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Research paper

CW-MFC technology for wastewater remediation: Antibiotic reduction, gas emission mitigation, and bioenergy production

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ABSTRACT

This study aimed to remove antibiotic residues from wastewater and assess greenhouse gas emissions, energy generation, and chlorophyll production using microbial fuel cells combined with constructed wetlands (CW-MFC). Two hydraulic retention times (HRT) of one and two days, two types of electrodes—graphite and bioelectrodes—and a control with no electrodes were employed. The results indicated complete removal of Tetracycline (TET) within three days and over 98.0 % removal of Norfloxacin (NOR) in all treatments, with no significant differences in their removal ($p > 0.05$). For Chloramphenicol (CHL), the two-day HRT was more effective (85.2 %) than the one-day HRT (68.1 %) ($p = 1.11e\text{-}06$). Significant differences were observed regarding ammonium, with better results for the two-day HRT and in the order control > graphite > bioelectrode ($p = 0.00758$). Chlorophyll production was higher in the wetlands with bioelectrode and two-day HRT (55.6 SPAD) than in the graphite electrode. Regarding gas emissions, CW-MFC treatments reduced CO_2 and H_2S emissions more effectively than the control group, which released lower oxygen levels. Lastly, wetlands with bioelectrodes achieved the highest potential differences: bioelectrode2 (0.22 ± 0.12 mV) and bioelectrode1 (0.16 ± 0.02 mV). These findings demonstrate the potential of CW-MFC technology, optimized with HRT and bioelectrodes, for efficient antibiotic removal, greenhouse gas mitigation, and bioenergy generation. Integrating microbial fuel cells with constructed wetlands achieved over 98.0 % antibiotic removal, enhanced ammonium reduction, and increased chlorophyll production. CW-MFC systems emerge as a sustainable and effective alternative to conventional wastewater treatment.

1. Introduction

Antibiotics are widely recognized for their detrimental impact on aquatic ecosystems, primarily through their persistence in wastewater and surface waters, including drinking sources [1,2]. Compounds such as NOR, TET, and CHL exemplify this issue, as their extensive use in human and veterinary medicine contributes to their pervasive presence in aquatic environments [3–5]. Particularly concerning is the recalcitrant nature of certain antibiotics like CHL, which resist environmental degradation, posing long-term ecological and health risks [6]. These antibiotics are commonly found in wastewater and aquatic ecosystems, representing significant risks to human health [7–12]. Unfortunately, the contamination of ecosystems with wastewater not only exposes us to antibiotic residues but also causes other effects, such as disruption of photosynthesis [13,14], generation of foul odors [1,15], greenhouse gas emissions [16–20], and eutrophication [1,21].

Constructed wetlands are often presented as nature-based technologies [22,23] designed to mitigate contamination in aquatic environments [1]. Despite their long-standing use, their effectiveness remains constrained by significant limitations, requiring ongoing optimization of various parameters to address their inefficiencies in wastewater treatment and removing toxic elements [19]. Moreover, their well-documented greenhouse gas emissions [18,19] expose a concerning contradiction: a technology intended to protect the environment inadvertently contributes to its degradation [24]. This paradox underscores the urgent need for critical reassessment and innovation to ensure these systems are more efficient and sustainable.

Integrating constructed wetlands with microbial fuel cells (CW-MFC) offers a promising alternative to removing antibiotics [25–27]. CW-MFC systems have enhanced capabilities in oxidizing organic compounds,

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eliminating antibiotic contaminants, and generating electricity, presenting a dual benefit for wastewater treatment and energy production [19,22,23,28]. Additionally, this system has demonstrated the ability to reduce greenhouse gas emissions. Previous studies have shown that CW-MFC systems effectively treat wastewater while reducing greenhouse gas emissions by an average of 15.0- 23.0 % [16,28]. This combination enhances the efficiency of wastewater treatment and contributes to greenhouse gas mitigation [19]. However, while a previous study has addressed this topic, aspects still require further evaluation to fully understand the impact of antibiotic pollution on this process [28].

On the other hand, the potential to reuse wetland biomass to produce bioelectrodes introduces an additional layer of sustainability [29]. Hirose et al. [30] emphasize that these bioelectrodes, owing to their biocompatibility, high electrical conductivity, and low production cost, represent an innovative solution that maximizes the use of system by-products, reduces environmental impact and enhances its feasibility as a sustainable technology. However, developing effective procedures to optimize biomass conversion into bioelectrodes remains a significant technical challenge that must be addressed to establish this technology as an integrated and scalable solution [31].

The electrodes play a crucial role in electrochemical technologies, facilitating electron transfer and energy generation [32]. To optimize their performance, various electrode materials have been investigated, enabling applications such as the removal of antibiotics [33], the reduction of gas emissions [34], and the analysis of chlorophyll in plants [35]. However, these characteristics have been studied in isolation and still require significant improvements [36]. Evaluating all aspects comprehensively is challenging, as the design approach must vary depending on the specific priorities being addressed [36].

