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**RESEARCH ARTICLE**

# EVALUATION OF BIOELECTRICTY PRODUCTION FROM THE ELECTROGEN *Proteus* sp. BVB09 IN THE TWO CHAMBER MICROBIAL FUEL CELL

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**ABSTRACT**

This study attempted to monitored the effect of various parameters which effect the bioelectricity generation by the nano-wire forming electrogen *Proteus* sp. BVB09 isolated from the cow dung in two chambers MFC having cellophane membrane as PEM. Experimental results concluded that mineral media M6 produced highest bioelectricity of 988.32mW/m$^2$ (397.8mV, 0.004mA, and 2.48mA/m$^2$) on the 6th day of the MFC operation at highest external resistance of 100KΩ whilst on the optimization of the necessary factors, a significant bioelectricity generation of 244363.97mW/m$^2$ (198.70mV, 1.98mA and 1229.81mA/m$^2$) on the 26th day of the MFC operation at 100Ω was recorded. These results obtained were also directly proportional to the bio film and biomass accumulated at the anode and anolyte of the MFCs.



## INTRODUCTION

Microbial fuel cells (MFCs) are Bio-Electrochemical Systems (BESs) (Rabaey *et al*., 2007), which utilize bacteria to oxidize organic matter and transfer electrons to the anode, where they flow to the cathode and react with protons and oxygen to form water (Logan *et al*., 2006). Electricity generation in a mediator less microbial fuel cell (MFC) is linked to the ability of certain bacteria, called exoelectrogens ("exo-" for exocellular and "electrogens" for the ability to transfer electrons to insoluble electron acceptors), to transfer electrons outside the cell to the anode in an MFC (Logan, 2008). Different genetic groups of bacteria have shown exoelectrogenic activity in MFCs, including β-Proteobacteria (*Rhodoferax*) (Chaudhuri and Lovley, 2003.), ϒ-Proteobacteria (*Shewanella* and *Pseudomonas*) (Rabaey *et al*., 2004.), δ-Proteobacteria (*Geobacter*) (Bond and Lovley, 2003). The mechanisms used for exocellular transport of electrons by these bacteria are still being studied. It has been demonstrated that cell-bound outer membrane cytochromes and conductive pili (nano-wires) may play a key role in electron transfer for some *Geobacter* and *Shewanella* species (Gorby *et al*., 2006, Myers and Myers, 1992; Reguera *et al*., 2005).

Alternatively, some exoelectrogens, such as *Pseudomonas aeruginosa* (Rabaey *et al*., 2004) and *Geothrixfermentans* (Bond and Lovley, 2005), excrete mediators to shuttle electrons to anode surfaces. Also, it was reported that the MFCs should be optimized in terms of reactor configuration and electrolyte to reduce the internal resistance and enable operation to the full microbial catalytic potential (Liu *et al*., 2005). Hence, in the present study a nano-wire forming electrogen *Proteus* sp. BVB03 isolated and identified in the initial phase of the study from the anodic biofilm of cow dung was employed in the two chambers MFC with PEM whilst different factors affecting its efficiency for bioelectricity productions, in terms of power generation, were determined.

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## METHODS AND MATERIALS

### Electrogenic bacteria and MFC fabrication

The nano-wire forming electrogen *Proteus* sp. BVB09 was taken from the bacterial cell collection of Bio Genics Lab which was isolated from the anodic biofilm of MFC operated with the cow dung as anolyte while identified using 16S rDNA technique and stored for the following study. The dual-chambered MFC was fabricated using PVC bottles (100mL capacity) provided with wire inputs from the top with inlet and outlet ports. The anode and cathode solutions were separated with the aid of cellophane membrane (0.35nm pore size) procured from Himedia, Mumbai, India used as proton exchange membrane (PEM) for the MFC. The graphite pencils (Apollo pencil manufacturers, Mumbai, India) were used as electrodes (specific surface area of 0.0014m$^2$) and positioned at a distance of 3cm on either side of the PEM. The electrodes were inserted into respective chambers after aiding with initial wetting (Allen and Bennetto, 1993) while circuit connections were made with the copper wires fixed into the drilled holes of electrodes and sealed with epoxy resin to avoid corrosion of copper wire (Zou *et al*., 2007). The MFC setups were also sterilized with 70% ethanol and dried under UV light for 20min. The selected electrogen *Proteus* sp. BVB09 was grown overnight in the standard LB media, inoculated with 1% inoculum, prior to inoculation (25%, v/v) in the 75mL mineral media as anolyte for the anodic compartments while the 100mM phosphate buffer enriched with 100mM of potassium hexacyanoferrate was used as catholyte (Kassongo and Togo, 2010). Initially, MFCs were operated for ten days on the closed circuit (100KΩas external resistance) for all the parameter studyand later for one month after the optimization while polarization curves on external resistance were drawn for the Power density (PD) (mW/m$^2$) and Current density (CD) (mA/m$^2$) normalized to the projected surface area of the anode where power generation was calculated using the equation; P = VI by measuring the voltage (V) and current (I) after an observation of 15min for bioelectrochemical analysis, using the digital multimeters (UNI-DT830D, Uni-Trend Group Ltd., Kowloon, Hong Kong) following every 24h.

**Screening of different growth media as anolyte**

The isolated nano-wire forming electrogen BVB09 was inoculated (1%) in 25mL of different mineral media were (g/L): M1 ($KH_2PO_4$, 1.5; $K_2HPO_4.3H_2O$, 2.9; $(NH_4)_2.SO_4$, 1.3; $MgCl_2.6H_2O$, 0.1; $CaCl_2$, 0.02; Yeast extract, 5.0; $FeSO_4$ solution, 5%; Resazurine, 0.2%; Cysteine hydrochloride, 0.5 and Na CMC, 0.1); M2 ($CaCl_2.2H_2O$, 0.04; $MgSO_4.7H_2O$, 0.1; $NaHCO_3.H_2O$, 1.8; $KH_2PO_4$, 0.42; $NH_4Cl$, 0.22; KCl, 0.38; Vitamin/Mineral, 10mL; $CH_3COONa$, 10mM; Fumarate, 40mM and Cysteine, 1mM); M3 ($NH_4Cl$, 1.05, KCl, 0.1; $NaH_2PO_4.H_2O$, 4.90; $Na_2HPO_4$, 9.15; Yeast extract, 0.2; Vitamins/Minerals, 10mL and Na CMC, 1.0); M4 ($NH_4Cl$, 1.05, $KH_2PO_4$, 1.5; $K_2HPO_4$, 2.9; Yeast extract, 0.2; $NaOOCH_3$, 10mM and Vitamin/Minerals, 10mM); M5 ($K_2HPO4$, 0.13; $MgCl_2$, 4.0; $ZnCl_2$, 0.034; Vitamin, 7.5mL; Starch, 10.0; Beef extract, 10.0; DL-alanine, 10.0; Glycerol, 20.0mL and Seawater, 10%) and M6 (Tryptone, 10.0; NaCl, 10.0; Yeast extract, 5.0 and Sodium acetate, 10.0) served as anolytes in the fabricated two chambers MFC. The 25mL of 24h old bacterial culture in all the types of mineral media were then poured into the anodic chambers of MFCs containing respective 75mL sterilized media as anolyte while 100mM potassium hexacyanoferrate enriched in 100mM phosphate buffer as catholyte (Kassongo and Togo, 2010). The electricity generation was monitored from the MFC setups and media which produced more electricity was taken as standard media for further study.

