International Biodeterioration & Biodegradation 102 (2015) 94–99

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Effect of organics on nitrous oxide emission during wastewater nitrification with enriched nitrifiers

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ARTICLE INFO

Article history:
Received 26 December 2014
Received in revised form
5 March 2015
Accepted 5 March 2015
Available online 22 March 2015

Keywords:
Enriched nitrifiers
N_2O emission
Nitrification
Denitrification
Organics
COD/N

ABSTRACT

Nitrous oxide (N_2O) is a potent greenhouse gas emitted during wastewater nitrification and denitrification. Batch nitrification with enriched nitrifying sludge and municipal wastewater was conducted in a laboratory reactor (working volume: 0.5 L) to investigate the impacts of organic components on N_2O emissions at different aeration rates (10 and 20 mL/min) and $NH_4^+ - N$ concentrations (25, 50, and 100 mg/L). Maximum $NH_4^+ - N$ removal efficiencies reached 95 and 99% at the 10 and 20 mL/min aeration rates, respectively. At the 10 mL/min aeration rate, cumulative N_2O-N emissions reached 0.44, 1.74, and 2.89 mg, while 0.79, 2.80, and 3.79 mg N_2O-N were emitted at the 20 mL/min aeration rate with $NH_4^+ - N$ concentrations of 25, 50, and 100 mg/L, respectively. N_2O emission yields (kg N_2O-N per kg processed $NH_4^+ - N$) were 0.039–0.076 and 0.064–0.109 at the 10 and 20 mL/min aeration rates, respectively, whereas the yields during nitrification without organics were 0.016–0.058 and 0.029–0.088 under the same experimental conditions. Overall, the municipal wastewater containing organics produced greater N_2O emissions and yield than the wastewater without organics under the same operating conditions.

Introduction

Among the greenhouse gases, nitrous oxide (N_2O) is the third most important after CO_2 and CH_4, having a global warming potential that is 296 times that of CO_2 (over a 100-year time horizon) (IPCC, 2001; Sovik et al., 2006). N_2O accounted for 7.9% of global anthropogenic greenhouse gas emissions in 2004, and its emission from wastewater accounts for approximately 2.8% of total anthropogenic sources of N_2O (IPCC, 2007). It can also disturb the stratospheric ozone layer via a photochemical reaction that produces nitric oxide (NO), which catalyzes the destruction of ozone (Conrad, 1996). Global N_2O emissions from wastewater treatment are expected to increase by 13% between 2005 and 2020 (IPCC, 2001).

N_2O emission is associated with biological nitrification and denitrification in wastewater treatment during the transformation of ammonium (NH_4^+) to nitrogen gas (N_2) (Hanaki et al., 1992; Zheng et al., 1994; Sümer et al., 1995). Biological nitrification consists of the oxidation of NH_4^+ or ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-), whereas denitrification is the reduction of nitrate to N_2 via several intermediates such as nitrite, NO, and N_2O. These transformations are performed by different bacterial taxa (Wrage et al., 2001). N_2O is an obligatory intermediate of denitrification (Tallec et al., 2006b). During denitrification, the sequential reduction of nitrate to N_2 is catalyzed by four enzymes: nitrate reductase, nitrite reductase, nitric oxide reductase, and N_2O reductase (Wrage et al., 2001). The release of N_2O is usually attributed to the inhibition of N_2O reductase, which catalyzes the reduction of N_2O to N_2. This inhibition depends on several conditions including low pH (Knowles, 1982) and the presence of nitrite and dissolved oxygen. A low carbon-to-nitrogen ratio also leads to N_2O emission during denitrification (Alinsafi et al., 2008). In nitrification, dissolved oxygen (aeration) and nitrite are the important factors determining N_2O emission (Zheng et al., 1994; Yin et al., 2002).

Recent reports have shown that apart from the conventional pathway, other pathways such as denitrification by autotrophic nitrifiers can also cause N_2O emission (Wrage et al., 2001). In this alternative pathway, ammonia is first oxidized to nitrite and then reduced to N_2O by denitrification with ammonium or hydrogen as

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http://dx.doi.org/10.1016/j.ibiod.2015.03.011
0964-8305/

the electron donor (Kampschreur et al., 2009). The enzymes required by the ammonia oxidizers that perform nitrifier denitrification are believed to be essentially the same as for ammonia oxidation and denitrification (Wrage et al., 2001). In this pathway, ammonia oxidizers can shift to nitrifier denitrification when oxygen is limited in the environment, and nitrite acts as the final electron acceptor. The end product of this pathway is gaseous forms of nitrogen, including N_2O (Tallec et al., 2006a).

The efficiency of biological nitrogen removal processes depends primarily on the composition of the wastewater and the environmental conditions of the process. Even though dissolved oxygen (aeration) is considered the most important operation factor that affects N_2O emission, inconsistent results have been reported in the literature regarding the role of aeration. Specifically, results from different studies have indicated that either high aeration (Itokawa et al., 2001; Ahn et al., 2010) or low aeration (Zheng et al., 1994; Okayasu et al., 1997) results in higher N_2O emission.

