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Constructed Wetlands as Nature-Based Solutions for the Removal of Antibiotics: Performance, Microbial Response, and Emergence of Antimicrobial Resistance (AMR)

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Abstract: Antibiotics and antibiotic resistance genes (ARGs) have been regarded as emerging pollutants and pose significant threats to the aquatic environment and to human health. This study aimed to investigate the removal of nutrients, antibiotics, and the emergency of ARGs in domestic sewage by means of constructed wetlands (CWs) filled with an electroconductive media, i.e., coke. In this study, the antibiotics removal efficiencies ranged from 13% to 100%, which were significantly higher in the system filled with coke compared with the CWs filled with common quartz sand (7~100%). Moreover, the presence of wetland plants could also significantly improve the removal of nutrients and tetracyclines. The results also demonstrated the importance of substrate selection and wetland plants in CWs on the alternation of microbial communities and structures, where the electroconductive media showed a promising effect on increasing the removal of antibiotics in CWs. In terms of the emergency of ARGs, the CWs filled with coke retained the most ARGs (10,690 copies/g) compare with the control groups (8576–7934 copies/g) in the substrate. As the accumulated ARGs could be released back to the watercourse due to the environmental/operation condition changes, the application of such an advanced substrate in CWs may pose a more significant potential threat to the environment. With these results, this study provided new insight into selection of the substrates and plants for wastewater treatment to achieve a sustainable and secure water future.

Keywords: antibiotic resistance genes (ARGs); electroconductive media; emerging contaminant; micropollutant; treatment wetland

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1. Introduction

Emerging contaminants, such as antibiotics, have been widely found in natural and artificial ecosystems [1,2]. Antibiotics have been used for human treatment, animal disease control, and as agricultural feed additives [3]. The excretion and residual antibiotics could pose a significant threat to the environment after entering the natural water body. Aside from their toxic effect on the aquatic biota [4], previous studies also reported that some antibiotics can reduce the diversity of microorganisms in biological treatment systems and affect the removal of nutrients [5]. Moreover, the abuse of antibiotics can result in the emergence of antibiotic resistance of bacterial strains [6], which has become a worldwide environmental challenge.

Traditional sewage treatment processes showed less efficiency in the removal of antibiotics; on the contrary, they showed effects on facilitating the growth of antibiotic resistant

bacteria and genes [7,8]. As a nature-based solution, constructed wetlands (CWs) have been used for various types of wastewater treatment [9–11], and are able to play the role of the last barrier for antibiotic treatment before entering into natural systems [12,13]. Previous studies have reported that CWs could effectively remove various antibiotics, such as tetracyclines [14], sulfonamides [15], quinolones [16], ibuprofen [17], and macrolides [18], with the removal efficiencies in the range of 75.8~98.6%, through the mechanisms of adsorption, plant uptake, and biodegradation. In order to enhance and secure a sustainable effective treatment performance, further intensification approaches to CWs need to be explored.

The substrate in CWs is regarded as one of the most important factors affecting antibiotics removal through either direct adsorption [19] or biodegradation processes [20]. The use of microporous zeolite as the CW medium could obtain a 95% removal of tetracyclines compared with that (83.2%) of the system filled with normal quartz sand [19]. Moreover, the use of manganese ore can also alter microbial community structure and activities in CWs, leading to significantly higher removal of amphoteric ciprofloxacin (69.4%) compared with the CWs filled with biochar (56.6%) [21]. Recently, electrically conductive materials, a never-ending source of terminal electron acceptors, were developed to support the microbial catabolism of pollutants [22]. The materials, such as coke and electrically conductive biochar, have been used in CWs to enhance the removal of nutrients and micropollutants [23]. Nevertheless, the contribution of such specific media to the removal of tetracyclines and relevant microbial responses has not yet been studied.

Wetland plants also play an important role in removing pollutants, including antibiotics, in CWs. The macrophytes can release oxygen and root exudates in the rhizosphere area and accelerate the growth of rhizosphere microorganisms [24]. The presence of *Juncus effuses* and *Canna indica* have increased the 21–54% removal of macrolides in CWs compared with the system without plants [25]. Moreover, plants also update and metabolize some antibiotics, such as quinolones, tetracyclines, and sulfonamides, inside the plant tissue [26]. After integrating microbial electrochemical technologies (MET) within CWs, wetland plants can facilitate electron transfer and increase the harvest bioelectrical voltages by 20% compared with the unplanted CWs [27]. Therefore, we hypothesized that CW configuration with both electroconductive media and plants could benefit the biogeochemical reactions processes in CWs and further increase the remediation of antibiotics.

It should be noted that along with the remediation of antibiotics in CWs, the emergency and transport of antibiotic resistant genes (ARGs) may occur in systems [15,28]. Previous studies have reported that the majority of ARGs were stored in substrate-associated microorganisms, and the horizontal and vertical gene transfer processes could accelerate the spread of ARGs [29]. Under certain conditions, such as changing temperature, the ARGs could be released back into the watercourse and pose risks to the environment and public health. Fang et al. (2017) investigated a field-scale CW (0.13 ha) for domestic sewage treatment with over 10 years of operation [30]. The removal efficiencies of those 14 ARGs were 77.8% and 59.5% during winter and summer, respectively. However, concentrations of five ARGs (sul1, tetA, tetC, tetE, and qnrS) in the winter and of six ARGs (sul1, sul3, tetA, tetC, tetE, and qnrS) in the summer were increased throughout the treatment process, indicating that CWs can act as a reservoir and release ARGs from sediments under heightened water agitation levels. Therefore, the monitoring of ARGs is crucial to evaluate the proposed CWs technology for the removal of antibiotics.

This study aims to evaluate the effects of electroconductive media and wetland plants on the removal of antibiotics and the potential risk of ARGs generation in CWs. Four tetracycline compounds, i.e., oxytetracycline, tetracycline, doxycycline, and chlortetracycline, were selected as the model antibiotics. Coke, a common industrial waste, was used as the electroconductive medium in CWs. At the end of the study, the microbial community structures in different systems and the emergency of ARGs in CWs were investigated. This study re-evaluated the trade-off between selecting special wetland media for the intensification of pollutant removal and their potential effects on accumulating/spreading more ARGs. With these results, this study could provide evidence and recommendations for

using CWs as a nature-based solution for the removal of micropollutants and to minimize the risk of ARGs.

