Effect of C/N Ratio on Nitrogen and Phosphorus Removal from Low-Pollution Water by Water Celery Floating Bed System (Post-print)

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Abstract

With the rapid development of China's economy, the problem of agricultural non-point source pollution has become increasingly serious, exacerbating the trend of freshwater quality deterioration. This study investigated the purification effects of a floating bed water celery (Oenanthe javanica) system and a plant-free control on nitrogen (N), phosphorus (P), and chemical oxygen demand (COD) in rural low-pollution water with different carbon-to-nitrogen ratios [defined as wastewater generated during agricultural production or rural living processes, rich in nutrients such as nitrogen and phosphorus required for plant growth and various trace elements, and meeting the Grade B requirements of the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants (GB 18918–2002 Grade B: TN 20 mg·L-1, TP 1 mg·L-1)]. The low-pollution water used in the experiment consisted of two types: domestic wastewater (TWW) and domestic wastewater with external carbon source addition (high carbon-to-nitrogen ratio, TWW-HC). The entire experiment lasted 82 days, with one water change in the middle. The experimental results showed that the water celery floating bed system receiving low-pollution water with high carbon-to-nitrogen ratio achieved better nitrogen and phosphorus removal effects. The addition of external carbon source could rapidly reduce the concentrations of N and P in low-pollution water within a short time; at 3 days of the experiment, the removal rates of total nitrogen (TN), ammonium nitrogen (NH_4^+-N), and total phosphorus (TP) in TWW-HC reached 40.8%, 38.4%, and 62.8%, respectively. Throughout the experimental period, the TN removal rate of the TWW-HC group was 73.9%~96.0%, higher than that of the TWW group (60.6%~85.9%); the TP removal rate of this group was 68.0%~81.1%, higher than those of the TWW group and Control group (TP removal rates were 21.3%~54.9% and 19.2%~58.1%, respectively). At harvest, the water celery biomass, average plant height, and relative growth rate in the TWW-HC treatment were significantly higher than

those in the TWW treatment ($P<0.05$). The N and P removed through plant uptake in the TWW-HC treatment accounted for 58.2% and 37.6% of the system' s N and P removal, respectively, which were significantly higher than those in the TWW treatment (corresponding proportions were 8.7% and 11.0%, respectively). This indicates that adjusting the influent C:N ratio through external carbon source addition promoted water celery growth and nutrient uptake from wastewater, which is beneficial for N and P removal by the water celery floating bed system.

Full Text

Effect of Carbon/Nitrogen Ratio on Nitrogen and Phosphorus Removal in a Floating-Bed Oenanthe javanica System

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Abstract

With China' s rapid economic development, agricultural non-point source pollution has become increasingly severe, exacerbating the deterioration of freshwater quality. This study investigated the purification efficiency of a floating-bed Oenanthe javanica system and a plant-free control for nitrogen (N), phosphorus (P), and chemical oxygen demand (COD) from rural low-pollution wastewater at different carbon-to-nitrogen ratios. Low-pollution wastewater is defined as sewage generated during agricultural production or rural living that is rich in nutrients such as nitrogen and phosphorus and various microelements, and meets the Class B Level 1 requirements of the Pollutant Discharge Standard of Municipal Wastewater Treatment Plant (GB 18918–2002 Level 1B: TN 20 mg · L ^{1}, TP 1 mg · L ^{1}). Two types of low-pollution wastewater were used: domestic sewage (TWW) and domestic sewage with external carbon source addition (high C:N ratio, TWW-HC). The 82-day experiment included one water exchange at the midpoint. Results demonstrated that the Oenanthe javanica floating-bed system receiving high C:N ratio wastewater achieved superior nitrogen and phosphorus removal. External carbon addition rapidly reduced N and P concentrations within a short period, with removal rates of total nitrogen (TN), ammonia nitrogen (NH -N), and total phosphorus (TP) reaching 40.8%, 38.4%, and 62.8%, respectively, after 3 days. Throughout the entire experimental period, the TWW-HC treatment achieved TN removal rates of 73.9%-96.0%, significantly higher than the TWW treatment (60.6%-85.9%). TP removal rates in TWW-HC reached 68.0%-81.1%, also exceeding both the TWW and control groups (21.3%-54.9% and 19.2%-58.1%, respectively). At harvest, plant biomass, average height, and relative growth rate in the TWW-HC treatment were significantly higher than in the TWW treatment ($P < 0.05$). Plant uptake accounted for 58.2% of N removal and 37.6% of P removal in the TWW-HC system, substantially higher than the TWW treatment (8.7% and 11.0%, respectively). These findings indicate that adjusting the influent C:N ratio through external carbon addition promoted Oenanthe javanica growth and nutrient uptake, thereby enhancing N and P removal efficiency in the floating-bed system.

