

THE INFLUENCE OF pH ON BIOELECTRICITY AND TREATMENT OF LOCUST BEAN WASTEWATER IN MICROBIAL FUEL CELLS

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Abstract. This study investigated the influence of pH on the treatment efficiency and electricity generation of locust bean wastewater using double-chamber microbial fuel cells (MFCs) inoculated with *Clostridium ramosum*. Four pH conditions (3.4, 7.0, 9.0, and 11.0) were tested over a ten-day period with voltage, current, and wastewater treatment parameters measured daily. Results demonstrated that neutral pH (7.0) consistently produced optimal bioelectricity, with voltage increasing from 585 mV to 730 mV and current reaching 63 mA. Acidic conditions (pH 3.4) yielded significantly lower outputs (130-240 mV), while alkaline environments showed initial tolerance but declining performance over time. At pH 7.0, wastewater treatment efficacy was substantial, with 48.6% reduction in electrical conductivity, 34.7% decrease in total dissolved solids, 25.6% reduction in chemical oxygen demand, and significant decreases in nitrate (13.4%) and sulfate (38.7%) concentrations. The study highlights the critical importance of pH control in MFC operations, with neutral conditions providing the optimal environment for microbial metabolism, electron transfer mechanisms, and overall system performance. These findings provide valuable insights for optimizing MFC applications in wastewater treatment facilities where pH fluctuations may occur, emphasizing that maintaining near-neutral conditions substantially enhances both electricity generation and contaminant removal efficiency in bio-electrochemical systems.

Keywords: Microbial Fuel Cells, pH, Bioelectricity

1. Introduction

Treating wastewater using microbial fuel cells (MFCs) has emerged as a promising technology that addresses wastewater management challenges while offering the potential for energy recovery. MFCs utilize electroactive microorganisms to convert organic matter in wastewater into electrical energy, facilitating simultaneous wastewater treatment and power generation [1], [2]. Among various wastewater types, locust bean wastewater presents unique challenges due to its high organic content and variable pH levels, which can significantly influence the performance of MFCs. Previous studies have indicated that the pH of the wastewater can affect microbial activity, electron transfer processes, and overall system efficiency [3], [4].

Research has shown that MFCs can effectively reduce chemical oxygen demand (COD) and total suspended solids (TSS) in wastewater while also generating electricity [1], [5]. The efficiency of these systems is often contingent upon operational parameters, including pH, which can alter the metabolic pathways of the microorganisms involved. For instance, studies have demonstrated that maintaining optimal pH levels can enhance the performance of MFCs by promoting the growth of specific microbial communities that are more efficient at degrading organic substrates [6]. Furthermore, the coupling of MFCs with other treatment technologies, such as membrane bioreactors, has been explored to improve treatment efficiency and reduce operational costs [7].

The influence of pH on MFC performance has been a focal point in recent research. For example, [3] found that utilizing a high-pH anode and a low-pH cathode configuration could significantly enhance electricity generation in two-chamber MFCs. This finding underscores the importance of optimizing pH conditions to

maximize the electrochemical reactions occurring within the cell. Additionally, the interaction between pH and microbial community dynamics has been highlighted, suggesting that different pH levels can lead to shifts in microbial populations, affecting the overall efficiency of the MFC [8].

This study aims to investigate the influence of pH on the treatment efficiency and electricity generation of locust bean wastewater using MFCs. By systematically varying the pH levels and analyzing their effects on microbial activity, electricity output, and pollutant removal rates, this research seeks to provide insights that could enhance the practical application of MFCs in wastewater treatment. This study will contribute to the growing knowledge on optimizing MFC performance and addressing the specific challenges of locust bean wastewater.

2. Materials and Method

A double-chamber horse-shoe-shaped microbial fuel cell (MFC) was constructed to evaluate the influence of pH on voltage and current generation using locust bean wastewater as the anodic substrate. The MFC consisted of two chambers separated by a salt bridge consisting of a composite mixture of Agar-agar (5% in solution) and potassium chloride (1 M)) which was subsequently heated and allowed to cool to form a gel, facilitating the transfer of protons while preventing the mixing of the anode and cathode compartments. The anode chamber was filled with locust bean wastewater, while the cathode chamber contained a potassium ferricyanide solution as the electron acceptor.

