Modular bioelectrochemical wetland: A demonstration study for treating urban wastewater

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Abstract:

The integration of microbial electrochemical technologies (MET) into treatment wetland (TW) led to a new generation of nature-based solution so-called METland®. In this context, METland® concept was further expanded to modular METland® while a comprehensive evaluation of a demo scale is reported for the treatment of real domestic wastewater. The overall treatment system included the following METland biofilter configurations: i) a horizontal flow modular unit, ii) a downflow unit made of EC coke, and iii) a downflow unit made of EC biochar. This hybrid treatment system aims to enhance pollutant removal efficiency through MET, leveraging the conductive properties of substrates to optimize microbial metabolic processes. The system exhibited high COD removal efficiency (>90%) regardless of high feeding rate (ca. 0.5m^3/m^{2}day) and significant nitrogen removal, with the ECBB unit showing high ammonia removal efficiency (90%). Standard treatment wetlands do not incorporate tools for monitoring the *in situ* performance of the systems. However, the electrochemical nature of the METland® allows continuous monitoring by measuring electrochemical parameters. In this context, electric potential (EP) measurements revealed spatial variations in electron utilization within the wetland, correlating with pollutant degradation. The electron current density (J=43.99 mA/m^2) within the system decreased along the flow path, indicating a consistent electrochemical activity aligned with the treatment process. High correlations between J values and COD concentrations suggest the potential use of electrochemical indicators as proxies for pollutant levels in wastewater treatment. This study gives insights into the electrochemical behaviour of the system to provide a foundation for future optimization.

Keywords: Modular constructed wetland; urban wastewater; electrobioremediation, METland, Microbial electrochemical technology; Electroconductive substrates

1 Introduction

Recent studies about water scarcity indicate that half of the world's population will live in water-stressed areas very soon. Thus, in this scenario there is an urgent need develop decentralized wastewater systems capable of generating reclamed water ready to be used [1]. In this context, treatment wetlands (TWs), also known as constructed wetlands

(CW), are a nature-based and cost-effective solution capable of treating wastewater by using a biofilter inspired by natural elements (gravel, plants and microorganisms) [2–5]. The core of the treatment wetland is the bed material, so researchers and engineers in recent years have sought and tested various innovative substrates to elevate pollutant removal efficacy in TW systems [6–8].

Among all materials, the use of those with electroconductive nature triggered the born of a new variety of TW where electromicrobiology concepts are integrated with the purpose of boosting biodegradation or harvesting energy [9–11]. Thus, electrodes can be allocated into classical TW inert beds to generate electrical energy from microbial metabolism of organic pollutants as part of devices so-called microbial fuel cells (MFCs) [12,13]. Actually, the new generation of TW capable of generating energy is named TW-MFC [14,15]. According to the previous studies, the range of bioelectricity power generation by various TW-MFCs is approximately 0.11-452.24 W/m^2 [16–18]. Alternative to power production, other researchers have merged electrochemistry and TW in a very different scenario where classical electrochemical terms like anode and cathode were replaced by the use of a single electroconductive material operated as microbial electrochemical snorkel. Indeed, the new configuration so-called METland was conceived after replacing all inert material such as gravel by electroconductive granular material [19,20]. Among materials used in the METland bed, authors have mainly reported conductive coke [19,21] or more sustainable materials such as conductive biochar obtained after high-temperature pyrolysis of wood [22–24]. In addition to its conductive nature, electroconductive biochar can also exhibit substantial electron storage capacities, thanks to their redox moieties acting act as a redox buffer when there is limited availability of electron donors or acceptors [25]. Thus, regarding materials two different mechanisms can be identified in METland: geoconductor mechanism and geobattery mechanism [23]. The current understanding of how electroactive microbial communities operate in METland suggests that microbial extracellular electron transfer, either in form of DIET (Direct Interspecies Electron Transfer) [26] or CIET (Conductive-particle-mediated Interspecies Electron Transfer) [27,28], eventually, allow interconnection between microbial communities through the role bacteria from *Geobacter* genus, obtaining optimal synergies leading to efficient removal from wastewater [10,29,30].

Moreover, the microbial electrochemical activity inside METland was demonstrated by measuring electrochemical parameters like electric potentials (EPs) and electron current density, allowing the operator to monitor the performance and efficiency of the process in real time [21–23,31].

Constructed METlands followed similar design methods to traditional TWs. Indeed, they have been applied under different environmental and operating conditions in diverse geographic regions while achieving COD removal efficiencies of ca. 90% [31] including emerging pollutants [32]. Furthermore, a life cycle assessment (LCA) study suggested that they are indeed an environmentally sustainable wastewater treatment technology [33]. Larger constructed METland designs are currently treating wastewater from a Camping site for 1000 inhabitants (Los Escullos, Spain) in just 50 m^2 while fulfiling all demanding discharge limits of a Natural Park. However, regardless of its high footprint efficiency, exploring new configurations of this novel technology is a must.

In this context, the concept of modular constructed wetlands (MTWs) emerges as an innovative enhancement upon conventional treatment wetland (TW) systems, embodying a strategic adoption of pre-designed and pre-fabricated modular units to rapidly build the wetland system and augment wastewater treatment ability [34]. The predefined modules, constructed from robust materials such as wooden or plastic, are meticulously designed to host specific substrates per unit, wetland vegetation, and microbial communitie, thereby simulating natural wetland processes for wastewater purification in a more controlled and efficient manner. The inherent modularity and scalability of MTWs demonstrate superior adaptability, especially in contexts constrained by construction limitations or diverse treatment requisites, offering a symbiotic blend of physical filtration, biological degradation, and chemical transformations to synergistically mitigate pollutant concentrations in wastewater. Similar to traditional TWs, MTWs utilize specific substrate as a pivotal component to enhance pollutant removal efficiency [35], or even nutrient recovery by Wei et al., (2024) from wastewater [36].

The objective of this study was to validate for first time modular concepts with METland® technology to implement a demo scale of modular METland® for treating real domestic wastewater treatment. This novel strategy will create new solutions to enhance the ability to effectively treat real wastewater and explore the applicated possibility for different communities or regions by the scalability and design optimization of the system.

2 Materials and methods

2.1 Site description

The modular METland® units were built in hybrid system, and located in the Foundation Centre for New Water Technologies (CENTA) at Carrion de Los Céspded, (Sevilla, Spain) (Fig. 1(a)). The province of Sevilla is the western area of the Autonomous Community of Andalusia. Meanwhile, Seville has a warm Mediterranean climate with an annual average temperature of 18.5 °C. Winters are generally mild while summers are hot. The maximum temperatures in summer often surpass 40 °C [37]. This scientific centre of CENTA stands as a pivotal research institution in the field of water/wastewater technologies, and it has 41,000 m^2 area for experimental treatment plant that exemplifies the fusion of cutting-edge and natural water treatment systems. Integral to CENTA's mission is the real-world validation of water treatment solutions. Before entering the market, a multitude of on-site water treatments, tailored for both individual households and smaller communities, undergo rigorous testing and verification at the facility. Moreover, this extensive technological platform serves as the nexus for research endeavors undertaken by CENTA and offers collaboration opportunities for other scientific entities and corporations in the water sector.

