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Design and evaluation of concentric cylinder bio fuel cell with proton exchange membrane-graphite powder–Glucose oxidase anode assembly

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Abstract

The current research is based on Mediated electron transfer in novel electrode assembly. Glucose oxidase was mixed with graphite powder and stuffed into a compartment with dialysis membrane on one side while Nafion 117 on the other side. The compartment was meshed with stainless steel wire. The dialysis membrane (Molecular weight cut-off > 12000 Da) was used to create a barrier between glucose solution and anode assembly. Hydroquinone was used as the mediator. The assembly generated a current density of 160μA/cm^3 and had a power density of 33μW/cm^3. The design is robust and can be used to overcome substrate inhibition.

Key words: Bio-fuel cell, Glucose Oxidase anode, Mediated Electron Transfer, Direct Electron Transfer, Dialysis membrane

Introduction

Biofuel cells (BFCs) are gaining attention as a possible alternative to the traditional chemical fuel cells. BFCs may utilize microbial cells (Karmakar et. al., 2010) or enzymes as such as catalysts (Pizzariello et.al, 2002) for oxidation of fuels. Among the two, the enzyme based fuel cells are more similar to traditional chemical fuel cells. Enzyme based BFCs are very much similar to chemical fuel cells in construction. But in contrast, they usually employ oxidases and dehydrogenases as anode catalysts for oxidation of the fuel handle and maintain than chemical fuel cell. Majority of the enzyme substrates, which is usually glucose, but can theoretically be any substrate based on the enzyme used. The cathode catalysts that have been used for reduction of dioxygen are mostly multi-copper oxidases such as fungal laccases (Habrioux et al., 2002). Considering the fact that BFCs work at physiological temperatures and pH, they are easier to BFC designs use mediated electron transfer (MET) type biocatalysis where electron transfer mediators are used to facilitate electron transfer between the enzyme and the electrode (Barton et.al, 2004, Kamitaka et.al, 2007, Zhu et al 2011, Leech et al 2012). The MET type system can be used with most of the redox enzymes. Since the mediators shuttle the electrons, it is necessary to keep the anode and cathode side separated using a suitable membrane. This can be avoided if both the enzyme and mediator are immobilized on to the electrode; but mediator leakage has been reported even in this case (Kim et al 2012). Layer-by-layer (LBL) assembly of alternate osmium redox polymers and glucose oxidase developed by a group of Irish researchers showed promising result (Rengaraj et al 2011). Another promising setup is the direct electron transfer (DET) type system (Ivnitski et.al, 2006). This system depends on close association of the enzyme with the electrode, such that the electron transfer site of the enzyme is in contact with the electrode allowing direct electron transfer. In the current research, the idea is to maximize the electron capturing, using Glucose oxidase in compressed graphite powder confined in stainless steel meshed compartment, with increased surface area and porosity. Instead of increasing the thickness of layers, the idea is to increase the effective area of contact. It improves the handling of the whole assembly generating higher current density. Since work is done on a large vessel; the effective current production is also high. Graphite powder being conductive can pass the electrons to mesh-electrode and let the H^+ ion to move towards Proton exchange membrane (PEM). It also prevents excessive motility of mediators, resulting in increasing of current density. A recent research on powdered graphite based miniature electrode showed good results (Zebda A et al 2012). However, a large enzyme based fuel cell with powdered graphite assembly is yet to be tested. The reaction mixture with Hydroquinone facilitated MET which further enhanced the Fuel cell activity. This approach to balance the glucose flow to the system, based on solubility gradient, with powdered graphite assembly is a new way to tackle the substrate inhibition and excessive mediator motility. This indigenous design also allows easy refuelling of glucose compartment needing no anaerobic pre-treatment.

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Materials and methods

Bio-Fuel cell Physical construction

Three cylindrical PTFE containers of same height (8 cm) but different diameters (10 cm, 5cm and 3 cm) were taken. These containers were assembled together to form concentric cylindrical vessel. Whole length slits were made in the inner two cylinders. The middle cylinder slits were covered with dialysis membrane (Sigma-

Aldrich MW cut off > 12000 Da) and adhered with epoxy adhesive. (Araldite). The inner cylindrical slits were covered with Nafion™ 117 proton exchange membrane. The assembly design is shown in Figure 1. The containers were washed with alcohol followed by double distilled water to remove out all salt based contamination. The middle cylinder was meshed with stainless steel wire of diameter of 30 µm to act as electron collector, connected with a graphite electrode. The inner cylinder was meshed with platinum black coated stainless steel wires connected with a platinum electrode of diameter of 2mm (CH Instruments).

