (2012) 204–208.
[18] L. Lei, Z. Jun, W. Yuxin, J. Membr. Sci. 226 (2003) 159–167.
[19] A. Rinaldi, B. Mecheri, V. Garavaglia, S. Licoccia, P.D. Nardo, E. Traversa, Energy Environ. Sci. 1 (2008) 417–429.
[20] A.H.M. Yusof, M.A.M. Amin, A.F. Ismail, M.N. Anam, N.A.N. Mahmud, Int. J. Sustain. Construct. Eng. Technol. 4 (2013) 63–74.
[21] K.N.T. Do, D. Kim, J. Power Sources 185 (2008) 63–69.
[22] Z. He, F. Mansfeld, Energy Environ. Sci. 2 (2009) 215–219.
[23] S. You, Q. Zhao, J. Zhang, J. Jiang, C. Wan, M. Du, S. Zhaob, J. Power Sources 173 (2007) 172–177.
[24] X. Zhang, S. Cheng, X. Huang, B.E. Logan, Biosens. Bioelectron. 25 (2010) 1825–1828.
[25] H. Liu, B.E. Logan, Environ. Sci. Technol. 38 (2004) 4040–4046.
[26] T.H. Choi, Y.B. Won, J.W. Lee, D.W. Shin, Y.M. Lee, M. Kim, H.B. Park, J. Power Sources 220 (2012) 269–279.
[27] Y. Yang, M. Xu, J. Guo, G. Sun, Process Biochem. 47 (2012) 1707–1714.
[28] Y. Qiao, S.J. Bao, C. Ming Li, X.Q. Cui, Z.S. Lu, J. Guo, ACS Nano 2 (2008) 113–119.
[29] S.B. Velasquez-Orta, I.M. Head, T.P. Curtis, K. Scott, Bioresource Technol. 102 (2011) 5105–5112.
[30] H.J. Kim, H.S. Park, M.S. Hyun, I.S. Chang, M. Kim, B.H. Kim, Enzyme Microb. Technol. 30 (2002) 145–152.
[31] Y. Fan, H. Hu, H. Liu, J. Power Sources 171 (2007) 348–354.
[32] J. Sun, Y. Li, Y. Hu, B. Hou, Q. Xu, Y. Zhang, S. Sizhe Li, Biotechnol. Lett. 34 (2012) 2023–2029.