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Bioelectricity Production from Various Feedstocks Using Pure Strain of *Bacillus firmus*

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ABSTRACT. Microbial fuel cells (MFCs) are bio-electrochemical devices that exploit microorganisms for producing electricity from a variety of materials, including complex organic waste and renewable biomass. In this study, the heterotrophic microbe, *Bacillus firmus* was used as the active bacterial component with synthetic waste waters for bio-electricity production. Three identical mediatorless and membraneless single chambered microbial fuel cells (MFCs) without catalyst was fabricated with different carbon source and operated in batch mode. The performance of these MFCs with glucose, hydrolyzed potato peel and hydrolyzed cyanobacterial biomass substrates were comparatively evaluated. Among these substrates hydrolyzed cyanobacterial biomass was found to be the favorable substrate for electricity production whereas potato peel was unable to construct a well-established MFC. The maximum power density of 16.46 mW/m^2 at 62.48mA/m^2 was achieved using cyanobacterial mass as the substrate. A current density of 53.47mA/m^2 appeared to characterize the maximum power produced from a polarization test was 5.85mW/m^2 for glucose substrate.

Keywords: microbial fuel cell, *Bacillus firmus,* single chamber, membrane-less, potato peel and cyanobacterial biomass

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1. Introduction

Electricity is a major commodity for industrialization, urbanization, economic growth and improvement of quality of life in society (Yavari et al. 2013). The fossil fuels like coal, oil, and gas, the major source of electricity generation, are limited and world may be faced with serious shortage of energy in a near future. The combustion of these fossil fuels release many pollutants such as carbon dioxide, carbon monoxide, oxides of sulphur and nitrogen (SOx and NOx) to atmosphere which cause to climate changes, global warming, greenhouse effect and adversely affected to the human health. To alleviate the hazardous environment concerns, future energy sources should be renewable and carbon neutrals with minimal negative environmental impact (Milner, Davies and Wilkinson, 2012). Microbial fuel cells offer the possibility of harvesting green electricity from organic waste and renewable biomass with low environmental foot print (Lovley, 2006).

Microbial fuel cells (MFCs) are bio-electrochemical systems which convert chemical energy of organic or inorganic substrates into electrical energy that generate electricity using viable electrochemically active microorganisms (Shukla et al. 2004; Logan and Regan, 2006). Microorganisms are self-regenerating systems that can oxidize substrates such as glucose, sucrose, alcohols, grape juice, or wastewaters from different origins as starch, beer brewery, chocolate industry, food processing and sewage sludge wastes from food industry (Allen and Bennetto, 1993; Behera et al. 2010; Herrero-Hernandez, Smith and Akid, 2013; Moqsud et al. 2013). The electrons resulting from these microorganism metabolisms are generally transferred to a high potential electron acceptor such as dissolved

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oxygen in the medium whereas in MFCs, electrons are transferred to an anode and if the electrons are led to the cathode through an external circuit, a voltage drop and an electrical current are produced (Li et al. 2014; Hernández-Fernández et al. 2015).

MFCs are classified into two types according to the transfer of electrons, from the bacteria to the anode (mediator-based or mediatorless). In mediator-based MFCs mediators such as neutral red, methylene blue, thionine, humic acid or sulphate/sulphide (Park and Zeikus, 2000; Scott and Murano, 2007; Gunawardena, Fernando and To, 2008; Rossi, Fedrigucci and Setti, 2015), depending on the species of microorganism used, are added which act as intermediaries between the microorganisms cell membrane and the anode to transfer electrons. But most available mediators are expensive and toxic. Mediatorless MFC functions without exogenous electron carriers and require some form of carbohydrate. Metal-reducing bacteria, such as *Shewanella, Rhodoferax* and *Geobacteraceae*, which transfer electrons directly to the anode via redox enzymes in their outer membrane are the most used species in mediatorless MFC (Moon, Chang and Kim, 2006; Liu and Li, 2007).

Some researchers have reported the generation of electricity using pure culture, such as *Geobacter* sp. (Bond and Lovley, 2003; Poddar and Khurana, 2011; Kim, Chang and Kim, 2012; Bond and Lovley, 2003) and *Shewanella* sp. (Watson and Logan, 2010; Nimje et al. 2012; Jain et al. 2012). On the other hand, many studies have shown that the performance of MFCs with mixed species consortia and sewage sludge is superior than using pure cultures, due to their stability, robustness, nutrient adaptability, stress resistance and higher current densities (Zain et al. 2011; Yang, Du and Liu, 2012; Choi and Ahn, 2014). However, in mixed-culture MFC, the electrochemical activity of a few bacterial species enhances the power output of the whole system. Hence, it is difficult to analyze the mechanisms and influence of the individual microorganisms contributing to power generation.