In response, this study proposes a holistic assessment that incorporates multiple parameters. It examines two types of electrodes alongside a control group (without electrodes). It evaluates two hydraulic retention times (one and two days) within an integrated constructed wetland system combined with microbial fuel cells (CW-MFC). The objectives were to assess the system's potential for antibiotic remediation in wastewater, measure the chlorophyll content of *Schoenoplectus tatora*, analyze gas emissions (CO_2, O_2, H_2S) from the CW-MFC, and record the potential difference in millivolts (mV). This study proposes using bioelectrodes derived from biomass in CW-MFC systems as a sustainable alternative to conventional carbon-based electrodes. These bioelectrodes enhance energy efficiency and reduce implementation costs [23]. Furthermore, the research expands on previous knowledge by analyzing the removal of recalcitrant antibiotics, gas emissions, and energy generation under different hydraulic retention times, addressing key gaps in optimizing CW-MFC performance for integrated wastewater treatment.

2. Methodology

2.1. Construction of CW-MFCs and system operation

The microbial fuel cell was installed in a PVC tube with an internal diameter of 10 cm and a height of 40 cm [37]. The reactor comprised three substrate layers: 8 cm of gravel, 8 cm of coarse sand, and 14 cm of fine sand (Fig. 1). The anode was placed between the gravel and coarse sand layers, while the cathode was positioned at the top. Wastewater entered from the top, following a vertical flow with an outlet valve at the bottom. Two types of electrodes were used: one made of carbon graphite and the other derived from *Lemna gibba* biomass, connected by a copper wire to complete the circuit. The system was inoculated with 20 mL of anaerobic sludge from a pig farm [38] to introduce electroactive anaerobic bacteria.

There were four treatments and a control group, each duplicated. Two HRTs were considered (one and two days), along with two types of electrodes: graphite and electrodes produced from *Lemna gibba* biomass

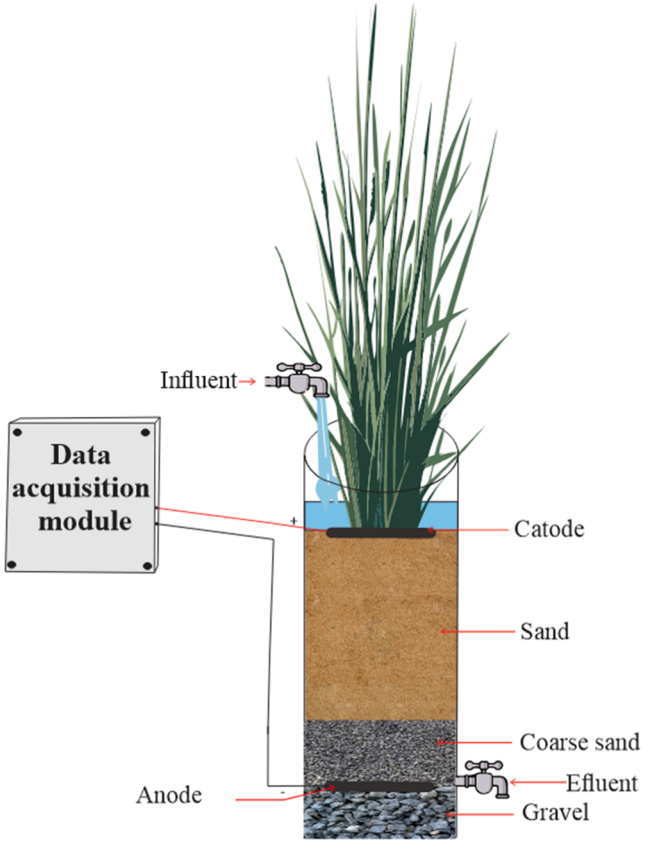


Fig. 1. Constructed wetland design.

(bioelectrodes). Graphite was chosen as the electrode material due to its wide availability in the market, cost-effectiveness, and high conductivity, making it a practical and accessible option. L. *gibba*, on the other hand, was selected for its prolific growth and ease of manipulation, which facilitate its use and transformation into biochar. This combination provides a sustainable and efficient alternative to other materials, promoting both environmental and economic benefits in bioelectrode design. All experimental units included *Shoenoplectus tatora* as a phytoremediation plant. The combinations were: Graphite + one-day HRT, Graphite + two-day HRT, Bioelectrode + one-day HRT, Bioelectrode + two-day HRT, and a control group (without electrodes) for one- and two-day HRTs, totaling six combinations. The experiment was conducted under controlled environmental conditions with artificial lighting, using 50 W LED bulbs with a 12-hour light cycle [39]. Average temperature and humidity values were 20.53 °C and 63.7 %, respectively.

After a 4-month adaptation phase, the experiment commenced and lasted for 33 days. The constructed wetlands operated with synthetic wastewater composed of glucose (300 mg/L), NH_4Cl (80 mg/L), K_2HPO_4 (12.8 mg/L), $FeCl_3$ (0.05 mg/L), $MgSO_4{\cdot}7H_2O$ (4.5 mg/L), and $CaCl_2{\cdot}2H_2O$ (7.3 mg/L) (Li et al., 2017). Initial antibiotic concentrations were 17.5, 19.37, and 20.52 mg/L for Norfloxacin, Tetracycline, and Chloramphenicol. The wetland's water replacement volume was 500 mL, considering one- and two-days HRT. Additionally, given that maintenance requirements for CW-MFC systems are minimal [21], the experiment primarily focused on monitoring the electrical circuits and electrodes to ensure their proper functioning.

