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Microbial Fuel Cell: Harnessing Bioenergy from Yamuna Water

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Abstract: ***Microbial Fuel Cells (MFCs) hold a great potential in generation of electricity and has proved as an alternative source of renewable energy. In the present research work, a mediator-less MFC with an anaerobic anode & aerobic cathode chamber linked with a salt bridge for the exchange of generated protons is constructed. Yamuna water from four different locations in Delhi viz., Palla, ITO, Nizamuddin Bridge and Okhla Barrage were collected to study the generation of electricity and pure culture of Escherichia coli & Bacillus subtilis were taken as controls. Glucose is used as substrate, which is considered as an instantaneous source of energy for biological systems. The pure cultures of E. coli and B. subtilis generated electric current varying from 326 - 522 mV and 260 - 580 mV respectively at different cellular concentrations. An increasing trend is observed when the cell counts are doubled. A variable increase in generation of electricity is obtained ie., 80 - 360 mV (Palla), 400 - 620 mV (ITO), 280 - 360 mV (Nizamuddin Bridge) and 320-480 mV (Okhla Barrage) respectively. This is higher as compared to the pure cultures used. The Yamuna water samples are deemed to contain a consortium of bacteria namely E. coli, Bacillus subtilis, Lactobacillus plantarum, L. casei, Streptococcus lactis, Rhodopseudomonas palustris, Rhodobacter sphaeroides, S. cerevisiae, Candida utilis, Streptomyces albus, S. griseus, Aspergillus and Mucor hiemalis. Thus implying that, in consortium the other microbes enhance the performance of the MFC. Secondly, chemical compounds (Fe^{+++}, NO_2^-, SO_4^-) present in the Yamuna water may act as mediators of the electrons transporter to the anode and hence increasing the potential difference.***

Keywords: Microbial Fuel Cells (MFCs), Yamuna River, Microbial consortium, Electrical energy, Mediators

1. Introduction

The fast depletion of the fossil fuels and an ever increasing demand of energy, renewable sources of energy appear to be an attractive option. The Microbial Fuel Cells (MFCs) provides an attractive option for the production of clean energy by the use of river water or other sources of water with high organic content (Du *et al*, 2007). MFC is a bio-electrochemical system, which converts the chemical energy stored in the biodegradable substrate to electrical energy via microbial catalyzed redox reaction (Lovely, 2006; Davis and Higson, 2007). In the MFC, bacteria are separated from the terminal electron acceptor at the cathode in a manner so as to ensure that the only means for respiration is to transfer electrons to the anode (Du *et al*, 2007). The electrons flow to the cathode due to the electrochemical potential which develops between the respiratory enzyme and the electron acceptor at the cathode. The transfer of electrons must be matched by an equal number of protons moving between the electrodes in order to preserve an electroneutrality (Logan and Regan, 2006). Generation of electric current is made possible by keeping microbes separated from oxygen or any other end terminal acceptor other than the anode and this requires an anaerobic anodic chamber (Park and Zheikus, 2000).

The earliest MFC concept was demonstrated by Potter in 1910 (Leropoulos, 2005). Electrical energy was produced from living cultures of *Escherichia coli* and *Saccharomyces* by using platinum electrodes (Potter, 1911). This didn't generate much interest until 1980s when it was discovered that current density and the power output could be greatly enhanced by the addition of electron mediators. Recent advances have shown rapid developments in MFC research, leading to higher levels of electricity generation. Changes have been made in the MFC designing, materials used as electrodes, methods of exchange of protons and the mediators used for the exchange (Du *et al*, 2007). The MFC has also been used successfully as a method of removal of organic matter from wastewater and complete elimination of microorganism, along with improved power output (Jadhav *et al*, 2014).

The substrate plays a significant role in the amount of electrons being generated in a fuel cell. Some substrates are capable of producing a higher columbic yield as compared to some of the more common sugars used. Glucose is the most commonly used substrate for bacterial culture. As glucose ($C_6H_{12}O_6$) is the most simple sugar which is an important carbohydrate and an instantaneous source of fuel (energy) in biological systems, ranging from bacteria to humans. Use of glucose may be either in aerobic respiration, anaerobic respiration, or fermentation.

