

Biological Nitrous Oxide Abatement by *Paracoccus denitrificans* in Bubble Column and Airlift Reactors

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Nitrous oxide (N₂O), with a global warming potential 300 times higher than that of CO₂, represents 6.2 % of the total greenhouse gas emission inventory worldwide. Furthermore, N₂O is considered the most critical O₃-depleting substance emitted in this XXI century. In spite of the environmental relevance of this pollutant, very little research on biotechnologies for the treatment of N₂O emissions has been conducted. In this study, the potential of a bubble column (BCR) and an internal loop airlift (ALR) bioreactors of 2.3 L was evaluated for the abatement of N₂O from industrial emissions from nitric acid plants along 62 days of operation. The systems were inoculated with a methylophilic *Paracoccus denitrificans* strain (DSM 413) and continuously supplied with methanol as a carbon and electron donor source for the anoxic reduction of N₂O. The simulated waste gas consisted of a N₂ gas stream containing 1 ± 0.1 % of O₂ and 3377 ± 312 ppm_v of N₂O at the inlet of the BCR and 1 ± 0.1 % of O₂ with N₂O concentration of 3617 ± 342 ppm_v at the inlet of the ALR. This N₂-laden stream was supplied at a constant flow rate of 110 ml min⁻¹ in each reactor. The performance of the BCR was characterized by a steady state N₂O removal efficiency (RE) of 87 ± 3 % with CO₂ productions of 308 ± 36 g m⁻³ d⁻¹ and total suspended solid (TSS) concentrations of 867 ± 109 mg L⁻¹. On the other hand, the ALR showed a N₂O RE of 88 ± 2 % with productions of CO₂ of 346 ± 28 g m⁻³ d⁻¹ and TSS concentrations of 874 ± 88 mg L⁻¹. This work constitutes, to the best of our knowledge, the first systematic study of a biotechnology for the continuous abatement of N₂O from nitric acid plants.

1. Introduction

The increasing concern about climate change and the steady rise of global temperature have attracted much attention in the scientific community. Scientists have confirmed that these environmental problems are produced by the rapid increase in atmospheric concentrations of greenhouse gases (GHGs), whose concentrations are 45 % higher than those prevailing in the preindustrial era (IPCC, 2014). Nitrous oxide (N₂O), the third most important GHG with a global warming capacity 300 times larger than that of CO₂ due to its larger atmospheric persistence (150 years), accounts for 6.2 % of the total GHG emitted globally. N₂O is also one of the major source of stratospheric NO_x and is considered as the most important ozone depleting substance emitted in this XXI century (Ravishankara et al., 2009). Agriculture is the most important source of anthropogenic N₂O emissions, followed by industrial emissions and waste management process. The major N₂O source in industrial processes is the production of nitric acid, whose global emissions can reach up to 400 Kton of N₂O per year (Pérez-Ramírez et al., 2003). The typical composition of a waste gas from nitric acid production can be represented by 100-3500 ppm_v of NO_x, 300-3500 ppm_v of N₂O, 1-4 % of O₂ and 0.3-2 % of H₂O.

Several physical-chemical technologies are applied for the treatment of N₂O emissions from nitric acid plants as end of pipe mitigation strategy. Nonselective catalytic reduction (NSCR) (Lee et al., 2011) is a typical technology applied in nitric acid plants nowadays. However, this system entails the consumption of a reducing agent such as hydrocarbons or ammonia and high temperature for N₂O destruction. Furthermore, there are novel catalysts technologies that usually require the use of precious metals (Inger et al., 2013) and do not need a reducing agent, but the temperature required for its operation is higher than in NSCR. Thus, all those

physical-chemical technologies require the preheating of the tail gas to be treated, resulting in a considerable energy consumption since nitric acid waste gas is typically emitted at ambient temperature (Wu et al., 2015). Furthermore, some reports have pointed out that NSCR technology can even emit CH₄ as a result of an incomplete fuel burning for the treatment of N₂O emissions in nitric acid plants (Environmental Protection Agency, 2010).

