Ecological Engineering 102 (2017) 145–151

Contents lists available at ScienceDirect

Ecological Engineering

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

Quantitative responses of nitrous oxide accumulation to genetic associations across a temperature gradient within denitrification biofilters

Yan Zhang, Guodong Ji*, Rongjing Wang

Key Laboratory of Water and Sediment Sciences, Ministry of Education, Department of Environmental Engineering, Peking University, Beijing 100871, China

ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 3 January 2017
Accepted 3 February 2017
Available online 21 February 2017

Keywords:
Nitrous oxide
Denitrification biofilter
Molecular mechanism
Genetic association
Temperature

ABSTRACT

Increased nitrous oxide (N_2O) in the atmosphere is of global concern. Biofiltration has been studied for gaseous nitrogen treatments in drinking water environments; however, the molecular mechanisms mediating N_2O accumulation in denitrification biofilters have not been quantified. Five denitrification biofilters were developed in this study and all achieved high removal efficiencies for total nitrogen (TN: 72.4–92.7%), nitrate nitrogen (NO_3^--N: 88.6–98.2%) and chemical oxygen demand (COD: 79.3–90.0%) across a low-temperature gradient (5–25 °C). Denitrification coupling with anaerobic ammonium oxidation (ANAMMOX) and dissimilatory nitrate reduction to ammonium (DNRA) processes yielded the presently robust treatment performance. Nitrite availability limited TN removal at 5–15 °C. Our findings indicate that temperature affected N_2O accumulation indirectly by controlling the balance of nitrite versus N_2O reductase carrying microorganisms. In addition, our results demonstrated that genetic association was an important index reflecting the relative intensity of N_2O accumulation at low temperatures.

1. Introduction

The increasing levels of nitrogen and organic matter in drinking water due to pollution caused by agricultural intensification, industrialization, and urbanization have been associated with health problems (Directive.2006/ 118/EC, 2006). This issue has become a matter of particularly great concern in regions with water shortages in China (Wang and Yu, 2014). Water polluted primarily with organic carbon and ammonia compounds is usually referred to as micro-polluted water (Sagbo et al., 2008). Pre-treating micro-polluted water is crucial for removal of excess nitrogen and other contaminants. In this context, there has been growing interest in the development of low-cost and environmentally friendly systems for remediation of micro-polluted water.

Biofilters have been developed for eliminating contaminants from various forms of wastewaters, including stormwater, agricultural runoff, municipal sewage, and industrial wastewater (Andrus et al., 2014; Ge et al., 2014; Payne et al., 2014; Wang et al., 2015a). Meanwhile, biofiltration system designers have begun to consider the importance of potent greenhouse gas emission byproducts of water filtration systems, such as nitrous oxide (N_2O) (Maia et al., 2012; Grover et al., 2013; Bollon et al., 2016).

The production and emission of N_2O in denitrification reactions are problematic and may contribute to ozone destruction and global warming (Ravishankara et al., 2009). Biological denitrification is an enzymatically catalyzed sequential reductive process, involving several critical functional genes in microbial organisms whose products are already being used as molecular markers. Correlations between the abundance of these genes and total denitrification rates in natural settings have been reported (Pan et al., 2015). Zhi et al. (2015) reported the genetic drivers for nitrogen removal in a constructed wetland system with a 24–12 h flood-and-drain flow cycle and demonstrated quantitative relationships between nitrogen cycling rates (NO_3^- and NH_4^+) and the presence of particular functional genes. Wang et al. (2015b) reported gene groups that drive the transformation of nitrogen compounds (NO_3^-, NO_2^-, and NH_4^+) during coupled nitrification and denitrification pathways in a trickling filter under hydraulic retention time constraints. However, little is known regarding the causal relationships among genetic and environmental variables and their quantitative impacts on N_2O accumulation in denitrification biofilters. The exact modulators for N_2O accumulation in denitrification biofilters under low temperature constraints are uncertain. Estimating N_2O accumulation on a molecular level is important not

* Corresponding author.
E-mail address: jiguodong@pku.edu.cn (G. Ji).

http://dx.doi.org/10.1016/j.ecoleng.2017.02.008
0925-8574/

only for protecting local soil environment, but also for the global environment at large.

Certain temperature conditions have been shown to be conducive to N_2O production in lake and agriculture ecosystems (Benoit et al., 2015; Fan et al., 2015; Paudel et al., 2015). However, these correlations suggest only that temperature affects N_2O accumulation, but do not provide information about whether these effects result from the adaptation or inhibition of microbial communities via changes in their genetic composition. Pang et al. (2015) showed that cold temperature constraints denitrification in tidal flow constructed wetlands, and that the acclimatization capacity of denitrifiers changes with temperature under long-term anaerobic-aerobic alternating conditions. Although denitrifiers in both natural and controlled environments have been examined in previous studies, little conclusive evidence is available regarding the question of whether variations in N_2O accumulation are directly dependent upon temperature or indirectly effects by way of genetic variables.

In this study, we investigated genetic variables that may modulate the accumulation of N_2O across a temperature gradient in five denitrification biofilters. The objectives of this present work were: 1) to investigate gene abundance and N_2O accumulation rates; 2) to quantify the relative importance of and casual correlations of gene abundance and temperature in N_2O accumulation rates; and 3) to reveal the major molecular mechanism of nitrogen removal in denitrification biofilters, and identify the molecular drivers of N_2O accumulation under low temperature constraints.

