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Research

Evaluating biochar-filled normal and electrode-embedded constructed wetlands: The impact of loading rates and plant diversity on septic effluent treatment

Tanveer Saeed[1†], Md. Abdus Salam[1], Asheesh Kumar Yadav[2]

[1]*Department of Civil Engineering, University of Asia Pacific, Dhaka 1205, Bangladesh.*
[2]*Department of Environment and Sustainability, CSIR-Institute Minerals and Materials Technology, Bhubaneswar, India*

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ABSTRACT

This study evaluated the effectiveness of biochar-filled, electrode-integrated constructed wetlands using *Phragmites* and *Canna indica* plants for treating septic tank effluent. Systems operated across varied hydraulic loads (0.25–1.0 m/d) and showed 93–98% chemical oxygen demand, 69–91% nitrogen, and 88–99% phosphorus removals. Electrodes enhanced pollutant removal by initiating bioelectrochemical reactions, while biochar media supported nutrient adsorption. Rhizosphere-dependent oxygen leakage capacities of the *Phragmites* and *Canna indica* plant species induced redox potential variations inside the wetland media. Microbial-based degradation primarily contributed to organic and nitrogen removals. A maximum of 4400 mg/kg nitrogen and 1400 mg/kg phosphorus concentrations were quantified with the wetland biochar, exceeding the fresh media's composition. These data profiles imply the influence of adsorption on nutrient removals. Plant-based nutrient accumulation percentages were negligible, ranging between 0.01 and 3%. Organic and nutrient removal percentage increase or decrease magnitude was ≤11% because of input load increment. The power density production with the *Phragmites* and *Canna indica*-based electrode-integrated wetlands ranged between 2674 and 63288 milliwatts (mW)/m^3; the *Phragmites*-based system showed greater power density production. The findings of this study will allow the design of low-cost, natural systems to produce better effluent and energy recovery in decentralized clusters.

Keywords: Adsorption, Biochar, Bioelectrochemical oxidation, Natural technologies, Removal stability

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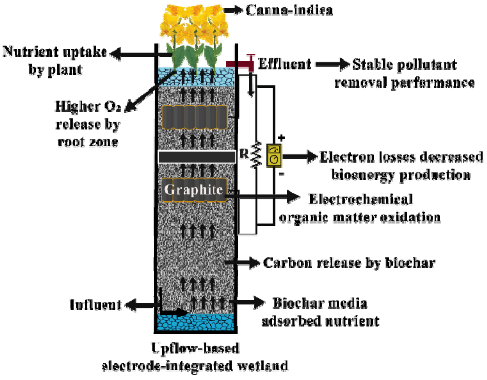
Graphical Abstract



[†] Corresponding author
E-mail: dr.tanveer@uap-bd.edu
Tel: +8802-58157091-4 Fax: +8802-5815709
ORCID: 0000-0003-4434-3710

1. Introduction

Oxide or nanostructure-based photocatalysts, waste stabilization or oxidation ponds, and constructed wetlands are being employed worldwide to a significant extent because of their dependency on atmospheric forces, environmentally friendly characteristics, exclusion of chemical agents, and minimal requirement of fossil energy [1-9]. The latter system, i.e., constructed wetlands, are nature-based treatment technologies that utilize its major components, i.e., filler media, plants, and microbial population, to remove pollutant from the incoming wastewater in an artificially controlled environment [10-13]. Because of low cost and simple operational protocols, these technologies are often integrated with septic tanks to treat wastewater in decentralized areas [14-16]. Literature review indicates global organic, nitrogen, and phosphorus removal percentages of 54-99%, 31-82%, and 27-90%, respectively, with the integrated septic tank and constructed wetlands [10, 15, 17-22]. These reported removal performances indicate wider removal percentage deviations, particularly nutrient (nitrogen and phosphorus) removals with such integrated treatment systems.

The previously reported overall performance deviations could be linked to the partial pollutant removal capacity of the septic tank systems, which is primarily restricted to organic matter [23-25]. The limitations of septic tanks often necessitate a greater reliance on subsequent-stage constructed wetlands to achieve effluent quality that meets acceptable standards. The common wetland components, such as media and plants, affect incoming wastewater treatment performance due to their influence on chemical and microbial-based pollutant removals [26-28]. Comparative experiments with different plant species were reported with constructed wetlands designed to polish the effluent produced from the septic tank [17, 29]; differences in pollutant removal performance were mostly observed in these studies due to root-based oxygen release rates and nutrient uptake capacity variations. Regarding the other wetland component, i.e., media gravel had been preferred for the wetland systems combined with septic tank [10, 18, 19]. The inefficiencies of gravel in supporting chemical and microbial-based pollutant removal (with constructed wetlands), specifically nutrient removal, are documented primarily because of their chemical composition [30].

The replacement of the traditional gravel with different media and its effect on pollutant removal improvement of the septic tank effluent treatment-based constructed wetland was demonstrated by Koottatep et al. [31]; the reported wetland was filled with multiple alternate media, i.e., iron-rich soil, sawdust, charcoal, and zeolite. The authors reported greater than 70% COD, NH_4-N, and TP removals (from septic tank effluent) with the developed constructed wetland; the iron-rich soil and zeolite adsorbed nutrient, whereas with the carbon structure of the employed organic materials supported microbial decomposition. The authors did not present TN removal performance data. Other organic materials, such as biochar, have also been used as the primary media with constructed wetlands (for removing different pollutant) due to their abundant carbon composition, cation exchange capacities, large surface area, low density, and cost-effectiveness [32-37]. Hence, additional studies with septic effluent treatment-based wetlands filled with organic media such as biochar are required to understand its influence on pollutant removal, particularly under variable input loading conditions. A positive association between input load intensification and pollutant removal decrease has been reported with non-carbon media (gravel)-based constructed wetland designed for septic tank effluent treatment [18]. Therefore, an experimental design and execution with biochar-filled constructed wetlands to treat septic tank effluent under variable loading conditions will extend the current knowledge limit on the performance efficiencies of such low-cost, natural systems at decentralized locations.

Electrode-integrated constructed wetlands, or intensified treatment technologies, include additional components, i.e., embedded electrodes [38, 39]. These electrodes catalyze electrochemical oxidation (of organic matter), concurrent pollutant removal, and bioenergy generation [40-43]. The literature analysis specifies the application of these intensified technologies to treat synthetic and real wastewater [44-47]; however, only one study [48] employed such a media-based (packed with commonly chosen gravel) novel bioenergy-producing system for septic tank effluent treatment. The authors reported COD and NH_3 removal percentages of 94 and 100%, respectively. TN removal percentages were comparatively low, i.e., ranging between 31 and 63%.