2.2. Antibiotic analysis in water samples

Antibiotics were obtained from Sigma Aldrich with 98.0 % purity. Water samples were taken every 3 days until the experiment concluded. Two milliliters of influent water were collected, filtered through a 0.22

μm x 13 mm PTFE syringe filter, and injected into a 2 mL vial. These samples were analyzed directly using an Ultra High-Performance Liquid Chromatography system (UHPLC-DAD) [5]. Chromatographic conditions were as follows: 0.8 μL of sample was injected into a Poroshell 120 EC—C18 column (2.1 × 150 mm × 1.9 μm, Agilent, USA) at a temperature of 35 °C. The mobile phase consisted of 0.1 % formic acid in water (B) and 0.1 % formic acid in acetonitrile (C). Elution was performed using a gradient: starting with 95.0 % B at 0 min, decreasing to 80.0 % B at 5 min, and increasing to 90.0 % B at 10 min. The mobile phase flow rate was 0.250 mL/min, with a post-run time of 2 min. The column effluent was monitored at 270 nm for antibiotic detection. The limits of detection (LOD) and quantification (LOQ) for the antibiotics were 0.044 and 0.145 μg/mL for NOR, 0.043 and 0.142 μg/mL for TET, and 0.054 and 0.177 μg/mL for CHL. A five-point calibration curve (0.5, 1, 2, 4, and 8 μg/mL) was established before analysis [40] and showed linearity for NOR ($R^2 = 0.997$), TET ($R^2 = 0.992$), and CHL ($R^2 = 1$) within the expected sample concentration range. (Supplementary material).

The following formula was used to calculate the antibiotic removal percentages:

$$R = \left(1 - \left(\frac{Cf}{Ci}\right)\right) * 100 \quad (1)$$

Where Ci represents the initial concentration, and Cf refers to the final concentration [13].

The water's physicochemical parameters (pH, dissolved oxygen, temperature, ORP, turbidity, conductivity, salinity, nitrates, and ammonium) were monitored with a HANNA HI 9829 multiparameter probe every three days until the experiment concluded.

2.3. Chlorophyll measurement

Chlorophyll measurements were taken using a Konica Minolta digital chlorophyll meter [41,42]. This device measures chlorophyll in SPAD units, a numerical expression based on the spectral absorbance ratio in two regions of the electromagnetic spectrum (600–700 nm and 700–1400 nm) [41,42]. Since *S. tatora* has a thick stem, only the outer layer of the stem was sliced from 10 different sections to assess chlorophyll content. An average chlorophyll value per treatment was then calculated based on these 10 measurements [42].

2.4. Gas measurement

Gas measurement was performed using a Shiantech multigas detecting device, model IRCD4, which measures CO_2 (carbon dioxide), H_2S (hydrogen sulfide), O_2 (oxygen), and CH_4 (methane). CO_2 and O_2 were measured as a percentage of the total volume, while H_2S was measured in parts per million (PPM). Measurements were taken in the final days of experimentation, as the experimental units needed to be sealed in a transparent acrylic static chamber (12.6 cm in height and 10.8 cm in diameter) for three days to allow gas accumulation [28]. A rubber pump was installed on top of the chamber, into which a syringe was inserted to draw out the accumulated air. The air was then passed through tubing connected to a suction device and into the measuring device, where the increase or decrease in gas levels was observed. The highest readings were recorded for H_2S and CO_2, while for O_2, the lowest readings were considered.

2.5. Electricity measurement

The data acquisition system was designed to read and store the potential differences in the cells. For this purpose, an INA3221 sensor with 12-bit resolution was used, providing voltage resolution up to 80 μV and an error margin of 0.25 % of the measured value, ensuring precise measurements to three decimal places. The acquisition and storage stage included a Data Acquisition System (DAQ) with an ATmega328p microcontroller, which communicated with the internal data storage and logging module. The system also featured a USB port for real-time data visualization through a computer, enabling efficient and accurate monitoring of the status of each cell.

2.6. Statistical analysis

A two-factor ANOVA was applied to assess the effect of HRT and electrodes on removing antibiotics, ammonium, and nitrate. A Principal Component Analysis (PCA) was also conducted to evaluate the relationship between physicochemical parameters and antibiotic removal. Descriptive statistics were employed for chlorophyll, gas measurements, and electricity. The software used for the analysis included R Studio and Excel spreadsheets.

3. Results and discussion

3.1. Antibiotic removal

3.1.1. TET removal based on HRT and electrodes

High removal percentages of antibiotics were achieved, with TET being completely removed in all treatments by day three, highlighting the system's efficiency under the evaluated conditions. No further statistical analysis was conducted due to this result (Fig. 2). This finding is particularly significant as TET is commonly found in wastewater and poses health risks to humans [3,13].

The high solubility of TET (231 mg/L) [13] may have also contributed to making it easier for plants to absorb water-soluble compounds like Ciprofloxacin [14]. In chemical systems, TET is best degraded at acidic pHs (5) [10], whereas in this type of system, neutral pH favors removal [43], mainly because living organisms require neutral pHs for their development. In a two-cell microbial fuel cell system, a 79.1 % removal of TET (50 mg/L) was reported over seven days, with initial lower values that improved towards the end, suggesting a recovery of the bacterial population [44]. The higher concentration of TET (50 mg/L) may have extended the removal period, while this study used 19.37 mg/L. The combination with constructed wetlands significantly enhanced the process, achieving up to 99.85 % removal in a single day, owing to adsorption at the cathode and bacterial degradation at the anode [29,45]. Bacterial degradation is crucial for TET removal [46,47], being more effective in the liquid medium than in the substrate [2]. Additionally, phytoremediation improves system efficiency [13], proving more effective than treatments such as ozonation, which has limitations in eliminating this recalcitrant contaminant [48].