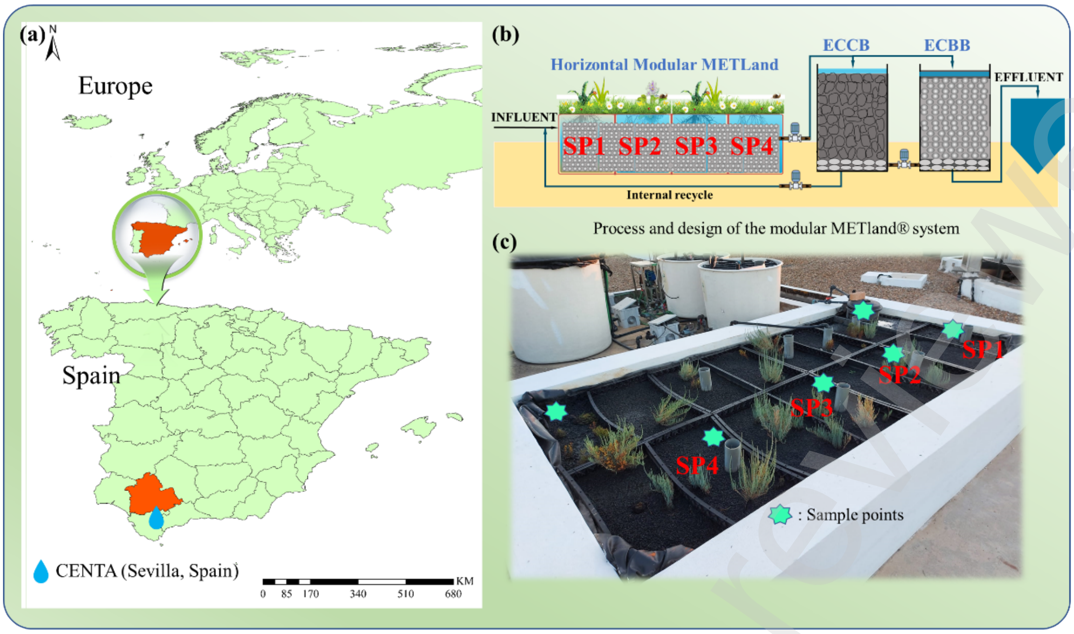


Fig. 1 Modular METland® system in the study: (a) Location map of site; (b) Process and operation of the system; (c)The *in situ* photograph of the system

2.2 Designation, construction and operation of the overall treatment system

The process of the modular METland® system is shown in Fig. 1(b). It was operated in combination with consisted of a three stages solution: i) horizontal flow electroconductive MTW (HF-EC-MTW), ii) vertical downflow EC coke-based biofilter (ECCB) and iii) EC biochar-based biofilter (ECBB), built in the year of 2020 at CENTA to treat real domestic wastewater (Fig. 1(b&c)).

The worktable dimension of the modular METland® system is 4.8m in length, 2.4m in width, and 0.6m in depth (with the volume and the surface area are 6.91m^3 and 11.52 m^2, respectively), and the main structures were installed by 16 plastic modules with both solid and hollow pieces of plastic boards. The detail of the plastic modules can be referred to Wei et al., (2024) [38]. The CAD drawing of the system is shown in Fig. 2a,b. Additionally, the concrete brick walls were built as the external reinforcements for adequate support. The bed of the modular METland® system was filled with 0.5m-deep electroconductive coke supplied by METfilter (Spain), and 5 cm of gravel at the bottom, engulfing the drainage system via Ø75 mm PVC perforated pipes. The sampling points were distributed over the system by Ø75 mm PVC perforated pipes for monitoring both water quality and bioelectrochemical activities. The schematic diagram for water flow path of the modular METland® system is shown in Fig. 2(a,b), while the *in situ* assembling scenario is shown in Fig. 2(c) .

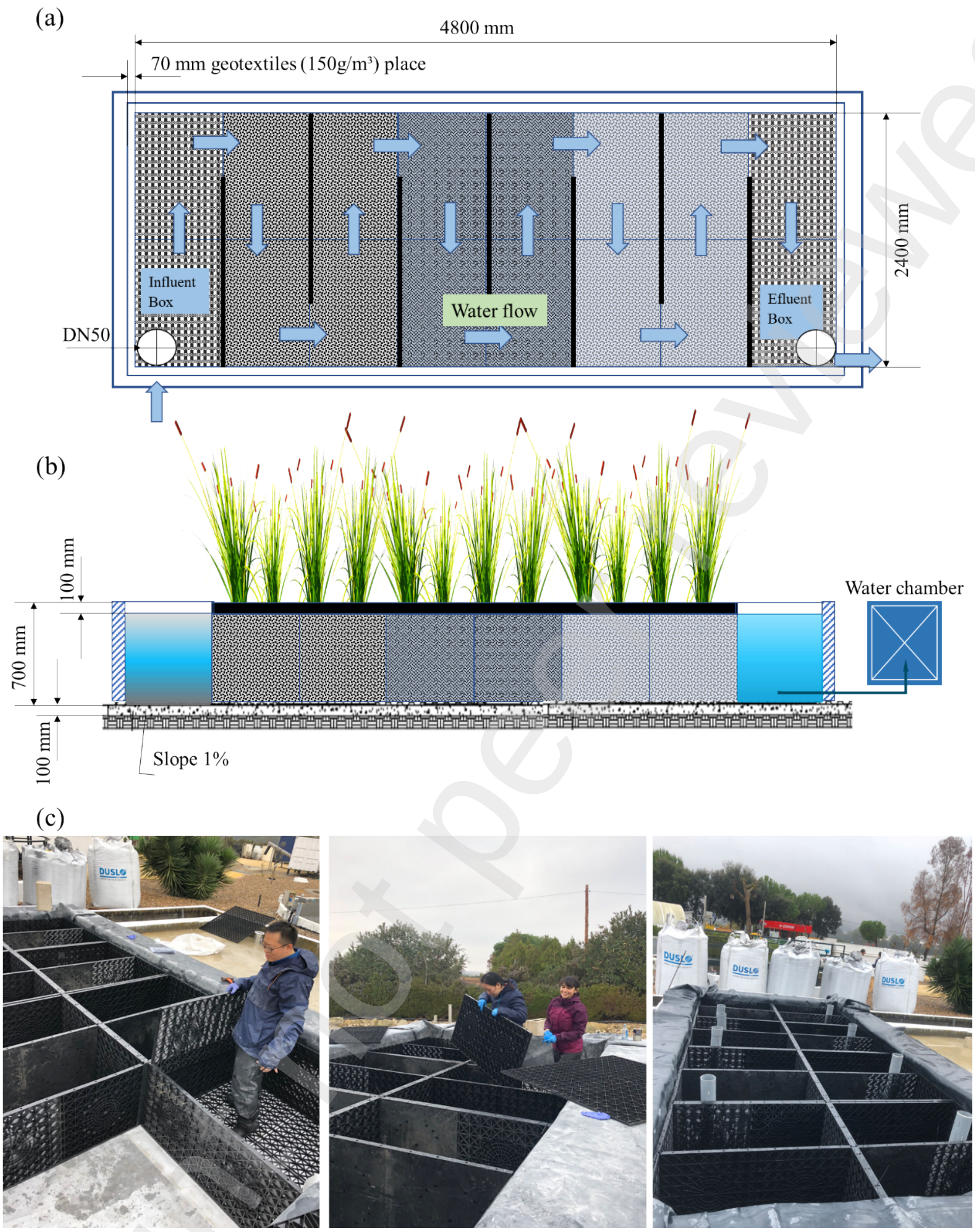


Fig. 2 Design and assembling of the modular METland®: (a) Top view; (b) Profile view; and (c) the *in situ* assembling scenario

