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ENHANCING ELECTRICITY GENERATION USING FUNGAL LACCASE-BASED MICROBIAL FUEL CELL

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ABSTRACT

Microbial fuel cells (MFCs) are very important source to obtain green electricity and also for decontamination of waste water. Bioelectricity yield from biofuel cells is still needed for maximizing. Microbial laccases, especially those produced by fungi, are currently considered to be one of the most promising biocatalyst for bioelectricity production and also purification of water from the different pollutant, especially phenolic compounds. In the present work, different electrolyte solutions used in anode and cathode chambers to evaluate efficiency of each to produce voltage & current and also to prove that using economical electrolytes, which were agro-industrial waste called el-ghasheem at anode and only tap water at cathode, achieve good results in comparison with other commonly used electrolytes which were glucose, sodium nitrate, mono-potassium phosphate, di-potassium phosphate, ammonium chloride and magnesium sulfate. The use of El-ghasheem in the economic MFC showed power improvement results when fungal laccase, produced from *Monodictys castaneae* fungus, had been used as cathodic reaction catalyst to increase voltage production from 0.466±0.003 V to 0.807±0.002 V and current from 0.025±0.003 A to 0.09±0.003 A at 37 °C, anolyte pH 6 and catholyte pH 5 for 10 days incubation period. It was noticed that this laccase enzyme had the 98.38±0.264 % phenol removal activity from anode chamber through indirect effect and 99.69±0.276 % phenol removal activity from cathode chamber through direct effect when El-ghasheem was used as the organic fuel at the anode side. In this study using unstudied agro-industrial waste, Electricity was produced by the new fungal laccase which showed the high performance in electricity production enhancement and also phenol compounds removal through low cost MFC.

Keywords: Laccase, Microbial fuel cell, Bioelectricity, Agro-industrial waste, Phenol removal

INTRODUCTION

Renewable, sustainable energy plays an important role in maintaining our environment as clean and safe for all humankind and also provides us with a stable supply of energy. Among the wide range of emerging renewable technologies, a microbial fuel cell system protrudes as it has a high ability to obtain electricity through direct energy conversion with an efficiency reach of up 81% **(Rabaey *et al.*, 2004).** Microbial fuel cells (MFCs) use various microorganisms as a biocatalyst to obtain bioelectricity from chemical energy. This biotechnology has attracted global attention as a novel way to acquire energy from wastewater **(Zhang *et al.*, 2012).** A MFC simply consists of an anodic chamber that contains an anode and anolyte and a cathodic chamber containing the cathode and catholyte. Both the anodic and cathodic chambers are connected to each other by a salt bridge. There are different types of salt bridge that are widely used in MFC, such as filter paper, tissue paper, or porous material soaked in electrolyte. The salt bridge is replaced with a proton exchange membrane (PEM) in the PEM fuel cell **(Du *et al.*, 2007)**. The microorganisms that are present in the anodic chamber are provided with favorable growth media and physiological conditions. These microorganisms degrade organic matter anaerobically producing electrons which are transported from the anode to the cathode through the external circuit, and the protons produced are selectively passed through the salt bridge. The resultant products from this degradation process by the action of the microorganisms in the anodic chamber travel to the cathode and react with oxygen to produce water **(Sharma & Li 2010)**. There are different materials that are used to construct MFC chambers such as glass and polycarbonate. Various materials can be used as anode and cathode electrodes, such as carbon cloth, carbon paper, graphite, and graphite felt **(Zhang *et al.*, 2011)**. Microbial fuel cells based on enzymatic reactions have a great ability to obtain significant current densities in the order of 1 mW/cm^2. There are different enzymes that have vital roles at the anode of enzymatic fuel cells, such as glucose oxidases and dehydrogenases. **(Leech *et al.*, 2012).** At the cathode of a bio-electrochemical cell, there are also various enzymes used as catalysts of electron acceptors, normally O_2, reducing and eliminating the need for an expensive catalyst, normally platinum. Laccases and bilirubin oxidases are examples of enzymes that are commonly used in oxygen bio-cathodes **(Lalaoui *et al.*, 2013)**. Bio-electrochemical cells (whole microbe or enzymatic) can also be used in bio-electrolytic cells with a biological substrate in the anode, cathode, or both. In this setup, the Microbial fuel cell is powered, and a reaction occurs, therefore the system can be tailored to generate valuable products (bio-electro synthesis) **(Rozendal *et al.*, 2008a)**. Hydrogen has been produced in the cathode of a microbial fuel cell, bio-anode and bio-cathode, of a microbial fuel cell (abiotic anode). Methane has also been produced using a microbial fuel cell where both bio-cathode and bio-anode are inoculated with microorganisms **(Villano *et al.*, 2010)**. Although biocatalyst cost at enzymatic microbial fuel cells involving production, co-factors, extraction, and purification of enzymes reduce the cost efficiency of these fuel cells, once enzymes or membrane fractions are acquired, electrodes can be gathered with a high biocatalyst density per surface area, implying that higher current densities can be achieved. It was reported at a previous study a current densities increasing to 335 mA/ mg for H_2 oxidation using *Aquifex aeolicus* hydrogenase as a bio-catalyst, and 488 mA/mg for O_2 reduction by *Bacillus pumilus* bilirubin oxidase at 25 °C, which increased to 1.08 and 0.84 A/mg respectively at 50 °C **(Mazurenko *et al.*, 2017)**. The stability of enzymatic electrodes is another issue to overcome **(Leech *et al.*, 2012)**. Several studies concentrated on an increase in electricity generation and lowering operation costs toward large-scale or real-world applications. For example, a novel design of MFC called Up-flow Bio-filter Circuit (UBFC) was developed to treat different types of industrial wastewater such as biodiesel and palm oil mill wastewater without chemical pretreatment and exogenous nutrient supplements **(Sukkasem & Laehlah, 2013)**. Generally, white-rot fungi produce and secrete extracellular laccase to reclaim nutrients from the plant material to the soil by lignin degradation. This copper-containing oxidoreductase enzyme plays an important role to transfer electrons from phenolic compounds and aromatic amines to atmospheric oxygen (O_2), so laccase has the ability to transfer electrons from the cathode to O_2, contributing degradation of recalcitrant aromatic compounds **(Lee et al., 2014).** Laccases play an important role as cathode biocatalysts in microbial fuel cells because of their ability for reversible adsorption to carbon electrodes. Laccases catalyze the one-electron oxidation of diverse chemical substrates at a single-copper-containing site near the surface of the protein **(Christwardana &Kwon, 2017)**. At the same time, laccases are used as a biocatalyst to reduce dioxygen to water at a tri-copper site in the interior of the protein without the production of superoxides or peroxides. In spite of their promise, laccase cathodes are upset by short enzyme lifetimes, suboptimal enzymatic reaction velocities, enzyme inactivation, and low enzyme adsorption **(Rubenwolf *et al.*, 2010)**. Various trials have been made to engineer laccases with faster reaction velocities, but further effort is required to address losses due to other factors. Filamentous fungi conquer short laccase lifetimes and laccase inactivation in nature by secreting laccases to catalyze the breakdown of lignin. Fungi can defeat laccase inactivation

