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Influence of Aquatic Plants and Electrode Spacing on Chemical Oxygen Demand Removal and Bioelectricity Generation

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Wastewater treatment presents a significant challenge, particularly in rural areas where treatment capacity is insufficient. This study explored the potential of microbial photosynthetic cells (MPFCs) for chemical oxygen demand (COD) removal and simultaneous electricity generation. Six MPFCs were operated for 60 days with wastewater, using the plants *Pistia stratiotes* and *Eichhornia crassipes*, with varying spacing between the graphite electrodes. The best COD removal (90.80%) and bioelectricity production (4.21 MW/m²) were obtained with *Pistia stratiotes*. These results highlight the relevance of PMFCs as a sustainable alternative for energy recovery and wastewater treatment.

1. Introduction

Water pollution from untreated municipal wastewater discharge represents a critical global challenge, particularly in regions with limited sanitation infrastructure (Albini et al., 2023). In Peru, this issue is especially concerning, as existing treatment plants lack the capacity to process the generated volumes, leading to detrimental impacts on aquatic ecosystems and public health (Nieto-Juárez et al., 2021). Microbial fuel cell (MFC) technologies have emerged as a sustainable alternative, enabling simultaneous wastewater treatment and bioenergy generation.Photosynthetic microbial fuel cells (PMFCs) incorporating microalgae offer additional operational advantages by eliminating the need for external aeration and allowing biomass reuse. This synergy between photosynthetic and electrogenic systems presents a promising paradigm for sustainable sanitation, particularly in regions with limited infrastructure (Meher and Sharma, 2022). Previous studies have demonstrated that species such as *Pistia stratiotes* and *Eichhornia crassipes* exhibit effective phytoremediation capabilities due to their high nutrient and heavy metal absorption capacity (Buta et al., 2023). However, their performance in PMFC systems may vary depending on plant species, planting density, and reactor configuration (Ntakiyiruta et al., 2022).Electrode spacing represents another critical factor influencing PMFC efficiency, as it affects internal resistance and electron transfer (Wang et al., 2018). Recent research indicates that smaller anode–cathode distances enhance bioelectricity production, although their impact on chemical oxygen demand (COD) removal requires further investigation (Ullah et al., 2023).

Despite growing evidence supporting PMFCs as dual-function systems for wastewater treatment and energy generation, understanding remains limited regarding how aquatic plant species (*Pistia stratiotes* and *Eichhornia crassipes*) and electrode spacing jointly affect COD removal and bioelectricity output under real municipal wastewater conditions.

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Therefore, the central research question is: How do aquatic plant species (*Pistia stratiotes* and *Eichhornia crassipes*) and electrode spacing (9 cm, 12 cm, and 14 cm) influence COD removal efficiency and bioelectricity generation in photosynthetic microbial fuel cells (PMFCs) treating municipal wastewater from the Covicorti Treatment Plant (Trujillo)? It is hypothesized that the combined effect of aquatic plant species and electrode spacing significantly influences both COD removal efficiency and bioelectricity generation in PMFCs treating municipal wastewater. Microbial fuel cell (MFC) technologies have emerged as a sustainable option capable of addressing two pressing needs: wastewater treatment and renewable energy generation. However, these dual objectives often compete in design priorities, as optimizing organic matter removal does not always coincide with maximizing bioelectricity output. Therefore, this study primarily seeks to enhance wastewater treatment efficiency while secondarily assessing the potential for energy recovery through photosynthetic microbial fuel cells (PMFCs). By clearly defining this hierarchy of goals, the research aims to provide evidence for the feasibility of PMFCs as an eco-efficient alternative for municipal wastewater management.

