Chemical Engineering Journal 321 (2017) 358–365

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Distinctive NO and N_2O emission patterns in ammonia oxidizing bacteria: Effect of ammonia oxidation rate, DO and pH

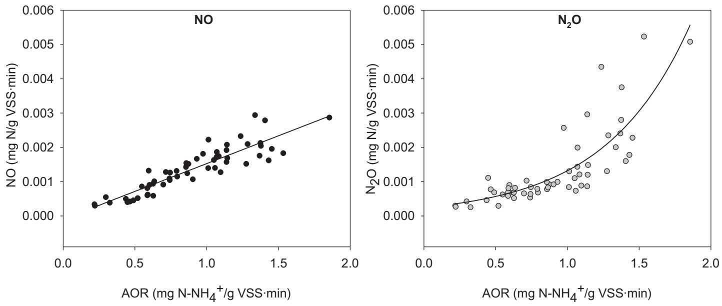
Anna Ribera-Guardia, Maite Pijuan *

Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

HIGHLIGHTS

- Relationship between NO and N_2O production rate and AORsp in an enriched AOB culture is studied.
- Effect of pH and DO on N_2O and NO production are assessed.
- NO linearly correlates with the AORsp while N_2O correlates exponentially.
- N_2O and NO can be produced under anoxic conditions.
- NO is chemically produced when HCl is added.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form 20 December 2016
Accepted 26 March 2017
Available online 29 March 2017

Keywords:
Ammonia oxidizing bacteria
Ammonium oxidation rate
Nitric oxide
Nitrous oxide

ABSTRACT

This study aims at investigating the relationship between the nitric oxide (NO) and nitrous oxide (N_2O) production rates with the ammonia oxidation rate (AORsp) in an enriched AOB culture. Different concentrations of ammonia were applied in a sequential batch reactor (SBR) performing partial nitritation in order to determine the effect of AORsp on N_2O and NO production rates. Results showed that NO linearly correlates with the AORsp whereas N_2O presents an exponential relationship. The effect of changes on the dissolved oxygen (DO) concentration on the overall NO and N_2O emissions was assessed by increasing and decreasing the DO maintaining a constant pH at 7. When DO decreased the AORsp was maintained at the level achieved with the starting DO and led to lower NO and N_2O emissions than when DO was increased. Finally, the effect of pH on N_2O and NO was also tested by maintaining the DO at 1.5–2 mg O_2/L while pH was gradually decreased from 8 to 6.5. Results show that NO was chemically produced due to the addition of HCl when decreasing the pH whereas N_2O was only produced biologically and was not affected by the addition of HCl.

1. Introduction

Ammonia oxidation in wastewater treatment plants (WWTP) is generally carried out by ammonia oxidizing bacteria (AOB). They perform the first step of nitrification where ammonia is oxidized to nitrite. During this process nitrous oxide (N_2O) and nitric oxide (NO) can be produced and emitted to the atmosphere [1]. N_2O is a potent green house gas with a global warming potential over 100 years, 265 times higher than carbon dioxide [2]. On the other hand, NO is an important compound that can cause depletion of the ozone layer [3] and it is toxic for living organisms [4]. In order to minimize the N_2O and NO emissions it is very important to understand the characteristics of their production. N_2O and NO are produced through two different routes: (i) the hydroxylamine pathway: N_2O and NO are intermediates of the hydroxylamine (NH_2OH) biological oxidation or produced by chemical decomposition of hydroxylamine and (ii) the nitrifier denitrification pathway: reduction of nitrite by AOBs under oxygen-limiting conditions or elevated nitrite concentrations [5].

---

* Corresponding author.
E-mail address: mpijuan@icra.cat (M. Pijuan).

http://dx.doi.org/10.1016/j.cej.2017.03.122
1385-8947/

There have been many studies reporting the factors affecting N_2O production in AOB. Law et al. [6] studied the effect of pH on N_2O production and revealed that the N_2O production rate of an enriched AOB culture was dependent on the pH which in turn, affected the ammonia oxidation rate. They studied this effect on the range of 6–8.5 and found that the relationship between N_2O production rate and AOR in this range of pH was linear. In another study, the same authors revealed that the relationship between N_2O production specific rate (N_2Osp) and ammonia oxidation specific rate (AORsp) was exponential in an enriched AOB culture [7]. Peng et al. [8] studied the effect of dissolved oxygen (DO) on N_2O production and their results showed that as DO increased the N_2O production rate also increased. Later on Peng et al. [9] reported the combined effect of DO and NO_2^- concentrations on the N_2O production of a nitrifying culture. Results showed that at each DO level, as NO_2^- concentration increased so did the N_2O production rate. On the other hand at each NO_2^- level, N_2O production rate decreased as DO concentrations increased. Moreover other factors such as nitrite [10] and salinity [11] apart from pH and DO also affect N_2O emissions from nitrifying systems.

On the other hand, reports on NO production have been very scarce. Rodriguez-Caballero and Pijuan [12] studied the N_2O and NO emissions in a partial nitrification reactor using different cycle configurations to minimize these emissions and concluded that NO should be also taken into account when implementing mitigation strategies to reduce N_2O, since some of these strategies might result in increased NO emissions. Yu et al. [13] also studied the production of NO and N_2O under transient anoxic conditions in a pure culture of AOB and reported N_2O emissions during transient conditions (from anoxic to aerobic) when ammonia had been accumulated. However, NO was mainly produced during anoxic conditions. The relationship between the ammonia oxidation rate and the NO production rate was found to be linear for a pure culture of *Nitrosomonas europaea* using synthetic wastewater [14]. Kampschreur et al. [15] studied the NO and N_2O emissions in a full-scale wastewater treatment plant treating reject wastewater in a two-reactor nitritation-anammox process. The NO emissions from the nitritation reactor were 0.2% of the N-load and denitrification by AOBs was considered to be the most probable cause of NO and N_2O emission from the nitritation reactor.