confrontation through certain endocytic pathways which facilitate the absorption of inactive enzymes for breakdown and reuse **(Lin *et al*., 2017)**. In the present study laccase extracted from fungi was used as a cathodic reaction catalyst to enhance bioelectricity production from different MFCs.

MATERIAL AND METHODS

Construction of the microbial fuel cell

The MFC was used in the study was the 'H'-type reactor which designed as MFC prescribed by **Kamau *et al*.** (2017). Two 1L containers were prepared as an anode chamber and a cathode chamber. Two small pores were made in the bottles' covers to insert wire through. One end of the copper wire was attached to 4 cm long and 0.5 cm diameter graphite rod electrodes. The other two terminals of wires were connected to a digital multi-meter (model, VEYON-VL9205A). A low-cost salt bridge was made from lamp wicks which were boiled in the 1M KCl solution, then kept in the freezer for solidification. The solidified salt bridge was passed through a rubber tube and attached to the chambers

Comparison between different anolytes and catholytes

Different electrolyte solutions used in anode and cathode chambers to evaluate efficiency of each to produce voltage & current and also to prove that using economical electrolytes achieve good results in comparison with other commonly used electrolytes. MFCs operated using these electrolytes and incubated at 37 °C. Produced voltage and current measured daily using multi-meter. There are three MFCs were designed: -

a-MFC1

i- Anode chamber composes of 10 ml soil extract, 20 ml agro-industrial waste called El-ghasheem which is produced from boiling of Sugarcane juice, 1 g glucose, 0.3 g sodium nitrate & distilled water up to 200 ml
ii- Cathode chamber composes of 0.2 g magnesium sulfate and distilled water up to 200 ml

b-MFC2

i- Anode chamber composes of 50 ml soil extract, 0.5 g sodium acetate, 3.6 g mono-potassium phosphate, 6 g di-potassium phosphate, 0.6 g ammonium chloride & distilled water up to 600 ml.
ii-Cathode chamber composes of 35 ml soil extract, 2.4 g mono-potassium phosphate, 4 g di-potassium phosphate & distilled water up to 400 ml.

c- MFC3

i- Anode chamber composes of 100 ml soil extract, 150 ml El-ghasheem & tap water up to 600 ml.
ii-Cathode chamber composes of 600 ml tap water only.

Optimization of physical conditions for voltage and current maximization

The best MFC was selected from previously designed the three MFCs and determined its performance according to produced voltage, then this best one operated under different temperatures (20, 28, 37 & 42 °C) and different anolytes pH (4, 5, 6 & 7) for 14-day incubation time. Voltage and current were measured daily to determine the optimum condition to obtain the maximum power.

Effect of laccase enzyme on the enhancement of bioelectricity

Both crude enzymes with 3U/ml activity which were detected before spectrophotometrically using guaiacol as substrate and also the fungal filtrate which contains the laccase were added to catholyte to determine its efficacy as a cathodic reaction catalyst and compare between voltage and current resulted from control MFC (without laccase) and MFC include laccase at its cathode. This Laccase had been obtained from *Monodictys castaneae* at the previous work **(Tammam *et al.*, 2023)**.

