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Development of microbial fuel cell as biosensor for detection of organic matter of wastewater

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Abstract

The removal of biodegradable organic matters (BOM) is a very important aspect of evaluating the treatment efficiency in a wastewater treatment plant. However, conventional Biochemical Oxygen Demand (BOD) method is time consuming (3 or 5 days) and not suitable for online process monitoring. Instead biosensors can be used to measure BOD. Microbial Fuel Cell (MFC) biosensor which uses electroactive biofilms as sensing element has the advantage of long-term stability and minimizes the replacement of sensing elements. BOM could be directly converted to electricity via MFC, where MFC itself is an integration of signal generator and transducer. Proton Exchange Membrane (PEM) is a very important component of MFC and the most widely used Nafion PEM (NPEM) is costly (*Jurado and Colomer, 2002; Liu et al., 2006, Jana et al., 2010*). Previously, researchers have successfully used low cost clayware separators as PEM (CWPEM) with improved performance of MFC (*Behera et al., 2009*, *Jana et al., 2010)*. Comparative studies has been carried out between MFC-1 (NPEM) and MFC-2 (CWPEM) to evaluate the performance of MFC as biosensor using mixed anaerobic culture with synthetic wastewater containing acetate as source of carbon. MFC-1 biosensor responds linearly between COD (Chemical Oxygen Demand) concentration of 22 mg/L and 51 mg/L (R^2=0.954) with a response time between 120 min and 210 min. Similarly, MFC-2 biosensor responds linearly between a concentration 64 mg/L and 212 mg/L (R^2=0.949) with a response time between 310 min and 120 min. The variation in rate of proton conductivity (PC) and thickness of the separators is suspected to be the cause for variation in range of detection and response time. The current market price of NPEM is very high i.e. Rs. 4000/10 cm^2 and that of CWPEM is Rs. 4/10 cm^2. With improvement in PC of CWPEM, low cost MFC biosensor can be successfully developed. Once successfully developed, such low cost MFC based sensors can be calibrated for BOD.

Keywords: Biodegradable Organic Matters, Biochemical Oxygen Demand, Microbial Fuel Cell, Biosensor, Proton Exchange Membrane.

INTRODUCTION

A MFC is a device that converts chemical energy to electrical energy with the aid of the catalytic reaction of microorganisms. The MFC system often consists of an anaerobic compartment with a negative electrode (anode) and an aerobic compartment with a positive electrode (cathode) separated by a PEM. In the anaerobic compartment, microorganisms oxidize the substrate (e.g. acetate) resulting in the generation of electrons and protons (eq.1.1). The generated protons migrate from the anaerobic compartment to the aerobic compartment through the PEM. The produced electrons are first transferred to the anode and then pass through an external electric circuit to the cathode, where they reduce oxygen forming H_2O (eq.1.2). The flow of electrons through the circuit generates current (*Kumlanghan et al., 2007*).

Anodic reaction: $CH_3COO^- + 2H_20 \rightarrow 2CO_2 + 7H_20 + 8e^-$ (1.1)

Cathodic reaction: $4H^+ + O_2 + 4e^- \rightarrow 2H_2O$ (1.2)

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The major applications of MFCs include electricity generation, biohydrogen production, wastewater treatment and biosensor. A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to the concentration of the analytes (*Cunningham., 1998; Su et al., 2011).* When the organic component concentration is constant, bacteria will produce a constant electrical current. However, when their metabolism is affected by the change in organic component concentration, the substrate consumption rate will change and with that the electrical current. In this way, at varying substrate concentration, MFC can act as a biosensor (*Stein, 2011)*.

Mediator-less MFC biosensors had been invented by *Kim et al. (1999)* as a lactate biosensor. The current was in proportion with lactate concentration up to 30 mM with R^2 = 0.84. It has been demonstrated that BOD of wastewater is linearly related with the total Coulombs produced (*Kim et al., 2003, Wang et al., 2013)*, which had been utilized in the measurement of BOD in real wastewater, as a low BOD sensor or as an organic carbon sensor (*Kang et al.,2003; Peixoto et al.,2011*).

A MFC meets many requirements of the perfect sensor: (a) it can be used as an early warning signal because it is sensitive to many components (*Tront, 2008*), (b) it can be low in cost, simple in operation and can monitor continuously, (c) bacteria grow on the anode and the signal produced is an electrical signal. This means that no transducer is needed to translate the signal to a readable signal (*Kumlanghan et al., 2007),* and (d) they use naturally available

microorganisms. No genetically modified bacteria are needed as is the case in some other types of biosensors (*Stein, 2011*).