2. Materials and Methods

2.1. Experimental Setup and Operation

Three mesocosm-scale CWs were made of PVC columns with a diameter of 0.3 m and a height of 1 m. Two of the columns were filled with coke, and the other system was filled with clean quartz sand (particle size: 2~5 mm). The inlet and outlet areas were paved with gravel (thickness: 5 cm) (Figure 1). Apart from one coke-filled system, the other two test systems were planted with *Pontederia cordata* L. (density: 40 plants/m^2), which is one of the most common wetland plants in the local area. The CWs were named Quartz Sand + Plant Constructed Wetland (QS-CW), Coke + Plant Constructed Wetland (CP-CW), and Coke + No Plant Constructed Wetland (CN-CW). All systems were operated as up-flow CWs, as tetracyclines have been proven to have a better removal performance in anoxic/anaerobic conditions [31]. Peristaltic pumps were used to pump water from the schoolyard's domestic sewage in order to acclimatize the plants for the first three months as the stabilization period. The systems were placed outdoors (Figure 1b) with a shield to avoid the effect of precipitation.

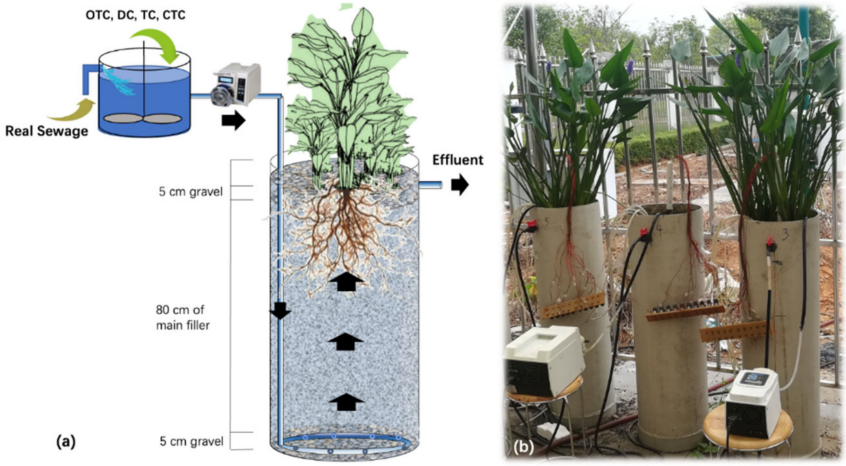


Figure 1. Schematic diagram of each system (**a**) and the photo of the experimental setup (**b**).

During the experimental stage, original schoolyard domestic sewage was used as the influent for all systems for three months. At the next stage, oxytetracycline, tetracycline, doxycycline, and chlortetracycline, with average concentrations of 254.53, 228.53, 160.49, and 94.70 μg/L, respectively, were spiked into the sewage as the influents for three months. The average pH and concentrations of COD, NH_3-N, and TP in the domestic sewage were 7.9, 160 ± 5 mg/L, 48 ± 17 mg/L, and 4.7 ± 1.4 mg/L for the whole experiment. Hydraulic retention time (HRT) was controlled by peristatic pumps and set to four days in all systems. The ambient temperature for the whole period was in the range of 26–34 °C.

2.2. Sampling and Data Analysis

2.2.1. Analysis of Water Samples

Water samples from the inlet and outlet of all systems were collected every four days. Dissolved oxygen (DO), pH, and redox potential (Eh) were measured on the spot with a portable multi-function probe (Hach, HQ40D, Loveland, CO, USA) at the time of sample collection. According to Chinese standard methods, NH3-N (HJ-535-2009), COD (HJ/T-399-2007), and TP (GB11893-89) were determined with an ultraviolet spectrophotometer (METASH, UV-6100A, Shanghai, China). Four tetracycline compounds were analyzed by HPLC (Agilent, 1200, Santa Clara, CA, USA), equipped with a C18 column (4.6 mm × 250 mm i.d., 5 μm particle size). The mobile phase was a mixture of methanol, acetonitrile, and 0.01 M oxalic acid (pH = 4) in a volume ratio of 10:20:70. Each sample was

injected twice with 10 µL at a flow rate of 0.8 mL/min and an operating temperature of 30 °C [32]. All samples and tests were conducted in triplicate.

2.2.2. Quantification of ARGs

At the end of the experiment, DNA was extracted from 10 g substrate samples taken from each system. After separation and purification by centrifugation, Qubit 4.0 (ThermoFisher, Q33226, Waltham, MA, USA) was used for quantitative detection of DNA sample concentration. The concentrations of the target ARGs were quantified using quantitative PCR (qPCR) [33]. Briefly, the sample was placed into the PCR instrument (Thermal Cycler, T100™, Foster City, CA, USA) for amplification. Temperature procedures for ARGs quantification included denaturation at 95 °C for 4 min, keeping the temperature at 95 °C for 20 s for 12 cycles, 58 °C for 20 s for 12 cycles, 72 °C for 30 min for 12 cycles, and 72 °C for 5 min. Copy numbers of bacterial 16S rRNA genes and the relative abundances of genes were calculated based on the mixing sample of copy numbers per gram.

2.2.3. Microbial Community Analysis

At the end of the study, 10 g of substrate samples were collected in order to determine the microbial community structure using the high-throughput sequencing method. The V3-V4 region of the bacterial 16S rRNA gene was amplified with forwarding primer 515F (GTGCCAGCMGCCGCGGTAA) and the reverse primer 909R (CCCCGYCAATTCMTTTRAGT) [9]. The filtered high-quality sequence units clustered the operational units (OTUs) at a 97% sequence similarity threshold.

2.3. Statistical Analysis

The difference in pollutant removal efficiency between different systems was obtained through Kruskal–Wallis one-way analysis of variance by SPSS 26.0 (IBM, Armonk, NY, USA) software at a 95% confidence level. The difference significance of the target item was represented by $p < 0.05$. All of the figures were prepared using Origin 2018 (OriginLab, Northampton, MA, USA).