Perform phenol standard curve using guaiacol

Standard curve acquired by graphical representation of the absorbance which measured at 540 nm versus different phenol concentrations. Prepare various concentrations of guaiacol (125, 250, 500 ppm), 2ml from each concentration added to 10 ml distilled water, 1 ml $FeCl_3$ (1%), then add water up to 25 ml. Using a spectrophotometer (model: JENWAY 6305) measure absorbance at 540 nm **(Alexandra *et al*., 2018).**

Laccase ability to remove phenol using MFC

Phenol concentrations at anolyte and catholyte of MFC were measured using the **Alexandra *et al.* (2018)** method and phenol standard curve. Phenol content was detected before and after the operation of MFC, with and without laccase to determine laccase efficacy for phenol removal.

Optimization laccase activity for electricity production

Different MFC temperatures (20, 28, 37, and 42 °C) and different catholyte pH (3, 4, 5, 6, 7, and 8) were performed for 10 days of incubation time. Voltage and current were measured to determine the optimum condition to obtain the maximum power and phenol removal ability using laccase as a biocatalyst.

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 voltage in volts. The power and current per surface area of the anode (6 cm^2) was used to calculate the power and current density. 1kOhm resistance was used as external load.

RESULTS AND DISCUSSION

Perform phenol standard curve using guaiacol

The standard curve for phenol detection is shown in the figure 1 which shows the increase in the absorbance value from zero to 0.9 corresponding to increasing in phenol (guaiacol) concentration.

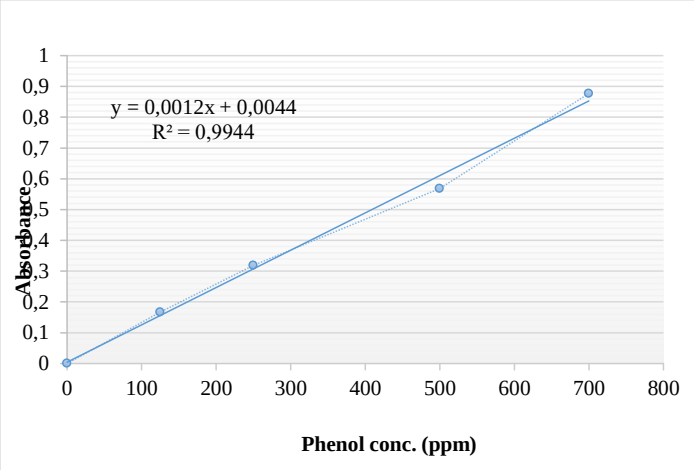

Figure 1 Phenol standard curve using different concentrations of guaiacol

Laccase ability to remove phenol using MFC

The anode of MFC3 contains El-ghasheem which is agro-industrial waste containing plant phenols. It was used in the anode chamber as an organic matter source. El-ghasheem phenol content in anolyte and catholyte was measured before and after MFC3 performing and the results were as below in the following table (Tab 1). When phenol concentration values in the case of control MFC (without laccase enzyme) were compared with those in the case of test MFC (with laccase) we found that laccase enzyme had 98.38±0.264 % phenol removal activity from the anode chamber through indirect effect and 99.69±0.276 % phenol removal activity from cathode chamber (direct effect), where a significant amount of phenols could transfer and diffuse through the used salt bridge.

Table 1 Phenol concentrations at anode and cathode of MFC3

Phenol source	Anode at day 0	Cathode at day 0	Anode at day 10 (without laccase)	Cathode at day 10 (without laccase)	Anode at day 10 (with laccase)	Cathode at day 10 (with laccase)
Conc. (ppm)	37.13±0.306	0.001	25.33±0.153	9.7±0.265	0.6±0.1	0.03±0.026

Compare the three types of MFC performance

Voltage, current, power, power density, and current density of MFCs (control, without laccase) were measured under the same physical conditions (37 °C, 10 days, and electrolytes pH 7). The results in the figure 2 show that MFC3 achieved the best voltage with 0.43±0.026 V in comparison to 0.36±0.015 V using MFC2 finally MFC1 with the lowest voltage value which was 0.23±0.02 V, there is a little difference between the MFC1 current value, 0.012±0.001 A and the achievable current in the case of MFC2 and MFC3 which have almost same produced current, 0.021±0.003 A, and 0.023±0.004 A, respectively. MFC3 did not only achieved the best performance, but also based on economical electrolytes which contain different nutrients and salts that suitable for obtaining power from the microorganisms. MFC3 was used to remove phenols from agro-industrial waste (El-ghasheem) and also produce significant electrical power. Using this waste instead of other electrolytes gave economic advance by lowering the cost of MFC organic feed, at the same time using laccase in this MFC showed double important actions which were bioremediation of this waste and also enhancing bioelectricity production resulted by MFC3.