The performance of MFCs depend on several important factors, such as nature of inoculum (biocatalyst), characteristics of carbon source, nature and coating of electrodes, cathode and anode electrode assembly, electrode surface area, the composition and the thickness of the ion-selective membrane between the two electrodes, operating conditions (loading rate, pH, temperature, retention time, etc.), and the value of external resistance (Rext) (Logan et al. 2006; Winfield et al. 2011; Velasquez-Orta et al. 2011 and Jia et al. 2014). Further, the use of separator causes several problems such as retarded transfer of proton from the anodic chamber to the cathode, increases the overall internal resistance of an MFC, lower the system stability and bioelectrochemical performance. In addition, it also increases the overall costs of an MFC. Therefore, performance of membraneless MFC was demonstrated by many researchers (Liu and Logan, 2004; Jang et al. 2004; Aldrovandi et al. 2009; Zhu et al. 2011; Nimje et al. 2012 and Choi and Ahn, 2014). Other researchers have suggested that MFC startup is most successful when biofilm harvested from the anode of an existing MFC is applied to a new MFC (Kim, Min and Logan, 2005; Mohan, Raghavulu and Sarma, 2008; Santoro et al. 2012 and Jain et al. 2012).

The objective of our study was to examine the feasibility of bioelectricity generation in membraneless single chambered MFC (mediatorless (anode); air cathode by *Bacillus firmus* NMBL-03 using three different synthetic wastewaters (glucose, hydrolyzed potato peel and hydrolyzed cyanobacterial biomass). Strains of *Bacillus* which had been reported earlier for electricity production are *B. subtilis* (Nimje et al. 2009) and other *Bacillus sp.* (Lu et al. 2009; Zhang et al. 2012).

2. Materials and Methods

2.1 Microorganism preparation

The *Bacillus firmus* –NMBL-03 was isolated from municipal sludge purified by clonal selection method. The strain was routinely maintained on nutrient agar slants and stored at 4^{o}C. Nutrient agar slants were prepared by suspending 28 g of nutrient agar (Himedia) in 1.0 L of double distilled water and autoclaved at 103.5 KPa (15 psi) pressure and at 121 ^{o}C for 15 min. Nutrient broth containing minerals, yeast extract, glucose and vitamin was used as the growth medium for inoculum. Bacterial cells were scrapped from the surface of nutrient agar slants and aseptically transferred to 9.0 ml nutrient broth. The inoculum was grown in this media for 16 h at 30 ^{o}C with continuous stirring.

Mineral solution in a liter constitutes; NH_4Cl, 8.1 g; KH_2PO_4, 9.4 g; K_2HPO_4, 19.3 g; NaCl, 0.4 g; $CaCl_2.2H_2O$, 0.5 g; $MgCl_2.6H_2O$, 0.93 g; $FeSO_4.2H_2O$, 13.9 µg; $NiCl_2.6H_2O$, 60.0 µg; $NaMoO_4$, 90.0 µg; $CoCl_2.6H_2O$, 200.0 µg; $MnCl_2.4H_2O$, 300.0 µg; H_3BO_3, 90.0 µg; $ZnSO_4.7H_2O$, 300.0 µg. Yeast extract 4 g/L and vitamin (becosule capsule), 510.0 mg/L were used for growth medium for inoculum. Vitamin solution was prepared by dissolving vitamin B complex capsule in 1 L autoclaved double distilled water and sterilized by Millipore filter. The mineral solution, glucose substrate at 1% (w/v) and yeast solution were autoclaved separately at 103.5 KPa (15 psi) pressure and at 121 ^{o}C for 15 min and mixed along with vitamin solution to prepare the nutrient broth. The pH of mineral solution was adjusted at 6.5 (Sinha and Pandey, 2014).

2.2 Substrates

The three carbohydrate substrates from different generation feedstocks were used as fuel for the three

MFCs in the batch mode experiment. The glucose from first generation feedstocks, potato peel from second generation feed stock and cyanobacterial biomass from third generation feed stock were taken as substrates for MFCs. The potato peel and cyanobacterial biomass were initially hydrolyzed with 0.5% (v/v) H_2SO_4 in an autoclave for 60 min. Thereafter hydrolyzed content was filtered and pH maintained to 7.0. These substrates were prepared and sterilized at 103.5 KPa (15 psi) pressure and at 121 ^{o}C for 15 min separately and mixed with mineral solution.

