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COMPARATIVE ANALYSIS OF ELECTRIC CURRENT PRODUCTION BY *Saccharomyces cerevisiae* USING A DUAL CHAMBER MICROBIAL FUEL CELL

BRISHNA KHAN[1], AYMEN RATHORE[1], AMAL ADNAN[1], SAIRA YAHYA[1*] AND M. L. MOHAMMED KALEEM ARSHAN[2]

[1]Department of Biosciences, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology, Karachi, Pakistan.

[2]Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi, Tamilnadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors BK, AR and AA designed the study and managed the literature search. Authors BK and SY wrote the first draft of manuscript. Authors BK, SY and MLMKA analyze the study and wrote the final draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Microbial Fuel Cell is a promising tool that utilizes microorganisms as biocatalysts to transform chemical energy into electrical energy. The particular microbe is able to utilize different substrates via metabolic activity resulting in the generation of electric current. The reason for the growing popularity of MFC is due to its multifaceted applications attributed to its eco-friendly nature. Even though the current generation is in milliampere (mA) scale, MFCs have unlimited benefits for future purposes. Fungi are among the microorganisms able to generate electricity as a result of their metabolic processes. The microorganism used in this study is *Saccharomyces cerevisiae* (baker's yeast). Electrons are diverted from the electron transport chain of this single-celled eukaryote which therefore helps in the conversion of chemical energy into electrical energy. The point of this examination is to measure the amount of electric flow produced by *Saccharomyces cerevisiae*, when using copper electrodes with mediator and sources of a substrate on the production of current. For the reaction to be carried out, Potassium permanganate ($KMnO_4$) was used as a mediator. The best results (105.3 mA) were obtained from maltose as substrate. Thus, here we describe the main findings, which can be used as the starting point for future investigations. We show that fungi have the potential to act as electrogens.

Keywords: Microbial fuel cell; metabolic activity; *Saccharomyces cerevisiae*; electrodes; potassium permanganate; mediator.

1. INTRODUCTION

A microbial fuel cell (MFC), is a device that converts chemical energy released as a result of the oxidation of complex organic carbon sources used as substrates by microorganisms into electrical energy, showing to be a cost-effective and sustainable energy source [1-7]. MFCs are mainly used for energy production and waste water treatment. Electricity is generated within the cell completely via microbes in order to disintegrate the organic compounds and the energy produced can be reused. Since there is a global energy crisis around the world thereby alternative sources have been built in terms to make energy [8].

**Corresponding author: Email: saira.yahya@yahoo.com;*

The current production in almost all microbial fuel cells are low and the only factor that pushes this cell to produce enough current is this type of microorganism used as well as different electrode materials. *Geobacter sulfurreducens* has the highest ability to produce electric current inside the cell along with the anode films and the type of anode in response to any organic waste being used (Strycharz & et.al., 2011). Oxygen and hydrogen percentages also vary as they can either help the microorganism to generate more current or shorten its production [9].

Microbes are placed in an anodic chamber of the MFC where oxidation takes place and substrates are added and there is generation of electrons and protons in the cell. The generation of carbon dioxide in the cell is an oxidative product. Once the salt bridge is attached between both chambers, all the protons go inside the cathodic compartment where they join with the O_2 in order to make H_2O molecules. Whereas the microorganisms that are placed inside the anodic chamber help extract all the electrons as well as protons during this procedure. Current production is possible in this cell only if the microbes are kept apart from the oxygen source, except at the anode end terminals as it is an anaerobic process. The microbial fuel cell, also known as a bioreactor can also produce electric current from the electrons that are found in the anode to cathode while they are in flow towards the external circuit. Previously this MFC was being used as a biocatalyst and this concept was introduced by Potter back in 1910 and the production of electric current was achieved by living cultures such as *Escherichia coli, Saccharomyces sp.* These two cultures when used alongside platinum electrodes were not able to generate enough current until the new MFC design which was introduced in 1980 [10].