3. Results

3.1. The Removal of COD and Nutrients

Before the addition of the mixture of antibiotics, CWs filled with coke (CP-CW) showed significantly higher removal of COD (77%) and TP (66%) compared with those (67% and 31% for COD and TP, respectively) in CWs filled with normal sand (QS-CW, Figure 2a,c). However, the removal of NH_3-N (19.6–21.7%) in these two systems was not significantly different (Figure 2b). Interestingly, the presence of the wetland plant in electroconductive media-filled CWs (CP-CW) did not significantly improve the removal of all pollutants compared with the system without a plant (CN-CW). After adding antibiotics to the influent, the removal of COD and TP decreased from 67–77% to 48–70% and from 30–71% to 11–52%, respectively, in all systems (Figure 2a–c). Nevertheless, in the presence of antibiotics, the removal efficiency of NH_3-N was significantly increased by 31.80% in QS-CW, 44.12% in CP-CW, and 18.62% in unplanted CN-CW.

3.2. The Removal of Antibiotics

The average concentrations of the four types of tetracycline compounds in the influent were 254.53, 228.53, 160.49, and 94.70 µg/L for oxytetracycline, tetracycline, doxycycline, and chlortetracycline, respectively (Figure 3). CWs with coke and a plant (CP-CW) showed the highest removal rates of oxytetracycline (91%), tetracycline (90%), and doxycycline (85%) compared with those (39–80%) in CWs with normal sand (QS-CW). In the absence of plants, CWs filled with coke (CN-CW) showed lower removal capabilities of tetracycline (82%) and doxycycline (61%). However, the removal efficiencies were still higher than those in the QS-CW system (Figure 3b,c). For oxytetracycline (Figure 3a), the removal efficiency (78%) was lower in CN-CW compared with that (91%) in CP-CW, but did not

show better performance compared with QS-CW (80%). Moreover, all three systems showed similar removal rates of chlortetracycline (60–65%) regardless of the types of media and the presence of plants (Figure 3d).

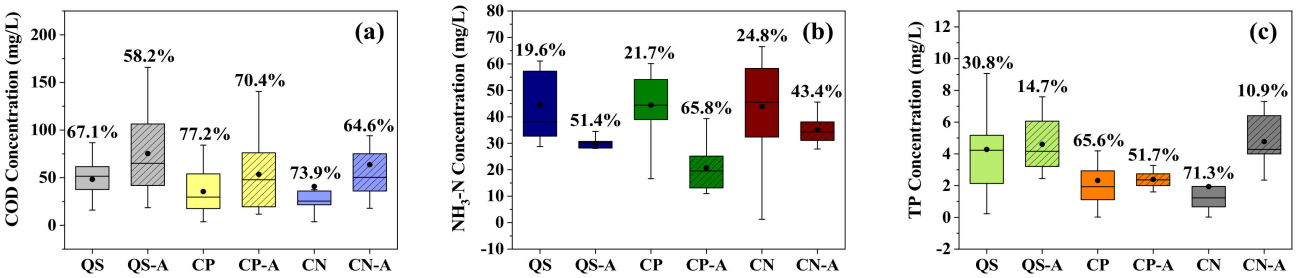


Figure 2. The effluent concentrations of COD (**a**), NH_3-N (**b**), and TP (**c**) from each system before and after the addition of antibiotics in the influent. The numbers above the bars represent the removal efficiencies of different pollutants. In X-axis, QS, CP, and CN represent QS-CW before adding antibiotics, CP-CW before adding antibiotics, and CN-CW before adding antibiotics, respectively. QS-A, CP-A, and CN-A represent QS-CW after adding antibiotics, CP-CW after adding antibiotics, and CN-CW after adding antibiotics, respectively.

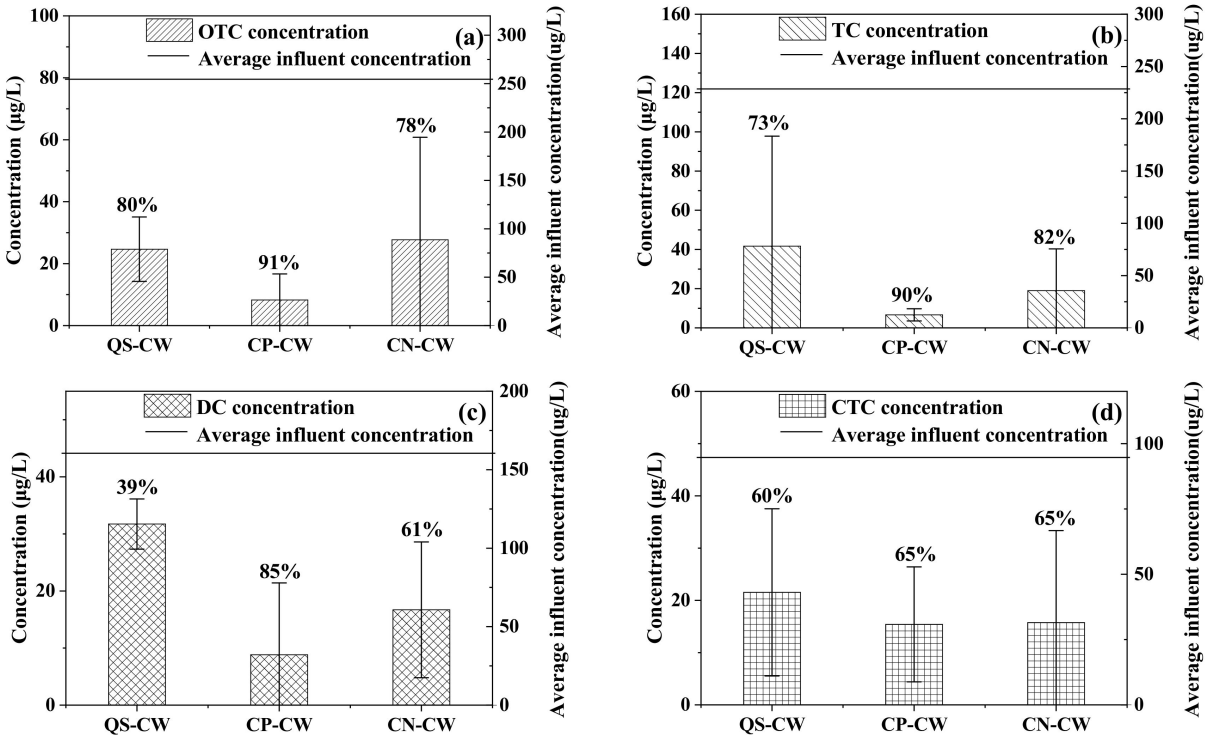


Figure 3. The average influent and effluent concentrations of oxytetracycline (**a**), tetracycline (**b**), doxycycline (**c**), and chlortetracycline (**d**) from each system. The numbers above the bars represent the removal efficiencies in different systems. OTC, TC, DC, and CTC represent oxytetracycline, tetracycline, doxycycline, and chlortetracycline, respectively.