2.3 Microbial fuel cells configuration

Three identical single chamber air-cathode MFCs without a membrane were designed and fabricated using 50 ml falcon tubes with wire input point (at top), inlet port, outlet port and cathode fixing port (Fig. 1). Graphite electrode without any coating was used for anode. The shape of anode was rectangular with 54 holes (0.1 cm diameter) on the surface. The overall surface area of anode was 64 cm^2. The carbon cloth was used as air cathode that was loaded with four PTFE diffusion layers [Santoro et al. 2011], without a platinum catalyst. The anodes were fully dipped inside the liquid substrate of 35 ml in each MFC with a distance of 1.6 cm between the anode and cathode. The electrodes were connected to the external electrical circuit through silver paste and copper wires. Leak proof sealing was provided at joints to maintain anaerobic microenvironment inside the MFCs.

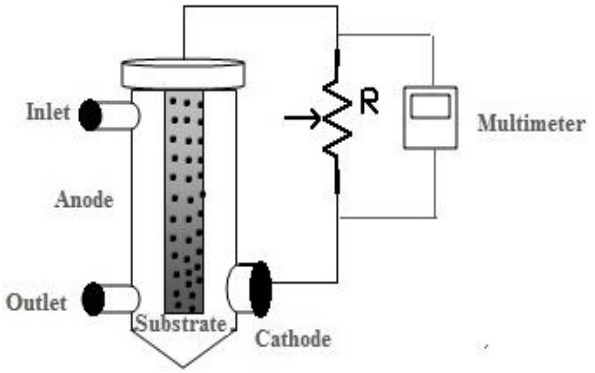

Fig. 1 Single chambered membrane-less Microbial fuel cell.

2.4 Biofilm growth

Prior to the biofilm growth on anodes, the inoculum was grown in 500 ml Duran with 350 ml nutrient broth and 35 ml prepared inoculum for 16 h at 30 ◦C with continuous stirring at 100 rpm speed. For the development of biofilm, all three anodes were completely suspended in 500 ml Duren filled with 400 ml inoculum. The Duren mouth was sealed with parafilm and placed on a magnetic stirrer with 100 rpm speed at room temperature (25 ± 2 ◦C). After 30 min of inoculation, the anodes were removed and placed in three MFCs separately.

2.5 MFCs operation

The three single chambered MFCs were operated separately with three different substrates viz., glucose (MFCgl), potato peel hydrolysate (MFCpp), and hydrolyzed cyanobacterial biomass (MFCal) at room temperature after adjusting substrates pH to 7. The MFCs were operated in three steps and prior to the beginning of each step, the following procedure was done (a) MFCs and electrodes were washed with 70 % (v/v) ethanol followed by distilled water (b) electrodes were soaked in deionized water for 24 h. (c) MFCs and electrodes were put in UV chamber for 20 min (d) the biofilm was grown on the surface of anodes (e) the MFCs were configured as mentioned in section 2.3 and sparged with argon gas to create the anaerobic environment. In the first step, the open circuit potential (OCV) of the three single chambered MFCs were recorded for 5 days. For second step, 1 kΩ resistance was connected to each MFC separately and the closed circuit voltage was observed for 15 days in three cycles of 120 hrs and each cycle started with fresh substrate. In last step, the MFCs were kept in open circuit mode for 24 hrs and then the polarization and power density characteristic was observed by connecting different resistance Rext.

2.6 Analysis

The MFCs potential (V) was recorded after every 10 min using a digital multimeter. The values of the potential were averaged on 18 data points (3 h). Current (I, in amperes) was calculated as

$$I = \frac{V}{R} \quad \textbf{(1)}$$

Where R is the external resistance (Ω), and V is the voltage in Volts.

The power (in watts) was calculated using

$$P = V \times I \quad \textbf{(2)}$$

The current density I_A (in mA/m^2) was calculated based on the anode area (A in m^2) as

$$I_A = \frac{I}{A} \quad \textbf{(3)}$$

Similarly, the power density P_A (in mW/m2) was obtained by dividing the measured power with the anode area as

$$P_A = \frac{P}{A} \quad \textbf{(4)}$$

From equation (1), (2) and (4), the power density can be calculated as

$$P_A = \frac{V^2}{RA} \quad \textbf{(5)}$$

The polarisation curve was obtained by changing the external resistance Rext (4.7 K, 3.3 K, 2.2 K, 1.5 K, 1 K, 680, 470, 330, 220, 150, 100 Ω). Each value of the R was maintained for 15 min and the total procedure required 6 h.

