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Research

Electrochemical technologies and phytoremediation: Strategies for antibiotic waste removal, electricity generation, and water quality improvement

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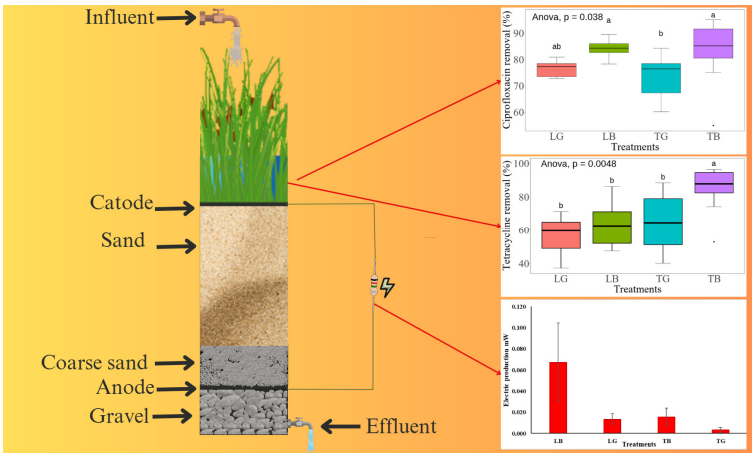
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ABSTRACT

This study evaluated the capacity of constructed wetlands combined with microbial fuel cells to remove antibiotics from wastewater. Two macrophyte species (*Lemna gibba* and *Schoenoplectus tatora*) and two types of electrodes (graphite and a bioelectrode derived from biochar produced from *L. gibba* biomass) were utilized. The experiments were conducted over three treatment cycles, measuring the removal of Ciprofloxacin (CIP) and Tetracycline (TET) in the influent and effluent sections of the wetland. Results showed that, in the influent, the second cycle exhibited more pronounced differences between treatments and time points for CIP and TET ($p < 0.05$), while cycles one and three showed similar results. In the effluent, high removal percentages were achieved across all three cycles, reaching 98.9% for CIP and 100.0% for TET. Additionally, physicochemical parameters such as pH, oxidation-reduction potential (ORP), and dissolved oxygen (DO) improved in all treatments, with initial and final values ranging from 4.7 to 7.2, -414.5 to -69.4 mV, and 0.3 to 3.2 mg/L, respectively. Regarding electricity production, the combination of *Lemna gibba* and the bioelectrode generated the highest output (0.067 mW) compared to other treatments. This study demonstrates that the system using a bioelectrode can effectively remove antibiotics from water within 60 minutes.

Keywords: CIP, Constructed wetlands, Microbial fuel cells, Organic contaminant, Phytoremediation, TET

Graphical Abstract



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1. Introduction

Among the numerous antibiotics reported in environmental samples, TET and CIP are the most frequently detected [1–3]. They are widely used in both humans and animals, exhibit low degradability, and possess high toxicity, with even potential carcinogenic effects [4,5]. Furthermore, they disrupt bacterial communities, leading to the development of antibiotic resistance [6–9]. Hence, there is an urgent need to adequately treat these contaminants to prevent their release into the environment.

Numerous technologies are employed for the removal of antibiotic residues, such as the Fenton system ([10], membrane processes, adsorption, reverse osmosis, electrocoagulation, and advanced oxidation [11,12]. However, most of these have associated drawbacks, as some are expensive, and others generate secondary waste that may be even more toxic than the initial contaminants or require additional treatment [13].

Constructed wetlands, known for their eco-efficiency, effectively remove contaminants from wastewater [14,15]. However, their main disadvantages are time, space, and the plant biomass they generate during phytoremediation. In this context, an innovative solution will be to use this biomass to manufacture electrodes for electrochemical technologies [16,17], solving biomass production this way.

Integrating constructed wetlands with microbial fuel cells (CW-MFC) emerges as a promising strategy for contaminant removal [6,18]. This combination optimises space and time usage and significantly enhances the wetlands' treatment capacity [7,19–21]. In CW-MFCs, redox gradients throughout their depth create ideal conditions for integrating microbial fuel cells, with anaerobic and aerobic zones functioning optimally as the anode and cathode, respectively [4,22].