Keywords: Rural low-pollution wastewater; Domestic sewage; C:N ratio; Floating-bed system; Oenanthe javanica; Nitrogen; Phosphorus; Removal rate

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Introduction

Rapid economic development in China has intensified agricultural non-point source pollution, causing declining water quality in many rivers and lakes and exacerbating water scarcity issues. According to reports, domestic sewage and livestock wastewater represent two major pollution sources in rural areas [1,2]. Unlike livestock wastewater with high nutrient concentrations, rural domestic sewage contains relatively low nitrogen and phosphorus levels—typically 30–40 $mg \cdot L^{1}$ for total nitrogen (TN) and 2.5–3.5 $mg \cdot L^{1}$ for total phosphorus (TP) in the Taihu Lake basin [3]—yet its large total volume, dispersed distribution, and intermittent discharge pose potential threats to rural ecological environments and public health [4]. This study categorizes such wastewater, which meets irrigation standards but causes pollution if discharged directly, as rural low-pollution wastewater. Defined as sewage generated during agricultural production or rural living that is rich in nitrogen, phosphorus, and various microelements while meeting the Class B Level 1 requirements of GB 18918—2002 (TN 20 $mg \cdot L^{1}$, TP 1 $mg \cdot L^{1}$), this low-pollution wastewater primarily includes treated rural domestic sewage tailwater, farmland drainage, and polluted river water. Effective treatment and utilization of such wastewater represents an urgent challenge in rural water environment management.

Floating-bed systems are widely used for ecological restoration of polluted rivers due to their low cost, simple operation, and environmental friendliness [5–8]. Zhang et al. [9] successfully applied plant floating-bed systems to domestic sewage treatment with favorable purification results. Current approaches to enhance floating-bed system efficiency primarily involve aeration enhancement, biofilm carrier installation, immobilized microbial inoculants, and other combined technologies [5,8,10]. Typically, pretreated wastewater enters floating-bed systems directly, with few studies focusing on influent quality adjustment [6,9]. The carbon-to-nitrogen ratio (C:N) serves as a crucial wastewater characteristic parameter that significantly influences nitrogen and phosphorus removal efficiency in treatment systems [11–13]. Puig et al. [13] demonstrated that a sequencing batch reactor (SBR) treating synthetic wastewater achieved optimal

COD, nitrogen, and phosphorus removal at a C:N:P ratio of 100:12:1.8. Hou et al. [14] reported that denitrification efficiency increased with C:N ratio at influent COD concentrations of approximately 150 mg · L 1 and 200 mg · L 1. Zhao et al. [12] found that vertical-flow constructed wetlands treating simulated wastewater achieved highest COD and TP removal at a C:N ratio of 5:1, with optimal nitrogen removal occurring at C:N ratios between 2.5 and 5. However, research on C:N effects remains limited in plant-microorganism coupled floating-bed systems.

This study examined differences in TN, TP, and COD purification efficiency from low-pollution wastewater at varying C:N ratios using a plant floating-bed system, and evaluated plant contributions to nitrogen and phosphorus removal under different influent conditions. By effectively utilizing nutrient resources in low-pollution wastewater and appropriately adjusting the C:N ratio, this research aims to enhance pollutant purification efficiency in floating-bed systems and provide technical support and application cases for rural low-pollution wastewater treatment.

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1. Materials and Methods

1.1 Experimental Materials

Oenanthe javanica was selected as the plant material for the floating-bed system. Seedlings were purchased from the Suqian Northern Jiangsu Flower Base, cleaned with deionized water, and pre-cultured in experimental water. After approximately three weeks, when seedlings reached 16 cm in height, uniform and healthy plants were selected for the experiment. The test water consisted of septic tank sewage from the Institute of Food Crops, Jiangsu Academy of Agricultural Sciences. The water was settled, UV-sterilized, and supplemented with glucose to modify the C:N ratio. Water sample properties are presented in .