**Optimization of standard anolyte**

The standard media selected as standard anolyte was further optimized against 100mM potassium hexacyanoferrate as catholyte in order to assess maximum bioelectricity production from the nano-wire forming electrogen *Proteus* sp. BVB09 by analyzing the following parameters.

**Addition of carbon and nitrogen supplements**

Five 1% different carbon sources (Na CMC, Lactose, Glucose, Starch and Sucrose) and 1% different nitrogen sources (Ammonium acetate, Urea, Ammonium chloride, Ammonium sulphate and Potassium nitrate) were added separately in the selected anolyte to study their effect on the bioelectricity production from the isolated electrogen in the MFCs.

**Optimization of catholyte**

The 100mL of standard catholyte was also manipulated by using different concentrations (25, 50, 75, 100 and 125mM) of potassium hexacyanoferrate enriched in 100mM phosphate buffer in the cathodic chambers against the standard anolyte M6 in the fabricated MFC setup to check their effect on the net bioelectricity generation from the isolated electrogen *Proteus* sp. BVB09.

**Optimization of distance between the electrodes**

In the constructed MFC setups, the effect of distance (1.5, 3 and 5cm) between the electrodes placed in the respective chambers containing 100mL of standard anolyte and standard catholyte was also monitored for the bioelectricity generation. The distance between the electrodes was varied by changing the length of clamps holding the PEM while keeping PEM at the locus point of the electrodes.

**Optimization of surface area of PEM**

The surface area of the PEM was also varied by changing its radius to check their effect on the bioelectricity generation from the fabricated MFC setup. The two circular pieces of PEM of radii (1 and 2cm) were fixed in the clamps attached to the chambers of the MFC filled with standard anolyte and catholyte were also analyzed for the bioelectricity generation.

**Optimization of electrode surface area**

The graphite electrodes used in the MFCs were varied in their superficial surface area to observe their effect on the bioelectricity generation. The MFC setups were constructed keeping the surface areas of the both the electrodes $0.0014m^2$; anode surface area of $0.0028m^2$ and cathode surface area of $0.0014m^2$ as well as anode surface area of $0.0014m^2$ and cathode surface area of $0.0028m^2$ in combination for the MFC setups for the bioelectricity generation.

**Optimization of external resistance**

The five new MFC setups were fabricated and bioelectricity generation was recorded on the external resistances of 10Ω, 100Ω, 1K, 10KΩ and 100KΩ in the circuit. The external resistance value at which maximum bioelectricity generation was recorded was then opted as the optimized external resistance for the fabricated MFC with electrogen *Proteus* sp. BVB09.

**Analysis of bioelectricity generation from optimized MFC**

Following optimization of different parameters essential for the enhancement of bioelectricity generation from the MFC with *Proteus* sp. BVB09, the new MFC setup was fabricated and operated for one month to check the effect of optimized factors on the bioelectricity production, where following every ten days interval 75% anolytes and 100% catholytes were replaced from the anodic and cathodic chamber respectively.

**Other analysis**

**Biomass measurement**

Biomass (protein content) attached to the electrodes was determined according to Bradford (1976) using Bovine Serum Albumin (BSA) as standard obtained from Aldrich Sigma (Mumbai, India). Immediately after one month of electrochemical analysis with the optimized parameters for the MFC, anode was removed and dipped in the growth medium to remove planktonic cells. It was further incubated in 1mL of 0.2M NaOH at 96°C for 20min to remove the attached biomass. This solution served as protein sample. Further, 0.5mL of 1X Bradford reagent [20mg Coomassie Brilliant Blue G-250 dissolved in 10mL of 95% (v/v) alcohol and 20mL of o-phosphoric acid, volume made up to 200mL with distilled water] was mixed with the 0.5mL protein sample and incubated for 5min at room temperature. The color change was monitored for its absorbance at 595nm against Bradford reagent as blank. The absorbance obtained was compared with the standard curve of BSA, prepared by using different concentrations of BSA (0-500µg) mixed with 5mL of Bradford reagent and absorbance was taken at 540nm. The biomass obtained was then expressed in mg/mL.

**Biofilm formation assay**

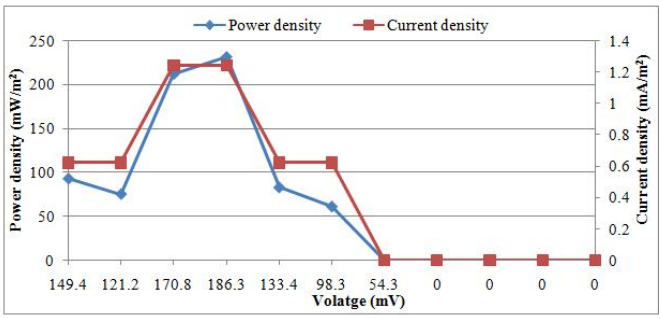
In order to examine the biofilm formation, crystal violet (CV) bio film assays were performed using a modification of a previously described protocol (O'Toole *et al*., 1999). The electrogen *Proteus* sp. was inoculated (1%) in the 300µL optimized media, further incubated at 35°C for 72h in the 92 well microtitre plates. Following incubation, the cultures were pipette out gently and 300µL of a 0.01% (w/v) CV solution was added to stain the cells attached to the walls. It was allowed to incubate for 15min before wells were rinsed to remove unattached cells. Remaining cells were dried for 20min at room temperature and then the CV solution was solubilized with 300µL of 100% dimethyl sulfoxide. The $OD_{600}$ was taken to monitor the cell attachment on the walls of well using spectrophotometer.

