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Enhancing nitrogen removal via the complete autotrophic nitrogen removal over nitrite process in a modified single-stage tidal flow constructed wetland

Zhen Wang*, Menglu Huang, Ran Qi, Yumeng Zhang

School of Resources and Environment, Anhui Agricultural University, Hefei 230036, China

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ABSTRACT

This study attempts to achieve a high-rate nitrogen removal via the complete autotrophic nitrogen removal over nitrite (CANON) process in a modified single-stage tidal flow constructed wetland (TFCW) with step-feeding, and nitrogen transformation pathways in the TFCWs treating domestic wastewater were explored under shunt ratio constraints. Shunt ratio significantly affected nitrogen transformation pathways in the TFCWs throughout the experiment. The anammox bacteria were enriched most effectively at the shunt ratio of 1:1, and then the initiation of a CANON process was accomplished in the TFCW under the appropriate limited oxygen condition. The mean TN removal rate reached up to $(127.00 \pm 13.78)\,mg\,(L\,d)^{-1}$ correspondingly. It could be concluded that autotrophic nitrogen removal via CANON process developed in the TFCW with optimized microenvironment that developed as a result of appropriate shunt ratio. The optimal shunt ratio of the TFCW for nitrogen removal was 1:1.

1. Introduction

Constructed wetlands (CWs) have proven to be an efficient ecological technology for the treatment of various kinds of contaminated waters. In comparison with conventional treatment systems, constructed wetlands are more easily maintained and operated, require reduced input, and consume less energy (Vymazal, 2010). Nevertheless, nitrogen removal in CWs exhibited substantial fluctuations and was often unsatisfactory (Sun et al., 2005). Therefore, the nitrogen removal capacity of CWs must be improved because nitrogen is a major contributor to water eutrophication.

Classical nitrogen removal route, known as conventional nitrification-denitrification, was once thought to be the major nitrogen removal route in subsurface flow CWs (Saeed and Sun, 2012). Nevertheless, this route is often impaired in a CW system due to either lack of organics or inadequate dissolved oxygen (DO). Apart from conventional nitrification-denitrification process, the autotrophic nitritation-anammox process also exists in CWs as an unconventional pathway, which requires less oxygen, eliminates the need for organics, generates less sludge, and reduces greenhouse gas emission (Sun and Austin, 2007). Although distinct conditions are required for nitritation (aerobic) and anammox (anoxic) respectively, it is feasible to integrate these two autotrophic nitrogen conversion processes within a single aerobic reactor under oxygen-limiting situation, referred as complete autotrophic nitrogen removal over nitrite (CANON) (Sliekers et al., 2002). Hence, enhancing nitrogen removal via the CANON process will be conducive to realize effective nitrogen removal in a single-stage CW when treating wastewater with low C/N ratio.

As a passively-aerated biofilm system, CWs possess natural advantages (limited oxygen supply, redox stratification and high biomass retention, etc.) to facilitate the CANON process. Although nitrogen removal via CANON process has been reported in several studies with different types of CWs (Dong and Sun, 2007; Hu et al., 2014; Sun and Austin, 2007; Tao and Wang, 2009; Tao et al., 2011), achieving stable and high-rate autotrophic nitrogen conversion is still a challenge in such systems. One major challenge is that it is extremely difficult to control oxygen supply and maintain appropriate level of DO in CWs. On the other hand, CWs are usually operated with a relative low nitrogen load, which is unfavorable to maintaining anammox (Joss et al., 2009).

Recently, tidal flow constructed wetlands (TFCWs) has been proposed to enhance the removal effect of nitrogen because its oxygen supply can be greatly strengthened by the "tidal" operation (Wu et al., 2011). As mentioned, the level of DO in bed has substantial impacts on activities of nitritation and anammox in a CW. Hence,

* Corresponding author.
E-mail addresses: zwang@ahau.edu.cn, russellvanhalen@gmail.com (Z. Wang).

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appropriate microenvironment in the system should be created for CANON process on the premise that the TFCW receives some modifications. Hu et al. (2014) reported that oxygen supply in TFCWs could be weakened with adoption of up-flow mode instead of the original down-flow mode, which could trigger CANON process in the system. It was found in our previous study that ananmox bacteria in TFCW could be enrichment to some extent by adopting step-feeding with up-flow mode, which proved to be conducive to enhance nitrogen removal (Wang et al., 2017). Hence, it was expected that step-feeding with up-flow mode could be an important controlling factor for the CANON route, since this modification may have significant impact on the nitrogen conversion pathway.