3.1.2. Removal of CHL and NOR based on HRT and electrodes

The two-factor ANOVA reveals that the electrodes, HRT, and their interaction have no significant impact on the removal of NOR, suggesting that other unassessed factors may be responsible for its removal (Table 1). The mechanisms of antibiotic removal in a CW-MFC system are diverse, such as the action of plants, microorganisms, substrate, HRT, and other electrochemical components [28].

For CHL, the HRT exhibited a significant difference ($p = 1.11E-06$), with a 2-day HRT (85.2 %) proving more effective than a 1-day HRT (68.1 %), indicating that a longer retention time enhances its removal. Although there were no statistically significant differences between the types of electrodes, bioelectrodes (78.5 %) showed a slight advantage over graphite electrodes (76.4 %) and the control (73.2 %), which could be relevant in applications aiming to optimize efficiency. While CHL is a recalcitrant antibiotic, previous studies have demonstrated that systems such as CW-MFC can achieve 100.0 % removal within 40 days [13], highlighting the role of electrochemical components in its treatment. Furthermore, the chemical characteristics of CHL may also have influenced these results (Log Kow=1.14; Pka=5.5 and Solubility 2.5) [13]. Being a compound of low solubility, its degradation could have been affected, which is why it is considered a recalcitrant compound [4].

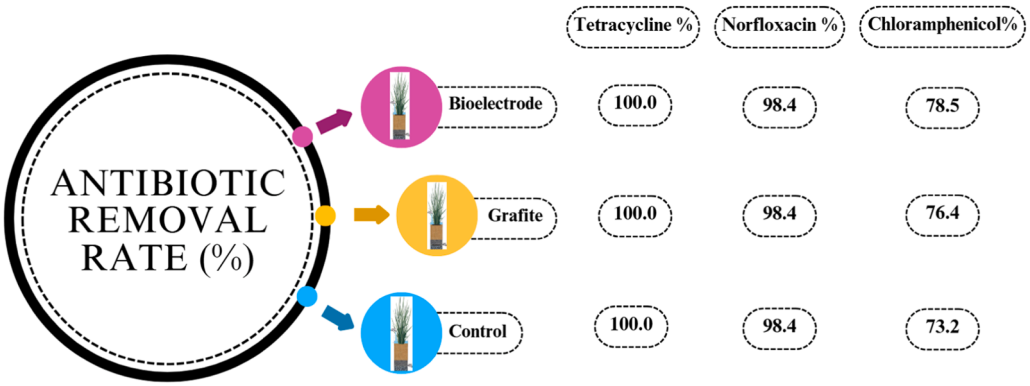


Fig. 2. Antibiotic removal rates as a function of CW-MFC treatments.

Table 1
Analysis of variance for Norfloxacin and Chloramphenicol based on the factors (HRT, Electrode).

ANOVA FOR NORFLOXACIN						ANOVA FOR CHLORAMPHENICOL					
	Df	Sum Sq	Mean Sq	F value	Pr(>*F*)	Df	Sum Sq	Mean Sq	F value	Pr(>*F*)	
Electrode	2	0.124	0.06197	0.297	0.744	2	410	205	0.685	0.506	***
HRT	1	0.314	0.31416	1.506	0.222	1	8026	8026	26.797	1.11E-06	
Electrode: HRT	2	0.126	0.06298	0.302	0.740	2	850	425	1.419	0.247	
Residuals	104	21.689	0.20855			104	31,148	299			

Additionally, Fig. 3A illustrates the removal of NOR throughout the experiment (33 days), showing a consistent removal pattern. Similarly, Ondon et al. [40] reported a 65.0 % removal of 128 mg/L of NOR, with the noteworthy aspect being that the MFC system was still generating electricity, indicating that the bacteria could tolerate these concentrations. Therefore, in this case, we could also attribute the high removal percentages to both the bacteria and the system as a whole.

Fig. 3B shows the removal of CHL. In this case, there is greater variability in the removal averages over the days. Likewise, it can be seen that at the beginning, there is a decrease in removal, increasing towards the last days of the experiment. This is evidence of a recovery of the system, which could be attributed to the bacterial community, given that after the course, it tends to recover its population [44].

3.1.3. *Influence of physicochemical parameters on antibiotic removal*

Additionally, a PCA was conducted to relate the percentages of CHL removal with the physicochemical parameters. The two dimensions shown explain 29.1 % and 22.0 % for dimensions 1 and 2, respectively, accounting for a total of 51.1 % of the variability explained by the data. It is also observed that the data corresponding to the Bioelectrode (Bioe), Graphite, and Control groups show some overlap between the Bioe and Control groups, indicating a similarity in the data distribution. On the other hand, the overlapping ellipses demonstrate a relationship among the analyzed data.

Fig. 4 demonstrates that NOR removal is closely associated with

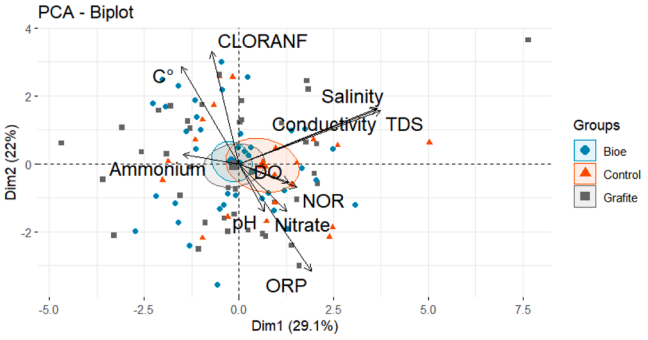


Fig. 4. The physicochemical variables and antibiotic removal percentages were analyzed using a principal component approach.

