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Influence of MLSS Concentration and *Aspergillus niger* on Microbial Fuel Cell Performance and Wastewater Treatment Efficiency

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ABSTRACT

Aims: In this study, we evaluated the performance of direct microbial fuel cells using citric acid wastewater as a substrate under different concentrations of MLSS 1000 mg.L-1 and 3000 mg.L-1.
Materials & Methods: Aspergillus niger yeast was used as a microorganism over 4 days and nights of this experiment. A Nafion membrane was used for proton transfer, and graphite plates were used for electron transfer. COD removal efficiency, maximum open circuit voltage, power, and current density were evaluated.
Findings: The general trend of energy production and removal efficiency showed that energy production increased with increasing MLSS. The maximum of these variables was recorded for MLSS of 3000 mg.L-1, achieving a removal efficiency of 93%, an open circuit voltage of 500 mV, and power and current density of 24345 µW.m-2 and 444 mA.m-2, respectively.
Conclusion: Our results showed that the designed MFC suits wastewater treatment and energy recovery.

Keywords: *Aspergillus niger*; Citric Acid; Microbial Fuel Cell; Wastewater Treatment.

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Introduction

In today's world, the energy supply of most countries relies on fossil fuels, which, in addition to being a non-renewable resource, produce greenhouse gases and other pollutants, posing risks to the environment. Therefore, one of the most critical problems in the world is the crisis and lack of energy, especially in developing countries. Since the balance between energy production and consumption is one of the most essential factors in the socio-economic development of a country, finding ways to provide energy through renewable sources is significant [1,4].

On the other hand, industry development following the Industrial Revolution has increased wastewater production. Although scientists are trying to find a suitable process for wastewater treatment through various research, current processes have many disadvantages, including low yield and efficiency, high cost, the need for solvents and reagents, and the production of secondary pollutants.

Statistics show that over 80% of produced wastewater is released into the environment without sufficient treatment. Therefore, the global community is trying to find a low-cost, high-efficiency, environmentally compatible method [5,7].

Today, microbial fuel cell (MFC) technology has attracted significant attention from scientists because this system is a promising approach to producing energy while treating wastewater. MFCs do not use chemicals, so their performance is environmentally friendly and natural.

An MFC system is typically designed as a two-distinct or single-chamber. In an anaerobic chamber, the anode electrode is located, and microorganisms act as biocatalysts to decompose the substrate and produce electrons and protons. In the other chamber, where the cathode electrode is located and designed aerobically, reduction reactions occur. These two chambers are connected through an electric wire circuit containing resistance to transfer electrons. Additionally, to transport the protons produced in this process, both chambers are connected through a proton exchange membrane, which allows only the passage of protons produced during substrate oxidation. In the cathode section, the electrons and protons that have been transferred react with oxygen to generate water. Consequently, electricity is created by the movement of electrons within the electric circuit. In summary, the anode functions as a recipient of electrons, whereas the cathode acts as a source of electrons in microbial fuel cells (MFCs) [8,13].

Compared to other advanced wastewater treatment processes, one of the critical advantages of the MFC system is its ability to treat all kinds of substrates. During their research, scientists have purified simple liquid substrates, such as alcohols, glucose, and volatile fatty acids. Also, by using complex substrates, such as domestic wastewater, brewery, dairy, winemaking, soybean oil refinery, distillery, and food processing effluents, researchers have been able to obtain promising results [14].

In addition, past research has proven that this technology can remove a wide range of pollutants, including mercury, ammonia, nitrite, iron, copper, nitrate, perchlorate sulfide, and chlorine compounds [15,16].

The concept of MFC was first introduced by Potter in 1910 with the production of electricity by *Escherichia coli*, and this technology was first researched in 1991 for wastewater treatment. Today, scientists are widely studying and researching this technology, so with more interested scientists entering this field, the number of journal publications has doubled in the last three years [2,17,19]. During the research conducted until today, scientists have paid much attention to improving and increasing

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the production power in MFCs, leading to much information about the factors affecting the parameters in these systems.