3.3. The Emergence of ARGs in the Substrate

Previous studies have reported that the ARGs may be generated and stored in the wetland substrate during the bioremediation of antibiotics. Song et al. (2018) quantified that the relative abundance of *sulII* and *tetA* in the substrate increased from 0 to 0.0586 and 0.05, respectively, after 120 days of operation [34]. In this study, 308 types of ARGs were identified in the substrate at the end of the experiment (Table S1). The top 20 ARGs with the highest abundances (Figure 4a) were identified for further analysis. The coke-filled

CW with the plant (CP-CW) retained the most ARGs (10,690 copies/g) compared with the coke-filled CW without the plant (QS-CW, 7934 copies/g) and the control (CN-CW, 8576 copies/g). Moreover, *MacB* and *BcrA* were in the dominant position in all systems, which accounted for approximately 50% of the top 20 gene abundances. Regarding the six tetracyclines ARGs, i.e., *otrA*, *tetW*, *tetT*, *tetPB*, *tetM*, and *tetC* (Figure 4b), the levels of abundance in QS-CW, CP-CW, and CN-CW were 838, 899 and 690 copies/g. Apparently, the types of substrate and plants could alter the emergence of ARGs in CWs. In general, all systems accumulated an obvious amount of ARGs and may pose further risks of releasing them back to the watercourse.

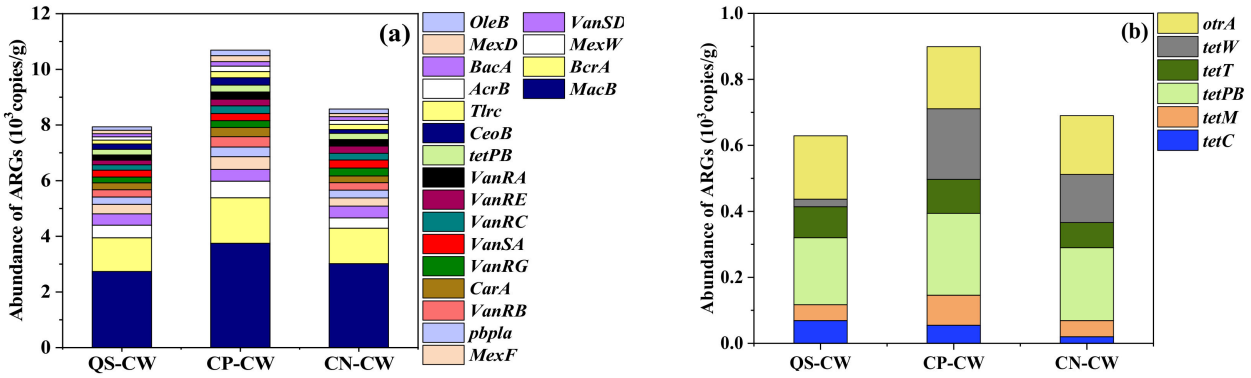


Figure 4. The abundance of top 20 ARGs (copies/g) (**a**) and tetracycline ARGs (copies/g) (**b**) from the substrate in QS-CW, CP-CW, and CN-CW.

3.4. The Responses of the Microbial Community

After the experiment, the microbial communities in all systems were measured in order to indicate the microbial response due to the effects of the wetland substrate and the presence/absence of plants. In total, 2229, 2228, and 2237 genera were observed in QS-CW, CP-CW, and CN-CW, respectively (Table 1). Moreover, the Shannon–Wiener index and Simpson's diversity index were used to estimate the richness and diversity of the bacteria community. CN-CW had a higher diversity index compared with the other two wetlands. Among the top 24 bacterial genera (Figure 5), the relative abundance of microorganisms in QS-CW and CP-CW was higher (~35%) than that in CN-CW (~20%). The presence of plants could benefit the growth of microorganisms, which might induce the lower microorganisms (20%) in CN-CW. Compared with the other two CWs, the microorganisms in CP-CW were more evenly distributed in quantity and had more dominant bacteria, which brought stronger adaptability to changes in the external environment. *Brachymonas*, *Methanosaeta* (anaerobic), and *Arenimonas* (denitrifying bacteria, anaerobic) were the predominant genera in QS-CW; *Flavobacterium* (aerobic), *Phenylobacterium* (aerobic), and *Arenimonas* (aerobic) were the predominant genera in CP-CW; *Geobacter* (aerobic) and *Thiobacillus* (aerobic) were the predominant genera in CN-CW. More *Arenimonas* grew in the wetlands filled with coke, and plants were also able to promote the growth of this denitrifying bacteria.

Table 1. Diversity index (Shannon–Wiener index and Simpson's diversity index) of QS-CW, CP-CW, and CN-CW at the genus level.

	Observed Genus	Diversity Index	
		Shannon–Wiener Index	Simpson's Diversity Index
QS-CW	2229	4.98	0.9843
CP-CW	2228	4.90	0.9903
CN-CW	2237	5.16	0.9932

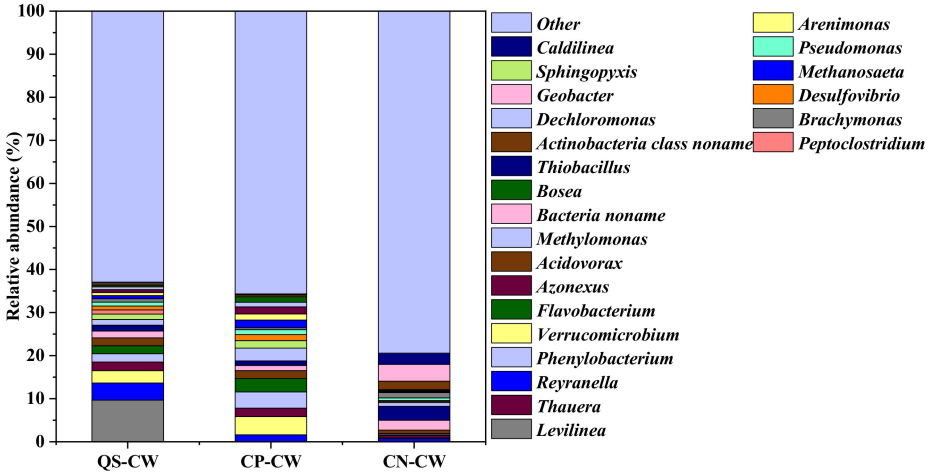


Figure 5. Relative abundance (%) of microorganisms in QS-CW, CP-CW, and CN-CW at the genus level.