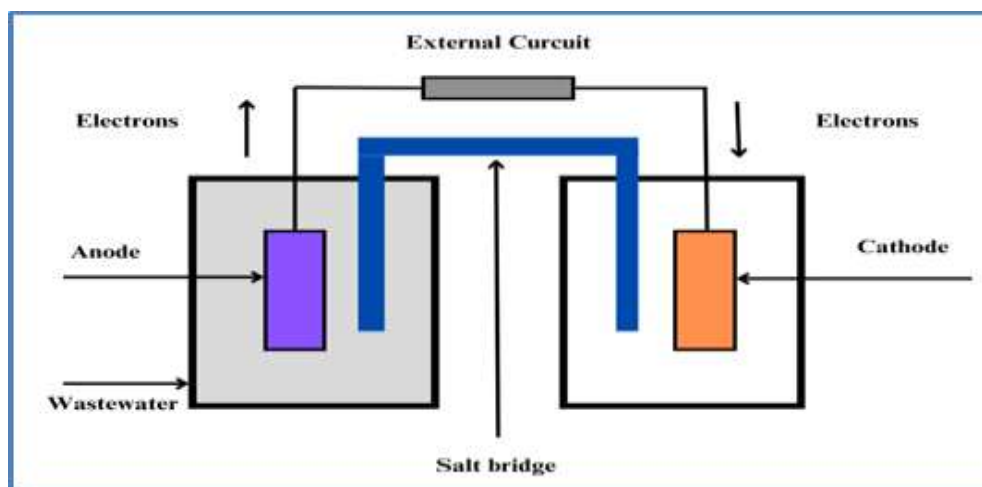


Fig. 1: A Horseshoe-shaped Double Chambered Microbial Fuel Cell

2.1. Inoculum Preparation

The inoculum used for the MFC consisted of *Clostridium ramosum*, a known exoelectrogenic bacterium. The inoculum was cultured in a nutrient-rich medium under anaerobic conditions to promote growth and enhance electroactivity. Before the experiment, the inoculum was harvested and then re-introduced into the anode chamber containing fresh locust bean wastewater that had previously been autoclaved to ensure that only the single strain of the selected inoculum was available to enhance the microbial activity for electricity generation.

2.2. Experimental Setup

The MFC was operated under controlled laboratory conditions at a constant temperature of 30°C. The pH levels of the locust bean wastewater were adjusted to four different values: 3.7, 7.0, 9.0, and 11.0, using hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions. Each pH condition was tested in separate MFCs, with three replicates for each pH level to ensure statistical validity.

2.3. Voltage and Current Measurements

Voltage and current readings were taken daily over ten days using a digital multimeter connected to the MFC. The voltage was measured across the external circuit, while the current was calculated using Ohm's

law ($I = V/R$), where R was the resistance of the external load connected to the MFC. The readings were recorded simultaneously each day to minimize variations due to environmental factors.

2.3 Data Analysis

The voltage and current data collected over the ten days were analyzed to determine trends and correlations with the varying pH levels. Statistical analysis was performed using graphical representations, including bar charts depicting daily voltage measurements, to facilitate clear visual interpretation of the experimental results. The results were presented in tabular format, summarizing each day's voltage and current measurements at each pH level.

3. Results and Discussion

3.1 pH Influence on Voltage Generation in Microbial Fuel Cells

The pH of the electrolyte environment in microbial fuel cells (MFCs) significantly influences voltage generation and overall system performance, fundamentally through its impact on microbial activity and electron transfer efficiency. This phenomenon is particularly evident when examining the performance patterns of *Clostridium ramosum* across a broad pH spectrum ranging from 3.4 to 11.0.

3.1.1 Acidic Conditions (pH 3.4)

The voltage generation performs poorly under acidic conditions by producing outputs ranging from 130 mV to 240 mV. The performance of MFCs suffers significant reduction because low pH inhibits both microbial metabolic processes and intrinsic electron transfer mechanisms. The bioelectricity production decreases because low pH values lead to proton accumulation at the anode electrode and hydroxide ion accumulation at the cathode which creates an environment that hinders essential enzymatic activities needed for substrate oxidation (Rojas-Flores et al., 2022). The scientific community has established that low pH conditions create metabolic barriers which require specific pH thresholds to maintain both microbial survival and MFC operational efficiency [9].

