A MICROBIOLOGICAL PROCESS FOR COMBINED BIOELECTRICITY PRODUCTION AND WASTEWATER TREATMENT USING *Staphyllococcus* Sp.

A.V. Pethkar*[1], Kalyani Kale[1], Priyanka Belgaonkar[1], Vaishali Bagul[1], V. S. Kale[2] and Sucheta N. Patil[3]

1. Deptartment of Biotechnology, KTHM College, Gangapur Road, Nashik, Maharashtra, (INDIA)
2. Deptartment of Electronics, KTHM College, Gangapur Road, Nashik, Maharashtra, (INDIA)
3. Deptartment of Microbiology, KTHM College, Gangapur Road, Nashik, Maharashtra, (INDIA)

*E-mail: avpethkar@yahoo.com

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ABSTRACT

The dwindling supply of energy resources and the concomitant rise in cost of fuels pose severe problems for mankind. Moreover, there are concerns about environmental harm either during production or use of the energy sources. Significant advances have been made in the search for new methods of tapping energy that will be cheaper, sustainable and environmentally safe. Bioelectricity which can be generated through the metabolic activities of microorganisms has been recognized as a potential source of renewable energy. A large number of research papers have been published on the topic and the design of Microbial Fuel Cells (MFCs) for bioelectricity production has been described. The MFC may well become the Mantra of the modern time. MCF is an electrolytic cell with two chambers connected by a salt bridge. One chamber has microbial cells at high density and an anode, while the other chamber contains electrolytic buffer with cathode. Microbes utilize a substrate provided in the anodic chamber and generate electrons as a byproduct of metabolic activity. Under anaerobic conditions (absence of electron acceptor such as oxygen) the electrons are trapped by the anode. The electrons are subsequently transferred through an external circuit to the cathode. Protons that are also generated in the anodic chamber are carried through the salt bridge to the cathodic chamber, where they interact with O_2 and electrons to generate water molecules. Efforts were undertaken in order to use MFC for treatment of wastewater coupled to bioelectricity production. Simulated wastewater and real wastewater of a paper industry were treated in anodic chamber of MFC that contained either a mixed bacterial consortium or pure isolate identified as *Staphylococcus sp*. that was isolated from a municipal anaerobic sludge sample. Initially, the organisms were studied for the ability to produce bioelectricity from simulated wastewater by optimizing different parameters viz. electrode surface area, distance between electrodes, resistance of external resistor, glucose concentration, type of salt bridge, etc. It was found that Staphylococcus sp. generated more electricity than the mixed consortium. The optimized parameters for a 1liter MFC viz. surface area- 100 m^2, distance between electrodes- 6 cm, external resistor- 50 ?, glucose concentration- 10g/L could generate 7.2 mV of electric potential and the peak level was reached in a period of 5 h. Subsequently, experiments were repeated with real wastewater sample obtained from a paper industry and it was found that an electric potential of 3.6 mV was generated in 3-5 h. Bioelectricity production was accompanied by simultaneous reduction (78%) in COD levels of wastewater. The experiment was continued for up to 21 days under controlled conditions with intermittent substrate addition and it was found that the levels of electricity generation and COD reduction remained constant during this period. Further experiments are underway in order to make the design of the MFC more efficient and handy for fruitful applications.

Key Words : Microbial fuel cell, Wastewater treatment, Bioelectricity, *Staphyllococcus*, Microbiological

*Author for correspondence

INTRODUCTION

There is a growing concern about the availability of energy resources across the world. According to the recent statistics, the world's fossil fuels would get depleted in a matter of few years. United Arab Emirates, one of the major oil exporting countries, would fail to meet the oil and natural gas demand by 2015 and 2042 respectively[1]. The fossil fuel reserves in Egypt would be exhausted within one to two decades. There is an urgent need, therefore, to find out new cost effective and renewable sources of energy to bridge the demand-supply gap. Coupled with this is the problem of environmental damage resulting from production or burning of the fuels and CO_2 release that leads to global warming. Various alternatives are being explored in order to develop clean, green and renewable energy sources that could supplement or even replace the existing ones. Bio-based systems are attractive, for example, anaerobic degradation of organic matter produces methane gas which is used as fuel. However, there are a number of issues that limit the use of such alternatives[1].