4. Discussion

4.1. The Effects of Substrate on the Removal of Nutrients and Antibiotics

Substrate adsorption and microbial degradation significantly contributed to pollutant removal in CWs for wastewater treatment [20]. The dense micropores of coke provided a larger specific surface area and a wider site for the adsorption of pollutants and microorganisms. Such higher abundant microorganisms and greater adsorption have led to better removal efficiency of COD, NH_3-N, and TP. This superior performance was also supported by the enhanced electron transfer and the induced higher biogeochemical reactions in CWs filled with electroconductive media [35]. The contribution of coke in terms of the enhancement of pollutant removal was not so obvious when compared with the other studies, where tailored electroconductive media was used [22]. This may be due to the better electroconductivity of the synthesized material compared with the coke.

The adsorption of antibiotics is related to the physical structure characteristics of the substrate. Materials with a larger specific surface area tend to have higher treatment efficiency for antibiotics (Table 2). The adsorption effect is achieved through surface complexation, ion exchange, and electrostatic interaction [3]; in this study, the presence of oxygen-containing functional groups on the surface of coke could also play a positive role in the removal of antibiotics. An abundance of oxygen-containing functional groups promoted the adsorption of tetracyclines [36]. Moreover, greater electron conduction density of electroconductive media accelerated electron transfer among bacteria [22]. The enhanced microbial metabolic activity (Figure 5) may benefit the biodegradation of tetracyclines (Figure 3). In summary, the difference in the removal of tetracyclines between CP-CW and QS-CW ($p < 0.05$) confirmed the importance of substrate in the removal of antibiotics.

4.2. The Effects of the Plant on the Removal of Nutrients and Antibiotics

The change in the microbial community caused by plant rhizosphere exudates was one of the key factors affecting the treatment of pollutants [16]. The removal efficiency of TP by planted CWs was more stable under the interference of antibiotics ($p > 0.05$). The absence of plants might be one of the reasons for the significant change in TP removal efficiency in CN-CW after the addition of antibiotics. This result indicated that the existence of plants could improve the removal efficiency of nutrients and the tolerance of antibiotics in CWs.

The previous study reported that plant accumulation accounts for approximately 2% of antibiotic removal. The influence of plants on the removal of antibiotics lies more in the response of microorganisms to plants [26]. Man et al. (2020) found that the rhizosphere of plants could enrich microorganisms that degrade antibiotics [37]. *Flavobacterium* and *Arenimonas*, antibiotic-resistant bacteria, often exist as the hosts of resistance genes [38,39], and their enrichments in QS-CW and CP-CW were much higher than those in CN-CW (Figure 5). In this study, the existence of plants retained the higher relative abundance

of microorganisms in CP-CW compared with CN-CW (Figure 5). The removal of all four types of tetracyclines was significantly higher in CP-CW compared with CN-CW (Figure 3). This agreed with the results summarized from numerous previous studies (Table 2). These enriched bacteria in planted CWs have great potential in the treatment of antibiotics.

4.3. Microbial Responses of CWs to Antibiotics

Antibiotics entering the ecosystem could be regarded as the driving force for the evolution of microbial communities [4]. As is shown in Table 2, the difference in pollutant removal under the stress of antibiotics with different physical and chemical properties further proved that there were various selection pressures of antibiotics to bacterial communities [21]. For microorganisms, the inhibitory or promoting effects of different types of antibiotics were discrepant. Compared with quinolones, sulfonamides had a greater negative impact on wastewater treatment [35]. Under the condition of low C/N (0.2) in this experiment, tetracycline might exist as a carbon source for denitrifying bacteria and result in higher efficiency of NH_3-N removal. Moreover, the disturbance of tetracyclines on microbial community structure might enrich nitrifying bacteria and denitrifying bacteria, resulting in an improvement of NH_3-N treatment efficiency, which is similar to the findings of previous research [40]. Nutrition removal may change significantly with the addition of antibiotics due to the reduction in microbial activity. Antibiotics have an inhibitory effect on phosphate-accumulating organizations (PAOs), and it has been reported that the specific release rate and specific absorption rate of phosphorus decreased after adding antibiotics [41]. Tetracyclines caused the death of a large number of PAOs in the substrate, and the effusion of dead bacteria outflowed with the water leads to an increase of total phosphorus content in the effluent.

4.4. The Potential Risks of ARGs Accumulation

Lower oxygen content of the substrate and lower bacterial proliferation rate suppressed the propagation rate of tetracycline resistance genes in up-flow CWs. The treatment effect of up-flow vertical flow constructed wetlands on antibiotics and ARGs was better than that of down-flow CWs [30]. Compared with down-flow vertical flow constructed wetlands, horizontal constructed wetlands, and surface flow constructed wetlands, the three CWs in this experiment had significantly lower abundance of ARGs [32,42–44].

Adsorption may be the main mechanism for ARG elimination [41]. The experimental results show that more tetracycline resistance genes were retained in CP-CW and CN-CW. The abundant active sites of coke provide a strong adsorption capacity, which offered a better attachment site for ARGs in sewage. The superior antibiotic adsorption performance in CP-CW and CN-CW determined more tetracycline and ARG residues in the substrate. More tetracycline resistance genes were induced and stored under the effects of tetracycline, which could also be the potential source of resistance gene transfer. Rhizosphere processes of plants can also promote the storage of more ARGs [36], and the presence of more microorganisms in the rhizosphere creates space for the existence of more ARGs. The addition of coke and plants is conducive to the removal of ARGs; however, it could also highlight the potential risk of the residues of ARGs in CWs.