The microorganisms work by oxidizing organic wastes to CO_2 and produce electrons and protons [11-18]. These electrons are the key reason for producing current while traveling from cathode to anode. The main principle through which CO_2 electrons, and protons are produced is Electron Transport Chain. The ability to produce current varies with microorganisms and the substrates that are provided as nutrient sources mainly organic substrates. Fungi have been used in the MFC systems in two main modes. In the anode (electron transfer is realized directly through redox-active fungal proteins or through chemical mediators facilitating the electron transport) or in the cathode (fungi are the source of enzymes catalyzing the reduction of a terminal electron acceptor mainly oxygen). Yeasts are more capable of producing electric current as compared to bacteria because of the difference in their structure and metabolic pathways, as yeast have complex structure due to the presence of a nucleus which leads to the complex pathways of metabolism [19]. Yeast has evolved to be exceptionally proficient at enduring sudden and gradual harsh changes in the environment outside the cell [16-18,20-22]. The most common yeast, *Saccharomyces cerevisiae* have been found on the plants surfaces, the GITs, and body surfaces of insects and warm-blooded animals. Yeast can also be found in the soil from all over the world in aquatic environment and most often in areas where fermentation can occur such as on the surface of fruit storage container and on the equipment used during fermentation process.

2. METHODOLOGY

2.1 Materials

2.1.1 Salt bridge preparation

Agar (2.5 g) and sodium chloride (2 g) were weighed and suspended in 100 ml of distilled water. The medium was continuously stirred and heated on a hotplate stirrer until all the ingredients were subsequently dissolved.

2.1.2 Nutrient mineral buffer (pH 5.6)

Desired amount of $NaHCO_3$ (3.13g/L), (0.1g/L) NH_4Cl, (0.05g/L) NaH_2PO_4, (0.10g/L) $CaCl_2$, (0.12g/L) $FeSO_4$ and (0.13g/L) KCl were weighed and suspended in 1000 ml of distilled water. The medium was continuously stirred and heated on a hotplate stirrer until all the ingredients were subsequently dissolved and autoclaved.

2.1.3 Electrodes and multimeter

A set of copper (152 mm x 51 mm x 3 mm) was purchased from a local electrical store. A multimeter was obtained from the mechatronics laboratory in SZABIST (KHI) for current detection.

2.1.4 Mediator preparation

Desired amount of (3.3 g) $KMnO_4$ was weighed and suspended in 100 ml of distilled water. The medium was continuously stirred and heated on a hotplate stirrer until all the ingredients were subsequently dissolved. The $KMnO_4$ mediator that was prepared was 208.8 mM.

2.1.5 Substrate collection and preparation

Desired amount (20g) of glucose, maltose and sucrose were weighed and suspended in 100 ml of distilled water separately. The medium was continuously stirred and heated on a hotplate stirrer until all the ingredients were subsequently dissolved. For banana

peels and potato peels, 26g were weighed and added to 100 ml distilled water respectively. Nutrient mineral buffer (2:1:3) was then added to the substrates and the medium was autoclaved.

2.1.6 Inoculum preparation

Packet form of baker's yeast (*S. cerevisiae*) was obtained from the local grocery store and 5 grams of it was added to the test tubes containing the YEPD broth (yeast extract: 1 g peptone: 2 g and dextrose: 2 g) and incubated to proliferate and grow.

2.2 Construction of Microbial Fuel Cell

The microbial fuel chamber was constructed by joining two 1000 ml bottles with the help of a plastic pipe that contained the salt bridge that passed through the top of both bottles. The salt bridge acted as a proton exchange membrane. One bottle was used as an anodic chamber (anaerobic chamber) and the other bottle was used as an aerated cathodic chamber (aerobic chamber). On each side of salt bridge two sterilized copper electrodes for both cathode and anode were provided in their respective bottles for their separate set of experiments with the respective substrates.