Of particular interest is the idea of using physiological processes in living microbial cells to generate bioelectricity, an idea originally conceived by M. C. Potter in 1911[2] and later developed by various researchers. Bioelectricity can be generated through the use of a special type of an electrochemical fuel cell, viz. Microbial Fuel Cell (MFC). The MFC is in principal similar to other fuel cells such as phosphoric acid fuel cell, proton exchange membrane fuel cell, molten carbonate fuel cell, solid oxide fuel cell, alkaline fuel cell, direct methanol fuel cell, zinc air fuel cell, etc. These fuel cells are used in automobiles, buildings, rechargeable batteries, etc. but have severe limitations such as need of high operating temperatures, fossil fuels and certain chemicals. MFCs, on the other hand have less exacting demands and proffer promising options[3].

MFC is a device which converts chemical energy to electrical energy during substrate oxidation with the help of microorganisms[4,5]. Like a normal fuel cell, MFC has both anode and cathode chambers. The anaerobic anode chamber harbors a microbial culture at a high density and is connected internally to the cathode chamber by an ion exchange membrane or a salt bridge. The cathode chamber contains electrolytic buffer and is externally connected to the anodic chamber by an external wire. MFCs use the catalytic reaction of microorganisms to convert (oxidize) virtually any organic material (glucose, acetate and some components of wastewater) into fuel. As part of the digestive process, electrons are pulled from the compound through a cascade of oxidation-reduction reactions and stored as intermediates (NADH, quinines). In the absence of an electron acceptor such as oxygen (anaerobic conditions), the electrons are trapped by the anode and then transferred through an external circuit to the cathode, a process which sometimes requires inorganic mediators such as neutral red, methylene blue, thionine, humic acid, etc.[6-7]. However, on account of the higher expenses and toxicity associated with the mediator, more emphasis is given on the development of mediatorless MFCs[8-10].

It has been demonstrated that an iron-reducing bacterium *Shewanella putrefaciens* which is electrochemically active acts as a catalyst in a mediatorless MFC[11-12]. Also, bacteria of the family Geobacteraceae can directly transfer electrons to electrodes using electrochemically active redox enzymes, such as cytochromes on their outer membranes[13]. Some of the important parameters that dictate overall efficiency of MFCs are: fuel oxidation at the anode, electron transfer from microorganisms to the anode, presence of active redox enzymes, resistance of the external circuit and proton transfer through the membrane to the cathode[9].

The acceptability of MFC for wastewater treatment would increase, if use of membrane can be eliminated using some alternative. A Membrane Less Microbial Fuel Cell (ML-MFC) was used successfully for conversion of organic contaminants to electricity[3], pointing towards potential use of an MFC for wastewater treatment. The present work was initiated in order to explore the possibility of using membraneless MFC for the wastewater treatment. The electric current generated in an MFC may be negligible, but it offers the possibility of generating clean water through microbiological degradation of organic pollutants.

AIMS AND OBJECTIVES

The objectives of the present research were:
(i) to develop a simple low-cost bacterial biofuel cell, (ii) to optimize the parameters such as geometry of the biofuel cell, electrodes, use of resistors, salt bridge, etc. for optimal electricity generation, (iii) to carry out media optimization studies in order to enhance the growth of bacteria for high efficiency of electricity production, (iv) to test the feasibility of the fuel cell as a method of sewage treatment with electricity as a by-product using mixed bacterial culture, (v) to isolate bacterial cultures for comparative evaluation of pure cultures and mixed bacterial populations with an aim of deriving maximal amounts of bioenergy.

MATERIAL AND METHODS

Microorganisms

Different materials such as anaerobic sludge and cow dung samples were procured from Up-flow Anaerobic Sludge Blanket (UASB) sewage treatment plant (under Godavari action plan, Tapovan, Nashik) and local animal farms, respectively. Enrichment of bacteria was carried out by inoculating samples in liquid thioglycollate medium overlaid with liquid paraffin. The cultures were incubated in a dessicator at room temperature for a week until turbidity was seen in the tubes. For mixed consortia, the enriched cultures were inoculated into synthetic wastewater containing glucose (2 g/L), NH_4Cl (0.5 g/L), KH_2PO_4 (0.20 g/L), K_2HPO_4 (0.20 g/L), $MgCl_2$ (0.25 g/L), $CoCl_2$ (20 mg/L), $ZnCl_2$ (10 mg/L), $CaCl_2$ (4 mg/L) and $MnCl_2$ (10 mg/L) and incubated at RT for 48 h. The cultures were subsequently used for inoculation in the anode chamber of MFC at the level of about 10% v/v. The composition of media used in the experiments was essentially taken from the available literature[7,14,15]. Pure isolates of bacteria were obtained by plating out suitable dilutions of the enriched broths on synthetic wastewater agar plates containing thioglycolate. Isolates were subsequently preserved on synthetic wastewater agar slopes containing thioglycolate at 2-8^{0}C.