2. Materials and Methods

2.1 Wastewater sampling and Plant Incorporation

A sample of wastewater was taken from the municipal area that discharges into the Covicorti Wastewater Treatment Plant in the city of Trujillo. The sampling point is located at the coordinates East: 714,844.58 m and North: 9,101,703.22 m. The study employed two plant species: *Eichhornia crassipes* in PMFC2, PMFC4, and PMFC5, and *Pistia stratiotes* in PMFC1, PMFC3, and PMFC6. *Eichhornia crassipes* specimens were collected from the wetlands of Víctor Larco District, while *Pistia stratiotes* plants were obtained from a Trujillo aquarium, with size-standardized selection to ensure morphological consistency. Although these species are not native to Trujillo’s coastal zone, both are well-established in Peruvian aquatic systems. They were chosen for their adaptability to eutrophic waters, rapid growth, and capacity to support electroactive biofilms that enhance bioelectric generation in plant–microbial fuel cells (PMFCs). To ensure uniformity, plants were size-standardized and complete surface coverage was maintained following Novelendah et al. (2018).

2.2 PMFC design

The schematic design, adapted from Venkata Mohan et al. (2011), utilized a rectangular PVC chamber (13 L total volume, 9.7 L working volume) equipped with two graphite electrodes (30.34 cm^2), a 30 cm copper wire, and a 1000 Ω resistor. Six PMFCs (PMFC1–PMFC6) were constructed using *Pistia stratiotes* and *Eichhornia crassipes* at heights of 14, 12, and 9 cm in PVC containers (31 × 21 × 20 cm). Electrons generated at the anode were transferred to the cathode through the external circuit (Kabutey et al., 2019). The electrodes were positioned near the rhizosphere: the anode was placed 2 cm below the sediment under anaerobic conditions, while the cathode was half-immersed in wastewater and half-exposed to air to facilitate aerobic reactions.

2.3 Sediment Collection and Inoculation

The sediment composition consisted of concentrated anaerobic sludge and gravel, commonly used as inoculum in photosynthetic microbial fuel cells (Fang et al., 2015). For this study, a total of 27 L of sediment was collected, comprising 6 L of anaerobic residual sludge from the cooling tower of the Cartavio sugar refinery according with the Monitoring Protocol RM-093-2018-Vivienda (Ministerio de Vivienda, Construcción y Saneamiento, 2018) and 0.02 m^3 of fine gravel. Microorganism identification was carried out through Gram staining and phenotypic characterization. Subsequently, 0.0035 m^3 of fine gravel was added to the mixture, followed by inoculation with 1 L of sludge for each of the six photosynthetic microbial fuel cells.

2.4 Wastewater Collection and Addition

A total of 31.20 L of influent wastewater was collected from the Covicorti Wastewater Treatment Plant in Trujillo, at coordinates East: 714,844.58 m and North: 9,101,703.22 m, following the effluent quality control protocol for domestic and municipal wastewater treatment plants (Ministerio de Vivienda, Construcción y Saneamiento, 2018). Subsequently, 5.20 L of wastewater (Venkata Mohan et al., 2011) was added as substrate to each PMFC unit.

2.5 Operation, measurement and monitoring of the PMFC

The PMFC system operated in batch mode for 60 days under ambient conditions, with 5.20 L of wastewater and 4.50 L of sediment per unit, resulting in a total working volume of 9.70 L for each photosynthetic microbial fuel cell. Initial characterization of both sludge and wastewater was performed, followed by periodic supplementation of approximately 100 mL of wastewater every three days to each PMFC to compensate for evaporative losses.

Daily voltage measurements were recorded throughout the experimental period to calculate power output (P, in mW), power density (mW/m²), and current density (mA/m²). After each treatment cycle, final chemical oxygen demand (COD) was analyzed for all PMFC units (Venkata Mohan et al., 2011). Additional control parameters, including temperature, conductivity, salinity, and pH, were also measured (Sharma et al., 2021).The following equations in Table 1 summarize the main electrochemical and performance parameters used to evaluate Plant Microbial Fuel Cells (PMFCs).

