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Optimized Design of Modular Constructed Wetland for Treating Rural Black–Odorous Water

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Abstract: In recent years, the phenomenon of black–odorous water has occurred frequently, and constructed wetlands have been widely used as an effective means of treating black–odorous water. In order to achieve the goal of low-carbon and high-efficiency long-term clean-up of black–odorous water, the modular constructed wetland system was optimized in this study. The optimized modular constructed wetland consisted of aeration, denitrification, and phosphorus removal, of which the denitrification module was a sulfur–iron autotrophic denitrification unit and the phosphorus removal module was a polyaluminum chloride composite filler phosphorus-removal unit. Experimental findings indicated that modular systems with layout ratios of 1:3:1 (A) and 1:2:2 (B) exhibit outstanding performance in remediating contaminants from black–odorous water. Notably, system B demonstrated superior treatment efficiency. Under conditions of high pollution loading, system B consistently achieved stable removal rates for COD (95.79%), TN (91.74%), NH_4^+-N (95.17%), and TP (82.21%). The combination of along-track changes and high-throughput sequencing results showed that the synergies among the units did not produce negative effects during the purification process, and each unit realized its predefined function. Changes in the substrate and internal environment of the wetland units caused changes in the microbial populations, and the unique microbial community structure of the units ensured that they were effective in removing different pollutants.

Keywords: black–odorous water; modular constructed wetland; microorganisms; pollutant removal

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1. Introduction

With the rapid development of the social economy, the state and people attach great importance to the protection of the ecological environment. Water pollution control has become the focus of environmental protection at present. Among various current concerns, the phenomenon of black–odorous water is widely considered a serious problem in water pollution prevention and control. The black–odorous phenomenon is a water pollution problem in which water bodies are contaminated, resulting in a black color and an unpleasant smell. Black–odorous water is common in developing countries and developed countries [1], especially in rural areas. Many places lack suitable sewage treatment facilities [2], which leads to a large amount of untreated sewage entering the natural environment. The contents of N, P, and water-soluble organic matter in water far exceed the self-purification ability of water bodies, algae multiply in large quantities, dissolved oxygen in water is consumed in large quantities, and the water ecosystem is destroyed [3]. Some studies have shown that when the concentration of organic matter reaches 1.0 g/L, the water will turn black [4]. Additionally, organic matter, nitrogen, phosphorus, heavy metals, and other pollutants deposited at the bottom of bodies of water contribute to endogenous pollution sources, exacerbating the black odor phenomenon upon re-release from sediments [5].

Black–odorous water has a serious impact on the normal life and health of residents and poses a threat to the local ecosystem, causing widespread concern among the masses, which is a prominent environmental problem around the masses [6]. Compared with urban black–odorous water, the causes of black–odorous water in rural areas are complicated, involving multiple factors, such as domestic sewage, domestic garbage, livestock and poultry breeding, planting pollution, and so on [7]. It is also more difficult to manage because the majority of black–odorous water is in static or circulating states in the form of pits, ditches, tiny rivers, etc. Furthermore, the area is limited, the hydrodynamic conditions are poor, and the water cycle's power is insufficient [8]. A challenging issue that needs to be resolved quickly is how to preserve the long-term efficacy of governance outcomes in addition to governance challenges.

Commonly used methods for treating black–odorous water, including artificial aeration, sediment dredging, microbial-enhancement technology, and constructed wetlands, exhibit varying effectiveness under different conditions [1]. Among these, constructed wetlands emerge as a promising option due to their green and sustainable ecological water-purification technology, offering low investment, low energy consumption, and convenient operation and maintenance [9]. However, traditional constructed wetlands face challenges, such as poor nitrogen-removal performance, lengthy construction periods, and high construction costs, limiting their application in rural areas [10]. Therefore, in order to meet the needs of the treatment of black–odorous water in rural areas, a new type of modular constructed wetland came into being. The modular constructed wetland (MCW) simplifies the traditional laborious and complicated civil engineering by prefabricating modular structures, which can be assembled off-site and installed on-site. This forward-looking method improves the removal efficiency of pollutants, reduces land use, and solves the blockage problem by quickly replacing modules. It is a cost-effective and environmentally friendly wastewater-treatment technology [11]. Choi et al. studied the pollutant-removal efficiency of modular horizontal subsurface flow (HSSF) and found that the system removed more than 90% of all total suspended solids and more than 50% of both total phosphorus (TP) and Zn [12]. Cong also found that the modular constructed wetland has an excellent treatment effect on rural sewage, and the average removal rates of NH_4^+-N, total nitrogen (TN), and TP are 70%, 66.9%, and 68.3% [13]. From the current study, it can be found that the modularly constructed wetland has a better pollutant-removal effect compared to the conventional constructed wetland, but some metrics still do not meet the treatment expectations, and the denitrification effect needs to be strengthened. Moreover, most studies of modular constructed wetlands at this stage have focused on structural and operational conditions and have not investigated in depth the optimization of the combination of different substrate modules within a modular constructed wetland system.

Therefore, based on a comprehensive analysis and experimental data, this study combined an aeration unit, a nitrogen-removal unit, and phosphorus-removal unit to construct a new modular constructed wetland system. It also aimed to determine the unit arrangement order of aeration unit → sulfur–iron autotrophic denitrification unit → phosphorus-removal unit with polyaluminum chloride composite filler. In order to maximize the purification efficiency of each unit, two systems, A and B, were constructed according to the experimental data of each unit. A comparative analysis of two systems, A (1:3:1) and B (1:2:2), was conducted to select the wetland unit system with a more reasonable layout based on the removal efficiency of COD, TN, TP, and NH_4^+-N. Additionally, microbial colonies in the wetland system were measured to assess the microbial situation of each unit, providing further insights into the optimization effect of the modular constructed wetland and the rationality of unit arrangement.

This study aims to furnish a theoretical foundation and engineering reference for the treatment and widespread adoption of modular constructed wetlands in addressing black–odorous water issues in rural areas.

2. Materials and Methods

2.1. Material

The sludge inoculated in the experimental system was sourced from the Wulongkou Water Branch in Zhengzhou City, Henan Province, China. The aeration module utilizes ceramsite (8–10 mm) as the matrix, derived from the preliminary experimental outcomes of our research group. In the denitrification module, the design is grounded in the principles of sulfur autotrophic denitrification, employing a matrix consisting of pyrite (2–4 mm), volcanic rock (5–8 mm), and bluestone (12–15 mm). These three fillers were procured in Luoyang City and Zhengzhou City, Henan Province, China, and combined in a volume ratio of 4:3:3. The matrix of the phosphorus-removal module is polyaluminum chloride composite filler, which comes from the early development results of the laboratory. The wetland's packing configuration is illustrated in Figure 1. The reagent purity in the experiment adheres to standard requirements.

Figure 1. Wetland fillers, in order of ceramic, volcanic rock, lapis lazuli, sulfurous iron ore, and polyaluminum chloride composite filler.

2.2. Simulated Wastewater

The experiment's stability is ensured by simulating the black–odorous water in rural areas using laboratory water distribution. High concentrations of nutrients and low concentrations of dissolved oxygen (DO) are the usual physical and chemical features of black–odorous water. Primary sedimentation tanks are mostly used to remove solids in wastewater treatment, and they are considered the basic part of the combined operation of biological and sludge-treatment processes. At present, conventional constructed wetlands are all equipped with pre-treatment units, which can fully remove suspended particles and have little influence on subsequent filler treatment [14]. And, there have been many improvements in sedimentation tanks at home and abroad, which can effectively remove suspended solids [15,16]. A comprehensive analysis of the existing research on constructed wetlands shows that the removal rate of high-concentration pollutants in black–odorous water is poor; some indicators still cannot meet the treatment expectations, and the nitrogen-removal effect needs to be strengthened. Therefore, the research goal of this paper is to improve the removal rate of common high-concentration pollutants.

