

*Research Article*

## ELECTRICITY PRODUCTION FROM WASTE WATER USING MICROBIAL FUEL CELL

Mannarreddy Prabu\*, Munireddy Durgadevi, Manavalan Tamilvendan, Puthupattu Thangavelu  
Kalaichelvan and Venkatesan Kaviyarasan

*Centre for advanced studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India.*

\*Corresponding Author e-mail: praboj@gmail.com

Received 20 July 2012; Revised 4 July 2012; Accepted 13 August 2012

### **Abstract**

Microbial fuel cells (MFCs) an electricity producing device using waste-water treatment, biosensor, eco-friendly and low cost management of energy production. In this study, investigation power generation from waste water compared with their pure culture, mixed culture and different medium ingredients with microorganism. Enhance the power production with different ingredients like monosaccharide's, nitrogen source and amino acids, these sources increasing the electron shuttle in the medium. Glucose (0.98 V), beef extract (0.85 V) and Leucine (0.92 V) exhibited maximum power production with the anodic chamber. Different electrode was used; platinum showed that maximum electron capturing in the anodic chamber. The SEM photography clearly showed that biofilm formation of microorganism on the electrode. The output power was compared with mixed culture to pure culture and different ingredients, thus bio electric power was retained maximum 1.03 V in pure culture from *Morganella morganii* and 1.2 V in mixed culture.

**Keywords:** Fuel cell, Waste-water treatment, Electricity production, *Morganella morganii*

### **INTRODUCTION**

Microbial fuel cells (MFCs) can provide an answer to several of the problems which traditional wastewater treatment faces. They enable the recovery of energy out of the wastewater, while limiting both the energy input and the excess sludge production (Rabaey *et al.*, 2005). Microorganisms that can oxidize substrates such as glucose, acetate, butyrate or wastewater to produce electricity have been reported (Chaudhari and Lovely 2003; Lovley 2006; Rabaey and Verstraete 2005). There is an increasing need in the world today for alternative forms of energy production. Microbial ecology and environmental biotechnology are inherently tied to each other. The electricity produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material containing a resistor or operated under a load (Logan 2006).

A different type of MFC has been described (Habermann and Pommer 1991), designed for the

treatment of sewage and landfill effluent wastewater. This was based on the sulphate reducing species *Desulfovibrio desulfuricans* mixed with four other species, namely *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The role of these other species was to utilize a wide range of sugars and other organic substrates and convert these into end products, including lactate, *D. desulfuricans* was capable of utilizing lactate as its carbon source and use sulphate (SO<sub>4</sub>) found in wastewater as its end terminal electron acceptor, from which it reduced to sulphide (S<sub>2</sub>-) (Habermann and Pommer 1991; Bond and Lovely 2003).

Renewable energy is an increasing need in our society. MFC technology represents a new form of renewable energy by generating electricity from what would otherwise be considered waste. This technology can use bacterium which already present in wastewater as catalysts for generating electricity and simultaneously treats wastewater (Lui *et al.*, 2004; Min and Logan 2004). Biological fuel cell is a bio-electrochemical system

converts that chemical energy to electrical energy by the catalytic reaction of microorganism (Allen and Bennetto1993).

## MATERIALS AND METHODS

### Sample collection

Water samples were collected from different places of Chennai, Tamil Nadu, India (Coovam river-Coovam, Adyar river- Saidapet, Adyar river- Adyar, sea water-Marina beach).

### Screening of high electricity production from different waste water samples

Four different waste water sample 100 ml were taken into an anode chamber. Using salt bridge anode and cathode chamber was connected. Potassium Ferricyanide was used in the cathode chamber. Graphite electrode inserted in both chamber and the voltage was measured using multimeter (DT830D) and the output power was taken on a daily basis.

### Isolation of culture for electricity production

Coovam sample (10 mL) was transferred to 100 mL of nutrient broth and kept at 37°C for 120 rpm for 24 h. After incubation each sample was serially diluted and inoculated onto the Nutrient agar medium in order to isolate viable bacterial strain. Bacterial strains were purified and maintained on Nutrient agar at 37°C. All the Isolated organisms were separately inoculated into the nutrient broth medium.