Table 1: Summary of Equations Used in PMFC Performance Evaluation

Parameter	Equation	Description
Current intensity (I)	$I = \frac{E_{cell}}{R_{ext}}$ (1)	*I* is the current intensity (mA), E$_{(}$cell$_{)}$ is the measured voltage (mV), and R$_{(}$ext$_{)}$ is the external resistance of the cell (Ω).
Power (P)	$P = I \times R_{ext}$ (2)	*P* is the power output generated by the PMFC system (mW) (Nguyen & Nitissaravut, 2019).
Power density (P_{an})	$P_{an} = \frac{(E_{cell})^2}{A_{an} \times R_{ext}}$ (3)	P_{an} is the power density (mW/m²) normalized to the anode surface area A_{an} (m²) (Logan & Regan, 2006).
Current density (d_a)	$d_c = \frac{I}{A_{an}}$ (4)	d_c is the current density (mA/m²).
COD removal efficiency	$\%Removal = \left(\frac{COD_{initial} - COD_{final}}{COD_{initial}}\right) \times 100$ (5)	$COD_{initial}$ and COD_{final} represent the chemical oxygen demand (mg/L) before and after treatment, respectively (Venkata Mohan et al., 2011).

Figure 1 shows how the sediment was loaded, the microscopic evaluation and the cells with *Eichhornia crasippes* and *Pistia stratiotes*.

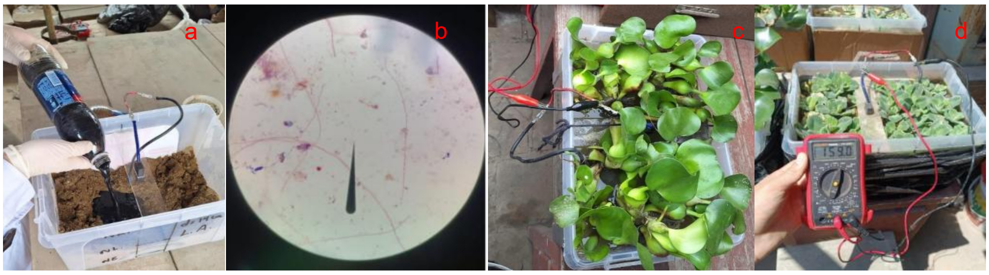

Figure 1: (a) Sediment load with microorganisms (b) Microscopic observation of the sediment by Gram stain. (c) PMFC cell with Eichhornia crassipes (d) *PMFC cell with Pistia stratiotes*

3. Results and Discussions

3.1 Initial effluent characterization

Table 2 presents the physicochemical characteristics of the wastewater before and after treatment. The observed increases in pH, conductivity, salinity, and temperature in the PMFC effluents result from the combined action of bioelectrochemical and photosynthetic processes. Microbial oxidation and cathodic oxygen reduction release heat and hydroxyl ions, while CO_2 uptake by *Pistia stratiotes* and *Eichhornia crassipes* increases alkalinity (Logan and Regan, 2006). The release of ions enhances conductivity and salinity, thereby improving proton transport and electron flow (Rozendal et al., 2008). The initial COD was 743.4 mg/L.

Table 2: Pre- and post-treatment control parameters

Control Parameters	Influent	Effluent					
		PMFC1	PMFC2	PMFC3	PMFC4	PMFC5	PMFC6
Temperature (°C)	21.8	27.4	25.1	25.3	25.3	25.5	26
pH	7.9	8.5	8.3	8.5	7.9	8.1	8.5
Conductivity (µS/cm)	1858	2094	3029	2076	3015	3027	2086
Salinity (%)	0.8	1.4	1.6	1.3	1.5	1.5	1.3

3.2 Bioelectricity production

Figure 2 shows voltage peaks occurring every 15 days following wastewater addition, which compensates for evaporation and supplies organic matter to electrogenic bacteria (Logan and Regan, 2006). *Pistia stratiotes* outperformed *Eichhornia crassipes*: PMFC6 (9 cm) generated 159.40 mV under closed-circuit and 539 mV under open-circuit conditions, compared to PMFC5 (9 cm) with 129.27 mV and 467 mV, respectively. The fibrous roots and biodegradable exudates of Pistia promote biofilm formation and enhance electron transfer (Kaku et al., 2008). Its dense root structure maintains anaerobic zones that improve anode performance, unlike the oxygenated aerenchyma of Eichhornia (Strik et al., 2008). The periodic addition of wastewater restores conductivity and voltage following evaporative losses (Rozendal et al., 2008).