In the preliminary study, we investigated a rural black–odorous water in Xingyang City, Henan Province, China, with water quality indicators of 102.7 ± 5.9 mg/L COD, 16.22 ± 3.71 mg/L TN, 9.73 ± 4.61 mg/L NH_4^+-N, 8.61 ± 1.98 mg/L NO_3^--N, 2.98 ± 0.37 mg/L TP, and 60.17 ± 0.17 mg/L SO_4^{2-}. Therefore, with reference to the data from this survey, and in conjunction with the different levels of pollution suffered by rural black–odorous water, the experiment simulated two types of synthetic water, each representing different pollution loads, as detailed in Table 1. The synthetic wastewater was composed of key components, including $(NH_4)_2SO_4$, $CH_3COONa{\cdot}3H_2O$, KNO_3, $CO(NH_2)_2$, and KH_2PO_5, with specific dosage information provided in Table 2. To supplement trace elements in the water, $FeSO_4$, $ZnSO_4$, $MgCl_2$, $CuSO_4$, and H_3BO_3 were incorporated. These measures aimed to accurately replicate the diverse composition and pollutant loads observed in rural black–odorous water, thus ensuring the experiment's robustness and reliability.

Table 1. Experimental inlet water quality.

Water Quality Index	COD (mg/L)	TN (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	Organic Nitrogenous (mg/L)	TP (mg/L)
Low	200	25	15	7	3	4
High	400	51	30	15	6	7

Table 2. Dosage of chemicals.

Chemicals	$CH_3COONa{\cdot}3H_2O$ (g)	$(NH_4)_2SO_4$ (g)	KNO_3 (g)	$CO(NH_2)_2$ (g)	KH_2PO_5 (g)
Low	29.7625	4.949	3.5385	0.4513	1.1923
High	59.535	9.9	7.5825	0.9026	2.1496

2.3. System Construction

Two laboratory-scale modular constructed wetland systems, designated as A and B, were constructed at the Joint Laboratory of Zhengzhou University, China, using prior findings of the study group [17]. The modular constructed wetland in this study was divided into three parts, namely, the aeration unit, the sulfur–iron autotrophic denitrification unit, and the polyaluminum chloride composite filler phosphorus-removal unit. The experimental setup is schematically shown in Figure 2.

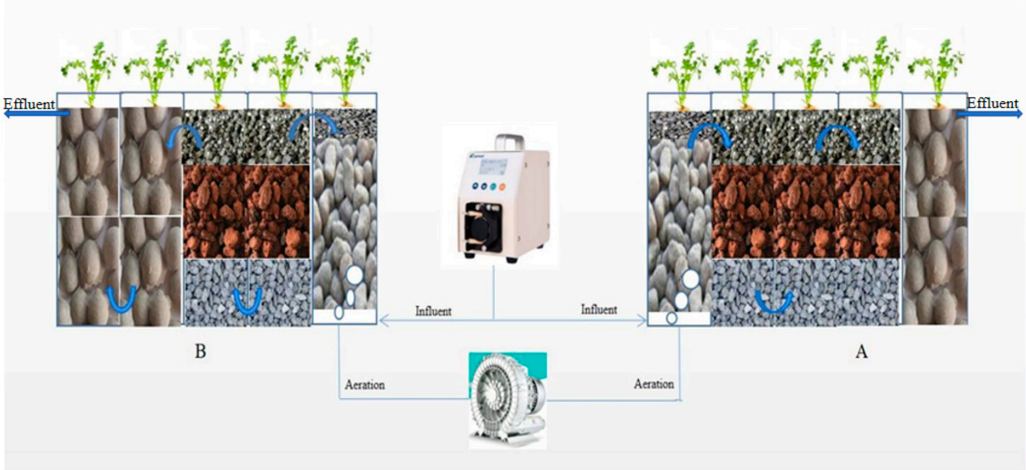


Figure 2. Schematic diagram of experimental device (The aeration, nitrogen-removal, and phosphorus-removal modules in the two systems is A (1:3:1) and B (1:2:2), respectively).

The aeration module employs a PVC column reactor with an inner diameter of 150 mm and a height of 36 cm. The bottom is equipped for aeration and oxygenation and interconnected to the subsequent module via plastic connecting pipes. The nitrogen- and phosphorus-removal modules are constructed from transparent plexiglass, measuring 57.6 cm in length, 27.5 cm in width, and 36 cm in height. The device is compartmentalized into four units utilizing the folded plate within the middle of the apparatus, creating a baffling state for water within the device. In this experiment, the arrangement ratio of the aeration, nitrogen-removal, and phosphorus-removal modules in the two systems is A (1:3:1) and B (1:2:2), respectively. The water enters at 3 cm from the bottom of the aeration module, and the outlet is positioned at 28 cm within the phosphorus-removal module. The water flow direction is depicted in Figure 2. The filling height of each module is 30 cm, and the bottom bearing layer comprises gravel (8–10 mm, h = 2 cm).

The water celery planted in both systems had the same size and growth. The experiment started in May 2022, and the temperature was 25–35 °C. The experiment is divided into three stages: the start-up stage, the low-pollution load stage, and the high-pollution load stage. The system residence time (HRT) was 24 h.

2.4. Sampling Analysis

2.4.1. Sampling and Analysis of Water

The experiment sampled and analyzed the influent and effluent of the two modular wetland systems every three days at 8:00 a.m. First, 500 mL samples were taken from each sampling port, and the experiment lasted for a total of 120 days. Using a portable water-quality analyzer (DZB-712, LEICI, Shanghai, China), the pH, DO, and temperature were measured. All water quality indicators were tested using standard laboratory methods. The COD was detected using a COD detector (DR 1010, HACH, Loveland, CO, USA). The NH_4^+-N was determined using Nessler's reagent spectrophotometry. The TN was determined through alkaline potassium persulfate digestion and ultraviolet spectrophotometry. And, the TP was detected through the potassium persulfate oxidation–ultraviolet spectrophotometer method [18].

2.4.2. Sampling and Analysis of Microorganisms

After the actual operation of the two systems for a period of time, the pollutant removal and changes along the way of the two systems were compared and analyzed, and the microbial situation in each unit of system B was selected for analysis. Samples were taken from the inoculated sludge, the aeration unit, the denitrification unit, and the phosphorus-removal unit of the system on the substrate surface of the biofilm, which are named S1, M1, M2, and M3, respectively. In the sampling process, multi-point mixed sampling was adopted for the same unit to avoid accidental errors as much as possible. After sampling, the samples were stored in sterile plastic bags and sent to Shanghai Shenggong Bioengineering Co., Ltd. (Shanghai, China) for Qualcomm analysis and determination through dry-ice preservation.

GDNA was extracted using an extraction kit, and then the target sequence was enriched using highly specific primers. Finally, the data obtained through sequencing were analyzed through bioinformatics. PCR carried out two rounds of amplification. In the first round of amplification, the genomic DNA was accurately quantified using the Qubit3.0 DNA detection kit to determine the amount of DNA added in the PCR reaction. The primer 341F/805R(CCTACGGGNGGCWGCAG/GACTACHVGGGTATCTAATCC) used in PCR was fused with the primer 16SV3-V4 of the sequencing platform [19].

2.5. Statistical Analyses

All data in this experiment were collected and sorted using Excel 2016. The data were analyzed using SPSS 26 with a significance level of 0.05. Origin 2021 was used for the painting. The pollutant-removal efficiency of the constructed wetland system was calculated using the formula

$$\text{Removal efficiency} = \frac{\text{Ci} - \text{Ce}}{\text{Ci}} \times 100\% \quad (1)$$

where Ci and Ce are inlet and outlet concentrations in mg/L.

3. Results and Discussion

3.1. Construction of Wetland System Units

The research on rural black–odorous water indicates that insufficient dissolved oxygen and excessive nitrogen and phosphorus pollution are primary contributors to the phenomenon. A comparative analysis of water-pollution control and remediation technologies reveals that aeration technology significantly enhances water quality and improves pollutant-removal efficiency in constructed wetland systems [20,21]. Therefore, based on aerobic aeration techniques, this experiment constructed a bottom-aerated wetland with ceramics as a substrate. The experimental results showed that COD and NH_4^+-N were removed above 80% in the aeration unit when HRT was higher than 12 h but only about 20–30% for TN and TP. Therefore, nitrogen- and phosphorus-removal units were added later to ensure purification.