The production of ARGs could not be separated from the induction of antibiotics, and the abundance of ARGs was positively correlated with the concentration and time of antibiotics [34]. *MacB* and *BcrA* played a dominant role in urban sewage, natural water bodies, and sediments [44]. These might have accumulated since the beginning of the experiment. The shorter period of the tetracyclines experiment caused less ARGs accumulation, resulting in more *MacB* and *BcrA* in the substrate of all CWs. The results agreed with the previous studies shown in Table 2. The CWs would have the risk that harboring the ARGs may lead to increased ARGs in the effluent. Notably, the majority of previous studies in Table 2 were conducted in the lab under a short period of investigation. We hypothesize that a higher risk of the release of ARGs could be present in an applied system under the change in ambient environment, including the variations of temperature,

inflow, water level, and vegetation growth. This study demonstrated that electroconductive media, i.e., coke, could benefit the pollutant, including antibiotics removal. However, it may retain a higher amount of ARGs and thus induce further risks to the environment.

Table 2. Treatment of antibiotics and ARGs in various types of constructed wetlands.

Wetland Type	Substrate	Plant	Antibiotic Type	Removal (%)	ARGs Abundance	References
VFCW	volcanic rocks	*hybrid pennisetum*	Oxytetracycline	91	decrease	[45]
VFCW	zeolite	*hybrid pennisetum*	Oxytetracycline	95	increase	[45]
VFCW	sand	*Phragmites australis*	Lincosamides Clindamycin	<−200	decrease	[18]
VFCW	zeolite	*Phragmites communis*	Sulfamethazine	49	decrease	[21]
VFCW	biochar	*Phragmites communis*	Sulfamethazine	57	decrease	[21]
VFCW	sand	*Phragmites australis (Cav.) Trin. Ex Steud.*	CIP	95	-	[26]
VFCW	sand	*unplanted*	CIP	93	-	[26]
HSCW	light expanded clay aggregates	*unplanted*	Tetracyclines	-	decrease	[30]
HSCW	-	*Phragmites australis*	Oxytetracycline	28~100	decrease	[46]
HSCW	oyster shell	*Cyperus alternifolius* L.	ETM-H_2O, MON, OFX, SMR, SMZ, NOV	10~60	decrease	[33]
HSCW	zeolite	*Cyperus alternifolius* L.	ETM-H_2O, MON, OFX, SMR, SMZ, NOV	66~99	decrease	[33]
HSCW	zeolite, gravel	*Iris pseudacorus*	Sulfamethazine	69	decrease (69%)	[16]
HSCW	zeolite, gravel	*Phragmites australis*	Sulfamethazine	65	decrease (65%)	[16]
SFCW	-	*Phragmites australis*	-	-	decrease (60~78%)	[43]
SFCW	heavy clay	*Phragmites australis, Typha latifolia*	Ciprofloxacin	59	unchanged	[28]
SFCW	sandy clay loam	*Typha orientalis Presl*	Monensin, Salinomycin and Narasin	27	-	[47]
SFCW	sandy soils	*Typha orientalis Presl*	Monensin, Salinomycin, and Narasin	32	-	[47]

5. Conclusions

The results of this study show that the addition of electroconductive media and plants can effectively improve the treatment of tetracyclines and nutrients in CWs. Antibiotics enhanced the potential of tetracyclines removal by promoting the enrichment of adaptable microorganisms, and this enrichment was more significant in planted CWs. The overall antibiotic tolerance of microorganisms in the substrate and the removal of tetracyclines and nutrients was promoted with the addition of plants and coke. However, the existence of electroconductive media and plants may highlight the potential risk of ARG enrichment in the substrate and pose a potential threat to public health. Further research is still needed to investigate the transformation mechanism and destination of antibiotics and ARGs in constructed wetlands.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142214989/s1, Table S1: The relative abundance of 308 antibiotic resistance genes (ARGs) in QS-CW, CP-CW and CN-CW.

