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Optimization of Agro-Industrial Waste Bioconversion into Bioelectricity

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Abstract

Bioelectricity is considered one of the most important sources of clean green energy that has attracted the attention of many scientists to discover some of its secrets. Moreover, agro-industrial wastes are widely used in the production of alternative energy in general and electricity in particular. There is an important agro-industrial waste produced during the molasses (black honey) industry in Upper Egypt, that waste is called (El-ghasheem). In the present work, El-ghasheem was used in an economical-designed microbial fuel cell (MFC) to obtain cheap green electricity. The 'H'-type MFC was operated with a capacity of 600 ml in each chamber, and copper wire was used to link the two graphite rod electrodes. Lamp wicks that had been soaked in a 1M KCl solution were used to create an inexpensive salt bridge. Factors affecting the production of bioelectricity from this MFC were optimized to get the optimum power. Bioelectricity production was enhanced using crude microbial laccase enzyme as a cathodic reaction biocatalyst. Laccase produced from *Penicillium chrysogenum* was used to increase voltage and current from 0.40±0.011 V, 0.02±0.002 A to 0.608±0.002 V and 0.05±0.003 A, respectively at 37 °C, anolyte pH 6, and catholyte pH 5 for a 10-day incubation period.

Keywords: Microbial fuel cell; Bioelectricity; Agro-industrial waste; Bioconversion; Laccase.

1. Introduction

Various human and industrial activities put extreme strain on non-renewable energy supplies. As a result of this urbanization, the non-renewable fossil fuel sources are rapidly depleted causing an environmental pollution. The combustion of fossil fuels releases a huge amount of different harmful greenhouse gases, which are the main cause of climate change and hazards for human health and the surrounding environment [1]. There are massive amounts of waste burned every year around the world. Occasionally, this waste is buried to keep it out of the environment. The majority of the garbage is burned on site, which contaminates the surrounding area. In order to be environmentally sustainable, an alternate approach must pair non- renewable energy sources with waste management. Using MFCs (Microbial Fuel Cells), the microorganisms generate ecologically friendly energy by utilizing different types of these wastes, is one such creative tactic [2]. MFCs are microbial reactors that use microorganisms to digest organic substances to produce green electricity. Different wastes resulting from agricultural and industrial activities can be used as feedstock for MFCs to convert stored chemical energy into electrical power. MFC is a cutting-edge technology that offers green and environmentally friendly energy generation, waste recyclability, and by-product utilization from a variety of sources. MFCs have been the subject of considerable research in recent years, with an emphasis on electrode development and utilization of various urban and rural wastes. The concept of MFC in large-scale applications is one of the best technologies for waste management and is also used to generate electricity to meet the needs of rural and remote areas not connected to the grid [3]. There are several applications for MFC; the most common one is wastewater purification in combination with electricity production. Valuable components production at the cathode, like H_2 and H_2O_2, is represented as another important application of MFC [4]. The MFC system is a technology that is recommended for producing energy due to its versatility in handling a wide variety of bacterial genera and their distinct waste products. There are different microbial genera, the majority of which are bacteria that are purported to produce electricity. Electrical current is known to be produced by a variety of bacteria. Yeast, microalgae, and fungi have also been employed to support the electrode in MFCs [5,6]. The most known design of MFC is the double chamber design, which consists of anode and cathode compartments that are separated by a salt bridge or a cationic membrane. The main action of microorganisms is the metabolization and digestion of organic compounds that are found in the anode chamber to generate electrons and protons. Electrons will be transferred after that to the cathode through an electrical circuit. Both electrons and protons are consumed in the cathode by the reduction of soluble electron acceptors such as oxygen [7]. El-ghasheem, or El-Reem, is an agro-industrial foamy liquid waste that resulted in huge amounts while boiling sugarcane juice to produce cane syrup (black honey) in Upper Egypt. Most of this