### MFC Design

Many different configurations are possible for MFCs. A widely used and inexpensive design is a two-compartment MFC built in a traditional “H” shape, consisting usually of two bottle connected by a tube which is usually a cation exchange membrane (CEM) such as nafion (Bond *et al.*, 2002; Park *et al.*, 1999; Logan *et al.*, 2005; Min *et al.*, 2005) or Ultrex, or plain salt bridge (Min *et al.*, 2005).

A novel single compartment of MFC was designed by combining the salt bridge into the cathode chamber itself and the graphite sheet were encircled around the combined salt bridge and cathode system (Figure 1). This combined novel MFC device directly inserted into the testing sample to measure the electricity. This novel MFC system shows a highly reproducible result like a normal one.

## Materials used for MFC construction

### Anode and cathode

Anode and cathode materials are must be nonconductive, biocompatible, and chemically stable in the reactor solution. We used Scot Duran 100 ml glass bottle and nonconductive plastic 60 ml bottle, Metal anodes consisting of non-corrosive stainless mesh can be utilized (Tanisho *et al.*, 1989) however copper is not useful in due to the toxicity of even trace ions to bacteria. The most versatile electrode material is carbon, available as compact graphite plates, rods or granules, fibrous material, and as glassy carbon (Bruce *et al.*, 2006). We used four different anode electrode to measure the electricity namely Platinum, Graphite sheet, Graphite rod, and carbon rod. The different electrode surface area was measured using following formula.

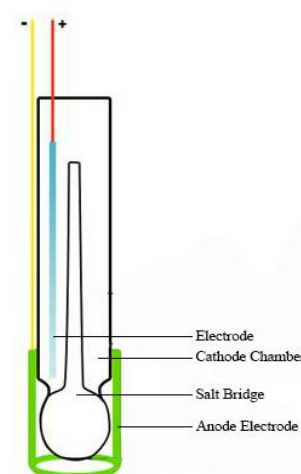


Figure 1: Novel MFC System

Total surface area of platinum electrode and graphite sheet =  $2(L \times B + L \times T + B \times T)$ . The entire formula for the surface area of a carbon rod and graphite rod is  $2r^2 + 2rh$ , where L-length, B-breadth, T-thickness, R-radius and H-height.

Electrical outer connect to connect the electrical cable. 0.5 mM of ferricyanide and Potassium permanganate was used in the cathode chamber. Potassium Ferricyanide  $K_3[Fe(CN)_6]$  is very popular as an experimental electron acceptor in microbial fuel cell (Gil *et al.*, 2003).

### Electrode Preparation

The new electrodes were activated by soaking in 100% ethanol for 30 minutes and in 1M HCl for 1 hour. After each use the electrodes were washed in 1.0 M HCl followed by 1.0 M NaOH, each for 1 hour to remove

possible metal and inorganic contamination, and stored in distilled water until use.

### **Salt Bridge and CEM Preparation**

The majority of MFC designs require the separator of the anode and the cathode compartment by a CEM. Exceptions are naturally separated system such as sediment MFCs (Reimers *et al.*, 2001) or specially designed single-compartment MFC (Liu *et al.*, 2004; Cheng *et al.*, 2006). The most commonly used CEM is Nafion (Dupont Co., USA), which is available from numerous suppliers (e.g., Aldrich and Ion Power, Inc.) (Bruce *et al.*, 2006). The salt bridge was used instead of CEM, the salt bridge was prepared using 2% agar and saturated KCl (37g in 100 ml) solution (Mohan *et al.*, 2007). We used nafion and salt bridge (KCl and NaCl) as a cation exchanger.

### **Enhancement of electricity production using monosaccharide**

Different monosaccharide such as glucose, galactose, mannose, fructose, fucose, rhamnose, xylose, ribose, arabinose, galactouranic acid, glucouranic acid, and gluconic acid at 5 mM concentration and minimal medium containing Sodium acetate (2 g L<sup>-1</sup>), NH<sub>4</sub>Cl (0.31 g L<sup>-1</sup>), NaH<sub>2</sub>PO<sub>4</sub> (5.8 g L<sup>-1</sup>), Na<sub>2</sub>HPO<sub>4</sub> (15.47 g L<sup>-1</sup>), KCl (0.13 g L<sup>-1</sup>), mineral solution (12.5 ml) and vitamin solution (12.5 ml) were added to sterile Coovam water. Pure culture *Morganella morganii*-PTK2 was inoculated to the above medium.