## RESULT AND DISCUSSION

**Screening of anolytes**

For an anolyte in a microbial fuel cell to work properly it has to satisfy a twofold criterion. The first has to do with the bacteria and the second with ion conductivity (Yuan and Kim, 2007). Hence, various mineral media were screened as anolytes for the electrogen *Proteus* sp. BVB09 in the anodic chamber of fabricated MFC for the proper conduction of ions. It was observed that the mineral media; M1

showed 231.42mW/m$^2$ (186.3mV, 0.002mA and 1.24mA/m$^2$) on the 4$^{th}$day (Fig.1A), M2 reflected 235.03mW/m$^2$ (189.2mV, 0.002mA and 1.24mA/m$^2$) on the 3$^{rd}$ day (Fig.1B), M3 presented 280.86mW/m$^2$ (226.1mV, 0.002mA and 1.24mA/m$^2$) on the 4$^{th}$ day (Fig.1C), M4 displayed 558.63mW/m$^2$ (299.8mV, 0.003mA, 1.86mA/m$^2$) on the 3$^{rd}$ day (Fig.1D), M5 showed 514.84mW/m$^2$(276.3mV, 0.003mA and 1.86mA/m$^2$) on the 6$^{th}$ day (Fig.1E) and M6 presented 988.32mW/m$^2$ (397.8mV, 0.004mA, and 2.48mA/m$^2$) on the 6$^{th}$ day (Fig.1F); the highest bioelectricity production profiles for *Proteus* sp. BVB09 inoculated in the respective MFC setups. Based on the obtained results, it was deduced that with the mineral media M6 electrogen generated maximum bioelectricity which must be because of its ideal composition for the proper growth of the electrogen *Proteus* sp. BVB09. Hence, mineral media M6 was selected as the standard anolyte for *Proteus* sp. BVB09.

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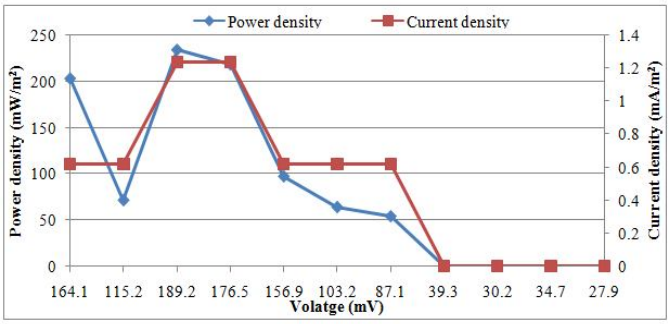


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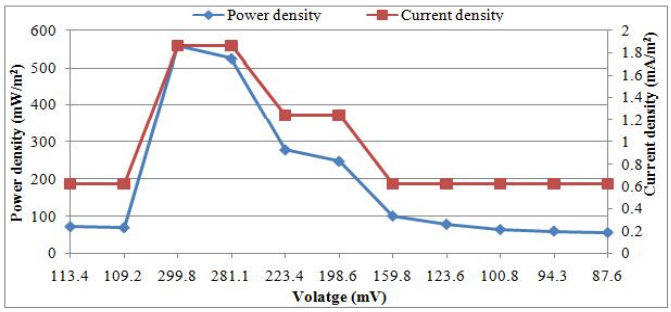


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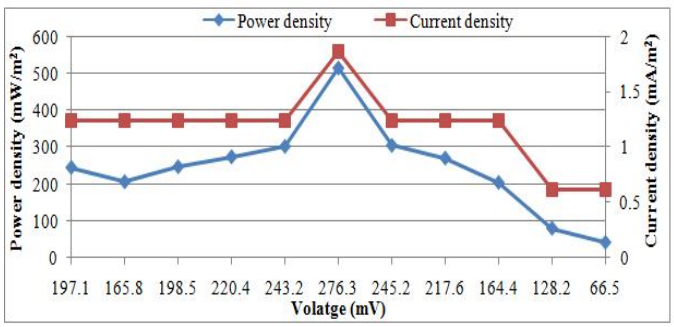


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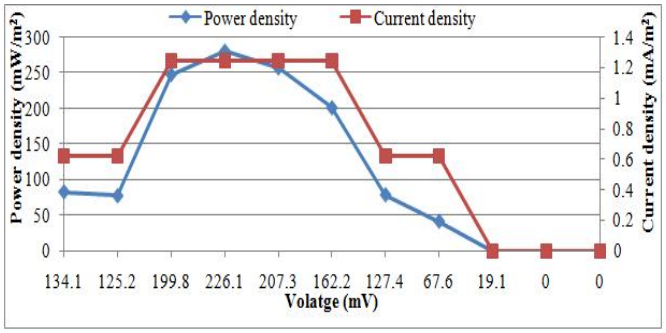


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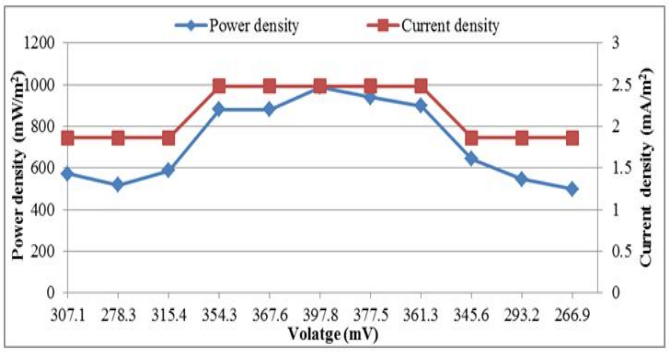



F

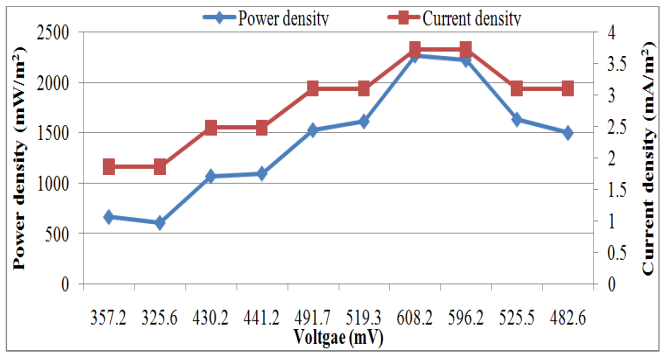
**Fig.1. Screening of mineral media M1(A), M2(B), M3(C), M4(D), M5(E) and M6(F) for bioelectricity production from the electrogen *Proteus* sp. BVB09 in MFC**

## Analysis of media optimization

The standard media M6 was further optimized in order to evaluate the maximum electric potential of the selected electrogen in the MFC by optimizing the following different parameters.