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References

1. Gothwal, R.S.; Shashidhar, T. Antibiotic Pollution in the Environment: A Review. *CLEAN Soil Air Water* **2015**, *43*, 479–489. [CrossRef]
2. Jeong, J.; Song, W.; Cooper, W.J.; Jung, J.; Greaves, J. Degradation of tetracycline antibiotics: Mechanisms and kinetic studies for advanced oxidation/reduction processes. *Chemosphere* **2010**, *78*, 533–540. [CrossRef] [PubMed]
3. Xu, L.; Zhang, H.; Xiong, P.; Zhu, Q.; Liao, C.; Jiang, G. Occurrence, fate, and risk assessment of typical tetracycline antibiotics in the aquatic environment: A review. *Sci. Total Environ.* **2021**, *753*, 141975. [CrossRef] [PubMed]
4. Weber, K.P.; Mitzel, M.R.; Slawson, R.M.; Legge, R.L. Effect of ciprofloxacin on microbiological development in wetland mesocosms. *Water Res.* **2011**, *45*, 3185–3196. [CrossRef] [PubMed]
5. Lv, T.; Carvalho, P.N.; Zhang, L.; Zhang, Y.; Button, M.; Arias, C.A.; Weber, K.P.; Brix, H. Functionality of microbial communities in constructed wetlands used for pesticide remediation: Influence of system design and sampling strategy. *Water Res.* **2017**, *110*, 241–251. [CrossRef]
6. Taubes, G. The bacteria fight back. *Science* **2008**, *321*, 356–361. [CrossRef]
7. Nnadozie, C.F.; Kumari, S.; Bux, F. Status of pathogens, antibiotic resistance genes and antibiotic residues in wastewater treatment systems. *Rev. Env. Sci. Biotechnol.* **2017**, *16*, 491–515. [CrossRef]
8. Teshome, A.; Alemayehu, T.; Deriba, W.; Ayele, Y. Antibiotic Resistance Profile of Bacteria Isolated from Wastewater Systems in Eastern Ethiopia. *J. Environ. Public Health* **2020**, *2020*, 2796365. [CrossRef]
9. Bai, S.; Lyu, T.; Ding, Y.; Li, Z.; Wang, D.; You, S.; Xie, Q. Campus Sewage Treatment in Multilayer Horizontal Subsurface Flow Constructed Wetlands: Nitrogen Removal and Microbial Community Distribution. *CLEAN Soil Air Water* **2017**, *45*, 1700254. [CrossRef]
10. Hijosa-Valsero, M.; Fink, G.; Schlusener, M.P.; Sidrach-Cardona, R.; Martin-Villacorta, J.; Ternes, T.; Becares, E. Removal of antibiotics from urban wastewater by constructed wetland optimization. *Chemosphere* **2011**, *83*, 713–719. [CrossRef]
11. Lyu, T.; He, K.; Dong, R.; Wu, S. The intensified constructed wetlands are promising for treatment of ammonia stripped effluent: Nitrogen transformations and removal pathways. *Environ. Pollut.* **2018**, *236*, 273–282. [CrossRef] [PubMed]
12. Santos, F.; Almeida, C.M.R.; Ribeiro, I.; Mucha, A.P. Potential of constructed wetland for the removal of antibiotics and antibiotic resistant bacteria from livestock wastewater. *Ecol. Eng.* **2019**, *129*, 45–53. [CrossRef]
13. Zhang, L.; Lyu, T.; Zhang, Y.; Button, M.; Arias, C.A.; Weber, K.P.; Brix, H.; Carvalho, P.N. Impacts of design configuration and plants on the functionality of the microbial community of mesocosm-scale constructed wetlands treating ibuprofen. *Water Res.* **2018**, *131*, 228–238. [CrossRef] [PubMed]
14. Huang, X.; Liu, C.; Li, K.; Su, J.; Zhu, G.; Liu, L. Performance of vertical up-flow constructed wetlands on swine wastewater containing tetracyclines and tet genes. *Water Res.* **2015**, *70*, 109–117. [CrossRef]
15. Zhang, S.; Lu, Y.X.; Zhang, J.J.; Liu, S.; Song, H.L.; Yang, X.L. Constructed Wetland Revealed Efficient Sulfamethoxazole Removal but Enhanced the Spread of Antibiotic Resistance Genes. *Molecules* **2020**, *25*, 834. [CrossRef]
16. Huang, X.F.; Ye, G.-Y.; Yi, N.K.; Lu, L.-J.; Zhang, L.; Yang, L.Y.; Xiao, L.; Liu, J. Effect of plant physiological characteristics on the removal of conventional and emerging pollutants from aquaculture wastewater by constructed wetlands. *Ecol. Eng.* **2019**, *135*, 45–53. [CrossRef]
17. Zhang, Y.; Lyu, T.; Zhang, L.; Button, M.; Arias, C.A.; Weber, K.P.; Shi, J.; Chen, Z.; Brix, H.; Carvalho, P.N. Microbial community metabolic profiles in saturated constructed wetlands treating iohexol and ibuprofen. *Sci. Total Environ.* **2019**, *651*, 1926–1934. [CrossRef]
18. Avila, C.; Garcia-Galan, M.J.; Borrego, C.M.; Rodriguez-Mozaz, S.; Garcia, J.; Barcelo, D. New insights on the combined removal of antibiotics and ARGs in urban wastewater through the use of two configurations of vertical subsurface flow constructed wetlands. *Sci. Total Environ.* **2021**, *755*, 142554. [CrossRef]
19. Du, L.; Zhao, Y.; Wang, C.; Zhang, H.; Chen, Q.; Zhang, X.; Zhang, L.; Wu, J.; Wu, Z.; Zhou, Q. Removal performance of antibiotics and antibiotic resistance genes in swine wastewater by integrated vertical-flow constructed wetlands with zeolite substrate. *Sci. Total Environ.* **2020**, *721*, 137765. [CrossRef]