### **Enhancement of electricity production using different nitrogen source**

Different nitrogen source such as malt extract, beef extract, yeast extract, peptone, sodium nitrate, potassium nitrate, casein, urea, ammonium molybdate, bacto tryptone and soya bean meal at 0.5% concentration and minimal medium containing Sodium acetate (2 g L<sup>-1</sup>), NH<sub>4</sub>Cl (0.31 g L<sup>-1</sup>), NaH<sub>2</sub>PO<sub>4</sub> (5.8 g L<sup>-1</sup>), Na<sub>2</sub>HPO<sub>4</sub> (15.47 g L<sup>-1</sup>), KCl (0.13 g L<sup>-1</sup>), mineral solution (12.5 ml) and vitamin solution (12.5 ml) were added to sterile Coovam water. Pure culture *Morganella morganii*-PTK2 was inoculated to the above medium.

### **Effect of different amino acid for electricity production**

Different amino acid such as cysteine, histidine, proline, tryptophane, alanine, leucine, methionine, glutamic acid, threonine and phenyl alanine at 0.1%

concentration and minimal medium containing Sodium acetate (2 g L<sup>-1</sup>), NH<sub>4</sub>Cl (0.31 g L<sup>-1</sup>), NaH<sub>2</sub>PO<sub>4</sub> (5.8 g L<sup>-1</sup>), Na<sub>2</sub>HPO<sub>4</sub> (15.47 g L<sup>-1</sup>), KCl (0.13 g L<sup>-1</sup>), mineral solution (12.5 ml) and vitamin solution (12.5 ml) were added to sterile Coovam water. Pure culture *Morganella morganii*-PTK2 was inoculated to the above medium.

### **Calculation and measurement**

Cell voltage of the system was monitored using a precision multimeter (DT-830D) and a data acquisition system. All data were manually recorded day basis. Power (P) was calculated according to  $P = IV$  ( $I = V/R$ ), where (I) is the current (A), V the voltage (V), and R is the resistance ( $\Omega$ ). Current density was calculated using the formula  $I/A$ , where I is the maximum current generated.

### **Scanning Electron Microscopy**

For scanning electron microscope (SEM) analysis, parts of the electrode were removed from the anode chambers, rinsed with a sterile medium, and immediately fixed using an anaerobic solution of 3% glutaraldehyde for 3hrs. Sample were then subjected to a serial dehydration protocol using increasing concentrations of ethanol (10%, 40%, 70%, 100 %; 30 minutes for each stage) and dried completely at room temperature. The desiccated samples were then visualized using scanning electron microscope (HITACHI, 5X TO 300000X) with gold sputter coating.

## **RESULTS**

### **Isolation of optimum electricity producing bacterial strains**

In the present attempt, four different waste water sample (Coovam river- Coovam, Adyar river- Saidapet, Adyar river- Adyar, sea water- Marina beach) tested for electricity production. Among the four samples Coovam waste water (Figure 2) shows maximum electricity than the rest of the waste water samples.

