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# The Improvement of Pollutant Removal in the Ferric-Carbon Micro-Electrolysis Constructed Wetland by Partial Aeration

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**Abstract:** Subsurface flow constructed wetland (SSFCW) has been applied for wastewater treatment for several decades. In recent years, the combination of ferric-carbon micro-electrolysis (Fe/C-M/E) and SSFCW was proven to be an effective method of multifarious sewage treatment. However, Ferric substrate created a relatively reductive condition, decreased the oxidation efficiency of NH<sub>4</sub><sup>+</sup>-N, and blocked the following denitrification process, which led to the low removal efficiencies of NH<sub>4</sub><sup>+</sup>-N and total nitrogen (TN). In this study, partial aeration was introduced into the ferric-carbon micro-electrolysis SSFCW (Fe/C-M/E CW) system to solve the problem above. The water quality and nitrogen-related functional genes of bacteria on the surface of substrate were measured for mechanism exploration. The results showed that, the removal efficiencies of NH<sub>4</sub><sup>+</sup>-N and total phosphorus (TP) in an aerated Fe/C-M/E CW system were 96.97%  $\pm$  6.06% and 84.62%  $\pm$  8.47%, much higher than 43.33%  $\pm$  11.27% and 60.16%  $\pm$  2.95% in the unaerated Fe/C-M/E CW systems. However, the TN removal in Fe/C-M/E CW system was not enhanced by aeration, which could be optimized by extending more anoxic section for denitrification.

**Keywords:** aeration; Ferric-Carbon Micro-Electrolysis; nitrogen removal; phosphorus removal; subsurface-flow constructed wetlands

# 1. Introduction

Constructed wetland (CW) for wastewater treatment is a competitive technology due to the low cost, easy operation and simple maintenance [1,2]. Nitrogen in wastewater can be removed by microbial ammonification, nitrification and denitrification, plant absorption and volatilization, matrix adsorption, filtration and precipitation in CW [3,4]. In addition, the removal mechanism of phosphorus includes the adsorption of the fillers, chemical action, absorption of plant and algae, combining with organics, normal assimilation and excessive accumulation of microorganisms, among which, phosphorus adsorption by fillers is considered the most effective pathway [5]. Subsurface flow constructed wetland (SSFCW) is applied for domestic sewage treatment [6,7]; however, it was reported that the denitrification efficiency was limited [8]. To enhance the efficiency of nitrogen and phosphorus removal, a new method, i.e., the combined SSFCW with ferric-carbon micro-electrolysis (Fe/C-M/E) was designed [9]. M/E technology was wildly used in a variety of industrial wastewater treatment processes [10]. Ferric-carbon micro-electrolysis played a role by galvanic cell reaction, reduction of hydrogen ions, complexation reaction, flocculation and precipitation, oxidation-reduction, among others [11].

In previous studies, a conclusion was found that the application of ferric-carbon micro-electrolysis SSFCW (Fe/C-M/E SSFCW) enhanced the removal performance in simulated domestic sewage water treatment, especially in the process of denitrification and phosphorus removal. Significant nitrogen and

phosphorus removal performance was also achieved, which were indicated by several studies [9,12–14]. The mechanism of these promoting effects was the supply of electrons by the galvanic corrosion of numerous micro-scale sacrificial anode in denitrification, and the combination of  $PO_4^{3-}$  with Fe<sup>2+</sup> or Fe<sup>3+</sup> to remove phosphorus [15].

The experimental results showed that, the removal efficiencies of  $NO_3^-$ -N and phosphorus by M/E-SSFCW were 99.54% ± 0.80% and 93.63% ± 5.30%, respectively, which were much higher than those of the ordinary SSFCW ( $NO_3^-$ -N: 37%; total phosphorus (TP): 39%) and biochar-added SSFCW ( $NO_3^-$ -N: 57%; TP: 21%) [9]. Another study by Zheng et al. [13] showed the similar tendency that the removal efficiencies of chemical oxygen demand (COD) and total nitrogen (TN) in iron-carbon micro-electrolysis vertical flow constructed wetland system increased by 10.16% and 13.72% respectively compared with the traditional constructed wetland treating tail water of a sewage treatment plant.