In recent years, the biochemical recycling of industrial wastewater using MFCs has been investigated, and a variety of single-chamber, double-chamber, and tubular MFCs with electrodes made from different materials such as carbon, graphite, stainless steel, or composites have been tested [14,20]. For example, to improve the process of industrial wastewater treatment and energy production, scientists prepared new anodes made of carbon cloth, carbon paper, and activated carbon. The results showed that the techniques significantly affected the system's performance in producing power and current density [10,21]. In another research, experimental results showed that MFCs can produce a maximum power density of 1771 mW.m^{-2} from industrial wastewater containing cyanide [22]. In their research involving wastewater samples from sectors such as vegetable oil production, metalworking, glass and marble manufacturing, chemical processing, and mixed industrial wastes, Abbasi *et al.* demonstrated that hydraulic retention time (HRT) plays a crucial role in the effectiveness of wastewater treatment [23].

In a study on food factory wastewater, Nimezh *et al.* recorded a power density of 150 mW.m^{-2}, compared to the power densities from paper, domestic, and agricultural wastewater [24].

In another study, electroplating industrial wastewater was used as a raw material to remove organic content and achieve the maximum power and current density of about 260 mW.m^{-2} and 364 mA.m^{-2}, respectively [25]. In various research, scientists have exploited the ability of certain types of microorganisms to carry out biochemical reactions, producing electrical energy from chemical energy or vice versa [26].

In the present research, we tested the feasibility of using a mediator-less MFC to treat industrial wastewater containing grape waste as a suitable carbon source in the anode, assisted by electron production using *Aspergillus niger* yeast as the microorganism. The COD removal rates and MFC electricity output were also evaluated. Laboratory experiments were carried out in a dual-chamber setup to optimize the anode and cathode conditions to improve the performance of the MFC reactor throughout wastewater treatment. The investigation focused on batch processes' power, current output, and the impact of different mixed liquor-suspended solids (MLSS) levels.

Materials & Methods

Wastewater Preparation and the Microorganism Source

The wastewater utilized in this study was sourced from the citric acid facility of Joven Agro-industry Company, situated in northeastern Iran. The characteristics of the effluent are presented in Table 1.

Table 1) Characteristics of the citric acid wastewater produced by Joven Agro-industry Company.

Characteristics	Unit	Level
Temperature	˚C	25
Electrical conductivity (EC)	μs.cm^{-1}	17500
COD	mg.L^{-1}	9740
BOD_5	mg.L^{-1}	4890
Nitrite	mg.L^{-1}	0.018
Nitrate	mg.L^{-1}	10.7
Volatile suspended solids (VSS)	mg.L^{-1}	21.5
Phosphate in terms of phosphorus	mg.L^{-1}	0.42
Sulfate	mg.L^{-1}	2>
Chloride	mg.L^{-1}	2835
Total Kjeldahl Nitrogen (TKN)	mg.L^{-1}	500<

The *Aspergillus niger* species present in the sewage itself was used. Citric acid wastewater has a high COD concentration in the range of 1000-10000 mg.L^{-1} and a low pH of 2-4, posing a threat to the ecological balance and human health [27]. Citric acid is a crucial product in global trade and one of the most extensive industrial biotechnology products, approved by the World Food and Health Organization for use in the food, pharmaceutical, and health industries. Currently, *Aspergillus niger* fungus is used for the commercial and successful production of citric acid [28,31]. The wastewater used in this research was stored in the refrigerator at 4°C before use.

Designing of the Microbial Fuel Cell

As illustrated in Figure 1, the microbial fuel cell (MFC) employed in this study was configured as a two-chamber arrangement comprising an anode compartment and a cathode compartment. The total volume of each chamber was 1 liter, and they were divided by a proton exchange membrane (Nafion 117). The size of the membrane sheet was 3×3 cm. Each chamber was designed from colorless plexiglass sheets with a diameter of 0.6 mm and dimensions of 6 ×13 ×13 cm. Before using the membrane, these steps were taken to remove impurities, increase the membrane porosity, and improve its performance in passing protons and protonizing them. The membrane was placed in a container of deionized water at approximately 80 ˚C for one hour. It was then placed in a 3% H_2O_2 solution by weight at about 80 ˚C for one hour, after which the first step was repeated. Next, the membrane was immersed in a 1 M sulfuric acid solution at 80 ˚C for one hour, and afterward, the first step was repeated. Following these steps, the membrane surface was activated and ready for use.

