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Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions

Pascal Wunderlin [a,*], *Joachim Mohn* [b], *Adriano Joss* [a], *Lukas Emmenegger* [b], *Hansruedi Siegrist* [a]

[a] *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, P.O. Box 611, 8600 Duebendorf, Switzerland*
[b] *Empa, Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Air Pollution and Environmental Technology, Ueberlandstrasse 129, 8600 Duebendorf, Switzerland*

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ABSTRACT

Nitrous oxide (N_2O) is an important greenhouse gas and a major sink for stratospheric ozone. In biological wastewater treatment, microbial processes such as autotrophic nitrification and heterotrophic denitrification have been identified as major sources; however, the underlying pathways remain unclear. In this study, the mechanisms of N_2O production were investigated in a laboratory batch-scale system with activated sludge for treating municipal wastewater. This relatively complex mixed population system is well representative for full-scale activated sludge treatment under nitrifying and denitrifying conditions.

Under aerobic conditions, the addition of nitrite resulted in strongly nitrite-dependent N_2O production, mainly by nitrifier denitrification of ammonia-oxidizing bacteria (AOB). Furthermore, N_2O is produced via hydroxylamine oxidation, as has been shown by the addition of hydroxylamine. In both sets of experiments, N_2O production was highest at the beginning of the experiment, then decreased continuously and ceased when the substrate (nitrite, hydroxylamine) had been completely consumed. In ammonia oxidation experiments, N_2O peaked at the beginning of the experiment when the nitrite concentration was lowest. This indicates that N_2O production via hydroxylamine oxidation is favored at high ammonia and low nitrite concentrations, and in combination with a high metabolic activity of ammonia-oxidizing bacteria (at 2 to 3 mgO_2/l); the contribution of nitrifier denitrification by AOB increased at higher nitrite and lower ammonia concentrations towards the end of the experiment.

Under anoxic conditions, nitrate reducing experiments confirmed that N_2O emission is low under optimal growth conditions for heterotrophic denitrifiers (e.g. no oxygen input and no limitation of readily biodegradable organic carbon). However, N_2O and nitric oxide (NO) production rates increased significantly in the presence of nitrite or low dissolved oxygen concentrations.

1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas, about 300 times more effective than carbon dioxide (CO_2), and a major sink for stratospheric ozone (Montzka et al., 2011; Ravishankara et al., 2009; IPCC, 2007). Limiting anthropogenic N_2O emission is thus an urgent requirement. It is estimated that about two thirds of the overall N_2O is emitted by

* *Corresponding author*. Tel.: +41 58 765 5037; fax: +41 58 765 5389.
E-mail address: pascal.wunderlin@eawag.ch (P. Wunderlin).
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microbial processes occurring mainly in agriculture, but also in biological wastewater treatment (USEPA, 2009). In fact, N_2O emissions have been shown to dominate total greenhouse gas emissions from biological wastewater treatment (Wunderlin et al., 2010). In addition to N_2O, nitric oxide (NO) could also be emitted, which is toxic for microorganisms (Zumft, 1993) and contributes to the destruction of the stratospheric ozone layer (Crutzen, 1979).

N_2O production in biological wastewater treatment is associated with autotrophic nitrification and heterotrophic denitrification. Nitrification is the stepwise autotrophic oxidation of ammonia (NH_4^+) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and further to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). Denitrification is the reduction of nitrate (NO_3^-) to atmospheric nitrogen (N_2) by heterotrophic denitrifiers (HET), with nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) as obligatory intermediates. According to Kampschreur et al. (2009), there are three main routes for N_2O production (Fig. 1):

