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Quantitative response relationships between nitrogen transformation rates and nitrogen functional genes in a tidal flow constructed wetland under C/N ratio constraints

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ABSTRACT

The present study explored treatment performance and nitrogen removal mechanisms of a novel tidal flow constructed wetland (TF CW) under C/N ratios ranging from two to 12. High and stable COD (83–95%), NH_4^+-N (63–80%), and TN (50–82%) removal efficiency were simultaneously achieved in our single-stage TF CW without costly aeration. Results showed that a C/N ratio exceeding six was required to achieve complete denitrification without NO_2^--N and NO_3^--N accumulation in the system. Molecular biological analyses revealed aerobic ammonia oxidation was the dominant NH_4^+-N removal pathway when the C/N ratio was less than or equal to six. However, when the C/N ratio was greater than six, anammox was notably enhanced, resulting in another primary NH_4^+-N removal pathway, in addition to the aerobic ammonia oxidation. Quantitative response relationships between nitrogen transformation rates and nitrogen functional genes were established, and these relationships confirmed that different nitrogen transformation processes were coupled at the molecular level (functional genes), and collaboratively contributed to nitrogen removal in the TF CW. Specifically, NH_4^+-N transformation rates were collectively determined by *amoA*, *nxrA*, anammox, *narG*, *nirS*, *nirK*, and *nosZ*; and TN removal was influenced primarily by *amoA* and anammox.

1. Introduction

Constructed wetlands (CWs) are engineered systems constructed for wastewater treatment. The systems are designed to utilize and replicate many physical, chemical, and biological processes that occur in natural wetlands, but do so within a more controlled environment (Vymazal, 2007). During the last five decades, CWs have evolved from empirical research into practical applications for treating various types of wastewater (Ji et al., 2007; Konnerup et al., 2009; Scholz and Hedmark, 2010; Zhou et al., 1996). Given the economic and ecological benefits, CWs have become a widely applied technique for economically underdeveloped areas and natural habitats, and a valuable complement to traditional sewage systems that dominate large cities (Zhi and Ji, 2012). However,

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under varying organic loading rate, nitrogen removal in CWs exhibited substantial fluctuations and was often unsatisfactory. The North America database documented an average of 44% nitrogen removal efficiency in CWs (Kadlec et al., 2000). In Europe, the nitrogen removal efficiency of a typical CW was only 35%, and could not exceed 50% even with an optimized design (Verhoeven and Meuleman, 1999). Therefore, how to improve nitrogen removal efficiency under varying organic loading rates have become an urgent issue and a research hotspot in the field of CWs.

Studies have demonstrated that nitrogen removal in CWs is often restricted by insufficient oxygen supply (EPA, 1993; Ye et al., 2012). Specifically, microbial ammonia oxidation, which is the first and rate-limiting step for subsequent nitrogen transformation and removal, is impaired by limited oxygen supply to the CW substrate (Caffrey et al., 2007; Francis et al., 2005). Insufficient nitrification is the key factor for improving NH_4^+-N removal efficiency, and therefore an increasing effort has made to increase the oxygen content in CWs. Vertical flow constructed wetlands (VF CWs), which increase oxygen transport capacity and therefore provide much better conditions for nitrification, have been extensively employed to enhance NH_4^+-N removal. However, total nitrogen (TN) removal is deficient in VF CWs due to the limited denitrification, agreeing with previous assessments that single-stage CWs cannot achieve high TN removal due to an inability to provide both aerobic and anaerobic conditions simultaneously (Vymazal, 2007). On the other hand, horizontal flow constructed wetlands (HF CWs), which suffer from a lack of oxygen in beds, provide suitable denitrification conditions. Therefore, a growing interest in integrating specific advantages of different CW types to achieve better TN treatment performance has been observed. The most common configurations of hybrid systems are VF-HF and HF-VF CWs (Vymazal, 2011). Some other hybrid systems consisting of more than two different CW stages are also widely implemented (Brix et al., 2007; Vymazal and Kröpfelová, 2011; Ye and Li, 2009).