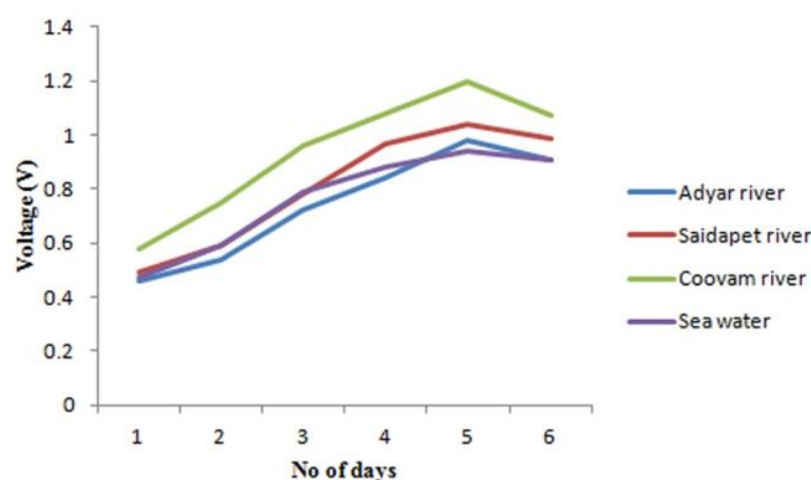


Figure 2: Maximum voltage production with different waste water samples

Four different bacterial strains *Bacillus cereus*-PTK1, *Morganella morganii*-PTK2, PTK3, and PTK5 were isolated from Coovam river water and subjected to electricity production (Figure 3). Among the four strains, *Morganella morganii*-PTK2 (1.21 V) shows maximum production of electricity.

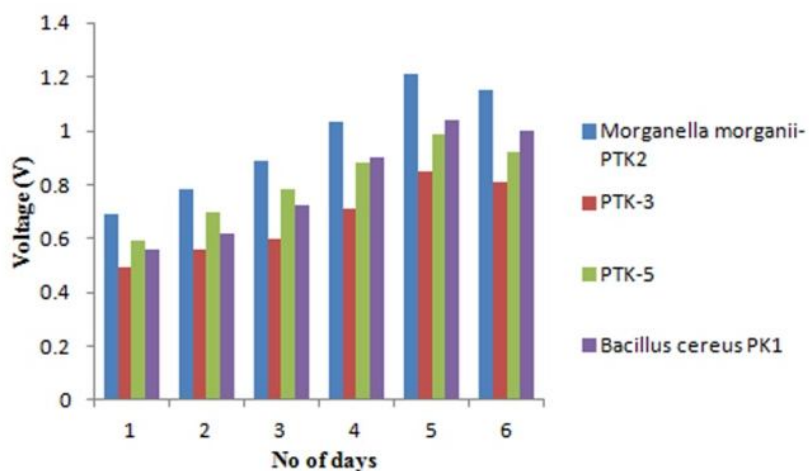


Figure 3: Voltage maxima comparison with four different bacterial strains

**Different types of anode electrodes**

Four different electrodes used to measure the electricity were platinum, graphite sheet, graphite rod, and carbon rod. Among the four different anode electrode, platinum electrode shows maximum electricity conduction followed by graphite sheet and graphite rod. Carbon rod shows poor electricity conduction (Figure 4).

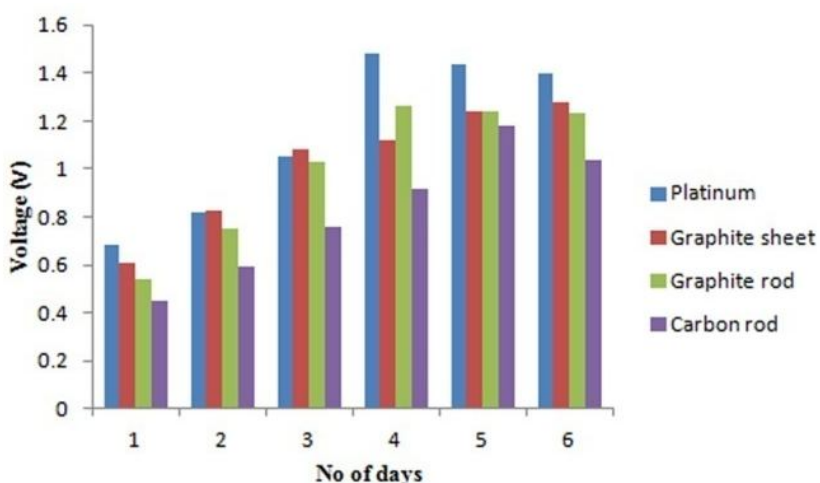


Figure 4: Voltage maxima comparison with different electrode

**Different types of cation exchanger**

Three different ion exchanger used to exchange proton form anode chamber to the cathode chamber were nafion, potassium chloride salt bridge, and sodium chloride salt bridge. Among the three different cation exchanger, nafion shows as a maximum ion exchanger followed by potassium chloride salt bridge, and sodium chloride salt bridge (Figure 5).