Nevertheless, the rapid decrease of dissolved oxygen (DO) in the system is a significant limitation for nitrification in constructed wetlands [9,16]. Moreover, Fe would compete for trace oxygen with NH<sub>4</sub><sup>+</sup>-N, which further inhibited the nitrification process [9]; while in the process of denitrification, NH<sub>4</sub><sup>+</sup>-N was produced as nitrate and reacted with Fe (0) directly [17]. Given concerns about the toxicity of ammonia nitrogen in a water environment [18,19], it is necessary to solve the problems of low nitrification efficiency and the NH<sub>4</sub><sup>+</sup>-N accumulation in ferric-carbon micro-electrolysis SSFCW. Cottingham et al. [20] indicated that there was less soluble ammonia at the outlet in aerated compared to non-aerated basins, confirming the positive effect of aeration on nitrifying bacteria. Another study [21] also showed a positive influence of appropriate aeration mode to improve the removal efficiency of sewage in constructed wetlands. Moreover, the removal of phosphorus in subsurface flow wetland is also affected by dissolved oxygen (DO). When DO increased in the system, phosphorus precipitation and adsorption to the substrate were also increasing [22]. Experimental results of Wang et al. showed that middle aeration allowed the CW to possess more uniform oxygen distribution and to achieve greater removals of COD and NH<sub>4</sub><sup>+</sup>-N [23].

Consequently, partial aeration of the substrate was chosen to increase DO content in the Fe/C-M/E SSFCW. To reduce the reaction of iron with oxygen and enhance the role of iron as an electronic donor for nitrate reduction, a system with upper aeration section and lower ferric-carbon micro-electrolysis section was set up to verify whether this structure can improve the efficiency of ammonia oxidation and the consequential total nitrogen removal in the practical application of Fe/C-M/E CW. The other three control systems were set up as whole-course Fe/C-M/E-coupled half-way aeration, whole-course Fe/C-M/E and traditional SSFCW.

In summary, there are three objectives of this optimization experiment: (1) investigate the effects of aeration on the removal efficiency of nitrogen and phosphorus in M/E-SSFCW; (2) explore the mechanism of pollutant removal influenced by aeration; (3) verify whether the structure of upper aeration and lower micro-electrolysis system could improve the removal efficiency of nitrogen.

#### 2. Experimental Design and Methods

#### 2.1. Experimental Facility

The schematic setup of four reactors is shown in Figure 1. Four light-proof laboratory-scale SSFCWs were set up in Qingdao, Shandong, China. These SSFCW cylinders were made of polyvinyl chloride, with the height of 600 mm and diameter of 200 mm. Quartz sand (diameter: 2–4 mm;), iron scraps (10–15 mm;) and biochar (10–15 mm) were three main substrates for the middle layer. The middle 450 mm layers of the four SSFCWs were installed with different substrate as follows: (1) Fe/C-M/E system ( $W_{ME}$ ): 20% biochar including 60% iron scraps and quartz sand; (2) whole-course Fe/C-M/E coupled half-way aeration system ( $W_{WME-HA}$ ); 20% biochar including 60% iron scraps and quartz sand; (3) half-way Fe/C-M/E coupled half-way aeration system ( $W_{HME-HA}$ ): upper part is quartz sand, lower part is 20% biochar including 60% iron scraps and quartz sand; (4) blank control system ( $W_C$ ): only quartz sand. At the bottom (50 mm) of the four systems, coarse gravel (20–30 mm) was filled

as the supporting layer and the top (100 mm) of CWs were also covered with quartz sand (diameter: 2–4 mm). The effluent water level was set at the height of 500 mm to guarantee the flooded status of most substrate in CWs and simulate the real function status of SSFCW. No plants were involved in these systems, since the contribution of pollutant removal by plants in vertical subsurface flow constructed wetland was reported to be less than 20% [8]. Three polyvinyl chloride (PVC) aeration pipes were each inserted in to the middle of substrates in  $W_{WME-HA}$  and  $W_{HME-HA}$  and providing the aeration with 1 L/min (as shown in Figure 1). In addition, a porous PVC pipe was inserted vertically through substrate to take out the samples from different heights and measure the indexes of temperature, pH, DO, oxidation reduction potential (ORP) and other physical and chemical properties of the systems in situ.