## Effect of carbon and nitrogen supplements

The bioelectricity generation was monitored from the MFCs with *Proteus* sp. BVB09 inoculated in the selected standard media M6 amended with five different carbon and nitrogen sources, respectively. It was recorded that significant bioelectricity productions of 2266.58mW/m$^2$ (608.20mV, 0.006mA and 3.72mA/m$^2$) by using CMC on the 7$^{th}$ day (Fig.2A), 2223.31mW/m$^2$(596.60mV, 0.006mA and 3.72mA/m$^2$) using Lactose on the 6$^{th}$ day (Fig.2B), 2101.49mW/m$^2$ (563.90mV, 0.006mA and 3.72mA/m$^2$) with Glucose on the 5$^{th}$ day (Fig.2C), 2158.88mW/m$^2$ (579.30mV, 0.006mA and 3.72mA/m$^2$) by Starch on the 7$^{th}$ day (Fig.2D) and 2152.91mW/m$^2$ (577.70mV, 0.006mA and 3.72mA/m$^2$) using Sucrose on the 8$^{th}$ day (Fig.2E) of MFC operation inoculated with electrogen *Proteus* sp. BVB09 were monitored. The readings obtained recommended the use of CMC as carbon supplement for the electrogen *Proteus* sp. BVB09 in the standard medium M6.

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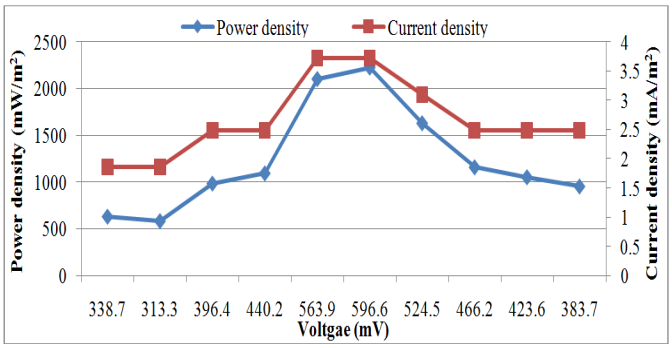


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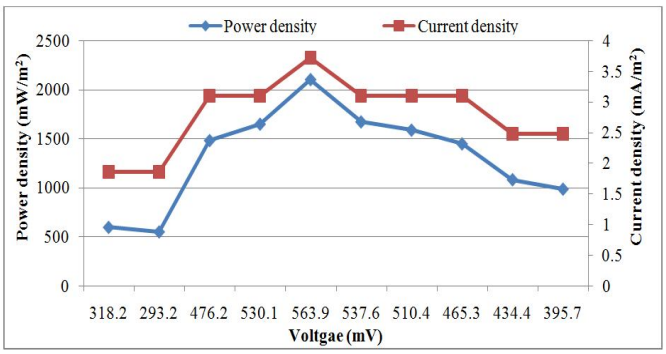


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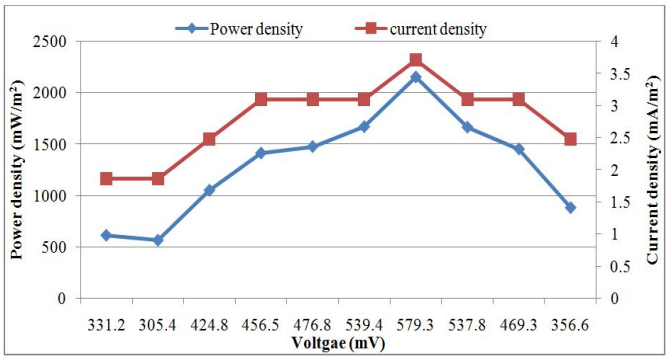


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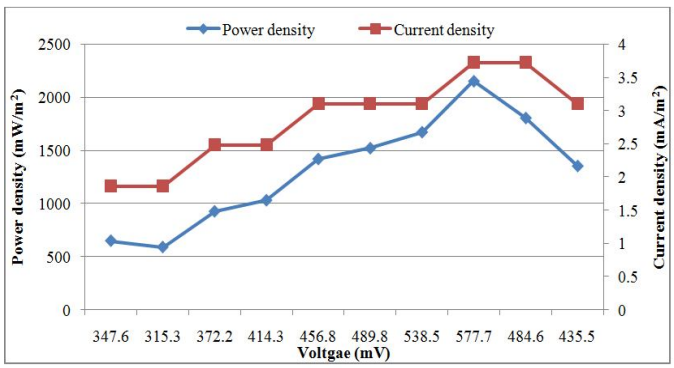
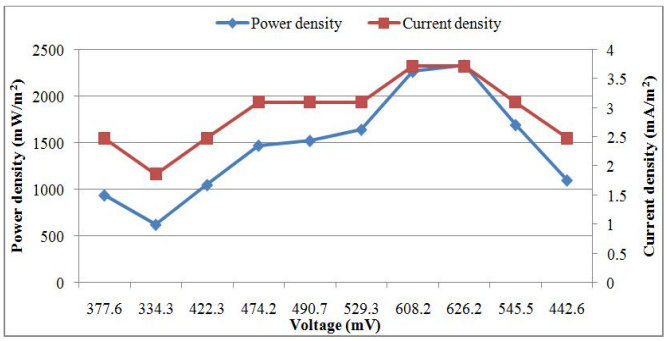



E

**Fig.2. Screening of bioelectricity generation from CMC(A), Lactose (B), Glucose(C),Starch (D) and Sucrose (E) using*Proteus* sp. BVB09 in the MFC setups**

Similarly, it was observed that among the different nitrogen sources amended in the standard media, the electrogen *Proteus* sp. BVB09 represented maximum bioelectricity production of 2266.58mW/m$^2$ (608.20mV, 0.006mA and 3.72mA/m$^2$) with Ammonium acetate on the 7$^{th}$ day (Fig.3A), 252.54mW/m$^2$ (203.30mV, 0.002mA and 1.24mA/m$^2$) with Urea on the 2$^{nd}$ day (Fig.3B), 268.57mW/m$^2$ (216.2mV, 0.002mA and 1.24mA/m$^2$) with Ammonium chloride on the 3$^{rd}$ day (Fig.3C), 268.94mW/m$^2$ (216.50mV, 0.002mA and 1.24mA/m$^2$) with Ammonium sulphate on the 4$^{th}$ day (Fig.3D) and