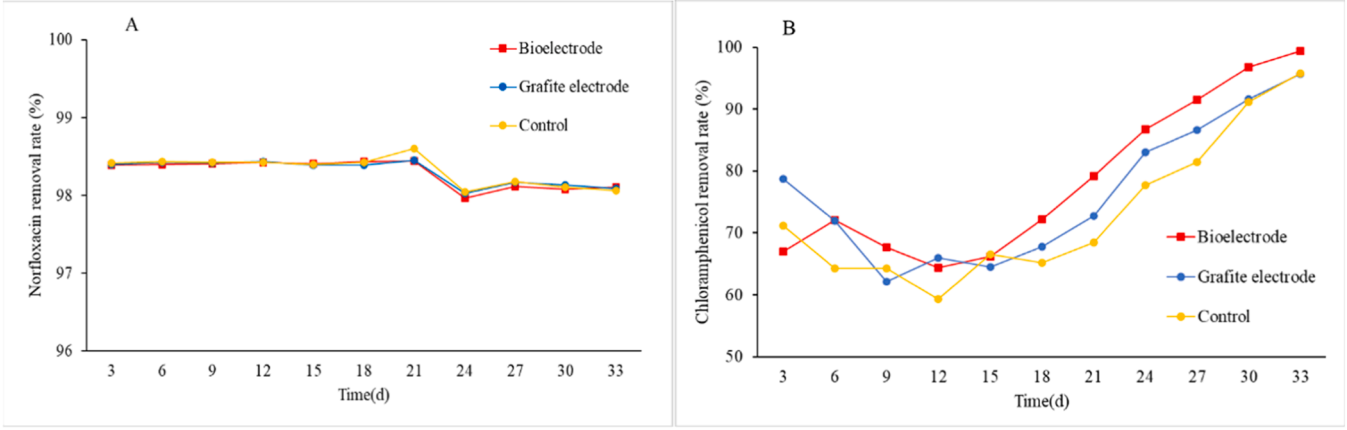


Fig. 3. Removal of NOR (A) and CHL (B) throughout the experiment for the two electrodes and the control group.

nitrate, pH, and ORP, as these variables influence the compound's biodegradability and physicochemical properties [3]. A previous study reported 84.0 % removal of 50 mg/L within 12 h, highlighting optimal removal conditions at 31.48 °C and pH 7.12 for an initial concentration of 106.37 mg/L [6]. In contrast, the present study's average temperature was 19.02 °C with a pH of 7.04. According to the PCA (Fig. 4), CHL is more strongly associated with temperature, indicating favorable conditions for its removal.

For ammonium, a significant difference was observed in the effects of the independent variables on its removal, with p-values of 0.00758 for electrodes and 0.00736 for HRT. A 2-day HRT exhibited greater efficiency (90.0 %) compared to 1 day (83.5 %), while the control treatment achieved the highest removal efficiency (92.4 %), surpassing graphite electrodes (86.9 %) and the bioelectrode (81.1 %) (Table 1). This finding suggests that longer retention times facilitate higher removal efficiencies, whereas shorter retention periods may lead to system saturation, affecting performance. In the case of nitrate, no significant differences were found among the evaluated variables (p-value > 0.05), with consistently high removal rates across all treatments, averaging 98.4 %. HRT plays a crucial role, as extended retention times promote nitrogen assimilation. A previous study on a CW-MFC system reported 35.0 % ammonium removal within three hours, with significant improvements after 12 h of treatment [49], highlighting the advantages of CW-MFC systems.

The higher nitrate removal efficiency compared to ammonium could be attributed to the fact that nitrate represents the final stage of the denitrification process, where it is reduced to N_2 by denitrifying bacteria in the anode under anaerobic conditions. In wastewater, nitrogen is predominantly present as ammonium or ammonia [1], which is converted into nitrate through nitrification in the cathodic zone, an aerobic process facilitated by nitrifying bacteria [16,21]. The presence of antibiotics in wastewater may inhibit the activity of these bacteria, temporarily affecting nitrogen removal; however, microbial communities can adapt and recover their functionality [37]. For instance, in an MFC system, nitrate removal of 96.45 ± 2.4 % and ammonium removal of 88.45 ± 1.4 % were reported, confirming the greater efficiency of nitrate removal over ammonium [27]. The role of plants with high root density is also crucial, as they release oxygen, enhancing nitrification in the cathodic zone [39]. Additionally, plants absorb nitrogen for growth, acting as a natural nutrient removal mechanism [1,20]. Consequently, CW-MFC technology accelerates and optimizes nitrogen removal by integrating biological processes, where various organisms and electrochemical technologies interact efficiently [1]. However, challenges persist, particularly concerning costs and scalability, mainly related to electrodes' large-scale availability and subsequent maintenance.