Due to the significant impact that electrodes have on the performance of these systems, scientists are always looking for materials with good chemical stability, low cost, high conductivity, and good biocompatibility. Carbon-based materials are one of the commonly tested materials that have been widely used [18,32]. In this experiment, graphite electrodes measuring 3×25×45 mm were employed for electron transfer. Prior to utilizing the system, the electrodes were rinsed

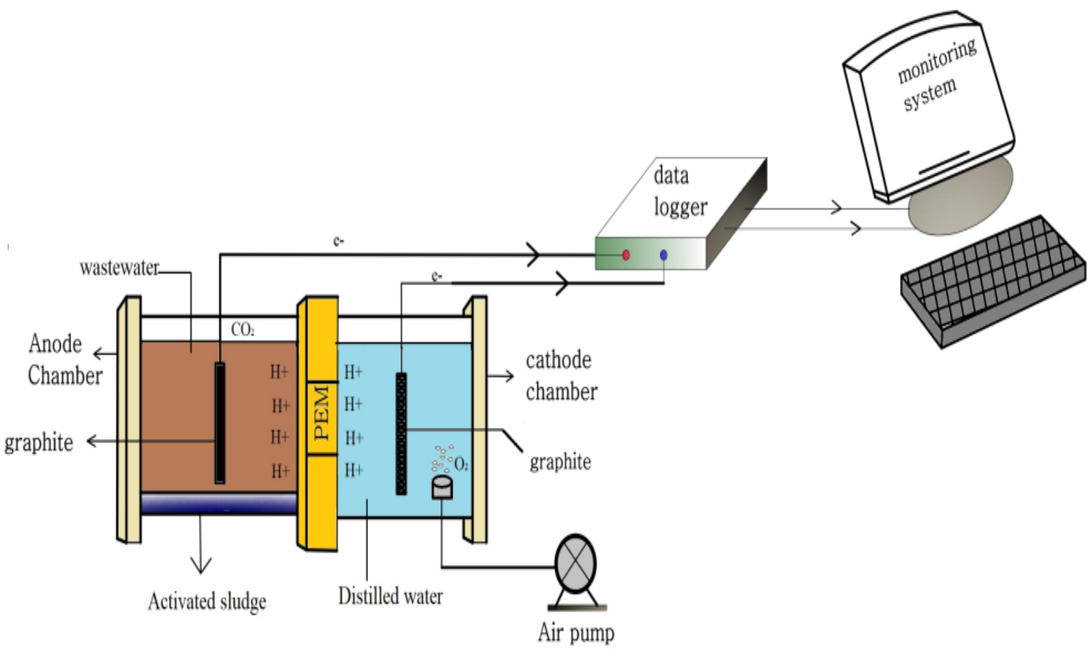

Figure 1) Schematic design of applied microbial fuel cell system.

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with 1 M hydrochloric acid and then followed by deionized water. The cleaned electrodes were kept in distilled water until they were needed. The electrodes were directly linked to a data logger system (model DL2114) to track the output voltage of the MFC reactor. An air pump was used to supply dissolved oxygen to establish aerobic conditions in the cathode. A magnet and stirrer were also used to stir the contents in the anode compartment. Since pH 7 is optimal for the activity of microorganisms under anaerobic conditions, the pH was adjusted to 7 [33].

Measurement of Voltage, Power, and Current Density

The MFC reactor was operated under open circuit conditions at different initial concentrations of MLSS. The voltage in mV was measured continuously at 1-minute intervals using a data logger. When different resistors with values of 10, 15, 22, 47, 68, 100, 150, 220, 330, 470, 680, 1000, 1500, 2200, 3300, 4700, 5600, 6800, and 10,000 ohms were added to the external circuit of the system, the voltage was stabilized. The current and power density were measured using Eqs. (1) and (2).

$$I = \left(\frac{E}{R_{ext}}\right) \quad \text{Eq. (1)}$$

$$P = I \times E \quad \text{Eq. (2)}$$

In this context, P denotes the production power, E represents the voltage, R_{ext} represents the external resistance, and I indicates the current value. To enhance electrical conductivity, the selected electrode for the fuel cell system should feature a high specific surface area, which increases the surface area available for reactions and reduces the system's internal resistance. In this investigation, the current and power measurements were normalized according to the cross-sectional area of the cathode, which was 22.5 cm^2.

