Bioresource Technology 233 (2017) 191–199

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Enhancement of the complete autotrophic nitrogen removal over nitrite process in a modified single-stage subsurface vertical flow constructed wetland: Effect of saturated zone depth

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HIGHLIGHTS

- SZD significantly affected N transformation in the VSSF.
- Suitable SZD benefited enhancement of CANON process in the VSSF.
- The VSSF with SZD of 45 cm achieved high-rate N removal via CANON route.
- Less N_2O emission was achieved owing to enhancement of CANON process in the VSSF.

ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form 19 February 2017
Accepted 22 February 2017
Available online 27 February 2017

Keywords:
Subsurface vertical flow constructed wetland (VSSF)
Saturated zone depth (SZD)
Complete autotrophic nitrogen removal over nitrite (CANON)
Anammox
Nitrogen transformation

ABSTRACT

This study was conducted to explore enhancement of the complete autotrophic nitrogen removal over nitrite (CANON) process in a modified single-stage subsurface vertical flow constructed wetland (VSSF) with saturated zone, and nitrogen transformation pathways in the VSSF treating digested swine wastewater were investigated at four different saturated zone depths (SZDs). SZD significantly affected nitrogen transformation pathways in the VSSF throughout the experiment. As the SZD was 45 cm, the CANON process was enhanced most effectively in the system owing to the notable enhancement of anammox. Correspondingly, the VSSF had the best TN removal performance [(76.74 ± 7.30)%] and lower N_2O emission flux [(3.50 ± 0.22) mg·(m^2·h)$^{-1}$]. It could be concluded that autotrophic nitrogen removal via CANON process could become a primary route for nitrogen removal in the VSSF with optimized microenvironment that developed as a result of the appropriate SZD.

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1. Introduction

Ammonium-rich wastewater with low C/N ratio is generated in a variety of sources such as animal feeding operations, landfills, and anaerobic digesters (Zhao et al., 2017). Recently, constructed wetlands (CWs) have been introduced to treat this kind of wastewater. In comparison with conventional treatment systems, CWs are more easily maintained and operated, require reduced input, and consume less energy (Vymazal, 2010). However, nitrogen removal in CWs exhibited substantial fluctuations and was often unsatisfactory (Wang et al., 2017). Therefore, the nitrogen removal capacity of CWs must be improved when treating ammonium-rich wastewater with low C/N ratio.

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 inadequate dissolved oxygen (DO) or lack of organics. 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

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http://dx.doi.org/10.1016/j.biortech.2017.02.100
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a single-stage CW during the treatment of ammonium-rich, organic-poor wastewater.

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 maintain anammox (Joss et al., 2009).

Ammonium in wastewater can be removed effectively by the down-flow subsurface vertical flow constructed wetland (VSSF) owing to its better nitrification ability, while the TN removal rates were not as great as expected because of a weak anoxic environment and inadequate organic carbon abundance, increasing the concentration of NO_x^--N in the effluent (Hu et al., 2016). Recently, studies report that oxygen supply in a VSSF can be regulated under the saturated zone depth (SZD) constraints, and the setting of saturated zone is subsequently proved to be effective on nitrogen removal in a single-stage VSSF as a result of the enhancement of anammox (Dong and Sun, 2007; Silveira et al., 2015). Hence, appropriate microenvironment in the system should be created for CANON process on the premise that a VSSF operated with an appropriate SZD. Heretofore, few efforts have been made to achieve satisfactory NH_4^+-N and TN removal in one single-stage VSSF via the CANON process, and the regulation of SZD still remains unclear as the VSSF is used in the degradation of ammonium-rich wastewater with low C/N ratio. So, it is quite necessary to qualify and evaluate the effect of the SZD in VSSF on wastewater treatment as this parameter can affect functional microorganisms involved in nitrogen transformation by changing the oxygen supply, more attempts should also been made to investigate nitrogen removal mechanisms at the molecular level in the system.

Accordingly, the modified single-stage VSSFs with saturated zone were constructed in this study, which was expected to enhance nitrogen removal via the CANON process. A comparison study of the VSSFs at four different SZDs to treat digested swine wastewater was carried out. Nitrogen transformation (including N_2O production) and treatment performances of the VSSFs were investigated under the SZD constraints. For the VSSFs, the absolute abundance of genes involved in nitrogen removal and their ecological associations were assessed to evaluate the relationship between the SZD and the microbial community performing nitrogen removal.

