CHAPTER 5

BIOSENSOR

Microbial fuel cell (MFC) based technologies are promising technologies for direct energy production from various wastewaters and waste streams. Beside electrical power production, more emphasis is recently devoted to alternative applications such as hydrogen production, bioremediation, seawater desalination and biosensors. Microbial fuel cells have been proposed in the past as biosensors for measuring environmental parameters such as biochemical oxygen demand (BOD). Although the technologies are promising, numerous hurdles have to be overcome before it’s field applications become economically feasible. The main purpose of this work was to improve the sensing range of MFC-based sensor, reduce the construction cost and expand the application scopes of MFC-based bio-electrochemical systems.

MFCs have been proposed in the past as biosensors for measuring environmental parameters such as BOD and water toxicity (Chang et al 2004, Kim et al 2003, Gil et al 2003, Tront et al 2008). Varieties of MFC based BOD sensors have been developed with the use of electron-mediator, where the mediators are used to facilitate electron transfer from the microbial cells to 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 that showed an operational stability of over 5 years with minimum maintenance (Chang et al
2004, Kim et al 2003). In addition, Kim et al reported that the performance of a 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 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 (Chang et al 2005). Lorenzo 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 do not require aeration and chemical regeneration of catholyte (Lorenzo et al 2009). Moreover the range of BOD concentrations detected by the biosensor increased because of a better oxygen supply to the cathode. Upon review of 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 Nafion117 membrane 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 (Kim et al 2005). The SCMFC biosensor output had a linear relationship with the BOD concentration up to 350 ppm (Lorenzo et al 2009). The limitation was attributed to system design parameters, like electrode surface area, microbial efficiency and mass transfer or cathode design. The power production in the MFC among many factors mainly depends on the reactor configuration and electrode material, performance of proton exchange membrane (PEM), 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.
This chapter 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).

5.1 SINGLE MEA-SCMFC BIOSENSOR RESPONSE AND CALIBRATION WITH DIFFERENT BOD CONCENTRATIONS 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 was slow and the current output rose after each batch feed, 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 both MFCs with different BOD concentrations ranging from 50 to 1100 mg/l at an external load of 500 Ω and the flow rate 0.43 cm³ min⁻¹. The steady-state current generation was monitored continuously. 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) was obtained.

Principle of BOD sensing is that the coulomb or current generated from a 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 (Kim et al 2003). Figure 5.1a 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%.

Figure 5.1 SPEEK-MEA-SCMFC sensor response to different BOD under concentrations with an external load of 500 Ω and flow rate 0.43 cm³ min L⁻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.

The variation in current with BOD concentration shows a linear response (Figure 5.1a) up to BOD values of 650 ppm (regression coefficient, r² = 0.97). The maximum current (0.88 mA) production 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. Figure 5.2 a, b shows a calibration of current generated versus BOD using Nafion 117. 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 SPEEEK.

![Figure 5.2 Nafion-MEA- SCMFC sensor response to different BOD under concentrations with an external load of 500 Ω and flow rate 0.43 cm$^3$ min L$^{-1}$: (a) Current generated under different BOD concentrations (b) Relationship between BOD value and steady-state current. Data are the average from two reactors.](image)

5.2 OXYGEN PERMEABILITY

The oxygen flux from cathode to anode through the SPEEEK and Nafion 117 membrane were evaluated using uninoculated MFC reactors by measuring $D_O$ accumulation in the solution of the anode chamber over time. When SPEEK membrane was used as a PEM in uninoculated MFC, the $K_O$ and $D_O$ were estimated to be $K_O = 2.60 \times 10^{-6}$ cm/s and $D_O = 5 \times 10^{-8}$ cm$^2$/s,
respectively. The MFC with Nafion showed higher values ($K_O = 2.80 \times 10^{-5}$ cm/s, $D_O = 4 \times 10^{-7}$ cm$^2$/s), indicating that the SPEEK can maintain the anaerobic condition of anode by preventing the oxygen diffusion from cathode to anode.

### 5.3 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 non sparged. To study the effects of oxygen diffusion on SCMFCs, AW with a BOD of 1000 ppm was used as fuel and external resistance $500 \, \Omega$ was selected and experiment was conducted in a batch mode. The current output of an SCMFC with Nafion 117 sparged with nitrogen gas was 0.58 mA and non-spared was 0.34 mA (Figure 5.3 a), producing CE values of 30.3% and 12.8%, respectively. Results of this study indicate that nitrogen gas sparged MFC displayed a higher current output than the non sparged one, due to 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 between the two MFCs, due to substrate loss in the non sparged 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 (Liu & Logan 2005). 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 (Chang et al 2005) resulting in inaccurate
measurement of BOD. Therefore high oxygen permeable system was not suitable for accurate measurement of BOD.

Figure 5.3 Comparison of current generation between a nitrogen gas sparged MFC and a non sparged MFC when BOD of 1000 ppm was used as the substrate at batch mode: (a) Nafion MEA SCMFC and (b) SPEEEK -MEA -SCMFC.

However, when nitrogen gas was sparged in the SPEEEK MFC, the current generated was 0.66 mA (Figure 5.3B) and in non sparged MFC it produced 0.60 mA and there was no large difference for both MFCs due to low oxygen permeability from cathode to anode. The CEs of SPEEEK with nitrogen sparged and non sparged were 35% and 32%, respectively. SPEEEK produced higher CE than Nafion because of low substrate losses. The improved dynamic range of the SCMFC with SPEEEK MEA studied here, was because of the use of SPEEEK 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.