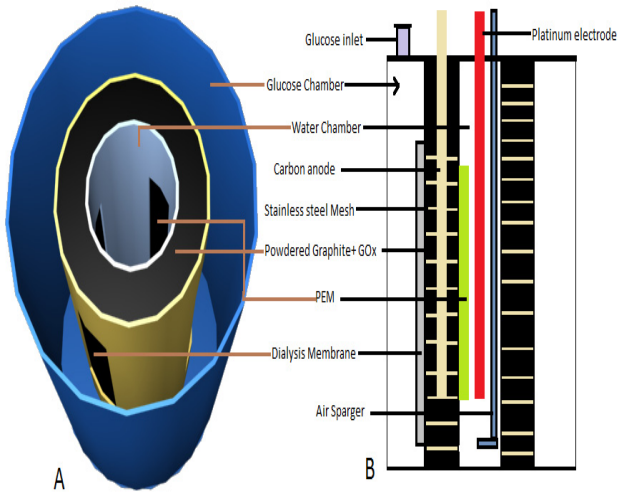

Figure 1: Schematic A) 3D view of the BFC and B) Front view of BFC designed.

Bio-electrode compartment preparation

0.5g Glucose oxidase (GOx) (Sigma Aldrich, EC: 1.1.3.4) was mixed with 50gm Graphite powder (Merck) and Potassium phosphate buffer (pH 7.0) (Himedia) to make slurry and poured in middle container and sealed, to cut off the oxygen supply. The effective volume of the middle compartment is 401.9 cm^3. The whole cylinder with conducting graphite powder and wire mesh acted as the anode as well as anodic chamber where the catalytic reaction took place. This not only increases the electron capturing but also a higher surface area for the reaction to continue.

The inner compartment, filled with distilled water and fitted with continuous air sparging unit, acted as cathode. The continuous air sparging elevated the Dissolved oxygen (DO) level of water and maintained the steady supply of electron acceptor catalyzed by laccases enzyme (Sigma Aldrich). The outer chamber was filled with 0.1 M glucose (Aldrich) solution and 1 mM hydroquinone (Sigma-Aldrich) in Potassium phosphate buffer (pH 7.0). The chamber was bubbled with nitrogen to remove out all the oxygen and sealed off. The outer chamber worked as the fuel reservoir.

Glucose diffusion rate measurement

The glucose from outer chamber was taken at fixed interval and analysed with GOD/POD standard reagent (Sigma-Aldrich) to ascertain the amount of glucose diffusing through the dialysis membrane inside the anodic compartment. Glucose is oxidized by glucose oxidase(GOD) to produce hydrogen peroxide and gluconate. The hydrogen peroxide is then oxidatively coupled with 4 amino- antipyrene(4-AAP) and phenol catalyzed by peroxidase (POD) to yield a red coloration that is measured at 505nm. The experiment was conducted in triplicate and the mean value was taken in consideration.

Cyclic Voltametry of the anodic compartment

Electrochemical measurements were performed with Potentiostat/Galvanostat with conventional three-electrodes, which included the working GOx-Graphite anode assembly, platinum net counter and Ag/AgCl reference electrodes (CH Instruments). The experiment was carried out with (1) Hydroquinone (HQ) 1 mM, (2) Glucose 10 mM and (3) 10mM glucose and 1 mM HQ, in 0.1 M phosphate buffer at pH 7.0 containing 20 mM KCl. (Pizzariello et.al, 2002). The cyclic voltammetry demonstrated the direct electron transfer and mediated electron transfer of the enzyme from the slurry to electrodes. The experiment was to access the electro-biochemical activity of the whole assembly.

Current Voltage Measurement of the BFC

The assembly was mounted. Outer chamber was filled with 0.5M glucose solution. Then the chamber was nitrogen bubbled and sealed off to create anaerobic environment. The air tight caps were sealed using silica gel to prevent any air passage. The electrochemical activity was measured using the Rishabh-15s multimeter, at different time intervals. The glucose were replenished from a large reservoir containing nitrogen bubbled sterilized 0.1 M glucose solution every 24 hours. The experiments were conducted in thrice and the mean value is taken into consideration to draw the current-time curve. The performance curve was evaluated using the standard bridge potentiometer (AmpTek) connected with multimeter, varying the current density to evaluate the power density and corresponding voltage.

Result and Discussion

Cyclic voltammetry gave a view of the electrochemical characteristics of the assembly. From Figure 2, it is seen for glucose and glucose+ hydroquinone, the current is high in the potential range of 200-300 mV with maxima around 250 mV; while for HQ only, the current is maximum around 200 mV. The curve rises sharply at first reaching its peak and then falling down following the same pattern.