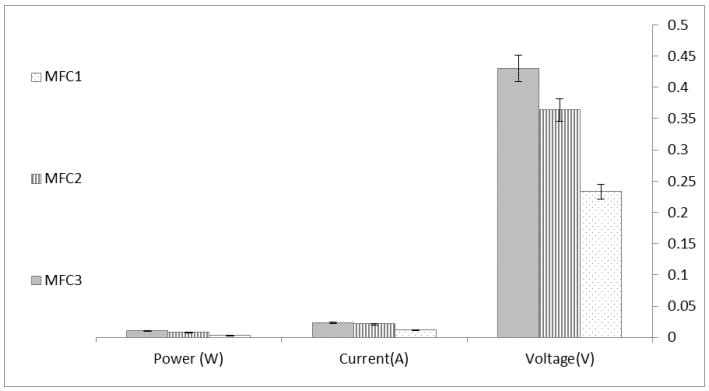

Figure 2 Voltage, current, and power of the three MFCs

Optimization of physical conditions for voltage and current maximization

Effect of different temperatures

Operating temperature affect directly the performance of any MFC. The temperature has a significant impact on many variables such as the conductivity of MFCs electrolytes, diffusion coefficient, charge transfer rates, activation energy, and microbial biochemical processes, so variation in temperature can significantly modify the MFC power output **(Nouri and Darzi, 2017).** In the present study, MFC3 achieved the highest voltage which was 0.43±0.026 V using 37 °C as incubation temperature for 10 days incubation period and pH 7 for both anolyte and catholyte, after that voltage showed depletion in its value. The lowest voltage was 0.283± 0.003 V which was achieved at 20 °C as shown in the figure 3. There is little change in the current at the different temperatures. The highest current was obtained at 37 °C then 28 °C which were 0.023±0.004 A, and 0.021±0.001 A, respectively. The lowest one, 0.011±0.0006 A was achieved using 20 °C. Similar temperature effect had been reported by **Moon *et al.* (2006)** who inspected the effect of temperature on the achievement of a "Sensor-type" two-chamber MFC used for wastewater treatment and observed a rather nonlinear trend. Both voltage and current of MFC showed a slight rise as the temperature was increased from 24 °C to 35 °C, but exhibited a decrease at higher temperatures, 38 °C, and 41 °C. This nonlinear behavior was related to ohmic overvoltage changes.

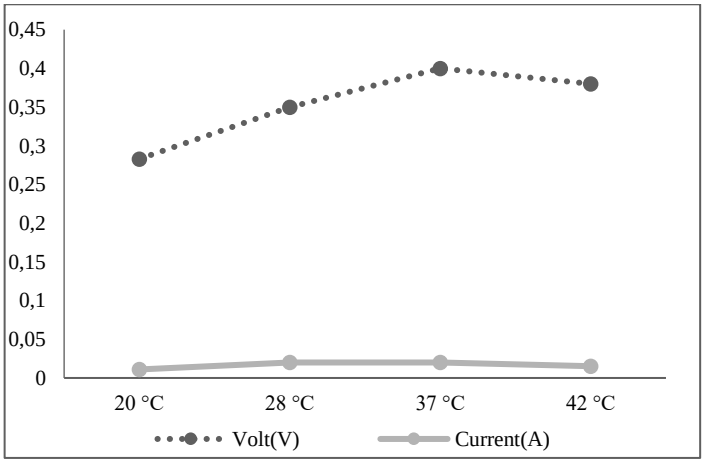

Figure 3 Voltage and current produced from MFC3 at different temperatures

Effect of different anolyte pH

In another study, it demonstrated that the MFC efficiency on the number of raw materials fed per day per unit volume of both chambers and also on the pH. The existence of the NADH/NAD+ and FADH/FAD+ confirmed using a certain electrochemical technique that measures the current that develops under conditions where voltage is in excess of that predicted by the Nernst equation **(Rabaey *et al.*, 2004)**. In this study, when MFC3 was incubated at 37 °C, catholyte pH7 for 10 days at different anolyte pH, it was found that anolyte pH 6 was the optimum pH for organic matter degradation. This pH achieved the highest voltage, 0.466±0.003 V, and current, 0.025±0.003 A. After that it was obtained by decreasing the voltage and producing electrical power, on the other hand, pH4 achieved the lowest voltage and current, 0.25±0.02 V & 0.011±0.002 A respectively, all these results are shown in figure 4.