- Hydroxylamine oxidation: production of N_2O from intermediates of biological hydroxylamine oxidation (e.g. HNO, $N_2O_2H_2$; Poughon et al., 2001), probably related to a highly imbalanced metabolic activity of AOB (Yu et al., 2010), or by chemical decomposition of hydroxylamine as well as by chemical oxidation with NO_2^- as an electron acceptor (chemodenitrification; Stüven et al., 1992; Ritchie and Nicholas, 1972).
- Nitrifier denitrification: reduction of NO_2^- by AOB in combination with ammonia, hydrogen or pyruvate as electron donors, e.g. at oxygen-limiting conditions or elevated nitrite concentrations (Wrage et al., 2001; Colliver and Stephenson, 2000; Stüven et al., 1992).
- Heterotrophic denitrification: production of N_2O by heterotrophic denitrifiers due to an imbalanced activity of nitrogen-reducing enzymes, e.g. due to oxygen inhibition (Lu and Chandran, 2010; Baumann et al., 1997), nitrite accumulation (von Schulthess et al., 1994), or a limited availability of biodegradable organic compounds (Itokawa et al., 2001).

In the last decade, significant efforts have been made to better understand the mechanisms of N_2O production (Rassamee et al., 2011; Yu et al., 2010; Tallec et al., 2006; Burgess et al., 2002; Itokawa et al., 2001; von Schulthess et al., 1994). As a result, several parameters favoring N_2O production were identified: low dissolved oxygen concentration, accumulation of nitrite, rapidly changing (dynamic) conditions or a low ratio of COD to N-compounds during heterotrophic denitrification (Kampschreur et al., 2009).

It is generally believed that nitrifier denitrification by AOB is the main N_2O production pathway in biological wastewater treatment under aerobic conditions (Colliver and Stephenson, 2000), but its importance with respect to hydroxylamine oxidation and heterotrophic denitrification remains unclear. Therefore, a better understanding of these mechanisms is essential to formulate operating strategies that minimize N_2O emissions.

In this study, we analyzed N_2O and NO production (emission patterns) from activated sludge under nitrifying and denitrifying conditions in a laboratory-scale batch reactor. A homogeneously mixed system was chosen to allow the selection of specific microbial activities (AOB, NOB, HET) without the use of (i) chemical inhibitors potentially interfering with activated sludge in a complex way, or (ii) pure cultures that may behave differently than mixed culture systems, such as activated sludge. This is the first study showing the combination of nitrifier denitrification by AOB, heterotrophic denitrification and hydroxylamine oxidation as relevant for N_2O (and NO) production in conventional activated sludge used to treat real municipal wastewater.

2. Materials and methods

2.1. Maintenance of sludge and experimental setup

Batch experiments were carried out with activated sludge taken from our pilot scale wastewater facility at Eawag, which was operated continuously for several weeks at the same sludge age before the experimental phase. It treats around 60

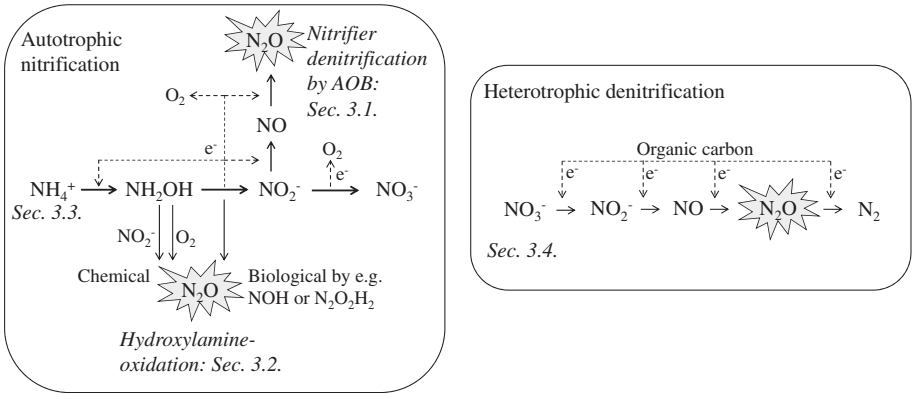

Fig. 1 – Scheme of relevant N_2O production pathways in biological wastewater treatment. During autotrophic nitrification (left figure), N_2O can be produced either via nitrifier denitrification by AOB or hydroxylamine oxidation (chemically as well as biologically). During heterotrophic denitrification (right figure), N_2O is an obligate intermediate in the nitrogen reduction chain.