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Effect of carbon source and competition for electrons on nitrous oxide reduction in a mixed denitrifying microbial community

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ABSTRACT

The competition for electrons has been recently demonstrated to affect the reduction rates of the nitrogen oxides in a methanol enriched denitrifying community. The aim of this study was to test if electron competition also occurred when other substrates were used for denitrification and if that could have an effect on the potential nitrous oxide (N_2O) production and subsequent consumption. A denitrifying culture was developed in a sequencing batch reactor using nitrate as electron acceptor and a combination of acetate, ethanol and methanol as carbon sources. Four sets of batch tests were conducted using acetate, ethanol, methanol and a combination of the three carbon sources respectively. For each set the effect of nitrate, nitrite and nitrous oxide on each other reduction rates when present individually or in combination was assessed. Results show that reduction rates are affected by the type of substrate added, probably due to different microbial populations specialized with consuming a particular substrate. Also, N_2O reduction rate is the most reduced under the different electron competition scenarios tested, which results in N_2O accumulation in some cases. The effect of substrate limitation on N_2O reduction was also assessed.

1. Introduction

Nitrous oxide (N_2O) is one of the most potent greenhouse gases, with a greenhouse effect around 265 times the one of CO_2 over a time frame of 100 years [1] and is also known to be involved in the depletion of the ozone layer [2]. Due to these detrimental environmental effects and its increased rate at around 0.31% per year N_2O has received an increasing attention in the recent years. N_2O is an intermediate of the denitrification process, being formed during the reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2). Four enzymes are involved in this reduction: the nitrate reductase (NaR) responsible for the reduction of the NO_3^- to NO_2^-; the nitrite reductase (NiR) responsible for the reduction of NO_2^- to nitric oxide (NO); the nitric oxide reductase (NOR) responsible for the reduction of NO to N_2O; and finally the nitrous oxide reductase (NoS) necessary for the reduction of N_2O to N_2 [3]. In fact, the rate in which N_2O is reduced will depend on the activity of NoS. Normally, under optimal conditions, N_2O does not accumulate since the N_2O reduction rate is always higher than the other nitrogen oxides reduction rates [4].

In wastewater treatment plants (WWTP) nitrogen removal occurs via nitrification and denitrification. Although the majority of the emissions are detected in the aerobic zones, where nitrification occurs, a fraction of this N_2O is being produced during denitrification and might accumulate in the anoxic zones, remaining dissolved until aeration starts. Several environmental factors have been suggested to affect N_2O reduction during denitrification. Among them, the effect of electron acceptors (oxygen, nitrite/FNA or nitric oxide), electron donors (type of organic carbon used for denitrification) or the relationship between COD/N in the wastewater have been capturing the majority of the attention of the research community [5–9].

In WWTP, external carbon is often added to biological processes to facilitate complete N removal when the wastewater COD is not sufficient. Methanol is commonly added as external carbon source for the denitrification process mainly because of its cheap cost. However, other substrates such as ethanol or acetate are often used as electron donors in order to enhance the denitrification rates of the process [10]. A study conducted by Lu and Chandran [6] investigated the N_2O emissions from two lab-scale denitrifying sequencing batch reactors (SBRs), one operating with ethanol and the other with methanol as sole carbon sources. Their results

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showed similar emissions when operating at steady state conditions. Only higher emissions were observed with ethanol on those tests that some oxygen was supplied. This was attributed to a lower tolerance to transient conditions of the ethanol denitrifying culture. Another study performed by Belmonte [11] explored the N_2O emissions using acetate and swine wastewater as carbon sources during the denitrification process and the results showed different N_2O productions depending on the carbon source used having more emissions for the latter. However, it is still unclear if the type of electron donor (carbon source) can have an effect on the N_2O reduction rate.

Recently, a study carried out by Pan [12] using a denitrifying culture developed with methanol as the only substrate suggested that a competition for electrons had an effect on the reduction rates of the different nitrogen oxides. Although N_2O did not accumulate in the majority of their experiments, they suggested that electron competition, even under non-limiting substrate conditions, could lead to N_2O accumulation. Since different carbon sources can have different oxidation rates, therefore providing different flows of electrons into the electron transport chain, the effect of electron competition on nitrogen oxides reduction when other substrates are used is still unknown.

The aim of this study was to investigate the impact of organic carbon sources and the competition for electrons on the N_2O reduction rate in a denitrifying culture developed with three carbon sources: acetate, ethanol and methanol. The effect of each carbon source on the different nitrogen oxides reduction rates was assessed and compared. Finally, the effect of carbon limitation on the nitrogen oxides reduction rates was reported using ethanol and acetate.

2. Materials and methods

2.1. Bioreactor set-up and operation

A cylindrical 6 L SBR was inoculated with activated sludge from Girona's wastewater treatment plant to develop a denitrifying culture. It was operated in a 6 h cycle, consisting of anoxic feed (5 min) where 1 L of synthetic wastewater was added, anoxic phase (5 h), aerobic mix (15 min), settling (20 min) and withdrawal (20 min).