Assembly and operation of MFC

The basic design of MFC was modeled as per descriptions in the literature[15-19].Two heavy duty plastic bottles (electrolytic chambers) of 1 liter capacity and having screw-capped lids were connected via PVC pipe as shown in **Fig. 1** and described in design criteria **(Table 1)**. The PVC pipe was filled with 10% agar containing predetermined concentrations of standard salts solution (containing K_2HPO_4, KH_2PO_4, $NaHCO_3$, NaCl, $CaCl_2$ and $MgSO_4$) to serve as a salt bridge for transfer of protons. The anode was made by placing a lead oxide plate (7 cm x 7 cm x 0.2 cm) in the anode chamber connected with a copper wire to the lead oxide cathode plate (7 cm x7 cm x 0.2 cm) in cathode chamber. The measured surface area of the two electrodes was 109.35 cm^2 and distance between the electrodes was approximately 6cm. Prior to start of the experiment, the electrodes were soaked in deionized distilled water for 24 h. Inoculum in the form of mixed bacterial consortium or pure isolates was added to synthetic wastewater (pH 5.5) in the anode chamber which was covered with a lid to prevent the entry of oxygen. Conducting solution (phosphate buffer, 50 mM, pH 7.5, DO 4-5 mg/L) was added to the cathode chamber which was sparged with compressed air. The external circuit was connected through a resistor and the MFC was tested with distilled water which showed zero deflection on the multimeter. The Chemical Oxygen Demand (COD) and voltage output were monitored at frequent intervals of time. A constant reduction in COD and stable voltage output indicated satisfactory running of the MFC. Reduction in the electricity output and constant COD signaled the need for the changing the feed. Prior to changing the feed, the contents of the anode chamber were allowed to settle down (30 min) and exhausted feed (950 ml) was pumped out under anaerobic conditions. The settled mixed inoculum (50 ml by volume) was retained in the chamber as inoculum for next cycle of operation of the MFC. After every feeding event, the anode chamber was supplemented with sodium thioglycollate (0.5gm/L) in order to maintain anaerobic environment. Performance of the MFC was evaluated at four substrate/ Organic Loading Rates (OLRs), viz. 0.517 kg

COD/m^3-day, 0.574 kg COD/m^3- day, 0.646 kg COD/m^3.day and 1.033 kg COD/m^3.day. The MFC was operated in batch mode at constant operating temperature of 28±2^{0}C.

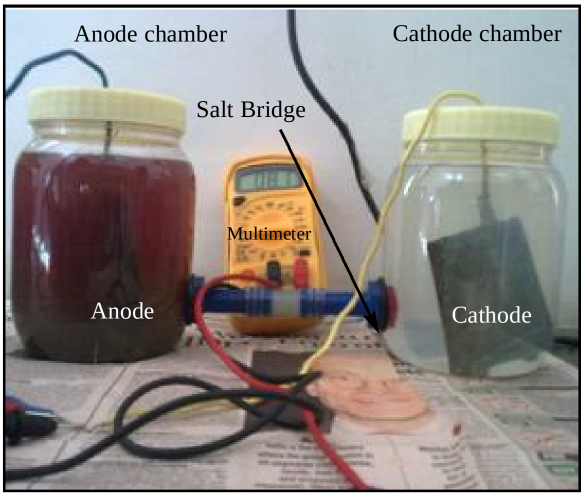

Fig. 1: Assembly of MFC used in present studies

Optimization of physical and biological parameters for bioelectricity production

Effect of nutrient loading on bioelectricity production (voltage) was tested by adding different concentrations of glucose (0.1%, 0.5 % and 1%) in the anodic chamber and monitoring the levels of electricity production using a digital multimeter. For experimental purpose, pure culture of bacteria that showed highest electricity production was used.