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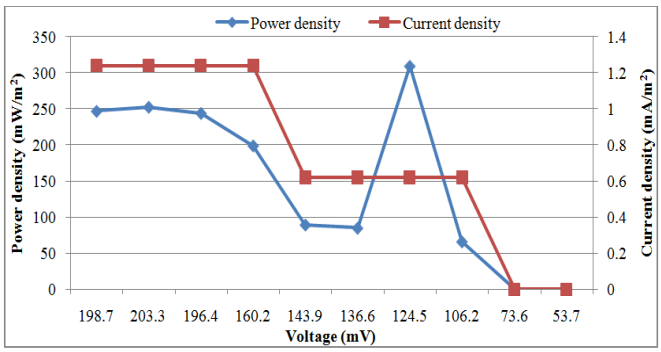


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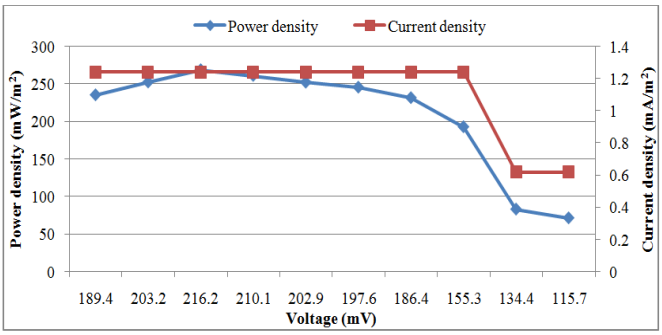


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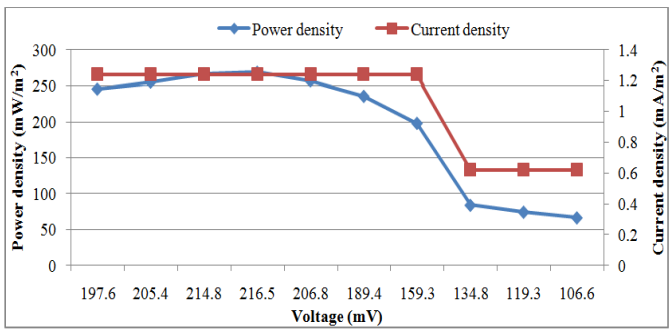


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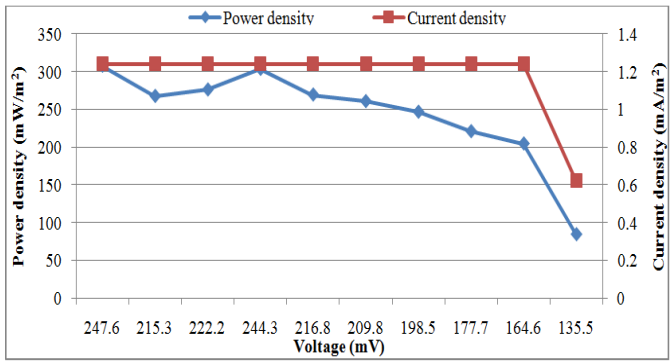


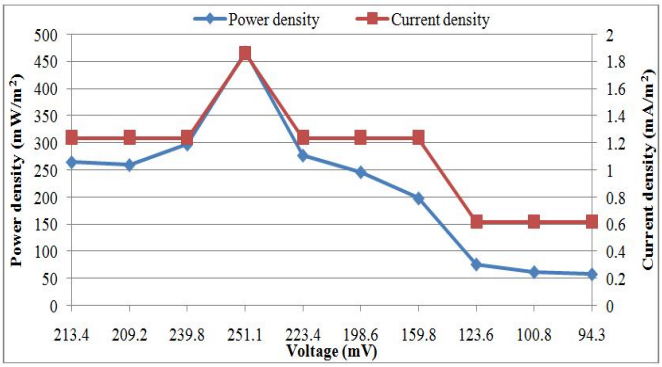

E

**Fig.3. Screening of bioelectricity generation fromAmmonium acetate (A), Urea (B), Ammonium chloride(C), Ammonium sulphate (D) and Potassium nitrate (E) using *Proteus* sp. BVB09**

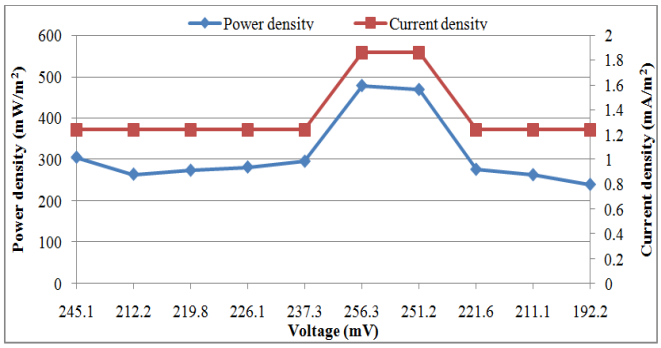
303.47mW/m$^2$(244.30 mV, 0.002mA and 1.24mA/m$^2$) with Potassium nitrate on the 4$^{th}$ day (Fig.3E) of the MFC operation from the respective MFCs. Hence, from the results obtained, it was concluded that Ammonium acetate was an ideal nitrogen source for *Proteus* sp. BVB09 in the MFC operation for bioelectricity generation.

### Optimization of catholyte

In the cathodic chamber, different concentrations of the potassium hexacyanoferrate enriched in potassium phosphate buffer were also screened. It was observed that the MFCs inoculated with *Proteus* sp. BVB09 produced highest electricity generation of 467.88mW/m$^2$ (251.10mV, 0.003mA and 1.86mA/m$^2$) on the 4$^{th}$ day against 25mM catholyte (Fig.4A), 477.57mW/m$^2$ (256.30mV, 0.003mA and 1.86mA/m$^2$) on the 6$^{th}$ day against 50mM catholyte (Fig.4B), 651.24mW/m$^2$ (349.50mV, 0.003mA and 1.86 mA/m$^2$) on the 7$^{th}$ day against 75mM catholyte (Fig.4C), 988.32mW/m$^2$ (397.80mV, 0.004mA and 2.48mA/m$^2$) on the 6$^{th}$ day against 100mM catholyte (Fig.4D) and 1436.64mW/m$^2$ (462.6mV, 0.005mA and 3.1mA/m$^2$) on the 7$^{th}$ day against 125mM catholyte (Fig.4E) from the MFC operation. It was concluded that with the increase in concentration of potassium hexacyanoferrate the electricity generation also increased. It has been documented that in mediator less microbial fuel cell losses occur in the cathode compartment due to activation over potentials which can be decreased by adding $K_3Fe(CN)_6$ to the liquid catholyte (Park *et al*., 2000).




