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Terzioğlu Kampüsü, 17100 – Çanakkale – TÜRKİYE
Telefon: +90 (286) 218 00 18 Dahili: 24006, Fax: +90(286) 218 19 48
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Microbial Biofuel Cell: Harnessing Bioelectricity from Fisheries Wastewater Using *Saccharomyces cerevisiae*

Maria Essa^{1,2} , Saima Mehar² , Mah Gunj^{1,2} , Haneef Ur Rehman¹

¹University of Turbat, Department of Natural and Basic Sciences, Kech Balochistan, Pakistan

²Sardar Bahadur Khan Women University, Department of Chemistry, Quetta Balochistan, Pakistan

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Abstract: Microbial fuel cell (MFC) technology offers an innovative and sustainable solution for energy production, particularly in electricity-deprived regions. The MFC system harnesses electrons released during biochemical reactions catalyzed by microorganisms. This study focuses on the design of a microbial biofuel cell that utilizes *S. cerevisiae* to generate bioelectricity from fisheries wastewater. Optimization of physical parameters was performed to maximize bioelectricity generation from fisheries wastewater. The results revealed that *S. cerevisiae*-based MFC achieved the highest bioelectricity production at 35 °C, pH 8, and an incubation period of 72 hours. To enhance performance, a flow rate of 50 mL/min of oxygen in the wastewater was found to be the most effective for bioelectricity generation. The findings demonstrate the practicality and sustainability of the *S. cerevisiae*-based MFC as a viable technique for both bioelectricity production and wastewater management in the fisheries industry. This innovative approach not only addresses the basic electricity needs of electricity-deprived regions but also helps mitigate wastewater pollution, presenting an environmentally friendly solution. The study highlights the potential of MFC technology to contribute to renewable energy generation and environmental sustainability in regions reliant on fisheries wastewater.

Keywords: Bioenergy, microbial fuel cells, *Saccharomyces cerevisiae*

✉ Correspondence: haneef.baloch@uot.edu.pk

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Introduction

The energy demands we have today relied heavily on fossil fuels, which unfortunately are finite resources and face the risk of depletion due to their limited availability (Amann, 1996; Das & Veziroglu, 2001). This reliance on fossil fuels has significant implications for the environment, particularly through the emission of CO₂, contributing to global climate change. It is crucial to recognize that a substantial portion of the global population lacks access to electricity for their daily needs. According to the United Nations, approximately 1.3 billion people are currently living without reliable access to electricity, and a significant proportion of these individuals reside in developing countries, including Pakistan. Additionally, the continued use of fossil-based fuels exacerbates the environmental concerns we face. The emission of CO₂ resulting from the combustion of these fuels contributes to the greenhouse effect and poses a threat to our planet's climate stability.

In light of these factors, it is imperative to explore alternative energy sources and transition towards more sustainable and environmentally friendly solutions to meet our energy needs. By investing in renewable energy technologies and promoting energy efficiency measures, we can mitigate the detrimental effects of fossil fuel consumption while ensuring access to electricity for all individuals, including those in underserved communities.

The growing energy demand and the urgency of addressing climate change have prompted researchers to seek alternative and sustainable techniques that can fulfill the basic electricity needs of people while being environmentally friendly (Logan, 2004). One such area of interest for many researchers is MFC, which utilize biological wastes to produce bioelectricity (Logan, 2004; Rabaey et al., 2003; Mohan, 2007).

Microbial fuel cells operate by harnessing biochemical reactions carried out by microorganisms in mild conditions, converting bioorganic wastes into electricity through biocatalysts (Logan, 2004). The MFC consists of separate anode and cathode compartments. In the anode section, microorganisms oxidize organic wastes, producing electrons and protons. The electrons then flow through a circuit system to the cathode compartment, where they combine with protons to form water molecules, generating electricity. Essentially, the MFC converts biochemical energy into electric energy, functioning similarly to traditional cells (Rabaey et al., 2003).