Coliforms are bacteria that are naturally present in the environment and used as an indicator of the presence of potentially harmful bacteria. The total coliform bacteria present in the water mainly consists of microbes belonging to the family *Enterobacteriaceae*, containing genera *Escherichia* (*E. coli*), *Klebsiella* (*K. pneumonia*), *Enterobacter* (*E. amnigenus*) and *Citrobacter* (*C. Freundi*) (Dufour *et al*, 2007; Allen and Edberg, 1995). These bacteria in consortium or individually may be involved in the generation of electricity under appropriate conditions.

A range of microbes have been reported to have the capability of generating electricity in self-sustaining microbial fuel cell, using a wide variety of organic compounds as substrates (Vega and Fernandiz, 1987). *E. coli* has been shown to generate substantial electric current in

MFC, with the magnitude depending on the substrate utilized. Generation of electricity was found to be higher when glucose was taken as the substrate (Bond and Lovely, 2003). Other microbes like *Bacillus subtillis* and *Saccharomyces cerevisiae* have also been shown to be useful for the generation of electricity (Sharma and Bulchandnani, 2012).

The Yamuna River harbors a host of microbes, some of which are present at levels high enough to deleteriously affect the quality of water. Some of the microbes present in the river include *Lactobacillus plantarum, L. casei, Streptococcus lactis, Rhodopseudomonas palustris, Rhodobacter sphaeroides, S. cerevisiae, Candida utilis, Streptomyces albus, S. griseus, Aspergillus* and *Mucor hiemalis* (Shrivastava *et al*, 2012).

The amount of dissolved oxygen in water bodies is dependent on the temperature, the quantity of sediment in the stream, the amount of oxygen taken out of the system by respiring and decaying organisms, and the amount of oxygen put back into the system by photosynthesizing plants, stream flow, and aeration. Dissolved oxygen is measured in milligrams per liter (mg/l) or parts per million (ppm). Biological oxygen demand (BOD) is a measure of the amount of oxygen that bacteria will consume while decomposing organic matter under aerobic conditions. Biochemical oxygen demand is determined by incubating a sealed sample of water for five days and measuring the loss of oxygen from the beginning to the end of the test. Chemical oxygen demand (COD) does not differentiate between the biologically available and inert organic matter, and it is a measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and the water. COD values are generally greater than BOD values, but COD measurements can be made in a few hours while BOD measurements take five days (Lenore *et al* 2005). Thus, the values of DO, BOD and COD are indicators of the levels of microorganisms present in a water body.

The current study is an attempt to estimate the electricity generated by specific microbes present in the prefixed locations of Yamuna river water and in pure cultures observed under laboratory conditions. A comparative research study is done with respect to electric current generated by the microbes in their natural profile in the river. Water samples were collected from four different points on the Yamuna namely; Palla, ITO, Nizamuddin Bridge and Okhla Barrage. The microbes are present in a consortium and are expected to have varying ratios depending on the collection point. Through our study we are ascertaining the viability of using Yamuna water for the generation of electricity, and studying the effect of microbial consortium on the magnitude of electricity.

2. Materials and Methods

2.1 Bacterial culture conditions

E. coli culture was used as one of the controls for this study. They were grown aerobically at a temperature of 37°C except when stated otherwise. The bacteria were first grown on Luria Bertoni (LB) agar plates (tryptone 10g/L, yeast extract 5g/L, NaCl 10g/L and agar 2g/L, adjusted to a pH of 7.2) for 18 hours. Single colonies were then picked and inoculated in broth followed by culturing them in a shaking incubator at a speed of 220rpm and 37°C for 12-14hrs. This was then used to inoculate 1L of LB broth in order to obtain the starter culture. *B. subtilis* was also used as a control in this study. They were grown aerobically at a temperature of 30°C. The bacteria were first grown on Beef agar plates (Beef extract 3 g, Peptone 3 g, NaCl 5 g, Agar 18 g, adjusted to a pH of 7.0) for 18 hours. Single colonies were then picked and inoculated in media and cultured in a shaking incubator at a speed of 220rpm and 30°C for 12-14hrs. This was then used to inoculate 1L of media to obtain the starter culture.