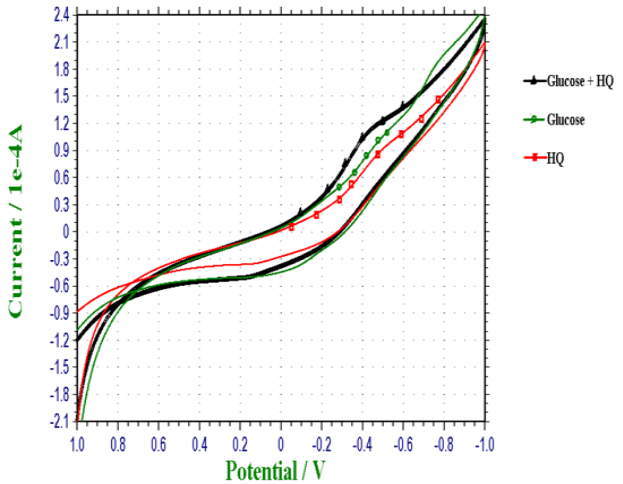

Figure 2. Cyclic Voltammetry of HQ, Glucose and HQ + Glucose with reference to standard Ag/AgCl electrode.

In negative range it is seen that the current is comparatively very low suggesting that the negative potential of the Glucose oxidase electrode assembly doesn't allow the reaction to go in the reverse direction. The three curves with 1) HQ, 2) Glucose and 3) HQ with Glucose are distinct. When the solution contains only the mediator, the characteristic is just due to difference in potential between anode and cathode solvents. When glucose is added, activity is clearly seen with a rise in current. This rise gives an idea that direct electron transfer from enzyme to electrode assemble is taking place. When glucose and HQ both are added, there is sharp rise in activity which infers the fact that in presence of mediators the assembly captures electrons better giving a sharp increase in current. This also clearly states that mediated electron transfer is better than direct electron transfer, though both the phenomena can generate detectable amount of current. DET and MET both are occurring in the anode assembly which consolidate the fact that graphite powder compresses in stainless steel meshed environment can capture the electrons to form current.

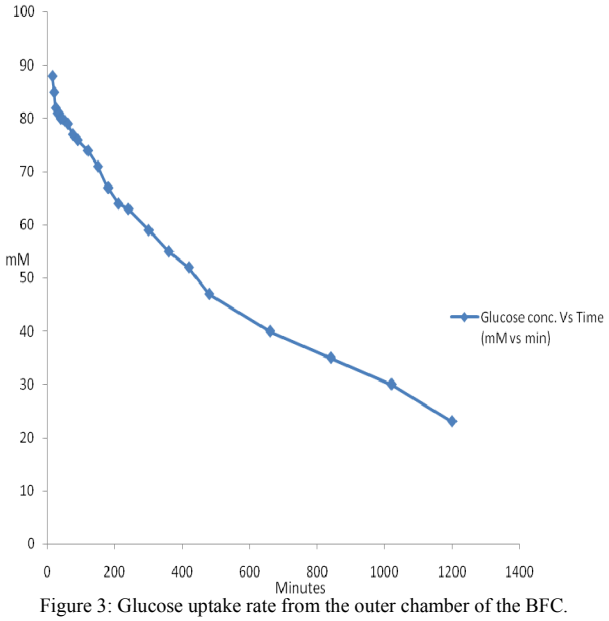

Figure 3: Glucose uptake rate from the outer chamber of the BFC.

The glucose depletion is an important characteristic to measure. This not only informs the rate of diffusion across the two membranes but also when the glucose is depleted enough to be supplemented. The glucose uptake from the outer chamber started faster but within minutes the rate of uptake dropped and soon it became steady. This may be due to the initial gradient prevailing across the dialysis membrane. Since the enzyme chamber is devoid of oxygen, the enzyme activity depended on the transfer of ions across the proton exchange membrane (PEM). Thus the PEM acted as the rate limiting step. The Figure 3 depicts the steady state glucose diffusion. The glucose uptake is approximately 3mM/hour after an hour or so. This clearly gives an idea that increasing the sugar concentration outside will not affect the enzyme activity and thus the overall power output of the cell. So a concentration above 3mM will be enough to let the fuel cell to work for an hour. Increasing the concentration above k_m value of enzyme (k_m= 110 mM) is not recommended as the activity deviates at higher concentration. 100mM concentration will let the cell to work efficiently for several hours without significant change in gradient and power. The fuel cell needed reloading glucose every 24 hours.

The performance curve in Figure 4 gives the peak power of 33μW/cm^3 at current density of 160 μA/cm^3 that is equivalent to 33 W/m^3 of the anodic compartment volume with equal scale up of enzyme load and graphite powder. Though the power density seems low but it is quite comparable enough when compared to the conventional microbial fuel cell. The power density is low, may be due to the H+ ion accumulation and PEM limitation in stopping oxygen diffusion across it. The power enhancement can be an excellent field in future research.

