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The combined effect of dissolved oxygen and nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge

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ABSTRACT

Both nitrite (NO_2^-) and dissolved oxygen (DO) play important roles in nitrous oxide (N_2O) production by ammonia oxidizing bacteria (AOB). However, few studies focused on the combined effect of them on N_2O production by AOB as well as the corresponding mechanisms. In this study, N_2O production by an enriched nitrifying sludge, consisting of both AOB and nitrite-oxidizing bacteria (NOB), was investigated under various NO_2^- and DO concentrations. At each investigated DO level, both the biomass specific N_2O production rate and the N_2O emission factor (the ratio between N_2O nitrogen emitted and the ammonium nitrogen converted) increased as NO_2^- concentration increased from 3 mg N/L to 50 mg N/L. However, at each investigated NO_2^- level, the maximum biomass specific N_2O production rate occurred at DO of 0.85 mg O_2/L, while the N_2O emission factor decreased as DO increased from 0.35 to 3.5 mg O_2/L. The analysis of the process data using a mathematical N_2O model incorporating both the AOB denitrification and hydroxylamine (NH_2OH) oxidation pathways indicated that the contribution of AOB denitrification pathway increased as NO_2^- concentration increased, but decreased as DO concentration increased, accompanied by a corresponding change in the contribution of NH_2OH oxidation pathway to N_2O production. The AOB denitrification pathway was predominant in most cases, with the NH_2OH oxidation pathway making a comparable contribution only at high DO level (e.g. 3.5 mg O_2/L).

1. Introduction

Nitrous oxide (N_2O), a potent greenhouse gas and a major sink for stratospheric ozone, can be produced and emitted from wastewater treatment plants (IPCC, 2007; Ravishankara et al., 2009). Ammonia oxidizing bacteria (AOB) are identified as the major contributor to N_2O production during wastewater treatment (Kampschreur et al., 2007; Yu et al., 2010; Law et al., 2012b). N_2O production by AOB occurs during nitrification via two different pathways: (i) the reduction of nitrite (NO_2^-) to N_2O via nitric oxide (NO), known as nitrifier or AOB denitrification (Kim et al., 2010; Chandran et al., 2011) and (ii) N_2O as a side product during incomplete oxidation of hydroxylamine (NH_2OH) to NO_2^- (Chandran et al., 2011; Stein, 2011; Law et al., 2012a). The AOB denitrification pathway is catalysed by a

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copper-containing NO_2^- reductase (*NirK*) and a haem-copper NO reductase (*Nor*) (Chandran et al., 2011). The NH_2OH oxidation pathway involves nitrosyl radical (NOH) or NO as the intermediates during the oxidation of NH_2OH to NO_2^-. NOH can decompose chemically to form N_2O (Anderson, 1964; Hooper, 1968), while the produced NO is further reduced to N_2O by alternative NO reductases, c′-beta or other homologue NO reductases such as *Nor*S (Stein et al., 2007; Chandran et al., 2011).

NO_2^- is a very important factor affecting N_2O production in nitrification. Varying observations have been reported in literature on the effect of NO_2^- on N_2O production by different nitrifying cultures. It has been demonstrated that the presence of NO_2^- leads to a significant increase of N_2O production in both full-scale and lab-scale studies (Tallec et al., 2006; Kampschreur et al., 2007, 2008; Kim et al., 2010; Wunderlin et al., 2012). It is proposed that N_2O production by AOB denitrification is dependent on the concentration of NO_2^- (Tallec et al., 2006; Kampschreur et al., 2007; Kim et al., 2010; Wunderlin et al., 2012). High NO_2^- concentration has been shown to have a stimulating effect on AOB denitrification by promoting the expression of the nirK gene (Beaumont et al., 2004). It is also found that the nirK and norB mRNA concentrations increase rapidly in the presence of high NO_2^- concentration (280 mg N/L) in an *N. europaea* batch culture (Yu and Chandran, 2010). However, Law et al. (2013) observed an inhibitory effect of high NO_2^- concentration (over 50 mg N/L) on N_2O production by AOB in a nitritation system treating anaerobic sludge digestion liquor. Further data analysis by a mathematical model revealed that the NH_2OH oxidation pathway became the primary pathway when the NO_2^- concentration exceeded 500 mg N/L (Ni et al., 2014).