It should also be mentioned that various factors influence the pollutant removal process in these hybrid systems [4]. These include the plant species, the time between the wastewater and the type of electrode material used, water flow, temperature, substrates, and other factors [23,24]. In this study, we seek to understand the influence of macrophyte species (*Lemna gibba* and *Schoenoplectus tatora*), as well as the impact of the use of different electrode materials (graphite and Bioelectrode from biochar of *Lemna gibba* biomass). *Lemna gibba* and *Schoenoplectus tatora* were selected due to their availability, adaptability to aquatic environments, and potential to enhance system performance. This addresses the scarcity of studies on floating species in this context. The bioelectrode, made from *Lemna gibba* biochar, was compared to graphite as a more sustainable and cost-effective alternative for system design and operation while promoting biomass utilisation. Furthermore, this study aims to identify the optimal treatment duration required for the effective removal of Tetracycline and Ciprofloxacin from wastewater, considering the variables examined in this study.

2. Materials and Methods

2.1. Construction of CW-MFCs

The bioelectrochemical device was designed in a single chamber in a PVC tube for the experiment. The inner diameter was 10 cm, and the height was 40 cm [25]. The reactor had three layers of substrates: 8 cm of gravel, 8 cm of coarse sand, and 14 cm of fine sand [22]. The anode was placed between the gravel and coarse sand layer on top and the cathode at the top of the wetland (Fig. 1). At the base, there was a water outlet tap used to take the water samples. The electrodes were of two types, one made of carbon graphite and the other from *Lemna gibba* (duckweed) biomass, called in this study bioelectrode. The process of obtaining the bioelectrode is shown in the supplementary materials (Text S1 and Fig. S1), which were connected with a copper wire to a resistance of 500 Ω. The system was inoculated with 20 mL of anaerobic sludge [4] to inoculate anaerobic bacteria into the system.

Four treatments were conducted in duplicate, considering two plant species and two types of electrodes: *Lemna gibba* with a graphite carbon electrode (LG), *Lemna gibba* with a biochar electrode derived from *L. gibba* biomass (LB), *Schoenoplectus tatora* with a graphite carbon electrode (TG), and *S. tatora* with a bioelectrode derived from *L. gibba* biomass (TB).

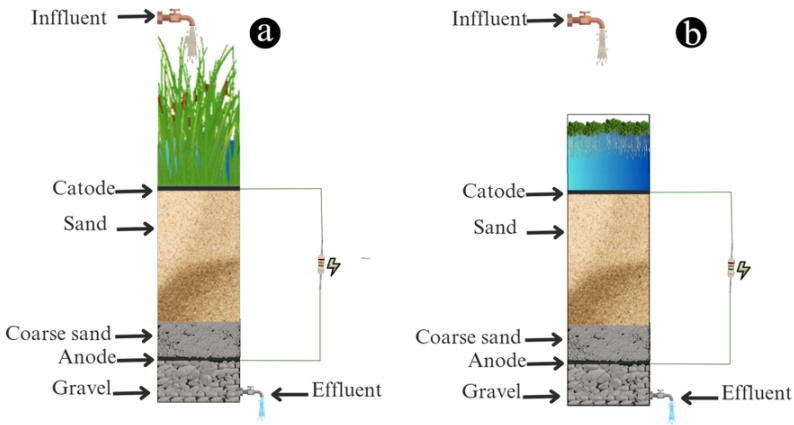


Fig. 1. Configuration of the artificial wetland. a) With *S. tatora*. b) With *L. gibba*.

2.2. External Conditions

The environment where the experiment was carried out had suitable environmental conditions simulating light and dark hours, with 12-hour cycles with 50 W LED bulbs [26].

2.3. Start-up and Operation of the CW-MFC Systems

The reactors underwent a two-month adaptation process, for which 20 mL of anaerobic sludge [12] from a pig farm was added. Synthetic wastewater with some plant nutrients was then added. The synthetic wastewater used in the experiment was composed of Glucose (300 mg/L), NH_4Cl (80 mg/L), K_2HPO_4 (12.8 mg/L), $FeCl_3$ (0.05 mg/L), $MgSO_4.7H_2O$ (4.5 mg/L), $CaCl_2.2H_2O$ (7.3 mg/L) [27]. Mixed wastewater was inoculated with 29.4 mg/L CIP and 27.6 mg/L TET. The experiment was carried out in three phases or cycles [28], with hydraulic retention time (HRT) of 4 days, 4 hours, and 120 minutes, and to differentiate them hereafter will be treated as days, hours, and minutes, respectively. Manual aeration was carried out only once, before sampling, and was restricted to the surface layer of the system. This process aimed to promote the movement and mixing of nutrients in the water column, particularly benefiting *Lemna gibba*, as nutrient availability near its roots tends to decrease over time. Additionally, aeration was essential to homogenize the water sample and ensure consistency in the experimental processes across all treatments. The average ambient temperature was 22.8 ±0.29 and a relative humidity of 61.5 ±2.54.