**Mixed culture**

The four different pure cultures were mixed at 1:1:1:1 ratio, the mixed culture was inoculated in anode compartment. The apparatus used in mixed culture is similar as pure culture; the values are noted in multimeter. The initial value 0.80 V and final value 1.20 V was obtained in mixed culture, which was 1.07 fold higher than pure culture (Figure 6).

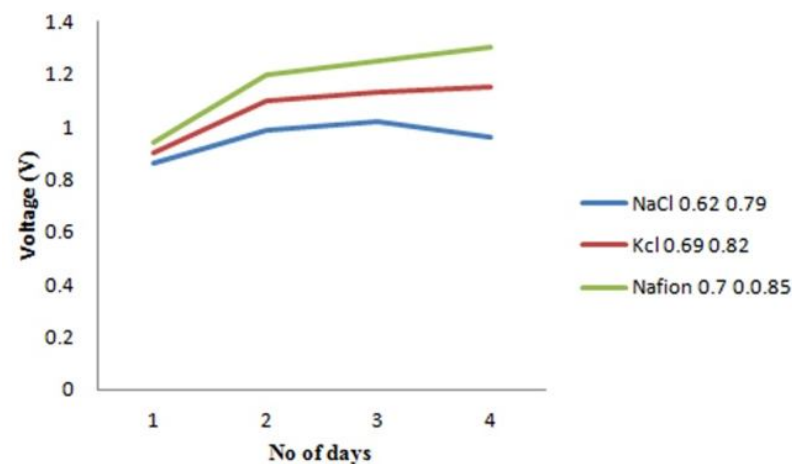


Figure 5: Maximum voltage compared different cation exchanger

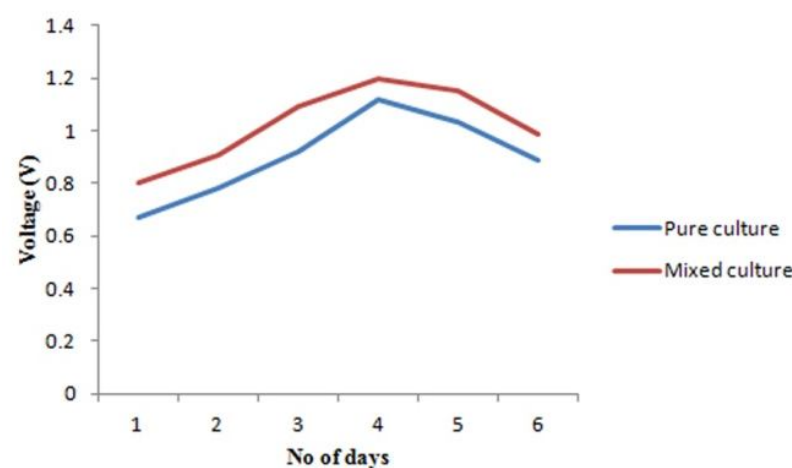


Figure 6: Voltage maxima comparison with pure and mixed cultures

**Effect of monosaccharide's on electricity production**

Twelve different monosaccharides were taken to enhance the electricity production. Among twelve, glucose shows maximum electricity production followed

by galactose. Moderate electricity production was found in ribose. Fairly lower electricity production with fructose and fucose (Figure 7).