3.1.2 Neutral pH Conditions (pH 7.0)

The voltage output shows its best performance at neutral pH conditions where it starts at 585 mV on Day 1 and reaches its maximum value of 730 mV by Day 10. Success rates among bacterial adaptability demonstrate their ability to form efficient electroactive biofilms that enable enhanced metabolic operation and electron transfer. Research shows that neutral pH represents an optimal environment for electroactive microorganisms to grow and function optimally inside MFCs [10], [11].

3.1.3 Alkaline Conditions

The alkaline conditions produce a multifaceted voltage response pattern. The voltage output decreased from 515 mV to 330 mV at pH 9.0 yet the pH 11.0 measurements showed a wider range of voltages from 395 mV to 650 mV. The data shows that *C. ramosum* maintains its alkaline tolerance at first but extended exposure causes severe metabolic strain and possible damage to cell membranes. The research by [12] supports the notion that pH stands as a fundamental operational parameter affecting MFC performance. Catalytic systems in MFCs strongly depend on pH equilibrium since both harsh acidic and alkaline conditions reduce microbial efficiency and decrease power output [13], [14]. Operational conditions must be carefully controlled because pH affects microbial metabolism and enzymatic activity and biofilm formation which determines the electricity generation potential.

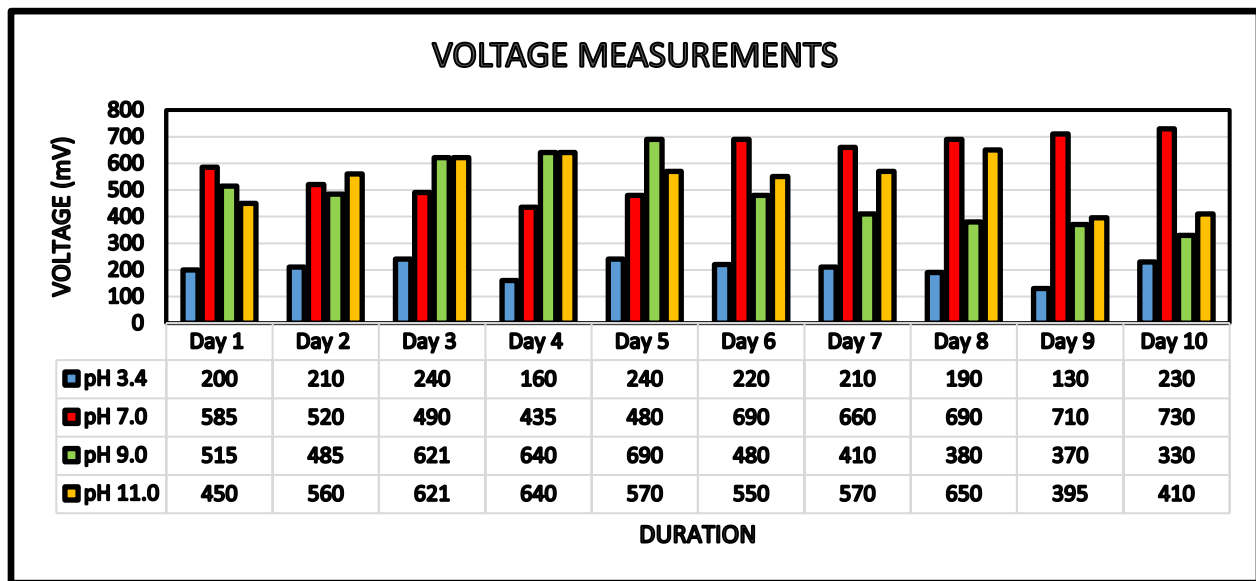


Fig. 2: Daily Voltage Readings at Varied pH Values

3.2 Performance of Microbial Fuel Cells in Current Production

The bioelectricity generation performance of microbial fuel cells (MFCs) using *Clostridium ramosum* strongly depended on electrolyte pH levels because this factor directly affects the electricity production process. The study investigates how *C. ramosum* behaves bioelectrically when exposed to acidic, neutral and alkaline pH solutions.