The dimensions of the additional biofilters were 1.5 m in diameter and 1.1 m in height, the surface area and the volume of each biofilter were ca. 1.7 m^2 and 1.9 m^3, respectively. Both the biofilters were filled with about 10-15cm (in height) gravel in bottom layer as foundation, then filled with electroconductive coke in ECCB and electroconductive biochar in ECBB, respectively, as main filter medium. The two biofilters were parallelly implemented in downflow mode after the modular METland® unit (Fig. 1(b)).

The modular METland® system was operated with real domestic wastewater after pre-treatment of septic tank. The purpose and the conditions of the operational processes were divided into five stages (Fig. 3). The first and second stages corresponded with 6 and 3 m^3/day of flow rates, respectively, for exploring the influence of hydraulic loading in the modular METland® system. In the third stage, two parallel biofilters (ECCB and ECBB) are connected after modular METland® system for considering the further treatment of ammonium. In the fourth and fifth stages, 100% and 200% rate of effluent recirculation, respectively, from ECCB to the modular METland® system were implemented for enhancing the TN removal.

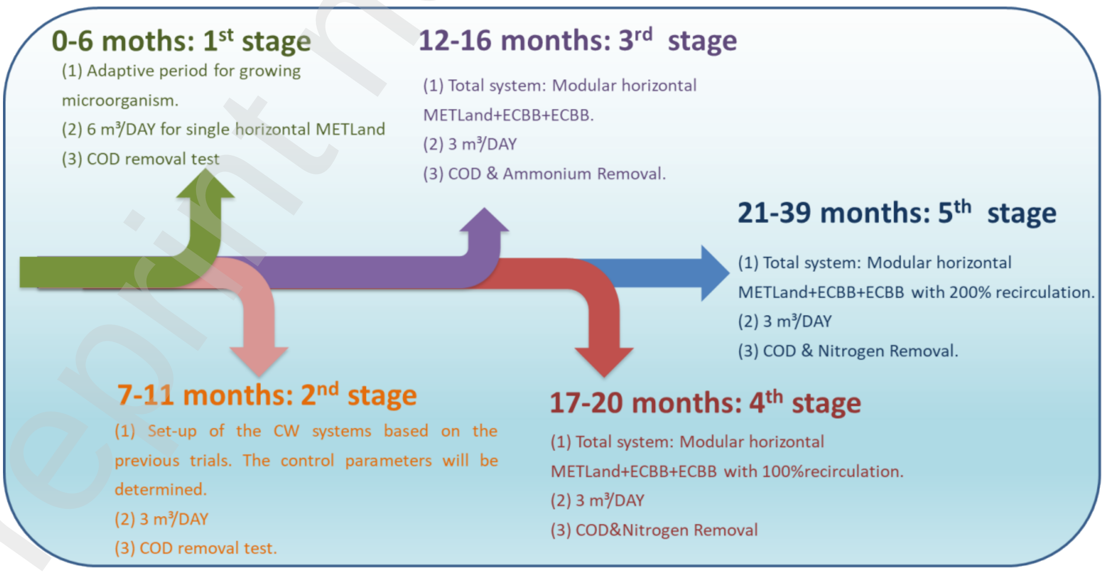


Fig. 3 The operational conditions in the overall trial period

2.3 Water source, sampling and water quality analyses

Urban wastewater source in continuous flow was from the municipality of Carrión de los Céspedes (2,500 inhabitants; Seville, Spain). The average pollutants concentration of wastewater were as follows: COD, NH_4^+ and TN were 419±54.1, 61.3±5.34 and 63.4±5.28, respectively. According to the wastewater quality, the influent corresponds to typical urban wastewater from a small agglomeration [39].

The water samples from influent and effluent chambers were taken weekly after 6 months of an initial acclimation period. The steady state was defined as constant removal efficiencies for pollutants without overflow and clogging events, using a flow rate of 2-3 m^3 per day. All the water samples were filtered and a completely physical-chemical analysis was performed by the Analytical Lab Service from CENTA, Sevilla, Spain. COD analysis was carried out by photometric evaluation (Hach LCK cuvette test + DR 3900 spectrophotometer). NH_4^+, NO_3^- and TN were measured with photometric evaluation (Hach APC and LCK cuvette test + DR 3900 spectrophotometer).

2.4 Electric potential measurement to monitor in situ microbial electrochemical activity

To evaluate the microbial electrochemical activity of the modular METland® system, EP sensors made of shielded silver/silver chloride (Ag/AgCl) were used. Construction of such custom-made sensor were inspired by previous design reported elsewhere by Damgaard et al., (2014) [40]. The sensors, with dimensions of 0.12 cm in diameter and 60 cm in height, were meticulously crafted to remain insensitive to redox-active compounds. The sensors with two electrodes were placed in different measuring spots in each plastic sampling column of the system, while the electrodes were connected by voltmeter. EP readings were taken every 1cm increments at a resolution of ± 45 seconds, and recorded for a duration of 30 seconds per spot [31,41]. To ease the graphical representation, the EP values (mV) were normalized using, as reference electrode, the water/atmosphere interface (0 mV at 0-cm depth). This adjustment rendered the EP profiles relative to the overlying water's potential, which served as a normalization reference. Comprehensive analyses of the EC-MCW systems' microbial electrochemical activity were then deduced from these EP measurements, along with estimations of ionic current densities (J) for forecasting the electron transfer activities.

The pollutants removal efficiency (RE, %) of the system was typically calculated as Eq. (1), and mass removal rate (MRR, $g/m^2 \cdot day$) was calculated as Eq. (2), where, C_{IN}, C_{OUT}, A and Q correspond to the pollutant concentrations of influent, effluent (mg/L), surface area (m^2), and flow rate (m^3/day), respectively:

$$\mathrm{RE} = \frac{C_{IN} - C_{OUT}}{C_{IN}} \times 100\% \qquad (1)$$

$$\mathrm{MRR} = \frac{C_{IN} - C_{OUT}}{\mathrm{A}} \times \mathrm{Q} \qquad (2)$$

The ionic current density was calculated with the Ohm's Law as Eq. (3) [42]:

$$J = -\sigma \times \frac{d\varphi}{dz} \qquad (3)$$

Where, J and σ represent the electron current density (A/m^2) and water electrical conductivity (S/m), respectively, and $\frac{d\varphi}{dz}$ is the EP gradient (V/m).

