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Evaluation and Exploration of Electricity Generation by Microbial Fuel Cell Isolated from Warje Waste Water Treatment Plant, Pune, India

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ABSTRACT

A microbial fuel cell (MFC) is bioreactor that converts chemical energy into electrical energy through metabolic activity of microorganisms under aerobic/anaerobic conditions. Study was carried out to test the performance of the cathodic electron acceptor and anodic electron donor which plays important role in microbial fuel cells for generation of electricity. The aim of our study is to explore these electricity generating bacteria. The two-chamber MFCs were used to conduct experiments. Total eight bacterial isolates were obtained from samples, out of which six isolates were prominent for generating electricity. The MFC was constructed and electricity generation was measured after various intervals. The effect of salt on MFC was studied, as well as source of carbon was altered, also the concentration of agarose was changed to study its effect. The bacterial isolate obtained from Warje waste water gave maximum 429mV. The series connection of five MFC chambers containing bacterial isolate from Warje waste water gave voltage of 65mV . The bulb (LED) glowed prominently at this voltage. Our results also indicated that searching and isolating bacteria that are more stable electricity producing bacteria could be a new strategy to for isolation and MFC from waste water treatment.

***Key words**: Microbial fuel cells, MALDI-TOF, Bioelectricity*

Introduction

The most crucial element in today's world is energy. The need for time is to identify new energy sources. As a result of rising human activity, natural energy sources are being depleted, which affects fossil fuels. Nearly all of the traditional energy generation methods used today, which call for the burning of harmful fossil fuels, are expensive and not regarded as environmentally friendly (Zain *et al.*, 2009; Mohan *et al.*, 2007). According to the US Department of Agriculture and Energy, biomass energy can replace fossil fuels for roughly 30% of the country's energy demands (Zhiyong *et al.*, 2007). There are numerous ways to generate electricity. Over the world, multidirectional research is being done on the production of electricity. Microbial fuel cells (MFCs) have become a promising but difficult technology in recent years. In an MFC, microorganisms communicate with electrodes by exchanging or supplying electrons via an electrical circuit. MFC is viewed as a viable sustainable technology to satisfy rising energy needs, particularly when wastewaters are used as substrates because it can generate electricity and treat wastewater at the same time, potentially reducing the operational costs of wastewater treatment facilities (Ionannis *et al.,* 2005).

Potter was the first to note that microorganisms may produce electric current. Yet, both in terms of the quantity of researchers and the applications for these systems, the real interest in MFCs has expanded significantly in recent years. Using platinum electrodes, Potter proved in 1910 how living cultures of either *Escherichia coli* or *Saccharomyces* may produce electrical energy (voltage and current). Since researchers had already shown how bacterial enzymes oxidise food, Cohen reintroduced Potter's MFC in 1931, bringing attention to this crucial discovery (the first reported MFC). Anodic and cathodic chambers of an MFC are divided by a proton exchange membrane (PEM). The semi permeable membrane or agar salt-bridge that joins the two chambers of the microbial fuel cell allows these released electrons to pass through (MFC). The transferred electrons produce a current that can power a variety of devices.

Many bacteria, including *Shewanella putrefaciens, Geobacter sulferreducens, G. metallireducens*, and *Rhodoferax ferrireducens*, use c-type cytochromes, biofilms, and highly conductive to transmit electrons from inside the cell to extracellular acceptors. These bacteria can develop biofilms on the anode surface, act as electron acceptors, and transmit electrons directly to the anode, increasing the amount of energy produced. They also have high coulombic efficiency. A microorganism's (*Shewanella oneidensis, Geothrix ferementans*) own mediator, which in turn facilitates extracellular electron transfer, or additional mediators are used to transfer electrons from microbial carriers onto the electrode surface. The bacteria can produce reduced products that are electrochemically active on a platform provided by mediators. As *Proteus vulgaris, Escherichia coli, Streptococcus lactis*, and *Pseudomonas species* cannot transmit electrons beyond the cell, mediators are necessary in MFCs using these bacteria.