In this context, the results obtained in this study regarding contaminant removal demonstrate that the use of plant biomass as an electrode source could be a cost-effective alternative for this technology. Furthermore, these findings contribute to the existing literature on its applicability in wastewater treatment [1], antibiotic and bacterial resistance gene removal [3,6,13], greenhouse gas reduction [16,17], azo dye removal [50], agricultural wastewater and nitrogenous effluent [21], wastewater treatment with microplastics [2], and energy production [15,27], among others. This highlights the potential of CW-MFC systems as an economically and environmentally sustainable solution for wastewater treatment and energy generation. Nevertheless, challenges remain regarding scalability, particularly in terms of long-term sustainability, installation complexity, and operational maintenance. These factors must be carefully considered to ensure the efficacy of these systems for large-scale applications.

3.2. Chlorophyll

Chlorophyll is widely recognized as a key parameter for assessing the stress levels of plants in response to specific environmental contaminants or nutritional deficiencies [42]. In this study, an increasing trend was observed in treatments with a 2-day HRT, averaging 48.2 SPAD compared to 47.4 SPAD for a 1-day HRT (Fig. 5). Regarding the electrodes, the best performance was recorded in units with bioelectrodes, achieving 53.6 SPAD, followed by the control group with 46.0 SPAD, and finally, the treatments with graphite, which recorded 42.8 SPAD. Antibiotics can have toxic effects on plants, as evidenced by reducing chlorophyll levels, particularly at elevated concentrations [13,14,51]. This phenomenon is evident in this study, where a shorter HRT corresponds to a more significant accumulation of antibiotics in plants, negatively impacting chlorophyll concentration. Furthermore, the decrease in chlorophyll may also contribute to reduced electrical production [39], leading to adverse effects on the entire system due to the increased accumulation of antibiotics.

It is also important to highlight that the integrated CW-MFC system contributed to reducing antibiotic concentrations, alleviating plant stress, and maintaining chlorophyll production. Low antibiotic concentrations do not negatively affect plants and may even have a stimulatory effect [13,14], a phenomenon known as hormesis. This effect has been reported in various studies [13,14,52,53] and primarily occurs at low antibiotic concentrations [52]. Therefore, the effects of the integrated system on chlorophyll production are also beneficial for plants, as the system operates as a whole.

3.3. Gases

It is widely acknowledged that one of the disadvantages of constructed wetlands is the emission of greenhouse gases [16–20]; however, the combination with microbial fuel cells could mitigate greenhouse gas emissions [19]. This study observed a slight emission trend of gases such as CO2 and H2S in the control group (Fig. 6), while a lower amount of oxygen was evident. In contrast, treatments that combined microbial fuel cells recorded higher oxygen values alongside lower CO_2 and H_2S concentrations. Although in natural wetlands, H_2S concentrations are lower (150 nmol/cm^2/d) [54], in constructed wetlands used for wastewater treatment, they may increase up to 2.5 mg/L [24]. This variation could be influenced by the high concentration of organic matter in wastewater and ambient temperature [20]. In a constructed wetland, it is well-known that plant respiration and the wetland contribute significantly to CO2 emissions [20]; even methanogenic bacteria can re-oxidize methane, producing CO2, a less harmful greenhouse gas [17,18]. However, the addition of electrochemical technologies may improve this deficiency; one study demonstrated a reduction of 5.9–32.4 % in CO_2 emissions compared to the control group, which was significantly influenced by the high organic load of the wastewater. An increase from 50 mg/L to 360 mg/L resulted in a CO_2 emission increase of 4.6 to 5.1 times, indicating that higher organic loads in the influent correlate with more significant CO_2 emissions [16].

H_2S can also be generated from the sulfates present in wastewater. Although it typically does not pose a significant health risk, under

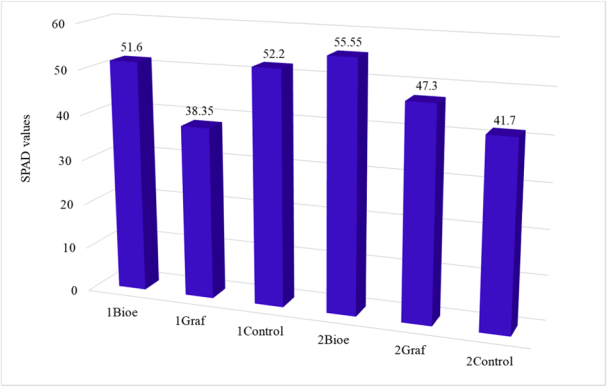


Fig. 5. Record of SPAD values in the applied treatments.

