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Electricity generation from bacteria *Staphylococcus aureus* and *Enterobacteriaceae bacterium* using microbial fuel cell - an alternative source of energy and its use application

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Abstract

Microbial Fuel Cells (MFCs) are devices that use bacteria to generate electricity from organic matter. In this study, the sewage waste was screened for pure cultures. Among the 10 isolated pure cultures, 2 showed best results (*Staphylococcus* and *Enterobacteriaceae bacterium* GP1). These were used in immobilized form as well to measure their electrochemical potential. These organisms are capable of transferring electrons to the anodic electrode of an MFC to generate an electric current. Further insights in to the anode reduction by these bio-film forming bacteria were gained through voltmeter. The redox, metabolites produced which varies with the different concentrations of ammonium and nitrogen sources was optimized. The power output was measured and compared among the organisms. 16s rRNA sequencing was done for the best strain after comparison. The bio-film formed on the anode for studied using scanning electron microscope.

Keywords: *Staphylococcus aureus*, fuel cells, electrochemical potential

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Introduction

Every year the global energy demand increases. While petroleum products currently supply much of this demand, the increasing difficulty of sustained supply and the associated problems of pollution and global warming are acting as a major impetus for research into alternative renewable energy technologies. Fuel cells offer a possible (and partial) solution to this problem. Among fuel cells, Microbial Fuel Cells (MFCs) are special types of bio-fuel cells. It is a device that converts chemical energy into electricity through the catalytic activities of microorganisms (Allen and Bennetto, 1993). MFC treatment can reduce the BOD in wastewater by degrading organic matter (Liu et al., 2004). While the detailed mechanism of generating electricity by MFC is not completely understood, many bacteria capable of generating electricity have been identified (Kim et al., 2002; Bond and Lovley, 2003; Rabaey et al., 2004). Microbial fuel cells seek to add the diversity of microbial catalytic abilities to this high-efficiency design, allowing organic compounds, from simple carbohydrates to waste organic matter, to be converted into electricity (Wingard et al., 1982). They are an alternative to conventional methods of generating electricity, for small scale applications (Bennetto, 1990). Microbial fuel cells have potential to generate electricity from a wide variety of organic wastes while oxidizing the waste to less harmful forms (Liu et al., 2004; Ieropoulos et al., 2005; Moon et al., 2006). In this article, we would see the maximum generation of electricity from bacterial cultures *Enterobacteriaceae bacterium* and *Staphylococcus aureus* isolated from Sewage waste water collected from anaerobic sludge at Koyembedu Sewage Treatment Plant, Chennai. Microbial fuel cell usually comprise of four major components-anode compartment, cathode compartment, ion exchange membrane and the electrodes.

Anode compartment forms the biological compartment of MFC, as it consists of microbes (biocatalyst) either in pure/mixed form (Kim et al., 1999; Chaudhuri and Lovley, 2003; Bond and Lovley, 2003, 2005; Holmes et al., 2004), which oxidizes the organic substances in the wastewater and releases free electrons. The bacterial growth in

this chamber produces the necessary protons and electrons through metabolic reactions. The metabolic reactions are not allowed to proceed to completion and the intermediate electrons are drawn from the cell to do the electrical work (Venkatamohan et al., 2007). Cathode compartment is the abiotic compartment of MFCs where the released electrons (from anode) are transferred to oxygen as a terminal electron acceptor. The ion exchange membrane helps in the transfer of protons from the anode compartment to the cathode compartment and helps to physically block oxygen diffusion into the anode chamber (Chae et al., 2008). Hence it is generally called Proton Exchange Membrane (PEM). Commonly used Proton Exchange Membranes are Nafion or Ultrex. Since PEMs are costly, here we have used salt bridges to maintain electro- neutrality and allow current to flow (Booki et al., 2005). The salt bridge contains a saturated solution of some inert salt, usually Sodium chloride. The salt is chosen specifically to be inert based on the rest of the reagents in the system. The major role of electron transfer is due to electrodes. Some commonly used electrodes are carbon rod, carbon/graphite sheet (Pham et al., 2004; Venkatamohan et al., 2008) stainless steel, glassy carbon, etc.