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el-ghasheem is discarded into sewage, although it contains valuable compounds and minerals [8]. This el-gasheem is similar to the scum layer that separated during the clarification process in cane sugar production. This layer contains sugars, nitrogen compounds, and others that are essential for microbial growth [9]. Recently, microorganisms have been widely used as biocatalysts to assist electron transfer and enhance cathodic reactions. In bio-cathodic system, different compounds are available to be used as terminal electron acceptors, such as oxygen, nitrate, sulfate, iron, manganese, selenate, and others [10]. These available varieties of electron acceptors make MFC important for wastewater treatment using bio-cathodes [11]. In this study, a double-chambered economical MFC was designed and fed with EL-ghasheem as the anodic organic matter. The physical and physiological conditions of this MFC were optimized to obtain maximum voltage. In the present work, microbial crude laccase produced from *Penicillium chrysogenum* was used as a cathodic reaction biocatalyst to increase the voltage and current produced from this MFC.

2. Material and Methods

2.1 Construction of the Microbial Fuel Cell

In this investigation, the 'H'-type MFC was designed as per Kamau et al. [12]. It was operated with 600 ml of electrolyte in both chambers. Two plastic containers were prepared as an anode chamber and a cathode chamber. Two small pores were made in the bottles' covers to introduce wire. The two electrodes were connected to each other using a copper wire. Both the anode and cathode were graphite rods with a diameter of 0.5 cm and a length of 4 cm. Lamp wicks soaked in a 1M KCl solution to be used as a low-cost salt bridge.

2.2 Performing economical MFC using El-ghasheem as organic feedstock

El-ghasheem was used in the anode chamber as organic matter feedstock, while the catholyte was tap water only. These MFC materials and electrolytes give it two advantages: lowering its design and operating cost and using agricultural waste instead of discarding it in the sewage. MFC chambers were fed as follows:

i- The anode chamber is made up of 90 ml of soil extract, various amounts of El-ghasheem, and up to 600 ml tap water. ii- The cathode chamber is composed of 600 ml of tap water only.

2.3 Optimization of bio-electricity production using El-ghasheem as the anolyte

Using different volumes of El-ghasheem (50, 100, 150, and 200 ml) with different anolyte pHs (4, 5, 6,7) at different operating temperatures (25, 30, 35, 37, and 42 °C). and different catholyte pHs (3, 4, 5, 6, 7) were performed for different incubation times (1-14 days). Voltage and current were measured to determine the optimum condition to obtain the maximum power.

2.4 Effect of laccase enzyme on the enhancement of bio-electricity

Crude enzyme with 1.4 ± 0.044 U/ml activity, which was detected before spectrophotometrically using guaiacol as substrate, was added to catholyte to determine its efficacy as a cathodic reactions bio-catalyst and compare the voltage and current resulted from control MFC (without laccase) and MFC including laccase at its cathode. This laccase had been obtained from *Penicillium chrysogenum* in the previous work.

2.5 Electrochemical Analysis

The power produced was calculated using the following formula: P = I x V, where P is power in Watts, I is current in amperes, and V is the electric potential in volts. 1K ohm resistance was used as an external load.

3. Results and Discussion

3.1 Performing MFC with different volumes of El-ghasheem

Using different volumes of El-ghasheem (50, 100, 150, and 200 ml), tap water was added up to 600 ml at the anodic chamber to determine the optimum concentration of organic material for microorganisms to liberate the highest number of electrons and obtain the maximum current density. It was shown that both voltage and current were the minimum values in the case of using 50 ml from El-ghasheeem, which produced 0.21±0.02V and 0.006±0.002A, respectively. When El-ghasheem volume was 150 ml in addition to tap water, it was 600 ml, MFC achieved the maximum voltage and current, 0.37±0.01 and 0.014±0.002, respectively **(Fig.1)**, while other conditions were 30°C incubation temperature, pH 7 for anolyte and catholyte, and ten days as the incubation period. Both the produced voltage and current became lower when the el-ghasheem volume increased by more than 150 ml. This may be due to the high concentration of sugar content, which directly affects microorganisms' physiological activities, while each microorganism has a certain organic matter tolerance. At the anode where el-ghasheem was used as organic matter material, microorganisms break down this organic substances and convert it bioelectricity as per the following chemical reaction:

$$CH_3COO + 2\,H_2O \longrightarrow 2CO_2 + 7H^+ + 8e^- \quad \text{(anodic reaction)}$$

In another study, Mohan et al. reported that a high electrolyte chemical oxygen demand (COD) concentration could lead to a reduction in electrical energy, and the flow rate of substances in the MFC IEM may be lowered [13]. Using domestic wastewater, at another work, the performance of MFC in the removal of COD was analyzed at various organic loading levels. It was noticed that the COD removal efficiency is greater than 90% as the organic loading rate increases. On the other hand, the COD removal efficiency declined to about 70% at a lower loading rate [14].