With the rapid increase in domestic and industrial wastewater resulting from higher water utilization, these waste streams often contaminate underground water systems. Developing countries face the challenge of municipal solid wastes constituting more than 60% of the total waste stream, making them a potential substrate for MFC (El-Chakhtoura et al., 2014). MFC technology not only aids in waste treatment but also simultaneously produces electricity (Jayashree et al., 2014). Additionally, untreated sewage and industrial effluents are polluting various natural water reservoirs. By utilizing wastewater as a substrate in MFC, it becomes possible to generate electricity while effectively managing wastewater, presenting a sustainable and cost-effective approach (Christwardana et al., 2020). A critical step in the MFC system is the selection of highly efficient microbial species, whether pure or mixed cultures. These microorganisms act as catalysts in transferring electrons from the substrate to the anode (Chaudhuri & Lovley, 2003; Logan et al., 2006; Saini et al., 2020).

In the cities of developing countries such as Pakistan, industrialization and urbanization pose a significant threats of wastewater pollution. To address this issue, a study was conducted to explore using the fisheries wastewater as a substrate for bioelectricity generation. The growth conditions of microorganisms were analyzed to maximize the production of bioelectricity using the MFC technology.

Materials and Methods

Microbial Fuel Cell (MFC) Reactor

The experimental setup involved designing a MFC reactor with a double-chamber configuration in the shaped liked letter 'H' Carbon electrodes and plastic bottles with a diameter of 15 cm were utilized for this purpose. The anode section of the MFC contained 500 ml of fisheries wastewater, while the cathode section was filled with distilled water. To establish a connection between the two sections, a plastic pipe measuring 2 cm in diameter and 14 cm in length was employed. This pipe was filled with a mixture of sodium chloride and agar in a 1:2 ratio. To evaluate the performance of the MFC, the voltage in millivolts (mV) was measured using a multimeter. The power (P) generated by the MFC was also calculated using the formula $P = I * V$, where I represents the current.

Effect of Incubation Period on Bioelectricity Production in a Microbial Fuel Cell (MFC)

To assess the impact of the incubation period on bioelectricity production, an investigation was conducted on a MFC. The study involved measuring the P generated at different time intervals, spanning from 24 to 120 hours. By analyzing the P output over this range of incubation periods, the relationship between time and electricity production could be determined.

Impact of Incubation Temperature on Electricity Production in a Microbial Fuel Cell (MFC)

An investigation was conducted to explore the impact of incubation temperature on electricity production in a MFC. The microbial fuel cell was subjected to a range of temperatures, varying from 25°C to 50°C. By studying the electricity generation under different temperature conditions, the relationship between temperature and bioelectricity production could be elucidated.

Influence of pH on Bioelectricity Production in a Microbial Fuel Cell (MFC)

An analysis was conducted to investigate the impact of pH on electricity production in a MFC. The pH of the microbial growth chamber was adjusted within a range of pH 5 to pH 10. By studying the electricity generation under different pH conditions, the relationship between pH and bioelectricity production could be examined.

Impact of Oxygen Flow Rate on Bioelectricity Production in a Microbial Fuel Cell (MFC)

An analysis was conducted to examine the influence of oxygen flow rate on electricity production in a MFC. The MFC cathodic chamber was equipped with a flow meter to maintain oxygen flow within a range of 10 to 70 psi. By studying the electricity generation under different oxygen flow rates, the relationship between flow rate and bioelectricity production could be investigated.

Results and Discussion

Isolation and Characterization of *S. cerevisiae* Strains from Yogurt Samples

Yeasts were extracted from yogurt samples to isolate strains of *S. cerevisiae*. To obtain pure cultures of *S. cerevisiae* strains from samples contaminated with bacteria, the samples were cultured in a medium containing antibacterial substances. A total of 61 pure isolates were obtained from the yogurt, and their morphological characteristics confirmed that these strains belonged to *S. cerevisiae*. Additionally, a fermentation capacity test, following the method described by Martin and Martin (1993), further confirmed the identification of the yeasts as *S. cerevisiae*.