Global energy demands are rising, and research programmes are concentrating on alternative, renewable, and carbon-neutral energy sources in an effort to support energy independence. Microbial fuel cells (MFCs), which use microorganisms to produce electrical energy, are one such renewable and sustainable technology that is regarded as one of the most effective and carbon-neutral energy sources. One such effort to offer a different and environmentally beneficial energy source is the use of wastewater to power microbial fuel cells. Waste water can be used by MFC to generate power, which serves the dual purposes of energy generation and waste water usage. So, the goal of the current study was to isolates the bacteria that produce electricity from different water sources and soil, identify them, optimise the medium, and investigate the effects of different parameter on production of electricity in MFC.

Materials and Methods

Collection of samples

Water samples were collected from various places in Pune in sterilized BOD bottles, the places were Pashan lake, Katraj lake, Fabrication industry waste water, Card coating industry waste water, soil samples from petrol pump and garage, waste water from Warje region.

Isolation and Identification of microorganisms

Enrichment of water samples and soil samples were carried out in nutrient broth. All flasks were incubated at 37 °C for 24h. The collected samples were inoculated in nutrient broth and were kept for incubation for 24h. Further the loopful culture was streaked on nutrient agar plate and kept for incubation for 24h. Well isolated and morphologically distinct colonies from these plates were transferred on the respective medium slants and maintained as stocks. Gram staining, motility and biochemicals test were performed for all the isolates.

MALDI-TOF

The identification of isolate was carried out by MALDI-TOF MS and MALDI Biotyper database. Among eight isolates one isolate was identified by MALDI-TOF by direct transfer and ethanol extraction method. MALDI Biotyper and Flex Analysis version 3.4 software were used to match the isolated organism. The isolated sample was given away to NCMR, Pune to carry out MALDI-TOF.

MFC assembly design and component

Electrode

Aluminium wire was used as electrode. It was used at both the ends of cathode and anode and tightly fixed with the containers containing medium, culture and distilled water.

Cathodic chamber

The cathode chamber of the MFC was made up of 400ml plastic jar filled with 150ml distilled water as catholyte.

Anodic Chamber

400ml plastic jars were used for this purpose. The bottles were surface sterilized by washing with 70% ethyl alcohol and followed by UV exposure for 15 minutes. Then 24h old culture of the desired bacteria was inoculated in Nutrient broth (150ml) of previously enriched broth culture of bacteria was added to it.

Salt bridge

The salt bridge was prepared by dissolving 3% agarose in 2M NaCl. The mixture was boiled for 2 minutes and poured in PVC pipes under aseptic condition. The salt bridge was properly sealed and kept in refrigerator for proper settling. Two holes were made in the lower side of bottles for the insertion of salt bridge.

Circuit Assembly and Measurement of potential difference and current

Two chambers were internally connected by salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes at its two ends and to the multimeter by another two ends. The potential difference generated by the Fuel Cell was measured by using multimeter from HAOYUE –DT830D.

Media optimization

Media optimization is a process where components of media and growth conditions are varied in concentration or changed respectively, so that we can get better growth of organism for high productivity. Carbon sources like Glucose, Xylose and Fructose utilization was checked for isolated bacteria, Effect of pH and temperature as well as various concentration of agarose and effect of NaCl and KCL on MFC were studied.

Negative Control

Sterile un-inoculated nutrient broth was used as negative control. For negative control, 150ml of nutrient broth was used in anode chamber instead of using inoculated culture of organisms.

Results and Discussion

Collection of Samples

Waste water and soil are rich source of microorganisms, wastes water samples were collected from fabrication industry and card coating industry. The microorganisms ability to oxidize the organic matter in water to perform its biological activities makes it potential to produce electricity. Total seven isolates showed electricity generation more than 150mV. Only one isolate showed less than 150mV electricity generation. The microbial fuel cell was observed upto 48h and the voltage was recorded at every 2h interval in all cases.