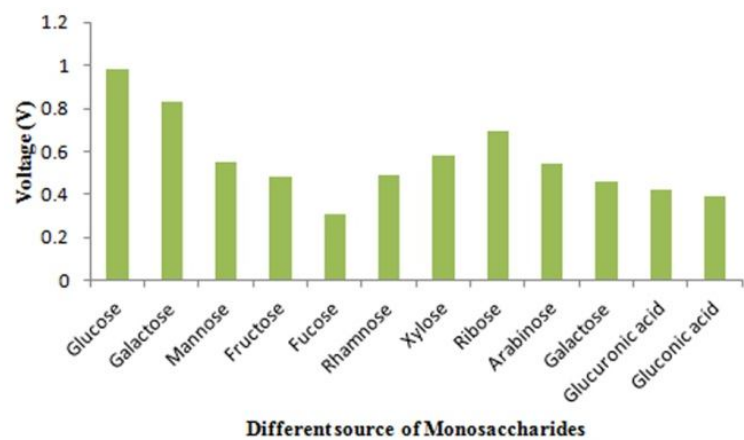


Figure 7: Voltage maxima comparison with different monosaccharides

### Effect of different nitrogen sources on electricity production

Ten different nitrogen sources were taken to enhance the electricity production. Among the 10 nitrogen sources, beef extract shows maximum electricity production followed by malt extract and yeast extract. Peptone and ammonium molybdate showed moderate electricity production. Fairly lower electricity production was found in bacto tryptone and soya bean meal (Figure 8).

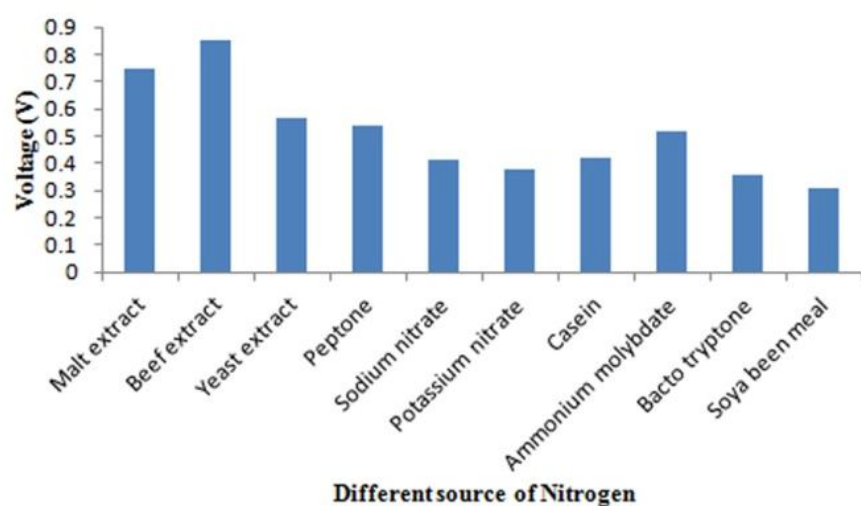


Figure 8: Voltage maxima comparison with different nitrogen sources

### Effect of different amino acid sources on electricity production

Ten different amino acids were taken to enhance the electricity production. Among the 10 amino acids, leucine shows maximum electricity production followed by

glutamic acid and threonine. Moderate electricity production was obtained with cysteine and phenyl alanine. Fairly lower electricity production in alanine and tryptophan (Figure 9).