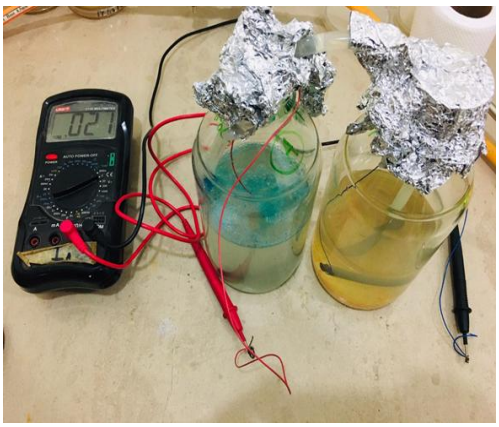

Fig. 1. Microbial fuel cell setup

2.3 Operation of Microbial Fuel Cell

A total of 14 experiments were set up with their respective requirements. For every run, both the anode and cathode chambers (aerobic and anaerobic) were filled with 500 ml of nutrient mineral buffer (NMB). 10 ml of yeast which was grown in the YEPD broth was added into the anode chamber with the subsequent substrate. 5ml of $KMnO_4$ was added as mediator. After completing the setup, both the chambers were sealed with aluminum foil to avoid contamination. When the yeast acted on the substrate, ethanol and CO_2 were produced with H^+ (proton), this process is known as fermentation. The H^+ moved across the salt bridge (PEM) from anodic chamber to cathodic chamber. The setup was operated for 3 days and the final results were measured on the 3rd day. Once the experiment was completed both the compartments were then washed and sterilized to start another cycle with different substrate.

3. RESULTS AND DISCUSSION

3.1 Profile of *S. cerevisiae* Mediated MFC with Simple Substrates

The simple sugars used in this research were glucose, maltose and sucrose. $KMnO_4$ was used as mediator in all the experiments. All experiments were performed with *Saccharomyces cerevisiae.* Maltose gave the highest current (105.3 mA) when paired up with *S. cerevisiae* alongside copper electrodes in the presence of $KMnO_4$ mediator.

Table 1. Current generated from *S. cerevisiae* mediated MFC with Simple substrates

Substrate	Result
Dextrose	56.9 mA
Maltose	105.3 mA
Sucrose	26.7 mA

3.2 Profile of *Saccharomyces cerevisiae* Mediated MFC with Complex Substrates

After simple sugars, complex substrates were run including potato peels and banana peels. $KMnO_4$ was used as mediator in all the experiments. All experiments were performed with *S. cerevisiae.* Potato peels gave the highest current (83.5 mA) when paired up with *S. cerevisiae* alongside graphite electrodes in the presence of $KMnO_4$ mediator.

Table 2. Current generated from *S. cerevisiae* mediated MFC with complex substrates

Substrates	Results
Potato peels	83.5 mA
Banana peels	0.97 mA

As stated before, the best results 105.3 mA were attained by maltose as a substrate as compared to any other substrate when used with copper electrode. According to hypothesis, complex substrate might produce better results when *S. cerevisiae* acts on it. The results that were obtained proved the hypothesis. All the previous studies were held with simpler substrate including sucrose, glucose or the

combination. According to Abdel-Naser Ahmed Zohri (2018) the result (0.175mA) was obtained with sugarcane molasses but the yeast was *Meyerozyma guilliermondii.*