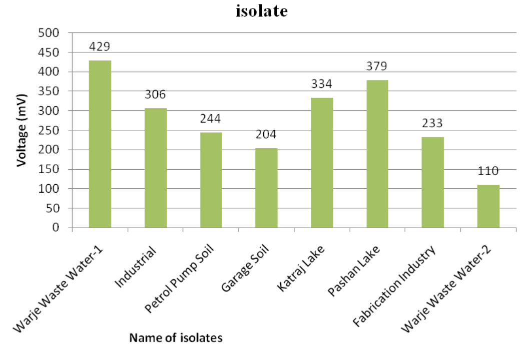
Isolation and Identification

Total nine samples were processed and eight isolates were obtained and all eight isolate were found electricity generating bacterial cultures. Standard biochemical tests were performed for all isolates. Each isolate in MFC was runned for upto 48h and voltage reading was noted down after every 2h using multimeter at 2000mV.

Maldi-t of MS Results

The graph (Fig. 1) below shows highest reading of each isolate and one isolate from Warje Waste water-1 was observe highest electricity generation than all other bacterial isolates. Warje Waste water-1 screened for identification based on MALDI-TOF MS and other MFC operation. The strains with more than 2.0 score values means reliable species-level identification, strains with score value ranging from 1.7 to 1.99 indicate genus-level identification, and the strains with score values less than 1.7 could not be identified by MALDI biotyper database. Warje

Fig 1: Electricity generation for MFCs with pure culture isolate

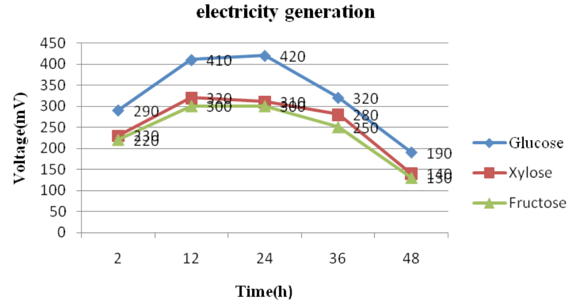


Waste water-1 bacterial culture identified as *Acinetobacter baylyi* DSM 14959 DSM. As *Acinetobacter* isolated from waste water gave maximum reading among the eight isolated organisms, it was selected for further MFC study. The bacterial isolate *Acinetobacter baylyi* DSM 14959 DSM from waste water showed voltage generation of 429mV after 2h of assembly set-up. This technology treats wastewater while also producing power by using the bacteria that are already present in it as catalysts. In a similar study carried out by Sabat *et. al.,* (2013) they had also isolated organism from waste water and showcased the ability of MFC along with waste water treatment. Isolated organism found and identified as *Acinetobactor baylyi* the best amoung the other isolates. The similar study was carried out by Dhundale *et al.,* (2017) they had isolated total twenty-eight bacteria from Lonar soda crater lake, they carried out certain cultural, morphological characteristics and standard biochemical test to avoid several identical isolates from total isolates. The electricity generated by ARS4 was 387mV after one hour.

Media-optimization

Media optimization was carried out in which pH of growth media was varied, different carbon source were used and temperature was varied. Media optimization is a process where components of media or different conditions either varied in concentration or changed so that can be get better growth of *Acinetobactor baylyi* for high productivity of MFC. In experiment carried out carbon sources like Glucose, Xylose and Fructose were studied and observed effect of these carbon sources on electricity generation. In the present studies it was revealed that, glucose as carbon source showed more electricity generation 420mV after 24h followed that than xylose was second carbon sources was more prominent for generating electricity (310mV) in MFC than fructose (300mV). This indicates that glucose was used by bacteria for growth and production of electricity in MFC. In similar study carried out by Shrivastava and Ayachi (2012), they had used waste water from bread factory containing organic matter like starch, glucose and sucrose. Different concentrations of carbohydrate solutions were made and filter sterilized by syringe filter method and pour into anode chamber. The amount of glucose was already present in bread factory wastewater is 3g/l and voltage generated by this concentration was 510mV. The concentrations used were 3g/l, 4g /l, 5g/l,6g/l , 7g/l, and 8g /l . It was found that maximum voltage (910mV) was generated when glucose was added in concentration of 5g/l. This showed that glucose is more preferred by bacteria over other sugars which were tested.