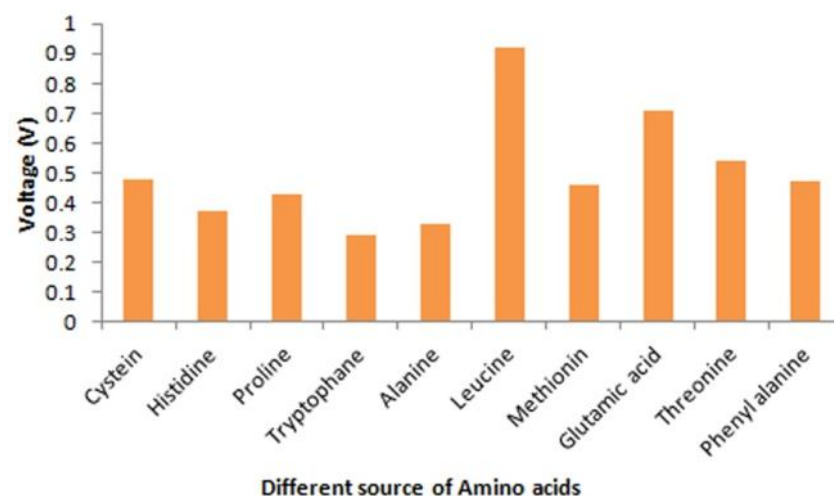


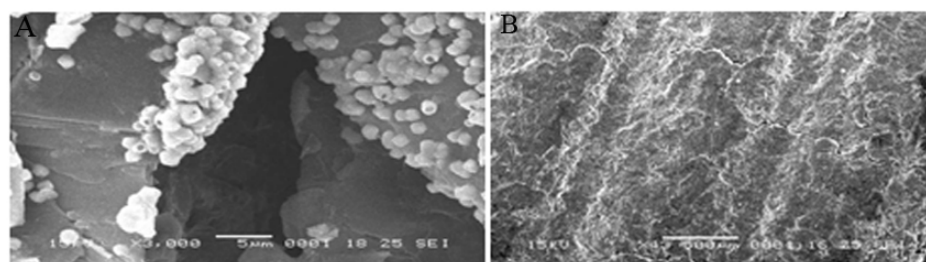
Figure 9: Voltage maxima comparison with different amino acid

### Comparison of electricity measurement using novel and conventional MFC device

Novel MFC system shows relatively equal electricity measurement with conventional one

### Scanning electron microscopy

The Scanning electron microscope (SEM) photos clearly show the deposition of bio-film on the electrode during the optimum electricity production period (Figure 10).



A. Anode electrode showing formation of bio-film and B. cathode electrode.

Figure 10: SEM analysis of MFC electrodes

### DISCUSSION

MFC's has a great potential for microbial ecology and environmental biotechnology. A fuel cell is an electrochemical device that continuously converts chemical energy to electrical energy for as loading as fuel and oxidants is supplied (Prasad 2006). Despite of four (two river, one sea and one waste water samples) waste water sample shows higher electricity production.

Similarly, Lee *et al.*, 2003 has used artificial waste water fed with acetate for increasing the current generation gradually.

Microbiologically catalyzed electron liberation at the anode and subsequent electron consumption at the cathode when both processes are sustainable are the defining characteristics of an MFC (Logan *et al.*, 2006). The construction of MFC requires a separating membrane usually called as proton exchange membrane (PEM) depending on the proton possessed between the anode to cathode compartment. In this study Nafion membrane, KCl, and NaCl salt bridge were used as separator where Nafion and KCl showed more or less equal chemical energy conversion (Min *et al.*, 2005). It has most commonly used PEM as Nafion in their research findings.

In the present study, when MFCs operated using mixed culture currently achieved substantially greater power densities than those with pure cultures isolated from the wastewater samples. Similarly, the investigation made by (Rabaey *et al.*, 2004; Rabaey *et al.*, 2005) has reported that mixed culture has dominated pure culture in electricity generation.

The increased electricity generation in the present study lies in the order Carbon > Graphite rod > Graphite sheet > Platinum. Power generation in this system is always found constructed by its high internal resistance (up to 1000  $\Omega$ ) (Min *et al.*, 2005). Moreover, the presence of an over potential between both electrodes may further reduce cell voltage to a lower load. The anode and cathode materials must be conductive, biocompatible and chemically stable in the reactor solution. The most versatile electrode material is carbon available as compact graphite plates, rods or granules, as fibrous material, out of different anodic materials used (Tanisho *et al.*, 1989).

MFC's can use bacteria from the natural environment to generate electricity from various substrates such as glucose, acetate, butyrate, lactate, ethanol, cysteine and bovine serum albumin as well as those from waste streams such as domestic waste waters and various food-industry waste waters (Rabaey *et al.*, 2005; Rezaei *et al.*, 2007; Liu and Logan 2004; Liu *et al.*, 2005; Logan *et al.*, 2005; Zuo *et al.*, 2006; Cheng *et al.*, 2006; Lovely and Phillips, 1988). An attempt was made to enhance electricity generation by incorporating different sugars, nitrogen sources and amino acids in the waste water sample. The monosaccharide amended in the

sample has slight increase but not up to the mark. Unfortunately better electricity production could not be obtained in case of nitrogen source and amino acid amended waste-water sample.

