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Enhanced nitrogen removal and associated microbial characteristics in a modified single-stage tidal flow constructed wetland with step-feeding

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HIGHLIGHTS

- Shunt ratio significantly influenced N transformation in TFCWs.
- The TFCW with a shunt ratio of 1:2 removed pollutants most effectively.
- Denitrification and anammox were intensified as the shunt ratio was increased.
- Multiple N removal processes were active in the TFCW at the optimal shunt ratio.

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ABSTRACT

This study was conducted to explore nitrogen transformation and associated microbial characteristics in a modified single-stage tidal flow constructed wetland (TFCW) at five different shunt ratios. Shunt ratio significantly affected nitrogen removal during operation of the TFCW with adoption of a modified step-feeding mode. When the shunt ratio was 1:2, TFCW had the best pollutant removal performance and was particularly effective for total nitrogen (TN). The abundance of nitrogen transformation microorganisms was also affected by the shunt ratio of the system. Molecular biological analyses demonstrated that both denitrification and anammox were enhanced when the shunt ratio was greater than 0:1, resulting in the development of multiple and complete nitrogen removal pathways in the TFCW. These results show that the optimal shunt ratio of the modified single-stage TFCW for effective nitrogen removal was 1:2.

1. Introduction

Constructed wetlands are effective means of treating various types of contaminated water. In comparison with conventional treatment systems, constructed wetlands are more easily maintained and operated, require reduced input, and consume less energy [1]. Nevertheless, CWs often provide inconsistent nutrient reduction, in particular for nitrogen [2]. Therefore, the nitrogen removal capacity of CWs must be improved because nitrogen is a major contributor to water eutrophication.

The primary nitrogen removal process in subsurface flow CWs is generally the microbial metabolic pathway, which removes approximately (89–96)% of nitrogen [3]. Several metabolic pathways, including conventional/partial nitrification–denitrification and anaerobic ammonium oxidation (anammox), are involved in nitrogen removal. Various environmental parameters and operating conditions influence the nitrogen treatment performance of CWs; the ratio of COD to TN (C/N) and DO concentration are crucial for nitrogen transformation [4,5].

Tidal flow constructed wetlands (TFCWs) are a fairly new technology that can be used to enhance nitrogen removal from the environment via a novel method of oxygen transfer [6–8]. However, many studies of TFCWs have found that total nitrogen (TN) removal rates were not as great as expected because of a weak anoxic environment or inadequate organic carbon abundance, increasing the concentration of NO_x^--N in the effluent [9].

Achieving stable and high-rate nitrogen removal is usually considered as a formidable challenge in TFCWs. One major challenge is that it is extremely difficult to regulate oxygen supply and maintain appropriate level of DO in TFCWs. On the other hand, competition for substrates (e.g. NO_x^--N, organics, etc.) usually occurs among the various species of microorganisms (e.g. anammox bacteria and denitrifiers) present in TFCWs [10–12]. As C/N ratio and DO content have substantial impacts on nitrification, anammox, and denitrification in a CW system, an appropriate microenvironment must be created for each microorganism involved in nitrogen transformation in TFCWs.

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Step-feeding is an effective method of improving TN removal in CWs [13]. In a previous study, we showed that the oxygen supply and C/N ratio in a subsurface vertical flow constructed wetland (VSSF) can be regulated by the adoption of step-feeding [14], enhancing nitrogen removal. Based on this finding, a single-stage TFCW with a modified step-feeding mode, which was expected to enhance TN removal, was established. Step-feeding in a CW is generally accomplished by installing a shunt pipe [15]. The shunt ratio of a CW is a crucial parameter that influences its TN treatment performance. Little effort has been expended to achieve satisfactory NH_4^+-N and TN removal in single-stage TFCWs; improved NH_4^+-N and TN removal have been achieved with multiple-stage TFCWs. The optimum shunt ratio for wastewater treatment by a single-stage TFCW with step-feeding remains unclear. Therefore, the effects of the shunt ratio on wastewater treatment in TFCWs and nitrogen removal mechanisms at the molecular level merit further investigation.

In this study, the domestic sewage treatment performance of a modified single-stage TFCW with step-feeding was assessed at five different shunt ratios. For each TFCW, the absolute abundance of genes involved in nitrogen removal and their ecological associations were assessed to evaluate the relationship between the shunt ratio and the microbial community performing nitrogen removal.

2. Materials and methods

2.1. System descriptions

TFCWs were constructed at the greenhouse of Anhui Agricultural University (AHAU) in Hefei, China, where the temperature was maintained at (25 ± 2) °C. Each single-stage TFCW (a polyethylene tank with a diameter of 20 cm and depth of 80 cm) was filled with 70 cm of oyster shell (particle size: 2–5 mm) as the substratum layer, as well as 10 cm of gravel (particle size: 10–15 mm) as the bottom under-drainage layer. The bed had a total volume of 25.12 L and a working volume of 10.12 L (initial porosity 40.30%).

