BIOTECHNOLOGY FOR ENERGY PRODUCTION: CONSTRUCTION OF A MICROBIAL FUEL CELL USING THE INDUS RIVER SEDIMENT SOIL AND WATER COUPLED WITH THEIR MICROBIAL FLORA

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Abstract
Current study provides new openings for the sustainable generation of energy by using Microbial Fuel Cells (MFCs) biotechnology. We constructed a MFC using the Indus River sediment soil and water with their microbial flora without adding any additional chemical source of energy. In resulted MFC, voltage readings were observed and their significance is calculated statistically. Furthermore, based on present findings we have proposed a microbial fuel generator model for the Indus River in Pakistan, which can be executed any where in the world.

Keywords: Microbial Fuel Cells, MFCs, Indus River, Microbial flora, Energy generation

Introduction
Energy has remained a priority area of research all the times in the world (Asif et al., 2007). At the movement, a range of energy sources are in use (Asif et al., 2007). However, a much focus is on renewable and sustainable energy solutions (Du et al., 2007; Lovley, 2006). In this regard, biotechnology has introduced MFCs as a candidate product for sustainable production of electricity (Potter, 1911; Lovley, 2006).

MFCs are bioelectrochemical systems that produce electricity from the catalysis of organic matter by the interplay of aerobic and anaerobic microbes (Logan, 2009). The decomposition of organic compounds leads to natural production of electrons that enter into the circuit via the anode (Potter, 1911). This transfer can happen in two ways: whether with the help of soluble chemical mediators or the electrons are directly deposited on the anode by bacterial biofilm. On other hand the protons, which are generated in the process, migrate to the cathode through the electrolyte and are reduced to form water (Bullen et al., 2006).

The first demonstrations of MFCs are credited to a professor of botany from University of Durham in 1911, who demonstrated the development of electric potential as a function of degradation of organic compounds by microbes in crude yeast cultures and cultures of E. coli. Potter also showed the production of 1.25 milli Amperes of current in a set up consisting of 6 cells connected in series, where copper plates were employed as electrodes (Potter, 1911). In early 1930’s, Cohen reported the production of up to 35 Volts from MFC units connected in series (Bullen et al., 2006). The research in this field was resurrected in the 1970’s, with researchers looking to improve the technology by studying different bacterial cultures and redox mediators (Tanaka et al. 1983; Delaney et al. 1984). In the light of these investigators findings it was observed that river sediments soils and water with their natural microbial flora are capable to host microbial electricity generators (Reimers et al., 2001), but little attention has been paid on the optimization and applicability.
MFCs may potentially be used to indefinitely power oceanographic instruments which can be deployed in situ (Reimers et al., 2001), in wastewater treatment (Yu et al., 2012) and as a power source in off-grid locations (Ajayi and Weigele, 2012). The simplest design among all MFCs is a sedimentary soil and water (along with their microbial flora) MFC, the principle of which is to harness electrical energy from microbes present naturally in river soil sediments under water and in flowing river water. The power generated in such a design is dependent upon electrode design, size and metal type, sediment composition, water quality and its microbial flora (Bandyopadhyay et al., 2013; Sacco et al., 2012). The other factors may include seasonal variations in environmental variables (Nielsen et al., 2007).

Scientists have tried MFC model in oceans. In these cells the anode plate is placed below the sediments and the cathode is made to float in top of seawater (Reimers et al., 2001). ‘Benthic’ MFCs were developed and deployed at sediment-water interface on experimental basis and showed significant potentials for their use (Reimers et al., 2006). Reimers et al. (2006) reported one such case of two MFCs deployed at Monterey Canyon, California, for studying voltage and power production and analyzing enriched microbial communities around the anode. Later on Reimers et al., (2006) and Mohan et al. (2009) showed stagnant water bodies with algal mats at their surfaces as better sites for benthic MFCs. A number of sedimentary systems have also been prepared and evaluated (Ajayi and Weigele, 2012; Song et al., 2011). The aim of such finding is to keeping the cost minimum as it could be and to provide an electrical source in off-grid areas. Ajayi and Weigele (2012) experimented upon bio-batteries for off-grid areas using terracotta flower pots and carbon electrodes without using any catalyst. They further enriched the microbial flora by river sediments inoculums and concluded that using different soil inocula give different results, due to differences in the composition of the sediments. The same evidence was observed in research of Song et al. (2011).

In Pakistan the science and technology of MFCs is a virgin field. Scientific reports are scanty for the evaluation and characterization of the local river soil sediments and water along with microbial communities for their potential use in microbial fuel generators. There are many studies on MFCs constructions in abroad in which researcher have proved experimental evaluation of MFCs developed from soil sediments and waters with their microbial obtained from different lakes and rivers of the world (Zhao et al., 2012; Hong et al., 2009) and evaluation of bacterial communities that play a major role in such complex systems (Mocali et al., 2013; Pisciotta et al., 2012; Song et al., 2011). In this paper, an effort has been made to evaluate the Indus river sediments soil and water for its potential use in sustainable generation of electricity. The specific objectives of this paper were to: (a) Construction of a MFC using the Indus river sediment soil and water along with their microbial flora without the input of any catalyst or external energy source and (b) preparation of a microbial fuel generator model for the Indus River.