Little is known about the factors affecting NO production and its relationship with N_2O. Kozlowski and co-workers [16] conducted a comparison of phenotypes of N. europaea lacking expression of NirK, Nor B and both enzymes. They found a clear implication NorB in N2O production, being this one significantly lower in those mutant strains without the expression NorB. More recently, an study with pure cultures of *N. viennensis* (in the pylum *Thaumarchaeota* from the Ammonia-oxidizing archea, AOA) and *N. multiformis* (from ammonia-oxidizing bacteria) indicated a different role of NO in the metabolism of both groups. While NO seems to play an essential role in the process of ammonia oxidation in AOA, stopping its oxidation if NO is absent, in the case of AOB NO would not be affecting their main metabolic pathway [17].

This study aims at investigating the effect of ammonia oxidation rate on NO production and assesses its relationship with N_2O. Also, the effect of pH and DO on the production of NO and N_2O was explored in an enriched AOB culture.

2. Materials and methods

2.1. Bioreactor set-up and operation

A cylindrical 8L SBR was inoculated with activated sludge from a local domestic WWTP located in Girona (Spain). The mixed liquor temperature was controlled at 30 °C using a water jacket, to mimic the common temperature conditions of reactors treating reject wastewater. The SBR was operated in cycles of 6 h, consisting of feed-1 (2 min), aeration-1 (105 min), feed-2 (2 min), aeration-2 (103 min), settling (132 min) and decanting (15 min). 1L of synthetic wastewater (prepared in the laboratory to maintain the same composition during the experimental period) was added in each feeding period, providing a hydraulic retention time (HRT) of 24 h. DO was controlled with a programmable logic controller (PLC) between 1.5 and 2.0 mg O_2/L by adding air or nitrogen gas at 5 L/min. The feed was prepared as to mimic the concentration of ammonia present in anaerobic digester liquor and is detailed below. 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 increased to 7.5 and decreased afterwards due to the nitrification reaction. When pH reached 7, it was automatically controlled by adding 1 M $NaHCO_3$ solution. Cycle studies were carried out on a weekly basis to monitor the nitrification activity of the reactor. Samples for the analysis of ammonia, nitrate and nitrite were taken along the cycle and filtered with 0.22 μm Millipore filters. At the end of the second aerobic phase mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were also analysed.

The synthetic wastewater had the characteristics of a typical anaerobic digester liquor. The wastewater composition was modified from Kuai and Verstraete [18]: 5.63 g/L of NH_4HCO_3 (1 g N-NH_4^+/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 solution included (g/L): 1.25 EDTA, 0.55 $ZnSO_4{}^*7H_2O$, 0.4 $CoCl_2{}^*6H_2O$, 1.27 $MnCl_2{}^*4H_2O$, 0.40 $CuSO_4{}^*5H_2O$, 0.05 $Na_2Mo_4{}^*2H_2O$, 1.37 $CaCl_2{}^*2H_2O$, 1.25 $FeCl_3{}^*6H_2O$ and 44.40 $MgSO_4{}^*7H_2O$.

2.2. Batch tests

Batch tests were conducted in the same parent reactor. Three sets of experiments were carried out. The first set consisted on adding a continuous feed (6.57 mg N-NH_4^+/min) followed with different ammonia concentration pulses to see the effect of the AOR on the N_2O and NO production. The DO and pH were controlled at the same values as in the parent reactor. Samples were taken every 30 min to analyse ammonia and nitrite.

The second set of experiments was conducted to explore the effect of DO on N_2O and NO emissions. Three different batch tests were conducted in this set of experiments. In the first batch (2.1) pH was maintained constant at 7 while DO was increased every 15 min from 0.5 to 3 mg O_2/L in a stepwise manner. The DO increased from 0.5–1 mg O_2/L to 1–2.5 mg O_2/L and 2.5–3 mg O_2/L. The second batch (2.2) mimicked the first but with DO decreasing every 15 min from 3 to 0.5 mg O_2/L in a stepwise mode. In this case the DO decreased in the ranges of 3–2.5, 2–1.5 and 1–0.5 mg O_2/L. A pulse of NH_4Cl (50 N-NH_4^+/L) followed by a continuous feed (6.57 mg N-NH_4^+/min) was added in the reactor. In the third batch test (2.3), DO was set at 0 mg O_2/L and pH was maintained at 7 to see the effect of anoxic conditions on the N_2O and NO emissions. No NH_4^+ was added in this test.

The third set of experiments consisted on exploring the effect of pH on N_2O and NO emissions. Five different batch tests were conducted (3.1–3.5). In the first batch (3.1), DO was maintained constant at 1.5–2 mg O_2/L while pH was gradually decreased 0.5 units every 15 min from 8 to 6.5. The other batch tests were conducted under the same conditions as batch 3.1. Batch test 3.2 was conducted without addition of ammonia. Batch test 3.3 was carried out without biomass and without the addition of ammonia. In the fourth batch test (3.4) RO water was used without biomass but with the addition of ammonia in the reactor. In batch test 3.5 NaOH was added. All the experiments lasted between 60 and 120 min.