Properties of the experiment waters

1.2 Experimental Methods

The plant floating-bed system (hydroponic) used 755 turnover boxes (outer dimensions: 790 mm × 565 mm × 505 mm; inner dimensions: 750 mm × 525 mm × 490 mm, length × width × height) as experimental containers. Each container held a PVC foam board (667 mm × 505 mm × 15 mm) with a 4×4 layout of 16 circular holes (4 cm diameter) spaced at 16.5 cm × 14 cm intervals. One Oenanthe javanica seedling was planted per hole and secured with sponge strips. A small tray (240 mm × 174 mm) placed at the bottom collected sediment deposits. Three treatment groups were established: (1) domestic sewage with floating-bed system (TWW), (2) domestic sewage with external carbon source addition (adjusted C:N ratio) and floating-bed system (TWW-HC), and

(3) domestic sewage without floating-bed system (Control). Each treatment had three replicates. The experiment was conducted in a greenhouse at the Jiangsu Academy of Agricultural Sciences in Nanjing from April 13 to July 4, 2014. Water was exchanged once on June 4, and plants were harvested on July 4. Water samples were collected every 7 days using a five-point sampling method (four corners and center of each tank), with 50 mL collected per sampling. Evaporation losses were compensated by adding tap water (see water properties in) the day before sampling.

1.3 Analytical Methods

Water samples were stored at 4°C after collection and analyzed using standard methods [15]: pH was measured with a pH meter (PHS-3C, Shanghai Leici); total nitrogen (TN) by potassium persulfate oxidation-UV spectrophotometry; ammonia nitrogen (NH -N) by Nessler' s reagent photometry; total phosphorus (TP) by potassium persulfate decomposition-molybdenum antimony spectrophotometry; and chemical oxygen demand (COD) using a COD analyzer (DR1010 COD, HACH, China). Removal rates were calculated as: Removal rate (%) = (C - C)/C × 100%, where C is the initial TN or TP concentration and C is the final concentration.

Plant height and weight were measured at the beginning and end of the experiment. Relative growth rate (RGR) was calculated as: RGR (%) = 100 × (W - W)/W , where W is the average fresh weight on day i and W is the initial average fresh weight.

After the experiment, water in each tank was drained to approximately 5 cm depth and air-dried. Sediment from the small trays was scraped off, weighed, and ground. Total nitrogen and phosphorus in sediments were determined using soil analysis methods [16]. Plant samples were collected, separated into roots, stems, and leaves, washed, oven-dried at 65°C to constant weight, ground, and digested with concentrated H SO -H O for subsequent analysis. TN and TP concentrations were determined by Kjeldahl digestion and molybdenum antimony colorimetry [16]. Plant nitrogen and phosphorus accumulation (PA) was calculated as: PA = PC × PB, where PC is plant N or P content ($mg \cdot g^{1}$, dry weight basis) and PB is plant biomass ($g \cdot m^{2}$, dry weight basis).

1.4 Statistical Analysis

All data are presented as means of three replicates. Comparisons of N and P removal rates among treatments were performed using one-way ANOVA (Duncan' s test). Independent samples t-tests were used to compare Oenanthe javanica growth status and tissue N and P contents between TWW and TWW-HC treatments. Data processing used Microsoft Excel 2010, and statistical analysis used SPSS 13.0.

2. Results

2.1 Effect of C:N Ratio on Nitrogen Removal from Low-Pollution Wastewater

Dynamic changes in TN concentration and removal rates are shown in [Figure 1: see original paper]. After initiation, a white, oil-film-like substance appeared on the TWW-HC water surface, subsequently decomposing as TN concentrations rapidly declined ([Figure 1: see original paper]A). On day 3, the TN removal rate in this group was significantly greater than in the other treatments ([Figure 1: see original paper]B, $P < 0.05$). This likely occurred because higher carbon content in the wastewater facilitated microbial denitrification while promoting nitrogen uptake by Oenanthe javanica. Zhao et al. [12] reported that vertical-flow constructed wetlands achieved highest nitrogen removal efficiency at C:N ratios of 2.5:1 to 5:1 when treating simulated domestic sewage. Xia et al. [11] found that TN removal efficiency increased with C:N ratios (3:1, 5:1, and 10:1) in a suspended carrier biofilm reactor (SCBR). In the first water exchange cycle of this study, TN removal rates were 85.9% and 96.0% in TWW and TWW-HC groups, respectively. During the second cycle after water exchange, removal rates were 60.6% and 73.9%, respectively, indicating that increasing the C:N ratio enhanced TN removal in the floating-bed system, consistent with Xia et al. [11].