**Figure 1.** Schematic diagram of experimental laboratory-scale subsurface flow constructed wetlands (SSFCW).

#### 2.2. Experimental Condition and Operation

The iron scraps were truncated to 10–15 mm, and the biochar was made of Arundo donax by heating in a muffle furnace at 300 °C for 1 h. The detailed preparation was described in our previous paper [9]. The size of iron scraps and biochar were both between 10–15 mm. The simulated primary effluent of treated domestic sewage as set out in Table 1. The experiment was conducted in continuous inflow mode, and the hydraulic loading rate (HLR) was set to 0.17 m<sup>3</sup>/(m<sup>2</sup>·d) for an average hydraulic retention time of 3 days. These four CWs were placed indoor with irradiation by high voltage sodium lamps. During the experiment, the temperature was kept between 20 °C and 25 °C. The whole experiment lasted from September 2018 to January 2019.

Indicator	Chemical Oxygen Demand (COD)	NH4 <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Total Phosphorus (TP)	
Setting Concentration (mg/L)	60	5	15	1	
Medicament	Sucrose (C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	$(NH_4)_2SO_4$	KNO3	KH <sub>2</sub> PO <sub>4</sub>	
Dosage (mg/L)	51	37.6	76.57	7	

Table 1. Influent water quality contents.

#### 2.3. Sample Collection and Analytical Methods

Water samples were collected every two days from the outlets, and six water quality indicators TP,  $NH_4^+-N$ ,  $NO_2^--N$ ,  $NO_3^--N$ , TN, and COD were measured according to Standard Methods for the Examination of water and wastewater of American Public Health Association. DO and temperature were determined by a DO meter (HQ30d, HACH, USA). The pH was monitored by a portable pH meter (PHS-3D, Rex Instrument, Shanghai, China). A portable ORP meter (ORP-BL, Shunkeda Instrument, Beijing, China) was applied for the ORP measurement.

Substrate samples were taken from four layers of 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm and mixed evenly, to analyze the nitrogen-related functional genes (i.e., ammonia monooxygenase (*amoA*), membrane-bound nitrate reductase (*narG*), copper-containing nitrite reductase (*nirK*), cd1-containing nitrite reductase (*nirS*), and nitrous oxide reductase (*nosZ*)) of the microbial community by quantitative polymerase chain reaction (qPCR) as in Shen [9].

Each measurement was performed in triplicate, and the results of each measurement were presented as mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was used to indicate the statistical differences between results in four SSFCWs, and the significance level of all analyses was set to p < 0.05.

### 3. Results and Discussion

#### 3.1. Nitrogen Removal Performance

#### 3.1.1. Overall Removal Performance and Removal Mechanism of Nitrogen

The physicochemical characteristics of the influent and effluent in four SSFCW systems are shown in Table 2. Nitrogen removal efficiency in SSFCW was expressed by items of  $NH_4^+$ -N,  $NO_2^-$ -N,  $NO_3^-$ -N and TN. The removal efficiency of  $NH_4^+$ -N in aerated M/E-SSFCW  $W_{WME-HA}$  and  $W_{HME-HA}$  presented excellent performance as 96.97% ± 6.06% and 95.66% ± 5.51% far more than (p < 0.05) the result of unaerated SSFCW  $W_{ME}$  (43.33% ± 11.27%) and  $W_C$  (22.41% ± 9.60%). It strongly demonstrated the aeration plays an important and positive role in  $NH_4^+$ -N removal (as shown in Figure 2). The effect of aeration on  $NH_4^+$ -N removal was also reflected in the studies of Lee et al. [24] and Cottingham et al. [20]. The higher DO content in  $W_{WME-HA}$  and  $W_{HME-HA}$  caused the better nitrification of nitrifying bacteria.