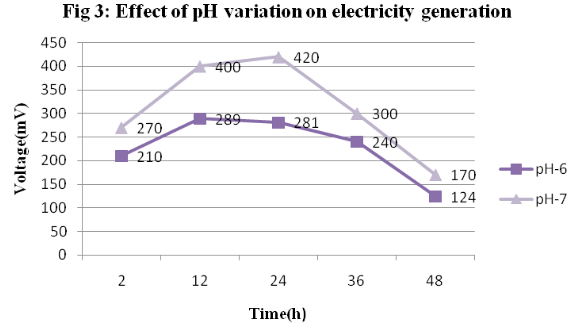
Fig 2: Comparitive study of effect of sugars on electricity generation



Effect of pH on Electricity generation

Media was prepared varying its pH to be 6,7 and 8. *Acinetobactor baylyi* showed very less or no growth at pH-8. Whereas *Acinetobactor baylyi* optimum grown at pH-7 and showed normal growth and higher electricity generation (420mV) but when pH of medium was 6 then electricity generation goes decline upto 33% (280 mV). This shows that pH-7 was the normal pH required by the bacteria to grow in the medium. Das (2013) in their study had grown organism at different pH range varying from 5.0-8.0 using bacterial isolate SM10. They found that pH-7 is the optimum for the growth of organism and also to perform its metabolic activity. The graph (Fig. 3) shows effect of pH variation on electricity generation.

Fig 3: Effect of pH variation on electricity generation



The isolated organism was grown at two different temperatures and was added to the anodic chamber of MFC, it was found that organism incubated at 37°C showed better electricity generation (420mV) then the electricity generation (301mV) at

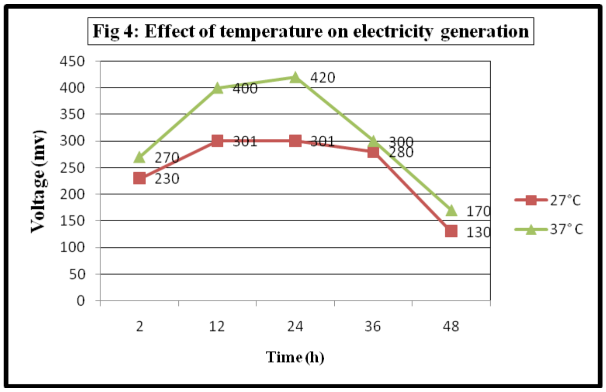


Fig 4: Effect of temperature on electricity generation

room temperature (27°C). This indicates that organism requires 37°C temperature for its growth and to perform metabolic activity. The graph plotted (Fig. 4) shows effect of temperature on electricity generation.

Effect of using NaCl and KCl in salt-bridge on electricity generation from *Acinetobactor baylyi*

Salt bridge is device used to connect the cell, made up of agarose and salt as electrolyte. It maintains the electrical neutrality within the internal circuit. The effect of NaCl and KCl used in salt bridge was studied. In the present study carried out it was found that use of NaCl in salt-bridge showed more electricity generation than KCl. 2M NaCl and 2M KCl was used as electrolyte in making of salt-bridge. It maintains the electrical neutrality within the internal circuit. In the similar studies on effect of NaCl and KCl carried out by Parkash *et al.*, (2015) involved Salt Bridge which is the most economical component in the dual chamber MFC. KCl and NaCl were compared for use as strong salt in salt bridge. Their study clearly showed that there was not much difference between these salts in terms of voltage output. Molar concentration of salt is critical since the

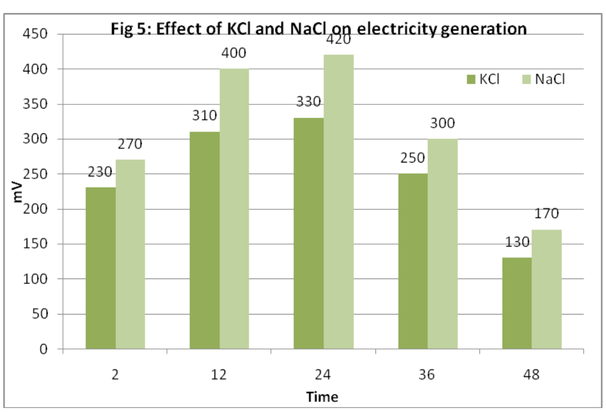


Fig 5: Effect of KCl and NaCl on electricity generation

transfer of protons through the salt bridge is facilitated by the dissociated ions in it. There experiments showed that, with increase in molar concentration the current decreases. Optimum results were obtained for salt bridge fabricated using 1M NaCl. It produced a maximum voltage of 0.372 V. The salt bridge MFC needs to be studied extensively, as the literature available on salt bridge based MFC is not sufficient.