3 Results and Discussion

3.1 Overall performance of modular METland® system for treating real urban wastewater

An electroconductive treatment wetland, so-called METland was design and constructed for the first time following a modular strategy for treating real urban wastewater while monitoring bioelectrochemical performance by means of electric potential profile and current density. The new configuration was designated as electroconductive modular treatment wetland (EC-MTW), and it was operated for two years along five stages for achieving a robust purification of pollutants removal (Fig. 3). i) The first and second stages were implemented in a range of 3-6 m3/day of flow rates for exploring the influence of hydraulic loading in MCW unit; ii) in a third stage, two parallel downflow electroconductive biofilters: EC coke-based biofilter (ECCB) and EC biochar-based biofilter (ECBB) were operated after EC-MTW unit for promoting ammonium oxidation. Finally, iii) 100% and 200% rates of recirculation from ECCB to MCW were implemented for enhancing the TN removal in the fourth, and fifth stages, respectively.

3.1.1 COD removal performance

In order to evaluate the performance of the EC-MTW in terms of removal efficiency (RE, %), and COD removal rate (MRR, $g/m^2/$ day), the systems were fed with a real URBAN wastewater under a continuous mode (Figure 4) Conventional horizontal subsurface flow treatment wetland (HSSF-CW) typically are designed to 3-5 m^2 per person equivalent (pe), while in this study, the hydraulic loading of EC-MTW system were significantly high (6 m^3/day), leading to footprint requirements in a 0.6 m^2/pe range. In the first and second stages with a single EC-MTW unit, the COD average removal rate was above 70%. Furthermore, the decrease in the second stage was attributed to a 50% reduction in flow rate. In the first two stages, the single EC-MTW unit revealed cod removal rates in the range of 200 g/m^2 per day leading to effluents with ca. 140 ppm COD. This value is consistent with removal rates observed elsewhere using wastewater from the same facility (Aguirre-Sierra et al., 2016). This is remarkable considering that EC-MTW was operating at TRH 20-fold higher than standard HSSF-CW demand for treating urban wastewater. To further enhance quality of the effluent, including the need for an extra nitrifying step, the EC-MTW was upgraded by adding two parallel downflown EC biofilters made of EC coke or EC biochar (stages 3-5).

Thus, this multi-biofilter hybrid system revealed COD removal efficiency above 90%. In particular, during the fourth and fifth stages, the additional recirculation process was implemented and significantly increased the removal rate of COD.

The process of removing COD from wastewater using conductive materials such as electroconductive coke or electroconductive biochar involved a unique combination of electrochemistry and biological mechanisms. Simultaneously, the role of electroconductive materials in facilitating biological degradation comes into play. The materials provided an ideal surface for the growth and activity of electroactive bacteria, a key component in the biological aspect of COD reduction [36,43]. These bacteria possess the unique ability to transfer electrons to and from the surface of substrates, biochar usually has a high surface area, porosity and a variety of functional groups on its surface, which has better electron transfer than coke and then potentially facilitates the degradation process for pollutants. Thus, the COD removal performance of ECBB was always better compared to ECCB (Fig. 5cc). Moreover, this electron transfer is vital for the oxidation-reduction reactions necessary for the removal of complex organic molecules. The conductive material serves as an electron mediator between microbial metabolism and passively-supplied oxygen, thereby optimizing the efficiency of bioremediation. Furthermore, conductive coke can exhibit catalytic properties to boost redox reactions, promoting the oxidation of organic pollutants. These species are highly reactive and play a critical role in removing persistent organic molecules that are typically resistant to biological degradation.

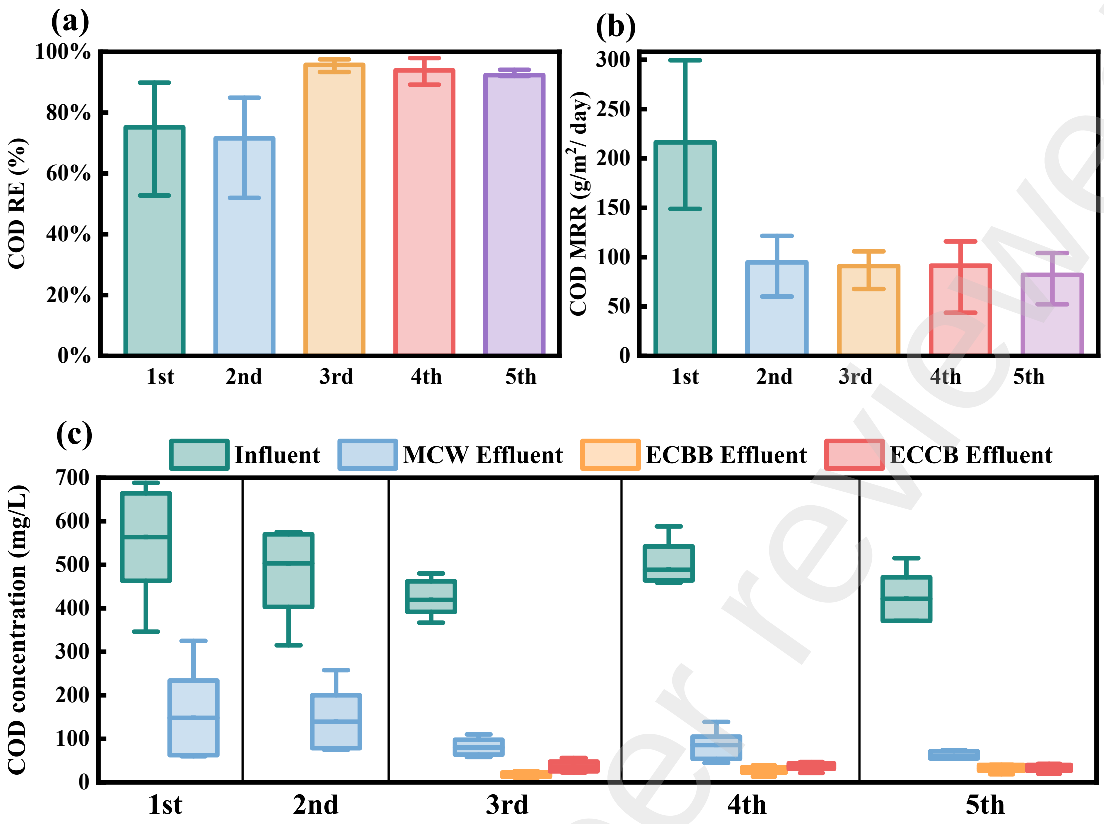


Fig. 4. COD removal performance of EC-MCWs in different conditions. 1st: EC-MTW1 fed with 3 m^3/day; 2nd: EC-MTW 2 fed with 6 m^3/day; 3rd: EC-MTW + EC DF corresponded to EC-MTW1 followed by ECCB and ECBB biofilters operated down in parallel; 4th and 5th:EC-MTW1 + EC DF +RC1 and EC-MTW1 + EC DF +RC2 corresponded to 100% (4th) and (5th) 200% rate of recirculation from ECCB to EC-MTW1.

