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Denitrification- and anammox-dominant simultaneous nitrification, anammox and denitrification (SNAD) process in subsurface flow constructed wetlands

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ABSTRACT

Simultaneous nitrification, anammox and denitrification (SNAD) process was developed in subsurface flow constructed wetlands (SFCWs) to treat polluted surface water. The effects of vegetation, hydraulic retention time (HRT), C/N, and influent nitrogen forms on nitrogen removal and microbial communities were investigated. Results showed that denitrification- and anammox-dominant SNAD corresponded to nitrate- and ammonia-dominant influent, respectively, and both could achieve more efficient nitrogen removal in planted SFCWs than the unplated. These higher efficiencies were due to the microbial growth, organic carbon release, oxygen supply and plant uptake promoted by vegetation. The electron donors accelerated denitrification but inhibited ammonia oxidation with deficient oxygen. Anammox contributed to nitrogen removal of 27.34% under oxygen-limited conditions without vegetation. Anammox combined with denitrification and plant uptake were over 90% in planted SFCWs. For the investigated factors, the ammonia, nitrate and C/N were the most significant ones influencing the microbial communities, further nitrogen removal pathways and performances.

1. Introduction

While the conventional aerobic nitrification and subsequent anaerobic denitrification process is quite efficient in removing nitrogen, extra oxygen is required for aeration and chemical oxygen demand coupling with nitrite accumulation and nitrous oxide emission (Du et al., 2014; Zhu et al., 2011). Simultaneous nitrification, anammox and denitrification (SNAD) process is now becoming a novel approach for nitrogen removal.

In the oxygen-limited conditions, inhibited nitrification leads to partial ammonia oxidized to nitrite by aerobic ammonia oxidizing bacteria (AOB), while the residual ammonia is oxidized with the produced nitrite as electron acceptors by anaerobic ammonia oxidizing bacteria (anammox bacteria). Subsequently, the inevitable by-product nitrate of anammox is reduced by denitrifying bacteria with carbon source (Wang et al., 2010). The SNAD process has been widely employed in bioreactors in wastewater treatment plants (WWTPs), especially in sequencing batch reactors. Further, the operational parameters of SNAD process have been investigated to enhance the nitrogen removal efficiencies (Chen et al., 2009; Du et al., 2014; Lan et al., 2011).

Nowadays, the surface water environment is significantly threatened by nitrogen from agriculture non-point sources and WWTPs effluent. These runoffs or effluents characterized with low concentrations and large quantities are commonly treated by constructed wetlands (CWs) (Fu et al., 2016; Zhi and Ji, 2014). CWs, composed of low-cost materials and ecological plants, serve the SNAD process with conducive conditions. The packing substrates and plant surfaces can provide immense interface for the attachment of microorganisms associated to nitrogen cycle. As the product of photosynthesis, oxygen is transported to the root zone by plant stomata. Root secreta of growing plants and litter leachate during decaying periods could release organic matters for denitrification (Bachand and Horne, 2000; Du et al., 2018; Vymazal, 2013). Note that previous studies mainly focused on treating wastewater with high concentration (up to 100–450 mg/L) of ammonia or urea in CWs, and the feasibility of SNAD has been confirmed in these cases (Liang et al., 2014; Paranychianakis et al., 2016; Wang and Li, 2011).

However, polluted surface water usually contains relative low concentrations of nitrogen contaminants and lacks organic carbon (Chen et al., 2015; Fu et al., 2017), whose water quality and

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composition are fluctuant with season meanwhile. Pathways regulating nitrogen removal treating this kind of water body in CWs have not been well understood. Additionally, the treatment performances, microbial communities and the link between them need to be investigated for both planted and unplanted CWs as well. Hence, this study is aimed to: (1) develop a SNAD process in laboratory-scale subsurface flow constructed wetlands (SFCWs) without seed sludge; (2) figure out the effects of vegetation, hydraulic retention time (HRT), influent forms and carbon addition, which are important factors influencing the SNAD; (3) analyze physicochemical responses and the microbial communities in detail, to further understand the nitrogen removal pathways.

2. Materials and methods

2.1. Experimental setup and operation

The laboratory scale experiment was conducted in a temperature controlled transparent greenhouse at Shanghai Jiao Tong University from October 2016 to August 2017. The scale-down SFCWs consisted of an inlet zone, a reaction zone and an outlet zone, and the adjacent zones were separated by clapboard with uniformly small holes for water distribution. The reaction zones (Length × Width × Height = 50 cm × 20 cm × 30 cm) were wholly filled with gravels (diameters of 20–30 mm). Reed, with an extensive rhizome system, was the most frequently used perennial plant for horizontal SFCWs (Vymazal, 2013). Iris pseudacorus was also a popular emergent aquatic plant for CWs in southern China (Dai et al., 2018). Consequently, two SFCWs (referred to as SFCW-R and SFCW-I) were transplanted with reeds and Iris pseudacorus at March 2016 and August 2016, respectively. The one (referred to as SFCW-G) without any plants was used as a reference case.