2.4. Analysis of Antibiotics in Water Samples

CIP and TET (98.0%) were obtained from Sigma-Aldrich-US. Methanol, acetonitrile, and formic acid of HPLC grade were purchased from BAKER ANALYZED. For water analysis, 1 mL samples were taken from the effluent and the upper part of the system (influent), which was deposited through a 0.22 μm x 13 mm PTFE syringe filter into a 2 mL vial for direct readings in an ultra-high-performance liquid chromatography (UHPLC-DAD). The chromatographic conditions were: two microlitres of the sample were injected onto an InfinityLab Poroshell EC-C8 column (2.1 × 150 mm, 1.9 micrometers, Agilent, USA) at 35°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% in acetonitrile (C). Elution was performed using a gradient: at start (0 min) with 95.0% B, at 5 min with 80.0% B, and at 10 min 90.0% B. The flow rate of the mobile phase was 0.250 mL/min. The column effluent was monitored at 280.4 nm to detect the antibiotics. The LOD and LOQ values for the antibiotics were 0.01 μg/L and 0.03 μg/L for CIP and 0.02 μg/L and 0.06 μg/L for TET. Calibration curves were constructed with five points (0.1, 1, 1, 5, 10, 20 μ g/mL) and showed linearity for CIP (R2 = 0.998) and TET (R^2 = 1) in the expected concentration range of the sample.

The following Eq. (1) was used to look at the antibiotic clearance rates:

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

where R is de antibiotic remotion, Ci represents the initial concentration, and Cf refers to the final concentration [29,30].

2.5. Bioelectricity Monitoring and Analysis

The reactors' voltage (V) and amperage (A) outputs were collected through a Trupper multimeter [12]. Measurements were taken daily before and during the experiment.

2.6. Water Quality Measurement

After taking the sample for antibiotic analysis, 30 mL of water was also taken for physicochemical parameter measurements [31], and the water sample was returned to the reactor not to alter the volume to be treated. The samples were immediately analyzed for pH, dissolved oxygen, temperature, ORP, turbidity, conductivity, total dissolved solids (TDS), ammonium, and nitrogen with the HANNA HI 9829 multiparameter [32].

2.7. Statistical Analysis

ANOVA and Kruskal Walis analyzed data related to removal rates in case they did not meet the required assumptions of the analysis. Also, the Duncan and Holm-Bonferroni tests were applied in cases with statistical significance, depending on the previous analysis applied in the free software R.

3. Results and discussions

3.1. Antibiotic Removal Efficiency

In the influent, during the first cycle, it was observed that a longer duration resulted in improved removal of both antibiotics (p<0.05).

Table 1. Percentage of antibiotic removal in the treatments used in the experiment.

Source	Treatment	First cycle		Second cycle		Third cycle	
		CIP	TET	CIP	TET	CIP	TET
Inffluent	LG	95.2	96.4	76.5	56.6	82.8	53.3
	LB	92.5	93.8	84.3	63.0	74.1	39.5
	TG	93.4	96.9	73.7	64.6	79.1	59.0
	TB	93.1	97.3	82.7	84.3	84.5	70.5
Effluent	LG	98.7	99.3	98.8	98.4	98.9	99.6
	LB	98.7	99.3	96.9	96.2	98.9	99.6
	TG	98.7	99.3	98.0	93.8	98.9	99.6
	TB	98.7	98.7	98.9	98.4	98.9	99.6

NOTE: *Lemna gibba* + graphite electrode (LG). *Lemna gibba* with bioelectrode from *Lemna gibba* (LB). *Schoenoplectus tatora* + graphite electrode (TG), another of *Lemna gibba* with bioelectrode from *Lemna gibba* (TB).

However, no significant differences were found between treatments for CIP and TET (Table 1).

In the second cycle, the removal of both antibiotics was significantly influenced by both the hours of treatment and the treatment applied ($p<0.05$) (Fig. 2). In particular, longer hours increased removal, and treatments with bioelectrode showed an advantage in contaminant removal, as illustrated in Fig. 2b,d. Finally, in the third cycle, only treatments significantly influenced TET removal ($p<0.05$), with *S. tatora* treatments showing an advantage over duckweed.

Additionally, during the third cycle, the influence of treatments on TET removal was observed ($p>0.05$), with the TB treatment achieving the highest TET removal, at an average of 70.5% (Fig. S2, supplementary materials).