Although the removal performance of the electrode-assisted bioenergy-producing wetland reported by Ebrahimi et al. [48] signifies the probable application of media-based electrode-integrated wetlands for septic tank effluent treatment, some research gaps exist in this domain. *First,* the reported higher pollutant removal performance was achieved due to external aeration that might incur additional functional costs and hinder its widespread application, particularly in decentralized setups. Therefore, examining such systems with plant variations, along with implementing organic media and assessing their impact on pollutant removal performances, might provide a more potential solution depending on removal performances. *Second,* the reported system received artificially prepared septic tank effluent. The composition of real wastewater is more complicated than that of synthetic solutions, which could influence pollutant removal of the electrode-integrated wetlands [45, 49]. Moreover, the reported system was operated under constant loadings, whereas the performance dependency of the bioenergy-producing wetland on variable loading conditions had been reported [44, 50, 51]. *Third,* the reported system was filled with gravel as the main media and activated carbon as the anode layer. The capacities of organic material, i.e., biochar, for improving functional capacities of the electrode-assisted wetland (that received strong wastewater) had been demonstrated [52] that might be extended for decentralized wastewater treatment. *Fourth*, the reported study did not include a control system, i.e., a wetland system without electrode integration. Such a comparison is required to precisely identify the electrodes' contribution to pollutant removal (from septic tank effluent) and the associated inducing factors. Hence, experimental exploration on the performance of biochar-filled electrode-assisted wetlands dosed with real septic tank effluent, planted with different species and the combined effect of plants, media, and electrode to produce better effluent quality in decentralized areas will increase the implementation boundaries of the novel bioenergy-producing systems.

To summarize, the literature review indicates shortcomings in knowledge on: (a) the effect of wetland components, i.e., organic

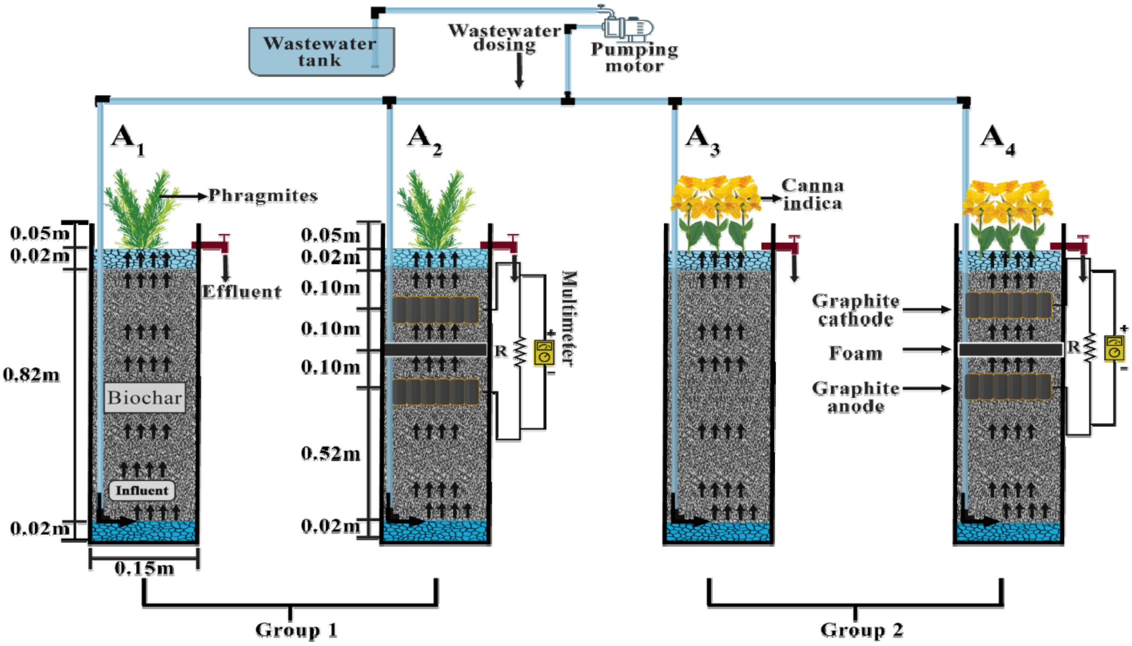


Fig. 1. Operational arrangement of the four wetlands. The symbols A_1 and A_2 denote *Phragmites*-based normal and electrode-coupled wetlands (Group 1). The symbols A_3 and A_4 denote *Canna indica*-based normal and electrode-coupled wetlands (Group 2).

biochar, plant species variations, and electrode-integration to treat real septic tank effluent; and (b) input load variants on pollutant removal of such systems in decentralized arrangements. This study was undertaken to address these information gaps by assessing the pollutant removal performances of the *Phragmites* or *Canna indica* plant-based and organic biochar-filled normal (without electrode) and novel electrode-coupled systems that received real septic tank effluent under variable hydraulic loading ranges. As such, this study aims to identify the pollutant removal mechanisms and their interaction with wetland components, the required factors to improve such removals from real septic tank effluent with normal and electrode-integrated wetlands, and the feasibility of a low-cost, recyclable material (i.e., biochar) for decentralized wastewater treatment.

2. Materials and Methods

2.1. Design of the Experiment: Wetlands Configurations

The experimental setup comprised four wetlands that were constructed with polyvinyl chloride (PVC) materials at the outdoor campus of the University of Asia Pacific, Dhaka, Bangladesh; the wetlands were symbolized as A_1, A_2, A_3, and A_4. The structural dimensions of a single wetland were a height of 0.91 m and a diameter of 0.14 m. Each of the four wetlands was packed with organic biochar produced from the pyrolysis of organic wood and bamboo [53], achieving an overall of 0.82 m depth. The physical properties of the chosen media were size: 9-13 mm and porosity: 34%; the likely chemical composition of the media is available in supplementary materials (Table S1). Previous studies reported the capacities of biochar to support pollutant removals through a combination of chemical and microbial-based pathways, primarily due to possessing unique elemental composition [54, 55]. Each wetland's inlet and outlet sections included stones of 0.2 m depth for wastewater distribution and disposal.

The A_2 and A_4 wetlands were integrated with graphite material-built anode and cathode electrodes. A single electrode occupied a volume of 0.0004 m^3. The anode was placed 0.52 m apart from the lowermost portion of the biochar. A separator foam was placed 0.1 m above the anode. The cathode was then laid at a distance of 0.10 m measured from the separator foam. The electrodes were connected by insulated copper wire circuits and completed with 820 Ω resistors (connected externally) and a multimeter. The other two wetlands, A_1 and A_3, functioned without electrode coupling, i.e., conventionally designed normal systems.