The TN removal efficiency in  $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$  reached 53.87% ± 4.60%, 55.92% ± 8.25% and 52.49% ± 10.29% respectively, showed a better effect than  $W_C$  (30.68% ± 9.03%) (p < 0.05). Enhanced nitrification in aeration groups led to high removal efficiency of  $NH_4^+$ -N. However, from the aspect of  $NO_3^-$ -N removal efficiency, the unaerated M/E-SSFCW  $W_{ME}$  was the best at 97.07% ± 1.78%, followed by the aerated M/E-SSFCW  $W_{WME-HA}$ ,  $W_{HME-HA}$  and the traditional CW  $W_C$  with the TN removal efficiencies less than 70% (p < 0.05). In M/E-SSFCW, the higher removal efficiency was caused by the ferric-carbon micro-electrolysis substrate in denitrification. Besides the electron donator COD in wastewater, Cathode Fe substrates can provide electrons to adjacent anode biochar (as shown in Figure 2), and  $NO_3^-$ -N in liquid phase could be directly consumed as electron acceptors in the anode compartment by the relative bacterial function on the surface of biochar, shown as Equations (1) and (2) [25] below.

$$5Fe + 2NO_3^- + 6H_2O \rightarrow 5Fe^{2+} + N_2 + 12OH^-$$
 (1)

$$10Fe^{2+} + 2NO_3^{-} + 6H_2O \rightarrow 10Fe^{3+} + N_2 + 12OH^{-}$$
(2)

Items	Influent (mg/L)	Effluent (mg/L, except pH)				Removal Efficiency (%)			
		W <sub>ME</sub>	W <sub>WME-HA</sub>	W <sub>HME-HA</sub>	W <sub>C</sub>	W <sub>ME</sub>	W <sub>WME-HA</sub>	W <sub>HME-HA</sub>	W <sub>C</sub>
pH	$7.60 \pm 0.03$	$7.42 \pm 0.05$	$7.49 \pm 0.06$	$7.46 \pm 0.06$	$7.39 \pm 0.04$	/	/	/	/
Dissolved Oxygen (DO)	$7.53\pm0.05$	$0.64 \pm 0.32$	$3.30\pm0.04$	$3.64\pm0.04$	$0.43 \pm 0.04$	/	/	/	/
COD	$59.09 \pm 8.39$	$31.47 \pm 8.39$	$25.07 \pm 7.11$	$26.44 \pm 7.66$	$38.13 \pm 5.679$	$43.64 \pm 9.72$	$54.98 \pm 6.63$	$51.78 \pm 6.48$	$32.73 \pm 7.32$
NH4 <sup>+</sup> -N	$5.99 \pm 0.63$	$3.79\pm0.78$	$0.16 \pm 0.32$	$0.27 \pm 0.35$	$4.50\pm0.65$	$43.33 \pm 11.27$	$96.97 \pm 6.06$	$95.66 \pm 5.51$	$22.41 \pm 9.60$
NO <sub>3</sub> <sup>-</sup> -N	$15.04 \pm 1.04$	$0.44\pm0.27$	$5.27 \pm 1.01$	$6.70 \pm 1.45$	$7.32 \pm 0.86$	$97.07 \pm 1.78$	$64.94 \pm 5.35$	$55.48 \pm 11.93$	$51.35 \pm 7.58$
$NO_2^{-}-N$	$0.19\pm0.22$	$0.60\pm0.18$	$0.27\pm0.09$	$0.40\pm0.09$	$2.13\pm0.87$	/	/	/	/
TN	$17.27 \pm 1.62$	$7.92 \pm 0.51$	$7.41 \pm 1.43$	$7.91 \pm 1.33$	$11.58\pm0.55$	$53.87 \pm 4.60$	$55.92 \pm 8.25$	$52.49 \pm 10.29$	$30.68 \pm 9.03$
TP	$1.04\pm0.37$	$0.47\pm0.11$	$0.15\pm0.08$	$0.41 \pm 0.05$	$0.89 \pm 0.13$	$60.16 \pm 2.95$	$84.62\pm8.47$	$63.28 \pm 4.16$	$23.27 \pm 6.35$

**Table 2.** The physicochemical characteristics of influent and effluent and related removal efficiency (mean ± standard deviation (SD), n = 30).



Figure 2. The mechanism of ferric-carbon micro-electrolysis (Fe/C-M/E)-coupled half-way aeration system.