Similarly, NH -N concentrations in the TWW-HC group decreased substantially during the first 3 days ([Figure 2: see original paper]A). Before water exchange, NH -N removal rates exceeded 98.0% in both treatment groups and the control ([Figure 2: see original paper]B). After water exchange, the TWW-HC group showed greater NH -N concentration reduction than the other groups, with a final removal rate of 89.3%, higher than the TWW group (80.1%) ([Figure 2: see original paper]B).

COD concentrations in the TWW-HC group gradually decreased from 0–38 days, reaching a minimum of 47.3 mg · L ^{1} on day 38 before increasing slightly. The TWW and control groups showed similar COD trends, decreasing to minimum values on day 3 then gradually increasing. After water exchange, COD in the TWW-HC group declined while TWW and control groups showed increases on day 66 before gradually decreasing ([Figure 3: see original paper]A).

During the first water exchange cycle, C:N ratios increased in all groups. However, the non-carbon-added treatments maintained low C:N ratios (<3) from days 3–17, which likely limited denitrification ([Figure 3: see original paper]B). As C:N ratios gradually increased, TN and NH -N concentrations in the TWW group decreased substantially after 20 days, with corresponding increases in removal rates ([Figure 1: see original paper], [Figure 2: see original paper]). This demonstrates that wastewater C:N ratio significantly influenced TN and NH -N removal efficiency. After water exchange, C:N ratios in the TWW-HC group remained relatively stable, while those in TWW and control groups first increased then decreased ([Figure 3: see original paper]B).

2.2 Effect of C:N Ratio on Phosphorus Removal from Low-Pollution Wastewater

The TWW-HC group also demonstrated superior TP removal efficiency. On day 3, TP concentration decreased from 1.01 mg · L ¹ to 0.38 mg · L ¹, achieving a 62.8% removal rate significantly higher than TWW and control groups ([Figure 4: see original paper]). Although TP concentrations increased slightly thereafter, they remained lower than the TWW group. On day 45, TP removal rates reached 81.1% in TWW-HC, compared to 21.3% and 15.7% in TWW and control groups, respectively ([Figure 4: see original paper]B). During the second cycle after water exchange, TP removal rates were 68.0% in TWW-HC, 54.9% in TWW, and 58.1% in the control.

2.3 Role of Floating-Bed Plants in Nitrogen and Phosphorus Removal

Under suitable environmental conditions, plant uptake represents an important pathway for nitrogen and phosphorus removal in floating-bed systems. Post-harvest measurements revealed no significant difference in average root length between treatments. However, biomass in the TWW-HC group (100.1 g) was significantly higher than in the TWW treatment ($P < 0.05$). Average plant height and relative growth rate were also significantly greater in the TWW-HC group (, $P < 0.05$). Tissue nitrogen content showed no significant difference between treatments ($P > 0.05$), while stem phosphorus content in the TWW-HC group was significantly lower than in the TWW group ($P < 0.05$), with no significant differences in root or leaf phosphorus content (, $P > 0.05$).

Calculations of nitrogen removal pathways showed that plant uptake accounted for only 8.7% of total nitrogen removal in the TWW group but increased to 58.2% in the TWW-HC group. Over 80% of nitrogen was removed through other pathways in both TWW and control groups, compared to 37.3% in the TWW-HC group. Sediment nitrogen represented 16.0% of removed nitrogen in the control group but only 8.5% in TWW and 4.6% in TWW-HC groups ([Figure 5: see original paper]A). For phosphorus removal, other pathways accounted for 80.9%, 60.5%, and 84.2% in TWW, TWW-HC, and control groups, respectively, while plant uptake contributed 11.0% and 37.6% in TWW and TWW-HC groups ([Figure 5: see original paper]B). These results demonstrate that the plant floating-bed system significantly reduced nitrogen and phosphorus in sediments and other pathways compared to the control, and that C:N ratio adjustment through carbon addition substantially promoted plant growth and system nutrient removal.