Phragmites and *Canna indica* plants were selected for the four wetlands of this study as these species promote the growth of the microbial population inside the wetland bed [56, 57]. Two groups were formed based on plant species type. Each group included two wetlands: a normal and an electrode-coupled wetland. *Phragmites* were planted into the normal (A_1) and electrode-coupled (A_2) wetlands of group 1. *Canna indica* were planted into the remaining normal (A_3) and electrode-coupled (A_4) wetlands of group 2. After the plantation, the pore spaces of the media in the four wetlands were filled with fresh water for 12 weeks for plant establishment. As the experimental wetlands were constructed outdoors, they were exposed to natural forces, i.e., wind, temperature, and sunlight. The atmospheric temperature within the experimental period ranged between 28°C and 35°C, favorable for plant growth. Fig. 1 illustrates the operational diagram of the four wetlands.

Table. 1. Mean composition of the septic tank effluent. SD resembles standard deviation.

Parameters	Units	Mean ± SD
pH	---	7.7 ± 0.5
Eh	mV	109.4 ± 31.4
NH_4-N	mg/L	46.5 ± 22.2
NO_2-N		0.4 ± 0.2
NO_3-N		39.4 ± 39
TN		156.2 ± 50.8
TP		328.1 ± 161.8
BOD_5		358.7 ± 102.3
COD		1947.5 ± 878.4
Coliform	CFU/100 mL	224833 ±162269

2.2. Wastewater Dosing Protocol

The influent wastewater of the experimental wetlands, i.e., septic tank effluent, was collected from the BCIC housing colony of the Mirpur area, Dhaka, Bangladesh; the collected septic tank effluent was stored in containers. The composition of the septic tank effluent is provided in Table 1.

The four wetlands received septic tank effluent for 34 weeks; the dosing period included 10 weeks of system adaptation followed by 24 weeks of experimental run. The experimental protocol included three phases: I, II, and III. The hydraulic load (across each system) differed within the three phases: 0.25 m/d in Phase I, 0.5 m/d in Phase II, and 1.0 m/d in Phase III. Each phase was continued for 8 weeks. During these three phases, the four wetlands received septic tank effluent 5 days a week (Sunday-Thursday) and were kept at resting mode (wastewater was not fed) during the remaining two days (Friday and Saturday). The septic tank effluent (of the storing facilities) was pumped into the bottom portion of the four wetlands. The transferred septic tank effluent was forced to pass from the anode compartment towards the overlying cathode compartment (with the electrode-integrated wetlands: A_2 and A_4) until it reached the effluent collection valve (of A_2 or A_4 wetland) positioned at 0.86 m distance (measured from the bottom portion of each system). A similar dosing procedure was followed for the normal wetlands A_1 and A_3. In these systems, wastewater was pumped from the lower to the upper portion of the employed media until it reached the effluent collection valve, positioned at a distance similar to the electrode-integrated wetlands. Hence, the four wetlands functioned as upflow-based systems.

2.3. Samples Analysis

Influent (i.e., septic tank effluent) and effluent (produced from the four wetlands) samples were collected weekly and analyzed in the Environmental Engineering Laboratory of the Department of Civil Engineering, University of Asia Pacific, to measure the concentration profiles of the following environmental and common pollutant parameters: pH, E_h, NH_4-N, NO_2-N, NO_3-N, TN, TP, BOD_5, COD, and coliform numbers.

The environmental parameters, i.e., pH and E_h of the wastewater samples, were measured with an HQ 40d multi-parameter and LDO101, PHC3OH, and MTC101 probes (provided by HACH company, USA). The common pollutant concentration of the samples, i.e., NH_4-N, NO_2-N, NO_3-N, TN, TP, and COD, were measured with an Ultraviolet-visible (UV-VIS) spectrophotometer (HACH DR 6000, USA), reactor blocks (HACH DRB 200, USA), and a Kjeldahl digestion-distillation unit (provided by VELP Scientifica, Italy), according to the procedures of the instrument manuals. The five-day biochemical oxygen demand (BOD_5) concentration of wastewater samples was measured with a manometric instrument (HACH BOD TRAK II, USA) and an incubator operated at 20°C. The coliform number was measured with Macconkey agar and an incubator operated at 37°C.

The biochar (referred to as used media) was extracted from two depths (measured from the top of the media): 0.11 and 0.31 m, from each of the four wetlands after the experiment terminated; the plants were harvested concurrently. The underground (UG) and aboveground (UG) portions of the harvested plants were separated. The nutrient composition of the media and plants was quantified following the digestion-distillation and vanadomolybdo phosphoric yellow color methods [47, 58]; the detailed analytical protocols of such methods are available in supplementary materials (Test S1).

To assess the nutrient (i.e., NH_4 and P) adsorption rate of the biochar (unused or fresh), standard nutrient solutions were prepared from the appropriate chemical compounds, i.e., ammonium chloride (NH_4Cl) and potassium dihydrogen phosphate salts (KH_2PO_4) with concentration ranges of 50-800 mg/L. Three grams of unused (fresh) biochar media was exposed to a 20 ml volume of the standard solution of a specific concentration of nutrient (for example, 50 mg/L) at different time intervals such as 0.5, 1, 2, 3, 4, 5, 6, 24, and 30 hrs to quantify the nutrient adsorption rate of such material. A detailed description of the media-based adsorption rate measurement protocol is available in supplementary materials (Test S2).

2.4. Bioenergy

The connected ammeter measured the voltage production of the A_2 and A_4 wetlands coupled with electrodes. Eq. (1) and Eq. (2) were used to calculate current and power density.

$$I = \frac{U}{VR} \tag{1}$$

$$P = \frac{U^2}{VR} \tag{2}$$

where. U=voltage (mV) across the A_2 or A_4 wetland; R= external resistance (Ω); V= anode volume (m^3) of the A_2 or A_4 wetland; I= current density (mA/m^3); and P=Power density across the A_2 or A_4 wetland (mW/m^3).

2.5. Statistical Test

The mean effluent pollutant concentration difference between normal and electrode-integrated wetlands of *Phragmites*-based group 1 (A_1 vs. A_2) or *Canna indica*-based group 2 (A_3 vs. A_4) was assessed through a statistical t-test. The effluent pollutant concentration difference between the normal (*Phragmites-based*: A_1 vs. *Canna indica-based*: A_3) and electrode-integrated wetlands (*Phragmites-based*: A_2 vs. *Canna indica-based*: A_4) of the two groups was also evaluated with a t-test. The statistically significant difference was accepted when $p < 0.05$.