Recently, tidal flow constructed wetlands (TF CWs) have been proposed to enhance treatment performance (Leonard et al., 2003; Sun et al., 1999, 2005). The "tidal flow" refers to an operation strategy that repeatedly allows CWs to be filled with wastewater, and then completely drained. During the filling phase, the CWs are gradually flooded and air in bed is continuously squeezed out and consumed. In the draining phase, fresh air is drawn into CWs and the bed is replenished with oxygen. During the rhythmic cycle of "flood/wet" and "drain/dry" phase, the wastewater acts as a passive pump to expel and draw air into the CWs, and consequently oxygen supply and consumption are substantially improved in the system. Hence, both nitrification and denitrification are intensified in TF CWs, and high TN removal can be achieved. Cui et al. (2012) used a two-stage TF CW, and accomplished maximum NH_4^+-N and TN removal of 72.7% and 53.2%, respectively. In two separate studies of four-stage CWs with tidal flow operation under different and varying chemical oxygen demand (COD) loading rates, Zhao et al. (2004, 2011) reported NH_4^+-N (TN) removal efficiency ranged from 49 to 93% (11–78%) and 30–91%. To date, only a few studies have been published on TF CWs. While these studies obtained some promising results in terms of improved NH_4^+-N and TN removal with multiple-stage TF CWs, no efforts have been made to achieve satisfactory NH_4^+-N and TN removal in one single-stage CW with tidal flow operation. In addition, previous studies have primarily focused on optimizing configurations and operational parameters to improve treatment performance under varying loading rates, and few attempts have been made to investigate nitrogen removal mechanisms at the molecular level in TF CWs. Therefore, research targeting nitrogen removal processes and pathways with functional genes under varying loading rates is much needed to optimize the design, operation, and application of TF CWs. Increased efficacy in treating wastewater will have notably positive environmental impacts, considering the ecological benefits these novel CWs can bring at such effective economic costs.

In one of the first attempts to achieve satisfactory nitrogen removal in one single-stage CW with tidal flow operation, the overall goal of this study was to understand nitrogen removal mechanisms at the molecular level under different C/N ratios, and the role of microbes in nitrogen transformation processes. The following four specific objectives were pursued: 1) evaluate the treatment performance under different C/N ratios; 2) quantify the absolute abundance of functional genes involved in nitrogen removal, and investigate ecological associations among these functional genes; 3) determine quantitative response relationships between nitrogen transformation processes and functional genes; and 4) identify key functional genes that determine the treatment performance of nitrogen removal in TF CW. Establishment and analysis of the quantitative response relationships could also aid in our future efforts to: 1) quantify the relative contribution of functional genes to nitrogen removal; 2) regulate nitrogen removal processes at the molecular level with specific functional gene enrichment; and 3) estimate the dynamics of nitrogen transformation rates using functional gene data.

2. Materials and methods

2.1. *Tidal flow constructed wetland*

2.1.1. *Experimental set up*

One lab-scale vertical CW with 40 cm length × 20 cm width × 120 cm depth dimensions (working volume of 40 L) was built with PVC and organic glass (one facet for observation). *Iris pseudacorus* was planted on surface of the CW at an initial density of 22 plants/m^2. The CW bed consisted of three functional layers, the water distribution layer (0–20 cm), the treatment layer (20–100 cm), and the water collection layer (100–120 cm). The treatment layer was filled with lava rock with a particle diameter of 8–10 mm, and the water collection layer was filled with gravel of 10–20 mm in diameter.

2.1.2. *Start-up and operation strategy*

C/N ratio was applied as an indicator to control organic loading rates in the influent. The experiment began on 5 December 2011, and involved the following six stages (total 245 days): Start-up stage (C/N = 2.0) from 5 December to 21 February; Stage I (C/N = 4.0) from 22 February to 28 March; Stage II (C/N = 6.0) from 29 March to 1 May; Stage III (C/N = 8.0)