Materials and methods

Samples used

Anaerobic sludge was collected from Sewage Treatment Plant, Corporation of Chennai, Tamil Nadu, India.

Isolation of pure culture

Pure cultures were isolated using serial dilution method followed by the spread plate technique on nutrient agar plates. Isolated colonies were further maintained in different nutrient agar slants for future work and stored at 4°C.

MFC set-up construction

A two chambered fuel cell was constructed. Two plastic containers each with diameter 20 mm were taken and marked cathode and anode. Two holes of diameter 6mm and 1.5 mm were made on each of the lids for the insertion of the salt bridge and electrodes. In the anode container, 100 mL of the anodic inoculation was used and in the cathode container 100 mL Potassium permanganate solution was used and the container lids were closed and sealed with tape as shown in Fig. 1.

Fig. 1. An MFC model

Salt bridge preparation

Salt bridge was made with 5mm diameter level tube. The salt bridge contained a mixture of 1M Potassium chloride with 5% Agar. The mixture was sucked into the level tube. This salt bridge was inserted into both the containers through one hole on both containers and sealed with tape.

Electrodes used

Graphite sheet with 0.5 mm thick, 1 cm width and 5 cm length was used as electrodes to collect the electrons in both anode and cathode with copper wire connections at the other hole on both the containers and sealed with tape. These electrodes were relatively inexpensive and available easily. The electrodes were first soaked in 100% ethanol for 30mins. After this the electrodes were washed in 1 M hydrochloric acid followed by 1M Sodium hydroxide, each for 1hr to remove possible metal and inorganic contaminations and to neutralize them. They were then stored in distilled water before use.

Anodic inoculation

100 mL of 24 h broth culture or cultures immobilized in a mixture containing 3 % sodium alginate and 0.2 M calcium chloride further suspended in the nutrient broth.

Cathode chamber preparation

For the cathode chamber, 0.1M Potassium permanganate solution was prepared. The voltage was checked with a Multimeter (UNITY DT-830D).

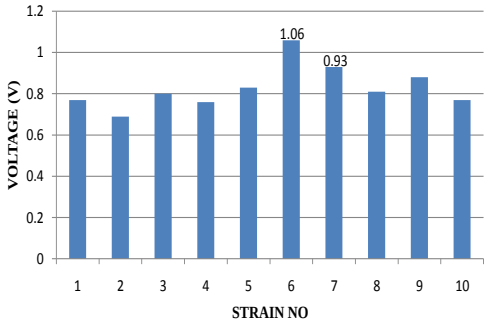
Operational conditions

All the operations were carried out with different electrodes and electrolytes. The MFCs were operated for 15 days at a room temperature of 28°C. The specific MFCs with various electrodes and various electrolytes which showed the maximum electricity generation were combined and connected in series and parallel connections to give higher electricity. The voltage generation was noted. It was also connected to LED to see if it glowed.

Results

In Fig. 2, the comparison of voltage in all strains those were isolated from the sewage sample. The strains 6 and 7 were found to produce highest voltage as well as they both had maintained the voltage stable for a long period of time.

Fig. 2. Highest voltages of all strains



In order to increase the survival and longevity, the organisms were immobilized in sodium alginate beads. The MFC set up was build by adding 10 g of immobilized beads in 100 mL of nutrient broth. From the Fig. 3, the immobilized strains showed a better result compared to normal Nutrient broth culture. The voltage of Nutrient broth cultures 6 & 7 at 3rd day produced 1.06 V and 0.93 V and then got decreased up to a level of 0.72 V and 0.71 V. In contrast, the immobilized strains produced 0.99 V and 0.89 V at the same 3rd day. It was maintained at the same potential for nearly about two weeks. Hence the immobilized cells gave a constant voltage for a long time compared to non immobilized strains. The Fig. 4 shows the growth phase study carried out for the strain 6 and effect of growth phase on voltage generation. In this study, the optical density value of the bacteria gradually increased from 4th hr to 24th hr which is known to be logarithmic phase (bacterial multiplication). After 24th hr it remained at the same value for several hours known to be stationary phase. The MFC set up was constructed using 24 hr culture, were the voltage began to increase. It was concluded that, during the stationary growth phase of bacteria the voltage keeps on increasing up to several days.