In CW-MFC systems, various mechanisms contribute to antibiotic removal. In a combined system, such as the one used in this study, elements, including plants, the substrate, and electrodes, work complementarily to optimize removal outcomes [7,19]. This interaction activates multiple processes, such as plant uptake, substrate adsorption, bacterial degradation, hydrolysis, and photolysis, among others [1,4,7,28,33], all of which contribute to treatment efficiency.

The results indicate that the duration of treatment cycles had a more significant impact on antibiotic removal than the treatments themselves, suggesting that the overall system plays a critical role in the process [20]. In the influent, plants appear to be the primary factor in removal due to their direct contact with the contaminants, supported by additional mechanisms such as photodegradation and oxidation. Conversely, effluent removal is influenced by the interaction of all system components, including sand, plants, and bacteria [1,28,34].

These results demonstrate that electrochemical technologies associated with microbial fuel cells accelerate antibiotic removal [6]. Similarly, in the case of Sulfamethoxazole, it was observed that during the first two hours of treatment, there was a rapid decrease in the compound, with values ranging from 22.5% to 59.4%, reaching up to 90.0% [35]. In another similar study, a 75.0% removal of Sulfamethoxazole was achieved within 16 hours, with the percentage decreasing as the HRT shortened, reaching 48.0% after four hours of exposure [9]. Additionally, in a study applying a microbial fuel cell, Penicillin was removed by 98.0% within 24 hours [36].

These findings suggest that the combinations employed in the system used are adequate, as high removal percentages of up to 100.0% for TET and 98.9% for CIP were achieved within 60 minutes of experimentation, surpassing results from other studies [2,37–39]. Understanding how these elements interact and the factors influencing system efficiency is essential and will be addressed in detail in the following paragraphs.

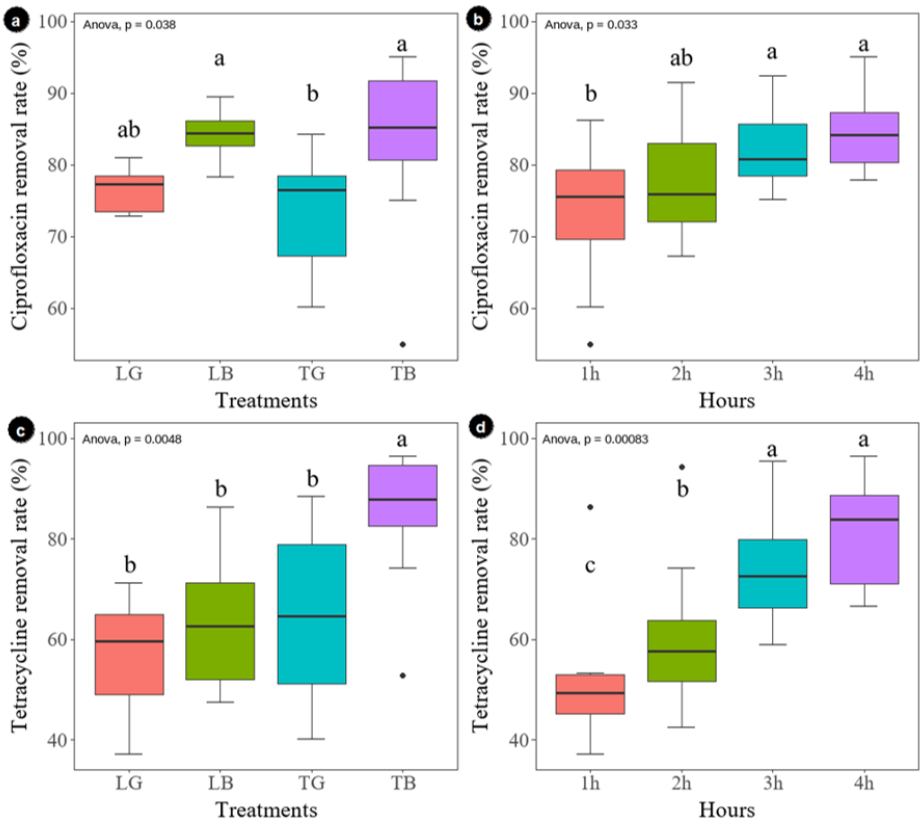


Fig. 2. The elimination efficiency of Ciprofloxacin (a, b) and Tetracycline (c, d) was determined according to treatment method (a, c) and operation time (b, c) (hours).