Effect of variation in agarose concentration on electricity generation from *Acinetobactor baylyi*

In study on effect of agarose percentage on electricity generation, different concentration agarose (4%, 5%, and 6%) were used in salt-bridge. The different concentration agarose did not help much in electricity generation, however when 5% agarose was used (400mV at 12h), it was seen that the electricity generation was increased than the 4% and 6% agarose were used (Fig. 6). When 6% agarose was used the electricity generation decreased than that of the 4% agarose. Similar study was carried out by Renganathan *et al.* (2013) in which they had used agarose concentration ranging from 7% to 12%. Their study confirmed the effect of agarose concentration on the performance of the microbial fuel cell as the agarose concentration indicates the permeability of the gel facilitating the transfer of proton.

They found that optimal concentration of agarose for the fabrication of salt-bridge is 10% concentration as it showed maximum power density. Rengathan *et al.* (2013) revealed that, internal resistance hinders the production of electricity as higher the grade of polymerization of the gel, internal resistance builds up inside the cell. Thus an optimal concentration of agarose is preferred while constructing a salt-bridge as it enhances the voltage production as well as production of bioelectricity.

Series connection of five MFCs

The current in series circuit goes through every component in the circuit. Therefore all the components in a series connection carries the same current. The principal characteristics of series connection were that it has only one path in which current can flow. The anode of one MFC was connected to the cathode of next MFC using copper wire, likewise five MFC assemblies were connected in series. The cathode of first assembly and the anode of last assembly were kept free. These inter-connected assemblies acted as whole cell and the free cathode terminal

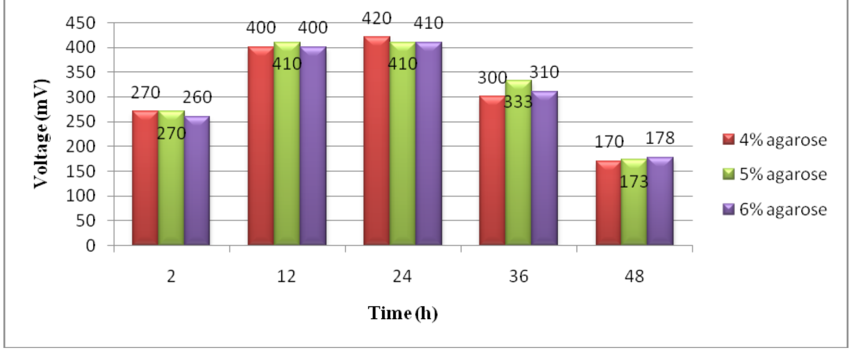


Fig 6: Effect of variation in agarose percentage on electricity generation

acted as positive terminal and anode as negative terminal. When we connected five separate cells (using isolated *Acinetobactor* from waste water) in series, a voltage of 0.65 V was generated. The two copper wires one end was connected to cathode and anode of cell respectively and other end to the positive and negative terminal of the LED. LED bulb glowed for sometime when connected to this circuit.

Conclusion

A total eight bacterial isolates obtained in the isolation exercise, cultural, morphological characteristics and standard biochemical test were performed for of all the isolates. Out of eight, seven bacterial cultures were selected for the electricity generation; out of seven *Acinetobacter baylyi* was selected for the further MFC studies. The MFC was constructed and measure the electricity generation after various intervals, 429mV was electricity was generated after 2h..The effect of salt on MFC studied, NaCl enhanced electricity generation than the KCl, indicating a bacterium *Acinetobacter baylyi* required NaCl for the MFC, sugar had an even greater effect on execute the electricity generation by MFCs. LED bulb glowed when five MFC were connected in series connection. Our results suggest that maintaining proper growth conditions for bacteria increases electricity generation and that MFC could be a new strategy to generate electricity.

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