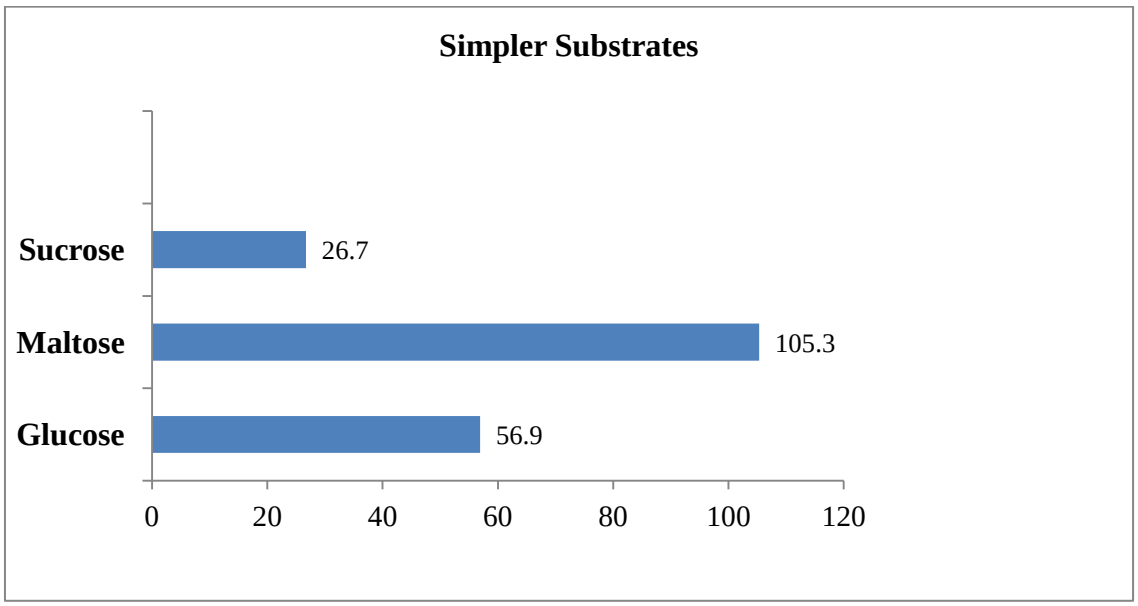

Fig. 2. Profile of *S. cerevisiae* mediated MFC with simple substrates

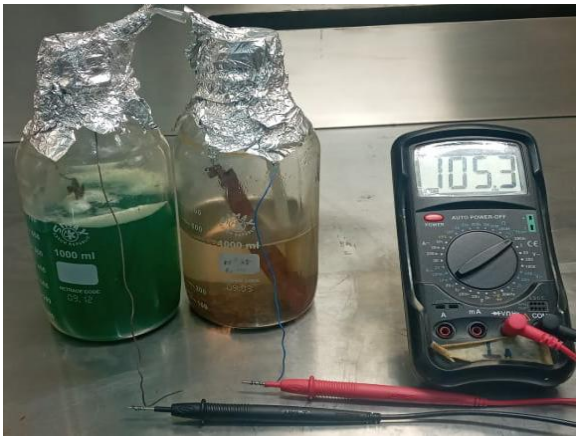

Fig. 3. Current generated using maltose, mediator and copper as an electrode with *S. cerevisiae*

Fig. 4. Current generated using sucrose, mediator and copper as an electrode with *S. cerevisiae*

Fig. 5. Current generated using glucose, mediator and copper as an electrode with *S. cerevisiae*