3.1.2 Nitrogen removal performance

The removal of N from wastewater involves a two-stage process: nitrification and denitrification, which mainly occur under aerobic and anoxic conditions, respectively [44]. Our modular METland® system (EC-MTW) operated under anoxic conditions due to its horizontal flow design, so it is not optimal for nitrification. Therefore, to enhance

N removal efficiency, additional vertical flow biofilters (ECCB and ECBB) were added after the EC-MTW system to provide the oxidative conditions necessary for effective nitrification. Then, N removal was investigated under continuous wastewater feeding for a whole year and notable difference in efficiency was observed in the reduction of NH_4^+-N and TN levels in the effluent (Table 1).

Table 1 Removal efficiency of NH_4^+-N and TN in 3rd, 4th and 5th stages of the treatment system

Treatment system	NH_4^+-N removal efficiency (%)			TN removal efficiency (%)		
	3rd	4th	5th	3rd	4th	5th
EC-MTW	12.5±24.6	31.4±23.9	33.9±26.8	20.0±20.3	29.9±22.2	31.6±24.3
ECCB	57.3±16.4	62.5±17.3	63.2±18.1	42.8±15.6	49.0±18.3	46.8±17.1
ECBB	90.9±5.62	87.3±6.82	86.3±5.17	48.9±12.8	49.6±17.8	45.1±17.2

Upon integrating the modular METland® EC-MTW with the ECCB biofilter, the NH_4^+-N removal efficiency significantly increased, starting at 57.3% in the 3rd stage and peaking at 63.2% in the 5th stage. On top of the nitrification role, the downflow ECCB biofilter revealed a capacity for removing total nitrogen (ca. 45%). This unexpected result considering the inhibitory impact of oxygen on denitrification was indeed previously reported [20,31], suggesting that electroconductive material may stimulate the electron transfer to nitrate in anoxic internal layers of electroctive biofilm. In contrast, the combination of modular METland® system EC-MTW with ECBB biofilter made of electroconductive biochar exhibited an even more pronounced improvement in nitrification. The effluent NH_4^+-N values from the overall treatment system drop from 53 mg/L in modular METland® system to 5.5 mg/L in ECBB unit, which can fully meet the wastewater discharge standard of Dir. 00/60/EC of 23 Oct 2000 regarding ammonium (Fig. 5(a)). The system achieved a remarkable high NH_4^+-N removal efficiency higher than 90% in the 3rd and 4th stages just with passive aeration and no energy cost associated with blowers. However, the effluent TN concentrations of the overall treatment system could be significantly lowered if effluent from biochar biofilter ECBB would be also recirculated. Due to management issues inside wwtp we could not perform such test. However, real METland operating in an urban wastewater (Otos municipality, Murcia, Spain) currently operates at 300% recirculation from EC downflow biofilters to successfully remove TN levels from 100ppm TN influent (Esteve-Núñez, personal communication).

The results clearly demonstrated that both ECCB and ECBB units significantly enhance the N removal with ECBB showing remarkable effectiveness, particularly for NH_4^+-N removal.

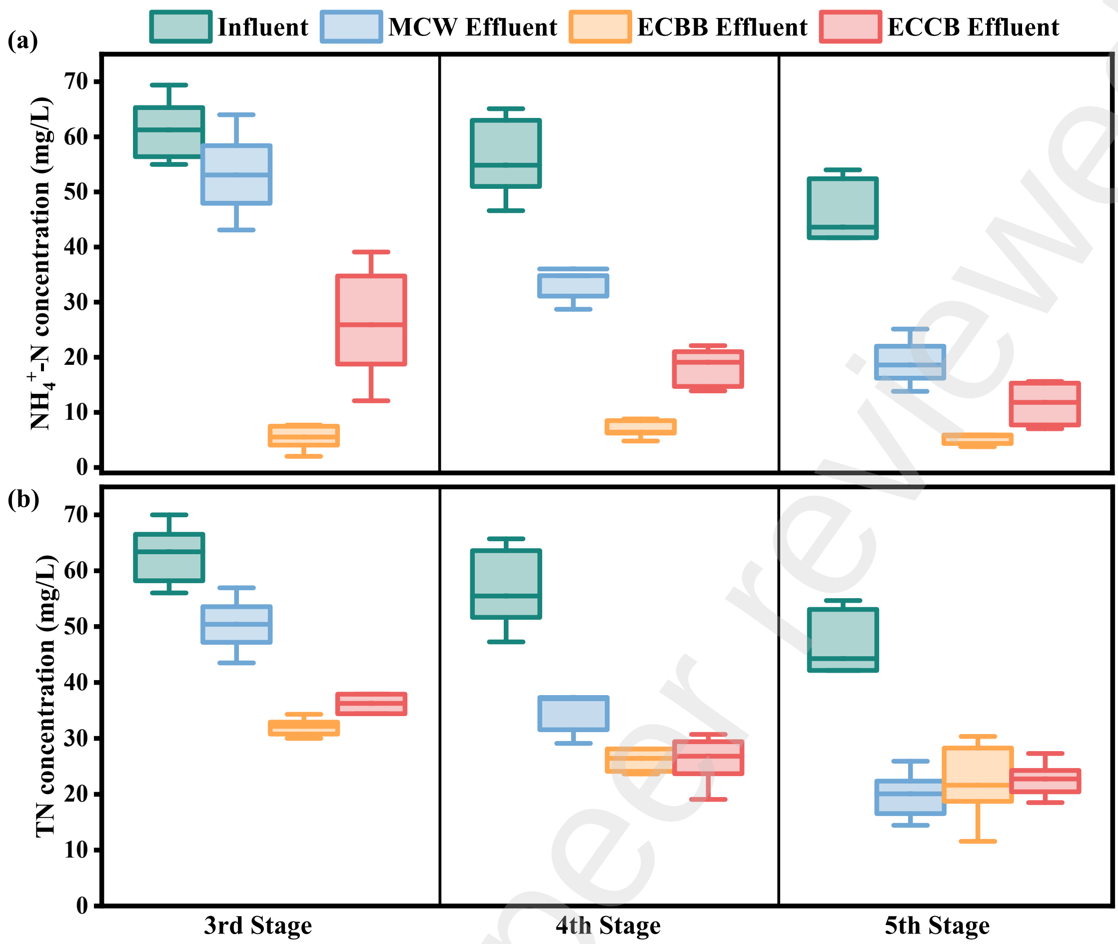
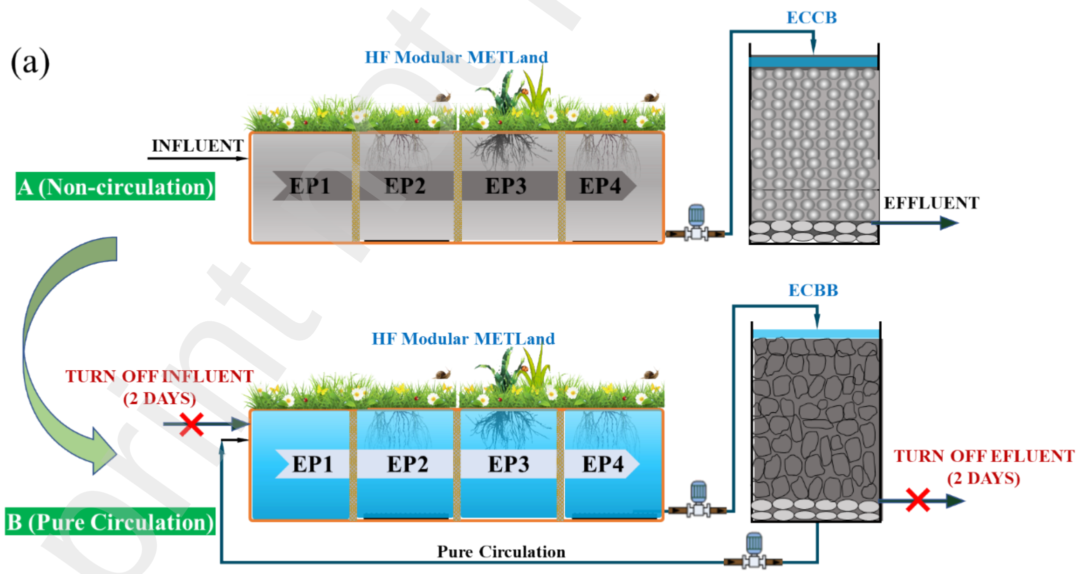