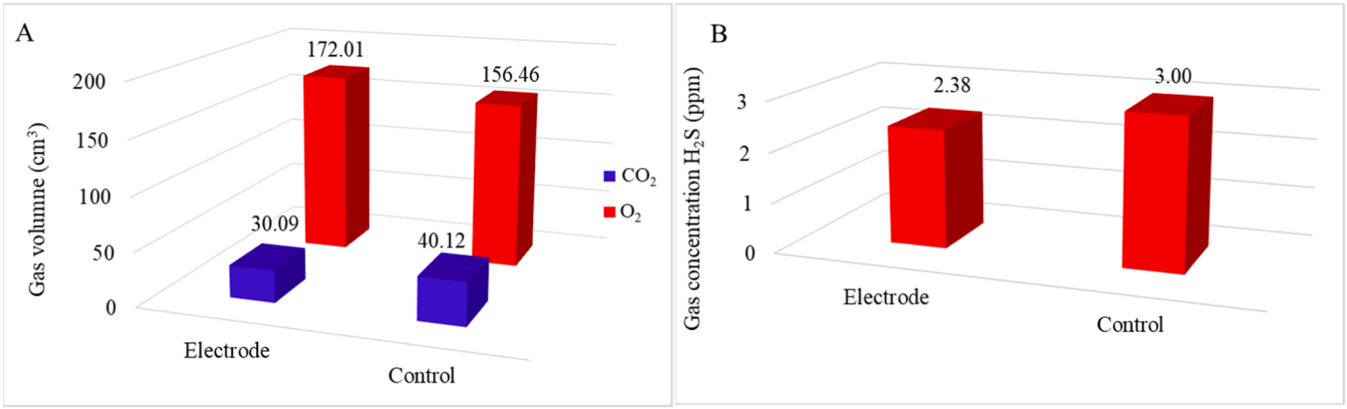


Fig. 6. A. Carbon Dioxide (CO_2) and Oxygen (O_2) Measurements. B. Hydrogen Sulphide (H_2S).

specific circumstances, it may cause problems [15]. It can become hazardous, not only to human health but also to ecosystems, as it may acidify the environment; unfortunately, the emission of this gas can be exacerbated by elevated temperatures [20]. Although it is not a greenhouse gas, it can impact environmental quality and potentially affect the dynamics of other greenhouse gases.

Furthermore, in a CW-MFC system, electrogenic bacteria modify their metabolic pathways by preferentially using the electrode as an electron acceptor under anaerobic conditions, thereby reducing the utilization of sulfates as electron acceptors [20]. This process results in lower formation of gases such as H_2S and CO_2. Additionally, the plants in the system can absorb part of the CO_2 produced during the process, incorporating it into photosynthesis and thereby contributing to reduced emissions [19]. Notably, this study represents one of the first attempts to address H_2S emissions in an integrated CW-MFC system in the presence of antibiotics. In parallel, previous studies investigating antibiotic removal have also reported satisfactory results in reducing methane, a potent greenhouse gas [28]. In this context, CW-MFC technology demonstrates significant potential for mitigating gaseous emissions and offers promising perspectives for sustainable wastewater treatment.

3.4. Electricity

The variability in bioenergy production is evident across the treatments analyzed. Bioelectrode2, which operates with a 2-day HRT, generates the highest average voltage (0.22 ± 0.12 mV) but exhibits high variability (Fig. 7). This could pose a significant challenge for practical applications requiring a stable energy supply. The high variability may be related to the 48-hour interval between stress cycles, allowing bacteria to partially recover before being exposed again to the stress of contaminated water replacement with antibiotics. In contrast,

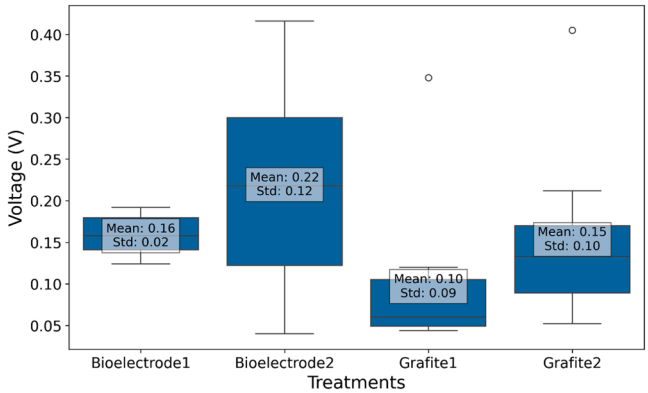


Fig. 7. Potential Difference by Treatments.

Bioelectrode1 (with a 1-day HRT) demonstrates a more stable energy output (0.16 ± 0.02 mV) with a low standard deviation and a narrow range. This makes it more suitable for applications demanding consistent and predictable energy performance, such as powering devices that require a reliable energy source.

On the other hand, the graphite electrodes, Grafite1 (0.10 ± 0.09 mV) and Grafite2 (0.15 ± 0.10 mV) exhibit lower voltages and lower energy production efficiency compared to the bioelectrodes. Although Grafite2 has a broader voltage range, its performance remains inferior to that of the bioelectrodes. Therefore, while Bioelectrode2 generates the highest voltage, its high variability may be a drawback for applications requiring stable energy production. Conversely, Bioelectrode1, with lower variability, is better suited for systems that prioritize stability and consistency in energy output.

Regarding the experimental days, Bioelectrode exhibits a general increasing trend in voltage, particularly in the second half of the observation period, suggesting improved stability and the potential to maintain consistently high energy levels (Fig. 8). In contrast, Graphite shows more significant fluctuations and a less predictable trend, with sharp peaks and drops. Although it ends at a lower level than Bioelectrode, it increases slightly in the final days. Therefore, Bioelectrode appears more effective in sustaining continuous energy production over time. In contrast, Graphite demonstrates higher variability and lower stability, which could limit its efficiency in applications requiring a sustained voltage output.

Therefore, bioelectrodes demonstrate better performance than graphite. In this regard, a study comparing rice husk biochar with carbon cloth electrodes found that bioelectrodes produced 12.3 A m^{-3}, whereas carbon cloth electrodes only generated 5.3 A m^{-3} [31]. This could be explained by the formation of carbon layers during graphitisation, which increases the specific surface area and facilitates electron flow [55]. Another study using loofah electrodes coated with electrically conductive ink reported a 5260.0 % increase in power density when adding rice husk charcoal [30]. These findings demonstrate that biochar-based bioelectrodes are viable for microbial fuel cells.