Impact of Fermentation Period on Electricity Production in a MFC

The impact of fermentation period on electricity production in a MFC was investigated by subjecting the MFC to various fermentation periods, ranging from 24 to 120 hours (Figure 1). The MFC exhibited an initial onset of electricity production at 24 hours and reached a peak of 492 mV after 72 hours. However, beyond 96 hours, the electricity production declined, with more than a 50% decrease observed after 120 hours. Within a span of four days, the *S. cerevisiae* effectively utilized organic wastes in the MFC.

In contrast, in a microbial-operated energy chamber with *E. coli*, a sustained higher voltage output was observed for an extended period of four days. However, in this case, the voltage output decreased after the first day (Ankur & Shipra, 2018). This could be attributed to the presence of additional substrates and the growth of diverse types of microbes, belonging to different genus/species/strains, under natural conditions to utilize those substrates. As a result, MFCs hold potential for waste management in addition to bioelectricity generation (Ankur & Shipra, 2018).

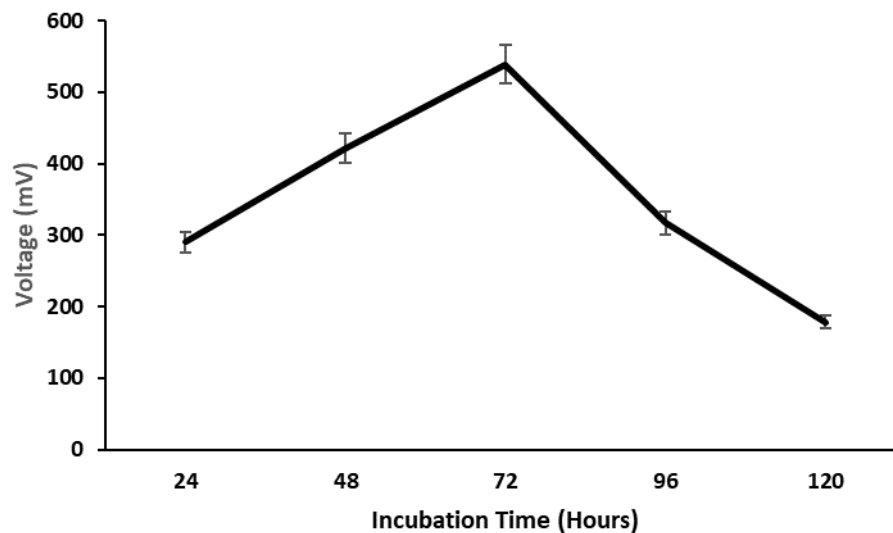


Figure 1. Effect of incubation time on the production of electricity by MFC using wastewater (means \pm S.E., n = 6)

Impact of Temperature on Electricity Production in a *S. cerevisiae*-based MFC

The impact of temperature on electricity production in a MFC utilizing *S. cerevisiae* was analyzed by subjecting the yeast to a range of temperatures from 25 °C to 50 °C (Figure 2). Temperature was found to have a significant influence on electricity production, with the highest levels observed at 35 °C. However, as the temperature increased to 50 °C, a decrease in bioelectricity production was observed. This decline in bioelectricity production at higher temperatures can be attributed to the temperature's effect on microbial growth and metabolic processes.

The performance of the MFC in response to temperature was further characterized by internal resistance, where higher internal resistance corresponded to lower P density obtained (Li et al., 2013). These findings highlight the ability of MFCs to effectively function across a broad temperature range, with the optimal electricity production occurring at 35°C.