Fig. 5 NH_4^+-N (a) and TN (b) concentration for the three stages of overall treatment system. 3[rd]: EC-MTW + EC DF corresponded to EC-MTW1 followed by ECCB and ECBB biofilters operated down in parallel; 4[th] and 5[th]: EC-MTW1 + EC DF +RC1 and EC-MTW1 + EC DF +RC2 corresponded to 100% (4[th]) and (5[th]) 200% rate of recirculation from ECCB to EC-MTW1.

3.2 Bioelectrochemical Behavior

3.2.1 Electric potential profiles

The design of the modular METland® (EC-MTW) consists of a single electrode, unlike the traditional microbial electrochemical systems that utilize two independent electrodes and an external circuit. Thus, the electrical current cannot be tested directly in this single electroconductive bed [45]. In order to gain insights into the electron flow occurring within the vertical axis of conductive substrate, an electrochemical parameter so-called electric potential (EP) was measured at this demo unit. Indeed, the electric potential profile is a useless parameter in conventional non-conductive biofilters like treatment wetlands since no variations can be monitored regarding EP [23,41]. In contrast, electron transfer along electroconductive bed from biofilters like METland® generates a measurable electric potential in the water column [31]. Thus, EP was monitored at 4 independent sampling points along the horizontal flow bed. Furthermore, EPs were monitored under two typical conditions for exploring the correlation between electroactive metabolic activity and wastewater contaminants (Figure 6).



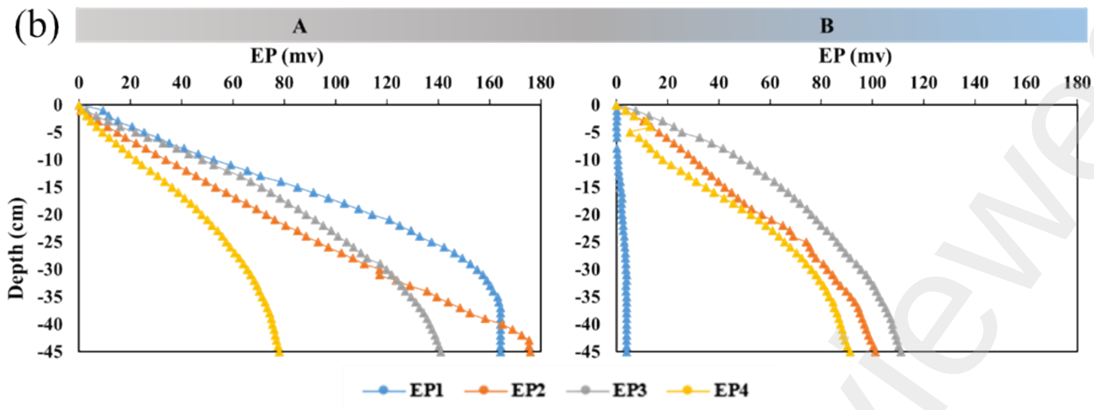


Fig. 6 Electron flow along EC-MTW: (a) Operational conditions where A is continuous mode; B is internal purely circulated mode; and and sample points (EP1, EP2, EP3, EP4 from left to right), (b) EP profiles of tested conditions (A, B) along depth (from top) at each sampling point

The horizontal subsurface flow EC-MTW system was operated under two conditions: (A) raw wastewater under normal operational mode, (B) treated raw wastewater under re-circulation operation. Variations in ww composition inside the bed can be monitored through the different EP profiles (Fig. 6(b)), revealing the impact of soluble electron acceptors (eg. nitrate or oxygen) on electron flux along the electroconductive bed., which can eventually correlate with electroactive bacteria capacity for removing pollutants.

Condition A correspond to raw wastewater, leading to higher COD concentration (Table 2 and Fig. 7). The EP profiles in this condition revealed how electrons from COD degradation were transferred to the EC material at 45cm depth zones and they were eventually consumed in the upper zones where more oxidative conditions were reached due to the presence of oxygen as electron acceptor. Such profiles were measured at different locations inside the modular METland®. Under standard operation (A), the curve of EP1 location (close to influent chamber with maximun COD) revealed the max variation in mV vs height, confirming the electron transfer along bed. Electron transfer along bed is typically directly correlated with COD removal rate values because electrons are generated by microbial oxidation of COD. Such variation was diminished as far as we move away from influent (EP2 to EP4) revealing a lower electron transfer due a lower COD values at such locations. (Fig. 6). Moreover, this can serve as a strong indication of the spatial distribution changes of COD degradation in a horizontal flow METland system.

In order to evaluate shifts in EP in response to operation variations, a new condition B, was tested. Inflow and outflow were closed so water was recirculating between horizontal modular METland® EC-MTW and the downflow biofilter ECBC. This operational change generated the main impact at the EP1 sampling point due to the recirculation of i) oxygenated water from down-flow ECCB, and ii) 15.6ppm nitrate-containing water, both from ECBC outlet. The presence of such soluble electron acceptors (especially oxygen) should negatively alter the vertical electron flow through the bed by accepting those electrons generated in the microbial metabolism of COD even in lower zones of bed. Such hypothesis was confirmed by monitoring EP values after recirculation (Figure 6) where EP1 drastically changed after recirculation showing a flat-like appearance very similar to the one reported in gravel [23,41]. As we monitor EP away from EP1, then oxygen from recirculation stream should be consumed and

EP2, EP3 and EP4 recover the classical fitting of an EC bed under anoxic conditions [31].