Traditional constructed wetland processes often fall short in denitrifying sewage. Low-valent sulfur in iron sulfides, such as FeS_2 and FeS, can serve as an electron donor for sulfur autotrophic denitrification, thus enabling long-term deep nitrogen removal [22]. Moreover, autotrophic denitrification is especially suitable for treating the shortage of biodegradable organic matter in the tail water of sewage treatment plants. It has the advantages of not needing to add a carbon source, a low sludge yield, a low operating cost, no secondary pollution in the effluent, a stable N_2 product, and so on, and it has little impact on the actual environment [23]. In this experiment, an autotrophic denitrification system was constructed with pyrite as the electron donor and bluestone as the acid–base-balance-regulating material. The highest removal rates can be achieved above 90% under the conditions of elevated TN and NO_3^--N of 12.88 mg/L and 7.73 mg/L, respectively. And, the pH of the system remains stable for a long time under the action of bluestone, which ensures the denitrification effect of the system.

The sulfur–iron autotrophic denitrification system effectively addressed the issue of black and odorous water in rural areas due to excessive nitrogen. The aeration unit supplied sufficient dissolved oxygen, promoting the enrichment of organic degradation bacteria and nitrifying bacteria. This transformation of NH_4^+-N into nitrate nitrogen, along with COD degradation, reduced the pressure on subsequent denitrification units and provided nitrate for sulfur autotrophic denitrification. The sulfur-autotrophic-denitrifying bacteria in the denitrification unit converted nitrate nitrogen into nitrogen, thus achieving deep denitrification and enhancing nitrogen removal efficiency. Finally, a phosphorus-removal unit, based on a polyaluminum chloride composite filler, was added to improve phosphorus-removal efficiency.

The phosphorus-removal unit utilized a polyaluminum chloride composite filler, a prior research outcome of the group [17]. The composite non-burning material, made of PACR, slag Portland cement, and bentonite, demonstrated excellent phosphorus-adsorption capacity in constructed wetlands. Experimental results showed a 90% removal rate, reducing the phosphorus concentration from 1 to nearly 0.1 mg/L. This filler has been successfully applied in engineering, with a monitored removal rate reaching 84.36% at Qingyuan Wetland Park over 306 days. In addition, the research group analyzed the leaching toxicity of heavy metals in the composite filler and found that only trace amounts of Pb, Cd, and Cr were detected, and their concentrations were far below the limit of the identification standard for hazardous wastes, which would not cause secondary pollution to the water body in practical applications.

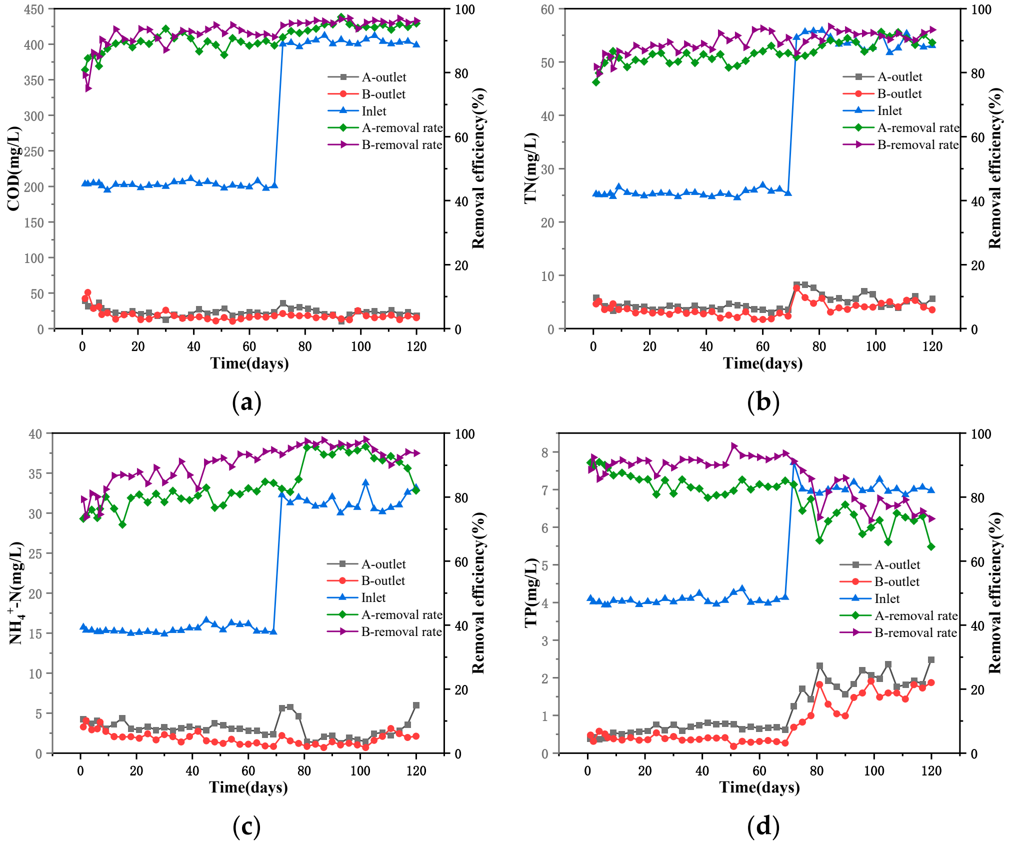
Following a comprehensive analysis and study of the three unit modules, the sequential arrangement was determined as follows: aeration unit → sulfur–iron autotrophic denitrification unit → phosphorus-removal unit with polyaluminum chloride composite filler.

The primary purpose of the aeration unit is to provide sufficient dissolved oxygen for the system. Nitrogen and phosphorus removal, however, require specific anaerobic and anoxic conditions, making a single aeration unit adequate for dissolved oxygen needs in the experiment. Considering the excellent adsorption effect of the PAC composite filler, one or two units can meet phosphorus-removal requirements [17]. Hence, this study focused on the number of nitrogen-removal units, and two systems, A and B, were selected for comparative analysis. The corresponding proportions of the aeration, denitrification, and dephosphorization modules are A (1:3:1) and B (1:2:2), respectively.

3.2. Contaminates' Removal

3.2.1. Removal of COD

In the experimental period, modular constructed wetland systems A and B were subjected to high and low pollution loads (Figure 3). The average influent COD during the low-pollution-load phase was 200 ± 7.8 mg/L. After the unit's start-up, the COD-removal rate steadily increased, stabilizing at around 90% after 15 days, indicating a successful initiation of the unit. Under this pollution load, the removal rates for wetland systems A and B were essentially stable at 89.62 ± 4.1% and 91.86 ± 3.0%, respectively. In the high-pollution-load phase, with an average influent COD of 401.4 ± 4.9 mg/L, the average removal rates were 94.23 ± 3.16% and 95.79 ± 1.05% for systems A and B, respectively. The COD-removal rates of both systems did not exhibit significant changes with the increase in the influent pollution load. Figure 3a illustrates the purification effect on COD, with system B showing slightly better performance than system A, though the difference is small.



(**a**) (**b**)

(**c**) (**d**)

Figure 3. Contaminant-removal performance of constructed wetland under different pollution loads: (**a**) COD; (**b**) TN; (**c**) NH_4^+-N; (**d**) TP.

The variation of the COD-removal rate with the pollution load in Figure 3a shows that the purification effect of the pollution load is not greatly affected. This is also consistent with the current mainstream research results, and the increase in the influent pollution load intensity will not affect the degradation of COD in two modular constructed wetland systems [24]; on the contrary, under the high pollution load, the COD-removal effect of the A and B modular constructed wetland systems was improved to some extent [25]. Presently, in research on treating black–odorous water in rural areas, where the COD of most water bodies falls between 100 and 500 mg/L, modular constructed wetlands exhibit robust impact-load resistance during the purification of black–odorous water in rural areas. They maintain a high COD-degradation rate even under high pollution loads.