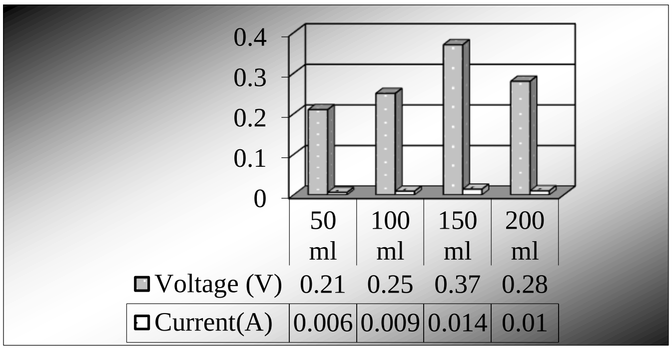


Fig.1:- Voltage and current production using different volumes of El-ghasheem

3.2 Performing MFC at different operating temperatures

MFC was employed at different temperatures using 150 ml of El-ghasheem, pH 7 for anolyte and catholyte, and a ten-day incubation period. It was shown that the optimum incubation temperature was 37 °C, which achieved the maximum voltage and current of 0.39±0.01 V and 0.017±0.001 A, respectively. The lowest voltage and current were 0.28±0.009 V and 0.011±0.001 A, respectively which were produced at 25°C **(Fig. 2)**. In another study, Liu et al. reported a 9% decrease in maximum power density when the MFC incubation temperature was lowered from 32 °C to 20 °C. Liu et al. suggested that this voltage and current are decreasing because of the reduction in cathode potential [15]. Moon et al., also observed an increase in the produced voltage and current when the MFC operating temperature was increased from 24 °C to 35 °C [16].

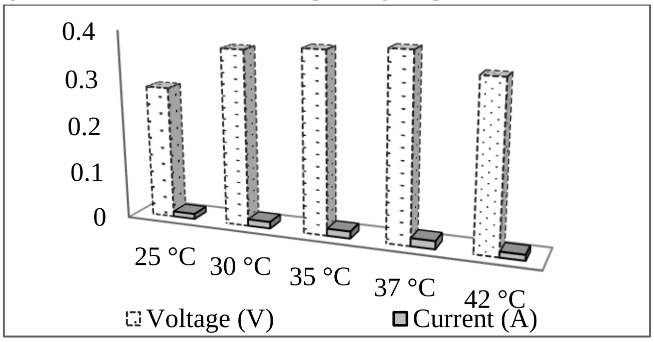


Fig.2:- voltage and current production at different incubation temperatures

3.3 Performing MFC at different anolyte pHs

Microbial physiological activities such as their metabolizing ability are attributed to the pH of the surrounding environment, so the organic matter digestion in the anode chamber is directly affected by the pH of the anolyte. At the present investigation, it was observed that the produced electricity increased by increasing pH from 4 to 6.The MFC produced maximum voltage and current, 0.4±0.011 and 0.02±0.002, respectively, at anolyte pH 6, then slightly decreased again when pH reached 7 **(Fig. 3)**. Rittmann and McCary stated that pH affects the microbial growth and activity in MFC chambers. They said that most microorganisms have a narrow pH range for growth and other physiological activities, which is between 6 and 8 [17].