Table 2 COD and nitrate concentration in different operational conditions.

Overall treatment system	COD concentration (mg/L)		Nitrate concentration (mg/L)	
	A	B	A	B
S-EP1	565±21	36.4±3	0.62±1	15.4±1
S-EP2	476±15	34.5±8	0.79±2	13.2±2
S-EP3	252±9	28±5	0.66±1	10.8±3
S-EP4	191±14	28.5±2	0.42±1	9.09±2

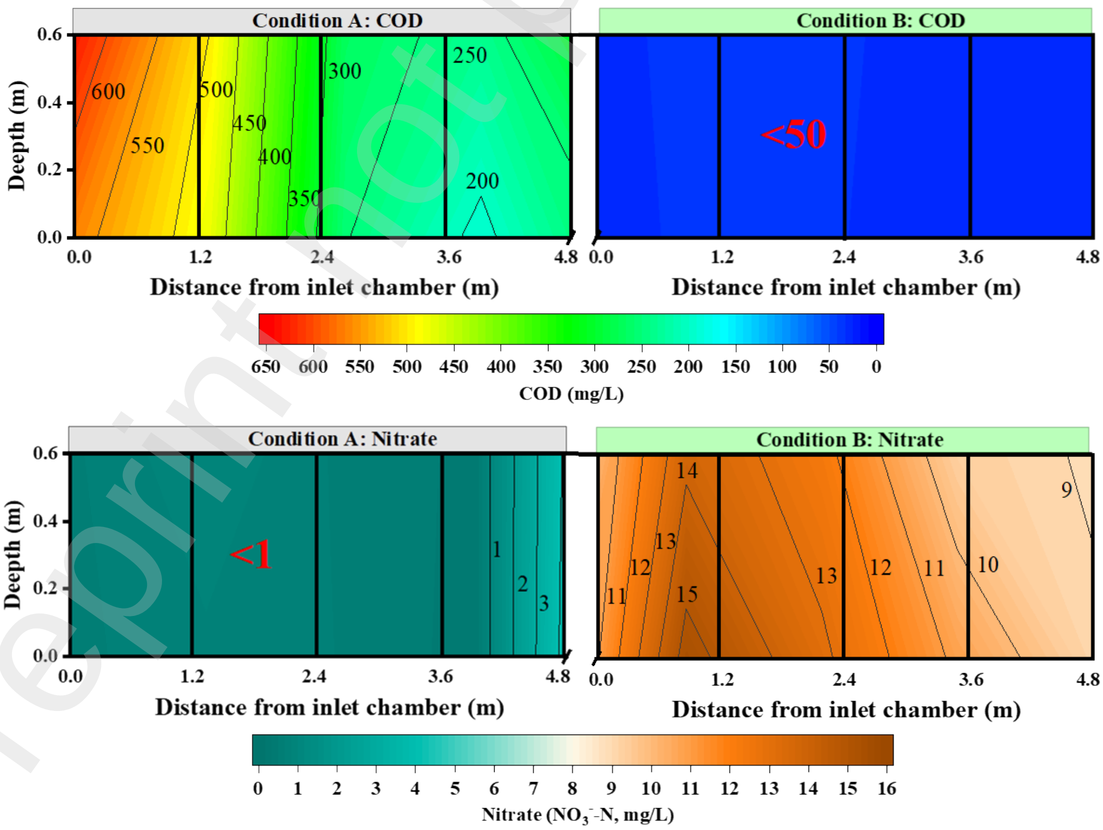


Fig. 7 Concentration distribution of COD and Nitrate along the horizontal flow modular METland® system (EC-MTW) under continuos feeding (A) and recirculation (B)

Overall, these EP profiles served as strong evidence for spatial variations in electron utilization for pollutants degradation within a horizontal flow modular METland® system. The distinct trends under each operational condition reflect the system's dynamic response to changes in wastewater characteristics and internal processing strategies.

3.2.2 Electron current density and response to organic pollutants

In this study, horizontal flow modular METland® system was a complete electric circuit, leveraging the natural process of organic matter oxidation by electroactive bacteria [33,45]. In the system, the bacteria oxidize organic pollutants, releasing electrons that travel through an electrically conductive bed till they find a suitable electron acceptor to be consumed. Simultaneously, this oxidation process generates ion-flows in the water, creating currents. The electron flow through the conductive bed and the ion-flow through the water are equal in magnitude but opposite in direction [40].

This section mainly explored the correlations between COD and electron current density (J). Thus, under continuous feeding (condition A)— there was pure recirculation without influent and effluent due to complete internal circulation overnight—the system's COD was nearly depleted. Therefore, Condition B is not considered suitable for establishing a correlation between COD and J in this part of the study. But this observation underlines that, despite the absence of COD under Condition B, there might still exist a notable correlation between nitrate (N) concentration and J, necessitating further research. Moreover, this suggests a competitive relationship between nitrate and electroconductive bed for accepting electrons from COD which needs additional investigation to fully understand the dynamics of pollutant removal and electrochemical activity within CW systems.

Thus, the two most commonly used operational conditions (non-recirculation or recirculation with both influent and effluent) were studied in this section (Figure 8). Electron current density (*J*) in the system was calculated by the gradient of EP profiles and wastewater conductivity in four sample points (Table 3). These two typical operational scenarios were involved to investigate the characteristic variations in electron current density (J) and to elucidate the linear correlation between electrochemical indices and changes in pollutant levels.

Table 3 Water conductivity (σ) of each chambers in two commonly used operational conditions.

Water conductivity (μS/cm)	S-EP1	S-EP2	S-EP3	S-EP4
Non-recirculation	1112	983	922	871
Recirculation	994	794	774	748

The J values distribution at four sampling points were calculated in the presence and in absence of recirculation (Fig. 8). In the horizontal flow modular METland® system, J values were generally decreasing from the sampling point near the water inlet chamber (S-EP1) to the sampling point near the water outlet chamber (S-EP4) under both recirculation and non-recirculation conditions. The J values for the recirculation condition decreased from inlet zone (J=43.99 mA/m^2) to outlet zone (J=7.08 mA/m^2), and from 49.38 to 15 mA/m^2 for the non-recirculation conditions. For a CW treatment system to daily treat a large volume of influent wastewater, recirculating the effluent is highlighted as a routine yet effective operational strategy to enhance the efficiency of pollutant removal. The systems with recirculation in place generally exhibit a substantial reduction in the overall concentrations of pollutants compared to those without recirculation [46]. In this study, the pollutant concentrations, such as COD, ranged from 20-270 mg/L under recirculation conditions. This is significantly lower than the COD concentrations under non-recirculating conditions, which ranged from 191-565 mg/L. This significant variability not only points to the efficacy of recirculation in pollutant reduction, the feedback effect can also be seen in the distribution curve of the current density J. Under recirculation conditions, with lower COD levels, the overall average distribution value of J is significantly lower than that under non-recirculating conditions (higher COD values). Moreover, this positive correlation trend is also evident in the horizontal flow wetland from the inlet to the outlet. This correlation is consistently observable along the flow path of the wetland, as the concentration of pollutants decreases from the inlet to the outlet, the J value distribution also decreases and shows a linear relationship.