The contradictory results for the effect of aeration are caused by differences in the operating conditions of the studied wastewater treatment plants and in the wastewater's composition. The latter plays an important role because wastewater is the source of substrates and nutrients for the microorganisms present in the sludge. The nutrients required by each group of microorganisms to perform their activities efficiently vary. Wastewater rich in organic components (organics) aids heterotrophic denitrification and the reduction of N_2O emission by complete denitrification, whereas the use of organics-limited wastewater leads to N_2O accumulation. Furthermore, the organics-limited condition establishes a situation of competition between the nitrate and nitrite reduction processes for electron supply (Almeida et al., 1995; Itokawa et al., 2001). To the authors' knowledge, the effect of the concentration of organics on N_2O emission during wastewater nitrification with enriched nitrifying sludge has not previously been reported. This study will verify the effect of organics on N_2O emission during nitrification using enriched nitrifiers. This study will also reveal the potential of enriched nitrifying sludge for assessing any other pathways involved in N_2O emission during nitrification.

Materials and methods

Sequencing batch reactor for the enrichment of nitrifiers

A sequencing batch reactor (SBR) with a total volume of 11 L (height: 40 cm, diameter: 20 cm) and a working volume of 9 L was used for the growth of nitrifiers and their enrichment. All the SBR operations (aeration, mixing, feeding and drawing, pH) were controlled by a computer program (Lab-view®, National Instruments, USA). The schematic diagram of the laboratory SBR system can be found elsewhere (Toor et al., 2014b). The initial inoculum for SBR operation was taken from a municipal wastewater treatment plant and nitrifiers were enriched by feeding the ammonium containing synthetic wastewater. Composition of the synthetic wastewater and the detailed cyclic operational parameters the SBR can be found elsewhere (Toor et al., 2014b). The SBR operation was carried out more than 3 months and the microbial communities of the enriched nitrifiers were regularly monitored by fluorescence *in situ* hybridization (Kim et al., 2012). Microbial community analysis showed that more than 30% of the total bacteria were nitrifying bacteria.

Batch reactor for the estimation of N_2O emission

In a batch reactor (working volume: 0.5 L), nitrification was carried out with enriched nitrifiers taken from SBR for the estimation of N_2O emission. The batch reactor was aerated for 2 h with 0.4 L of enriched nitrifiers from the SBR to remove the background ammonium. After 2 h of aeration, 0.1 L of municipal wastewater (the effluent of the primary sedimentation tank of a municipal wastewater treatment plant of Chuncheon, Korea) was added. Characteristics of the municipal wastewater used in this study were given in Table 1. Various concentrations (25, 50 and 100 mg/L) of $NH_4^+ - N$ in the municipal wastewater were deliberately adjusted for the batch experiments. Pre-calculated volume of the NH_4^+ stock solution (5 g/L $NH_4^+ - N$) was taken into the 0.1 L municipal wastewater. The batch reactor was mixed with a magnetic stirrer and air was provided for nitrification.

Analytical methods

All the analytical methods were performed based on the Standard Methods (APHA, 2005). $NH_4^+ - N$ was measured using the nesslerization method by reading the absorbance at 425 nm by UV-visible spectrophotometer (UV 1601, Shimadzu, Japan) after filtering through GF/C filter (Whatman, USA) (Sumner, 1918). $NO_2^- - N$ and $NO_3^- - N$ were measured by ion chromatograph (DX 500, Dionex, USA) after passing through 0.45-ìm and 0.22-ìm cartridge filters. N_2O was measured by gas chromatograph (6890, Agilent, USA) using HP-FFTP column and electron capture detector (ECD). N_2 gas was used as a carrier gas. The temperature of oven, injection, and detector were set at 50 °C, 100 °C, and 250 °C, respectively.

For the estimation of N_2O emission from the batch reactor, off-gas samples were taken every 30 min and directly injected to a gas chromatograph. Liquid samples were collected every hour for the measurement of $NH_4^+ - N$, $NO_2^- - N$, and $NO_3^- - N$. The N_2O yield and nitrogen removal efficiency were also calculated. All the wastewater nitrification and N_2O emission measurements were carried out in duplicate and the average values were used for the analysis.

Results and discussion

Nitrifier enrichment in SBR

The SBR was used to establish and enrich nitrifiers sludge in order to carry out batch reactor for the estimation of N_2O emission during wastewater nitrification. The SBR was stabilized within 2 months and obtained stable nitrification to nitrate. Nitrogen profiles in the SBR showed excellent nitrification of a paradigmatic SBR cycle. It can clearly be inferred that all the input NH_4^+ (1.8 L of 240 mg N/L) was completely oxidized into NO_3^- via NO_2^- in a cycle (6 h). In SBR operations maximum $NH_4^+ - N$ and $NO_2^- - N$ concentrations were maintained less than 5.0 and 2.3 mg/L, respectively, due to wastewater nitrification during the aeration time. Similar pattern for total nitrogen (TN) and $NO_3^- - N$ in the SBR was observed as described in the previous report (Toor et al., 2014b).

Table 1
Characteristic of the wastewater used in this study.

Characteristic	Wastewater (S.D.)
COD (mg/l)	85 (0.36)
$NH_4^+ - N$ (mg/l)	[a]125–500 (NA)
$NaHCO_3$ (mg/l)	0.53 (0.02)
NO_2-N (mg/l)	0.53 (0.04)
NO_3-N (mg/l)	0.0 (0.1)

S.D.: standard deviation.
NA: not applicable.
[a] Used for batch reaction.