Waste treatment coupled with bioelectricity generation

Efforts were undertaken in order to use MFC for treatment of real industrial wastewater and to generate electricity. For this, wastewater from a paper industry was treated in anodic chamber of MFC by utilizing pure culture of *Staphylococcus sp*. isolated from an anaerobic sludge sample. The MFC was operated for 21 days under optimized conditions as described earlier. Substrate was added at frequent intervals in order to grow bacteria at maximum levels and also retain the optimal concentration for growth of bacteria and bioelectricity production.

Table 1 : Design criteria of dual chambered mediatorless microbial fuel cell

Criterion	Description
Anode chamber	Suspended growth
Anode inoculums	Mixed anaerobic consortia, single isolates
Mediator-anode	Nil
Mediator-cathode	Air
Volume of anode and cathode chambers	1 litre
Anode and cathode material	Lead oxide plates
Surface area of electrodes	109.35 cm^2 (anode and cathode)
Distance between electrodes	6 cm
Proton conduction pathway	Salt bridge
Feeding nature	Batch (1l/feeding cycle)
Organic loading rate	10g per week
Operating temperature (^{0}C)	28 ± 2
Operating pH	5.5 (anode), 7.5 (cathode)

RESULTS AND DISCUSSION

Microorganisms

According to published literature, both pure cultures and mixed consortia of bacteria have been used in MFCs with varying results[10,19,20]. In the present studies, both mixed and pure cultures were studied for their ability to generate bioelectricity. In most papers, the necessity of maintaining anaerobic conditions in anodic chamber has been highlighted. Therefore attempts were made to isolate organisms from anaerobic environments and the MFC was designed accordingly. Four cultures were isolated in pure and each isolate was individually tested for the ability to generate electricity. Isolate that showed the highest activity was selected for further experiments. It was identified by routine microbiological and biochemical methods as a species of *Staphylococcus*. Since, bioelectricity production by the pure isolate of *Staphylococcus* was at a similar level as mixed population, pure culture was used for subsequent experiments for better control over MFC operations.

Optimization of physical and biological parameters for bioelectricity production

The anode chamber containing synthetic wastewater was inoculated with either the anaerobic mixed consortia or *Staphylococcus* sp. and the assembly was incubated at room temperature (c.a. 25^{0}C). Growth of bacteria in terms of visible turbidity was observed after overnight incubation. A constant substrate (COD) removal and voltage output were considered as indicators of the stable performance of the MFC. The fuel cell was operated continuously for 21 days with

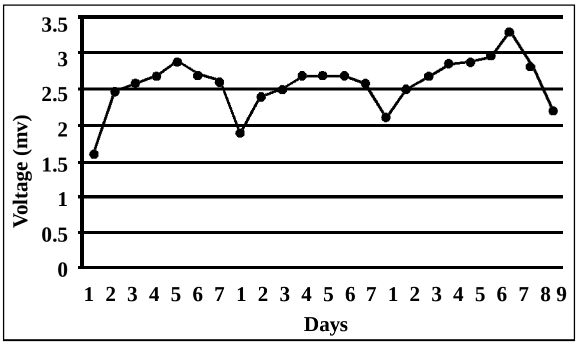

Fig. 2a : Bioelectricity using mixed population of bacteria from anaerobic sludge grown in a synthetic wastewater sample. Drop in voltage output signals need for addition of substrate.

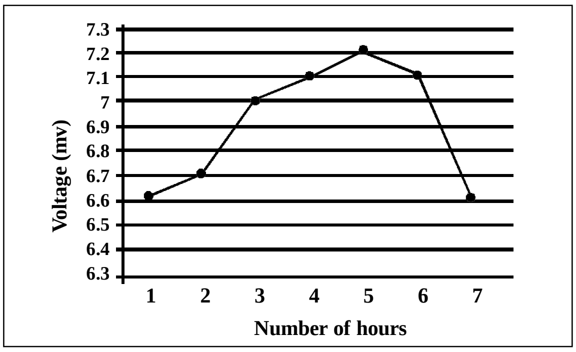

Fig. 2b : Generation of bioelectricity by *Staphylococcus sp*. isolated from anaerobic sludge and grown in a synthetic wastewater sample.