A "Ω"-shaped perforated inlet pipe was installed on the top of each TFCW, whereas another perforated pipe was installed at the bottom of the system as the shunt pipe. A vertical perforated PVC pipe (80 cm in length and 3.5 cm in diameter) was inserted into the bed in the middle of each TFCW to measure various physical and chemical parameters of wastewater *in situ*. Four orifices (25 mm internal diameter for each), which were used for collecting substratum samples, were respectively excavated in a line at different depths (14, 28, 42, and 56 cm) of side wall below the top of the bed. Every orifice was sealed by a rubber plug. The emergent plant employed in this study was reed (*Phragmites australis*), which is usually considered as a popular wetland plant [16]. Four reeds (initial height of approximately 30 cm) were planted in each system, and each of them has a main stem and two or three new shoots.

2.2. Experimental conditions

The TFCWs received schoolyard domestic sewage from AHAU after the sedimentation pretreatment. The water quality parameters of the sewage were as follows: TSS, (65.12 ± 24.51) mg L^{-1}; COD, (299.40 ± 17.72) mg L^{-1}; BOD, (117.83 ± 12.57) mg L^{-1}; NH_4^+-N, (34.17 ± 5.11) mg L^{-1}; NO_2^--N, (3.37 ± 1.67) mg L^{-1}; NO_3^--N, (1.72 ± 0.38) mg L^{-1}; TN, (43.56 ± 7.47) mg L^{-1}; TP, (13.64 ± 2.39) mg L^{-1}; and pH, (7.74 ± 0.58).

Each TFCW was operated in a modified step-feeding mode (designated as the modified "tidal flow" principle using a two-time feeding mode) in this study. Specifically, the entire operation cycle, which occurred every 12 h, was divided into 6 phases in chronological order, consisting of 10 min Feeding Phase 1, 4.50 h Flood Phase 1, 20 min Feeding Phase 2, 3.00 h Flood Phase 2, 15 min Drain Phase, and followed by 3.75 h Rest Phase (as shown in Fig. 1). (*a*) Feeding Phase 1: a portion of wastewater was rapidly loaded into the TFCW in batch mode via the inlet pipe; (*b*) Flood Phase 1: after the first feeding, the lower section of the substratum layer was kept saturated for 4.50 h, while the upper section of the substratum layer was unsaturated; (*c*) Feeding Phase 2: another portion of wastewater was continuously fed to the TFCW via the shunt pipe by a peristaltic pump, in order to reduce oxygen transfer, wastewater was fed into the TFCW in an up-flow pattern rather than the original down-flow pattern; (*d*) Flood Phase 2: the entire bed was kept saturated for 3.00 h after the second feeding; (*e*) Drain Phase: all wastewater was drained rapidly via the outlet pipe installed at the bottom of the TFCW; (*f*) Rest Phase: the entire bed was allowed to rest in an unsaturated state for 3.75 h.

Ten liters of wastewater were added to each TFCW for each cycle [corresponding to an HLR of 0.64 $m^3 (m^2 d)^{-1}$], including the wastewater added to the TFCW through the shunt pipe. The shunt ratio was defined as the ratio between the inflow volume through the shunt pipe and the inflow volume through the inlet pipe. In our study, five different shunt ratios were adopted: 0:1, 1:4, 1:3, 1:2, and 1:1. Correspondingly, heights of water level in the systems were respective 79, 63, 59, 53, and 40 cm above the bottom of the bed during Flood Phase 1 at the five different shunt ratios. Notably, the TFCWs with a shunt ratio of 0:1 were regarded as the control group with an operation mode representing that of conventional TFCWs.

Prior to the experiments, five groups of TFCWs with different shunt ratios were fed wastewater for three months to allow the development of plants and biofilms in the bed. The experimental period was one year.

2.3. Analytical procedure

Water samples were collected in triplicate once per week from the inlet and outlet of each TFCW and analyzed immediately. In order to provide insight into pollutant conversion mechanisms, a cyclic study was performed when the system was considered to be at a steady state. Water samples, extracted at the midpoint of the water depth from the vertical perforated pipe, were taken for chemical analysis with sampling intervals of 30 min during Flood Phase 1 and each 30 min during Flood Phase 2. As all the systems were operated for 720 cycles, each TFCW was excavated to allow substratum samples to be collected for experiments in the laboratory. During the sampling event, substrate samples excavated from the sampling orifices were mixed evenly, placed in an ice incubator, and immediately sent to the laboratory for DNA extraction. Soil DNA kits (D5625-01; Omega, USA) were used to extract and purify total genomic DNA. The genomic DNA extracted from the soil samples was detected by 1% agarose gel electrophoresis and stored at −20 °C. Besides, the average height of the plants in each TFCW was measured every week throughout the experiments.

2.3.1. Water quality analyses

Water quality analyses were conducted for temperature, pH, DO, ORP, TSS, COD, BOD, TN, NH_4^+-N, NO_2^--N, NO_3^--N, and TP. The analyses (including TSS, COD, BOD, TN, NH_4^+-N, NO_2^--N, NO_3^--N, and TP) were performed according to standard methods for assessing water and wastewater [17] and pH values of the water samples were determined by a digital pH meter (PB-10, Sartorius, Germany). During a typical cycle of each TFCW, water temperature, DO, and ORP were all measured *in situ* at the midpoint of the water depth from the vertical perforated pipe, using a portable HACH HQ30d Multi-Parameter analyzer.

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