3.2.1 Acidic Conditions (pH 3.4)

The bioelectricity output of *C. ramosum* decreased to between 9 mA and 18 mA when the solution reached pH 3.4. The research papers show that acidic conditions negatively affect microbial metabolism by impairing both essential enzymatic processes for substrate oxidation and disrupting electron transfer pathways. *C. ramosum* experiences cellular membrane damage and enzymatic dysfunction at acidic pH 3.4 because these conditions are essential for proper energy conversion [9]. The evidence shows that cells need a balanced pH environment for their best performance because microbes react strongly to changes in pH.

3.2.2 Neutral pH Optimization (pH 7.0)

C. ramosum demonstrated its best performance at pH 7.0 which leads MFCs to peak currents that reached 63 mA. Under neutral conditions microbial metabolic activity continues through the establishment of effective electroactive biofilms and improved electron transfer processes. The research by Santoro *et al.* (2017) supports the importance of neutral pH conditions because they enhance the growth and electrochemical efficiency of electroactive microorganisms. The measurements of voltage and current at neutral pH show a positive relationship which indicates *C. ramosum* achieved its best energy production rates under these conditions.

3.2.3 Alkaline Conditions (pH 9.0 and 11.0)

The effects on MFC performance become intricate in alkaline conditions. The measurements at pH 11.0 showed transitional stability through a current that reached its peak at 52 mA before experiencing a major decrease. The observed pattern demonstrates how *C. ramosum* demonstrated adaptive responses to different pH conditions. The prolonged exposure to extreme alkaline conditions causes metabolic stress and damages cell membranes according to research on microbial communities under different pH conditions. The microorganism shows limited alkaline tolerance but its long-term operational capability will decrease because metabolic processes become increasingly impaired.

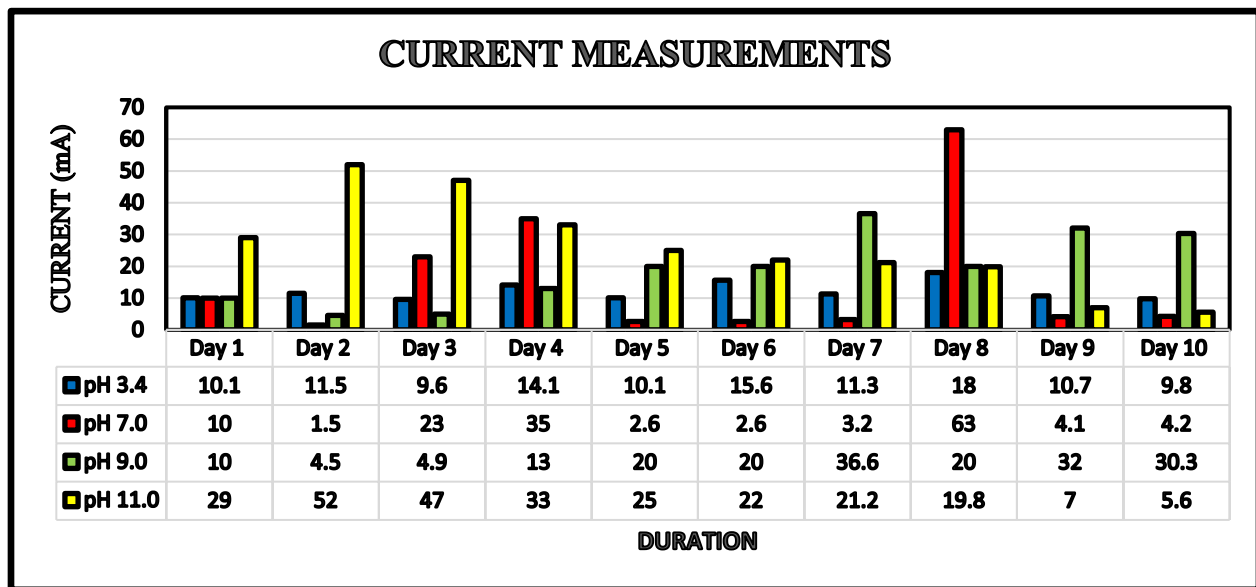


Fig. 3: Daily Current Readings at Varied pH Values

3.3 Microorganism-Specific Considerations

The pH-dependent behavioral responses of *Clostridium ramosum* directly impact the performance results of MFC systems. The microorganism demonstrates metabolic flexibility to adapt at different pH levels but requires neutral conditions for sustainable energy conversion operations. The pH sensitivity of *C. ramosum* requires researchers to choose appropriate microbial strains and optimize operational parameters in bioelectrochemical systems for maximum energy generation efficiency. Future investigations must explore the fundamental processes behind MFC operation to optimize design features for better bioelectricity production.