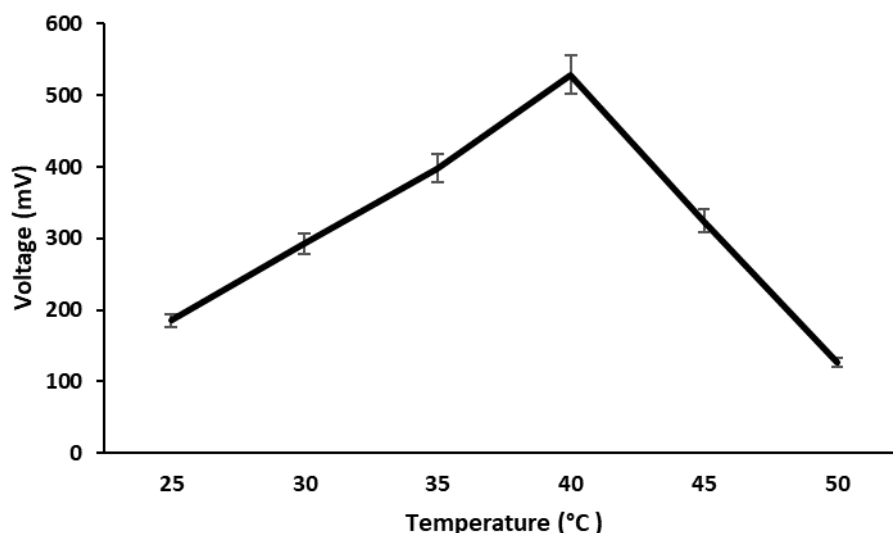


Figure 2. Influence of temperature on the production of electricity by MFC using wastewater (means \pm S.E., n = 6)

Impact of pH on Bioelectricity Production in a MCF

Investigating the effect of pH on the bioelectricity production in a MFC, the pH of the growth medium was carefully controlled within a range of pH 5 to pH 10 using a pH meter (Figure 3). The results demonstrated a

significant impact of pH on bioelectricity production in the MFC, with the highest levels observed in a slightly alkaline range at pH 8. The variation in voltage output can be attributed to the changes in ionic concentration within the chamber due to different pH levels. The pH not only influences the flow of current but also affects the metabolic reactions of microorganisms.

In comparison to previous studies involving MFCs with switchable P release, which were controlled by combinations of physiologically important parameters, the observed changes in pH were found to be significant, particularly when pH was decreased from 7.0 to 5.0. The switch ability of the MFC was attributed to the activity of the microbial anode, which was affected by the combined temperature and pH of the medium. Changes in pH caused reversible activation-inactivation of the bioanode, thereby impacting the overall activity of the MFC (Tang et al., 2014).

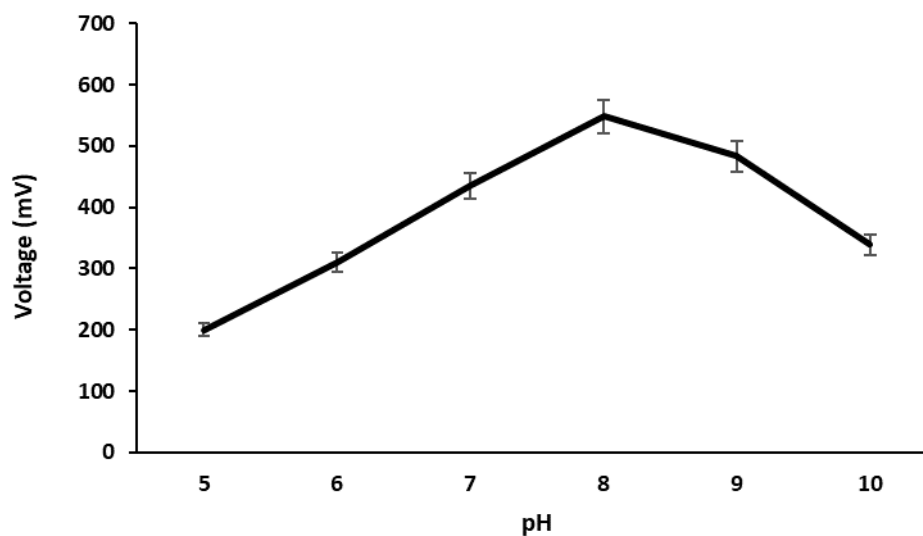


Figure 3. Effect of pH on the production of electricity by MFC using wastewater (means \pm S.E., n = 6)