3. Results and Discussion

3.1. Organic Matter Removal

3.1.1. Overall removal performance

The mean output or effluent organic (BOD_5 and COD) concentration produced by the four wetlands with respect to operational phases is depicted in Fig. 2; the associated mean removal organic removal percentages are also integrated with Fig. 2. The A_1, A_2, A_3, and A_4 wetlands produced mean effluent BOD_5 concentration ranges of 32-41 mg/L, 25-29 mg/L, 15-30 mg/L, and 14-28 mg/L, respectively. The overall mean effluent COD concentration ranges were 77-103 mg/L, 51-80 mg/L, 37-97 mg/L, and 30-83 mg/L, respectively. The four wetlands in this study achieved higher COD removal percentages than BOD_5 removals; a similar trend was reported previously with constructed wetlands [52, 59]. COD measures the composition of non-degradable particulates or dissolved organic matter (along with degradable particulate or dissolved organic matter of BOD_5). Such non-degradable organic components are often removed through physical (filtration, sedimentation) and chemical (adsorption) routes induced by plant roots and wetland media that the BOD_5 parameter could not capture.

3.1.2. Impact of electrode-coupling

The *Phragmites*-based electrode-integrated wetland A_2 achieved lower mean effluent organic concentration ranges than the normal wetland A_1 (statistically significant: $p< 0.05$) in group 1. The cohabitation of electrochemically active and inactive routes and their synergistic impact on organic removal are salient characteristics of the electrode-integrated wetlands [47, 60]. Such co-occurrence improves overall organic removals of the novel systems compared to the normal wetland (without electrode coupling); organic removal in the traditionally designed systems is primarily achieved through electrochemically inactive routes [47, 61]. Statistically insignificant ($p>0.05$) lower mean effluent organic concentration ranges were also observed with the *Canna indica*-based electrode-integrated wetland A_4 than the normal A_3 system in group 2. Hence, the effect of electrode integration in improving organic removal of the A_4 wetland could not be confirmed in this study, probably due to the influence of root-based oxygen leakage from the *Canna indica* plants, which will be discussed in the following paragraph.

3.1.3. Root-based oxygen leakage

The organic removal pattern demonstrated by the *Canna indica*-based normal wetland A_3 demands particular attention, as such a system also produced statistically significant ($p<0.05$) lower mean effluent organic concentration than the *Phragmites*-based normal wetland A_1. The normal wetlands support organic matter removal through electrochemically inactive anaerobic and aerobic pathways [62]. Oxygen leakage from the plant root zone is a major catalyst that supports aerobic organic removal pathways; such oxygen release rates could differ depending upon the root porosity of plant species [63, 64]. The overall mean effluent redox concentration profiles across the *Canna indica*-based wetlands (A_3 and A_4) ranged between 107 and 114 mV (within the three operational phases: supplementary materials (Fig. S1) that were higher compared to the ranges, i.e., 97-101 mV of the *Phragmites*-based wetlands (A_1 and A_2); similar findings, i.e., variable environmental conditions development (inside the wetland media) depending on plant species were also observed by Oodally et al. [65]. Therefore, higher effluent redox concentration of the *Canna indica*-based wetlands (compared to *Phragmites*-based systems) might be linked to more oxygen accessibility from the respective rootzone. Such supplementary oxygen might have intensified organic removal through electrochemically inactive aerobic pathways, particularly in the *Canna indica*-based normal A_3 system, resulting in more efficient organic removal performance than *Phragmites*-based normal wetland A_1 or statistically insignificant ($p>0.05$) organic removal differences between the *Canna indica*-based normal (A_3)

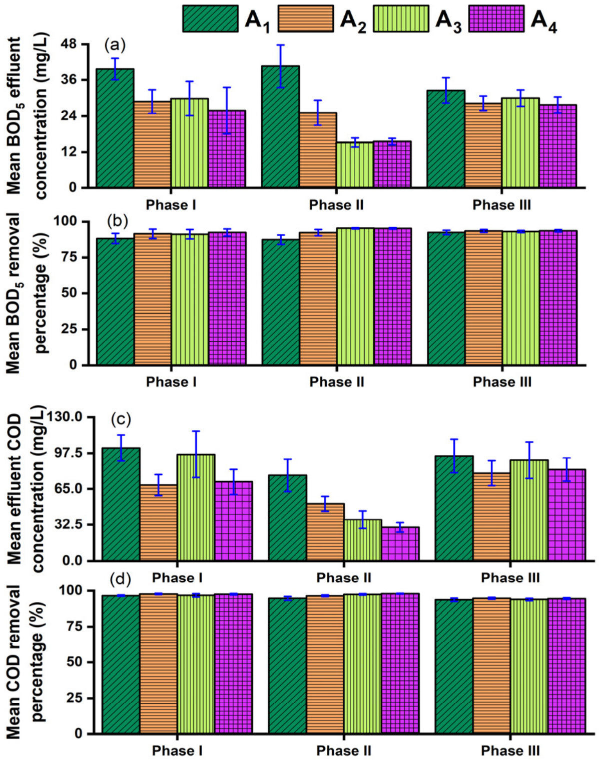


Fig. 2. Organic removal achieved by the four wetlands within three operational periods: mean effluent concentration (a and c); and (ii) mean removal percentages (b and d). Standard error values are presented with the bars. The symbols A_1 and A_2 denote *Phragmites*-based normal and electrode-coupled wetlands (Group 1). The symbols A_3 and A_4 denote *Canna indica*-based normal and electrode-coupled wetlands (Group 2).

and electrode-integrated (A_4) wetlands. The mean effluent organic concentration variations between electrode-integrated wetlands (of the two groups): A_2 (*Phragmites*-based) and A_4 (*Canna indica*-based), were statistically insignificant ($p > 0.05$). These trends suggest that the developed redox slope within the electrodes favored electrochemical oxidation [38] in these wetlands (A_2 and A_4), probably due to the positive effect of biochar in improving the oxygen concentration around the rhizosphere, thus increasing the stratified redox potential between the electrodes and associated electrochemical oxidation of organic matter[66].

3.2. Nutrient Removal: Impact of Plants and Media

The mean output or effluent nutrient (i.e., NH_4-N, TN, and TP) concentration produced by the four wetlands with respect to operational phases is depicted in Fig. 3; the associated mean removal percentages are also integrated with Fig. 3. The mean effluent NH_4-N concentration limits of the A_1, A_2, A_3, and A_4 wetlands were 9-22 mg/L, 11-18 mg/L, 7-14 mg/L, and 6-9 mg/L, respectively; 34-58 mg/L, 27-40 mg/L, 19-28 mg/L, and 13-21 mg/L, respectively, for TN; 7-44 mg/L, 6-47 mg/L, 4-32 mg/L, and 6-27 mg/L, respectively, for TP.