Biological technologies have demonstrated promising features such as robustness, cost efficiency and environmentally friendliness for the treatment of industrial waste gases (Estrada et al., 2011). In spite of the above advantages, no biotechnological system has been ever evaluated for the abatement of N₂O emissions from nitric acid plants. N₂O denitrification seems to be the most favorable biological pathway for degradation of N₂O, where this GHG is an obligate intermediate in the reduction steps of NO₃⁻ or NO₂⁻ to N₂ during organic matter oxidation in wastewater treatment plants. Thus, since nitric acid emissions are mainly composed of N₂O, N₂ and trace levels of O₂, denitrification can be an attractive alternative for the abatement of N₂O provided a cheap source of organic carbon and electron donor for heterotrophic bacteria (Frutos et al., 2016, 2014).

Bubble column (BCR) and internal loop airlift (ALR) bioreactors have been consistently proven as low cost alternative technologies for N₂O abatement. These bioreactor configurations are pneumatically agitated by a gas phase bubbled from the bottom, resulting in a low energy consumption. Moreover, simplicity in construction with no moving parts and high gas-liquid mass transfer rates constitute also key advantages over conventional stirred tank reactors (Chisti and Moo-Young, 1989; Fu et al., 2007; Merchuk et al., 1994). ALRs differ from BCRs in the presence of an inner tube that separates the gas bubbling in the inner part (riser) from an external part (downcomer) promoting liquid recirculation. The ALR configuration has been previously studied for the removal of nitrogen in a sequential nitrification-denitrification process (Guo et al., 2005) where aerobic-anoxic environments are combined in a single reactor.

In this context, a BCR and an ALR inoculated with a denitrifying strain of *Paracoccus denitrificans* (DSM 413) were studied and compared systematically for a continuous N₂O abatement in a simulated emission of a nitric acid plant for a period of 62 days.

2. Materials and Methods

2.1 Chemicals and mineral salt medium

All chemicals for mineral salt medium (MSM) preparation were purchased from PANREAC (Barcelona, Spain) with a purity of at least 99%. The MSM used in the experimentation was composed of (g L⁻¹): Na₂HPO₄·12H₂O 6.16, KH₂PO₄ 1.52, MgSO₄·7H₂O 0.2, CaCl₂ 0.02, NH₄Cl 1.5, and 10 mL L⁻¹ of a trace element solution (containing per liter: EDTA 0.5 g, FeSO₄·7H₂O 0.2 g, ZnSO₄·7H₂O, 0.01 g, MnCl₂·4H₂O 0.003g, H₃BO₃ 0.03 g, CoCl₂·6H₂O 0.02 g, CuCl₂·2H₂O 0.001 g, NiCl₂·6H₂O 0.002 g, NaMoO₄·2H₂O, 0.003 g). The final pH of the MSM was 7. A cylinder of 40 L of 50,000 ppm_v of N₂O in N₂ was purchased from Abelló Linde S.A. (Barcelona, Spain) as well as the 40 L cylinder of pure N₂ needed to create the simulated emission.

2.2 Microorganism cultivation

A lyophilised methylotrophic strain of *Paracoccus denitrificans* (DSM 413) was purchased from DSMZ (Braunschweig, Germany). The bacterium was cultivated in 2 sterilized flasks with 0.5 L of MSM with methanol (1 %v/v) as the sole carbon and energy source under aerobic conditions for 3 weeks.