3. Results and discussion

In this study, bioelectricity production from three different substrates in air cathode microbial fuel cells using pure strain of *Bacillus firmus* –NMBL-03 has been investigated, including:

1) Glucose substrate from 1st generation feed stock.
2) Potato peel from 2nd generation feed stock.
3) Cyanobacterial biomass from 3rd generation feed stock.

The experiments were performed using three similar MFCs at room temperature (30 ± 2° C).

3.1 Electricity production using glucose substrate

On the initial construction of each microbial fuel cell, the open cell voltage (OCV) was monitored. After inoculation of glucose substrate in anode chamber of microbial fuel cell MFCgl with *B. firmus* NMBL-03, open circuit voltage (OCV) 64mVwas recorded. An infinite resistance was used to obtain OCV. The OCV of MFCgl reached the maximum value of 588mV, 36 hrs after the inoculation. However, after 2 days from the maximum value, the OCV decreased slowly with time, indicating depletion of organic carbon substrate (Fig. 2). The OCV of all MFCs was observed for 120 hrs.

For the second step, the MFCgl was reinstalled with fresh media of glucose substrate and new film of *B. firmus* NMBL-03. In order to obtain close circuit voltage, a 1 KΩ resistance was fixed in external circuit and the system worked at this situation for three cycles. The duration of each cycle was 120 hrs and started with fresh media of glucose. In first cycle, power output started immediately with fresh wastewater and a voltage of 120 mV which correspond to 2.3mW/m^2 power density was attributed.

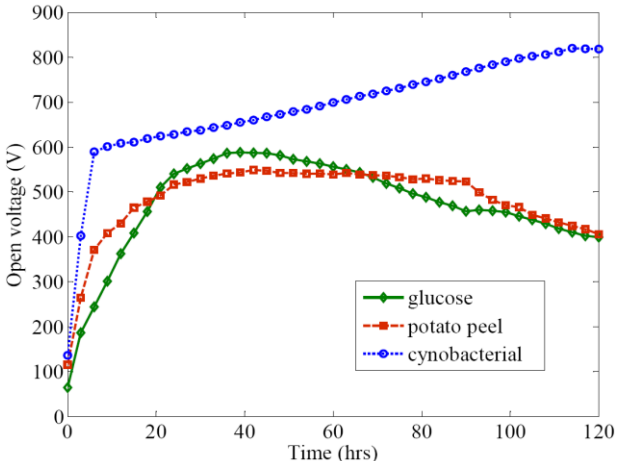

Fig. 2 Open circuit potential (OCV) for glucose substrate MFCgl, hydrolysate potato peel extract MFCpp and hydrolyzed cyanobacterial biomass MFCal.

As a consequence of the thick biofilm formation by *B. Firmus*, the power density was gradually increased to maximum value of 5.5 mW/m^2 (Fig. 3). Since bacteria in a biofilm have comparatively different properties than planktonic bacteria. The bacteria can cooperate and interact by various physical and chemical means in dense and protected environment of the film. Subsequently a gradual decrease was observed in power density and a stable plateau was observed after 60 hr, due to the metabolic inhibition of the cells due to a build-up of acid or other toxic by-products from bacterial activity, which resulted in a drop in pH from 7 to 4.8 during the experiment. The response of second and third cycle was nearly same and the maximum power output fell to about 40% from the first cycle of the experiment. The high electrical output in first cycle corresponded to the periods of maximum growth rate. After 60 hr, the response of first and second cycle was almost same with nominal difference and response of third cycle was slightly less.

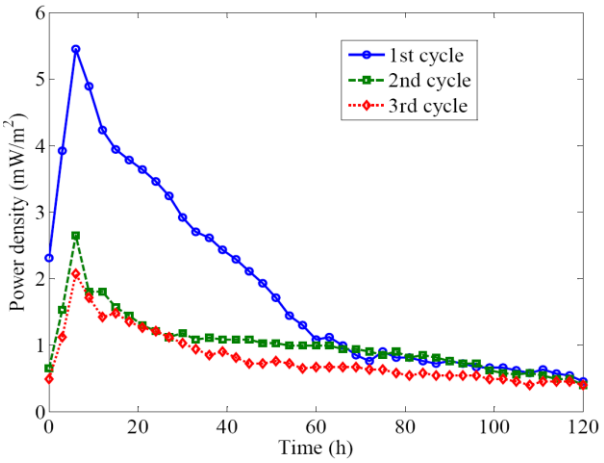

Fig. 3 Power density in glucose substrate MFCgl at external resistance 1000 Ω for three cycle.