The results that removal efficiency of NO<sub>3</sub><sup>-</sup>-N in the aerated group (i.e.,  $W_{WME-HA}$  and  $W_{HME-HA}$ ) were statistically significantly lower than that in unaerated group (p < 0.05), were consisted with the study of Pan et al. [26]. The removal efficiency of NO<sub>3</sub><sup>-</sup>-N in aerated group were 64.94% ± 5.35%, while in the unaerated systems, the removal efficiency of NO<sub>3</sub><sup>-</sup>-N up to 97.07% ± 1.78%. This phenomenon can be explained by the reduction of denitrification. On one hand, the amount of oxygen supplement by aeration can consume COD and activated hydrogen [H], leaving less COD for denitrification of nitrate/nitrite [13,27]. On the other hand, aeration formed an aerobic condition with DO above 4 mg/L in the whole system, which inhibited the growth and function of denitrifying bacteria [28]. As can be seen from Table 2, three experimental systems ( $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$ ) with iron-carbon micro-electrolytic substrates caused a small accumulation of NO<sub>2</sub><sup>-</sup>N, which were all less than 1 mg/L; while the concentration of NO<sub>2</sub><sup>-</sup>-N in the effluent of  $W_C$  reached above 2 mg/L (p < 0.05).

# 3.1.2. Nitrogen Concentration Distribution in Different Heights of the SSFCWs

Figure 3 shows the variation curves of physical and chemical indexes along constructed wetlands. As a poisonous pollutant, the concentration of  $NH_4^+$ -N was of primary concern. The concentrations of  $NH_4^+$ -N in four CW systems (Figure 3c) were all reduced rapidly within the 0–10 cm interval. However, the removal efficiency of  $NH_4^+$ -N in aerated systems was significantly higher than that in unaerated systems in this section (p < 0.05). The removal efficiency of  $NH_4^+$ -N in the 0–10 cm section of aerated systems were close to 100%, while in the unaerated systems, the nitrification was inhibited because the DO concentration dropped below 2 mg/L in the 10–60 cm section (Figure 3e), then the  $NH_4^+$ -N nitrification was inhibited and became stable.

The concentrations of TN (Figure 3b) in 0–10 cm were all decreased in all four systems. It can be deduced that simultaneous nitrification and denitrification process occurred in the 0–10 cm section of all the four CWs. However, the TN concentration tended to be stable in  $W_C$  in 10–60 cm, while it gradually decreased in  $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$ . Among them, the removal effect in  $W_{ME}$  was slightly better than that of  $W_{WME-HA}$  and  $W_{HME-HA}$ .

 $NO_3^--N$  showed a similar trend to TN. The  $NO_3^--N$  concentration in  $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$  showed continuous downward trends, while the downward trend is gentler in  $W_C$  (Figure 3a). In  $W_{WME-HA}$  and  $W_{HME-HA}$ , the denitrification rate was higher than that of  $W_C$  due to the presence of iron-carbon micro-electrolytic substrate, but lower than that of  $W_{ME}$  owing to the high DO concentration. Though aeration was imported to strengthen the ammonia oxidation, excess aeration could inhibit the following denitrification process, which should be accurately adjusted.

ORP, oxidation-reduction potential, reflects the concentration of oxidizing substance and the activity of organisms in the systems, which has also been used to monitor nitrification and denitrification processes [29]. In 0–10 cm, ORP declined in four systems because of the oxidation of Fe and  $NH_4^+$ -N by DO. Then in 10–60 cm, ORP decreased in Fe/C-M/E systems ( $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$ ) along with

the process of denitrification. Interestingly, in 30 cm of  $W_{WME-HA}$  and  $W_{HME-HA}$ , there were sudden increases of ORP, which were due to the position of aeration. Although the relatively higher ORP in  $W_{WME-HA}$  and  $W_{HME-HA}$  improved ammonia oxidation, it inhibited the denitrification process, which needed further optimization.

In the study of Zhou et al., a proper gas-water ratio played a greater role in micro-electrolysis water treatment process, and the optimal ratio of gas to water is 5:1 under experimental conditions [30]. The relatively low concentration of  $NO_2^-$ -N and  $NH_4^+$ -N in  $W_{WME-HA}$  and  $W_{HME-HA}$  were attributed to a high concentration of DO (as shown in Figure 3e). On the other hand, the over-high concentration of DO in  $W_{WME-HA}$  and  $W_{HME-HA}$  destroyed the original anoxic condition, further inhibited the reduction of  $NO_3^-$ -N, and limited the TN removal. Hence, introducing partial aeration into the M/E CW system is necessary for the first step of TN removal, i.e. the  $NH_4^+$ -N oxidation, but the following anoxic condition for denitrification should also be guaranteed. In other words, the ratio of aeration section to non-aeration section.