Setting Up Microbial Fuel Cell

The MLSS variable was treated as a factor in every experiment to establish this system. Research indicates that MLSS is among the most significant and impactful parameters influencing MFC performance. MLSS serves as a measure of the active microbial mass [34]. The concentrations of this variable were 1000 $mg.L^{-1}$ and 3000 $mg.L^{-1}$. In this study, the adequate volume of each chamber was 675 ml, and the reactor temperature was kept at standard conditions, corresponding to the surrounding temperature and atmospheric pressure.

Findings

In this research, we examined how changes in MLSS affect microbial fuel cells utilizing citric acid industrial wastewater and *Aspergillus niger* at two different concentrations, specifically 1000 $mg.L^{-1}$ and 3000 $mg.L^{-1}$, for wastewater treatment and concurrent electricity generation. Consequently, we evaluated the voltage, power, current density measurements, and the percentage of COD removal, all affected by and contingent upon MLSS. The findings indicated that inoculating samples with various initial MLSS concentrations significantly impacted the system's performance, confirming the importance of this parameter. The performance of the microbial fuel cell, regarding the open circuit production voltage at different concentrations, is shown in Figures 2 and 3.

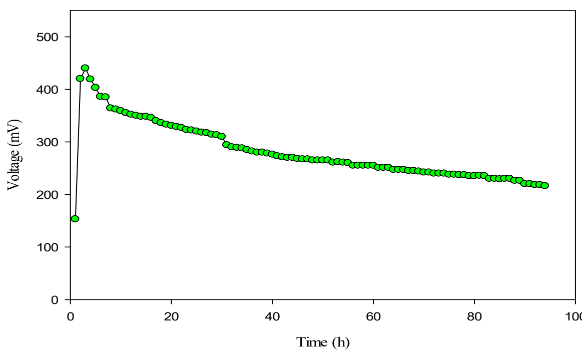

Figure 2) The effect of MLSS dose of 1000 $mg.L^{-1}$ on the production of open circuit voltage.

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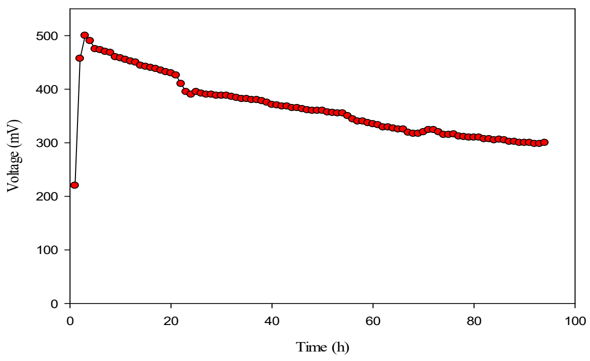

Figure 3) The effect of MLSS dose of 3000 mg.L^{-1} on the open circuit voltage production.

Figure 2 shows that the maximum open circuit voltage produced at the concentration of MLSS 1000 mg.L^{-1} is 440 mV. The highest production voltage of MLSS 3000 mg.L^{-1} concentration is 500 mV, as shown in Figure 3. Both Figures 2 and 3 demonstrate that electrical energy is produced directly from the biodegradable compounds in wastewater. After feeding, the system produces energy and reaches its maximum production voltage after 3 hours. However, the voltage decreases over time after reaching the peak point due to the lack of substrate and organic matter [35].

Figures 4 and 5 illustrate how MLSS variations impact the MFC system's polarization curve. As indicated by Figure 4, the peak current and power density for an MLSS of 1000 mg.L^{-1} are 11735 µW.m^{-2} and 244 mA.m^{-2}, respectively.

Figure 5 shows that the maximum power and current density at the MLSS of 3000 mg.L^{-1} are approximately 24345 µW.m^{-2} and 444 mA.m^{-2}, respectively. For systems that treat wastewater, the efficiency of COD removal stands out as a crucial factor in assessing performance and energy generation. The effectiveness of the MFC regarding wastewater treatment was also assessed at varying MLSS concentrations of 1000 mg.L^{-1} and 3000 mg.L^{-1}. The outcomes of the experiments examining the impact of the MLSS variable on electricity generation and COD removal rates in the MFC system are presented in Table 2.