Fig. 3. Highest voltage compared among different immobilized cells

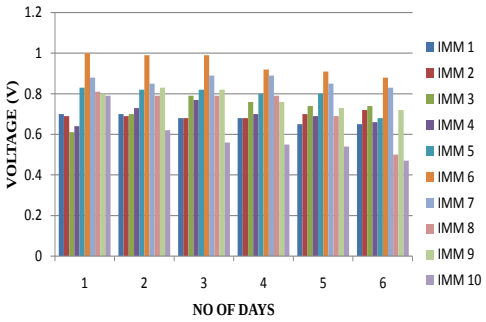
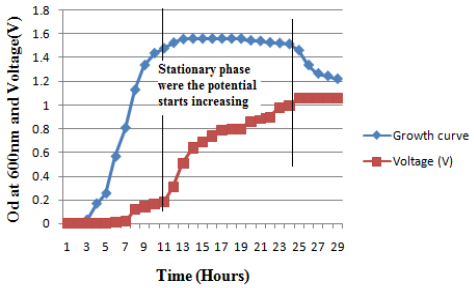


Fig. 4. Increase in voltage depending on the growth phase



One of the nitrogen sources, peptone supplies nutrients for the survival of bacterial growth. The decrease in voltage indicates that the bacteria are in to death phase. In Figure 5 the optimization of peptone was carried out. The concentration of peptone taken in nutrient broth was varied from 5 to 25 g / L. Generally for the bacterial culture the nutrient broth consists of 5 g / L. It showed increase in stability with increased peptone concentration. For the strain 6, the maximum stability was maintained at 10 g / L of sample produced voltage of 0.89 V at 6th day and it was maintained for several days. Thus concluded that, increase in peptone concentration increased the

current generation as well as increased the potential of organism in MFC. The strain 7 also showed gradual increase in voltage from the third day of MFC set up at 10 g / L concentration of peptone and had a better stability without decrease in voltage for several days.

Fig 5. Strains 6 and 7 optimized by peptone

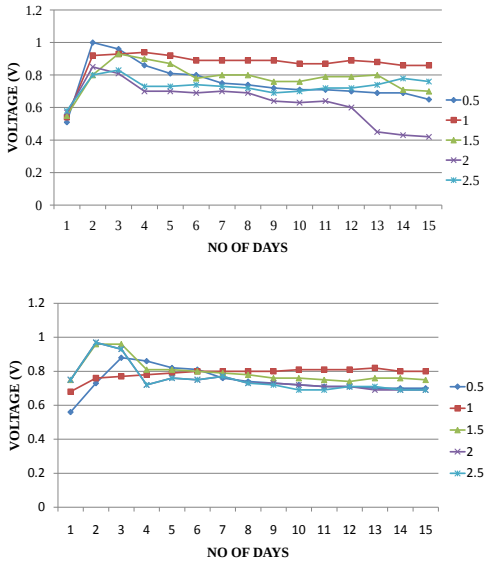


Fig. 6. Comparision of voltage using various ammonium salts for the strains 6 and 7