3.1.1. Effect of hydraulic retention times

This study demonstrated that the HRT employed during the cycles significantly influenced antibiotic removal more than the treatments applied. It was observed that longer retention times resulted in improved removal, which was most evident in the influent during the second cycle, as described in Fig. 2. Additionally, in the effluent of the third cycle, the effect of HRT on TET removal was significant (Kruskal-Wallis test, $P < 0.05$), showing that 100.0% removal was achieved after 60 minutes, compared to 98.4% at 30 minutes. Although more significant variability in antibiotic removal data was recorded in the influent during this cycle, ranging from 67.7% to 95.1% for TET and 31.4% to 89.7% for CIP, it was evident that removal improved over time, with better results observed from 60 minutes onwards for both CIP and TET.

This demonstrates that longer HRTs are associated with higher antibiotic removal percentages. For instance, one study reported 88.9-93.5% removal efficiencies for Sulfamethoxazole and 89.3-95.6% for Tetracycline over 40 days [38]. Similarly, another study found that an HRT of three hours allowed for removing 72 ± 2.4% of total organic carbon [40], further confirming the influence of time on process efficiency.

3.1.2. Effect of electrodes

Regarding electrodes, this study represents the first documented case in which an electrode derived from duckweed biomass was developed and utilised in a microbial fuel cell system. The results obtained were similar to, or slightly better than, those of a conventional electrode, highlighting the potential of bioelectrodes for treating environmental pollutants [8,41,42]. Similarly, a study employing electrodes made from rice biomass demonstrated that these were more efficient than commercial electrodes, achieving a 40.0 ± 1.2% increase in dissolved organic carbon removal during wastewater treatment [16]. Additionally, another combination of plant sponge and rice husk biochar achieved a 65.0% removal of organic carbon in wastewater [17].

These findings demonstrate that electrodes fabricated from plant biomass are an efficient alternative for wastewater treatment and antibiotic removal [42]. Their high porosity provides a larger surface area for bacterial colonization, facilitating antibiotic degradation and electron transfer through the electrode [38]. Furthermore, these electrodes significantly reduce system installation costs while achieving comparable or better performance than commercial electrodes.

3.1.3. Effect of species

Regarding the influence of species, *Schoenoplectus tatora* exhibited slightly better performance than *Lemna gibba* in the treatment process. This may be attributed to the high concentrations of contaminants evaluated, which could induce toxicity in *L. gibba* due to its constant exposure to pollutants through its roots and fronds. Such exposure affects its physiological and biochemical development, generating stress that temporarily compromises its growth [15]. Nevertheless, *L. gibba* demonstrates remarkable recovery capabilities. For instance, after exposure to TET, which interferes with electron flow in photosystems and impairs photosynthesis, it can recover within approximately one week [43]. This combination of initial sensitivity, resilience, and high growth rate makes *L. gibba* a key tool for the phytoremediation of contaminated water bodies [30,44].

Phytoremediation enhances contaminant removal [45,46] and operates through various mechanisms, such as phytoabsorption, phytostabilisation in plant roots, and phytovolatilisation of organic compounds, among others [23,47,48]. These mechanisms enable plants to play a crucial role in decontaminating aquatic environments.

On the other hand, electroactive bacteria also influence contaminant removal. Although bacterial populations are initially affected upon first contact with antibiotics [1,10,33], some develop resistance and carry out degradation processes as well as electricity production [6–9]. Consequently, antibiotic removal in the initial hours is often suboptimal; however, this improves over time, reaching 100.0%. It is well-established that bacterial biodegradation is one of the primary pathways for antibiotic elimination [49]. Bioelectrochemical systems promote the growth of bacterial biofilms on electrically conductive materials and enhance bacterial metabolism, which leads to more significant contaminant degradation [17,35,50]. The cathodic zone is also suitable for microorganism development due to releasing plant exudates, oxygen, protons, and electrons [1,51]. This environment supports the growth of specific bacterial groups, such as nitrifying bacteria [50]. Therefore, bacteria play a key role in remediation processes within bioelectrochemical systems.

3.2. Relationship Between Physicochemical Parameters and Antibiotic Removal Rate

Fig. 3 illustrates the correlation between antibiotic removal and the evaluated physicochemical parameters. Specifically, a moderate relationship is observed between TET and the ORP (0.56 mV). This finding suggests that an increase in ORP values is associated with a higher percentage of TET removal. It is worth noting that the ORP values obtained indicate a highly reducing environment, with an initial value of -414.55 mV, which decreased to -0.13 mV during the treatments. In a combined system with constructed wetlands, plants release oxygen into the cathodic zone through their roots, which helps improve ORP values [52]. Therefore, plants play a significant role in enhancing ORP levels in the treatment system [53].