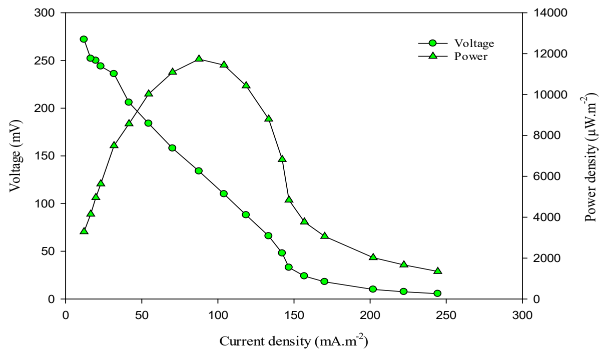

Figure 4) The effect of MLSS dose of 1000 mg.L^{-1} on polarization curve in MFC system.

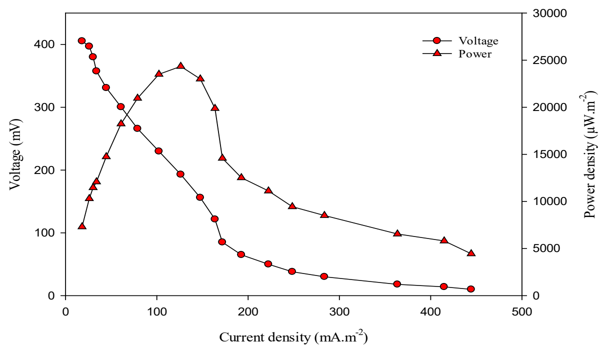

Figure 5) The effect of variable MLSS 3000 mg.L^{-1} on polarization curve in MFC system.

The results show that a high concentration of MLSS in the anodic chamber has increased microorganisms' substrate utilization, resulting in higher COD removal efficiency. The highest COD removal efficiency of 93% was obtained at the MLSS concentration of 3000 mg.L^{-1}; however, the COD removal percentage for the MLSS concentration of 1000 mg.L^{-1} was 71%.

Discussion

The data presented in Table 2 indicates a positive correlation between open circuit voltage generation and MLSS concentration during the operation of the MFC. It can be deduced that an increase in MLSS concentration enhances substrate

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Table 2) The findings from the tests examining the impact of MLSS variables on electricity production and COD removal percentage.

MLSS ($mg.L^{-1}$)	Maximum Voltage (mV)	Maximum Power Density ($\mu W.m^{-2}$)	Maximum Current Density ($mA.m^{-2}$)	COD Removal, %
1000	440	11735	244	71
3000	500	24345	444	93

Table 3) Power density produced in different MFCs using electrodes and microorganisms.

Anode	Cathode	Microorganism	Power Density	Reference
Graphite plate	Graphite plate	*Aspergillus niger*	24345 $\mu W.m^{-2}$	Current study
Plain carbon paper	Plain carbon paper	*C.acetobutylicum* and *C.Thermohydrosulfuricum*	7.18 $mW.m^{-2}$	(45)
Activated carbon	Hydrophobic carbon fabric	*Proteus hauseri* ZMd44	103 $mW.m^{-2}$	(46)
Graffiti pages	Platinum meshes	*Shewanella oneidensis*	1410 $mW.m^{-2}$	(47)
Activated carbon fabric	Graphite foil	*D. desulfurisers strain*	0.51 $mW.m^{-2}$	(47)
Graphite felt	Graphite rod	*Shewanella oneidensis* MR-1	32.5 $mW.m^{-2}$	(48)
Granular activated carbon	Stainless steel mesh	*Geobacter Sulfurreducens* and *Beta Proteobacteria*	610 $mW.m^{-2}$	(48)
Carbon fabric	Carbon cloth with platinum as a catalyst	*Shewanella oneidensis* MR-1	158.1 $mW.m^{-2}$	(49)
Magnetic composite electrode with a polymer coating	Mesh carbon paper	*Synechococcus sp.*	4.9 ±0.5 $W.m^{-3}$	(50)
Carbon fabric / reduced graphene/ polypyrrole	Carbon paper	*E. coli*	1068 $mW.m^{-2}$	(51)
Carbon fabric	Carbon fabric	*Klebsiella sp*. MC-1	412 $mW.m^{-2}$	(52)
Carbon/graphene fabric	Carbon fabric	*S. putrefaciens* CN32	679.7 $mW.m^{-2}$	(53)
Graffiti page	Graffiti page	*Acidithiobacillus sp.* and *Ferroplasma sp*	17.6 $mW.m^{-2}$	(54)
Porous carbon fabric	Porous carbon fabric	*Proteus hauseri*	83.4 $mW.m^{-2}$	(55)
Graphite Fe@Fe2O3	Graphite Fe@Fe2O3	*Saccharomyces cerevisiae*	30.46 $mW.m^{-2}$ 13.51 $mW.m^{-2}$	(56)