2. Materials and methods

2.1. System description and experimental conditions

VSSFs were constructed at the greenhouse of Anhui Agricultural University (AHAU) in Hefei, China, where the temperature was maintained at (25 ± 2) °C. Each VSSF (a polyethylene tank with a diameter of 20 cm and depth of 80 cm), which was shown in Fig. 1, 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%). Four orifices (25 mm internal diameter for each), which were used for collecting substratum samples, were respectively excavated in a line at different depths (16, 32, 48, and 64 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.

A "Ω"-shaped perforated pipe installed on the top of each VSSF was used as the inlet pipe, and an "L"-shaped pipe embedded at the bottom of each system was used as the outlet pipe. Water level in the system could be regulated via the valves installed on the "L"-shaped pipe at different depths. Regarding the VSSFs, four different water levels were adopted: 5, 30, 45, and 60 cm. Correspondingly, the SZDs in the systems were respective 5, 30, 45, and 60 cm above the bottom of the bed during the treatment.

Each VSSF was operated with a vertical down-flow pattern and received digested swine wastewater from an anaerobic digestion tank. In the process of anaerobic treatment, most of the biodegradable organics was consumed, resulting in a low BOD_5/TN ratio (≈0.47) in the wastewater. The water quality parameters were as follows: TSS, (185.12 ± 24.51) $mg \cdot L^{-1}$; COD, (654.24 ± 162.40) $mg \cdot L^{-1}$; BOD, (230.59 ± 92.57) $mg \cdot L^{-1}$; NH_4^+-N, (459.98 ± 36.88) $mg \cdot L^{-1}$; TKN, (478.78 ± 35.11) $mg \cdot L^{-1}$; NO_3^--N, (3.21 ± 1.71) $mg \cdot L^{-1}$; NO_2^--N, (1.75 ± 1.26) $mg \cdot L^{-1}$; TN, (487.60 ± 38.74) $mg \cdot L^{-1}$; TP, (36.64 ± 12.39) $mg \cdot L^{-1}$; and pH, (7.74 ± 0.58). Prior to the experiment, a biofilm was formed on the substrate using activated sludge collected from a secondary sedimentation tank in a continuous WWTP (Wangtang, Hefei, China) as the inoculum. Each VSSF was operated with an HLR of 0.02 $m^3 \cdot (m^2 \cdot d)^{-1}$. The experimental period lasted for 375 d and was divided into five periods: (1) Period A (adaptation for each VSSF with the SZD of 5 cm), lasted for 42 d; (2) Period B (SZD of 5 cm), lasted for 72 d; (3) Period C (SZD of 30 cm), lasted for 105 d; (4) Period D (SZD of 45 cm), lasted for 108 d; (5) Period E (SZD of 60 cm), lasted for 48 d.

2.2. Analytical procedure

Water samples were collected in triplicate every two days from the inlet and outlet of each VSSF and analyzed immediately. The N_2O emission flux of each VSSF was measured every two days. Substratum samples were collected from each VSSF at least two times during each phase. 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 VSSF was measured every week throughout the experiments.

2.2.1. Water quality analyses

Water quality analyses were conducted for temperature, pH, TSS, COD, BOD, TN, NH_4^+-N, NO_2^--N, NO_3^--N, and TP. The analyses were performed according to standard methods for assessing water and wastewater (APHA, 2002).

2.2.2. Measurement of N_2O emission flux

The close flux chamber technique was used to measure N_2O emission flux in each VSSF (Inamori et al., 2008). The temperature in the chamber was measured every time before sampling. The gaseous phase from each VSSF was disturbed firstly for 5 min using air pumps. Gas samples were then collected from closed gas chambers through sampling tubes into gas sampling bags. Five times of sampling following an interval of 10 min were carried out. The N_2O concentration was analyzed by means of gas chromatography (Shimadzu Co., Japan) equipped with an electron capture detector (ECD) and a Poropak Q column, using 40 $mL \cdot min^{-1}$ argon-containing 5% methane as the carrier gas, and the temperature of

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