2.2 Sampling of water from Yamuna River

Water samples were collected in sterilized bottles from four locations on the Yamuna river, Delhi; namely Palla, ITO, Nizamuddin Bridge, and the Okhla Barrage. While collection, it is important to ensure that the sample is collected from the appropriate collection site, away from the banks, to rule out water contamination due to human settlements on the banks. These regions receive a huge load of domestic and agricultural waste resulting in large scale deterioration of the water quality and affect the physico-chemical parameters of the water.

2.3 Physico-chemical analysis of Yamuna water

The water was processed immediately post collection, to ensure that the water profile is not affected by storage. The various parameters like pH, turbidity, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were estimated using pH meters, titrimetric methods (APHA, 2005). Total coliforms present in the water were also estimated by dilution method to estimate the most probable number (MPN) of bacteria in 100ml of culture. The content of iron, nitrates and sulfates were calculated using the Phenanthroline method, the Brucine method and the Turbidometric method (APHA, 20th edition, 1998).

2.4 Microbial fuel cell construction and operation

The microbial fuel cell was constructed using cost effective materials as follows: the cathode and anode chambers were constructed using plastic boxes which were sealed with sealing clay after the addition of the required cultures. The anaerobic conditions in the anode chamber were maintained by purging with nitrogen. A salt bridge was created using 10% agar and 5% NaCl, which were boiled together and filled in a PVC pipe and sealed with polythene sheets. The cathodes and anodes were prepared using materials like carbon cloth which was surrounded in an aluminum mesh. Substrate of glucose (3g/L) was added in the case of *E. coli* controls. The electricity generated was measured using a multimeter (Sanwa CD770) at a regular interval of 30 minutes.

3. Results

3.1 Physico-chemical analysis of samples

Water samples collected from afore mentioned locations were analyzed for various physico- chemical parameters (Table 1). The pH was found to range between 7 and 8, while temperature was found to vary between 26-27°C at the different locations of sample collection. Both the pH and temperature range detected is expected to be permissive for microbial growth. It was found that DO could be detected at

Palla only. The other three locations showed absence of any detectable DO Both COD and BOD was found to be the lowest at Palla, followed by Okhla Barrage, and high at both ITO and Nizamuddin Bridge (Fig. 1). Bacteriological water quality status in terms of total coliform count was studied. The coliform analysis of the four samples indicated that the most probable number (MPN) of coliforms is the highest at ITO, followed by Okhla Barrage and Nizamuddin Bridge, and was the lowest at Palla (Fig. 2).

Table 1: Characterization of physical and chemical parameters of the water samples collected from four different locations on the Yamuna river

Parameters	*Units*	*Results*			
		Palla	*ITO*	*Nizamuddin Bridge*	*Okhla Barrage*
Total coliform	MPN/ 100ml	40842	31786556	11786556	12986000
DO	mg/l	8.0	Nil	Nil	Nil
COD	mg/l	19.8	106	110	76
BOD	mg/l	2.41	30	29	23
Iron	mg/l	1.16	1.29	1.12	1.20
Nitrate	mg/l	1.4	1.5	1.9	1.7
Sulphate	mg/l	24	57	75	59