2.3 Experimental set up and operational conditions

A BCR of 42 cm of height (H) and 9 cm of inner diameter (ID), and an ALR of the same dimensions with a concentric draft tube (5.5 cm ID, 29.5 cm H) located at 4 cm from the bottom of the reactor were inoculated with 0.5 L of methylotrophic inoculum and filled with MSM to a working volume of 2.3 L, resulting in an initial total suspended solid (TSS) concentration of 56 mg L⁻¹ in both bioreactors. The simulated nitric acid gas emission was prepared by mixing 50,000 ppm_v of N₂O in N₂, air from a compressor and pure N₂. The gas mixture resulted in a BCR inlet gas N₂O concentration of 3377 ± 342 ppm_v with 1 ± 0.1 % of oxygen, while the inlet gas of the ALR was composed of 3617 ± 342 ppm_v of N₂O and 1 ± 0.1 % of oxygen. Both the BCR and ALR were supplied with a gas inlet flow rate of 110 mL min⁻¹, which correspond to a gas empty bed residence time (EBRT) of 17 ± 0.6 and 16 ± 1.2 min, respectively. Pure methanol (CH₃OH) was injected in the gas line by means of a syringe pump in a sample port filled with fiberglass wool to facilitate its evaporation at a flow rate of 1.9 mL d⁻¹, which resulted in a daily CH₃OH loading rate of 661 g m⁻³ d⁻¹. A detailed diagram of the experimental setup is presented in Figure 1. Prior to inoculation, an abiotic test was conducted under abiotic conditions with MSM in order to assess the potential abiotic elimination of N₂O by photolysis or adsorption. The concentrations of N₂O, CO₂, and O₂ were periodically monitored by GC-ECD and GC-TCD at both inlet and outlet gas sampling ports of the bioreactors. The total organic carbon (TOC), total nitrogen (TN), dissolved

CH₃OH and TSS were measured three times per week before the replacement of 300 mL of fresh MSM to replenish nutrient concentrations. The systems were operated in a controlled temperature room at 25 °C.