Nutrient removals in traditionally designed normal and bioenergy-producing wetlands are influenced by wetland components: plants and media [26, 38, 67, 68]. The interaction between wetland components and wastewater nutrient with the four wetlands (Fig. 3) will be discussed in the following paragraphs.

The critical wetland component, i.e., plants, uptake nutrient through the rhizosphere, thus contributing to nutrient removal [69-72]. Hence, nutrient accumulation of the segregated *Phragmites* and *Canna indica* plant biomass portions (section 2.3) was measured and presented in Table 2. The nutrient accumulation of the wetland plants in this study (Table 2) accords with the nitrogen and phosphorus concentration ranges of 6000 mg/kg-25000 mg/kg and 100 mg/kg-4500 mg/kg, respectively, which were reported previously with *Canna indica* or *Phragmites* plants of fecal sludge and landfill leachate treatment-based wetlands [52, 73]. Overall nutrient accumulation percentage ranges in segregated plant biomass portions (i.e., AG and UG) were 0.2-2.7% (nitrogen) and 0.01-0.2% (phosphorus). Similar lower nutrient accretion percentage ranges were reported previously in *Phragmites* and *Canna indica* plants of normal or electrode-integrated wetlands because of greater loading scales [49, 74]. Although the plant-based nutrient accumulation was low, overall removal percentages of the four wetlands were high (Fig. 3). Hence, the probable contribution of other nutrient removal pathways, i.e., chemical-oriented adsorption and microbial decomposition, is discussed in the following paragraphs.

The media's physicochemical structure could supplement the wetland systems' overall nutrient removal through retention [26, 32]. Table 3 summarizes the nutrient concentration data of the biochar media (unused and used) to assess its likely contribution to nutrient removals. According to the data sets, the used media's nutrient concentration profiles exceeded the corresponding concertation of the unused media. Such higher nutrient accumulation profiles in the used media (than the unused biochar) indicate that incoming nutrient were retained by the biochar media of the four wetland systems. The energy-dispersive spectroscopy (EDS) analysis, which quantifies the probable element structure of materials, was also conducted (supplementary materials: Table S1) to provide insight into the existence of media-induced adsorp-

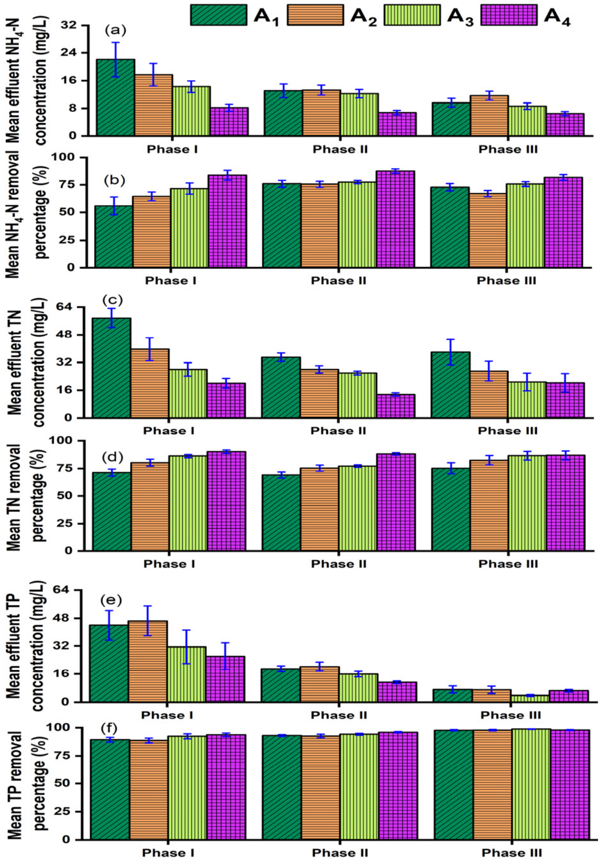


Fig. 3. Nutrient (NH_4-N, TN, and TP) removal achieved by the four wetlands within three operational periods: mean effluent concentration (a, c, and e); and mean removal percentages (b, d, and f). Standard error values are presented with the bars. The symbols A_1 and A_2 denote *Phragmites*-based normal and electrode-coupled wetlands (Group 1). The symbols A_3 and A_4 denote *Canna indica*-based normal and electrode-coupled wetlands (Group 2).

Table 2. Nitrogen (N) and phosphorus (P) concentrations in the segregated portions (aboveground: AG and underground: UG) of the *Phragmites* and *Canna indica* plants.

Nutrient	Concentration unit	Biomass portions	Group 1 *Phragmites*		Group 2 *Canna indica*	
			A_1	A_2	A_3	A_4
N	mg/kg	AG	15300	17000	10800	9800
		UG	13200	11100	12400	10900
P	mg/kg	AG	4400	2900	2400	1400
		UG	4700	5500	6000	1900

Table 3. Nitrogen (N) and phosphorus (P) concentration of the biochar media (unused and used). The used biochar media was extracted from two depths: 0.11 m and 0.31 m apart from the surface area.

Nutrient	Concentration unit	Unused media	Depth (m)	Used media			
				A_1	A_2	A_3	A_4
N	mg/kg	3100	0.11	3600	4400	4300	4200
			0.31	2700	3400	3600	2600
P	mg/kg	1080	0.11	500	1400	400	1300
			0.31	1100	1100	900	700

tion pathways and their influence on nutrient removals. According to the EDS analysis, media-based nutrient adsorption-favoring elements were probably present in the employed unused or used biochar media and contributed to nutrient composition increment in the used biochar of the four wetland systems (Table 3) through cation and anion exchange sites [26, 75-77].

The unused (fresh) biochar media's nutrient adsorption capacity was also analyzed (protocol available in section 2.3) to confirm the probable contribution of the specified route on overall nutrient removal (Fig. 3); the results are presented in Fig. 4. The unused biochar media exhibited greater nutrient adsorption capacity within 0.5-6 hrs contact periods that decreased when the contact periods were extended between 24 and 30 hrs for all examined standard nutrient solutions strength. The decrease in the adsorption rate of unused or fresh biochar media (at higher contact periods) could be attributed to reaching a saturation state. As such, the results of the adsorption rate analysis confirm the contribution of nutrient adsorption (triggered by the biochar media) on overall observed nutirent removals (Fig. 3) with the four wetlands of this study. A similar trend, i.e., the adsorption rate dependency of biochar (prepared from wetland plants or waste and modified by hydrogen peroxide or potassium phosphate) on initial pollutant concentration or contact periods, was also demonstrated in previous studies [78, 79].