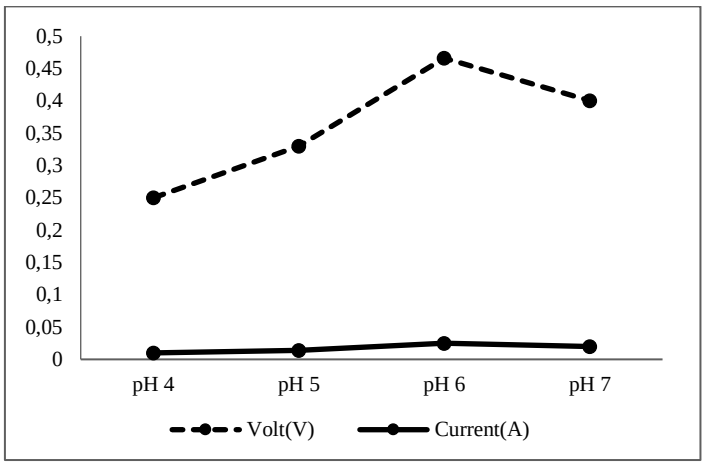

Figure 4 Voltage and current produced from MFC3 at different anolyte pH

Effect of laccase enzyme on enhancement bioelectricity

In several studies, it was proved that laccase enhanced the electrical power of different MFCs, **Sato *et al.* (2018)** stated that using laccase as biocathode of MFC produced 249.67 ± 3.21 mV open-circuit voltage (OCV) which was 23.8% more than the 202.33 ± 8.02 mV is obtained with the negative control (without laccase). **Wu *et al.* (2012)** used laccase produced by *Trametes versicolor* which is a laccase-secreting white-rot fungus. This laccase was immobilized on the cathode surface to obtain a maximum of 180 mV. **Lai *et al.* (2017)** mentioned that laccase secreted by the *Ganodium lucidum* fungus exhibited a high ability for removal of synthetic dye acid orange and production of electricity using the two-chamber MFC which produced 699 mV as a maximum voltage with 96.7% de-colorization performance. In this study using laccase at the cathode of MFC3 elevated the resulted voltage from 0.466±0.003 V, control voltage, to 0.805±0.004V, laccase voltage, (42.11% improvement). A 68.75% increase in the produced current from 0.025±0.003 A to 0.08±0.002 A and an 81.25% improve in the power from 0.012±0.001W to 0.064±0.002W were achieved with using MFC with laccase enzyme. All results are shown in figure 5 Both control MFC3 and laccase MFC3 were operated at 37 °C, anolyte pH6, and catholyte pH7 for 10 days incubation period.

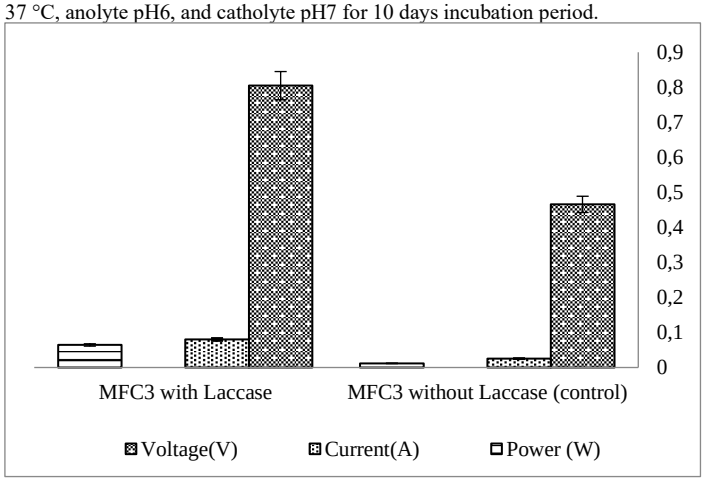

Figure 5 Voltage, current, and power of control MFC3 and laccase MFC3

Optimization of laccase activity for electricity production
optimum incubation period for laccase to improve bioelectricity

Because of the poor stability of the enzymes in the MFCs, their utilities in these cells are limited. In a microbial fuel cell, the lifetime of the enzymes typically

changes from 7-10 days **(Cooney *et al.*, 2008)**. **Rubenwolf *et al.* (2012)** stated that *T. versicolor* laccase activity decreased with the operating time, although other favorable conditions (pH and temperature) were applied. They noticed that the enzyme stayed stable for 2 days after which there was a constant activity rate decline with a half-life of 7 days. **Higgins *et al.* (2011)** mentioned that MFC operating with laccase biocathode had a 4% depletion in voltage after 4.75 days, although **Savizi *et al.* (2012)** reported that laccase in MFC was shown to conserve its activity for a period of 30 days. In the present work, laccase keep its activity for all of 14 days, the complete incubation period, and achieved the maximum stimulation for bioelectricity at the range day 10-12, after that its activity decreased slightly and gradually. The optimum incubation period for control MFC3 was 10 days at 37 ºC, pH 6 for anolyte, and pH7 for catholyte, at these conditions laccase at the cathode enhanced voltage production on day 10 from 0.466±0.003 V to 0.805±0.004V and increased the current from 0.025±0.003 A to 0.08±0.002 A. These results are found in the figures 6 & 7.