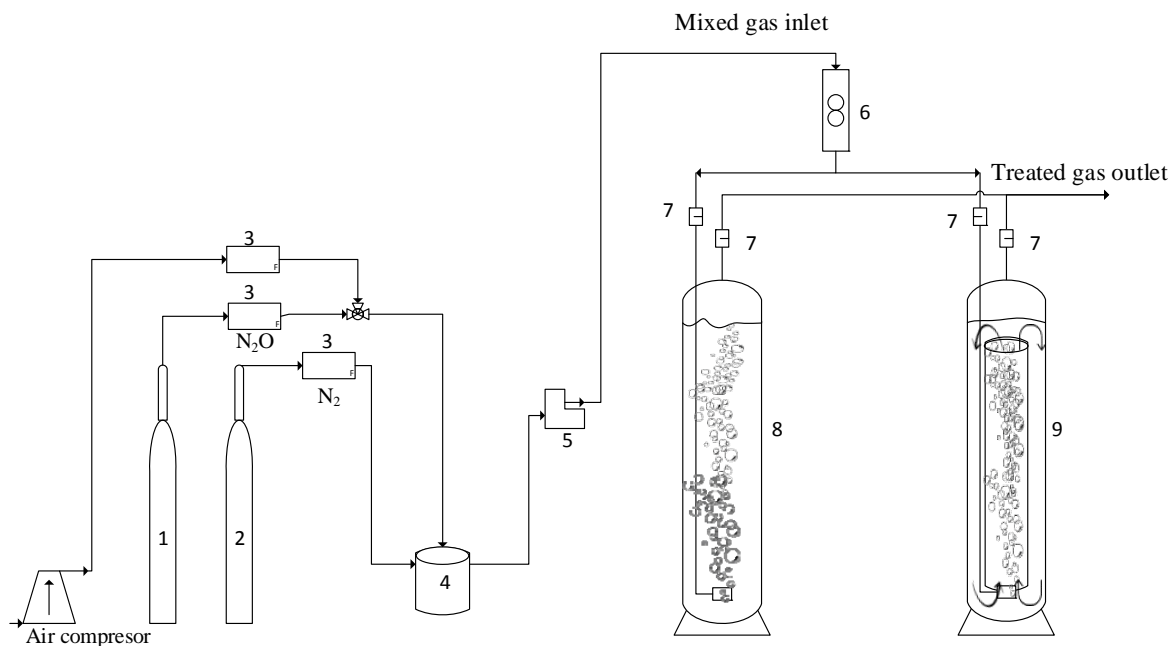


Figure 1: Schematic diagram of the experimental setup: (1 and 2) N₂O and N₂ gas cylinder, respectively, (3) mass flow controller, (4) mixing chamber, (5) methanol syringe pump, (6) gas flowmeter, (7) gas sampling port, (8) bubble column and (9) internal loop airlift reactor.

2.4 Analytical procedures

The concentration of N₂O was measured in a Bruker Scion 436 Gas chromatograph with an Electron Capture Detector (GC-ECD) (Palo Alto, USA) equipped with a HS-Q packed column (1 m x 2 mm ID x 3.18 mm OD) (Bruker, USA). Injector, detector and oven temperatures were set at 100, 300, and 40 °C, respectively. Nitrogen was used as the carrier gas at 20 mL min⁻¹. External standards prepared in volumetric bulbs (Sigma-Aldrich, USA) were used for N₂O quantification. The concentrations of CO₂ and O₂ were determined in a Bruker 430 gas chromatography (Palo Alto, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m x 0.53 µm x 15 µm) and a CP-PoraBOND Q (25 m x 0.53 µm x 10 x µm) columns. The oven, injector and detector temperatures were maintained at 40, 150 and 200 °C, respectively. Helium was used as the carrier gas at 13.7 mL min⁻¹, while external standards prepared from calibration mixtures were used for CO₂ quantification.

TOC and TN concentrations were measured using a TOC-VCSH analyser (Shimadzu, Tokyo, Japan) coupled with a total nitrogen chemiluminescence detection module (TNM-1, Shimadzu, Japan). Dissolved CH₃OH concentration was determined in a Gas chromatograph coupled to a Flame Ionization Detector (Bruker 3900, Palo Alto, USA) equipped with a SupelcoWax (15 m x 0.25 mm x 0.25 µm) capillary column. Injector and detector temperatures were maintained at 200 and 250 °C, respectively. Nitrogen was used as the carrier gas at 1 mL min⁻¹ while H₂ and air were fixed at 30 and 300 mL min⁻¹, respectively. N₂ was used as the make-up gas at 25 mL min⁻¹. The determination of TSS concentration was performed according to standard methods (APHA, 2005) and pH was periodically monitored with using a pH/mV/°C meter (pH 510 Eutech Instruments, Nijkerk, the Netherlands).

The results were statistically analysed to compare the performance of the bioreactors with an analysis of variance (ANOVA) with 95 % of confidence level and Tukey's honest significance test.

3. Results and discussions

The abiotic test conducted prior inoculation showed a negligible (<3 %) adsorption or photolysis of N₂O. The pH of the BCR remained at 6.65 ± 0.13 with a dissolved oxygen (DO) concentration of 0.11 ± 0.14 mg L⁻¹ for the entire experimentation period, while the ALR presented a pH of 6.64 ± 0.13 with a DO concentration of

$0.06 \pm 0.08 \text{ mg L}^{-1}$. The first ten days of operation were characterized by a gradual increase in the removal efficiency (RE) of N_2O in both systems (Figure 2A and 2B). This was likely due to the need for an adaptation period where the *P. denitrificans* synthesized the enzymes necessary for the anoxic degradation of CH_3OH . At this point, it is important to stress that the cultivation of the strain during inoculum preparation was carried out aerobically. The inlet and outlet N_2O concentrations of the ALR were 3617 ± 342 and $420 \pm 69 \text{ ppm}_v$, respectively (Figure 2A), which represented a steady state RE of $88 \pm 2 \%$. On the other hand, the BCR showed a steady state N_2O RE of $87 \pm 3 \%$ with inlet and outlet N_2O concentrations of 3377 ± 342 and $441 \pm 74 \text{ ppm}_v$, respectively (Figure 2B). Similar results were observed in a previous work (Frutos et al., 2016) where the abatement of diluted N_2O ($\approx 100 \text{ ppm}_v$) in air and simultaneous wastewater treatment were evaluated under a gas EBRT of 40 min in a bioscrubber. However, the results here reported represent the highest N_2O RE observed during continuous operation (50 days of steady state removal) in biological systems. The statistically evaluated data showed unexpected significant differences between both bioreactors, where slightly higher N_2O REs were observed in the ALR likely due to the lower DO (0.06 mg L^{-1}) recorded, which promoted the reduction of N_2O by the denitrifying bacteria.