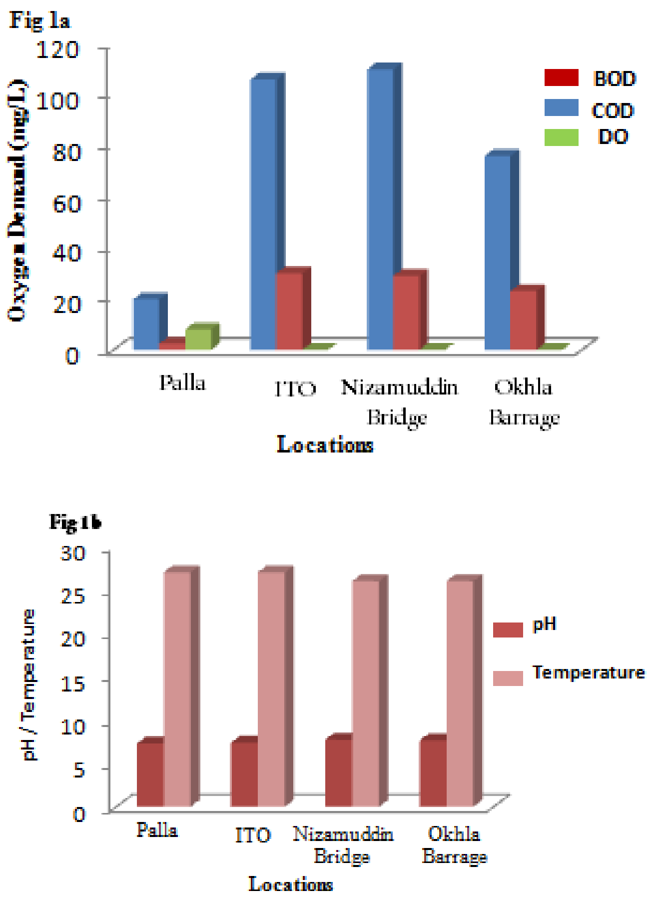


Figure 1: Comparative analysis of the physico-chemical parameters of water samples collected from four different locations on the Yamuna River

a. Dissolved Oxygen (DO) was measurable at Palla only. Biological Oxygen Demand (BOD) was found to be the highest in sample collected from Nizamuddin Bridge while it was lowest in Palla sample. The Chemical Oxygen Demand (COD) was found to be highest at ITO, followed by Nizamuddin Bridge and the lowest at Palla.

b. The pH and temperatures noted at the locations were found to be comparable.

Amongst the samples collected from the four defined locations, the amount of iron varied from 1.1-1.3mg/L (highest at ITO and lowest at Nizamuddin Bridge), that of nitrate ranged between 1.4-1.9 mg/L (highest at Nizamuddin and lowest at Palla) while the levels of sulphate varied between 24-75 mg/L (highest at Nizamuddin and lowest at Palla) (Table 1).

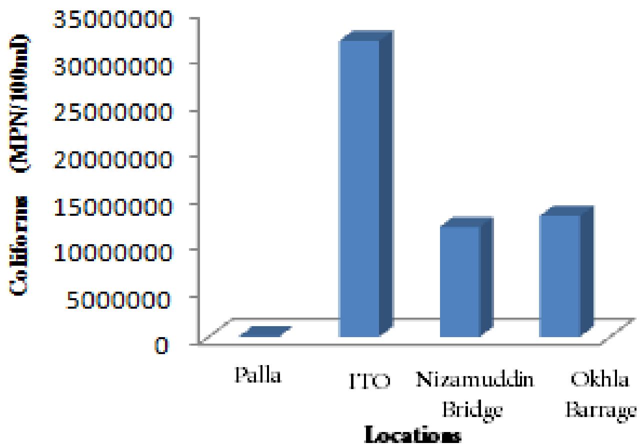


Figure 2: A comparative analysis of the coliforms detected in the water samples collected from the four different locations on the Yamuna River. The maximum numbers of coliforms were detected in the ITO sample, indicating a high microbial load. The lowest numbers of coliforms were detected at Palla, showing that among the four chosen locations, water is comparatively cleaner at Palla

3.2 Generation of electricity using pure cultures of *E. coli* and *B. subtilis*

Pure cultures of *E. coli* and *B. subtilis* were analyzed for their capacity to generate electricity. Two Overnight grown cultures of *E. coli* in log phase implying the presence of ~6.65 x 10^{11}(sample A) and ~1.27 x 10^{12} cells (sample B) in 500ml of the bacterial culture used for generation of electric current. The electric current generated was found to range from 220-320mV in case of sample A (data not shown here), and 280 – 520 mV in case of sample B. Thus, a direct correlation between the number of cells and electricity generated can be established. Sample B was further used as a control for the test samples collected from the Yamuna (Fig. 3).