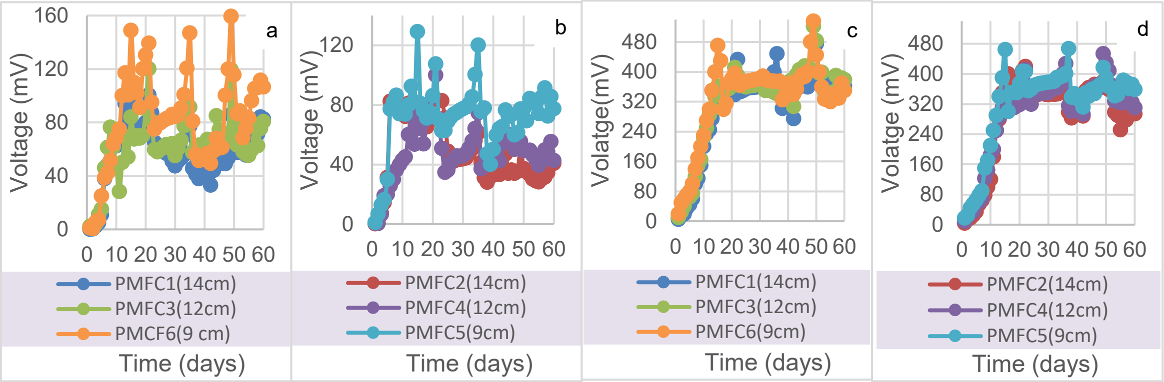

Figure 2: Voltage generation recorded during 60 days of operation with (a) Pistia stratiotes in closed circuit (b) Eichhornia crassipes in closed circuit (c) Pistia stratiotes in open circuit (d) Eichhornia crassipes in open circuit

The voltage output under the closed-circuit configuration exhibited moderate dispersion among the six PMFCs, with standard deviations of 22.6 mV (PMFC1), 22.0 mV (PMFC2), 22.5 mV (PMFC3), 17.8 mV (PMFC4), 22.9 mV (PMFC5), and 20.3 mV (PMFC6). Under the open-circuit configuration, the voltage values showed greater dispersion compared to the closed-circuit system, with standard deviations of 115.4 mV (PMFC1), 115.7 mV (PMFC2), 113.1 mV (PMFC3), 111.3 mV (PMFC4), and 111.5 mV (PMFC5). The substantially higher standard deviation (SD) values observed in the open-circuit mode are attributed to the absence of electron flow, which leads to unstable anodic potentials and large voltage fluctuations. In contrast, the closed-circuit configuration maintains a steady redox balance, resulting in lower dispersion.

3.3 Power, current density and power density evaluation

Figure 3 results indicate that Pistia stratiotes exhibits higher energy efficiency, reaching voltages of 225 mV in PMFC6 and outperforming Eichhornia crassipes. The maximum power density obtained was 4.21 mW/m² at current densities of 20–30 mA/m², which is characteristic behavior of PMFC systems. The polarization curves reveal significant activation losses followed by ohmic polarization. Electrode spacing proved to be a critical factor, with lower performance observed in the 14 cm configurations (PMFC1 and PMFC2) compared to those with 9–12 cm spacing. The superior performance of Pistia stratiotes is attributed to its greater root exudation, enhanced establishment of electroactive biofilms, and differences in the microbial rhizosphere. These findings highlight the importance of optimizing both plant species and system geometry to maximize energy efficiency in PMFCs. Polarization curves were obtained from mean voltage values under steady-state operation; therefore, dispersion values (SD) were not included.