3.2.2. Removal of TN

Figure 3b illustrates the variation in the TN (total nitrogen) concentration in two modular constructed wetlands under different pollution loads. It is evident from the figure that the TN-removal rates of both systems increased during the initial stages of start-up. In the low-pollution-load stage (TN = 25 mg/L), the average TN-removal rates for modular constructed wetlands A and B were 84.09 ± 0.19% and 88.02 ± 5.75%, respectively. The average TN concentrations in the effluent reached 4.0 ± 1.02 mg/L and 2.97 ± 1.77 mg/L, respectively. As the influent load increased to TN = 51 mg/L, both systems demonstrated a further improvement in removal rates, stabilizing at approximately 90%. The average removal rates for systems A and B were 89.21 ± 1.92% and 91.47 ± 1.88%, respectively. System B exhibited slightly better TN removal, which was similar to the pattern observed for COD, with no significant difference between them.

In traditional constructed wetlands, TN-removal rates often decrease with increasing influent load, affecting pollutant-removal efficiency [26]. This is attributed to the elevated pollution load leading to increased oxygen demand during the aerobic decomposition of organic matter. This, in turn, weakens the position of nitrifying bacteria, thus inhibiting the nitrification process and resulting in reduced total nitrogen-removal rates [27]. In contrast, the modular constructed wetland system, equipped with an aeration device at the front end, ensures sufficient dissolved oxygen for subsequent nitrification reactions. This design facilitates the normal operation of subsequent reactions, contributing to excellent nitrogen removal. As water enters the sulfur–iron autotrophic denitrification unit, pyrite serves as an electron donor for the denitrification reaction. Additionally, sulfur autotrophic denitrification bacteria attached to the substrate, with a large specific surface area, ensure the effective denitrification performance of the system.

3.2.3. Removal of NH_4^+-N

Figure 3c displays the concentration and removal rates of ammonia and nitrogen in the inlet/outlet water of the constructed wetland under different pollution loads. In the low-contamination-load phase, with NH_4^+-N at 15 mg/L, both systems exhibited an average removal rate that initially ranged around 70–80% during the early start-up phase, which was relatively low. As the operational time increased, the internal removal rate of both modular constructed wetland systems also improved, stabilizing above 80% after 15 days of official start-up. System A achieved an average removal rate of 80.7 ± 4.1%, while system B exhibited a higher removal rate at 89.37 ± 5.16%. The concentration of ammonia nitrogen in the effluent was 3.24 ± 0.96 mg/L for system A and 2.03 ± 1.23 mg/L for system B. When the influent ammonia nitrogen concentration increased to 30 mg/L, the removal rate of effluent ammonia nitrogen temporarily decreased and then gradually returned to normal. The average removal rate of NH_4^+-N in both modular constructed wetland systems A and B exceeded 90%. However, towards the end of the high-pollution-load stage, the effluent ammonia nitrogen increased in system A. The average effluent ammonia nitrogen concentration rose from 1.64 mg/L (81–102 days) to 3.25 mg/L (103–120 days). System B also experienced a slight increase in effluent ammonia nitrogen concentration, but the rate of increase was relatively low. A comparison of substrate conditions in each unit revealed a slight obstruction on the substrate surface of the aeration unit due to pollutant accumulation, which may explain the reduced removal rate at the end of the operation.

Ammonia nitrogen removal in constructed wetlands primarily relies on the absorption of nitrifying bacteria and plants [28]. The nitrification process, requiring substantial oxygen, competes with organic degradation bacteria [29]. In this experiment, dissolved oxygen was supplemented by the aeration device at the front end, and the water flow state was altered using a folding plate to ensure sufficient contact between the water body and the substrate in each unit. This continuous supply of dissolved oxygen enhances the activity of nitrifying bacteria, thus allowing the modular constructed wetland system to maintain excellent removal efficiency during the purification process of black–odorous water in rural areas under high-pollution-load conditions. This addresses the shortcomings of traditional constructed wetlands in this regard, meeting the treatment requirements for black–odorous water in rural areas with a superior nitrogen-removal effect.

3.2.4. Removal of TP

As depicted in Figure 3d, under low-pollution-load conditions, the average TP-removal rates for the two systems were 85.52 ± 5.4% and 90.88 ± 3.24%, respectively. The effluent concentration of system B was consistently lower than 1 mg/L, with the lowest reaching 0.17 mg/L. In contrast to other pollutants, the TP-removal rate reached 90% after the ninth day of start-up. However, with the increase in the influent load, the purification efficiency of both systems declined to varying degrees, and the decline was more noticeable with continuous device operation. System B maintained an average removal rate of 82.21 ± 9.0%, while system A was more affected, exhibiting the lowest removal rate at only 64.49%.

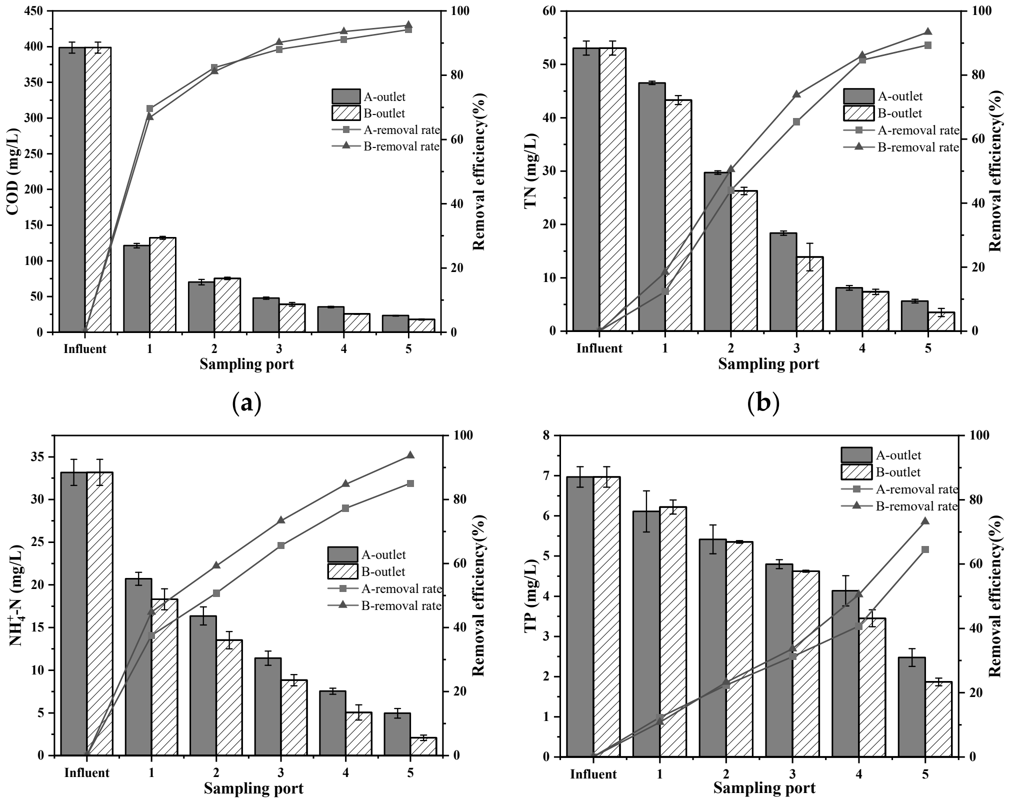
Upon analyzing the trends of total phosphorus in the inlet and outlet water of the two systems, it is evident that the phosphorus-removal effect of the modular constructed wetland is not consistently stable at the laboratory scale. System B consistently demonstrated excellent removal efficiency throughout the entire operation process, with removal rates higher than those of system A except during the initial start-up. Under high pollutant load, both systems experienced a decrease in removal rates, aligning with previous research trends [30]. Traditional constructed wetlands often exhibit less than 20% removal in high-pollution phases, and even systems with optimized substrates achieve only around 50%. This phenomenon may be linked to the mechanisms of phosphorus removal in constructed wetlands, where phosphorus removal primarily relies on plant absorption, microbial action, and substrate adsorption [31]. In this experiment, the removal of TP from sewage achieved excellent results at the initial stage of wetland operation. The reason for this phenomenon is that phosphorus in water was enriched on the surface of the substrate due to the adsorption of the substrate. With the increase in the operation time and the pollutant concentration, the adsorption capacity of the substrate and the release of calcium and aluminum plasma decreased to some extent, which led to the increase in the TP concentration in the effluent [32]. Nonetheless, under the combined action of plants, microorganisms, and substrates, the modular constructed wetland system still exhibited a superior phosphorus-removal effect compared to traditional constructed wetlands.