Moreover, a slight correlation was observed between CIP and TET (0.5), indicating that degradation processes apply to all antibiotics in the system. Additionally, there was a moderate positive correlation between CIP removal and pH (0.4), suggesting that as pH increases, so does the percentage of CIP removal. Similarly, another study found that the optimal pH for Chloramphenicol removal (96.5%) was 7.1 [8]. Furthermore, a CIP and TET removal survey found a positive relationship between antibiotic removal and pH values ranging from 2.0 to 10.0 [29]. Similarly, sulfamethoxazole removal ranged between 22.4% and 64.7% at pH values between 6.5 and 7.9, with values close to neutral [35]. These pH values also support the development of bacterial communities, contributing to contaminant degradation [10]. In summary, antibiotic removal improves at neutral and slightly alkaline pH levels.

On the other hand, elements such as salinity exhibit a strong positive correlation with total dissolved solids (TDS) (0.99 mg/L)

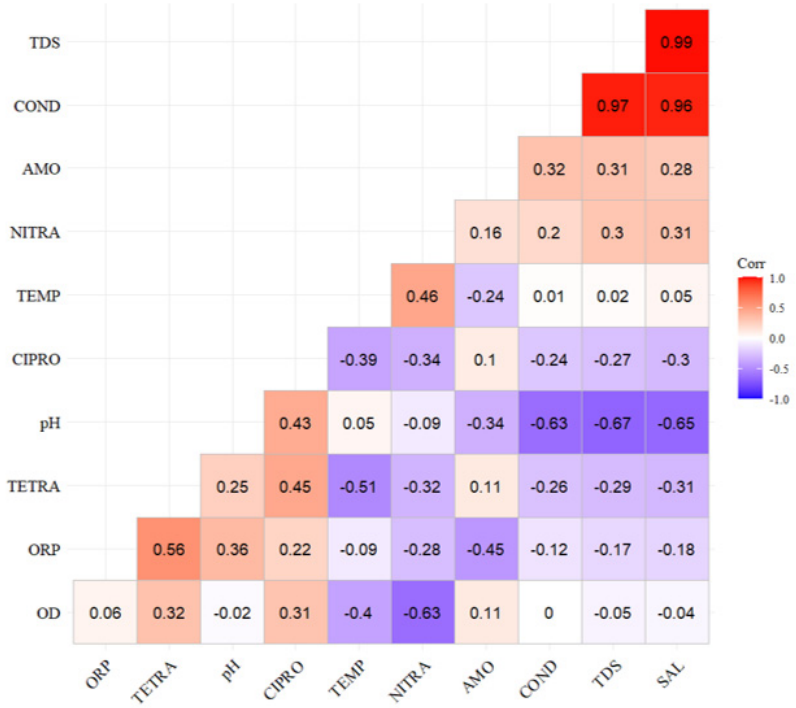


Fig. 3. Multiple correlation between physicochemical variables and antibiotic removal rates.

and conductivity (0.96 μS/cm). This is reasonable, as higher salinity leads to more significant dissolved solids and, consequently, higher conductivity in the inorganic aquatic [54,55].

3.3. Electricity Production

Concerning electricity production, electroactive bacteria are responsible for electricity generation, and the electrons transferred by these bacteria impact the increase of metabolic reactions in these bacteria [8]. Thus, apart from resisting and degrading antibiotics, they produce electricity [6,9,33,36]. In this study, it was observed that the *L. gibba* treatment and the bioelectrode produced higher amount of electricity compared to the other treatments (0.067 mW) (Fig. 4). Similarly, in one research, it was found that

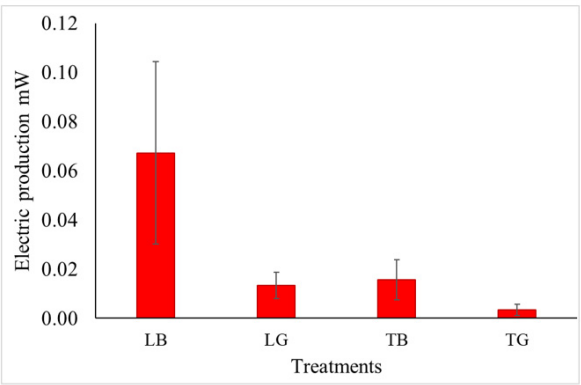


Fig. 4. Electrical production in the applied treatments.

the electrode obtained from rice husk showed better electrical generation results with a current density of 6.9 ± 3.1 W/m³ than the control group (electrode with carbon cloth) [16]. Another research using rice husk charcoal also found that charcoal material has a higher affinity for microorganisms, so they grow better on this surface, which results in higher pollutant degradation as well as electrical production, improving the current density up to 5260.0% [17]. This shows that plant biomass can be a raw material for obtaining bio-electrodes capable of generating energy and collaborating with the drug removal process.