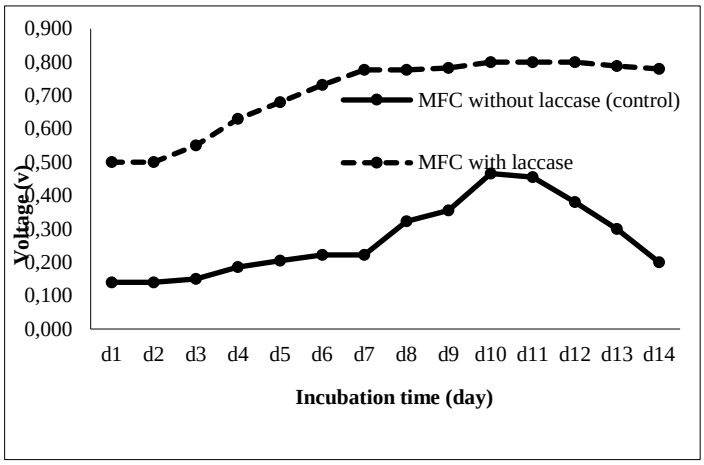

Figure 6 Voltage of control MFC3 and laccase MFC3 during incubation periods

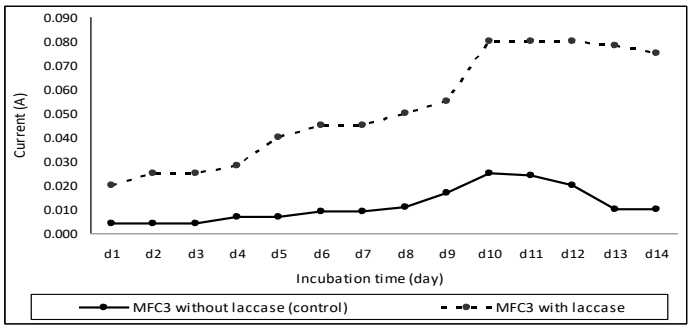

Figure 7 Current of control MFC3 and laccase MFC3 during incubation periods

Effect of different catholyte pH on laccase action as bioelectricity enhancer

pH gradients have a negative impact on MFC application by interfering with metabolic activity in the anode and potential losses at the cathode. Nernst equation shows that these pH gradients are the root cause of high anodic equilibrium potential and/or low cathodic equilibrium potential that cause significant cell voltage decreases, a loss of ~60 mV per 1 pH change **(Rozendal *et al.*, 2008b)**. It was stated in another study that an increase in pH by one unit causes oxygen reduction potential in the range of 30 – 80 mV in MFC using laccase as a cathode catalyst. pH changes can also affect enzyme properties and characteristics such as activity, stability, and solubility **(Fokina *et al.*, 2015)**. In this work using the optimum MFC3 physical conditions, it was noticed that catholyte pH7 achieved the highest voltage and current, 0.466±0.003 V and 0.025±0.003 A for control MFC3 (without laccase), and there is very little difference in the produced voltage and current at the range of catholyte pH 6-8, although there is a high decrease in the voltage and current at pH 3, which achieved the lowest electrical yield, 0.2±0.02 V and 0.01±0.001 A. When laccase was used at the MFC3 cathode, it was found that catholyte pH 5 was the optimum pH for laccase activity and achieved the highest voltage and current, 0.807±0.002 V and 0.09±0.003 A. After that, there are gradually decreasing in voltage and current with an increase in pH till pH 8 which achieved the lowest voltage and current values, 0.553±0.02 and 0.04±0.002 A. All these results are shown in figures 8 & 9.

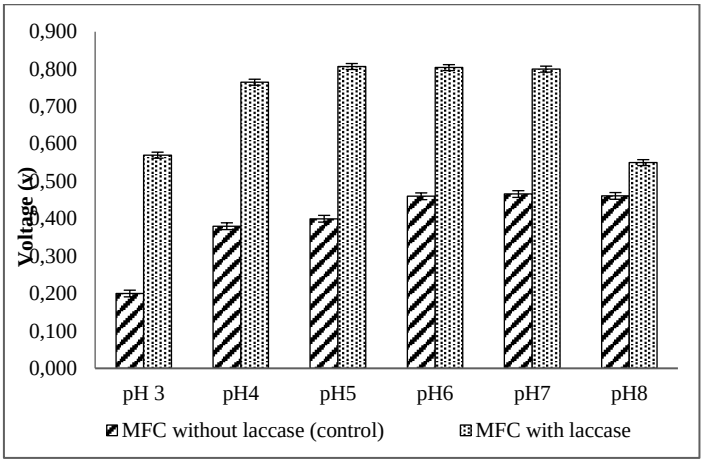

Figure 8 Voltage of control MFC3 and laccase MFC3 at different catholyte pH

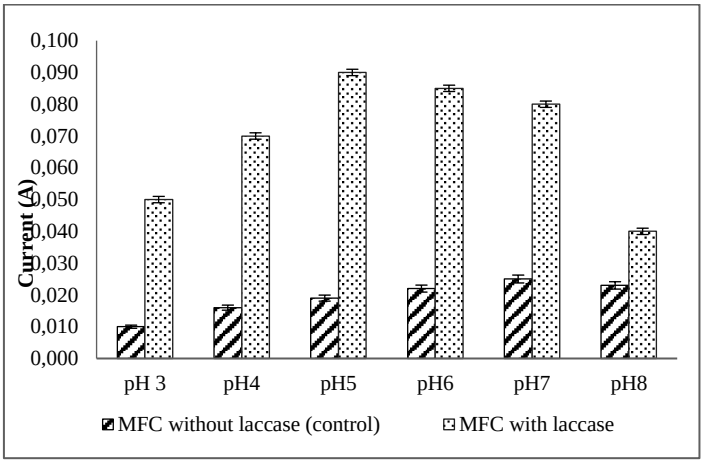

Figure 9 Current of control MFC3 and laccase MFC3 at different catholyte pH

Effect of different temperatures on laccase action as bioelectricity enhancer

Mani *et al.* (2016) stated that laccase secreted by *Trametes versicolor* showed the best bioelectricity enhancement at 30 °C through 10 days incubation period. **Li *et al.* (2013)** utilized a two-chamber MFC using a proton exchange membrane as a salt bridge and reported the effect of temperature over a wide range of temperatures from 10 °C to 55 °C. Li et al. recorded results similar to that of **Moon *et al.* (2006)** with the highest voltage and currently achieved at 37 °C, which decreased by 21% when the temperature increased to 43 °C. At the temperature of 55 °C, there was no steady power generation. In this work laccase at 37 °C showed the best performance for increasing MFC3 voltage from 0.466±0.003 V to 0.807±0.002 V and current from 0.025±0.003 to 0.09±0.003 A at other optimum conditions. This laccase showed the lowest activity and performance at 20 °C, while it showed the best activity to enhance electrochemical reactions at 37 °C, which start to decrease gradually when the temperature increased from 37 °C to 42 °C. All these data are found in the figure 10.