The membrane or the separator used in a MFC is a very important component. It acts as a separator for the aerobic and anaerobic compartments in a MFC as well as allows passage of ions through it (*Kim et al., 2009*). An ideal membrane must have the following characteristics: (a) a high proton transfer coefficient to ensure that it does not inhibit protons from reaching the cathode, (b) low oxygen transfer coefficient to improve Coulombic efficiency, and (c) relatively non-biodegradable (*Zhang et al., 2009*).

Commercially available polymer membranes make a MFC very costly to be used for wastewater treatment. Nafion is the most widely used membrane in MFCs. But it has certain disadvantages like (a) higher affinity to cations like Na^+ and K^+ than H^+ ions, (b) expensive material thereby increasing the cost, and (c) acidic in nature which reduces the growth of biomass (*Prakash et al., 2010*). *Behera et al*. (*2009*) used a clayware membrane and obtained a maximum power density of 16.8 W/m^3. This MFC with manufacturing cost less than 1 US$ gave a quiet good performance compared to other MFCs fabricated using expensive materials.

The main aim of the present research work is to explore the possibility of developing low cost organic sensors using MFC. A comparative study between MFC-1 (NPEM) and MFC-2 (CWPEM) has been made to evaluate the performance of MFC as biosensor using mixed culture with acetate containing synthetic wastewater. Performance characteristics of biosensor: (a) linearity (b) sensing time and (c) repeatability have been evaluated for MFC-1 and MFC-2 biosensors for the purpose of comparative study.

MATERIALS AND METHODS

Microbial fuel cell and its operation

NPEM (Nafion™ 117, 175 µ thick, DuPont Co.,USA) was used in MFC-1 and CWPEM (locally manufactured; 3 mm thick; elements present: Na-1.39%, Mg-1.12%, Al-23.90%, Si-51.52%, K-3.95%, Ca-1.05%, Ti-0.76%, Fe-16.31%) was used in MFC-2. NPEM and CWPEM were having a surface area of 6.25 cm^2. NPEM was pre-treated by immersing in 0.1N HCl for 4 h at room temperature (*Kumlanghan et al., 2007)* to remove any contaminants from the surface. The electrode system was compact to minimize the internal resistance and comprised of anode and cathode electrodes in direct contact with PEMs. Anode and cathode were of carbon felt of surface area 90 cm^2. The electrodes were connected to 10 Ω resistance using copper coated wires (Fig. 1).

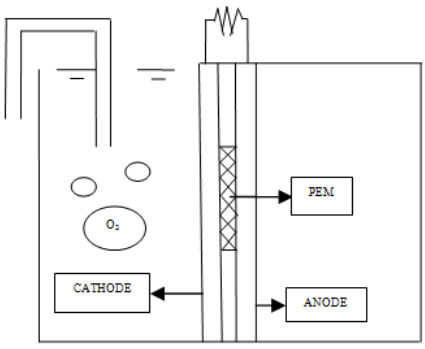

Fig 1. Schematic of MFC

Air-saturated tap water was fed into the cathode compartment as oxidant. The biosensors were operated in a batch mode at room temperature between 20 ^{0} C – 25 ^{0} C. After giving heat pre-treatment at 100 ^{0}C for 15 min to suppress the methanogens, 40 mL of sludge collected from bottom of a septic tank was added to the reactors to maintain the sludge loading rate (SLR) at more than 0.4 kg COD.kg $VSS^{-1}.d^{-1}$ during initial start-up.

Wastewater composition

Artificial wastewater (AW) containing acetate as a source of carbon was used. The acetate medium also contained (per gram of COD) $NaHCO_3$, 1500 mg; NH_4Cl, 318 mg; $CaCl_2.2H_2O$, 250 mg; $MgSO_4.7H_2O$, 64 mg; K_2HPO_4, 27 mg; and KH_2PO_4, 9 mg. Trace metals were added as $FeSO_4.6H_2O$, 10.00 mg/L; $MnSO_4$, 0.526 mg/L; $ZnSO_4.7H_2O$, 0.106 mg/L; H_3BO_3, 0.106 mg/L; and $CuSO_4.5H_2O$, 4.5 µg/L (*Behera et al., 2010*). The influent feed pH was in the range of 7.5-8.5 throughout the study.