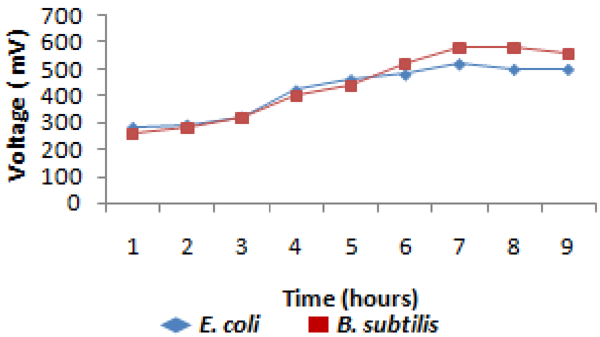


Figure 3: Generation of electric current using overnight grown pure cultures of *E. coli and B. subtilis*. The maximum voltage generated was found to be 520 mV and 580 mV respectively

Overnight grown culture of *B. subtilis* in log phase was taken, indicating the presence of 2.5 x 10^{11} cells in 500ml of the culture. The electricity generated ranged from 260mV to 580mV. This was further used as a second control for the test samples collected from the Yamuna (Fig. 3).

3.3 Generation of electricity using water samples collected from the Yamuna

The water of Yamuna has been reported to harbor plethora of micro-organisms, which also include *E. coli* and *B. subtilis.* The pure cultures of both were taken as controls for comparison with the tests samples collected at various locations. Water samples collected at the four chosen locations were then tested for their capacity to generate electric current in comparison with the pure culture controls. The potential difference generated ranged from 80-360mV (Palla), 400-620mV (ITO), 280-360mV (Nizamuddin Bridge) and 320-480mV (Okhla Barrage) (Fig.4). Thus, upon comparison of the four selected locations, ITO was shown to generate the maximum amount of electricity. They were then compared with the potential difference generated by the pure cultures, for a better understanding of the working of the microbial fuel cell.

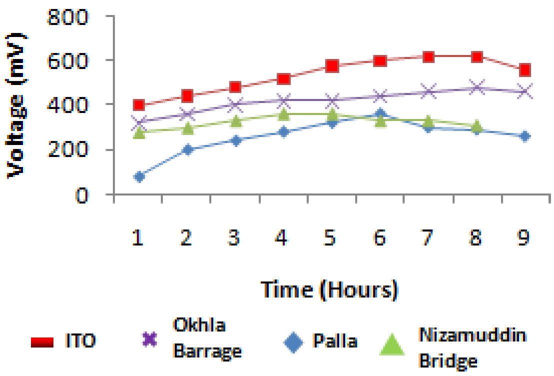


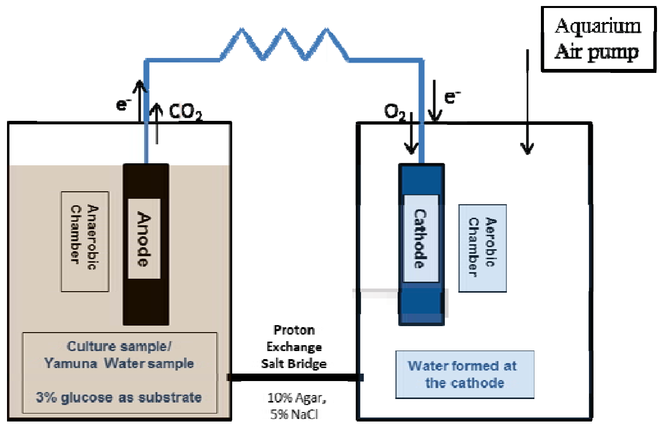
Figure 4: Generation of electric current using water samples collected from Palla, ITO, Nizamuddin Bridge and Okhla Barrage on the Yamuna River. The maximum voltage generated was 620mV, 480mV, 360 mV and 360 mV respectively

4. Discussion

Microbial population in terms of total coliform is contributed mainly through human activities which prevail in the entire stretch of Yamuna River in Delhi. The increased level of pollutants in the Yamuna water leads to a higher BOD and COD. The DO is a direct indicator of bacterial load. The maximum potential difference was generated by the ITO water sample which had the highest MPN of total coliform. The lowest potential difference was generated by the Palla water sample which had the lowest MPN of total coliform.