## REFERENCES

- Allen RM and Bennetto HP. 1993. Microbial fuel cells. Electricity production from carbohydrates. *Applied Biochemistry and Biotechnology* 39:27–40.
- Bond DR and Lovley DR. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Applied and Environmental Microbiology* 69:1548–55.
- Bond DR, Holmes DE, Tender LM and Lovley DR. 2002. Electrode reducing microorganism that harvest energy from marine sediments. *Science* 295:483–485.
- Bruce EL, Bert H, Rene R, Uwe S, Jurj K, Stefano F, Peter A, Willy V and Korneel R. 2006. American chemical society. Microbial fuel cells: Methodology and Technology. *Environmental science and Technology* 40:5181–5192
- Chaudhari SK and Lovley DR. 2003. Electricity generation by direct oxidation of glucose in microbial fuel cells. *Nature Biotechnology* 111–114.
- Cheng S, Liu H and Logan BE. 2006. Increased performance of single chamber microbial fuel cells using an improved cathode structure. *Electrochemistry Communication* 8:48–494.
- Gil GC, Chang IS, Kim BH, Kim M, Jang JK, Park HS and Kim HJ. 2003. Operational parameters affecting the performance of a mediator-less microbial fuel cell. *Biosensors and Bioelectronics* 18:327–334.
- Habermann W and Pommer EH. 1991. Biological fuel cells with sulphide storage capacity. *Journal of Applied Microbiology and Biotechnology* 35:128–33.
- Lee J, Phung TN, Chang IS, Kim BH and Sung HC. 2003. Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. *FEMS Microbiology Letters* 223:185–191
- Liu H and Logan BE. 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of the proton exchange membrane. *Environmental Science and Technology* 38:4040–4046.
- Liu H, Cheng S and Logan BE. 2005. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. *Environmental Science and Technology* 39:5488–5493.
- Liu H, Ramnarayanan R and Logan BE. 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environmental Science and Technology* 38:2281–2285

- Logan BE, Murano C, Scott K, Gray ND and Head IM. 2005. Electricity generation from cysteine in a microbial fuel cell. *Water Research* 39:942–952.
- Logan BE. 2006. Invited editorial: Energy diversity brings stability. *Environmental Science and Technology* 40(17):5161
- Lovley DR and Phillips EJP. 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron and manganese. *Applied and Environmental Microbiology* 54:1472–1480.
- Min B and Logan BE. 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environmental Science and Technology* 38:5809–5814.
- Min B, Cheng S and Logan BE. 2005. Electricity generation using membrane and salt bridge microbial fuel cells. *Water Research* 39:1675–1686.
- Mohan VS, Veer Raghavulu S, Srikanth S and Sarma PN. 2007. Bioelectricity production by mediatorless microbial fuel cell under acidophilic condition using wastewater as substrate: Influence of substrate loading rate. *Current Science* 92:12–25.
- Park DH, Laivenieks M, Guettler MV, Jain MK and Zeikus JG. 1999. Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production. *Applied and Environmental Microbiology* 65:2912–2917.
- Prasad D, Sivaram TK, Berchmans S and Yegnaraman V. 2006. Microbial fuel cell constructed with a micro-organism isolated from sugar industry effluent. *Journal of Power Sources* 160:991–996
- Rabaey K and Verstraete W. 2005. Microbial fuel cells: Novel biotechnology for energy generation. *Trends in Biotechnology* 23:291–298.
- Rabaey K, Boon N, Siciliano SD, Verhaege M and Verstraete W. 2004. Biofuel cells select for microbial consortia that self mediate electron transfer. *Applied and Environmental Microbiology* 7:5373–5382.
- Rabaey K, Clauwaert P, Aelterman P and Verstraete W. 2005. Tubular microbial fuel cell for efficient electricity generation. *Environmental Science and Technology* 39:8077–8082.
- Rezaei F, Richard TL, Brennan RA and Logan BE. 2007. Substrate enhanced microbial fuel cells for improved remote power generation from sediment-based systems. *Environmental Science and Technology* 41 (11):4053–4058.
- Tanisho S, Kamiya N and Wakao N. 1989. Microbial fuel cell using *Enterobacter aerogenes*. *Bioelectrochemistry and Bioenergetics* 21:25–32.
- Zuo Y, Maness PC and Logan BE. 2006. Electricity production from steam-exploded corn stover biomass. *Energy & Fuels* 20:1716–1721.