Analyses and calculations

The organic content of wastewater was assessed as COD using Standard Methods (*APHA, 1998*). The pH and dissolved oxygen (DO) was measured with a Thermo Fisher Scientific Advanced Electrochemistry meter (Chelmsford, USA). The potential difference (PD) between anode and cathode was measured using a multimeter and recorded every 5 sec through a data acquisition system (Agilent Technologies, 34972A Malaysia). The current was calculated using the relationship PD = current × resistance. The Coulombic efficiency (CE) of the MFC was calculated by integrating the measured current over time relative to the maximum current possible based on the observed COD removal (eq. 2.1). The CE evaluated over a period of time *t*, is calculated as (*Logan et al., 2006*)

$$CE = \frac{100\,M\int_0^t I\,dt}{F\,b\,V_a\Delta\,COD} \qquad (2.1)$$

Where, *M* is molecular weight of oxygen = 32 g/mol; *F* is Faraday's constant = 96485 C/mol; *b* is the number of electrons exchanged per mole of oxygen = 4; V_a is the volume of liquid in the anode compartment, and Δ*COD* is the change in COD over time '*t*'.

RESULTS AND DISCUSSION

Performance of MFC biosensor

The MFCs were inoculated with anaerobic sludge and enriched using AW with a BOD of 3000 mg/L as fuel. After 80 days of operation, an average current of 2.15 mA and 1.12 mA was generated in MFC-1 and MFC-2, respectively. The average CE obtained for MFC-1 and MFC-2 was 14.24 % ± 2.56 % and 15.91 % ± 1.67 %, respectively. Polarization studies were done for MFC-1 and MFC-2 by varying the external resistance from 5000 Ω to 10 Ω (Fig. 2). During polarization, current density was found to be increasing with the decrease in resistance which indicated the behaviour of a typical fuel cell (*Behera et al., 2009*).

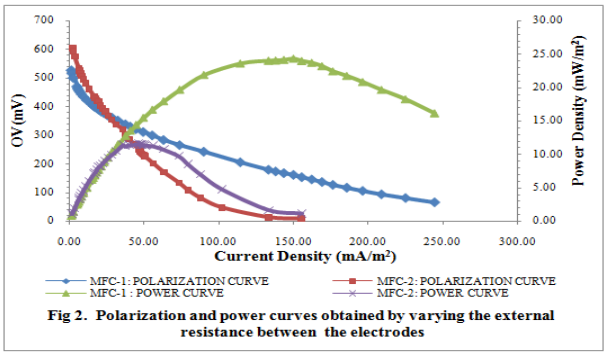

Fig 2. Polarization and power curves obtained by varying the external resistance between the electrodes

has low internal resistance (Table 1). The better performance of MFC-1 is attributed to high PC offered by NPEM. The PC of NPEM is 10^{-2} S cm^{-1} (*Jana et al., 2010*) while that of CWPEM is 2.57 x 10^{-4} S cm^{-1} (*Behera et al., 2009*). Average DO measured in MFC-1 and MFC-2 anode chamber was found to be 0.3 mg/L and 0.45 mg/L, respectively. Oxygen diffusion in MFC-2 is 50 % more than that in MFC-1. Oxygen acts as alternate electron acceptor and reduces the electrical performance of the system.

MFC-1 gave better electrical performance than MFC-2 and

Table 1. Electricity generated in MFC-1 and MFC-2

MFC	Operating Voltage at 10 Ω resistance (mV)			Open circuit potential (mV)			Volumetric Power Density (mW/m^3)		Sustainable Current Density at 10 Ω resistance (mA/m^2)	Internal resistance (Ω)
	Max.	Avg.	Standard deviation	Max.	Avg.	Standard deviation	Max.	Sustainable at 10 Ω resistance		
MFC-1	28.3	21.55	±1.98	640	579	±25	1090	400	239	153
MFC-2	13.3	11.25	±1.25	682	626	±22	520	200	125	484

Current generation at different COD concentration

Different concentrations of AWs ranging from 10 to 300 mg/L as COD was prepared and fed into the MFCs to monitor the current and steady-state response time. When the MFCs were operated under various conditions, the current generated was recorded, along with a COD analysis of the effluent and hence a calibration curve was plotted (Fig. 3). MFC-1 and MFC-2 biosensors responds linearly between a COD concentration of 22 mg/L and 51 mg/L (R^2 = 0.954) and 67 mg/L and 212 mg/L (R^2 = 0.949), respectively.