A

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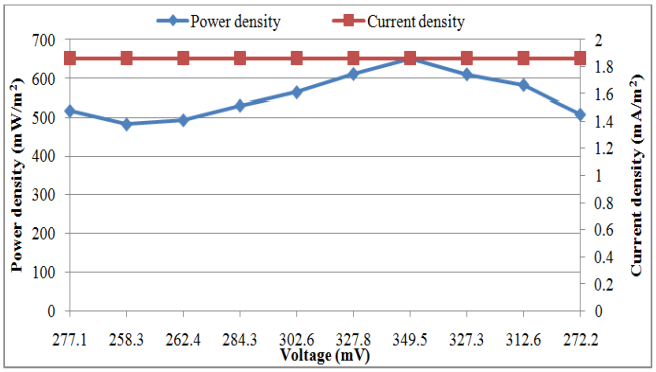


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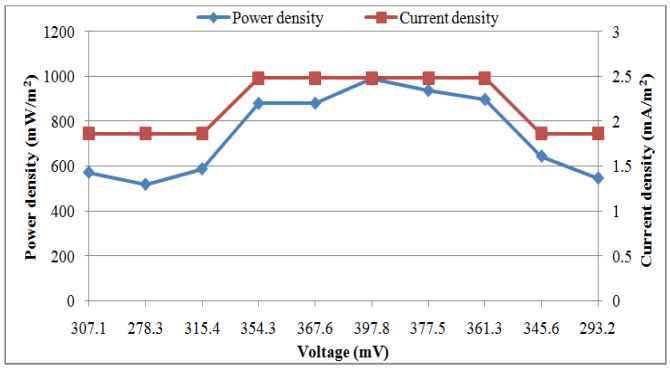
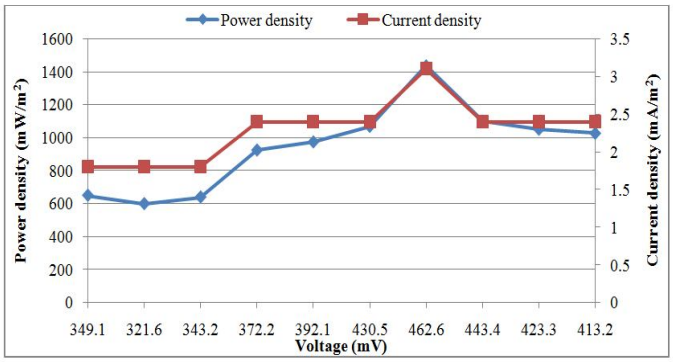



D

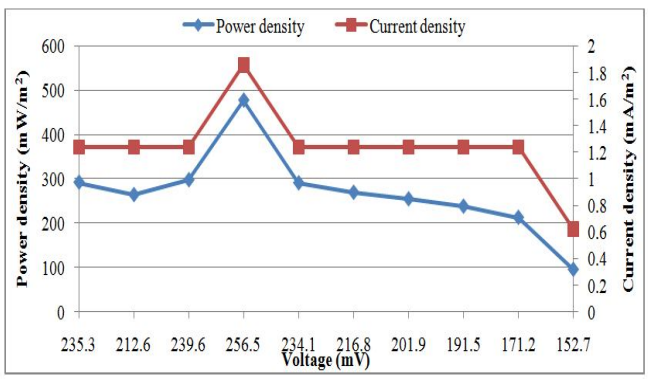



E

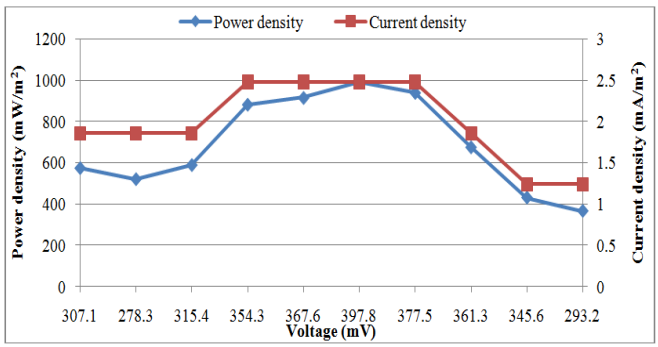
**Fig.4. Bioelectricity generation from 25mM (A), 50mM (B), 75mM (C), 100mM (D) and 125mM (E) of Potassium hexacyanoferrate as catholyte in the MFCs inoculated with *Proteus* sp. BVB09**

### Effect of distance between electrodes

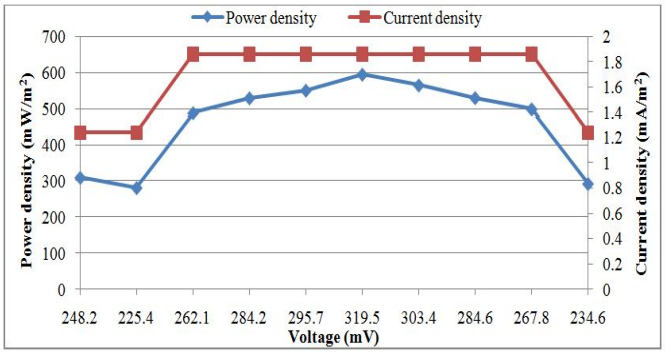
The bioelectricity generations from the isolated electrogens were also monitored with the change in distance between the electrodes placed in the chambers of the MFCs. It was recorded that the bioelectricity generation of 477.95mW/m$^2$(256.50mV, 0.003mA and 1.86mA/m$^2$) on the 4$^{th}$ day with electrodes at 1.5cm distance (Fig.5A), 988.32mW/m$^2$ (397.80mV, 0.004mA and 2.48mA/m$^2$) on the 6$^{th}$ day with electrodes at 3cm (Fig.5B), and 595.34mW/m$^2$ (319.50mV, 0.003mA and 1.86 mA/m$^2$) on the 6$^{th}$ day with electrodes at 5cm distance (Fig.5C), in the MFCs inoculated with *Proteus* sp. BVB09 in the anodic chambers were logged. These result suggested that the mass transfer between two electrodes is a limiting factor, probably proton transfer from the anode to the cathode. Since both the anode and the cathode solutions have resistance to proton transfer, therefore optimized distance between the electrodes reduced the resistance of the electrolytes that the protons have to overcome resulted in reduction of microbial fuel cell resistance and higher power output (Yuan and Kim, 2007).