The SFCWs were operated with continuous flow to simulate polluted surface water from secondary effluents of WWTPs. To minimize variability in the experiment, synthetic wastewater composed of KNO_3 and NH_4Cl (with target TN concentration at 15 mg L^{-1}) was pumped into each of the three SFCWs. Hogland's nutrition solution (containing $CaNO_3$, KH_2PO_4, $MgSO_4$, H_3BO_3, $MnSO_4$, $ZnSO_4$, $CuSO_4$, MoO_3, $MgCl_2$, $FeSO_4$ and EDTA-Na) was added to provide trace elements for plant growth (Dai et al., 2018). Two-month microbial acclimation of SFCWs started naturally without seeding sludge and carbon source before the experiment, maintaining the TN influent concentration of 15 mg/L and the hydraulic retention time (HRT) of 4 d. Five operational phases (phase I–V, as shown in Table 1) were applied in this study to investigate the effects of various parameters on SNAD through following comparisons: (1) In Phase I and II, the influence of HRTs was assessed while the influent concentration of nitrate and ammonia remained unchanged; (2) In Phase I, III and IV, the influence of C/N (TOC/TN) was investigated by adding different amount of glucose as carbon source; (3) In Phase I and V, the influence of influent nitrogen forms (nitrate and ammonia) was explored without additional carbon source.

2.2. Chemical analysis

Water samples were collected every two days from the SFCWs and then filtered through a membrane (0.45 μm pore size). Nitrate, nitrite and ammonia were analyzed according to the standard method with an ultraviolet spectrophotometer (UV-1800, SHIMADZU, Japan). Total nitrogen (TN) and total organic carbon (TOC) were measured with a TN/TOC analyzer (Multi N/C 3100, Analyticjena, Germany). Temperature, pH and dissolved oxygen (DO) were detected with a multiparameter water quality instrument (ProPlus, YSI, American). All chemical reagents were of analytical grade. All chemical analysis was conducted in duplicate.

Table 1
Operational scenarios of SFCWs to investigate the effects of HRT, influent form, and carbon addition.

Phase	Days (d)	C/N	NO_3^--N (mg/L)	NH_4^+-N (mg/L)	HRT (d)	Temperature (°C)
I	20	0	11–12	3–4	2	19.52 ± 1.79
II	24	0	11–12	3–4	4	18.34 ± 1.65
III	38	1.6	11–12	3–4	2	22.28 ± 2.20
IV	20	2.8	11–12	3–4	2	20.67 ± 2.42
V	40	0	1–2	12–14	2	24.95 ± 2.27

2.3. Microbial analysis

The microbial samples of SFCWs were collected from the gravel surface at the end of Phase I, III and V. The bacterial DNA was extracted with QIAamp DNA stool Mini Kit. (QIAGEN, Germany) following the manufacturer's protocol. The extracted DNA was amplified with the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) in the V3-V4 region of bacterial 16S rRNA genes. The PCR amplification started with the initial denaturation at 95 °C for 5 min, followed by 27 cycles of 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and end with the final elongation at 72 °C for 10 min. High-quality sequencing was conducted with Illumina PE250 by BIOZERON Biotechnology Co. Ltd. (Shanghai, China).

High-quality sequences were clustered into the operational taxonomic units (OTU) by setting a 97% sequence similarity with Usearch (version 7.1). The taxonomy was conducted using the RDP Classifier (version 2.2) on Qiime platform at 70% confidence threshold. Rarefaction curves, bacterial diversity (Shannon and Simpson) and community richness (Chao1 and Ace) were obtained with Mothur (version 1.30.1).

The principal coordinates analysis (PCoA) and redundancy analysis (RDA) were conducted in R software. The microbial samples were predivided into group 1, 2 and 3 representing different phases, respectively. The abbreviations G, R and I represent the wetlands with gravels, reeds and Iris pseudacorus, respectively. For example, G1 means the microbial sample of SFCW-G in Phase I. The similarity or dissimilarity of bacterial community was estimated by PCoA based on bray-curtis measure. The correlation between environmental factors and bacterial community was analyzed by RDA based on Euclidean distance at genus level.

2.4. Data analysis

All statistical analysis was conducted with SPSS (version 20.0) and was considered significant at 0.05 level. The differences of TN, nitrate, nitrite, ammonia, pH, DO were tested by one-way ANOVA test. The removal rate (RR, g m^{-2} d^{-1}) and removal efficiency (RE, %) were calculated as follows:

$$RR = q \times (C_{in} - C_{eff}) \quad (1)$$

$$RE = \frac{C_{in} - C_{eff}}{C_{in}} \times 100\% \quad (2)$$

where q is the hydraulic loading rate (HLR, m d^{-1}); C_{in} and C_{eff} are the influent and effluent TN concentrations (g m^{-3}), respectively. The effects of plants in SFCWs were evaluated as follows:

$$C_R = \frac{TNRR_{SFCW-R} - TNRR_{SFCW-G}}{TNRR_{SFCW-R}} \times 100\% \quad (3)$$

$$C_I = \frac{TNRR_{SFCW-I} - TNRR_{SFCW-G}}{TNRR_{SFCW-I}} \times 100\% \quad (4)$$

where C_R and C_I are the contribution of reeds and Iris pseudacorus; $TNRR_{SFCW-R}$, $TNRR_{SFCW-I}$ and $TNRR_{SFCW-G}$ are the TN removal rate of SFCW-R, SFCW-I and SFCW-G, respectively.