3.2.5. Variation in Pollutants along the Route

To explore the removal laws of pollutants in modular constructed wetlands, sampling ports were set up at the end of each unit's water flow. The opposite positions of the sampling ports 1, 2, 3, 4, and 5 are, for system A, the aeration unit, denitrification unit 1, denitrification unit 2, denitrification unit 3, and phosphorus-removal unit 1. For system B, they are the aeration unit, denitrification unit 1, denitrification unit 2, phosphorus-removal unit 1, and phosphorus-removal unit 2. The high-pollution-load phase was chosen for a comprehensive study of the variation in pollutants along the treatment pathway, as illustrated in Figure 4.

Following treatment of experimental wastewater using wetland systems, COD-removal rates exceeded 90% (Figure 4a). The primary contributor to COD removal was the aeration unit, largely influenced by the metabolic activities of aerobic organic degradation bacteria [33], which thrive in oxygen-rich environments. However, as wastewater progressed to the denitrification unit, COD-removal efficiency may decline due to reduced dissolved oxygen levels and competition from nitrifying bacteria. This observation is supported by COD-removal trends at subsequent sampling ports. Notably, the removal efficiencies of systems A and B remained relatively stable in subsequent modules, which is attributed to prior oxygen depletion by organic degradation and nitrifying bacteria in the preceding units.

The removal of TN reached 89.4% and 93.4% in systems A and B, respectively (Figure 4b), and the removal of NH_4^+-N was 85.08% and 93.72% (Figure 4c). Both total nitrogen and ammonia nitrogen concentrations decreased significantly, facilitated by the aerobic conditions in the aeration unit, where nitrifying bacteria converted ammonia nitrogen to NO_3^--N while denitrification was partially suppressed [34], which explains the large amount of ammonia nitrogen removed in this unit and the low decrease in total nitrogen. After the wastewater entered the denitrification unit, the dissolved oxygen concentration gradually decreased, and an anaerobic environment was formed inside of the wetland, which resulted in deep denitrification. The corresponding unit at A4 was the denitrification unit, while B4 was the phosphorus-removal unit, and the difference in the composition of the substrate led to the difference in the removal rate. However, from the point of view of the overall nitrogen removal of the system, the nitrogen-removal efficiency of the A4 and B4 units, although there is a certain difference, did not have a significant impact on the overall nitrogen-removal effect of the modular constructed wetland system. However, by comparing the concentrations of TN and NH_4^+-N in the fourth outlet of system A and system B, it can be seen that NH_4^+-N accounts for a large proportion of TN, and the relatively low NO_3^--N cannot make denitrification unit 3 in system A sufficiently play its role. This also shows that two denitrification units can meet the purification requirement.



(**a**) (**b**)

(**c**) (**d**)

Figure 4. Contaminant-removal performance of pollutants in each unit of the wetlands: (**a**) COD; (**b**) TN; (**c**) NH_4^+-N; (**d**) TP.

Figure 4d illustrates similar TP-removal rates and speeds in the initial three units of systems A and B. However, at sampling port 4, system B demonstrated significantly higher TP-removal efficiency (50.49%) compared to system A (40.66%). Final effluent TP concentrations were 2.48 mg/L and 1.87 mg/L, with removal rates of 64.49% and 72.21%, respectively. System B's superior phosphorus-removal efficiency is attributed to the use of polyaluminum chloride residue (PACR) composite filler in the phosphorus-removal unit, thus enhancing the chemical-adsorption capacity.

The main difference between experimental systems A and B studied in this paper is that the last two modules are set differently; therefore, it was expected that the results obtained at the outlet of the third module would be the same in both systems. However, according to the results shown in Figure 4, a difference was found to occur at the aeration module, which could be due to a difference in sludge domestication, but it can also be seen that the difference is smaller. Among the along-track changes in ammonia nitrogen, it was found that the difference in nitrification that occurred in the aeration module remained largely the same in the rest of the subsequent modules, which also proves that an additional denitrification unit has less of an impact on the overall removal of ammonia nitrogen. Observing the along-track variation in total phosphorus, the removal rate of the first three module units was similar, but the difference was small, and the phosphorus-removal efficiency increased significantly from the fourth unit, which proved the reasonableness of the system B arrangement.

In summary, the removal efficiency of COD and TN in system B was better than that of system A, but it was not significantly different. However, system B significantly outperformed system A in NH_4^+-N and TP removal, demonstrating greater stability and resilience to external influences. The presence of two denitrification and two phosphorus-removal units in system B ensures efficient nitrogen and phosphorus removal, thus validating the system's design. System B designed in this paper has also been applied to a village environmental improvement project in a city as a water-quality-purification facility to remediate the pit pond behind the temple. The modular constructed wetland design size is 10 m × 3 m × 1.5 m and the design scale is $30\text{m}^3/\text{d}$. It is divided into an aeration area, a denitrification area, and a phosphorus-removal area. The project effectively solves the problems of black odor and poor water mobility in the pit pond so that the water environment can be restored. The purified black and odorous water can also be reused for local irrigation areas through unified and reasonable deployment, which is also an application to promote the sustainability of the irrigation system through the implementation of the reclaimed wastewater system [35]. A comprehensive analysis shows that system B is more suitable for purifying the black–odorous water in rural areas, which can improve the effluent quality and promote the recovery of the internal circulation system of the water body, effectively improve its pollution capacity, and achieve the goal of "long-term clean-up" in the process of solving the phenomenon of black–odorous water in rural areas.

3.3. Bacterial Community Diversity and Composition

To explore the pollutant-removal dynamics within each unit of the modular constructed wetland, we selected system B, known for its superior purification effectiveness, for high-throughput sequencing analysis. Samples were collected from the aeration, denitrification, and phosphorus-removal units, as well as the inoculated sludge, designated as M1, M2, M3, and S1, respectively. This comprehensive analysis aimed to elucidate the role of microorganisms in the modular constructed wetland concerning species diversity, abundance, and community structure. The insights gained from this investigation will offer theoretical support for understanding the internal response of each unit within the modular artificial wetland system and ensure the synergy of each unit module to avoid the occurrence of antagonistic phenomena.

3.3.1. Alpha Diversity Analysis

The abundance and diversity of bacterial communities in the modular constructed wetland system were analyzed through Alpha diversity. The main indicators in this analysis method are the Chao, Ace, Shannon, Coverage, and Simpson indexes. To make the analysis of high-throughput sequencing results more convenient, an OTU cluster analysis was adopted in the experiment, and the cluster-similarity level was 97%. The values of the specific indexes of the Alpha diversity analysis of each microbial sample are shown in Table 3.

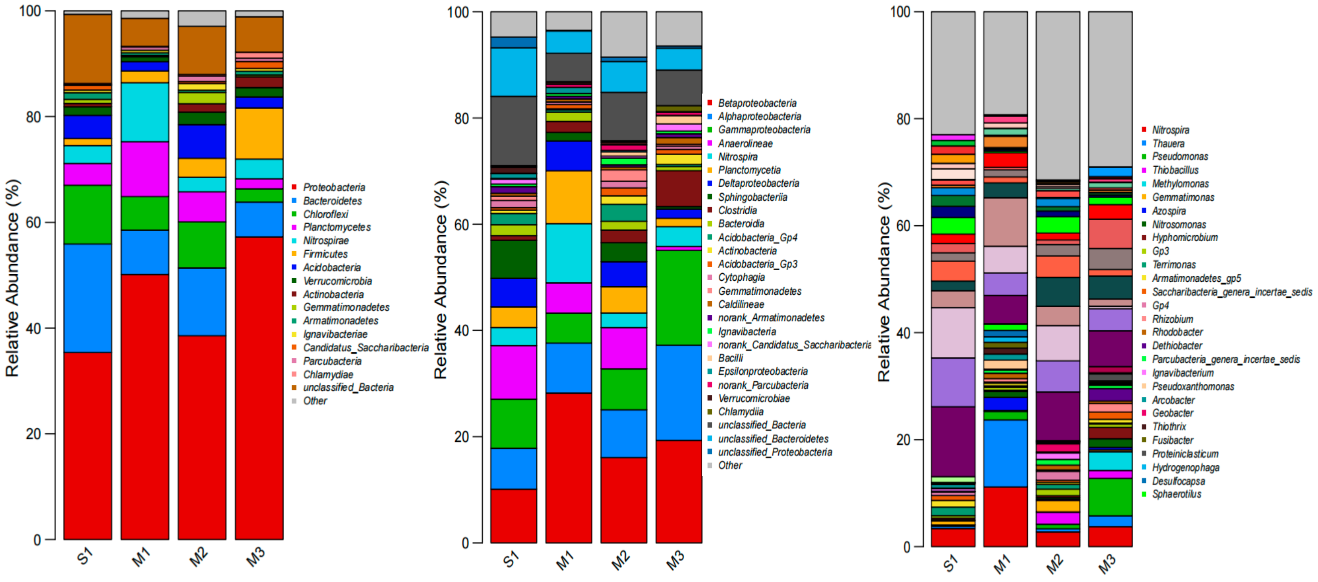
Table 3. Correlation index of Alpha diversity at the OTU level.