Although antibiotic residues may affect the process, bacteria can adapt and continue generating electricity. Wang et al. [44] showed that after acclimating bacteria to TET (10–50 mg/L), voltage production reached 606 mV, decreased to 157 mV at 50 mg/L, but recovered to 482 mV after 14 days, highlighting their adaptability and ability to use TET as a resource. Similarly, Li et al. [29] found that 10 mg/L of TET promoted electricity generation, while 20–30 mg/L concentrations reduced it. Wen et al. [38] also achieved the remediation of Sulfamethoxazole and TET, recording 412 mV, confirming that energy production is feasible even in antibiotics. Furthermore, in wastewater, typical antibiotic concentrations (0.01–125 μg/L) are significantly lower than those used in laboratory studies [3], reinforcing the viability of this technology.

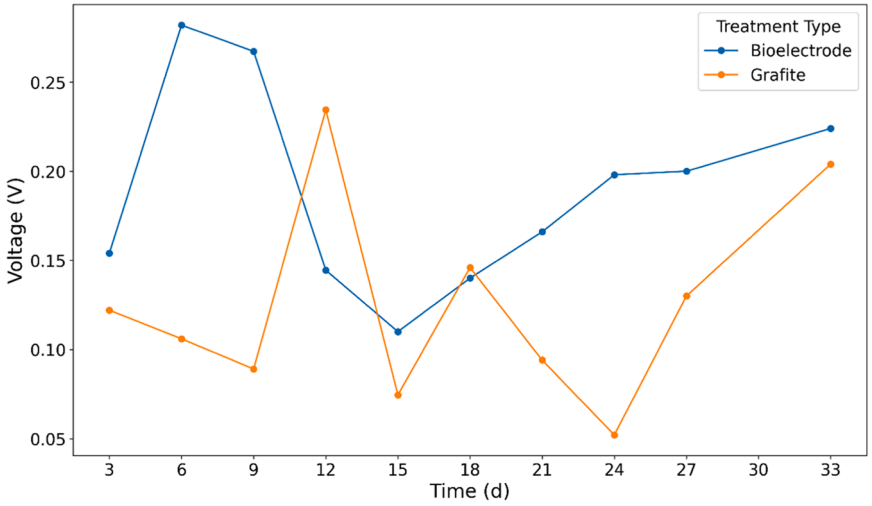


Fig. 8. Potential Difference During the Experimental Days.

While CW-MFCs can generate electricity, their scalability as a renewable energy source faces significant challenges, including high implementation costs, low current density, and high variability in energy production, which may limit performance and consistency [56]. This variability is associated with influent quality and contaminant concentrations, impacting microbial activity and system efficiency. This study addresses some of these challenges by reducing costs using bioelectrodes derived from plant biomass and demonstrating the viability of CW-MFCs under-treated wastewater conditions. However, stabilizing current density and improving energy production remain key areas for further research to optimize performance and scalability.

Although antibiotic removal is efficient, electrical energy generation via bioelectrodes remains low. This suggests that factors such as plant-substrate interactions and microbial dynamics [28], play a crucial role. In contrast, electricity production depends mainly on electrode properties, conductive materials, and bacterial activity. Addressing these aspects will help unlock the full potential of bioelectrodes, particularly for large-scale applications [56,57]. Additionally, the system tested in this study is a single-cell configuration that does not require proton exchange membranes. CW-MFCs utilize plant biomass-derived electrodes, a widely available resource. This makes them a promising technology for antibiotic removal, wastewater treatment, and energy production. However, further research is needed to enhance energy sustainability, efficiency, and competitiveness with other renewables. Optimizing designs, conductive materials, and operational configurations will be key to scaling up this technology and maximizing its energy potential for practical global implementation.

4. Conclusion

This study demonstrates the high efficiency of CW-MFCs in the removal of TET (100.0 %), NOR (98.0 %), and CHL (>80.0 %) throughout the experiment. The best results were achieved with the two-day HRT. Regarding electrodes, no significant effects were observed on antibiotic removal. The two-day HRT outperformed the one-day HRT, maximizing ammonium (90.0 %) and nitrate (98.5 %) removal. Additionally, graphite electrodes and bioelectrodes contributed to more excellent chlorophyll production and reduced emissions of CO_2 and H_2S, particularly with the two-day HRT. Regarding energy generation, bioelectrodes exhibited optimal performance in producing potential difference (mV), indicating their viability for future applications in microbial fuel cells. In conclusion, the CW-MFC system provides an effective and sustainable solution for contaminant removal from water, standing out for its potential in environmental treatment. Although there are still limitations to consider, it would be essential to extend the duration of the experiment and evaluate the system with real wastewater. This would allow testing its efficiency against a wider range of antibiotics and other emerging compounds. Also, Future research could focus on scaling up the system to assess its performance in larger, more dynamic environments.

CRediT authorship contribution statement

Ingrid Maldonado: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Saul Huaquipaco:** Supervision, Software, Methodology, Formal analysis. **Salomon R-Rivera:** Validation, Supervision, Methodology, Investigation. **Elvis Jack Colque Ayma:** Validation, Methodology, Formal analysis. **Franz Zirena Vilca:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rineng.2025.104314.

Data availability

Data will be made available on request.

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