Polarization and power density curves were obtained in third step with new biofilm and fresh media of glucose. Once the cell OCV was stabilized at maximum steady voltage, the polarized curve was obtained using the external electrical circuit which was subjected to loads of 100, 150, 220, 270, 330, 470, 680, 1000, 1500, 2200, 2700, 3300 and 4700 Ω. Fig. 4 illustrates the power density verses voltage curve and fig. 5 shows the polarization and power density curves as a function of current density. A polarization curve is a powerful tool for the analysis and characterization of MFC and describes voltage as a function of current. The polarization curve shows three region, where different phenomena are the main causes of the observed voltage drop. There was an initial abrupt decrease in voltage from OCV of 588mV at zero current to 220mV at current 0.18mA, which indicated activation losses. The activation losses are caused by the slowness of the reactions taking place on the surface of the electrodes. Also, a proportion of the voltage generated is lost in driving the chemical reaction that transfers the electrons to or from the electrode. The subsequent slope of the polarization curve was almost linear over the range of 0.11–0.33mA, which is due to ohmic losses. In the ohmic losses region the voltage drop is explained mainly by the resistance to the flow of electrons through the material of the electrodes, bacterial biofilm (if present) and DET; but perhaps the more important factors at MFCs are the electrolyte and PEM membrane (if present) capacity to transfer charge. The last part of the curve, denominated mass transport or concentration losses region, result from the change in concentration of the reactants at the surface of the electrodes (or at the surface of a biofilm) as the fuel is used. At higher current densities, no rapid fall in voltage was observed which indicates a lower mass transport limitation at the electrode. Furthermore, it implies that the bacterial biofilm is electrochemically competent and capable of solving diffusional and electrochemical limitations. The losses or polarization losses explain why the OCV potential never reaches the theoretical or calculated potential predicted considering the reduction potentials of cathodic and anodic reactions. Moreover compatible conditions with life limit strongly the temperature and chemical environment where microbial life is able to thrive. It is observed from power curve that there was no power produced when no current was flown under open circuit conditions, followed by an increase in power output to a maximum of 5.9mWm^{-2}, corresponding to a current of 0.330mA, at a potential of 109mV at 330Ω. Nimje et al (2009) studied the energy generation by glucose-fed MFC with M9 minimal medium in the anode chamber using stain of *Bacillus subtilis* which produced the maximum power of 115 mV at a resistance of 0.56 k Ω. In another study, Zhang et al. (2012) have isolated 74 bacterial strain and surveyed their electricity generation properties.

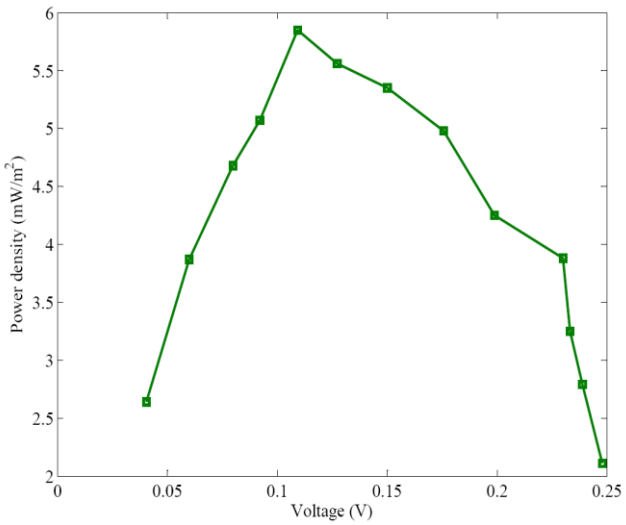

Fig. 4 Power density verses voltage curve for glucose substrate MFCgl with different resistances.

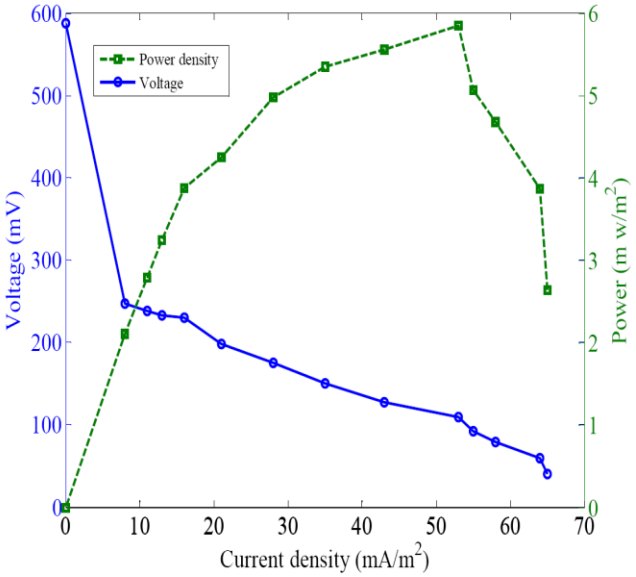

Fig. 5 Power and polarization curve for glucose substrate MFCgl.