intermittent addition of substrate whenever the COD reduction efficiency was lowered or the voltage was lowered. **Fig. 2(a)** and **Fig. 2(b)** show the result of experiment using mixed culture and individual isolate, respectively. It was observed that constant voltage was generated over a period of 21 days for mixed population. During the initial stage after inoculation, a gradual rise in the voltage was observed, which approached a maximum of 3.3 mV at 50 Ω on the 21st day. However, on the fifth day of operation, a drop in potential difference was observed indicative of the exhaustion of carbon source in the feed. Hence, fresh feed was supplemented into the anodic chamber in order to restore the potential difference. In case of the pure culture, maximum power output of about 7.2 mV was immediately observed in the 5th hr of operation followed by a decline by 7th day. Hence, the experiment was terminated at this stage. The COD removal efficiency in case of both the pure and mixed cultures was to the tune of 75%. COD removal values in the range of 70-90% have been reported earlier[9], indicating satisfactory performance of the MFC. The experimental results indicated that the anodic chamber of MFC doubled up as an anaerobic suspended growth reactor of a typical wastewater treatment plant for anaerobic treatment with electricity generation.

Waste treatment coupled with bioelectricity generation

The microbial fuel cells consisting of wastewater samples from a paper industry were operated for a period of 10 h with continuous one hourly monitoring of the voltage generated. In this case the pure culture of *Staphylococcus* was inoculated in the anodic chamber. The experimental data revealed that electricity was generated from the wastewater sample **(Fig. 3)**. The cultures started fermentation of the substrate and generated current after about 1 h of inoculation and reached the maximum voltage generation after 5 h (3.6 mV).

The use of MFC for treatment of wastewater has been previously reported[19]. As seen in **Fig. 3**, there was significant drop in voltage after approximately 6 h due to substrate exhaustion. As compared to data on synthetic wastewater, in

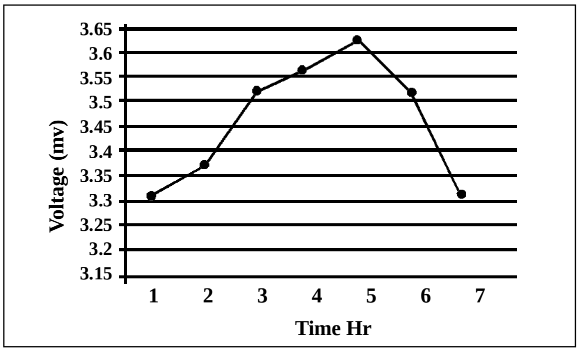

Fig. 3. Generation of bioelectricity by *Staphylococcus sp*. in a real wastewater sample obtained from paper industry. The experiment was carried out over a period of 7 days.

which case the voltage was higher (7.2 mV), the efficiency with real wastewater was lower(3.6 mV). This was probably due to the fact the substrate (mainly cellulose) was present in an insoluble particulate form and was not readily available to the growing culture. The precipitate of cellulosic material seen at the bottom of the anodic chamber was evident and it may be hypothesized that further adaptation of the culture to the new environment might significantly increase the power generation. A similar lessened power generation from wastewater compared to synthetic medium was reported earlier[21]. Perhaps an active cellulose degrading culture in association with the electricigens or more appropriately the exo-electrogens[20] may be used for enhancement of the electricity production. It also needs to be mentioned that electricity production in the present work is still not in tune with other published reports due to the very simple design of the MFC and attempts are underway to improve performance by screening for better organisms rather than complicating the design of MFC. An important aspect of the MFC was a significant reduction in the COD of the synthetic wastewater to the extent of 78%, an efficiency which was found to remain stable throughout the period of study i.e. 21 days. Similar observations have been reported by other researchers working in the area of microbial fuel cell technology[9,14,19]. Studies utilizing co-cultures of cellulolytic bacteria and electricigens are being undertaken in order to enhance bioelectricity production. Moreover, the possibility of biological treatment of a variety of wastewaters with corresponding electricity generation is also being investigated.

CONCLUSION

The study demonstrates the feasibility of bioelectricity generation from anaerobic wastewater treatment process using a low-cost MFC consisting of non-coated plain lead oxide electrodes and eliminating the need of any toxic mediators. A significant reduction in the COD during electricity production from real wastewater may be considered as the useful feature for clean and green power generation. The major problem encountered in the present work was that of incomplete cellulose decomposition, and further modifications would be required to enhance the efficiency of the device. Trials with other types of effluents may give encouraging results.

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