5.4 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 of 500 Ω was selected. In Figure 5.4, Nafion showed 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 that of Nafion, which was due to anaerobic metabolism of bacteria. 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 Nafion117, 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 was reported up to BOD concentrations of 150 ppm. Lorenzo et al reported a linear response up to a BOD concentration of 350 ppm for a SCMFC with Nafion (Lorenzo et al 2009). They suggested 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 lower internal resistance when compared with Nafion. The lower internal resistance was obtained due to lower oxygen crossover which attributed high electron flow from bacteria by the anaerobic metabolism.
Figure 5.4  Comparison of internal resistance distribution in Nafion 117 based MEA SCMFC and SPEEK based MEA SCMFC using 1000 ppm of BOD as the substrate and 1M phosphate buffer in batch mode

5.5 CYCLIC VOLTAMMETRY

In the cyclic voltamgram showed in Figure 5.5 the MFC with SPEEK showed the strongest activity (highest redox peak), whereas the MFC with Nafion117 exhibited lower electrochemical activities as evident from their lower peaks (oxidation and reduction peaks I and II, respectively). Each anodic peak indicated the presence of some redox species in the microorganisms. 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) (Choi et al 2012, Yang et al 2012). The redox potential range of the glucose oxidation is from -0.4 to -0.2V. 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.

Figure 5.5  Cyclic voltammetry of MFCs based Nafion and SPEEK with a scan rate of 10 mV s⁻¹

This redox reaction was quasi reversible since the oxidation peak current I was higher than the reduction peak current II in all cases (Figure 5.5)
SPEEK and Nafion 117). This correlation designated redox reaction to the diffusion of a redox compound rather than the compound or cellular component confined on 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 the figure 5.5, it was clearly seen that the SPEEK membrane showed higher anodic peak than Nafion 117 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 Nafion117. The observation of higher performance of SPEEK based MFC was supported by the works of Kim et al who demonstrated the higher oxidation peak being associated with Shewanella Putrefaciens grown in 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 (Kim et al 2002).

5.6 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 an active area of 25 cm². 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. Figure 5.6 a.
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 (Figure 5.6 b). The high response of the double MEA-SCMFC towards the high BOD concentration was attributed to its design configuration such as large surface area of electrode which provided high surface area for electrochemically active bacteria along with a large membrane area for the proton transport.

![Figure 5.6](image)

**Figure 5.6** (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

### 5.7 EFFECT OF FLOW RATE ON SCMFC

To study the effect of flow rate on SCMFCs, 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 two different reactors is given in Table 5.1.

Table 5.1  Change in steady state current, current response time and
coulombic efficiency on increasing the flow rate of AW in
both single and double MEA- SCMFCs

<table>
<thead>
<tr>
<th>Flow rate (cm$^3$ min$^{-1}$)</th>
<th>Steady-state current (mA)</th>
<th>Response time (min)</th>
<th>HRT (min)</th>
<th>Coulombic Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single MEA &amp; Double MEA</td>
<td>Single MEA</td>
<td>Double MEA</td>
<td>Single MEA &amp; Double MEA</td>
</tr>
<tr>
<td>0.25</td>
<td>0.26</td>
<td>0.33</td>
<td>420</td>
<td>240</td>
</tr>
<tr>
<td>0.38</td>
<td>0.35</td>
<td>0.45</td>
<td>300</td>
<td>180</td>
</tr>
<tr>
<td>0.46</td>
<td>0.42</td>
<td>0.60</td>
<td>240</td>
<td>120</td>
</tr>
<tr>
<td>0.54</td>
<td>0.56</td>
<td>0.72</td>
<td>220</td>
<td>100</td>
</tr>
<tr>
<td>0.65</td>
<td>0.57</td>
<td>0.88</td>
<td>200</td>
<td>80</td>
</tr>
<tr>
<td>0.73</td>
<td>0.58</td>
<td>0.90</td>
<td>190</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 5.1 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 upto 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 upto a flow rate of 0.65 cm³ 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 electrode. The double MEA was associated with a considerably faster response of the system for 0.65 ml/min 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 5.1 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 (Kim et al 2006). 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. 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 lower oxygen diffusion coefficient). The above mentioned effects directly increased 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 Nafion117 in SCMFC (Lorenzo et al 2009). A linear response was in fact observed up to a BOD concentration of 750 ppm, in contrast with SCMFC with Nafion117 in which linearity was observed upto a maximum of 350 ppm BOD as observed and reported by Millea et al. 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 upto 750 ppm (Sivasankaran & Sangeetha 2014).

5.8 BOD CONCENTRATION MEASUREMENT

The linearity between BOD concentration and current generation was observed beyond 15 ppm only in Nafion containing MFC (Figure 5.7). The limitation was attributed to system design parameters, such as electrode surface area, membrane, microbial efficiency, mass transfer, or cathode design. In case of SPEEK the linearity was observed beyond 5 ppm which is due to the low oxygen cross over properties of SPEEK (Figure 5.8). Both MFCs were tested for the monitoring of low BOD (up to 50 ppm) by using
AW, containing glucose as check solution. From the results, it is concluded that the SPEEK based MFC has better ability to measure the low BOD concentrations.

Figure 5.7  (a) Current generation from MFC-SPEEK fed with AW of different BOD concentration (up to 50 ppm) (b) correlation between steady-state current and BOD concentration

Figure 5.8  (a) Current generation from MFC-SPEEK fed with AW of different BOD concentration (up to 50 ppm) (b) Relationship between steady-state current and BOD concentration
This section demonstrates that a SCMFC with SPEEK MEA, had the potential to be used as a biosensor for labile BOD. SPEEK makes it suitable for maintaining 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 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 reducing the response time (80 min) which reduced the hydraulic retention time (HRT) of the biosensor (76.9 min) (Sivasankaran & Sangeetha 2014)