**Figure 3.** The  $NO_3^--N$  (**a**), TN (**b**),  $NH_4^+-N$  (**c**),  $NO_2^--N$  (**d**), DO (**e**), and Eh (**f**) concentration distributions in different heights of four subsurface flow constructed wetlands (SSFCWs).

## 3.2. Phosphorus Removal Performance

## 3.2.1. Overall Removal Performance of Phosphorus

As Table 2 shows, the phosphorus removal efficiency of  $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$  are all above 60%, obviously higher than that of  $W_C$  (23.27% ± 6.35%) (p < 0.05). It indicated that, Fe/C-M/E substrate played a positive role in improving phosphorus removal efficiency in CW. The dissolved Fe<sup>2+</sup> or Fe<sup>3+</sup> could co-precipitate with PO<sub>4</sub><sup>3+</sup>. In the experiment of Shang et al. [31], the TP removal efficiency of continuously aerated M/E-SSFCW was 3.74% higher than unaerated M/E-SSFCW, and 24.82% higher than traditional CW; these results are consistent with ours. Furthermore, research of Zhou et al. [32] also showed that the combination of ferric-carbon micro-electrolysis and subsurface-flow constructed wetlands enhanced the phosphorus removal performance. In our research, the combination of aeration and ferric-carbon material addition improved the TP removal, which showed a better performance than the independent effects of either aeration or ferric-carbon material.

3.2.2. Phosphorus Removal Mechanism and Concentration Distribution in Different Heights of the Subsurface Flow Constructed Wetland (SSFCW)

The improvement of TP removal efficiency may be due to two factors-aeration and F/C-M/E substrate. As Figure 4 shows, the TP removal efficiency of W<sub>C</sub> was ineffective, while other three M/E CWs (i.e., W<sub>ME</sub>, W<sub>WME-HA</sub> and W<sub>HME-HA</sub>) all presented a rapid downward trend. Among them, the removal efficiency of TP in W<sub>ME</sub> and W<sub>HME-HA</sub> was approximately coincident, while the removal efficiency of W<sub>WME-HA</sub> was higher than those of the former two (p < 0.05), which provided strong evidence that Fe/C-M/E coupled aeration could improve the removal efficiency of TP. The simultaneous appearance of oxygen and Fe in the upper layer of W<sub>WME-HA</sub> could form more Fe<sup>3+</sup> other than Fe<sup>2+</sup>, providing 50% more ionic bond for PO<sub>4</sub><sup>3-</sup> compared with Fe<sup>2+</sup> (as shown in Figure 2) [33]. That may be the reason for the highest TP removal efficiency in W<sub>WME-HA</sub> (as shown in Figure 1). The dissolved ferric ions were not detected, which guaranteed the effluent quality. Besides that, phosphorus-accumulating bacteria (PAOs) can adsorb the dissolved phosphorus and form polyphosphates to accumulate energy in the aerobic condition, and aeration can enhance the potential phosphorus absorption of PAOs [34].



Figure 4. The concentration distribution of phosphorus over different heights in four SSFCWs.

## 3.3. Microbial Community Analysis

The qPCR technique was applied to detect the effect of aeration and the Fe/C-M/E substrate on microbial functional gene abundance and the further pollutant-removal efficiency. The types of nitrogen-related genes were as follows: *amoA*, *narG*, *nirK*, *nirS* and *nosZ* [35], and bacterial 16S rRNA. The substrate from the upper layer and below layer were separately taken for functional gene detection.

As Table 3 shown, the addition of biochar in  $W_{ME}$ ,  $W_{WME-HA}$ , and  $W_{HME-HA}$  increased the total number of bacteria (in terms of 16S RNA). The abundance of nitrifying bacteria (in terms of amoA) in  $W_{WME-HA}$ was highest, followed by the  $W_{ME}$ ,  $W_{HME-HA}$ , and  $W_C$ , which confirmed the promoting effect of aeration in nitrification. In general, the abundances of denitrifying bacteria (in terms of *narG*, *nirK*, *nirS*, and *nosZ*) in ME-SSFCW ( $W_{ME}$ ,  $W_{WME-HA}$  and  $W_{HME-HA}$ ) were higher than that in  $W_C$ , which fitted with the better denitrification efficiency in Fe/C-M/E CW. Moreover, the nitrification related genes preferred to show in the upper layer for the better anaerobic condition, while the denitrification-related bacteria/genes preferred to show in the lower layer for the better aerobic condition according to the oxygen distribution as shown in Figure 3e.