They reported, strain similar to *Bacillus stratosphericus* was produced maximum power density of 87.5 mWm^{-2}, while strain similar to *Bacillus altitudinis* generated lower power of ~6mWm^{-2} in fumanic acid medium. Choi and Ahn (2014), reported maximum 0.16 to 2.0mW/m^2 power density with membraneless microbial fuel cell in air cathode and carbon cloth electrodes. Nimje et. al. (2012), explained the current production by MR-1 using paper industry wastewater (PWW), Agricultural wastewater (AWW), domestic wastewater (DWW) and food/dairy wastewater (FDWW). The maximum power density of 13, 36, 28 and 13 were reported with AWW, DWW, PWW and FDWW.

3.2 Electricity production using hydrolysate potato peel substrate

Potato peels are rich in starch and a co-product from the potato processing industry. The utilization of potato peels for the production of hydrogen and electricity would provide alternative for this by-product (Djomo, Humbert and Blumberga, 2008; Mars et al. 2010). Experimental results showed that the freshly inoculated aneaerobic biofilm of *B. firmus* NMBL-03, the MFCpp could generate electricity using hydrolyzed potato peel as substrates and an initial OCV of 115mV was immediately recorded (Fig. 2). Thereafter, the voltage increased because of biological activity, and stabilized at about 530±20mV for 70 hrs after 24hrs incubation. Following the steady phase, the voltage started to decrease which was essentially identical to the voltage observed with MFCgl.

When fresh media of hydrolysate potato peel was introduced into MFCpp with new biofilm, an initial circuit voltage of 103mV (power density 1.72mW/m^2) was immediately generated across the fixed external resistance of 1kΩ (Fig. 6), which might be due to the difference of the potential between the two electrodes based on both chemical and biological factors. Thereafter, the voltage increased because of biological activity, and reached a maximum of 150mV (power density 3.56mW/m^2) approximately 6hr after the start of the experiment. Thereafter a gradual decrease was observed in voltage, which reduced below 50mV. After 120 hr, the substrate in the anode was replaced and second cycle was initiated. A smaller amount of voltage was perceived in this cycle and ceased after 60 hr. Despite the addition of fresh media of hydrolysate potato peel, no change in voltage was achieved. Sinha and Pandey (2014), documented that potato peel extract was not found very suitable for the growth of microbe might be, due to release of the other inhibitory metabolites (Sinha and Pandey, 2014).

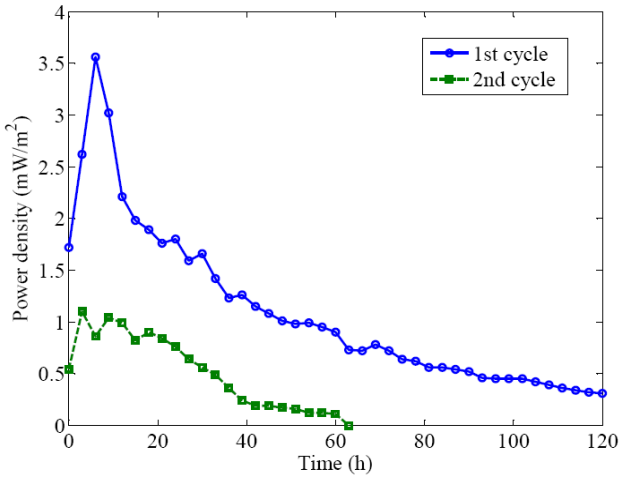

Fig. 6 Power density in hydrolysate potato peel extract MFCpp at external resistance 1000 Ω for two cycle.

The variation in power density with voltage at different external resistance is shown in fig. 7. The polarization and power density curve of MFCpp is shown in fig. 8. The maximum power output was 4.9mW/m^2, corresponding to a current of 49mA/m^2 at external resistance of 330Ω was achieved with hydrolysate potato peel substrate, and which was less than the output obtained from glucose substrate. It was observed that the activation loss region ranged from 0 – 0.07mA and ohmic loss region lied from 0.07–0.3mA for MFCpp. In the third part of the polarization curve the voltage decreased from maximum value to 26mV, which showed the concentration loss.