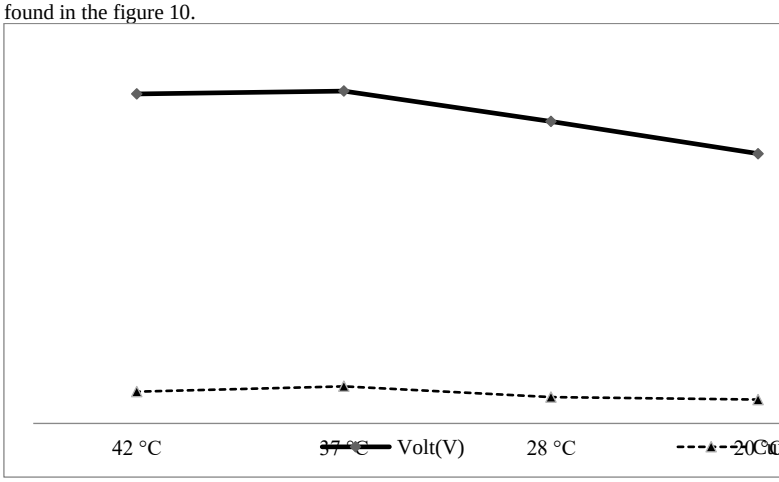

Figure 10 Voltage and current produced from laccase MFC3 at different temperatures

CONCLUSION

In this study using unstudied agro-industrial waste in Egypt which produced from (Black honey industry), Electricity was produced by the new fungal laccase which showed the good performance in electricity production enhancement and also phenol compounds removal through low cost MFC. Laccase produced from *Monodictys castaneae* fungus showed high ability to increase voltage production from 0.466±0.003 V to 0.807±0.002 V and current from 0.025±0.003 A to 0.09±0.003 A using this economic microbial fuel cell. In the present study, laccase activity was maximized using the optimum physical conditions which were 37 °C, anolyte pH 6 and catholyte pH 5 for 10 days incubation period.

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REFERENCES

Alexandra, G.A., Tatiana, I., Veronica, M.R. & Laura, B. (2018). Simple and rapid spectrophotometric method for phenol determination in aqueous media. Bulletin of the polytechnic institute of Jassy, Construction. *Architecture*, 64(3), 9-18.

Christwardana, M. & Kwon, Y. (2017). Yeast and carbon nanotube-based biocatalyst developed by synergetic 298 effects of covalent bonding and hydrophobic interaction for performance enhancement of membrane-less microbial fuel cell. *Bioresource technology*, 225, 175-182.

Cooney, M.J., Svoboda, V., Lau, C., Martin, G. & Minteer, S.D. (2008). Enzyme catalyzed biofuel cells. *Energy & Environmental Science*, 1(3), 320-337. https://doi.org/10.1002/Energy Environ. Sci.2008.1.3.320-337

Fokina, O., Eipper, J., Winandy, L., Kerzenmacher, S. & Fischer, R. (2015). Improving the performance of a biofuel cell cathode with laccase-containing culture supernatant from *Pycnoporus sanguineus*, *Bioresource Technology*, 175, 445-453. https://doi.org/ 10.1016/j.biortech.2015.175.445-453.

Higgins, S.R., Lau, C., Atanassov, P., Minteer, S.D. & Cooney, M.J. (2011). Hybrid biofuel cell: Microbial fuel cell with an enzymatic air-breathing cathode. *American Chemical Society Catalysis*, 1(9), 994- 997. https://doi.org/10.1021/ ACS Catal.2011.1.9.994-997

Kamau, J.M., Mbui, D.N., Mwaniki, J.M., Mwaura, F.B. & Kamau, G.N. (2017). Microbial Fuel Cells: Influence of External Resistors on Power, Current and Power Density. *Journal of Thermodynamics & Catalysis*, 8(1), 1-5. https://doi.org/10.4172/J.Thermodyn. Catal.2017.8.1.1-5

Lai, C.Y., Liu, S.H., Wu, G.P. & Lin, C.W. (2017). Enhanced biodecolorization of acid orange and electricity generation in microbial fuel cells with superabsorbent-containing membrane and laccase-based bio-cathode. *Journal of Cleaner Production*, 166, 381-386. https://doi.org/10.1016/j.jclepro.2017.166.381-386.

Lalaoui, N., Elouarzaki, K., Le Goff, A., Holzinger, M. & Cosnier, S. (2013). Efficient direct oxygen reduction by laccases attached and oriented on pyrene-functionalized polypyrrole/carbon nanotube electrodes. *Chemical Communications*, 49(81), 9281-9283. https://doi.org/10.1039/chem.commun.2013.49.81.9281-9283.