**Table 3.** The abundances of related functional genes in denitrifying bacteria and nitrifying bacteria (mean  $\pm$  SD, n = 30).

Functional	W <sub>ME</sub> (Copy Numbers/g Substrate)		W <sub>WME-HA</sub> (Copy Numbers/g Substrate)		W <sub>HME-HA</sub> (Copy Numbers/g Substrate)		W <sub>C</sub> (Copy Numbers/g Substrate)	
Genes	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
amoA	$2.29 \pm 0.06 \times 10^{7}$	$1.45\pm0.02\times10^{7}$	$3.50\pm0.04\times10^{7}$	$1.08 \pm 0.06 \times 10^{7}$	$2.20\pm0.04\times10^7$	$9.99 \pm 0.18 \times 10^{6}$	$9.73 \pm 0.34 \times 10^{6}$	$5.30\pm0.14\times10^{6}$
narG	$4.20 \pm 0.21 \times 10^{5}$	$5.61 \pm 0.33 \times 10^{5}$	$1.97 \pm 0.11 \times 10^{5}$	$5.49 \pm 0.45 \times 10^{5}$	$4.28 \pm 0.36 \times 10^{5}$	$8.57 \pm 0.10 \times 10^{5}$	$2.52 \pm 0.05 \times 10^{5}$	$1.53 \pm 0.09 \times 10^{5}$
nirK	$1.29 \pm 0.06 \times 10^{6}$	$1.67 \pm 0.04 \times 10^{6}$	$8.57 \pm 0.44 \times 10^5$	$1.65 \pm 0.10 \times 10^{6}$	$8.79 \pm 0.11 \times 10^5$	$1.36 \pm 0.01 \times 10^{6}$	$8.83 \pm 0.24 \times 10^5$	$6.24 \pm 0.74 \times 10^{5}$
nirS	$6.20 \pm 0.08 \times 10^4$	$2.15 \pm 0.06 \times 10^5$	$1.81 \pm 1.24 \times 10^{5}$	$2.35 \pm 0.02 \times 10^5$	$1.78 \pm 0.25 \times 10^{5}$	$2.06 \pm 0.03 \times 10^{6}$	$3.00 \pm 0.02 \times 10^4$	$8.06 \pm 0.17 \times 10^5$
nosZ	$2.21 \pm 0.11 \times 10^{8}$	$2.61 \pm 0.42 \times 10^8$	$2.36 \pm 0.17 \times 10^{8}$	$1.04 \pm 0.13 \times 10^{8}$	$1.08 \pm 0.06 \times 10^{8}$	$6.24 \pm 0.17 \times 10^{8}$	$3.48 \pm 0.49 \times 10^{7}$	$7.27 \pm 0.91 \times 10^{7}$
16S rRNA	$2.13 \pm 0.09 \times 10^{9}$	$2.38 \pm 0.06 \times 10^{9}$	$1.64 \pm 0.06 \times 10^{9}$	$1.99 \pm 0.06 \times 10^{9}$	$2.62 \pm 0.21 \times 10^{9}$	$3.30 \pm 0.06 \times 10^{9}$	$1.27 \pm 0.04 \times 10^{8}$	$1.44\pm0.01\times10^8$

#### 4. Conclusion

The combination of Fe/C-M/E substrate and half-way aeration demonstrated a great advantage in removing NH<sub>4</sub><sup>+</sup>-N and TP. The average removal efficiency of NH<sub>4</sub><sup>+</sup>-N and TP in the aerated Fe/C-M/E system were 96.97%  $\pm$  6.06% and 84.62%  $\pm$  8.47%, which were 61.41% and 24.46% higher than unaerated Fe/C-M/E system, respectively. The average TN removal efficiency of three Fe/C-M/E CWs were similar, shown as 52.49%–55.92%, approximately 25% higher than the traditional SSFCW. The structure of upper aeration and lower micro-electrolysis CW did not show the expected advantage of TN removal owing to the excess aeration, which can be enhanced by decreasing the aeration section and strengthening the anoxic iron-carbon micro-electrolytic substrate section.

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