Sample ID	OTUs	Shannon	Chao	Ace	Simpson	Coverage
M1	876	5.032578	988.286	965.121	0.22351	0.99678
M2	1247	5.961997	1374.86	1354.36	0.00591	0.99423
M3	624	5.411563	625.200	624.899	0.01161	0.99993
S1	942	5.664302	1087.32	1037.06	0.00852	0.99518

The data in Table 3 reveal that the coverage index for all four samples exceeded 99%, indicating an exceptionally low probability of undetected sample sequences and ensuring the reliability of the test results. Comparing the Chao index values among the samples, M2 > S1 > M1 > M3, suggests that microbial abundance is highest in the nitrogen-removal unit and lowest in the phosphorus-removal unit. Variances in microbial abundance may be attributed to significant differences in dissolved oxygen (DO), pH, electron donors, and the release of metal ions among different units within the wetland system [36]. The Ace index and the Chao index reflected the same situation, further indicating that microorganisms played an essential role in the organic-matter degradation and denitrification process of the modular constructed wetland. The microbial species diversity was negatively correlated with Simpson index and positively correlated with the Shannon index [37], and the specific data of the two indexes also proved that the microbial community diversity of M2 was the highest among the samples. From the above data, it can be seen that as the units in the modular constructed wetland changed, the microbial community also changed, which led to a difference in the removal rate of different pollutants per unit.

3.3.2. Bacterial Community Structure

The microbial community structure is a key item in microbial analysis, and Figure 5 shows the community structure of microbial samples from inoculated sludge as well as different units in the modular constructed wetland at three different levels of "phylum", "class", and "genus", respectively.



(**a**) (**b**) (**c**)

Figure 5. Relative abundance of different CW samples at the phylum (**a**), class (**b**), and genus (**c**) taxonomic ranks.

In Figure 5a, the relative abundance of microbial communities at the phylum level is depicted for the four sample sets, revealing 15 dominant phyla with relative abundances exceeding 1%. Among them, Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Nitrospirae, and Firmicutes dominated the wetland unit. The proportion of Proteobacteria was highest in the four sets of samples, with relative abundances of 35.36%, 50.15%, 38.53%, and 57.23% in S1, M1, M2, and M3. This result is consistent with previous studies [38]. In a related study, it was found that numerous microorganisms involved in the carbon, nitrogen, and sulfur cycles belong to the Proteobacteria, which have a better degradation capacity [39]. In addition, most of the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) also belong to the Proteobacteria. From Figure 5b, it can be seen that the relative abundance of Betaproteobacteria was the highest among the four samples, and the Alphaproteobacteria and Gammaproteobacteria, which also accounted for a large proportion of the samples, also belong to Proteobacteria. Betaproteobacteria contain a variety of bacterial groups that are effective in degrading nitrogenous pollutants in the water body [40]. The relative abundance of this bacteria from S1 (inoculated sludge) was high, and thus the group remained dominant in the subsequent units of the modular constructed wetland. *Thauera*, which was relatively abundant (0.42–12.53%), also belongs to the Proteobacteria, and it is an essential part of activated sludge and plays a vital role in the process of nitrogen removal. This genus is also capable of denitrification and phosphorus removal [41]. Pseudomonas, which had the highest relative abundance at 6.99% in M3, is also a Proteobacteria. This genus can denitrify under aerobic conditions using aerobic denitrifying enzymes, and it can also denitrify under low-oxygen conditions to reduce nitrate [42]. The high relative abundance of Pseudomonas in the phosphorus-removal unit demonstrated that the unit also had some denitrification effect, further justifying the unit arrangement.

Most Bacteroidetes are chemotrophic heterotrophic bacilli that mostly survive in anaerobic environments, and they are effective at degrading organic matter. Bacteria from this phylum are commonly involved in wastewater purification and treatment processes [43]. The bacteria in Chloroflexi are mostly parthenogenetic anaerobes that can use reduced sulfides as electron donors for non-oxygen photosynthesis. These bacteria are involved in the cycling of C, N, and S, and they can survive under high pollution loads. Anaerobic ammonia oxidation refers to the direct conversion of ammonia and nitrogen in water into nitrogen gas under anaerobic conditions, with ammonia and nitrite acting as the electron donor and the acceptor, respectively. Anaerobic ammonia-oxidizing bacteria are the main participant in the process. It is a representative bacterium in Planctomycetes.

Nitrospirae is also one of the phyla with nitrogen-removal capacity in constructed wetlands [44], which can further convert NH_4^+-N into nitrate nitrogen in aerobic environments. Related studies have demonstrated a significant relationship between Nitrospirae and nitrification efficiency, with differences in DO concentration resulting in a considerably larger abundance of this phylum in the M1 sample than in the other phyla. This is the reason for the substantial decrease in NH_4^+-N in the effluent of the aeration unit and the lower TN removal. The genus *Nitrospira*, which is typical of the Nitrospirae phylum, also had the highest relative abundance share in the system (Figure 5c), and it plays a key role in nitrification [45]. The M2 samples were taken from the denitrification unit, which carries out a predominantly sulfur-autotrophic denitrification reaction. This is the reason why it has the lowest relative abundance among the four sets of samples. The variation in the relative abundance of this genus across the sample also validates previous analyses of the along-range orbital variations of TN and NH_4^+-N.

Firmicutes can use nitrate nitrogen in the water column as an electron donor to complete the denitrification process in anaerobic environments, which further promotes the denitrification process in modular constructed wetlands [46]. Among them, the relative abundance of Clostridia in each unit showed an upward trend and was higher than that in inoculated sludge (Figure 5b). In this experiment, the iron sulfide in the nitrogen-removal unit and the composite filler in the phosphorus-removal unit could provide some iron ions to the system, and it was found that the iron atoms could promote the growth of microorganisms of Clostridia. A higher relative abundance of Clostridia can promote nitrate removal [47]. Similarly, Fe^{2+} has a significant promoting effect on *Thiobacillus*, resulting in its enrichment within the denitrification and phosphorus-removal system units. *Thiobacillus* is a common genus of microbial organisms in the sulfur-autotrophic denitrification process, which can utilize reduced sulfur as an electron donor for the conversion of nitrate nitrogen or nitrite nitrogen into N_2. It can also act as a Fe^{2+} oxidizing mediator of coupled NO_3^--N reduction [48]. In a related study, it was found that *Hyphomicrobium* can effectively degrade DMS and others in water, which can help to solve the problem of black–odorous water in rural areas [49].