3.3. Synthesis of Nutrient Removal Pathways

3.3.1. Impact of plant species variation (*Phragmites* vs. *Canna indica*)

The reduction in effluent TN concentration (as demonstrated by the four wetlands: Fig. 3) compared to the influent profiles (Table 1) indicates nitrogen disappearance inside the experimental systems. Effluent TN concentration ranges differed within the four wetland systems of the two groups. These variations suggest that media-based nitrogen adsorption (Table 3) was not the major contributing pathway, as the four wetlands were filled with biochar.

The constructed wetlands depend heavily on microbial nitrification, followed by denitrification to remove influent nitrogen [80, 81]. The mean NH_4-N, NO_2-N, and NO_3-N removal percentages within the four wetland systems were 56-88%, 43-94%, and 78-95%, respectively. Considering these profiles, the nitrogen removal pathways in this study were identified as (1) nitrification-denitrification, (2) plant uptake, and (3) biochar adsorption. Assuming that adsorption (by the biochar media) occurred at similar levels across all treatments, differences in nitrogen removal efficiency are likely attributable to variations in the performance of pathways (1) and (2). The *Canna indica*-based wetlands achieved lower mean effluent NH_4-N concentration ranges than the *Phragmites*-based systems. The results indicate that *Canna indica*-based wetlands removed

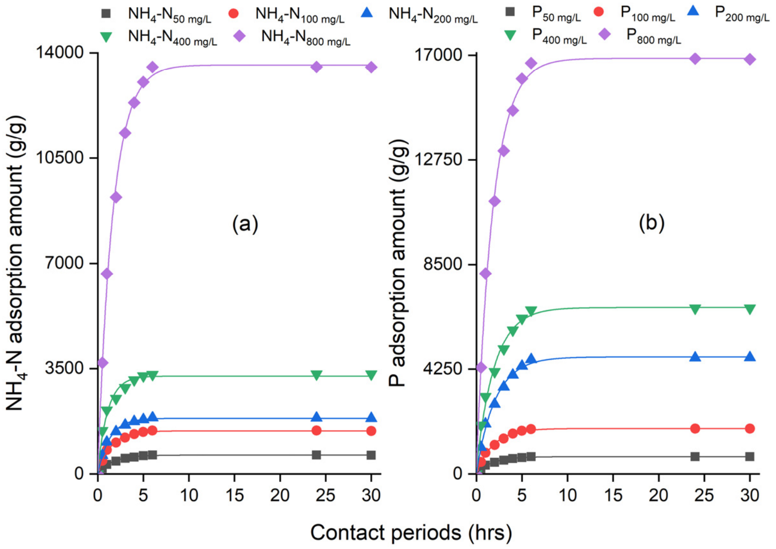


Fig. 4. NH_4-N (a) and P (b) adsorption amount of the biochar media (unused) with respect to variable contact periods.

more NH_4-N than *Phragmites*-based systems, suggesting that *Canna indica* was more effective in promoting nitrification (e.g., A_1 vs. A_3 or A_2 vs. A_4), likely due to its higher oxygen release capacity. This finding aligns with the previously discussed higher efficiency of organic matter removal.

The *Canna indica*-based wetlands produced statistically significant ($p< 0.05$) lower mean effluent TN concentration ranges than the *Phragmites*-based wetlands. The existence of more favorable environmental conditions to support nitrification and NO_3-N production as the end-products was another probable major factor that resulted in TN removal differences between the wetland systems of the two groups; TN removal via denitrification (will be further discussed in the following paragraph) is also interlinked with NO_3-N availability.

3.3.2. Effect of biochar on denitrification: carbon source

The four wetlands achieved a mean TN removal percentage of 69-91%; the mean removal percentages of the NO_X components ranged between 43 and 95%. These trends suggest the probable reduction of NO_3-N to N_2 gas, i.e., denitrification, which is more challenging to achieve due to its reliance on organic carbon accessibility [82, 83]. The two major carbon sources with wetland systems are the organic carbon composition of the wastewater and the filler materials, i.e., media [84, 85]. The biodegradable organic matter was not readily available in the septic tank effluent to support denitrification-based NO_3-N and TN removal, as signified by the wastewater's lower biodegradation ratio (Table 1): 0.2. Different types of organic media, including biochar reinforced denitrification-based nitrogen removal (with constructed wetlands) because of their porous structure for hosting substantial denitrifying microbial population and abundant carbon composition to fuel the connected removal route [32, 62, 82, 86, 87]. The EDS analysis of the employed unused and used biochar media (in this study) signifies the probable presence of organic carbon in substantial quantity (supplementary materials: Table S1); such enriched carbon structure of the biochar could have catalyzed observed nitrogen removal via denitrification in deeper compartments of the formed biolayer on media surface (supplementary materials: Fig. S2) with this study's four wetlands despite low biodegradability of the influent [40].

3.3.3. Electron availability from anode

Comparisons between wetlands with and without electrodes (A_1 vs. A_2 and A_3 vs. A_4) showed that electrode-integrated systems achieved statistically significant ($p <0.05$) higher TN removal efficiency. These trends suggest that electrode integration potentially enhanced denitrification. The contribution of electrons (produced during electrochemical oxidation) from the anode to reducing NO_3-N via denitrification with electrode-integrated wetlands had been reported previously [88]; such alternate electron availability (from the anode) could have fostered denitrification and TN removals of the electrode-integrated wetlands (A_2 and A_4).

3.3.3. Media-based phosphorus adsorption

The mean effluent phosphorus concentration deviations between the normal and electrode-integrated wetlands of *Phragmites* (A_1 vs. A_2) or *Canna indica* (A_3 vs. A_4)-based group were not broader. The *Canna indica*-based wetlands produced comparatively lower but statistically insignificant ($p>0.05$) effluent phosphorus concentration ranges than the *Phragmites*-based wetlands. The commonly reported media-based adsorption primarily contributed to phosphorus removal in the four wetlands of this study [26, 89]. The probable elemental structure of the biochar media that induces chemical-based adsorption (supplementary materials: Table S1) and experimental evidence, i.e., higher phosphorus concentration of the wetland biochar (compared to the fresh samples: Section 3.2 and Table 3), along with phosphorus adsorption rate of fresh biochar (Section 3.2 and Fig. 4) supports the existence of such chemical-dependent adsorption route.