The potential difference was also generated by the pure culture of *E. coli* and *B. subtilis* when glucose was used as a substrate. The no. of *E. coli* and *B. subtilis* (1.27 x 10^{12} and 2.5 x 10^{11}) cells used in MFC are much higher than the total coliform (3.1 x 10^{7} MPN per100 ml) reported in the ITO sample. Hence, the generation of a higher potential difference is observed in the ITO sample at a lower microbial count as compared to the controls.

This increase can be explained by the existence of other micro organisms in consortium which enhances the performance of MFCs (Fig.5). Secondly, the presence of metal ions- sulphate, ferric and nitrate in the Yamuna water may act as mediators of electron transport. It has been reported that the metal reducing bacteria can directly transfer electrons to electrodes using active redox enzymes like cytochromes on their outer membranes or together with electron shuttles dissolved in the solution (Du et al). *E. coli* genome contains seven putative c type cytochromes of these Nap-C despite its function in the nitrate respiratory pathway, is functionally equivalent to Cym A in its role in vivo as a soluble iron reductase (Gescher *et al* 2008).



Anodic reaction: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+$

Cathodic reaction: $12O_2 + 24e^- + 24H^+ \rightarrow 24H_2O$

Figure 5: Schematic representation of a dual chamber microbial fuel cell assembly. The anaerobic chamber contains the sample (Yamuna water or bacterial culture). The anaerobic conditions of the chamber were maintained by purging in nitrogen (at Anode). An aquarium air pump was introduced into the cathodic chamber to maintain aerobic conditions.

Figure 6 illustrates the chemical compounds like sulphate, ferric and nitrate mediating the electron transfer from the microbes directly to the anode. These mediators shuttle between the bacteria and the anode transferring the electrons. They take up the electrons from microbes and discharge them at the surface of the anode and thus may lead to enhancement of the current generation.

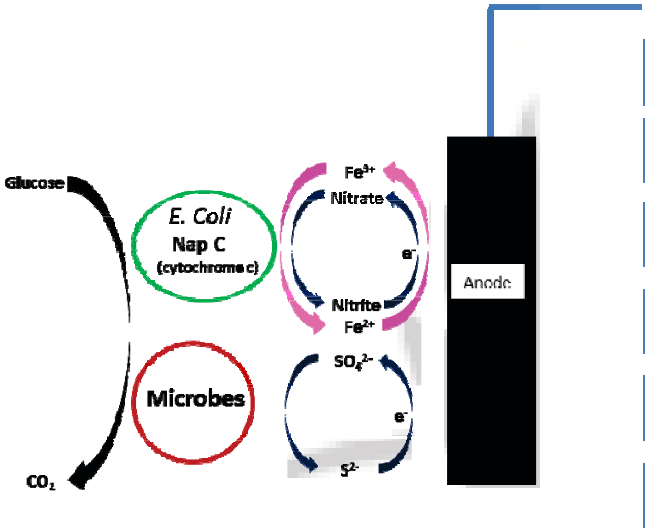


Figure 6: Model for ferric, nitrate and sulfate serving as electron shuttles between microbes and the anodes in a microbial fuel cell

5. Future Scope

Through our study we have shown that the water of the river Yamuna is also an excellent source for the generation of electricity. We are aware that the maximum power density produced by the MFC constructed can be increased substantially by carrying out minor modifications. Recent work has shown that mediators as electron transporters enhance the take up of the electrons from microbes and discharge them at the surface of the anode, thus leading to enhanced current generation. We have carried out preliminary work which is in support of these studies. By the use of nanotechnology in constructing electrodes, the surface area of the anodes can be increased markedly. This would increase the interaction between the anode surface and microbial biofilm and lead to generation of more electricity. As an improvement over our traditional carbon cloth electrodes, we intend to use modified nanofabricated carbon tubes for further study. We have currently used traditional methods of salt bridge for the transfer of electrons; the use of more advanced material like Nafion membranes will significantly aid our work. Further optimization studies are being attempted to significantly enhance the power density generated.

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