On the other hand, organic pollutants such as antibiotics also serve as a resource for developing microorganisms [33]. Penicillin was used as the sole resource in one study, generating 2.1 W/m³ with 50 mg/L of Penicillin. When glucose was added, production increased to 101.2 W/m³ [36]. This demonstrates that these microorganisms can tolerate and degrade high concentrations of antibiotics while producing electricity in the system.

3.4. Water Quality

Water quality improved in all experimental units compared to the initial value. In the case of pH, the initial value was 4.7, and most of the treatments were above 7. This shows that the treatments applied had positive effects from the first day of treatment (Fig. 5a). Concerning ORP, its initial value was -650 mV, representing a very reducing environment; in the applied treatments its value increased, approaching the 0 mV value on the fourth day of treatment in all the experimental units (Fig. 5b). Similar studies found negative results, associating these values to the lack of oxygen in the anodic zone, showing values of up

to -21.2 mV of ORP [53]. Although in the correlation (Fig. 3), there is no significant relationship; this could be because the water sample was taken from the complete circuit and not only from the anode zone, which could have influenced the mixing of water in the cathode and anode zone, where there is greater oxygenation [52]. Thus, mixing with oxygen could provide positive ORP values, as shown by a system coupled with artificial aeration [56].

Regarding the dissolved oxygen, from a value of 0.3 mg/L, it increased and decreased in the days of treatment (Fig. 5c) but remained above the initial value. It reached its optimum on the second day, decreased on the third day, and increased again on the fourth day, but it remained above the initial value, above 2 mg/L.

The initial conductivity was 362 μS/cm, reaching a maximum of 1998 μS/cm in the TB treatment on the first day. On this same day, TDS (1065.0 mg/L) and salinity (1.1 mg/L) exhibited similar patterns due to their close relationship, which was attributed to system components such as sand, whose composition likely increased the presence of salts in the system [57].

Regarding ammonium, its initial concentration was 12.7 mg/L, and all treatments successfully reduced this level, with the TG treatment being the most effective, lowering it to 0.8 mg/L in the final measurement (Fig. 5g). In contrast, nitrate showed an opposite trend: it started at 0.4 mg/L and increased across treatments, reaching a maximum of 5.6 mg/L. By the fourth day, however, nitrate levels began to decrease in all treatments (Fig. 5h). This behaviour may be attributed to the initial disruption of the bacterial community caused by antibiotics, followed by its gradual adaptation, which likely facilitated nitrate removal, as antibiotics are known to disrupt the nitrogen cycle [33]. Furthermore, this effect could be linked to the nitrification process, where bacteria convert ammonium into nitrate, contributing to the observed changes [18,35].