A




B

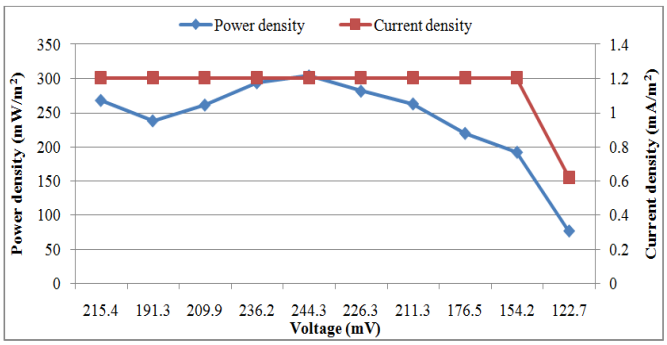



C

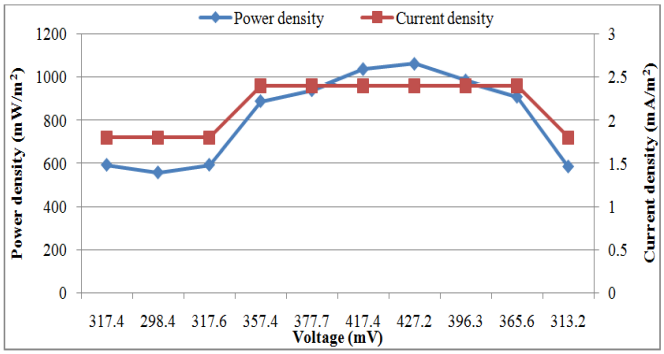
**Fig.5. Bioelectricity generation from the MFC having electrode separation 1.5cm(A), 3cm(B) and 5cm(C) inoculated with*Proteus* sp. BVB09**

## Analysis of surface area of PEM

The surface area of the membrane was also analyzed in the optimization of MFC and readings obtained revealed that *Proteus* sp. BVBO9 inoculated in the anodic chambers of MFCs having 1cm radius of circular membrane produced maximum bioelectricity generation of 303.47mW/m$^2$ (244.30mV, 0.002mA and 1.2 mA/m$^2$) on the 5$^{th}$ day (Fig.6A) and 1061.36mW/m$^2$ (427.2mV, 0.004mA and 2.4mA/m$^2$) with 2cm radius of circular membrane on the 7$^{th}$ day (Fig.6B).




A



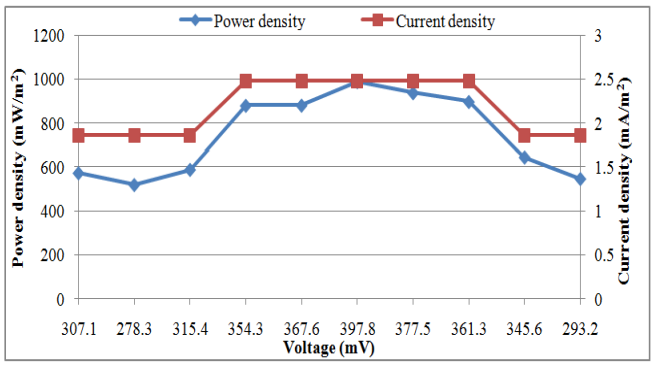

B

**Fig.6. Monitoring of bioelectricity generation from the MFC using PEM having 1cm (A) and 2cm (B) radius inoculated with *Proteus* sp. BVB09**

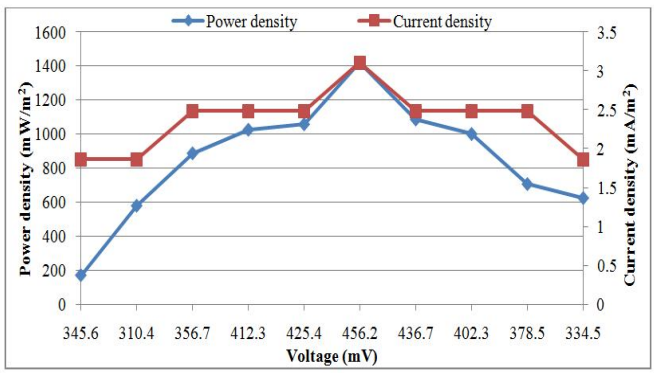
From the data, it was concluded that 2cm radius of membrane enabled greater electricity generation, suggesting that membrane with 1cm generating less electricity was probably because of the surface area of the membrane which did not provide enough proton flux through the membrane in the MFC while maximum surface area delivered maximum photons and hence resulted in maximum electricity generation.

## Effect of electrode surface area

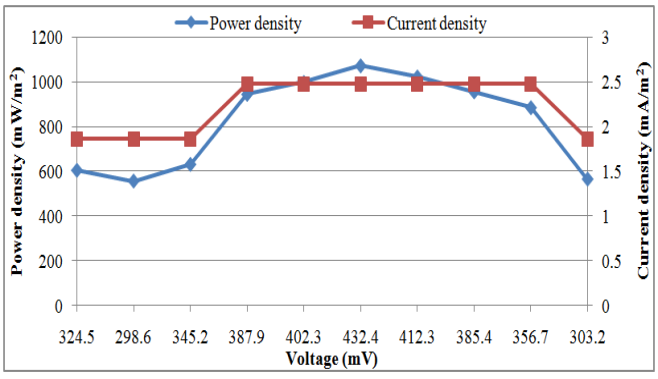
In the fabricated MFCs, graphite electrodes were varied in their apparent surface area and their effect on the bioelectricity generation was recorded from the respective MFCs inoculated with *Proteus* sp. BVB09. From the obtained data, it was concluded that *Proteus* sp. BVB09 produced maximum bioelectricity generation of 988.32m W/m$^2$ (397.80mV, 0.004mA and 2.48 mA/m$^2$) on the 6$^{th}$ day with both the electrodes surface area of 0.0014m$^2$ (Fig.7A), 1074.28mW/m$^2$ (432.40mV, 0.004mA and 2.48mA/m$^2$) on the 6$^{th}$ day with the anode surface area of 0.0028m$^2$ and cathode surface area of 0.0014m$^2$ (Fig.7B) whereas 1416.77mW/m$^2$ (456.20mV, 0.005mA and 3.1mA/m$^2$) on the 6$^{th}$ day with the anode and cathode surface area of 0.0014m$^2$ and 0.0028m$^2$ (Fig.7C), respectively.