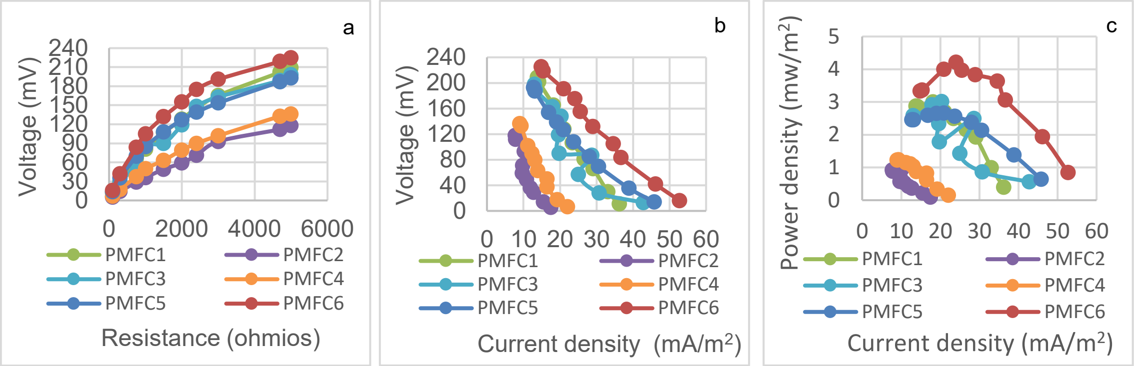

Figure 3: (a) Voltage vs resistance (b) Voltage vs current density (c) Power density vs current density

3.4 Removal of chemical oxygen demand from the effluent

According to Figure 4, the maximum removal efficiency was achieved by PMFC3 (*Pistia stratiotes* at 12 cm), reaching 90.80%. PMFCs with *Eichhornia crassipes* exhibited slightly lower efficiencies. This suggests that *Pistia stratiotes* may promote greater microbial activity in the rhizosphere compared to *Eichhornia crassipes*. The 12 cm electrode spacing resulted in optimal organic matter removal, indicating a balanced compromise between resistance and ionic conductivity. In the COD removal analysis, a single final value was obtained for each PMFC; therefore, standard deviation values were not included, as no replicated measurements were performed within individual systems.

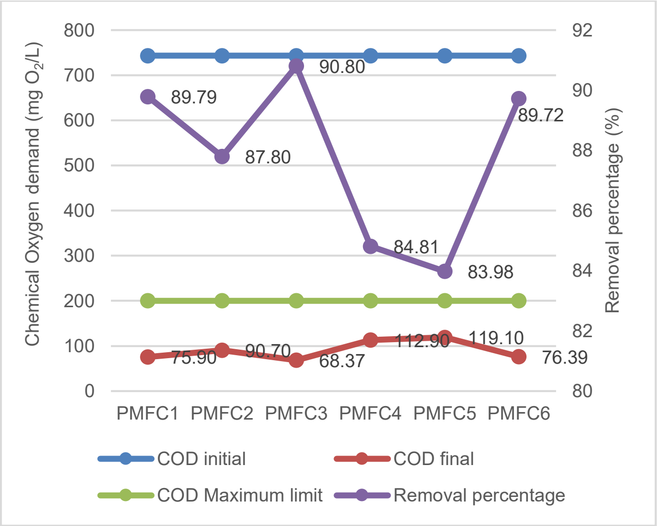

Figure 4: COD removal from wastewater in PFMC cells

4. Conclusions

The study evaluated bioelectricity generation and COD removal efficiency in photosynthetic microbial fuel cells (PMFCs) using wastewater from the Covicorti Treatment Plant. The best results were obtained with PMFC6, which exhibited the highest voltage (536 mV), and PMFC3, which achieved a 90.80% COD removal efficiency. Multimetric measurements were conducted to determine voltage, power, current, current density, and power density values. PMFC6 produced optimal results with values of 225 mV, 0.073 mA, 0.012 mW, 52.74 mA/m², and 4.21 mW/m², respectively. The optimal electrode spacing for maximum bioelectricity generation was identified as 9 cm for both *Pistia stratiotes* in PMFC6 and *Eichhornia crassipes* in PMFC5. *Pistia stratiotes* in PMFC6 was recognized as the best-performing plant species in the photosynthetic microbial fuel cells. These findings demonstrate that PMFC systems can significantly contribute to sustainable development by coupling wastewater treatment with renewable bioenergy production. This integration reduces environmental pollution and promotes eco-efficient resource utilization. Nevertheless, future research should assess the scalability of this technology at pilot and industrial levels to evaluate its long-term performance and economic feasibility.

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