Indeed, the J values in this study were generally lower than previously reported in non-modular METlands® (ranging from 45.09 to 223.84 mA/m^2) [31]. The main reason of such discrepancy could be due to the different feeding patterns between both studies: down-flow subsurface feeding distributed along the whole METland surface [31] versus standard horizontal subsurface flow where feeding occurs in a single point (this study). Such different feeding profiles would generate a totally different scenario regarding COD distribution and microbial activity so it is reasonable to observe a different electron flow profile along surface.

The linear regression results suggest a good fit for the J value change in relation to the position within the EC-MTW, with R^2 values of 0.83 for recirculation and 0.99 for non-recirculation conditions. This indicated a strong correlation between the electrochemical indicators and the wastewater treatment process, where the electric current decreased as wastewater progressed through the system. The analysis further correlated the average COD concentration at each sampling point with the average J values, exploring the relationship between organic pollutant load and electrochemical activity. This relationship was supported by R^2 values of 0.96 for recirculation and 0.92 for non-recirculation conditions, which indicates a strong correlation (Figure 8). The linear relationship between the COD removal and the J values throughout the system not only demonstrates the effectiveness of recirculation but also highlights the predictable nature of the treatment efficacy from the water inlet to the outlet in the EC-

MTW. Such clear linear relationships may provide significant evidence that can be used to indicate pollutant concentrations through electrochemical indicators in future system operations.

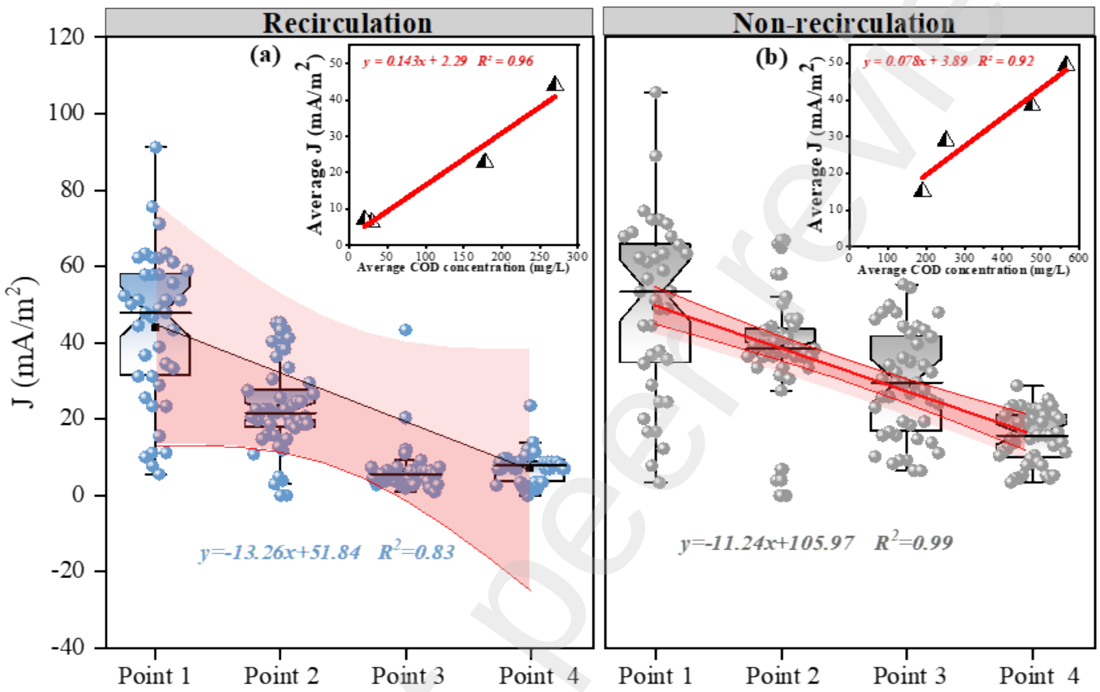


Fig. 8 Bioelectrochemical response through current density J (mA/m2) for horizontal flow EC-MTW system under (a) recurcualtion (b) non-recirculation

3.2.3 Perspective and recommendations based on electrochemical behavior

The investigation of EP and J across different sections of the modular METland® EC-MTW provides invaluable insights for design optimization. For instance, areas with higher electron utilization, as indicated by steeper EP gradients, suggest more active microbial degradation of pollutants. This understanding can guide the strategic placement and proportion of electroconductive substrates in various sections of the MCW. Optimizing the ratio of conductive substrate in the relatively active zone of system, based on the observed electric behavior, can enhance overall treatment efficiency. More significantly, the modular nature of the system allows for tailored configurations to suit specific treatment requirements, ensuring that each module is optimally designed for its role in the treatment process.

J is a critical indicator of microbial activity within the system. Observations of decreasing J values along the flow path indicate areas where organic matter oxidation is more pronounced. This information is pivotal in determining the HRT and flow rates of the modular METland® system, ensuring that wastewater remains in high-activity areas for optimal treatment time although flow rate seems not significantly impact the treatment efficiency in the current study. No doubt, adjusting the flow rate to maintain the balance between sufficient contact time for microbial action and efficient throughput can improve the system's performance. Furthermore, the EP measurements can inform the frequency and extent of recirculation within the system, which is crucial for enhancing N removal efficiency.

Understanding the electric behavior within the modular METland® system opens avenues for enhancing its sustainability and scalability. By identifying the most efficient substrate composition and arrangement through electric behavior analysis, the system's footprint can be minimized without compromising treatment efficiency. This aspect is particularly beneficial for scaling the system up for urban applications or down for rural settings. Additionally, insights gained from electric behavior studies can guide the development of more energy-efficient systems, potentially integrating with renewable energy sources, thereby contributing to the overall sustainability of wastewater treatment infrastructures.

4 Conclusions

The integration of electric conductive substrates within the horizontal flow MCW, connecting with both coke and biochar-based downflow biofilters in a hybrid system, has demonstrated a sustainable and efficient removal of pollutants. This system leveraged electrobioremediation field , utilizing the conductive properties of materials to enhance the metabolic processes of electro-active microbes, thereby facilitating increased degradation of organic pollutants and nitrogen compounds. Moreover, this study confirms that the J within the horizontal flow modular METland® system decreases along the flow path from the inlet to the outlet, suggesting a consistent electrochemical activity that aligns with the treatment process. Overall, the findings from this study underscore the potential of modular METland® system as a scalable and effective solution for wastewater treatment in various operational processes, providing a foundation for further exploration and optimization of these systems in real-world applications.

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