Impact of Oxygen Flow Rate on Bioelectricity Production in a MCF

Investigating the impact of oxygen flow rate on the Microbial Fuel Cell (MFC) cathodic chamber, the flow rate of oxygen entering the chamber was analyzed using a flow meter (Figure 4). The results revealed a positive correlation between the oxygen flow rate and electricity production in the MFC. As the oxygen flow rate increased, the production of electricity also increased, reaching a maximum at 50 ml/min.

The higher electricity production observed at higher flow rates of oxygen can be attributed to the increased acceptance of protons in the anodic chamber. Conversely, lowering the oxygen flow rate results in a decreased acceptance of electrons, leading to a reduction in electricity production. This highlights the importance of maintaining an optimal oxygen flow rate for maximizing bioelectricity generation in the MFC.

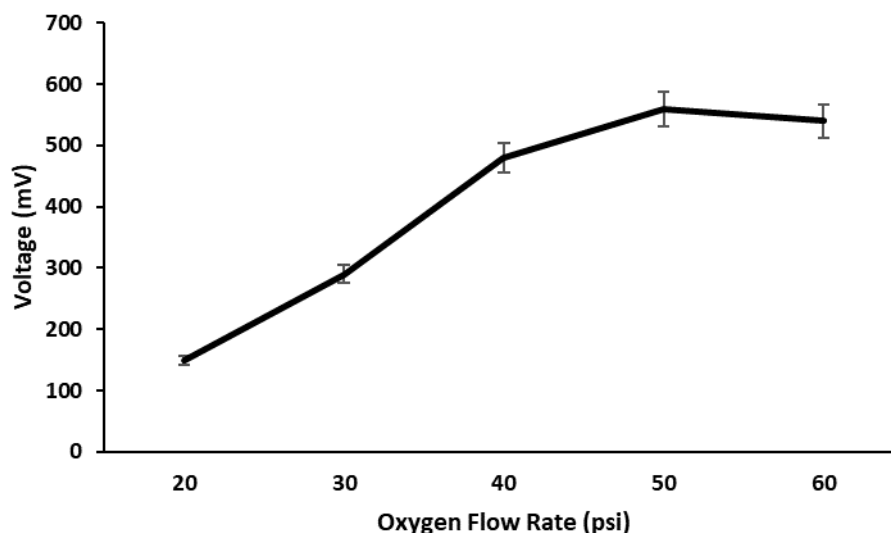


Figure 4. Effect of oxygen flow rate on the production of electricity by MFC using wastewater (means \pm S.E., n = 6)

Conclusion

Investigating the role of key physical parameters, such as incubation time, pH, temperature, and oxygen flow, in bioelectricity generation from a MFC revealed their crucial significance. In this study, it was observed that *S. cerevisiae* demonstrated the highest bioelectricity production when subjected to an incubation period of 72 hours, pH 8.0, temperature of 35°C, and an oxygen flow rate of 50 ml/min, utilizing fisheries wastewater as the substrate.

These findings underscore the effectiveness of fisheries wastewater as a suitable substrate for facilitating the biochemical reactions of microorganisms in MFCs to generate bioelectricity. Moreover, the results highlight the significant potential of MCF technology in developing large-scale systems for wastewater treatment, further emphasizing its application in sustainable and environmentally friendly approaches.

Additional Information and Declarations

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Authors' Contributions:

Maria Essa: Conducted the experimental work and prepared the initial draft of the manuscript.

Saima Mehar: Co-supervised the research by providing guidance on experimental procedures.

Mah Gunj: Assisted in the drafting and editing of the manuscript.

Haneef Ur Rehman: Supervised the overall research, including the design and planning of the experimental work.

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