In summary, Proteobacteria (35.36–57.23%) were the phylum with the highest relative abundance at the “phylum” level for all units in the system. The dominant phyla, such as Bacteroidetes (6.59–20.54%), Chloroflexi (2.50–11.13%), and Nitrospirae (3.39–11.15%), were the next most abundant. From the analysis at the “class” level, it was found that the relative abundance of Alphaproteobacteria, Proteobacteria, Gammaproteobacteria, and Deltaproteobacteria in each group of samples was high. From the "genus" level, *Nitrospira*, *Thauera*, *Pseudomonas*, and *Thiobacillus* are relatively abundant. The results of high-throughput sequencing showed that nitrifying bacteria and denitrifying bacteria were abundant in the wetland system, nitrification and denitrification were the main denitrification processes in the wetland, and wetland fillers also promoted the growth and reproduction of microorganisms to some extent. The analysis of the distribution of *Nitrospira*, *Thiobacillus*, and other bacteria in each group of samples can verify the previous judgment from the analysis of TN and NH_4^+-N along the way. Overall, the experimental results affirm the rationality of the wetland units' arrangement, showcasing their collaborative and synergistic efficacy in treating rural black–odorous water.

4. Conclusions

At this stage, modular constructed wetland research mostly focuses on operation and construction, while this study takes different combinations of unit module types as the starting point to optimize the modular constructed wetland system. In this study, two types of modular constructed wetland systems, A and B, were constructed to purify rural black–odor water. The findings demonstrate that both systems exhibit exceptional performance in handling different pollution loads associated with rural black–odorous water. A comparative analysis revealed that system B outperformed system A, displaying higher removal rates for various pollutants. Particularly, under conditions of high water-intake load, system B maintained a consistent removal rate with values of 95.79%, 91.74%, 95.17%, and 82.21% for COD, TN, NH_4^+-N, and TP, respectively. The pollutant variations observed across each unit's data further substantiated the rationality of the units' arrangement. The high-throughput sequencing results emphasized the significance of nitrification and denitrification as the primary nitrogen-removal processes in wetlands. Moreover, the wetland fillers were found to contribute to microbial growth and reproduction to some extent. The results of the study confirmed that the modular constructed wetland system has mutual synergy among the units, a reasonable arrangement, and a good effect in purifying black, smelly water. It is also an economical and applicable rural black-smelly-water treatment technology, which provides technical support and a theoretical basis for the future development of rural black-smelly-water treatment and modular constructed wetlands.

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References

1. Cao, J.X.; Sun, Q.; Zhao, D.H.; Xu, M.Y.; Shen, Q.S.; Wang, D.; Wang, Y.; Ding, S.M. A critical review of the appearance of black-odorous waterbodies in China and treatment methods. *J. Hazard. Mater.* **2020**, *385*, 121511. [CrossRef]
2. Gao, S.; Huang, Z.; Xia, X.; Liu, P.; Wang, H.; Li, Y. Investigation and countermeasures of rural domestic sewage treatment. *J. Agric. Resour. Environ.* **2022**, *39*, 276–282.

3. Xu, W.H.; Wang, W.W.; Deng, B.B.; Liu, Q.X. A review of the formation conditions and assessment methods of black and odorous water. *Environ. Monit. Assess.* **2024**, *196*, 42. [CrossRef]
4. Lu, X.; Feng, Z.-Y.; Shang, J.-G.; Fan, C.-X.; Deng, J.-C. Black water bloom induced by different types of organic matters and forming mechanisms of major odorous compounds. *Huan Jing Ke Xue= Huanjing Kexue* **2012**, *33*, 3152–3159.
5. Liu, Y.; Zhou, X.; Huang, Y.A.; Luo, J.W. Study on the present situation and prevention measures of water pollution in a drainage basin in Eastern Guangdong. *Desalination Water Treat.* **2022**, *249*, 174–182. [CrossRef]
6. Yu, S.W.; Bao, J.X.; Ding, W.; Chen, X.; Tang, X.N.; Hao, J.L.; Zhang, W.; Singh, P. Investigating the Relationship between Public Satisfaction and Public Environmental Participation during Government Treatment of Urban Malodorous Black River in China. *Sustainability* **2021**, *13*, 3584. [CrossRef]
7. Xiao, C.; Zhou, D.; Ma, H.; Guo, Y.; Sang, F.; Chang, S. Treatment of Black and Odorous Water Body in Old Urban Areas Based on the Improvement of the Quality and Efficiency of the Sewage System. *China Water Wastewater* **2021**, *37*, 23–27.
8. Xu, W.; Liu, Z.L. Evaluation on the Current Situation of Integrated Treatment of Rural Sewage in China: A Case Study of Anhui Province. *Water* **2023**, *15*, 415. [CrossRef]
9. Zhang, F.; Peng, Y.; Zhang, R. Experimental Study on the Black and OdorousWater Body in Southern Rural Areas by Tidal Flow ConstructedWetland. *Technol. Water Treat.* **2022**, *48*, 114. [CrossRef]
10. Zhu, H.; Yan, B.; Wang, X. Progress in Research and Applications of Constructed Wetlands in China and Suggestions for Future Development. *Bull. Natl. Nat. Sci. Found. China* **2022**, *36*, 391–397.
11. Wei, T.; Zhao, Y.Q.; Zhou, M.W.; Zhang, Z.Y.; Wei, Y.F.; Núñez, A.E. Initial concept and embodiment to develop modular constructed wetland: A unique and promising solution to sustainability transitions in water management. *J. Clean. Prod.* **2024**, *450*, 141912. [CrossRef]
12. Choi, J.Y.; Maniquiz, M.C.; Geronimo, F.K.; Lee, S.Y.; Lee, B.S.; Kim, L.H. Development of a horizontal subsurface flow modular constructed wetland for urban runoff treatment. *Water Sci. Technol.* **2012**, *66*, 1950–1957. [CrossRef] [PubMed]
13. Cong, C.S.; Wang, M.S.; Jin, L.; Ye, T. Design of modular constructed wetland and its effect on rural domestic sewage treatment. In Proceedings of the International Symposium on Energy Environment and Green Development (EEGD), Chongqing, China, 20–22 November 2020.
14. Jover-Smet, M.; Martín-Pascual, J.; Trapote, A. Model of Suspended Solids Removal in the Primary Sedimentation Tanks for the Treatment of Urban Wastewater. *Water* **2017**, *9*, 448. [CrossRef]
15. Wang, P.; Ye, N.; Han, Y.; He, X. Experimental Study on the Sedimentation Performance of an Arc-Plate Linear Sedimentation Tank. *Water* **2024**, *16*, 1075. [CrossRef]
16. Ochowiak, M.; Matuszak, M.; Wlodarczak, S.; Ancukiewicz, M.; Krupinska, A. The modified swirl sedimentation tanks for water purification. *J. Environ. Manag.* **2017**, *189*, 22–28. [CrossRef] [PubMed]
17. Li, Q.; Zhang, J.S.; Gao, J.Q.; Huang, Z.Z.; Zhou, H.X.; Duan, H.Y.; Zhang, Z.H. Preparation of a novel non-burning polyaluminum chloride residue (PACR) compound filler and its phosphate removal mechanisms. *Environ. Sci. Pollut. Res.* **2022**, *29*, 1532–1545. [CrossRef]
18. Wang, W.; Gao, J.; Guo, X.; Li, W.; Tian, X.; Zhang, R. Long-term effects and performance of two-stage baffled surface flow constructed wetland treating polluted river. *Ecol. Eng.* **2012**, *49*, 93–103. [CrossRef]
19. Zhu, T.; Gao, J.; Huang, Z.; Shang, N.; Gao, J.; Zhang, J.; Cai, M. Comparison of performance of two large-scale vertical-flow constructed wetlands treating wastewater treatment plant tail-water: Contaminants removal and associated microbial community. *J. Environ. Manag.* **2021**, *278*, 111564. [CrossRef] [PubMed]
20. Liu, G.; He, T.Y.; Liu, Y.H.; Chen, Z.Y.; Li, L.J.; Huang, Q.Q.; Xie, Z.H.; Xie, Y.F.; Wu, L.S.; Liu, J. Study on the purification effect of aeration-enhanced horizontal subsurface-flow constructed wetland on polluted urban river water. *Environ. Sci. Pollut. Res.* **2019**, *26*, 12867–12880. [CrossRef]
21. Liu, M.S.; Li, T.T.; Wang, Z.C.; Radu, T.; Jiang, H.Y.; Wang, L. Effect of aeration on water quality and sediment humus in rural black-odorous water. *J. Environ. Manag.* **2022**, *320*, 115867. [CrossRef]
22. Ge, Z.; Wei, D.; Zhang, J.; Hu, J.; Liu, Z.; Li, R. Natural pyrite to enhance simultaneous long-term nitrogen and phosphorus removal in constructed wetland: Three years of pilot study. *Water Res.* **2019**, *148*, 153–161. [CrossRef] [PubMed]
23. Tian, T.; Yu, H.Q. Denitrification with non-organic electron donor for treating low C/N ratio wastewaters. *Bioresour. Technol.* **2020**, *299*, 122686. [CrossRef] [PubMed]
24. Zhou, X.; Jia, L.X.; Liang, C.L.; Feng, L.K.; Wang, R.G.; Wu, H.M. Simultaneous enhancement of nitrogen removal and nitrous oxide reduction by a saturated biochar-based intermittent aeration vertical flow constructed wetland: Effects of influent strength. *Chem. Eng. J.* **2018**, *334*, 1842–1850. [CrossRef]
25. Wu, J.-m.; Wang, R.; Xu, D.; Zhang, X.-l.; He, F.; Wu, Z.-b. Long term performance of pilot-scale vertical flow constructed wetland using different substrates. *China Environ. Sci.* **2010**, *30*, 633–638.
26. Hoang, L.; Sukias, J.P.S.; Montemezzani, V.; Tanner, C.C. Quantifying the Nitrogen-Removal Performance of a Constructed Wetland Dominated by Diffuse Agricultural Groundwater Inflows Using a Linked Catchment-Wetland Model. *Water* **2023**, *15*, 1689. [CrossRef]
27. Saeed, T.; Sun, G.Z. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* **2012**, *112*, 429–448. [CrossRef] [PubMed]