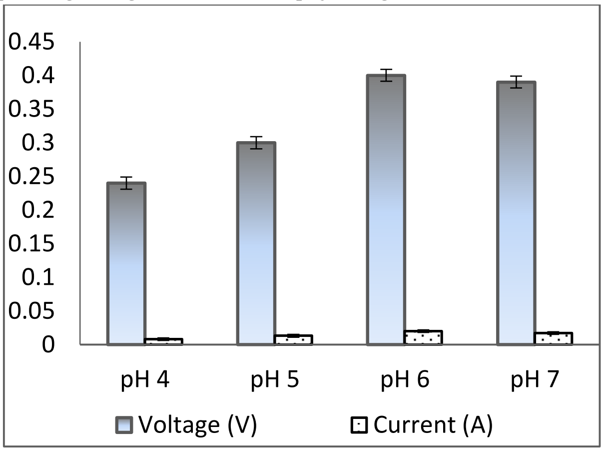
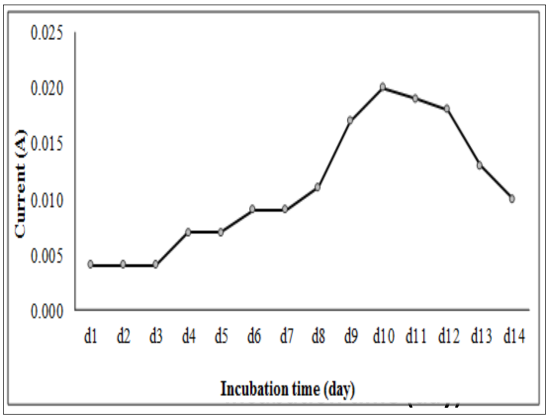


Fig.3:- Voltage and current production at different anolyte pHs

3.4 Monitoring of MFC performance during two weeks

Both voltage and current were recorded daily for fourteen days to determine the optimum incubation period. In our study, we obtained that MFC produced voltage and current after incubation for 24 hours at 37°C, pH 6 for anolyte and pH 7 for catholyte using 150 ml El-ghasheem, then both voltage and current increased until they reached maximum values, 0.4 V and 0.02, respectively, at the 10^{th} day, then decreased again **(Fig. 4.a,b)**. This change in MFC performance during the entire incubation period (14 days) is strongly attributed to the lag, log, stationary, and death phases of the anodic microorganisms. In this investigation, it was noticed that the lag phase of microorganisms was the longest phase, where they started from the 4^{th} day until the 9^{th} day, then began the log phase (the phase in which both growth and organic matter digestion increase rapidly) at the 10^{th} day. Microorganism growth and nutrient utilization start to decrease significantly after the 12^{th} day, when organisms enter their death phase. In another study, Hegazy et al. reported a lag phase of four days; the log phase was continued until the 11th day, before the start of the stationary phase. After twelve days, the organism entered the death phase with a gradual decrease in the voltage and current produced [18]. Several studies have recorded different optimum incubation periods. For example, when using a halo-alkaliphilic archaeon to generate electricity, Hegazy et al. stated that the maximum volt reading was recorded on the 24th of the incubation period [19].