The feed synthetic wastewater had a concentration of 90 mg NO_3^--N/L and 300 mg COD/L and included 900 mL of solution A and 100 mL of solution B. The composition of solution A was (per L): 0.55 g $NaNO_3$, 0.33 g $MgSO_4{\cdot}2H_2O$, 0.033 g $CaCl_2{\cdot}2H_2O$, 0.145 g K_2HPO_4, 0.01 g allythiourea 96% (ATU), 0.27 g NH_4Cl and 220 mL of trace elements solution (per L): 1.5 g $FeCl_3{\cdot}6H2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4{\cdot}5H_2O$, 0.18 g KI, 0.12 g $MnCl_2{\cdot}4H_2O$, 0.06 g $Na_2MoO_4{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.15 g $CoCl_2{\cdot}6H_2O$ and 10 g EDTA. Solution B contained 2.13 g/L sodium acetate, 0.634 mL/L ethanol (96%) and 0.896 mL/L methanol (99.9%), 100 mg COD/L of each carbon was added to the reactor. This solution was autoclaved to avoid any COD biodegradation.

The sludge retention time (SRT) was 20 days and the hydraulic residence time (HRT) was 36 h. Nitrate and COD were completely removed at the end of the anoxic phase. The pH was controlled at 7.5 ± 0.4 using 0.6 M hydrochloric acid. Dissolved oxygen (DO) concentration was also controlled with a programmable logic controller (PLC) between 2 and 2.5 mg O_2/L by supplying air at 5 L/min. Redox potential was also monitored.

Cycle studies were carried out on a weekly basis to monitor the denitrification activity of the reactor. Samples for the analysis of nitrate, nitrite, COD and acetate were taken every 60 min and filtered with 0.22 μm Millipore filters. At the end of the aerobic phase mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were also analyzed.

Table 1
Batch tests conducted for each set of experiments.

Batch test type	A	B	C	D	E	F	G
Electron acceptors used	NO_3^-	NO_2^-	N_2O	NO_3^- N_2O	NO_2^- N_2O	NO_3^- NO_2^-	NO_3^- NO_2^- N_2O

2.2. Batch tests

Batch tests were carried out to study the effects of nitrate, nitrite and nitrous oxide on each other's reduction rates using three different substrates independently (acetate, ethanol and methanol) and a combination of the three.

Four sets of experiments were conducted. The first three were carried out using the three different carbon sources separately (acetate, ethanol and methanol) and in the last set a combination of the three was used. Seven types of batch tests were conducted using different electron acceptors for the first three sets of experiments. For the last set, when a mix of the 3 carbon sources was added, only batch tests A–E were carried out (Table 1). All the batch tests were conducted in triplicate.

2.2.1. Batch reactors set-up and operation

A 330 mL sealable reactor was used for the batch tests. A 5 mL reservoir was connected to the lid of the reactor (Fig. 1). The reservoir avoided the entrance of air into the batch reactor when liquid samples were taken during the batch test for the analysis of nitrate, nitrite and acetate. Mixed liquor samples were taken using a syringe and immediately filtered through disposable Millipore filter units (0.22 μm pore size) and analyzed. N_2O was continuously monitored with an N_2O microsensor (Unisense A/S, Arhus, Denmark). All the batch tests were conducted taken the sludge from the SBR at the end of the anoxic phase to ensure that nitrate, nitrite and COD were removed completely. The sludge was pretreated with 1 h aeration to oxidize any internal COD that could be present, half an hour bubbled with nitrogen gas to ensure anoxic conditions and finally washed with a phosphate buffer solution (PBS) previously sparged with nitrogen and placed in the batch reactor. Since the reactor did

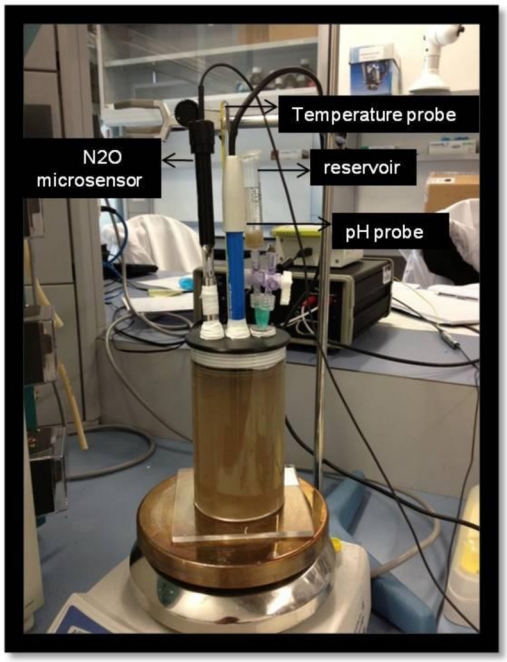

Fig. 1. Sealable reactor used for batch tests.