28. Ding, X.W.; Xue, Y.; Zhao, Y.; Xiao, W.H.; Liu, Y.; Liu, J.G. Effects of different covering systems and carbon nitrogen ratios on nitrogen removal in surface flow constructed wetlands. *J. Clean. Prod.* **2018**, *172*, 541–551. [CrossRef]
29. Bodelier, P.L.E.; Libochant, J.A.; Blom, C.; Laanbroek, H.J. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. *Appl. Environ. Microbiol.* **1996**, *62*, 4100–4107. [CrossRef]
30. Maina, C.W.; Mutua, B.M.; Oduor, S.O. Evaluating performance of vertical flow constructed wetland under various hydraulic loading rates in effluent polishing. *J. Water Sanit. Hyg. Dev.* **2011**, *1*, 144–151. [CrossRef]
31. Lu, S.; Jin, X.; Yu, G. Phosphorus removal mechanism of constructed wetland. *Ecol. Environ.* **2006**, *15*, 391–396.
32. Qiu, F.; Xu, Y.; Xu, J.; Fu, K. Research progress in influence factors of phosphorus removal in constructed wetland systems. *Sci. Technol. Rev.* **2017**, *35*, 23–29.
33. Chan, S.S.; Khoo, K.S.; Chew, K.W.; Ling, T.C.; Show, P.L. Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium—A review. *Bioresour. Technol.* **2022**, *344*, 126159. [CrossRef] [PubMed]
34. Miao, Y.Y.; Zhang, L.; Yu, D.S.; Zhang, J.H.; Zhang, W.K.; Ma, G.C.; Zhao, X.C.; Peng, Y.Z. Application of intermittent aeration in nitrogen removal process: Development, advantages and mechanisms. *Chem. Eng. J.* **2022**, *430*, 133184. [CrossRef]
35. Pérez-Sánchez, M.; Sánchez-Romero, F.J.; Zapata, F.A.; López-Jiménez, P.A.; Ramos, H.M. Zero discharge: An optimized strategy to improve the hydric deficit in the Mediterranean area by pumped hydro storage. Case study of Alicante, Spain. *Agric. Water Manag.* **2024**, *292*, 108684. [CrossRef]
36. Ma, S.C.; Fang, J.H.; Liu, J.; Yang, X.F.; Lyu, T.S.; Wang, L.D.; Zhou, S.Y.; Dou, H.S.; Zhang, H.H. Differences in sediment carbon-fixation rate and associated bacterial communities in four wetland types in Hulun Lake Basin. *Catena* **2022**, *213*, 106167. [CrossRef]
37. Zhang, L.L.; Zhang, C.; Hu, C.Z.; Liu, H.J.; Qu, J.H. Denitrification of groundwater using a sulfur-oxidizing autotrophic denitrifying anaerobic fluidized-bed MBR: Performance and bacterial community structure. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2815–2827. [CrossRef] [PubMed]
38. Xu, L.N.; Zhang, B.; Peng, X.W.; Zhang, X.P.; Sun, B.; Sun, H.S.; Jiang, C.C.; Zhou, S.N.; Zeng, X.G.; Bai, Z.H.; et al. Dynamic variations of microbial community structure in *Myriophyllum aquaticum* constructed wetlands in response to different NH_4^+-N concentrations. *Process Biochem.* **2020**, *93*, 55–62. [CrossRef]
39. Liu, C.J.; Li, X.; Yang, Y.L.; Fan, X.Y.; Tan, X.; Yin, W.C.; Liu, Y.W.; Zhou, Z.W. Double-layer substrate of shale ceramsite and active alumina tidal flow constructed wetland enhanced nitrogen removal from decentralized domestic sewage. *Sci. Total Environ.* **2020**, *703*, 135629. [CrossRef]
40. Kapley, A.; Prasad, S.; Purohit, H.J. Changes in microbial diversity in fed-batch reactor operation with wastewater containing nitroaromatic residues. *Bioresour. Technol.* **2007**, *98*, 2479–2484. [CrossRef]
41. Morgan-Sagastume, F.; Nielsen, J.L.; Nielsen, P.H. Substrate-dependent denitrification of abundant probe-defined denitrifying bacteria in activated sludge. *Fems Microbiol. Ecol.* **2008**, *66*, 447–461. [CrossRef]
42. Mozumder, M.S.I.; Shahreyar, S.; Islam, S.; Sen, T.; Dey, J. Functionality of denitrification with minerals composition, process conditions and microbial community. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 9691–9702. [CrossRef]
43. Silveira, N.C.; Oliveira, G.H.D.; Damianovic, M.; Foresti, E. Two-stage partial nitrification-Anammox process for nitrogen removal from slaughterhouse wastewater: Evaluation of the nitrogen loading rate and microbial community analysis. *J. Environ. Manag.* **2021**, *296*, 113214. [CrossRef]
44. Cao, Q.Q.; Wang, H.; Chen, X.C.; Wang, R.Q.; Liu, J. Composition and distribution of microbial communities in natural river wetlands and corresponding constructed wetlands. *Ecol. Eng.* **2017**, *98*, 40–48. [CrossRef]
45. Daims, H.; Wagner, M. Nitrospira. *Trends Microbiol.* **2018**, *26*, 462–463. [CrossRef] [PubMed]
46. Wang, Q.H.; Feng, C.P.; Zhao, Y.X.; Hao, C.B. Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor. *Bioresour. Technol.* **2009**, *100*, 2223–2227. [CrossRef] [PubMed]
47. Li, W.J.; Li, Y.J.; Lee, S.M.Y.; Mok, K.M.; Hao, T.W. Nitrogen Removal in Moving Bed Sequencing Batch Reactors with Polyurethane Foam Cube and Luffa Sponge Carrier Materials. *J. Environ. Eng.* **2019**, *145*, 04019025. [CrossRef]
48. Zhang, Z.W.; Han, Y.X.; Xu, C.Y.; Ma, W.C.; Han, H.J.; Zheng, M.Q.; Zhu, H.; Ma, W.W. Microbial nitrate removal in biologically enhanced treated coal gasification wastewater of low COD to nitrate ratio by coupling biological denitrification with iron and carbon micro-electrolysis. *Bioresour. Technol.* **2018**, *262*, 65–73. [CrossRef]
49. Hayes, A.C.; Zhang, Y.F.; Liss, S.N.; Allen, D.G. Linking performance to microbiology in biofilters treating dimethyl sulphide in the presence and absence of methanol. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1151–1166. [CrossRef]