3.4. Coliform Mortality

The mean output or effluent coliform concentration production of the four wetlands with respect to operational phases is depicted in Fig. 5; the associated mean removal percentages are also integrated with Fig. 5. The A_1, A_2, A_3, and A_4 wetlands produced mean effluent coliform concentration ranges of 21812-83250 CFU/100 mL, 8438-68125 CFU/100 mL, 9500-44375 CFU/100 mL, and 5375-35000 CFU/100 mL, respectively. The *Phragmites* (A_2) or *Canna indica* (A_4)-based electrode-integrated wetland achieved lower mean effluent coliform concentration ranges than the normal wetland (A_1 or A_3) within the same group. Electrochemical bioreactions were reported to improve coliform mortality with electrode-integrated wetlands [59]. The *Canna indica*-based wetlands achieved lower mean effluent coliform concentration ranges than the *Phragmites*-based systems. Plants supplement coliform removal due to physical filtration and toxin organic production induced by UG biomass, i.e., rootzone [90-92]. The coliform removal rates could be dependent on plant species and the developed root structure to intensify mortality, as observed by the mean effluent coliform concentration range deviations within the *Phragmites* and *Canna indica*-based wetlands in this study.

3.5. Input Load Variations

The four wetlands received influent at three loading rates: 0.25 m/d in Phase I, 0.5 m/d in Phase II, and 1.0 m/d in Phase III for assessing the effect of load variations on pollutant removal performances, which was an objective of this study. The four wetlands achieved mean COD removal percentages of 93-98% throughout the examined operational periods (Fig. 2). A gradual COD removal percentage decrease of ≤3% was observed because of hydraulic loading increment (from Phases I to III) with the *Phragmites*-based normal (A_1) and electrode-integrated (A_2) wetlands. The *Canna indica*-based normal (A_3) and electrode-integrated (A_4) wetlands achieved maximum COD removal percentage in Phase II when hydraulic loading was intermediate between the three applied ranges. When hydraulic loading was the greatest, these wetlands achieved minimum COD removal percentage in Phase III. However, such observed COD removal variations were not substantial, i.e., ≤4%. Srivastava et al. [44] reported ≤7% COD removal decreases in upflow-based normal and electrode-integrated hybrid wetland trains because of input load increase. This study's upflow-based single-stage wetlands achieved narrower COD removal variations (due to input load increment) despite being operated with real wastewater of a greater mean COD concen-

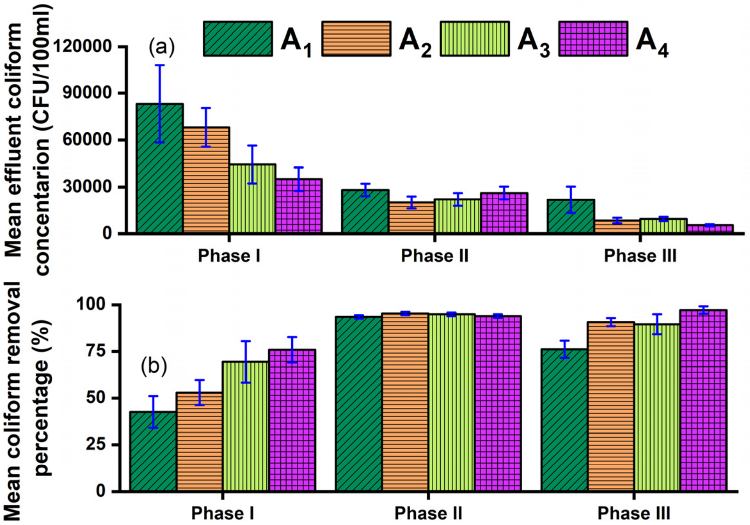


Fig. 5. Coliform removal achieved by the four wetlands within three operational periods: mean effluent concentration (a); and mean removal percentages (b). Standard error values are presented with the bars. The symbols A_1 and A_2 denote *Phragmites*-based normal and electrode-coupled wetlands (Group 1). The symbols A_3 and A_4 denote *Canna indica*-based normal and electrode-coupled wetlands (Group 2).

tration (i.e., 1948 mg/L) than the mean COD concentration (i.e., 267 mg/L) of the reported artificial wastewater [44]. It should be noted that the first stage upflow-based normal and electrode-integrated vertical flow wetlands of the previously reported hybrid trains were operated as unplanted units. The root of plants attenuates the adverse effect of hydraulic load increment [93]. Such a positive effect of the rhizosphere could have induced comparatively stable COD removal performances with this study's planted single-stage normal and electrode-integrated wetlands despite input load variations.

The *Phragmites*-based wetlands demonstrated mean TN removal percentage ranges of 69-83%; the *Canna indica*-based systems achieved TN removal percentage ranges of 77-91% (Fig. 3). TN removal percentage decrease ranges of 2-11% were observed with these wetlands when the hydraulic load increased from 0.25 m/d (Phase I) to 0.5 m/d (Phase II). However, hydraulic load increments from 0.5 m/d to 1 m/d (Phase III) resulted in 6-9% TN removal percentage increments over the preceding operational period. Overall TN removal percentage variations with the four wetlands (in this study) due to input loading changes were narrower compared to the reported performances (i.e., 4-26% nitrogen removal variations) of the wetland systems (without and with electrode coupling) operated under loading variations [44, 47]. As such, more stable nitrogen removal performances of the normal and electrode-integrated wetlands of this study could be linked to two factors: (a) the existence of a favorable environmental condition inside the bed matrix to support nitrification; and (b) the adsorption rate and carbon availability of the biochar media to trigger chemical adsorption and microbial denitrification.

The mean TP removal percentage ranges were 88-98% and 92-99% with the *Phragmites* and *Canna indica*-based wetlands, respectively, throughout the three experimental periods (Fig. 3). TP removal percentage increment ranging between 2 and 5% was observed with the four wetlands due to input load increment between the three phases, reflecting the better performance of the systems at higher loading. The unused biochar media's phosphorus adsorption rate increased during its exposure to synthetic phosphorus solutions of greater concentrations (Fig. 4). Such chemical properties of the biochar media might have improved phosphorus removals of the wetland systems under greater loadings.

The four wetlands achieved a mean 42-76% coliform removal percentage range in Phase I; removal percentages increased by 77 and 96% during the last two phases (Fig. 5). Better coliform removal performances during the latter operational periods could be attributed to more matured root establishment that triggered physical coliform separation, i.e., filtration.