3.4 Wastewater Treatment

Treating wastewater at near-neutral pH (specifically around pH 7) has demonstrated significant effectiveness across various water quality parameters, underscoring the importance of pH in biological wastewater treatment processes. The initial pH of 6.8 as depicted in Figure 1, which slightly increased to 7.4 by the end of the treatment, indicates a successful buffering capacity within the system, maintaining optimal conditions for microbial activity throughout the treatment process. This slight pH increase is crucial, as it aligns with the optimal pH range for many wastewater treatment microorganisms, which typically thrive between pH 6.5 and 7.5 [15].

The reduction in Electrical Conductivity (EC) from 1850 to 950 $\mu\text{S}/\text{cm}$, representing approximately a 48.6% reduction, suggests the effective removal of dissolved ions and charged particles. This substantial decrease correlates with Total Dissolved Solids (TDS) reduction from 1500 to 980 mg/L, indicating a 34.7% removal efficiency. Such parallel decreases in EC and TDS highlight the successful treatment of dissolved constituents under near-neutral conditions, essential for maintaining water quality standards [16].

Chemical Oxygen Demand (COD) showed a notable improvement, decreasing from 820.325 to 610.472 mg/L, reflecting a 25.6% reduction. The moderate COD reduction at pH seven can be attributed to optimal microbial metabolism and substrate utilization conditions. The enhanced microbial activity at this pH level facilitates the degradation of organic matter, which is a critical aspect of wastewater treatment. Studies have shown that microbial communities exhibit peak activity in the pH range of 6.5-7.5, further supporting the observed improvements in COD reduction [17].

Nutrient dynamics also exhibited favorable trends under near-neutral pH conditions. Nitrate (NO_3) levels decreased from 3.102 to 2.687 mg/L, indicating a 13.4% reduction. This moderate reduction suggests active denitrification processes, likely supported by the favorable pH conditions promoting denitrifying bacteria growth. Similarly, sulfate (SO_4) concentrations decreased from 4.512 to 2.765 mg/L, representing a 38.7% reduction. This reduction indicates the effective operation of sulfate-reducing bacterial communities, which thrive in near-neutral pH environments [18].

The slight temperature decrease from 28.5°C to 27.9°C, remaining within the mesophilic range optimal for biological treatment processes, further supports stable microbial populations and consistent treatment performance. Such minimal temperature variation, combined with the near-neutral pH, is conducive to maintaining microbial diversity and activity, which is essential for effective wastewater treatment [19].

The performance metrics indicate that the near-neutral pH environment supported effective biological treatment processes, particularly for organic matter removal and nutrient transformation. Maintaining pH within the optimal range throughout the treatment period suggests good system stability and buffering capacity, essential for consistent treatment efficiency. These results align with established principles of biological wastewater treatment, where near-neutral pH conditions typically support optimal microbial diversity and activity.

Table 1: Before and After Treatment at Near-Neutral pH

Parameter	Before Treatment	After Treatment	Percentage Reduction (%)
pH	6.8	7.4	-
Electrical Conductivity (EC)	1850 mg/L	950 mg/L	48.6
Total Dissolved Solids (TDS)	1500 mg/L	980mg/L	34.7
Chemical Oxygen Demand (COD)	820.33 mg/L	610.47 mg/L	25.6
Nitrate (NO ₃)	3.2 mg/L	2.69 mg/L	13.4
Sulphate (SO ₄)	4.51 mg/L	2.77 mg/L	38.7

4. Conclusion

This study demonstrates that pH significantly impacts MFC performance when treating locust bean wastewater, with neutral conditions (pH 7.0) providing optimal results for both electricity generation and contaminant removal. The established correlation between pH and bio-electrochemical performance provides critical insights for practical MFC applications. Maintaining near-neutral pH conditions is essential for maximizing electron transfer efficiency, promoting microbial metabolism, and ensuring consistent treatment outcomes. These findings contribute valuable knowledge for scaling up MFC technology in wastewater treatment facilities, particularly in environments prone to pH fluctuations, and highlight the importance of pH control strategies in bio-electrochemical system design and operation.

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