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Carbon source effects on nitrogen transformation processes and the quantitative molecular mechanism in long-term flooded constructed wetlands

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ABSTRACT

The effect of adding rice husk as an electron donor on the genetic and metabolic diversity of denitrifying communities in a long-term flooded constructed wetland (D1) was compared with the control group without external carbon source addition (D2). The underlying molecular mechanisms responsible for nitrate removal were quantified under different nitrate loading rates. The results showed that D1 achieved higher and more stable removal efficiencies for NO_3^--N (90–97%) and total nitrogen (73–87%) under different nitrate loading rates, which were approximately 10 times higher than those of D2. This corresponded with much higher absolute abundances of bacterial and nitrogen functional genes in D1 than D2. Additionally, the vertical distribution of microbial communities in D1 and D2 showed significant differences. This pattern suggested that adding rice husk in flooded CWs had beneficial effects on the denitrifying community. Quantitative response relationships and network analysis indicated that denitrification was the dominant nitrogen removal pathway. Moreover, the present study indicated that using rice husk as an external carbon source could be conducive to completing the nitrification process, which was first confirmed at the molecular level. The present study indicated that using rice husk as an external carbon source can significantly enhance denitrification rates and thus improve nitrogen removal in CWs for wastewater treatment process.

1. Introduction

Nitrogen compounds discharged into the environment can cause serious problems such as eutrophication of rivers and deterioration of water sources, as well as hazards to human health. Nitrate is a prior pollutant of concern for groundwater in many countries due to both its toxicity and universality. Additionally, nitrates can also transform into nitrosamines and nitrosamides, the potential carcinogenic compounds. As a result, nitrate contamination of water is becoming a severe environmental problem worldwide (Fernandez-Nava et al., 2010).

Nitrate removal technologies mainly include ion exchange, reverse osmosis, adsorption, electrodialysis and biological denitrification (Della Rocca et al., 2007; Ji et al., 2014). Among these, only ion exchange and biological denitrification are feasible on a large scale (Soares, 2000). Furthermore, the biological nitrogen removal process is considered to be the most appropriate technology when as compared to other techniques due to its effectiveness and relatively low cost (Koren et al., 2000).

Various biological processes have been developed which include anaerobic ammonium oxidation (Annamox), nitrification and denitrification, completely autotrophic nitrogen removal over nitrite (CANON) process, and oxygen-limited autotrophic nitrification-denitrification process with widespread applications throughout the world (Khardenavis et al., 2007; Peng and Zhu, 2006). Anaerobic ammonium oxidation (Anammox) is accomplished by the reactions of NO_2^- and NH_4^+ and does not require additional external carbon sources (Wu et al., 2018). Biological denitrification occurs naturally when certain bacteria use nitrate as the terminal electron acceptor in their respiratory process under anaerobic or anoxic conditions (Cervantes et al., 2001). Generally, biological denitrification involves both autotrophic and heterotrophic pathways, and both can be developed under anaerobic conditions, which consist of sequential reductive reactions from NO_3^- to NO_2^-, NO_2^- to NO, NO to N_2O, and, finally, N_2O to nitrogen gas (N_2) (Maia et al., 2012; Rich and Myrold, 2004; Wang et al., 2014).

Denitrifying bacteria are ubiquitous in nature. They require carbon

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and energy sources that may be organic or inorganic compounds (Soares, 2000). Autotrophic denitrification (AD), based on an inorganic carbon source, involves sulfur and hydrogen gas as the electron donor for the bacterial metabolic chain (Della Rocca et al., 2007). Heterotrophic denitrification is a respiratory process in which heterotrophic bacteria consume organic substances as carbon sources and nitrate as the terminal electron acceptor (Jafari et al., 2015). When nitrate-containing wastewater does not contain sufficient organic carbon, it is necessary to add an external source in order to achieve complete denitrification (Fernandez-Nava et al., 2010; Obaja et al., 2005; Shen et al., 2009; Sun et al., 2012). Characteristics of the added carbon source have been found to have major effects on the important parameters of denitrification process such as COD demand, denitrification rate, biomass yield and biomass composition (Obaja et al., 2005). In addition, the nature of the carbon source can influence both the nitrate reduction pathway and the carbon utilization patterns (Ramakrishnan and Gupta, 2008).

Generally, denitrifiers can be supplied by a soluble or insoluble carbon source. Soluble carbon sources, such as glucose, sucrose, glycerol, acetic acid, lactic acid, and methanol, are quite effective when used in nitrogen removal processes (Akunna et al., 1993; Constantin and Fick, 1997; Gomez et al., 2002; Mohseni-Bandpi et al., 1999). However, these external electron donors are high-cost and thus have limited utilization in concentrated wastewaters. Insoluble carbon sources, such as vegetable oil (Hunter, 2001), natural organic substrates such as sawdust (Schipper et al., 2004), licorice (Lee and Welander, 1996), wheat straw and cotton (Volokita et al., 1996), bio-polymer (Boley et al., 2002) and methane (Islas-Lima et al., 2004) are the more widely cited ones. Fernandez-Nava et al. (2010) used three alternative carbon sources: wastewater from a sweet factory, a residue from a soft drinks factory and a residue from a dairy plant in the denitrification of a high nitrate concentration wastewater, obtaining maximum specific denitrification rates of between 42 and 48 mg NO_3^--N/g VSS h. Cappai et al. (2004) employed two industrial wastewaters originating from an ice-cream production factory and a beet-sugar factory, obtaining a mean specific denitrification rate of 3.28 mg NO_3^--N/g VSS h and 2.72 mg NO_3^--N/g VSS h, respectively.