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3. Discussion and Conclusion

Organic carbon provides an energy source for denitrifying bacteria, and its content and availability are primary factors affecting microbial denitrification

[17,18]. Generally, typical domestic sewage has relatively high C:N ratios, which decrease after primary treatment and further decline after secondary and tertiary treatment. Denitrification is typically favored when wastewater C:N ratios exceed 3:1 [19]. While most primary-treated municipal wastewater meets this criterion, organic carbon may become a limiting factor for systems receiving secondary or higher-level treated effluent [20]. Xia et al. [11] reported that a suspended carrier biofilm reactor treating synthetic wastewater exhibited optimal total nitrogen removal at an influent C:N ratio of 10:1, outperforming ratios of 3:1 and 5:1. Conversely, Zhao et al. [12] found that vertical-flow constructed wetlands achieved higher TN removal at C:N ratios of 2.5:1 and 5:1 than at 10:1, regardless of whether carbon or nitrogen content was held constant. In this study, adjusting the low-pollution wastewater C:N ratio from 4:1 to 6:1 resulted in rapid TN concentration decline and significantly enhanced removal during the initial experimental stage ($P < 0.05$), indicating that higher carbon content facilitated nitrification-denitrification processes in the Oenanthe javanica floating-bed system. However, external carbon addition increases treatment costs, prompting researchers to explore cellulose-based natural organic materials as alternatives to traditional carbon sources like methanol and ethanol for denitrification [21,22].

Heterotrophic microorganisms comprise a large proportion of bacteria in wastewater treatment systems and compete with nitrifying bacteria (ammonia-oxidizing bacteria AOB and nitrite-oxidizing bacteria NOB). Their relative dominance is influenced by hydraulic retention time, C:N ratio, dissolved oxygen, and other conditions [23,24]. The C:N ratio plays a crucial role in nutrient removal, particularly affecting denitrification processes and associated microbial populations and abundances. Xia et al. [11] observed that total bacterial community diversity decreased while the proportion of nitrifying bacteria increased as C:N ratios decreased from 10:1 to 3:1, suggesting a successional trend between nitrifying and heterotrophic microorganisms with changing C:N ratios. We hypothesize that the white biofilm appearing on the water surface during the initial high C:N ratio treatment phase played an important role in rapid nitrogen removal, though its microbial composition and structure require further investigation.

Phosphorus removal mechanisms in ecological floating-bed systems include adsorption, complexation-precipitation, plant uptake, and biological assimilation [12,25]. Zhao et al. [12] attributed optimal phosphorus removal in vertical-flow constructed wetlands at a C:N ratio of 5:1 to the combined effects of carbon and nitrogen on phosphorus removal. Polyphosphate-accumulating organisms (PAOs) play important roles in biological phosphorus removal, excessively absorbing phosphorus from wastewater under aerobic conditions and storing it as polyphosphate, while hydrolyzing stored polyphosphate under anaerobic conditions to increase liquid-phase phosphorus concentrations [26]. In this study, the substantial TP concentration decrease on day 3 in the C:N = 6:1 treatment group, followed by a slight increase, may be related to PAO activity and changes in water physicochemical properties. During the mid-to-late experimental period, plant uptake became increasingly important for phosphorus removal as Oenanthe javanica grew, with the higher biomass TWW-HC group achieving greater TP removal efficiency.

Plants constitute an essential component of floating-bed systems. Unlike wetland systems, the well-developed root systems of floating-bed plants are fully exposed to water, providing ample hydraulic retention time and contact area for capturing and precipitating particulate pollutants [27]. Furthermore, plant rhizospheres provide favorable habitats for microbial survival and reproduction, facilitating nitrification and denitrification processes [5,28]. Previous studies reported that plant uptake accounted for approximately 20.2% of nitrogen and 29.4% of phosphorus removal in floating-bed systems treating eutrophic water, with over 60% of nutrients removed through sedimentation [27]. Li et al. [5] found that Oenanthe javanica in micro-aerated floating-bed systems removed 6.87% of nitrogen and 26.82% of phosphorus through plant uptake over 80 days. In contrast, this study found plant uptake contributed 8.7% and 11.0% to nitrogen and phosphorus removal in the TWW group, increasing to 58.2% and 37.6% in the TWW-HC group. These results demonstrate that C:N ratio adjustment through carbon addition significantly promoted plant growth and nutrient removal.

In summary, this study concludes: (1) The Oenanthe javanica floating-bed system exhibited different removal efficiencies for low-pollution wastewater at varying C:N ratios, with external carbon addition rapidly reducing nitrogen and phosphorus concentrations and improving system removal efficiency. (2) The carbon-added treatment maintained consistently high C:N ratios favorable for denitrification and plant growth, while the non-carbon-added treatment experienced rapid C:N ratio decline (<3) during the initial stage, limiting denitrifying bacterial proliferation and plant growth. (3) Adjusting the C:N ratio through external carbon addition significantly promoted Oenanthe javanica nutrient uptake from low-pollution wastewater, substantially increasing the contribution of plant absorption to nitrogen and phosphorus removal.

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Note: Figure translations are in progress. See original paper for figures.