A




B




C

**Fig. 7. Bioelectricity generation using combination of Anode (0.0014m$^2$) and cathode (0.0014m$^2$) (A); Anode (0.0028m$^2$) and cathode (0.0014m$^2$) (B) and Anode (0.0014m$^2$) and Cathode (0.0028m$^2$) (C) from MFCs inculated with *Proteus* sp. BVB09**

The results concluded that the combination of 0.0014m$^2$ and 0.0028m$^2$ surface areas of electrodes for the electrogen generated maximum bioelectricity. It was suggested that at the anode more surface area was available to accept the electrons from the solution and carry them to the external circuit. The bacteria produced (and may still be producing) more electrons than the uptake capacity of the cathode and when the surface area of cathode was increased the electrons up take was normalized that reduced the over potential at the anode while at the cathode the extra surface area provided the sufficient oxidant to reduce the incoming electrons from the electrode. This readily facilitated more flow of electrons and thus a larger power output (Cheng *et al*., 2006).

### Effect of external resistance

The external resistance on the circuit was also optimized for the system to evaluate maximum bioelectricity generation. It was noticed that with the decrease in the external resistance from higher value to lower value, increase in current density production while decrease in power density was recorded. The maximum power density of 147950.31mW/m$^2$ (158.80mV, 1.5mA and 931.67mA/m$^2$) was recorded for the *Proteus* sp. BVB09 at 100Ω resistance (Fig.8) from the respective MFC. It was also noticed that with the decrease in the external resistance from higher value to lower value, increase in current density production while decrease in power density was recorded. At 100Ω, maximum current density and power density normalized to the anode surface was recorded while further decrease in the external resistance caused decrease in both the current and power density. The obtained results deduced that 100Ω would be equal to or near to the internal resistance of the constructed MFC. It has been reported that the external electric circuit must have a resistance equal to the internal resistance of the microbial fuel cell for the cell to produce the maximum amount of power (Kim *et al*., 2008).

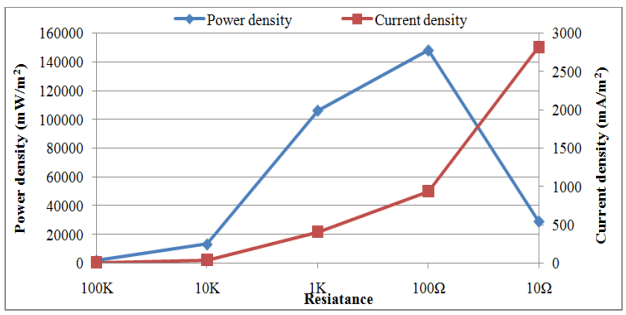



**Fig.8. Effect of different external resistance on the bioelectricity generation from MFC inoculated with *Proteus* sp. BVB09**

### Analysis of bioelectricity generation from optimized MFC

The MFC setup inoculated with the isolated electrogen *Proteus sp*. BVB09 showed significant bioelectricity generation of 244363.97mW/m$^2$ (198.70mV, 1.98mA and 1229.81mA/m$^2$) on the 26$^{th}$ day (Fig.85) of the MFC operation.

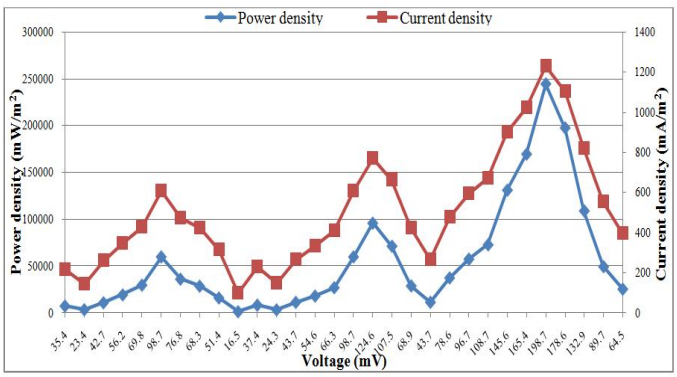



**Fig.9. Bioelectricity generation from the MFC under optimized parameters inoculated with *Proteus* sp.BVB09**

It was also recorded that the obtained maximum powers from the respective MFCs were constant for almost 1h followed by decrement in the output, suggested that the optimized parameters contributed to the reduction of the different resistances and over potentials necessary for the better efficiency of the fabricated MFC setup.

### Analysis of bacterial biomass and bio film formation

Following termination of the MFC operation after one month, the electrodes and the anolytes were analyzed for the biomass. The anodes were employed for estimation of attached protein in form of bio films whereas the anolytes were analyzed for the biomass productions using Bovine Serum Albumin (BSA) as standard curve (Fig.10). It was noted that Proteus sp. BVB09 represented 1.2mg/cm$^2$ and 4.77mg/mL of protein content and biomass, respectively from the anode and anodic chamber. The results for the bio film attachment assay of the isolated electrogen *Proteus* sp. BVB09 in the standard media (0.112 λ max) and optimized media (0.178 λ max) revealed that the bacteria showed maximum absorbance in the optimized media as compared to the standard media, suggested due to happy biofilm formation of the electrogen *Proteus sp.* BVB09 in the optimized media because of its optimized factors necessary for the electrogen bio film formation.

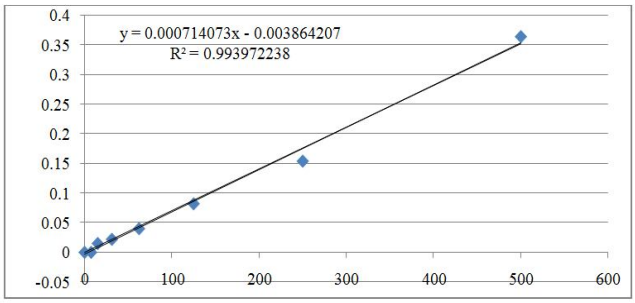



**Fig.10. Bovine Serum Albumin (BSA) as standard curve**

### Conclusion

The present research work emphases on the evaluation of bioelectricity generation from the isolated electrogen *Proteus* sp. BVB09 from the cow dung. The electrochemical analysis data obtained revealed that the isolated electrogen have the potential to generate maximum bioelectricity in the optimized MFC. Hence, the potential electrogenic bacteria *Proteus* sp. BVB09 procured from the cow dung biofilms and mineral media M6 can be used with necessary requirements for the better usage to understand the MFC technology as alternate energy to sustain the demand of future.

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