2. Materials and methods

2.1. Denitrification biofilter setup and operation strategy

Five laboratory-scale denitrification biofilters, with an effective volume of 8.4 L (inner diameter: 90 mm, height: 1800 mm) for each one, was constructed using plexiglass. The top of the reactor was sealed to form an anaerobic environment. A reticulated polyurethane foam, purchased from Joyce Foam Products, Australia, was fixed as the carrier material. The polyurethane foam was characterized as density, 28 kg/m^3; tensile strength, 150 kPa; tear resistance, 650 N/m; cells per 25 mm, 90 ± 10; and pore size, $300 - 500$ μm. The polyurethane foam was cut in shape with a height of 1200 mm in the biofilter. A sieve tray was installed at the top and bottom of the polyurethane foam. Temperature was controlled using a circulating water bath device. Synthetic wastewater was pumped continuously by a metering pump (ProMinent, China) set with a programmable timer at the bottom of the biofilter, and the treated effluent was discharged from the top the reactor by outlet pipe (Fig. S1).

The overall experiment was composed of acclimation and operation periods. Synthetic wastewater was prepared by adding 30 mg/L KNO_3, 300 mg/L CH_3COONa, 15 mg/L NH_4Cl, 3.0 mg/L KH_2PO_4, 100 mg/L $MgSO_4 \bullet 7H_2O$, 23 mg/L $CaCl_2$, 3.0 mg/L $FeCl_3 \bullet 7H_2O$, and 12 mg/L $NaHCO_3$ to the tap water of local water supply network (Table S1). The pH in the freshly prepared wastewater was adjusted to 7.2 ± 0.1. To minimize the impacts of current rush on the biofilm, hydraulic loading from days 0–42 was kept at 10 cm/d and increased to 40 cm/d from day 43. The influent concentration of nitrate nitrogen (NO_3^--N) increased from 30 mg/L to 100 mg/L to promote microbial growth. On the last day of acclimation period, the NO_3^--N removal efficiency reached 90%, and biofilm was visible at the surface of the polyurethane foam. Water temperature was maintained at 25 °C in the acclimation period. Afterwards, the temperature was gradually reduced, whereas the other variables were maintained as the experiment proceeded: Stage I (T = 25 °C) from April 28 to May 18; Stage II (T = 20 °C) from May 21 to June 10; Stage III (T = 15 °C) from June 13 to July 4; Stage IV (T = 10 °C) from July 7 to July 27; and Stage V (T = 5 °C) from July 30 to August 19.

2.2. Sample collections and analysis

Throughout the operation, water samples were collected at the inlet and outlet every three days and analyzed immediately at the Key Laboratory of Water and Sediment Sciences, Peking University. Triplicate water sample from every location was measured at each time to determine the water quality. A HACH DR2800 (HACH, USA) multi-functional water quality tester was used to determine variables, including total nitrogen (TN), NO_3^--N, nitrite nitrogen (NO_2^--N), ammonium nitrogen (NH_4^+-N) and chemical oxygen demand (COD) according to standard protocols (APHA et al., 2012).

Gas and microorganism sampling was conducted at the last day of each operation stage. Triplicate gas samples were collected with a vacuum and sampling bags at each time. The vacuum (Becker, Germany) operated continuously until the degree of absolute vacuum decreased to 0.03 MPa. The gas volume was measured and used to calculate the average production rate during the 24 h. Afterwards, gas samples were analyzed for N_2O concentrations in the laboratory (Kampschreur et al., 2008). Gas sample components were determined on a 6890 N gas chromatograph (Agilent, USA) with the following operating conditions: PEG–20 M capillary tube chromatographic column (0 m × 0.53 mm × 1.00 μm); column temperature 40 °C; detector temperature 250 °C, injection port temperature 150 °C, makeup gas flow 60 mL/min, column flow 5 mL/min, and injection column 100 μL.

Microorganism samples were collected at five locations at the end of each operation stage. The sampling locations were set at five depths (0–24 cm, 24–48 cm, 48–72 cm, 72–96 cm, and 96–120 cm, respectively). During each sampling, four or five samples were taken and mixed well. After each collection, the microorganism samples were stored in an ice incubator and sent to the Key Laboratory of Water and Sediment Sciences, Peking University for analyses. D5625-01 Soil DNA Kits (Omega, USA) were used to extract and purify total genomic DNA from the microorganism samples. Extracted genomic DNAs were detected by 1% agarose gel electrophoresis and maintained in a −20 °C freezer for further analysis.

2.3. Quantitative polymerase chain reaction (qPCR)

In order to compare the accumulation rates of N_2O with the presence of microorganisms under five temperature conditions, the abundance of several functional genes were quantified using qPCR. The 16S rRNA gene was used to assess the abundance of anaerobic ammonium oxidation (ANAMMOX) bacteria, archaea and total bacteria, whereas the *narG, napA, nirK, nirS, qnorB* and *nosZ* genes were used to assess different groups of denitrifiers.

The qPCR was performed on an ABI PRISM 7300 (Applied Biosystems, USA). The forward and reverse primers in Table S2 were used for amplification processes. We used the specific cloning enzyme TransTaq-T DNA Polymerase (TransGen Biotechnology Company, Beijing) as the polymerase. The PCR reaction mixture contained the following: 10 μL Power SYBR Green Mixture (Applied Biosystems, USA), 2.5–10 pmol primer pairs, 1.0 μL amplified DNA, and 0.625 U Taq polymerase. Sterile water was added until the reaction reached a total 20 μL volume. PCR products were detected by 1% agarose gel electrophoresis.

Plasmids containing functional genes were used as quantitative standards. Specific gene fragments obtained from PCR amplifications were connected to pEASY-T3 (Beijing TransGen Biotechnology Co. Ltd.) and subsequently cloned into Trans1-T1 competent cells (Beijing TransGen Biotechnology Co. Ltd.). The strains were then screened on ampicillin (50 mg/L) plates and incubated at 37 °C