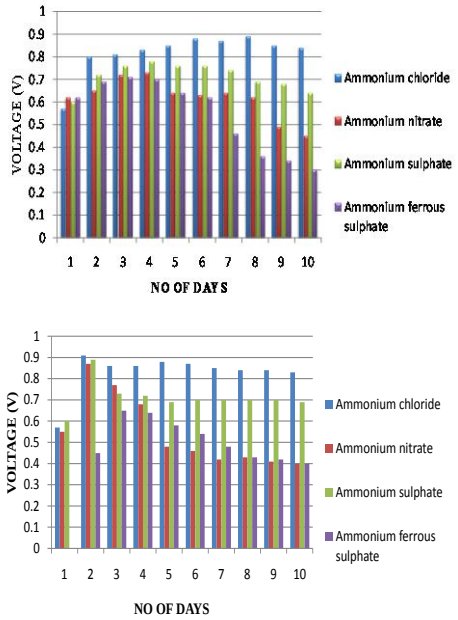


Fig. 6 shows the different ammonium salts used as nitrogen source for the bacterial strains 6 and 7. The concentration of these salts was 5 mM per litre. Of the different salts such as ammonium chloride, ammonium sulphate, ammonium nitrate and ammonium ferrous sulphate used, ammonium chloride was proved to be the best source.

The MFC was developed using the best optimized sources (10 g of peptone and 5 mM of ammonium chloride). In this the best sources were used to check the stability of the voltage of the 2 strains. The voltage was stable for longer time when compared to set up without these sources. The voltage remained constant for 12 days. Then it showed a very slight decrease.

Identification of strains 6 and *7*

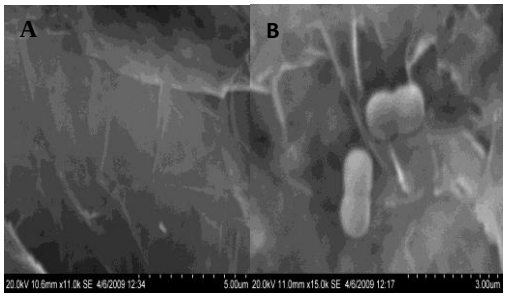
1. Red color ring formation - Indole positive
2. No color change - MR negative
3. No color change - VP negative
4. No color change or bubble (H_2S) formation - TSI negative
5. Color change from green to blue - Citrate positive
6. Color change from yellow to red – Urease positive

The strain 6 was identified preliminarily using biochemical test and further was done its 16s rRNA sequencing was carried out using BLAST and further it was identified as *Enterobacteriaceae bacterium* GP1. The strain 7 was a yellow colored culture which shows that was a color producing organism indicated it may be the *Staphylococcus*, *Micrococcus* sp etc.

Confirmatory test for Staphylococcus

For the confirmation of strain 7, it was streaked in MSA medium (red in color) containing mannitol salt. After incubation period the medium color was changed to yellow due to the presence of *Staphylococcus.* The organism itself utilizes the medium and changed them in to yellow color. This conformed that the organism found was *Staphylococcus aureus.* The electrodes were subjected for the SEM analysis to check for the formation of bio-film. Fig. 5b shows the bio-film formed on the anode electrode stating that the presence of bacteria in the anode and its growth over the electrode. Whereas fig. (5 a) shows the cathode electrode on which on bio-film formation was observed.

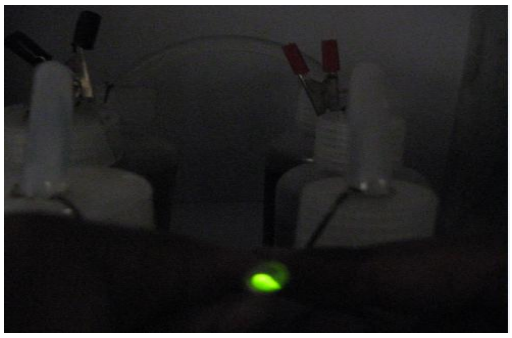
Fig. 5. SEM analysis of a. cathode electrode, b. Anode electrode showing formation of bio-film



Application using MFC

Numerous fuel cells have been shown to generate power by oxidation of compounds found in waste water streams. Two useful purposes can be realized by this procedure; (a) for the removal of organic compounds from the waste stream and (b) for the generation of electrical power. Thus, we show that the MFC is used for the Power generation using which an LED can glow (Fig. 6).