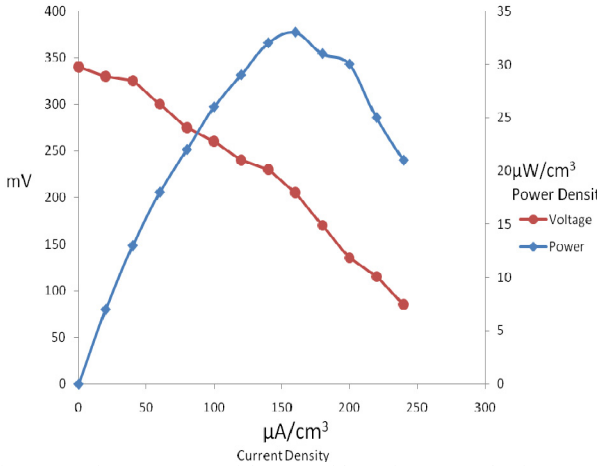

Figure 4: Performance curve of the BFC with varying current density

With the same setting of Potentiostat of 653mΩ for maximum power generation with varying current density, the current generated was recorded over time as shown in Figure 5. The current rose steeply at start and reached a peak value of about 70 mA within 10 minutes. The sudden rise in current is due to the addition of glucose. It soon dropped to around 64 mA where it stayed stable for 12 hours. The drop may be explained by the saturation of the electrode assembly, after which it remained stable for a long time before the drop. After that the current started dropping and it reached 42 mA by the end of 24 hours.

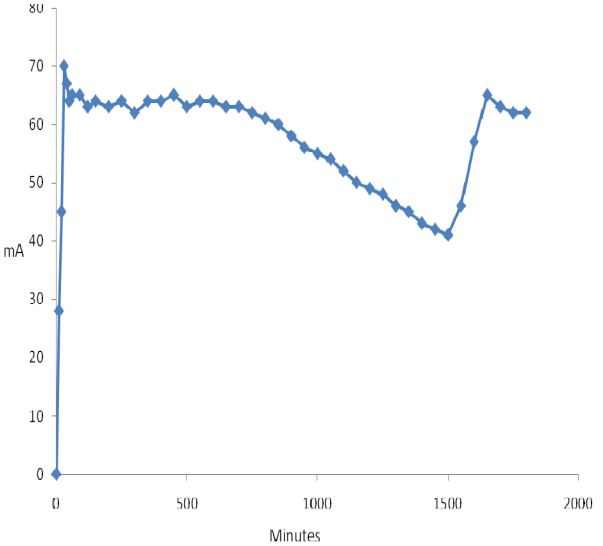

Figure 5: Current generated vs. Time Curve of Designed BFC.

The drop may be due to the combination of various effects. It may be due to drop in glucose gradient across membrane or/and accumulation of H^+ ions in the membrane electrode assembly arising due to saturation of Proton exchange membrane (PEM). PEM is also reported to have oxygen diffusion (Jung Chae, et.al, 2008). This also can give rise to accumulation of H_2O_2 which is GOx inhibitory in

nature. These effects alone or in combination can give rise to the current drop.

Glucose was supplemented at this point to reach 100 mM concentration in the outer chamber. The current value again increased to 65 mA and then stabilized at 62 mA. Rise in current depicts the positive role of glucose gradient in the current generation. But the current peak value decrease also reveals that the GOx is not functioning at its peak and this may be perhaps due to the accumulation of inhibitory H_2O_2.This approximates to 5% efficiency decrease in 24 hoursColumbic efficiency of the system was calculated using equation 1 with the data of 3mM/ hour of glucose depletion rate and 65mA of steady state current production.

$$\text{Columbic efficiency (C)} = N_e \times 100 / N_g \times N_{H+} \quad (1)$$

N_e = Total number of electron flowing for the current production; N_g= Number of molecules of glucose consumed during the actual reaction; N_{H+} = Number of H+ ions involved

The columbic efficiency is found to be 39.84 %. The efficiency of the system is quite high and quite comparable to the energy liberated in biological system but a lot of work is to be done to make the system as efficient as normal chemical fuel cell. However, the energy involved in heating a chemical fuel cell and its water management is very high than this form of BFC, that can make BFC an excellent candidate for future studies. But the power of the BFC can be a excellent measure to understand the performance. Higher electrode contact area can lead to a higher current value. So the design characteristics should be established keeping in mind the power requirement. This model is quite compact in nature. This can be designed as whole cell microbial fuel cell using different set of membranes with higher glucose permeability. This may lower the diffusion resistance of the assembly and also give higher half life of electrode assuming microbes can survive longer than the enzyme alone.

Conclusion

The concentric Bio-fuel cell is a viable design if we need to replace the conventional batteries. Maximum power density of 33µW/cm^3 was observed. The fuel cell also gave a steady state current value of 64 mA. The peak voltage is a relative phenomena and it depends on the load. So it is tough to ascertain a standard voltage for the cell

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