20. Bai, S.; Qin, L.; Liu, L.; Gao, X.; Ding, Y.; Li, Y. Effect of substrate types on contaminant removals, electrochemical characteristics and microbial community in vertical flow constructed wetlands for treatment of urban sewage. *J. Environ. Manag.* **2021**, *280*, 111682. [CrossRef]
21. Yuan, Y.; Yang, B.; Wang, H.; Lai, X.; Li, F.; Salam, M.M.A.; Pan, F.; Zhao, Y. The simultaneous antibiotics and nitrogen removal in vertical flow constructed wetlands: Effects of substrates and responses of microbial functions. *Bioresour. Technol.* **2020**, *310*, 123419. [CrossRef] [PubMed]
22. de Nicolás, A.P.; Berenguer, R.; Esteve-Núñez, A. Evaluating bioelectrochemically-assisted constructed wetland (METland®) for treating wastewater: Analysis of materials, performance and electroactive communities. *Chem. Eng. J.* **2022**, *440*, 135748. [CrossRef]
23. Lei, Y.; Langenhoff, A.; Bruning, H.; Rijnaarts, H. Sorption of micropollutants on selected constructed wetland support matrices. *Chemosphere* **2021**, *275*, 130050. [CrossRef] [PubMed]
24. Lv, T.; Zhang, Y.; Casas, M.E.; Carvalho, P.N.; Arias, C.A.; Best, K.; Brix, H. Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. *Chemosphere* **2016**, *148*, 459–466. [CrossRef]
25. Tai, Y.; Tam, N.F.-Y.; Dai, Y.; Yang, Y.; Lin, J.; Tao, R.; Yang, Y.; Wang, J.; Wang, R.; Huang, W.; et al. Assessment of rhizosphere processes for removing water-borne macrolide antibiotics in constructed wetlands. *Plant Soil* **2017**, *419*, 489–502. [CrossRef]
26. Wang, S.; Cui, Y.; Li, A.; Zhang, W.; Wang, D.; Ma, J. Fate of antibiotics in three distinct sludge treatment wetlands under different operating conditions. *Sci. Total Environ.* **2019**, *671*, 443–451. [CrossRef]
27. Wen, H.; Zhu, H.; Yan, B.; Xu, Y.; Shutes, B. Treatment of typical antibiotics in constructed wetlands integrated with microbial fuel cells: Roles of plant and circuit operation mode. *Chemosphere* **2020**, *250*, 126252. [CrossRef]
28. Berglund, B.; Khan, G.A.; Weisner, S.E.; Ehde, P.M.; Fick, J.; Lindgren, P.E. Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes. *Sci. Total Environ.* **2014**, *476–477*, 29–37. [CrossRef]
29. Wei, Z.; Feng, K.; Wang, Z.; Zhang, Y.; Yang, M.; Zhu, Y.G.; Virta, M.P.J.; Deng, Y. High-Throughput Single-Cell Technology Reveals the Contribution of Horizontal Gene Transfer to Typical Antibiotic Resistance Gene Dissemination in Wastewater Treatment Plants. *Environ. Sci. Technol.* **2021**, *55*, 11824–11834. [CrossRef]
30. Fang, H.; Zhang, Q.; Nie, X.; Chen, B.; Xiao, Y.; Zhou, Q.; Liao, W.; Liang, X. Occurrence and elimination of antibiotic resistance genes in a long-term operation integrated surface flow constructed wetland. *Chemosphere* **2017**, *173*, 99–106. [CrossRef]
31. Ma, J.; Cui, Y.; Li, A.; Zou, X.; Ma, C.; Chen, Z. Antibiotics and antibiotic resistance genes from wastewater treated in constructed wetlands. *Ecol. Eng.* **2022**, *177*, 106548. [CrossRef]
32. Pan, M.; Lyu, T.; Zhan, L.; Matamoros, V.; Angelidaki, I.; Cooper, M.; Pan, G. Mitigating antibiotic pollution using cyanobacteria: Removal efficiency, pathways and metabolism. *Water Res.* **2021**, *190*, 116735. [CrossRef] [PubMed]
33. Chen, J.; Wei, X.D.; Liu, Y.S.; Ying, G.G.; Liu, S.S.; He, L.Y.; Su, H.C.; Hu, L.X.; Chen, F.R.; Yang, Y.Q. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Optimization of wetland substrates and hydraulic loading. *Sci. Total Environ.* **2016**, *565*, 240–248. [CrossRef] [PubMed]
34. Song, H.L.; Zhang, S.; Guo, J.; Yang, Y.L.; Zhang, L.M.; Li, H.; Yang, X.L.; Liu, X. Vertical up-flow constructed wetlands exhibited efficient antibiotic removal but induced antibiotic resistance genes in effluent. *Chemosphere* **2018**, *203*, 434–441. [CrossRef] [PubMed]
35. Yang, H.; Chen, X.; Tang, J.; Zhang, L.; Zhang, C.; Perry, D.C.; You, W. External carbon addition increases nitrate removal and decreases nitrous oxide emission in a restored wetland. *Ecol. Eng.* **2019**, *138*, 200–208. [CrossRef]
36. Dai, X.; Su, C.; Chen, Z.; Li, X.; Lu, P.; Qi, Z.; Luo, Z.; Chen, M. Sulfonamide and quinolone antibiotics contaminated wastewater treatment by constructed rapid infiltration: Efficiency and microbial community structure. *Process Saf. Environ. Prot.* **2022**, *161*, 542–555. [CrossRef]
37. Man, Y.; Wang, J.; Tam, N.F.; Wan, X.; Huang, W.; Zheng, Y.; Tang, J.; Tao, R.; Yang, Y. Responses of rhizosphere and bulk substrate microbiome to wastewater-borne sulfonamides in constructed wetlands with different plant species. *Sci. Total Environ.* **2020**, *706*, 135955. [CrossRef]
38. Tian, Z.; Liu, R.; Zhang, H.; Yang, M.; Zhang, Y. Developmental dynamics of antibiotic resistome in aerobic biofilm microbiota treating wastewater under stepwise increasing tigecycline concentrations. *Environ. Int.* **2019**, *131*, 105008. [CrossRef]
39. Chen, H.; Chen, R.; Jing, L.; Bai, X.; Teng, Y. A metagenomic analysis framework for characterization of antibiotic resistomes in river environment: Application to an urban river in Beijing. *Environ. Pollut.* **2019**, *245*, 398–407. [CrossRef]
40. Islam, G.M.; Gilbride, K.A. The effect of tetracycline on the structure of the bacterial community in a wastewater treatment system and its effects on nitrogen removal. *J. Hazard. Mater.* **2019**, *371*, 130–137. [CrossRef]
41. Meng, F.; Gao, G.; Yang, T.-T.; Chen, X.; Chao, Y.; Na, G.; Ge, L.; Huang, L.-N. Effects of fluoroquinolone antibiotics on reactor performance and microbial community structure of a membrane bioreactor. *Chem. Eng. J.* **2015**, *280*, 448–458. [CrossRef]
42. Chen, J.; Ying, G.G.; Wei, X.D.; Liu, Y.S.; Liu, S.S.; Hu, L.X.; He, L.Y.; Chen, Z.F.; Chen, F.R.; Yang, Y.Q. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Effect of flow configuration and plant species. *Sci. Total Environ.* **2016**, *571*, 974–982. [CrossRef] [PubMed]
43. Nolvak, H.; Truu, M.; Tiirik, K.; Oopkaup, K.; Sildvee, T.; Kaasik, A.; Mander, U.; Truu, J. Dynamics of antibiotic resistance genes and their relationships with system treatment efficiency in a horizontal subsurface flow constructed wetland. *Sci. Total Environ.* **2013**, *461–462*, 636–644. [CrossRef]

44. Imchen, M.; Kumavath, R. Metagenomic insights into the antibiotic resistome of mangrove sediments and their association to socioeconomic status. *Environ. Pollut.* **2021**, *268*, 115795. [CrossRef] [PubMed]
45. Liu, L.; Liu, C.; Zheng, J.; Huang, X.; Wang, Z.; Liu, Y.; Zhu, G. Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. *Chemosphere* **2013**, *91*, 1088–1093. [CrossRef]
46. Sabri, N.A.; Schmitt, H.; van der Zaan, B.M.; Gerritsen, H.W.; Rijnaarts, H.H.M.; Langenhoff, A.A.M. Performance of full scale constructed wetlands in removing antibiotics and antibiotic resistance genes. *Sci. Total Environ.* **2021**, *786*, 147368. [CrossRef]
47. Hussain, S.A.; Prasher, S.O.; Patel, R.M. Removal of ionophoric antibiotics in free water surface constructed wetlands. *Ecol. Eng.* **2012**, *41*, 13–21. [CrossRef]