Lee, H., Jang, Y., Choi, Y.S., Kim, M.J. & Lee, J. et al. (2014). Biotechnological procedures to select white rot fungi for the degradation of PAHs. *The Journal of Microbiological Methods*, 97, 56-62. https://doi.org/10.1016/j.mimet.2014.97.56-62

Leech, D., Kavanagh, P. & Schuhmann, W. (2012). Enzymatic fuel cells: Recent progress. *Electrochimica Acta*, 84(1), 223-234. DOI: https://doi.org/10.1016/j.electacta.2012.84.1.223-234

Li, L.H., Sun, Y.M., Yuan, Z.H., Kong, X.Y. & Li, Y. (2013). Effect of temperature change on power generation of a microbial fuel cell, Environmental Technology, 34 (13), 1929-1934.

Lin, T., Bai, X., Hu, Y., Li, B. & Yuan, Y. J. et al. (2017). Synthetic *Saccharomyces 315 cerevisiae-Shewanella oneidensis* consortium enables glucose-fed high-performance microbial fuel cells. *American Institute of Chemical Engineers Journal*, 63(4),1830-1838.

Mani, P., Keshavarz, T., Chandra, T.S., Kyazze, G. (2016). Decolourisation of Acid orange 7 in a microbial fuel cell with a laccase-based biocathode: Influence of mitigating pH changes in the cathode chamber. *Enzyme and Microbial Technology*, 96, 170-176 https://doi.org/10.1016/j.enzmictec.2016.96.170-176.

Mazurenko, I., Monsalve, K., Infossi, P., Giudici-Orticoni, M.T., Topin, F., Mano, N., Lojou, E. (2017). Impact of substrate diffusion and enzyme distribution in 3D-porous electrodes: A combined electrochemical and modelling study of a thermostable H_2/O_2 enzymatic fuel cell. *Energy and Environmental Science*, 10, 1966–1982.

Moon, H., Chang, I.S. & Kim, B.H. (2006). Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. *Bioresource Technology*, 97(4), 621-627.

Nouri, P. & Darzi, G.N. (2017). Impacts of process parameters optimization on the performance of the annular single chamber microbial fuel cell in wastewater treatment. *Engineering in Life Sciences*, 17(5), 545-551.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M. & Verstraete, W. (2004). Biofuel cells select for microbial consortia that self-mediate electron transfer. *Applied and Environmental Microbiology Journal*, 70 (9), 5373–5382.

Rozendal, R.A., Jeremiasse, A.W., Hamelers, H.V. & Buisman, C.J. (2008 a). Hydrogen production with a microbial biocathode. *Environmental Science & Technology*, 42(2), 629-634.

Rozendal A, Hamelers HVM, Rabaey K, Keller J, Buisman CJN (2008 b). Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology*, 26 (8), 450-459. https://doi.org/10.1016/j.tibtech.2008.26.8.450-459

Rubenwolf S, Sané S, Hussein L, Kestel J (2012). Prolongation of electrode lifetime in biofuel cells by periodic enzyme renewal Theoretical background. *Bioenergy and Biofuels*, 96(3), 841-849.

Rubenwolf, S., Strohmeier, O., Kloke, A., Kerzenmacher, S. & Zengerle, R. et al. (2010). Carbon electrodes for direct electron transfer type laccase cathodes investigated by current density-cathode potential behavior. *Biosensors and Bioelectronics*, 26 (2),841-845.

Sato, C., Chaijak, P., Sukkasem, C., Lertworapreecha, M. & Boonsawang, P. et al. (2018). Enhancing Electricity Generation Using a Laccase-Based Microbial Fuel Cell with Yeast *Galactomyces reessii* on the Cathode. *Journal of Microbiology and Biotechnology*, 28 (8), 1360-1366. https://doi.org/10.4014/jmb.2018.28.8.1360-1366

Savizi, I.S.P., Kariminia, H.R. & Bakhshian, S. (2012). Simultaneous decolorization and bioelectricity generation in a dual-chamber microbial fuel cell using electropolymerized-enzymatic cathode. *Environmental Science & Technology*, 46(12), 6584-6593.

Sharma, Y. & Li, B. (2010). The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs). *Bioresource Technology*, 101, 1844-1850.

Sukkasem, C. & Laehlah, S. (2013). Development of a UBFC biocatalyst fuel cell to generate 259 power and treat industrial wastewaters. *Bioresource Technology*, 146(13), 749-753.

Tammam, A.M., Mohamed, M.S.M., Moubasher, H. (2023). Optimisation of Fungal Laccase Production from *Monodictys castaneae*. *ASM Science journal*, 18, 1-11.

Villano, M., Aulenta, F., Ciucci, C., Ferri, T. & Giuliano, A. et al. (2010). Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Bioresource Technology*, 101(9), 3085-3090.

Wu, C., Liu, X.W., Li, W.W., Sheng, G.P. & Zang, G.L. et al. (2012). A white-rot fungus is used as a biocathode to improve the electricity production of a microbial fuel cell. *Applied Energy*, 98, 594-596.

Zhang, F., Chen, M., Zhang, Y. & Zeng, R.J. (2012). Microbial desalination cells with ion exchange resin packed to enhance desalination at low salt concentration. *Journal of membrane science*, 417(1), 28-33. https://doi.org/ 10.1016/j.memsci.2012.417.1.28-33

Zhang Y, Mo G, Li X, Zhang W, Zhang J et al. (2011). A graphene modified anode to improve the performance of microbial fuel cells. *Journal of Power Sources*, 196(13), 5402-5407.