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Nitritation *versus* full nitrification of ammonium-rich wastewater: Comparison in terms of nitrous and nitric oxides emissions

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HIGHLIGHTS

- We compare N_2O and NO emissions from nitritation and full nitrification processes.
- N_2O and NO emissions were largely minimized under full nitrification conditions.
- Avoidance of NH_4^+ and NO_2^- accumulation contributed to gas emission minimization.
- Increasing ammonium loading presented no effects on N_2O emissions.
- Evaluation of nitritation is needed before implementation on real-scale.

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ABSTRACT

The processes of nitritation and full nitrification of synthetic reject wastewater were compared in terms of N_2O and NO emissions. Two lab-scale sequencing batch reactors (SBR1 and SBR2) were enriched with *Nitrosomonas* (ammonia-oxidizing bacteria) and *Nitrobacter* (nitrite-oxidizing bacteria), as shown by fluorescence *in situ* hybridization (FISH) and high-resolution 16S rRNA tag pyrosequencing. Stable conversion of ammonium to nitrite and nitrite to nitrate was achieved in SBR1 and SBR2 respectively. Biomass from SBR2 was added in SBR1 in order to achieve full nitrification. Under nitritation, 1.22% of the converted-N was emitted as N_2O, and 0.066% as NO. During the transition from nitritation to full nitrification, effluent nitrite concentrations decreased but nitrogen oxides were emitted at levels similar to the nitritation period. Gas emissions decreased sharply under full nitrification conditions (0.54% N_2O-N/converted-N; 0.021% NO-N/converted-N), probably as a result of the combined effect of lower nitrite and ammonium concentrations in the bioreactor.

1. Introduction

Production of nitrous oxide (N_2O), a potent greenhouse gas, and nitric oxide (NO), an ozone depleting agent, has been detected during nitrogen removal in wastewater treatment systems (Kampschreur et al., 2008a; Ahn et al., 2010). In particular, the process of nitrification is considered as a major source of the direct emissions of these gases (Foley et al., 2010). Nitrification is comprised by two coupled processes: first, ammonium (NH_4^+) is oxidized to hydroxylamine (NH_2OH) and nitrite (NO_2^-) through the process of nitritation by ammonia oxidizing bacteria (AOB). Then, NO_2^- is oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB) through a reaction known as nitratation.

In nitrification systems, N_2O and NO can be produced through different pathways. In one of them, the so called nitrifier denitrification, N_2O is produced by the activity of nitrifier-encoded NirK and Nor enzymes, responsible for the reduction of nitrite to N_2O (Wrage et al., 2001; Kampschreur et al., 2008b; Kim et al., 2010). Other suggested possibility involves NH_2OH chemodenitrification and autooxidation (Arp and Stein, 2003; Schmidt et al., 2004). Although the different mechanisms of N_2O production are not completely understood, it seems clear that parameters such as NO_2^-, NH_4^+ and dissolved oxygen (DO) concentrations affect N_2O and NO production during nitrification (Kampschreur et al., 2008b).

Wastewater treatment facilities performing anaerobic sludge digestion are in need to implement the treatment of reject wastewater effluents. The more stringent regulatory demands in terms of nutrient discharge from wastewater treatment facilities, as well as the need to reduce operational costs and energy consumption have resulted in nitritation or partial nitrification of reject wastewater being a common side-stream process. Reject wastewater is usually characterized by high NH_4^+ concentrations as well as low carbon content. When coupled with conventional denitrification or anammox, nitritation is considered as an attractive (low-cost) option for the treatment of NH_4^+-rich wastewater. However, the implementation of nitritation implies accu-

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mulation of NO_2^- and might increase N_2O and NO emissions. Thus, an evaluation on the potential effect of nitritation on nitrogen oxides emissions is needed. In a recent study, Ahn et al. (2011) compared N_2O and NO emissions from a lab-scale bioreactor operated sequentially in full-nitrification and partial-nitrification modes and found an increase in these emissions when operating in partial nitrification conditions. However, in their study, the transition from full nitrification to partial nitrification was achieved by reducing the DO concentration and the sludge residence time (SRT) which alone could have already caused an effect on N_2O production. They also reported a change on the predominant AOB population when operating under full and partial nitrification modes respectively which could also had an effect on the overall emissions detected. Further research is needed to establish the effect of partial or full nitrification on N_2O and NO emissions.

This study explores the different N_2O and NO emission profiles measured in lab-scale sequencing batch reactors (SBRs) performing nitritation, nitratation, and full nitrification of synthetic reject wastewater (1 g NH_4^+-N/L). A characterization of the different bacterial populations based on fluorescence *in situ* hybridization (FISH) and 16S rRNA tag pyrosequencing was performed. A rapid shift from nitritation to full nitrification as a result of the combination of two different enriched populations (AOB and NOB) followed by a change in the SBR cycle configuration allowed a precise comparison between both processes in terms of gas emissions. We tested the hypothesis that N_2O and NO emissions would be minimized during full nitrification due to NO_2^- consumption by NOB. Moreover, the effect of wastewater loading on total N_2O and NO emissions was further studied along individual experiments in the full nitrification system in order to evaluate its importance.

2. Methods

2.1. Enrichment of AOB and NOB populations

Two cylindrical 8L SBRs (SBR1 and SBR2) were inoculated with activated sludge from a domestic wastewater treatment plant (WWTP) located in Girona (Spain). In SBR1, the enrichment of AOB was promoted and nitritation was achieved. SBR2 was utilized to enhance the growth of NOB and the nitratation reaction was established. Both bioreactors were operated in cycles of 6 h, consisting of feed-1 (2 min), aeration-1 (120 min), feed-2 (2 min), aeration-2 (120 min), settling (10′) and decanting (15 min).