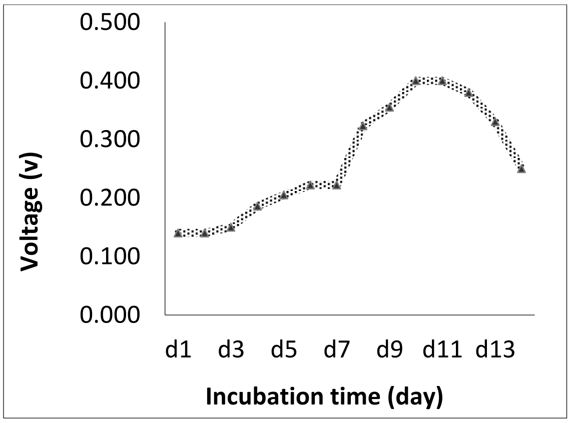


Fig.4a:- Current production from MFC daily during two weeks

Fig.4b:- Voltage production from MFC daily during two weeks

3.5 Enhancement of electricity generation using laccase enzyme

Laccases are widely used as cathodic reaction biocatalysts in different MFCs due to their ability for reversible adsorption onto carbon electrodes. Laccases are also used as a biocatalyst for the oxidation of several chemical substrates at a single copper -containing site [20]**.** In several studies, it was proved that laccase enhanced electricity production. Sato et al. recorded that using laccase as a biocatalyst in MFC produced 249.67 mV which was 23.8% more than the 202.33 mV resulted by the negative control (without laccase) [21]. In the present study, crude laccase enzyme with 1.4 ± 0.044 U/ml laccase activity was used to increase the produced voltage and current. This laccase produced from *Penicillium chrysogenum* Thom-AUMC 15310, which was isolated from arid soil in the vegetation area of the Western Desert. Using this laccase in the cathodic chamber as a biocatalyst showed an increase in voltage and current from 0.40±0.011 V, 0.02±0.002 A, to 0.608±0.002 V, and 0.05±0.003 A, respectively, at 37 °C, anolyte pH 6 for a 10-day incubation period **(Fig. 6)**, after the catholyte pH was,optimized for laccase activity, which showed the maximum activity at pH 5 **(Fig.7)**. This laccase catalyzes the cathodic chemical reaction that can be represented as the following diagram **(Fig.5)**:-

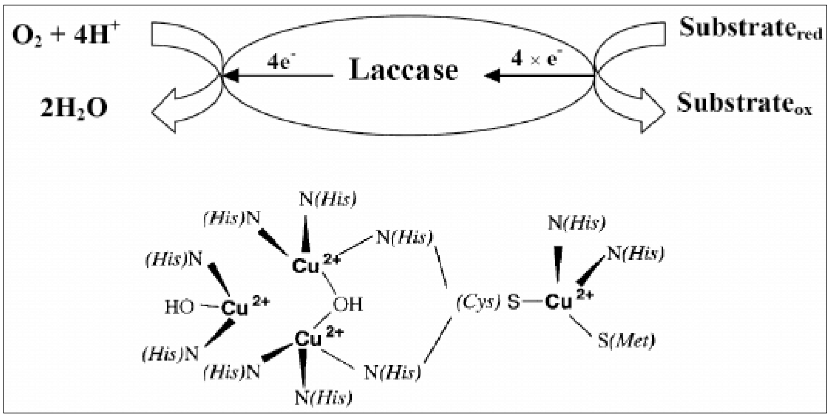


Fig.5:- Schematic diagram of catalytic reaction of laccase and proposed active site structur

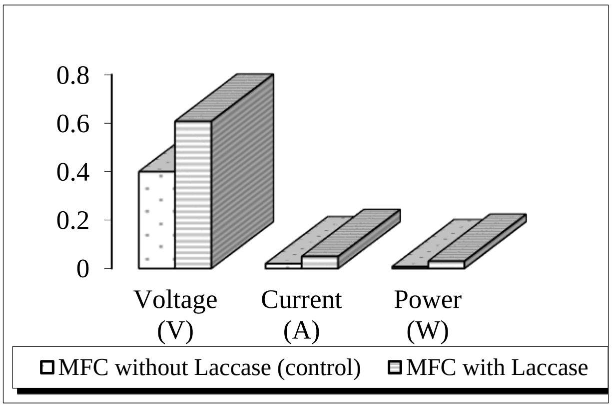


Fig.6:- Performance of control MFC and laccase-contained MFC

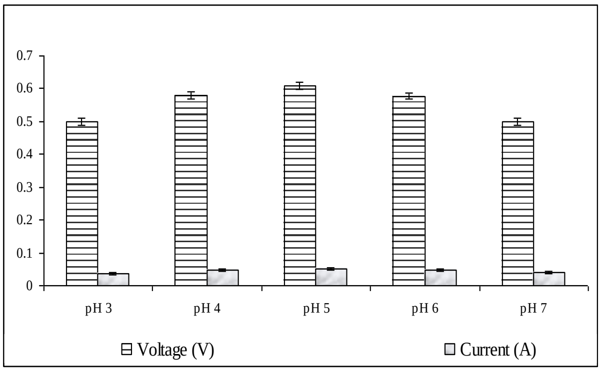


Fig.7:- Voltage and current production at different catholyte pHs

4. Conclusion

In the present work, un-studied agro-industrial waste, El-ghasheem resulting from *black honey* industry at upper Egypt was used in an economical-designed microbial fuel cell (MFC) to obtain cheap green electricity. Factors affecting the production of bioelectricity from this MFC were optimized to get the optimum power. Bioelectricity production was enhanced using crude microbial laccase as a cathodic reaction biocatalyst.

5. Conflicts of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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