As we know, step-feeding has been broadly demonstrated in conventional activated sludge process as an effective option to enhance TN removal by stepwise introduction of the influent to the nitrified liquid, thus making more efficient use of the influent carbon source for denitrification (Puig et al., 2004; Tang et al., 2007). Until recently, some studies are carried to investigate step-feeding in improving nitrogen removal in CWs and this setup is subsequently proved to be effective on TN removal in the systems (Hu et al., 2012; Fan et al., 2013). Since step-feeding is usually achieved by the installation of a shunt pipe in one location of a CW (Wang et al., 2014), the shunt ratio becomes a crucial parameter which can play a key role in forming effective anoxic conditions that are favorable for reducing oxidized-N to improve removal TN. Nevertheless, few efforts have been made to achieve satisfactory NH_4^+-N and TN removal in one single-stage TFCW via the CANON process, and the regulation of shunt ratio still remains unclear as the single-stage TFCW with the adoption of step-feeding is used in the degradation of wastewater that contains relative lower organic carbon concentration. So, it is quite necessary to qualify and evaluate the effect of the shunt ratio in TFCW on wastewater treatment as shunt ratio can affect functional microorganisms involved in nitrogen transformation by changing the oxygen transfer rate, and more attempts should also be made to investigate nitrogen removal mechanisms at the molecular level in the system.

Given this, the single-stage TFCWs with adoption of a modified step-feeding mode were established in our study, which was expected to enhance nitrogen removal via the CANON process. A comparison study of the modified TFCW at six different shunt ratios to treat domestic wastewater was carried out. Nitrogen transformation and treatment performances of the TFCW were investigated under shunt ratio constraints. For the 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. 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 10.50 L (initial porosity 41.80%). 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 the 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. 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 anaerobic pretreatment. In the process of anaerobic treatment, most of the biodegradable organic substrate was consumed, resulting in a low BOD_5/N ratio (≈0.88) in the wastewater. The water quality parameters of the sewage were as follows: TSS, (65.12 ± 24.51) mg L^{-1}; COD, (79.40 ± 17.72) mg L^{-1}; BOD_5, (37.83 ± 12.57) mg L^{-1}; NH_4^+-N, (35.54 ± 1.80) mg L^{-1}; NO_2^--N, (2.80 ± 0.57) mg L^{-1}; NO_3^--N, (1.72 ± 0.18) mg L^{-1}; TN, (42.97 ± 2.85) 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 in order to enhance nitrogen removal via the CANON process. Specifically, the entire operation cycle, which occurred every 6 h, could be divided into four phases in chronological order (as shown in Fig. 1): (*a*) Feeding Phase: part of wastewater (via the inlet pipe) was loaded to the TFCW in batch mode (t = 10 min), meanwhile, the rest of wastewater (via the shunt pipe) was continuously fed to the TFCW in 10 min by a peristaltic pump, in order to regulate DO level in the system, the wastewater via the shunt pipe was pumped in an up-flow pattern; (*b*) Flood Phase: since the ending of Feeding Phase, the whole bed was kept saturated for a certain period of time (t = 240 min); (*c*) Drain Phase: all the wastewater was drained out rapidly (t = 10 min) via the outlet pipe installed at the bottom of the TFCW. (*d*) Rest Phase: the whole bed was allowed to "rest" (unsaturated) for a while (t = 100 min).

Ten liters of wastewater were added to each TFCW for each cycle [corresponding to an HLR of 1.27 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, six different shunt ratios were adopted: 0:1, 1:4, 1:3, 1:2, 1:1 and 2:1. 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, all the TFCWs with a shunt ratio of 0:1 were fed wastewater for three months to allow the development of plants and biofilms in the bed. Thereafter, The experimental period lasted for 616 days and was divided into six periods: (1) Period A (shunt ratio of 0:1), lasted for 91 d; (2) Period B (shunt ratio of 1:4), lasted for 93 d; (3) Period C (shunt ratio of 1:3), lasted for 93 d; (4) Period D (shunt ratio of 1:2), lasted for 123 d; (5) Period E (shunt ratio of 1:1), lasted for 123 d; and (6) Period F (shunt ratio of 2:1), lasted for 93 d. Since the TFCWs were placed indoors, influents and effluents ranged in temperature from 21 to 26 °C throughout the study (data not shown), namely water temperature did not vary significantly over the 616 days of operation, which assured comparison of the shunt ratios across the six periods.

2.3. Analytical procedure

Water samples were collected in triplicate once every three days from the inlet and outlet of each TFCW and analyzed immediately. Substratum samples were collected from each TFCW at least two times during each period. During the sampling event, substrate samples excavated from the sampling orifices were mixed evenly,