SBR1 was fed with synthetic reject wastewater (adapted from Kuai and Verstraete, 1998), containing 5.63 g/L of NH_4HCO_3 (1 g NH_4^+-N/L), 0.064 g/L of each KH_2PO_4 and K_2HPO_4 and 2 mL of trace element stock solution. The trace element stock solution contained (per liter): 1.25 g EDTA, 0.55 g $ZnSO_4{\cdot}7H_2O$, 0.40 g $CoCl_2{\cdot}6H_2O$, 1.275 g $MnCl_2{\cdot}4H_2O$, 0.40 g $CuSO_4{\cdot}5H_2O$, 0.05 g $Na_2MoO_4{\cdot}2H_2O$, 1.375 g $CaCl_2{\cdot}2H_2O$, 1.25 g $FeCl_3{\cdot}6H_2O$ and 44.4 g $MgSO_4{\cdot}7H_2O$. The feed had a pH of 8 and a molar ratio of ammonium to bicarbonate of 1:1. After feeding, the pH of the reactor rose to 7.8 and decreased afterwards due to the nitritation reaction. When pH reached 6.7, it was automatically controlled with addition of 1 M $NaHCO_3$ solution. The mixed liquor temperature in SBR1 was controlled at 30 °C using a water jacket. 1 L of synthetic wastewater was added in each feeding period, providing a hydraulic retention time (HRT) of 24 h. 100 mL of biomass was removed at the end of the second aeration period providing a sludge retention time (SRT) of 20 d. Dissolved oxygen (DO) was controlled with a programmable logic controller (PLC) between 0.8–1.5 mg O_2/L by adding air or nitrogen gas at 5 L/min. After 4 months of operation, SBR1 achieved stable nitritation, with a 98% of NH_4^+ converted to NO_2^- and no NO_3^- detected in the effluent.

SBR2 was fed with synthetic wastewater which comprised the following composition per liter (adapted from Kuai and Verstraete, 1998): 4.93 g of $NaNO_2$ (1 g NO_2^--N/L), 0.4 g of $NaHCO_3$, 1 g of each KH_2PO_4 and K_2HPO_4 and 2 mL of a stock solution containing the same trace elements as for SBR1. 1L of synthetic wastewater was added in each feeding period, providing a HRT of 24 h. During the start-up (~3 month) of SBR2 no biomass was wasted due to the slow bacterial growth. Then, small volumes of biomass were intermittently removed following bacterial growth observations keeping the SRT over 20 d during the experimental period. This bioreactor was operated at room temperature (~23–25 °C). DO was controlled similarly to SBR1. There was no need for pH control as this parameter was around 7.5 and presented no significant changes during the operation of the reactor. SBR2 achieved stable nitratation after 9 months of operation, with a 99% of NO_2^- converted to NO_3^-.

2.2. SBR operation for full nitrification

Four liters of the mixed liquor enriched with AOB were removed from SBR1 (~0.8 gVSS/L) and substituted with the same amount of mixed liquor withdrawn from SBR2 (enriched with NOB), resulting in an equally mixed AOB/NOB community (~0.8 gVSS/L) (SBR1 MIX). After a first settling period, the supernatant was extracted and the biomass was washed with phosphate buffer solution (1 M) before the start of the next cycle. SBR1 MIX was then operated as SBR1 during 4 d (transition period). After this transition period, the cycle configuration was modified in order to achieve full nitrification. The feed volume was reduced to 0.5 L/cycle and the aeration time was extended. The new cycle configuration (480 min) consisted of feed (0.5 min), aeration (360 min), settling (104.5 min) and decanting (15 min). The composition of the synthetic wastewater used, the mixed liquor temperature as well as the pH and DO control were maintained as for normal operation of SBR1 (see Section 2.1). Mixed liquor was manually wasted at a rate of 100 mL/d to maintain a stable VSS concentration. Full nitrification was achieved during the following 30 d of the study.

2.3. Bacterial composition analyses

Mixed liquor samples were taken from SBR1 (during nitritation) and SBR2, along with a sample of the mixed bacterial population (SBR1 MIX) during the full nitrification period. Samples were then subjected to FISH analysis for evaluation of the AOB and NOB communities' enrichment, and to pyrosequencing of bacterial DNA in order to characterize each one of the bacterial populations.

FISH was performed as described in Nielsen et al. (2009) with Cy5-labeled EUBMIX probes and Cy3-labeled AOBMIX probes (for AOBs, comprising equal amounts of probes Nso1225, NEU, NmV, Cluster6a192) and Cy3-labeled Nso190. Cy3-labeled Ntspa662 and NIT3 were utilized for NOB cells. FISH preparations were visualized with a Nikon CS1 confocal laser-scanning microscope (CLSM) using Plan-Apochromat 63× oil (NA1.4) objective. Thirty images were taken from each sample for quantification. The area containing Cy3-labeled specific probe (AOBMIX + NSO190 for AOB and Ntspa662 + NIT3 for NOB) cells was quantified proportionally to the area of Cy5-labeled bacteria probe (EUBMIX) within each image using the daime software package (Daims et al., 2006).

Bacterial DNA was extracted with the FastDNA® SPIN Kit for Soil (MP Biomedicals) from each biomass sample (SBR1, SBR2 and SBR1 MIX) and amplified with primers 27F (3′-GAG TTT GAT CNT GGC TCAG-5′) and 519R (3′-GTN TTA CNG CGG CKG CTG-5′). The amplicons were then sequenced using Roche 454 GS FLX Titanium technology. All pyrosequencing reads were initially screened for quality and length of the sequences using the MOTHUR software package (Schloss et al., 2009). Sequences were trimmed to