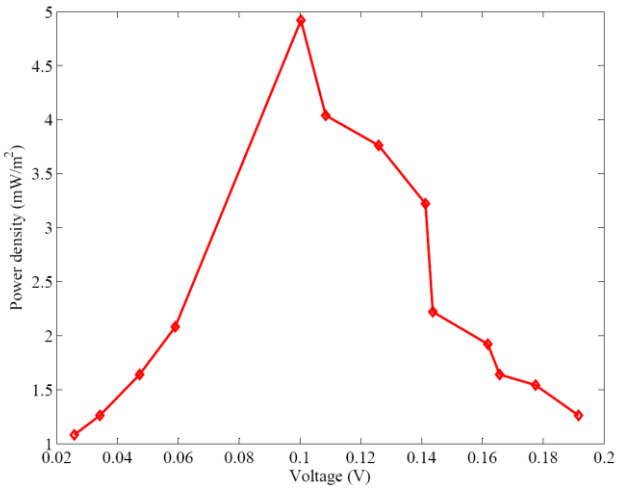

Fig. 7 Power density verses voltage curve for hydrolysate potato peel extract MFCpp with different resistances.

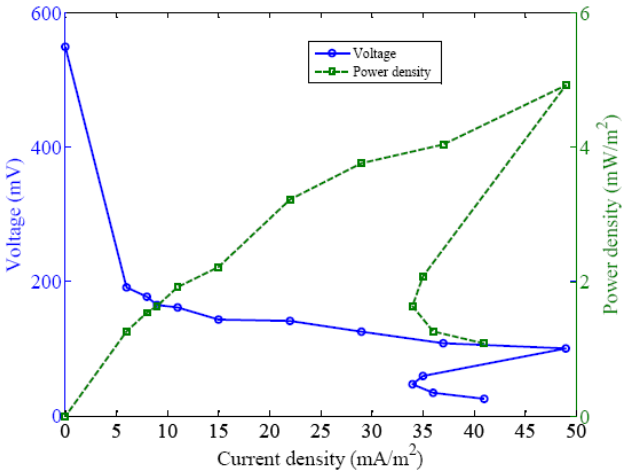

Fig. 8 Power and polarization curve for hydrolysate potato peel extract MFCpp.

3.3 Electricity production using hydrolysate algae substrate

Algae biomass display greater sustainability and are commercially advantageous feedstocks due to their

rapid growth rate, cultivability without soil, high capturing ability for CO2 and other greenhouse gases and very short harvesting cycle (1-10 days) (Parmar et al. 2011; Jones and Mayfield, 2012). It is composed of proteins, carbohydrates and lipids, serves as nutrient source for the microbes. Hence use of algae biomass in MFC is a promising approach to generate electricity (Strik et al. 2008; Velasquez-Orta, Curtis and Logan, 2009; Yuan et al. 2011; Lakaniemi, Tuovinen and Puhakka, 2012; Rashid et al. 2013; Kondaveeti et al. 2014; Gajda et al. 2015). Experimental data illustrated the feasibility and sustainability of using cynobacterial biomass as anodic substrate with microbial strain of B. firmus NMBL-03 in MFCal for bioelectricity generation. Among these three substrates, the highest OCV was recorded with cynobacterial biomass. The initial OCV of MFCal was 136mV, which rapidly increased to the maximum value of 820mV, 110 hrs after the inoculation and stabilized afterward on the same maximum voltage (fig. 2).

Fig. 9 shows the closed circuit characteristic (power density) of MFCal with time. The initial power density produced by MFCal was 5.13 mW/m^2 corresponding to the voltage 178mV, which gradually increased with time to maximum power density of 16 mW/m^2 (315 mV), 63hrs after the inoculation. The slow-release nature of this feedstock, indicates that cynobacterial biomass seem to be a more complex substrate due to its mineral composition. The voltage thereupon decreased to 100 mV (7mW/m^2) over the time period of 15 hrs. Afterward steady change in voltage was recorded. The second and third cycle followed the same pattern as MFCgl with the maximum power density 15.8mW/m^2 and 2.6 mW/m^2 respectively.

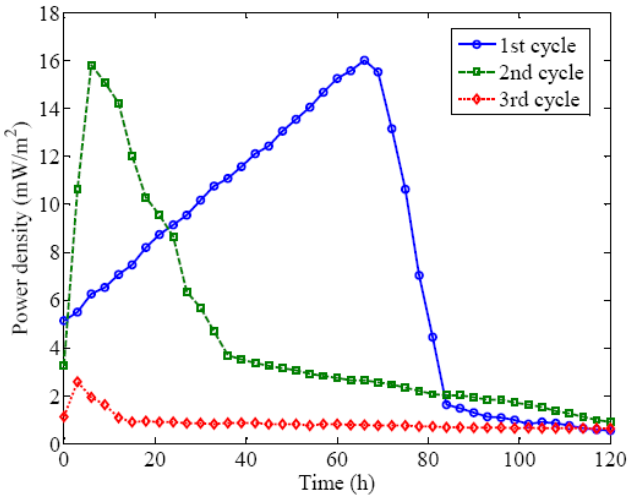

Fig. 9 Power density in hydrolyzed cyanobacterial biomass MFCal at external resistance 1000 Ω for three cycle.