Many researchers have considered waste products as possible carbon sources from economic and environmental perspectives, such as industrial wastes or municipal and agricultural effluents (Bilanovic et al., 1999; De Lucas et al., 2005; Elefsiniotis and Li, 2006; Elefsiniotis et al., 2004; Park et al., 2008; Sage et al., 2006). Their studies have primarily focused on optimizing configurations and operational parameters to improve treatment performance. Few attempts have been made to investigate carbon source effects on nitrogen removal mechanisms at the molecular level in long-term flooded constructed wetlands (CWs). Rice husk, the by-product of rice processing, is an abundant resource. The yield of rice husk in China is more than 1/3 of the world's supply, approximately 32 million tons. In addition to the main components, hemicellulose, lignin cellulose and silicon dioxide, rice husk contains plenty of lipids, proteins, mineral nutriments, vitamin B and vitamin C, etc. The use of rice husk as a source of easily biodegradable carbon for denitrification in high-strength wastewaters treatments is an attractive strategy.

The present study was aimed at exploring the effects of rice husk as the only carbon source on the long-term treatment performance and nitrogen removal pathway by using nitrate as the final electron acceptor in flooded CWs. The underlying molecular mechanisms of nitrogen transformation were also explored. Additionally, the key functional gene groups in denitrification processes were determined and their contributions were assessed along a depth gradient. The results of this study might have significant implications for nitrogen removal enhancement in flooded CWs by using crop waste as the carbon source.

2. Methods

2.1. Construction and operation of flooded CWs

Two lab-scale vertical flooded CWs with 0.4 m length × 0.2 m width × 1.0 m depth dimensions (working volume of 40 L) were built with PVC and organic glass (one facet for observation). *Iris pseudacorus* was planted on the surface of CWs with an initial density of 22 plants/m^2 (Zhi and Ji, 2014). A perforated PVC pipe (L × D = 1.0 × 0.05 m^2) was vertically preburied in the center of the CW, which was filled with rice husk. This CW was termed D1, and the other one without rice husk was termed D2. The CW bed was filled with 6–10 mm diameter lava rocks. In addition, a PVC pipe (L × D = 1.0 × 0.05 m^2), harboring a total of eight columns (filled with the same bed materials of CWs) that were wrapped by highly permeable nylon mesh, was pre-buried in the CW bed for microbial sampling (Zhi et al., 2015). The Schematic diagram of long-term flooded constructed wetland system was shown in Fig. S1 (Supporting Information, SI).

The CW was operated under long-term flooded conditions, fed with NO_3^--N wastewater to investigate the long-term treatment performance and NO_3^--N removal pathway. The experiment began on March 1, and involved the four following stages (total 112 days): Start-up stage from March 1 to March 28; Stage I (C/N = 1.0) from March 29 to April 26; Stage II (C/N = 0.5) from April 27 to May 24; and Stage III (C/N = 0.25) from May 25 to June 21. Synthetic wastewater was made from tap water (Beijing groundwater). Wastewater compositions in each operation stage are summarized in Table S1. NO_3^--N concentrations varied from 5.0 to 20.0 mg/L depending on the operation phase. The hydraulic loading rate in all phases was maintained at 0.3 $m^3/(m^2$ d). Synthetic wastewater was prepared daily in a feeding tank and then pumped into the CWs through flumes in the distribution layer. The flooded CWs were placed indoors where the temperature of influents and effluents ranged from 16.3 to 29.4 °C and the pH was between 6.3 and 8.4.

2.2. Sample collection and analysis

During the operation phase, water samples were collected from D1 and D2 at a depth gradient of 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m and 0.8–1.0 m every week except the first week of each stage, and analyzed immediately at the Key Laboratory of Water and Sediment Sciences at Peking University. COD, NH_4^+-N, NO_2^--N, and NO_3^--N determination were conducted according to standard analytical procedures (Apha, 1995). COD was measured using a HACH DR2800 (HACH, Loveland, CO). NH_4^+-N, NO_2^--N, and NO_3^--N were determined with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Dissolved oxygen (DO) and temperature of the solution were measured with a 52 YSI DO meter. The pH of the influent was measured by an 828 Orion pH meter. During the start-up stage of D1 and D2, a 100 g high efficiency compound microbial inoculum B350 M (Biosystems Co.) was inoculated into each CW. Microorganism samples were collected from pre-buried columns in D1 and D2 at the end of weeks 1, 2, 3, 4, 8, 12, and 16. They were collected at four locations (0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m, respectively) in the polyurethane foam. During each sampling process, four or five microbiological samples were taken from each location and mixed well (approximately 5.0 g). After each on-site collection, microbiological samples were stored in an ice incubator, and subsequently sent to the Key Laboratory of Water and Sediment Sciences, Peking University, for analysis. Total genomic DNA was extracted from microbial samples using the Soil DNA kits D5625-01 (Omega, USA) and then detected by 1% agarose gel electrophoresis. The extracted DNA samples were stored at −20 °C in a freezer until further processing.