The AOB denitrification is also promoted by oxygen limitation, as revealed by both pure and enriched AOB culture studies (Bock et al., 1995; Kampschreur et al., 2007). Recently, Peng et al. (2014) demonstrated that the increase of dissolved oxygen (DO) from 0.2 to 3.0 mg O_2/L decreased the contribution of AOB denitrification from over 90% to approximately 70% in a nitrifying culture with the aid of site preference measurement and model-based data analysis. In comparison, N_2O production via the NH_2OH pathway mainly takes place under aerobic conditions and is likely favoured by high DO concentrations (Chandran et al., 2011; Law et al., 2012a). Wunderlin et al. (2012) also showed that N_2O production via the NH_2OH pathway was favoured at high NH_3 and low NO_2^- concentrations, and in combination with a high metabolic activity of AOB.

However, some of previous studies were one-dimensional studies with one factor being varied and the other fixed. For example, Peng et al. (2014) varied DO levels in the range of 0.2–3.0 mg O_2/L, while keeping nitrite at very low levels (below 1.5 mg N/L). In contrast, Law et al. (2013) varied nitrite concentration in the range of 0–1000 mg N/L, while DO was fixed to 0.55 mg O_2/L in most experiments. The dependency of N_2O production by AOB in a two-dimensional (DO and nitrite) space may not be readily predicable from the one-dimensional studies. One of the key reasons is that DO influences the ammonia and NH_2OH oxidations rates, which is expected to not only exert a direct effect on N_2O production through the NH_2OH oxidation pathway, but also influences the nitrite reduction by AOB (and thus N_2O production by the AOB denitrification pathway) through impacting the electron flows.

Whilst in other studies both DO and nitrite concentrations varied simultaneously, they were not changed independently in most cases (Bock et al., 1995; Beaumont et al., 2004; Kampschreur et al., 2007; Yu and Chandran, 2010). In fact, the changes in nitrite concentration were induced by DO changes. As AOB oxidize ammonia to nitrite during nitrification, nitrite could accumulate and the level of accumulation would be dependent on the DO concentration. These conditions would therefore only represent some very limited 'snapshots' in the two-dimensional space of DO and nitrite, and therefore the results cannot be easily extrapolated to the entire two-dimensional space. In fact, it is hard to separate the effects of DO and nitrite in these cases, as the two factors were not independently varied.

In some other studies (e.g., Tallec et al. (2006), Yang et al. (2009), Kim et al. (2010) and Wunderlin et al., 2012), activated sludge comprising a large amount of heterotrophic biomass in addition to AOB and NOB was used. It is known that heterotrophic bacteria are able to produce and consume N_2O, which would not allow separation of the true effects of DO and nitrite on N_2O production by AOB.

Therefore, the aim of this study is to fully clarify the combined effect of DO and NO_2^- on N_2O production by AOB using an enriched nitrifying culture consisting of primarily AOB and nitrite-oxidizing bacteria (NOB). The effect of a relatively small amount of heterotrophic bacteria (growing on AOB and NOB cell lysate) on N_2O production has been identified to be negligible in a previous study (Peng et al., 2014). To reveal the combined effect of DO and NO_2^- on each of the two known pathways and provide further evidence of the relative contributions by both pathways, a N_2O model incorporating both pathways was employed to interpret the experimental data (Ni et al., 2014).

2. Materials and methods

2.1. Culture enrichment and reactor operation

A lab-scale sequencing batch reactors (SBRs) with a working volume of 8 L was operated in the laboratory at room temperature (22.0–23.0 °C) seeded with sludge from a domestic wastewater treatment plant in Brisbane, Australia. The SBR was fed with ammonium with the aim to obtain an enriched culture of AOB and NOB. One cycle of 6 h consisted of 260 min aerobic feeding, 20 min further aerating, 1 min wasting, 60 min settling and 19 min decanting periods. In each cycle, 2 L of synthetic wastewater (compositions are described below) was fed to the reactor, resulting in a hydraulic retention time (HRT) of 24 h. The solids retention time (SRT) was kept at 15 days by wasting 130 mL of sludge during the 1-min wasting period. pH in the reactor was measured with miniCHEM-pH metres and controlled at 7.5 by dosing 1 M $NaHCO_3$. Compressed air was supplied to the reactor during the feeding and aerobic phases. DO in the reactor was continuously monitored online using miniCHEM-DO2 metres and controlled between 2.5 and 3.0 mg O_2/L with a programmed logic controller (PLC).