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degradation, promoting the production of protons and electrons by microorganisms, ultimately leading to increased voltage output [36]. Also, by comparing the power density and the current density recorded in Table 2 for both MLSS concentrations, the substrate degradation rate is lower at the MLSS value of 1000 mg.L^{-1} due to fewer microorganisms in the anode chamber. Due to the substrate's bottom degradation, fewer electrons and protons are produced. As a result, the electron transfer rate decreases due to reduced production of electrons by microorganisms.

These conditions cause low decomposition of COD by microorganisms. This result aligns with previous MFC research that explained higher energy production associated with more biomass available for electron and proton generation, achieving higher electron transfer rates [36]. Our research indicates that elevated energy production at increased MLSS concentrations is connected to the system's food-to-microorganism ratio (F/M). Consequently, as the MLSS concentration rises, the F/M ratio declines, and previous studies have shown that a lower F/M ratio can enhance organic removal efficiency and sludge flocculation, leading to increased power generation [37]. On the other hand, at the high concentration of MLSS, the high growth of bacteria during the chamber activity can increase the electricity production. Stabilizing the sludge at the termination of the test run and reducing bacterial populations have led to a decrease in electricity production [35,38]. In conclusion, it can be observed that an increase in the initial concentration of MLSS leads to an enhancement in both power density and current density [39]. According to the results of other researchers, a thick biofilm on the surface of the electrodes does not cause a problem in the process of transferring electrons to the surface of the electrodes because the attached bacteria can form bridges of electron transfer [34].

The literature states that higher MLSS concentrations contribute to greater uptake and degradation of substrates and bacterial communities. However, it has been reported that electricity generation is limited by excess sludge. Wang *et al.* reported that as the MLSS concentration increased from 3400 mg.L^{-1} to 4340 mg.L^{-1}, the current output rose from 0.64 mA to 0.76 mA. However, with continued increases in MLSS over time, there was a subsequent decline in the current generation [38,40,41]. The results of Table 2 show that the MLSS concentration of 3000 mg.L^{-1} provides a sufficient amount of microorganisms for the decomposition of wastewater organic matter. Research has indicated that increased MLSS concentration is associated with a lower F/M ratio, which can enhance removal efficiency [34,37]. Our findings align with research reporting that increasing MLSS concentration improves removal efficiency [37,42,43]. It has also been shown that increasing MLSS beyond a specific range can negatively affect MFC removal performance and energy recovery [41,44]. As a result of this study, it is concluded that the level of MLSS influences microbial activity, and higher MLSS concentrations enhance the efficiency of COD removal. Considering the importance of the power density parameter in evaluating the performance of a microbial fuel cell system, Table 3 presents the maximum power density produced in several studies conducted by researchers using various types of microorganisms compared to the current research.

Conclusion

This research showed that the variable MLSS has an essential effect on the performance of the microbial fuel cell without an intermediary. The obtained data showed that COD removal efficiency and electricity

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generation in two experiments were affected by MLSS dosage, as MLSS of 3000 mg.L^{-1} in organic matter reduction, power, and voltage generation has a much better performance than MLSS of 1000 mg.L^{-1}. The findings indicated that the thick biofilm formed on the electrodes at elevated MLSS levels did not hinder the electron transfer process to the electrodes' surfaces, as the adhered bacteria could create electron transfer connections. As a result, significant voltage production can still be attained even with high MLSS concentrations. Overall, the findings regarding COD reduction and energy output demonstrate the satisfactory performance of microbial fuel cells in treating actual industrial wastewater, such as that from the citric acid sector, and biomass can serve as a viable source for renewable energy generation in bioenergy production. On the other hand, due to the widespread attention of scientists and their extensive investigations on the application of microbial fuel cells in the treatment of various types of wastewater, the prospects for developing this technology can be evaluated very clearly.

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Conflicts of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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