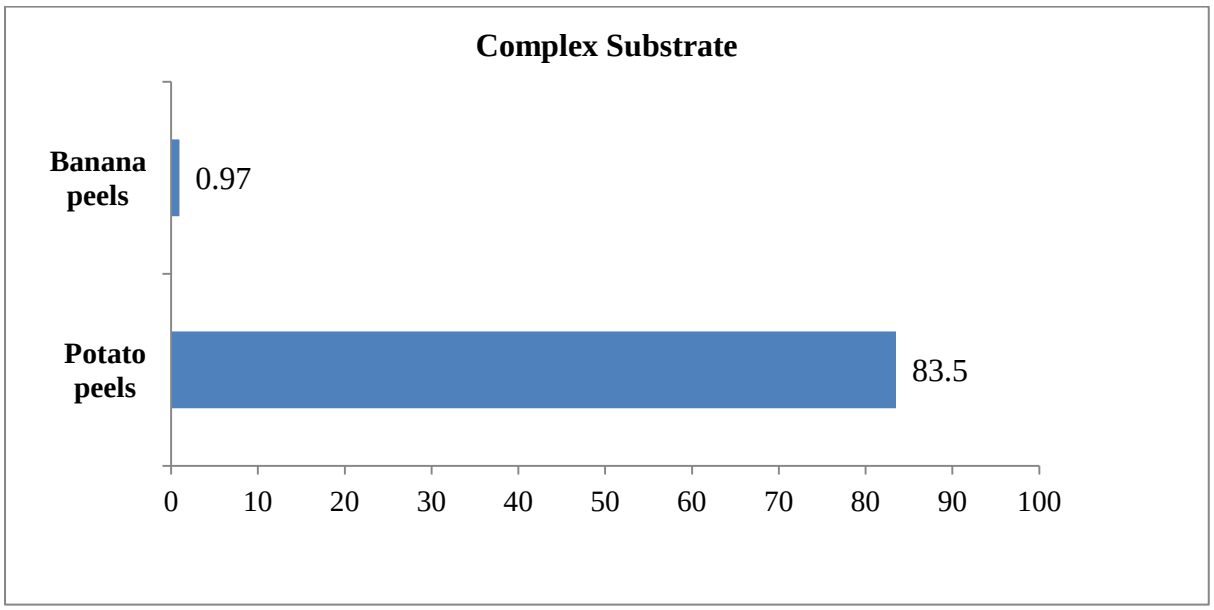

Fig. 6. Profile of *S. cerevisiae* mediated MFC with Simple substrates

Fig. 7. Current generated using banana peels, mediator and copper as an electrode with *S. cerevisiae*

Fig. 8. Current generated using potato peels, mediator and copper as an electrode with *S. cerevisiae*

Different microorganisms that are less harmful can be used as biocatalyst specifically bacteria and yeast. These microorganisms work by oxidizing organic wastes to CO_2 and produce electrons and protons. These electrons are the key reason for producing current while traveling from cathode to anode. The main principle through which CO_2 electrons, and protons are produced is Electron Transport Chain. The ability to produce current varies with microorganisms and the substrates that are provided as nutrient sources, mainly organic substrates. Yeasts are more capable of producing electric current as compared to bacteria because of the difference in their structure and metabolic pathways as yeast have complex structure due to the presence of a nucleus which leads to the complex pathways of metabolism [19]. Lee et al. [1] reported that several species of fungi to produce current in fungi-based MFC. Among these species, *S. cerevisiae* has been the most beneficial species. The power density produced by *S. cerevisiae* reached up to 1.5 Wm^{-2} when Methylene blue was added as a mediator.

Additionally different mediators can also be used to enhance the electron transfer via electrodes. These mediators include $KMnO_4$, Methylene blue, natural red, Potassium Ferro cyanide, etc. and among them, $KMnO_4$ and methylene blue show the best results in several experiments. In the present study, 208.8 mM of KMnO4 was used as a mediator. The reason for choosing $KMnO_4$ was to distinguish the results that were obtained from Methylene Blue in various studies. Dani Permanaa (2014) studied that 5mM of MB can produce 1700 mV of current when used as mediator. When $KMnO_4$ used as a mediator, 140 mV of current was produced by using kitchen wastes. A mediator-less microbial fuel cell was developed by Herrero Hernandez *et al.,* (2019) using *Escherichia coli* bacteria and platinized titanium mesh as electrodes, producing a maximum power density of 627 mW m−2, however the surface area and concentration of microorganisms affect the amount of current production. The higher concentration of *Shewanella putrefaciens* and larger surface area of electrodes (approx. 50 cm^2) produced current of about 3,000 mA within 12hrs (Hyung Joo Kim, et. al., 2002).

4. CONCLUSION

The study has shown that construction and working of dual chamber MFC using low cost materials (two media bottles, substrates, well known microorganism i.e. *S. cerevisiae* and electrodes is beneficial to produce energy. The variation in currents is due to the effects of different substrates and electrodes. Among all the substrates, maltose has shown the best result with the copper electrodes with the production of 105.3 mA. According to the experiments performed in this work, results from both the simple and complex substrates gives us the idea that indeed if we want to utilize our waste such as potato peels, we will be able to generate certain milliamperes worth of electric current which when implemented on a larger scale will benefit everybody, especially in the countries that have regular power outages. MFCs are one of the newest technologies to produce energy from different sources of substrates. Because of the promise of sustainable energy generation from different substrates such as organic wastes, research has been intensified in this field in the last few years. MFCs have different applications based on generated power. The generated power in MFC is still too low and researchers are working to improve it for commercial application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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