Materials and methods
The Indus River soil (Weaver and Mickelson, 1994) and water (APHA, 1995) samples was obtained from Kund Park (District Attock) at the point where the River Kabul falls in the Indus River (Fig. 1). We constructed lab scale MFCs in a 250 mL glass bottles (Fig. 2 & 3). Each bottle was filled up to 5-6 cm with river soil. A carbon electrode (anode) was buried in the soil for the hosting of anaerobic biofilm on its surface. For current flow an insulated copper wire was connected with this electrode. The rest of bottle is filled with river water. In the same way the second carbon electrode (cathode) was suspended in water for hosting of aerobic biofilm. During investigation, the whole apparatus was kept in room temperature and conditions. Voltages on anode and cathode terminals were recorded manually with Multi-Tester (Model: Samwa YX-360, China) on daily basis for 40 days. We performed our
experiment in triplicate. For voltage parameter variations standard deviation and average was calculated. Resultant data was presented graphically.

**Results**

All MFCs constructed during this study shown significant amount of voltage in their outputs. The time and average voltage relationships are shown in Fig. 4. During triplicate experiments the measurement of average voltage ranged between 0.4 V to 0.6 V and standard deviations vary along 0.07 to 0.14.

**Discussion**

MFCs are attractive sustainable energy generation sources because they are environment friendly, with zero-carbon emission (Lovley, 2006) and can be applicable to small and large scale processes (Bullen et al., 2006). Current study was focused on demonstrating the phenomenon potential in the Indus River sediment soil and water coupled with their microbial activity. All the MFCs which are constructed during this study showed the significant amount of electricity generation. During replication experiments we obtain very low value of standard deviation which increases our confidence on average voltage. Fig. 4 depicts that initially, form the day first, the voltage is very low but with the passage of time notable boost is observed. This explains the positive correlation exists between ‘the biofilm formation for electricity generation on anode and cathode terminals’ with the ‘time’. During this ‘time’ the biofilm stabilizes itself on the surface of carbon electrodes. The development of a biofilm on the anode in a sedimentary MFC is considered vital for the steady current production and maintenance of voltage (Franks et al., 2010).

Different studies have been conducted on sedimentary MFCs on different levels. Song et al. showed in 2012 the performance of six sedimentary samples obtained from different sites within the Lake Taihu. The maximum voltage that one of the samples in the study gave was 0.58 volts (employing a 1000 Ω resistance in the external circuit) equating to 112 mW/m² power density. Further in his studies some samples failed to produce any substantial voltage at all (Song et al., 2012). Reimers et al., 2006, demonstrated voltage production in deep-ocean cold seep at Monterey Bay, California in two benthic Microbial Fuel Cells, which were observed for their effects for a period of 125 days. They also conducted two lab scale experiments. a) The cathode of the deployed benthic MFC gave a maximum potential of 0.384 volts (against Ag/AgCl reference electrode) and the anodic potential dropped to 0.427 volts against the same reference. a) The lab scale set-ups gave 0.65 volts and 0.3 volts.

In our experiments, all MFCs gave voltages, the observed peak values being ranged between 0.4 and 0.6 volts. These voltage values were found to evolve over time till the stabilization of biofilm (Fig. 4). The voltage readings increased noticeably around 14 day of experimentation and were found to stabilize after 25 days of operation. Our developed MFCs are environment friendly as no catalyst or additive was used for controlling any of the biochemical reactions which are happening within the cells. Based on our lab findings, we have proposed a Microbial Fuel Generator model for the Indus River (Fig. 5). It depicts the feasibility of such a system at large scale. But before moving to this large scale mega investment, there are few new question which need to be addressed in advance. What kinds of materials are best for electrode manufacturing for hosting of biofilm for a particular river or lake for maximum production of electricity? Environmental factors which influence the production of electricity required to be identified. The pressure of seasonal variations along with climate change should be investigated. Similarly, the microbial diversity across the biofilm can be another aspect of the study.
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Conclusion
Form above discussion it is concluded that the MFC biotechnology is an emerging field for sustainable energy production. The Indus River natural microbial flora is capable of biotechnological production of electricity. There is immediate need of the time that more research and data should be generated by scientists for the practical execution of the project.

References:
Fig. 1. The Indus River sediment soil and water sampling site (Red dot).
Fig. 2. Lab scale MFC Construction
Fig. 3. Working scheme of lab scale MFC
Fig. 4. Electricity generation in MFCs during experimental period

![Graph showing electricity generation in MFCs over 40 days with lines for Experiment 1, Experiment 2, and Experiment 3.]

Fig. 5. Model of Microbial Fuel Generator for the Indus River

![Diagram of a microbial fuel generator with sections labeled for River, Aerobic Biofilm, Cathode, Electricity Output, Electric Circuit, Sediment Soil, Anode, Anaerobic Biofilm, and River Bottom.]

Legend:
- River
- Aerobic Biofilm
- Cathode
- Electricity Output
- Electric Circuit
- Sediment Soil
- Anode
- Anaerobic Biofilm
- River Bottom