The power density and voltage cure for hydrolyzed cyanobacterial biomass MFCal with different external resistance is given in fig. 10. The maximum power density 16.5mW/m^2 was obtained at current density 62.3mA/m^2 at resistance 680 Ω (fig. 11) which is much higher than the glucose substrate and hydrolysate potato peel extract. The polarization graph shows decrement in OCV to 393 mV, followed by a stable decrease to 126mV i.e. the activation loss and ohmic loss. After that concentration loss occurred in current range of 0.47–0.51mA.

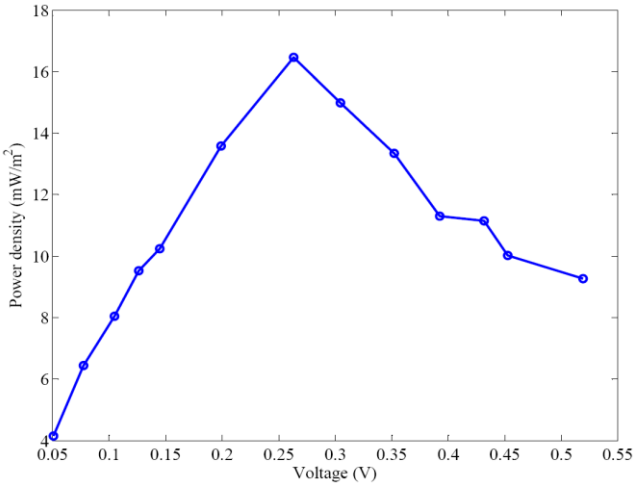

Fig. 10 Power density verses voltage curve hydrolyzed cyanobacterial biomass MFCal with different resistances.

Overall the power production from algae biomass is much higher than the glucose substrate and hydrolysate potato peel extract. During water treatment process, a large amount of algae residues is produced because algae are potential pollution vector in stored water causing eutrophication (Bubrick, 1991). Hence the use of algae biomass as a substrate can provide an opportunity for sustainable and low-cost production of electricity with waste management.

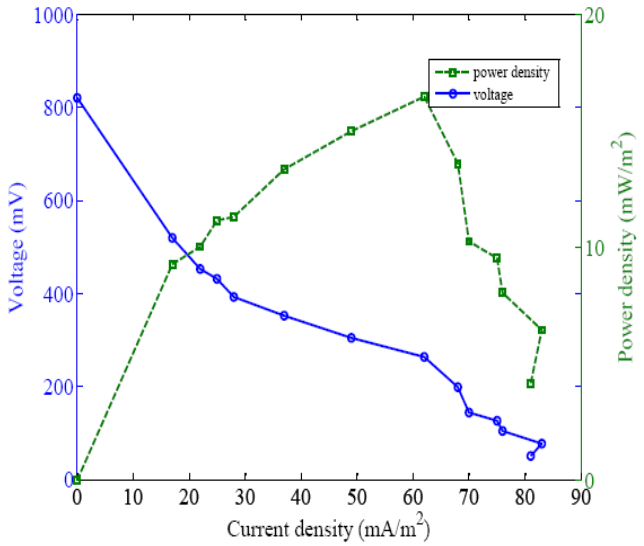

Fig. 11 Power and polarization curve for hydrolyzed cyanobacterial biomass MFCal.

4. Conclusion

We have demonstrated that *B. firmus* NMBL-03 has potential to metabolize a variety of substrate to produce a biofilm in a microbial fuel cell, which generated a long-term power output, due to which the isolate can be a member of an industrially important species. The *B. firmus* strain could be is one of the most commonly used hosts in fermentation production, because it is simple to cultivate. Electricity generation was observed with three synthetic wastewaters (glucose, hydrolyzed potato peel and hydrolyzed cyanobacterial (biomass) individually in mediatorless and membraneless single chambered microbial fuel cells (MFCs) without catalyst coating on electrodes. The power generated by the cynobacterial biomass was much higher than glucose substrate and hydrolysate potato peel substrate. The hydrolysate potate peel was not found suitable substrate for the growth of the *B. firmus*.

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