The reason for better performance of MFC-1 with NPEM, at low COD concentration, might be due to its better conductivity compared to that of MFC-2 with CWPEM. At low substrate concentration, less amount of protons are generated. Due to high conductivity of NPEM, these protons might reach the cathode successfully while the same is not possible in CWPEM due to its low conductivity. Limitation of protons at cathode decreases the performance of the MFC (*Gil et al., 2003)* thereby affecting the detection limit.

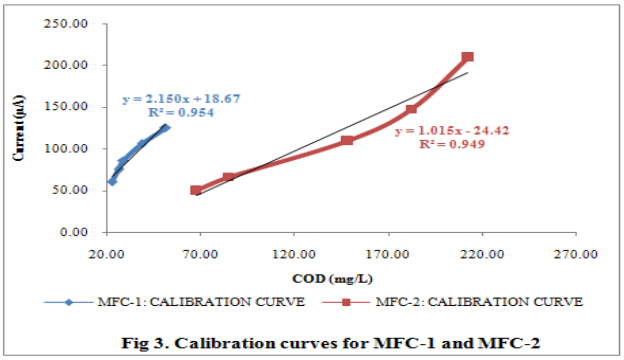

Fig 3. Calibration curves for MFC-1 and MFC-2

MFC-2 is found to have linear response in high concentration range. This is due to availability of sufficient protons at cathode in the determined range of detection. Below 67 mg/L, limitation of protons at cathode makes the sensor non-responsive to lower concentration of substrate. Addition of chemical catholyte might overcome the limitations at cathode and increase the range of detection.

Response time

Time required to attain maximum stable operating voltage (OV) upon change in organic matter concentration in anode chamber was recorded as the response time. MFC-1 required less time for sensing than MFC-2. As the concentration decreases, time of response also decreases for both the MFC biosensors. MFC-1 requires 120 min to sense a COD concentration of 22.43 mg/L while MFC-2 requires same 120 min to sense a higher COD concentration of 64.28 mg/L (Table 2).

Variation in PC and thickness of separators is suspected to be the cause of variation in response time. PC of NPEM is 40 times greater than that of CWPEM. The thickness of CWPEM is 17 times greater than NPEM. Due to increased thickness of CWPEM, it offers more distance of travel for protons. This means more time required for complete reaction to occur and hence more time to attain stable maximum OV.

Table 2. Response time for MFC-1 AND MFC-2 at different COD concentrations

MFC	MFC-1		MFC-2	
Sl.No	COD Concentration (mg/L)	Duration of response (min)	COD Concentration (mg/L)	Duration of response (min)
1.	51.26	210	212.27	310.00
2.	38.45	185	182.16	300.00

3.	28.84	165	148.00	290.00
4.	26.21	130	84.91	200.00
5.	22.43	120	64.28	120.00

Repeatability

The APHA standard method for a 5-day BOD (BOD_5) test allows a standard deviation of 30.5 mg/L using AW containing glucose, glutamate and acetate with an average BOD_5 value of 198 mg/L as the repeatability, which is equivalent to a ±15.4% variation in repeatability. The current generated from the MFCs fed with AW of COD in detection range for each MFC was observed to determine the repeatability. AW of COD concentration 30 mg/L and 100 mg/L was fed into MFC-1 and MFC-2 respectively to determine repeatability. Current corresponding to 30 mg/L in MFC-1 and 100 mg/L in MFC-2 was found to be 87.2 µA ± 8.3 µA and 143 µA ± 13.13 µA, respectively. The variation in repeatability was ± 9.5 % and ± 9.18 % in MFC-1 and MFC-2 respectively. The variation in repeatability is within ±15.4% for both the MFCs.

CONCLUSIONS

MFC-1 with NPEM can sense an organic matter of low COD concentration (22 mg/L to 51 mg/L) while MFC-2 with CWPEM is a good organic sensor at high COD concentration range (67 mg/L to 212 mg/L). MFC-1 has less time of sensing i.e. between 210 min and 120 min while that of MFC-2 is between 310 min to 120 min. Decrease in thickness of CWPEM with due consideration for oxygen diffusion and increase in proton conductivity are proposed to increase the sensitivity of CWPEM. Also chemical catholyte can be used to test the improvement in sensitivity. Cost of NPEM is 1000 times more than that of CWPEM. Though the detection limit of CWPEM is higher than that of NPEM, it is showing ability to detect the COD concentration generally encountered in wastewater treatment plant; and its application is no less than that of NPEM. Application of MFC in the field of biosensor is very limited due to limited research in its biosensor application area. Though range of detection, time of sensing, repeatability, stability etc., varies in each study, the results are encouraging to perform further studies.

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