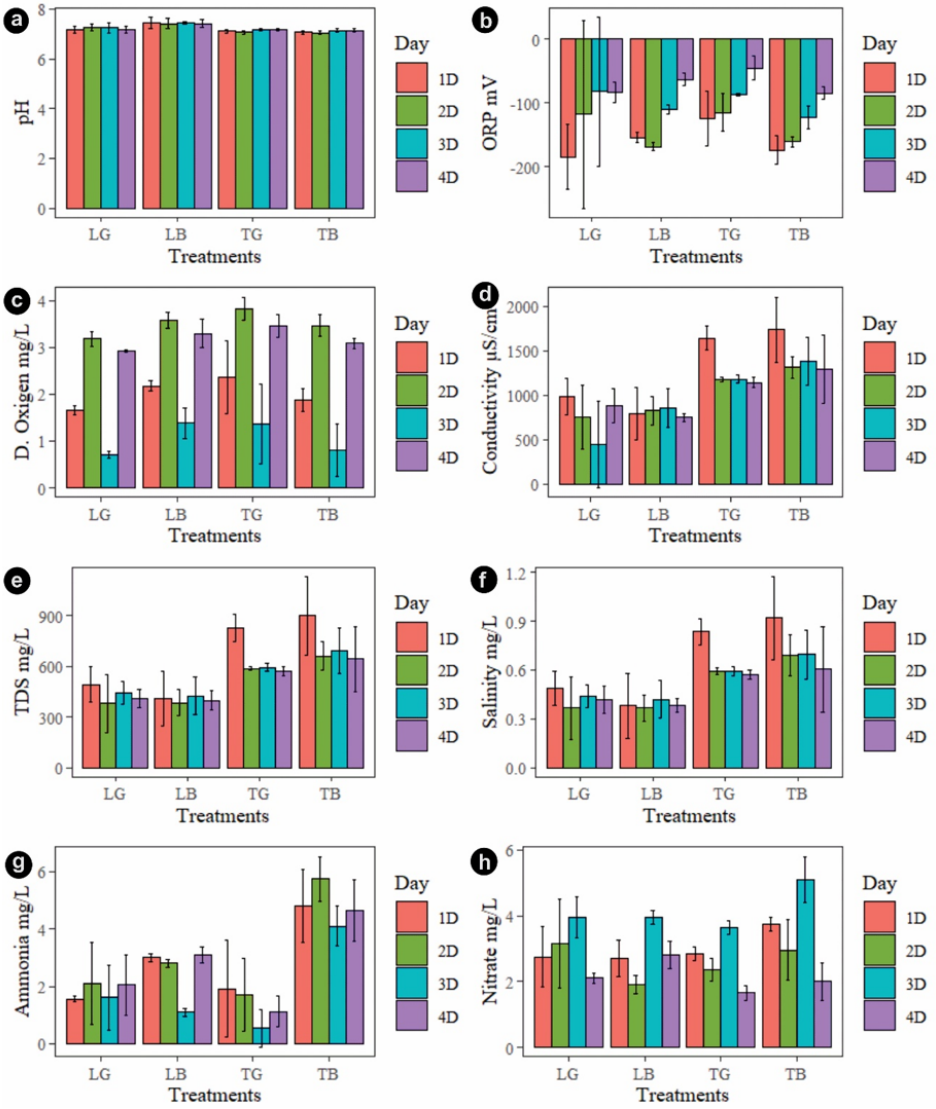


Fig. 5. Physico-chemical parameters in the different treatments tested and days of experimentation.

The contradictory results regarding the increase in salinity, conductivity, and TDS could be attributed to factors such as microbial processes involved in the mineralization of antibiotics, during which certain inorganic salts are generated [10,54,58] (Fig. 5d,e,f). Additionally, the presence of sand might also play a role, as its composition may contain salts [57]. A study found that conductivity increased with higher concentrations of Penicillin [36], suggesting that the high initial concentration of the antibiotic tested in this study could have similarly contributed to the rise in salinity and its associated variables. Another source of salts could be the nitrification process carried out by the bacterial community within the system [18,35]; this is supported by the observed decrease in ammonium concentration and the concurrent increase in nitrate, providing evidence of bacterial nitrification [1,25,54], which, in turn, contributes to water salinization [24,59].

4. Conclusions

The combinations of the four treatments positively affect the removal process of CIP and TET, evidencing a slight trend of improvement in the bioelectrode treatment with *Schoenoplectus tatora*. In general, removal percentages reach 100.0% for both antibiotics evaluated, particularly in the effluent. During the second cycle, the influence of exposure time and the treatments applied becomes evident, showing that longer exposure times result in higher removal percentages. In the third cycle, removal percentages of up to 100.0% for TET and 98.9% for CIP are achieved within just 60 minutes. A moderate relationship is observed between the increase in pH and CIP removal. Furthermore, among the treatments applied, the LB treatment (0.039 mW) achieves the best results in electricity production compared to the others. Regarding physicochemical parameters, the treatments generally improve water quality, particularly in pH, dissolved oxygen, ORP, and ammonium, demonstrating the effectiveness of the applied treatments. In summary, the treatments are efficient in removing contaminants and improving the physicochemical quality of wastewater. Additionally, the bioelectrodes demonstrate similar or slightly superior performance compared to conventional electrodes, contributing to the reutilization of waste as a resource for electrochemical technology.

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Conflict-of-Interest Statement

The authors declare that they have no conflict of interest.

Author Contributions

I.M. (postdoctoral fellow) conducted all the experiments and wrote the manuscript. Y.M.R. (Research assistant) helped during the experiment's execution. F.Z.V. (Principal professor) supervised the experiments and revised the manuscript.

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