3.6. Bioenergy

3.6.1. Voltage and power density production profiles

The mean bioenergy (voltage and power density) generation profiles across the *Phragmites* (A_2) and *Canna indica* (A_4)-based electrode-integrated wetlands with respect to operational phases are presented in supplementary materials (Fig. S3). The A_2 and A_4 wetlands produced mean voltage production ranges of 504-704 mV and 144-227 mV, respectively. Mean power density production ranges across the A_2 and A_4 wetlands were 32558-63288 mW/m^3 and 2673-6546 mW/m^3, respectively. These profiles indicate improved bioenergy production of the A_2 wetland compared to the A_4 system. Comparisons of organic matter removal profiles between these two wetlands reveal opposing phenomena; effluent organic concentration deviations between such systems were statistically insignificant ($p>0.05$). Such contradictory organic removal and bioenergy production with the two electrode-coupled wetlands suggest probable interfering with the completion of electrochemical reactions, which could have adversely impacted the A_4 wetland's bioenergy production.

3.6.2. Electron loss

Electron losses and internal resistance development are two factors that could hamper the electron (generated due to electrochemical oxidation) transfer from anode to cathode electrodes of the electrode-integrated wetlands and their bioenergy production performances [61]. The first factor, i.e., electron losses, is initiated by the consumption of electrons (generated via electrochemical oxidation) by the electrochemically inactive microbes, thus inhibiting the anode electrode's electrons capturing capacity [45]. It could be possible that the existence of **a** more aerobic environment inside the A_4 system's media (supported by higher effluent redox concentration of the A_4 system than the A_2: supplementary materials Fig. S1) could have triggered such electron losses through electrochemically inactive aerobic pathways, resulting in lower bioenergy production in the A_4 system compared to A_2 wetland. The positive correlation between bioenergy production decrease and input load increment (from Phases I-III: supplementary materials Fig. S3) of the A_4 wetland also suggests the probable influence of electrochemically inactive pathways on the completion of electrochemically active organic matter oxidation. Minor organic removal percentage deviations within the four wetlands due to input load increments reflect balanced removal performance despite unstable loading conditions (section 3.1). Therefore, organic matter removal through electrochemically active and inactive routes was probably intensified under greater loading conditions [38, 62] to maintain a stable performance with this study's normal and electrode-integrated wetlands. If such a hypothesis is true, more electrons were produced (due to intense electrochemical-based organic matter oxidation) under greater loading rates with the A_4 system. Such higher electron production could have increased electron losses through electrochemically active aerobic pathways due to increased metabolism under greater loadings, resulting in a positive correlation between bioenergy production decrease and input load increment (from Phases I-III) of the A_4 wetland. Another alternative hypothesis to explain the lower bioenergy production of the A_4 system compared to that of A_2 could be attributed to higher electron consumption by the denitrifying bacteria (in the A_4 system) before being captured by the anode [88]. Pollutant removal profile, i.e., higher mean TN removal of the A_4 wetland than A_2 (Fig. 3), also supports probable consumption of electrons (produced through electrochemical oxidation) by denitrifiers.

3.6.3. Internal resistance

The second performance-reducing factor with the electrode-integrated wetlands, i.e., internal resistance development, could be assessed by a polarization test [94, 95] that involves three steps: (a) connecting external resistors (of variable resistance) with electrode-integrated wetlands; (b) recording associated voltage production and calculating current and power density using the formulas specified in section 2.4; and (c) formulating polarization curves with the recorded data sets. The polarization test with the electrode-integrated wetlands A_2 and A_4 in this study was conducted by connecting variable resistors (100 Ω -33000 Ω); the data sets produced during the polarization test are presented in supplementary materials (Fig. S4). The polarization curves indicate the peak power density production of 2758 and 3677 mW/m^3 with the A_2 and A_4 wetlands, respectively, when connected with a resistor (of 1000 Ω), reflecting similar internal current resistance development with both systems. As such, the polarization curves further reflect that electron losses (induced by electrochemically inactive organic removal pathway) could be a significant factor in the difference in bioenergy production between the two systems.

3.7. Comparative Performance Evaluation and Research Implication

This study's organic biochar-based normal and electrode-coupled wetlands achieved overall organic (93-98%) and nutrient (69-91% nitrogen and 88-99% phosphorus) removal percentages despite being operated under unstable loading conditions. Such removal performances coincided with or exceeded the removal percentage ranges of the septic tank-constructed wetland systems employed in previous studies (supplementary materials: Table S2). Therefore, biochar could be a potential media for improving operational performances of the wetlands and achieving high effluent quality in decentralized areas; electrode integration and appropriate plant selection might further improve such effluent quality due to their observed impact on organic and nutrient removal improvement in this study. Bioenergy production of the electrode-integrated wetlands could be considered a supplementary advantage of such systems in decentralized areas.

4. Conclusions

The main findings of this study are:

- The *Phragmites*-based wetland systems attained 87-94% organic, 69-83% nitrogen, and 42-96% coliform removals; the *Canna indica*-based wetlands achieved better pollutant removal, i.e., 91-96% organic, 77-91% nitrogen, and 69-95% coliform removals.
- The normal *Phragmites* or *Canna indica*-based wetland achieved 87-95% organic, 69-87% nitrogen, and 42-95% coliform removals; the parallelly operated electrode-integrated system within the same group achieved 91-96% organic, 75-91% nitrogen, and 53-96% coliform removal. Electrode-dependent bioreactions improved organic removals, denitrification, and coliform mortality.
- The four wetlands achieved a mean phosphorus removal percentage of 88-99%, primarily through chemical route-dependent adsorption.
- The direct impact of plants on nutrient removal (through rhizosphere-dependent uptake) was negligible because of low accumulation profiles in biomass. However, plants indirectly influenced pollutant removal by controlling the redox potentials inside the media pores.
- Organic and nutrient removal increases or decreases ranged between 2% and 11% because of input load variations.
- The *Phragmites*-based electrode-integrated wetland produced power density in higher magnitudes (32558-63288 mW/m^3) compared to the profiles (2673-6546 mW/m^3) of the *Canna indica*-based electrode-integrated system. Bioenergy pro-

duction depended upon electron losses induced by electrochemically inactive routes.

- This study explored the interactions between organic biochar, plants, embedded electrodes, and wastewater pollutant, employing septic tank effluent treatment-based normal and electrode-embedded constructed wetlands under variable loading conditions. The experimental evidence provided in this study might extend the existing knowledge boundary with nature-based decentralized wastewater treatment systems.
- Future studies should design constructed wetlands with different organic media and electrode materials to treat septic tank effluent that will provide comprehensive data sets on the potential implementation of such low-cost, natural systems in decentralized areas. Operating under broader loading variation ranges will also further assist in designing more stable natural decentralized wastewater treatment systems.

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Author contributions

T.S. (Professor) developed the conceptualization, methodology, and wrote the manuscript. M.A.S. (M. Engg. student) completed the experiment and contributed to the writing. A.K.Y. (Professor) reviewed the manuscript and provided valuable insights.

Conflict-of-Interest Statement

The authors declare that they have no conflict of interest.

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