Fig. 6. LED glowing with power from MFC



Discussion

A redox potential of 0.82 V was observed against a natural hydrogen electrode at pH 7 when cell suspensions of electrochemically active bacterial species were tested by voltammeter (Kim et al., 1999a, 2006; Park et al., 2001; Pham et al., 2003). With the redox potential of oxygen (+ 0.82 V) at standard conditions, a maximum open circuit potential of 0.82 V is expected and infact a maximum open circuit potential of 0.8 V has been reported in a MFC (Liu et al., 2005). Youngin Choi and co workers reported power density of 563 mA / sq.m (10Ω) equivalent to 0.65 V in *Bacillus* sp. In the present study, effects of strain *Enterobacteriaceae bacterium* have been investigated for the maximum potential of fuel cells. This study documented the feasibility of bioelectricity generation from *Enterobacteriaceae bacterium* using a MFC fabricated with low cost anode materials (non coated plain graphite electrodes), without any toxic mediators. The procedure was cost effective and sustainable. The power generated by the *Enterobacteriaceae bacterium* was reported as 1.06 V. The chemically immobilized bacteria on to the surface of graphite felt electrodes supported production of continuous electric current and could be reused after storage. The response to additions of substrate when immobilized bacteria were used was faster than that achieved with freely suspended organisms (Robin et al., 1993).

Potential of 0.90 V was observed in immobilized *Enterobacteriaceae bacterium*. The non immobilized *Enterobacteriaceae bacterium* showed a retention time ranging between 6 to 8 days with respect to potential drop. The MFC with immobilized strain supported production of continuous voltage up to 12 days. This is attributed to the advantageous mass transfer kinetics resulting from the proximity of immobilized bacteria and the electrode surface. This study demonstrates the optimization of nitrogen sources to produce high power densities at enhanced voltages. The nitrogen sources peptone and different ammonium salts were chosen for the maximum production of voltage. The maximum voltage 0.92 V drawn for the strain6 (*Enterobacteriaceae bacterium*) was developed by 10 g / L concentration and the longevity was increased up to 15 days. The effects of various ammonium salts on strain 6 (*Enterobacteriaceae bacterium*) have been investigated, while the voltage from ammonium nitrate and ammonium sulphate produces 0.72 V and 0.78 V with the loss of potential after 5 days. The ammonium chloride provides maximum voltage of 0.89 V with sustainable voltage up to 10 days.

Conclusions

This study not only validates Microbial Fuel Cells as a renewable and alternative energy source, but also suggests that a very simple MFC design is capable of generating appreciable power.

The use of salt bridge may exhibited quite high internal resistance; however, connecting the immobilized strains (*Enterobacteriaceae bacterium*) in series allowed us to power small loads such as LED with the voltage of 2.3 V. It showed appreciable result compared to lightening of LED with 4.5 V (Josh McCready and Tess Edmonds, 2008). Bacteria use the anode in their metabolism; they strategically position themselves on the anode surface to form a bacterial community called bio-film. Bacteria in the bio-film produce a matrix of material so that they stick to the anode. The bio-film matrix is rich with material that can potentially transport electrons. The sticky bio-film matrix is made up of complex extracellular proteins, sugars, and bacterial cells. The matrix also has been shown to contain tiny conductive nano-wires that may help facilitate electron conduction. Clearly MFC technology is an emerging and very promising alternative to traditional energy sources. The simplicity of this design illustrates the potential for installing these devices in aqueous environments without disturbing the ecosystems. Furthermore, with sufficient cells and anode surface area, remote power from bacteria is a very real possibility. MFC technology is shown to be an alternative and promising way for the electricity generation. Salt bridge MFC is the simplest biological fuel cell that can be designed and studied (Mohan et al., 2008).

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