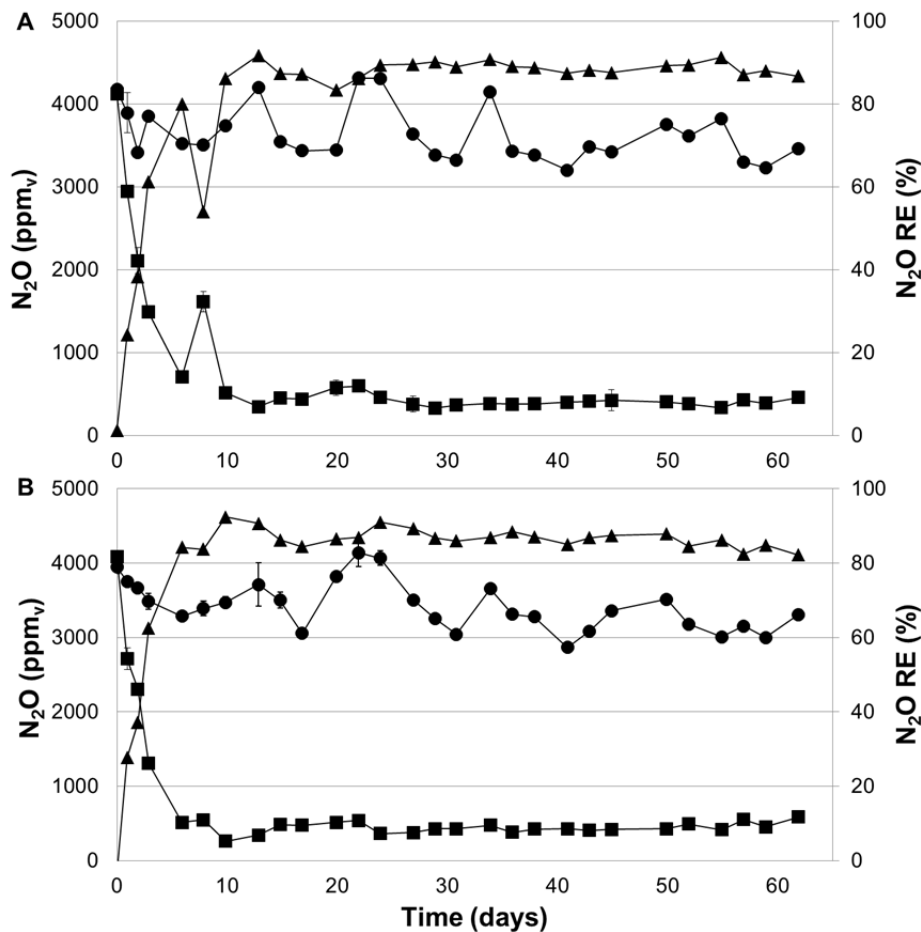


Figure 2: Time course of the Inlet (circle), outlet (square) and removal efficiencies (triangle) of N_2O in the ALR (A) and BCR (B). Verticals bars represent the standard deviation from duplicate measurements.

The steady state production of CO_2 was reached after 10 days of operation, when the systems reached stable N_2O REs (Figure 3A). The BCR showed a production of CO_2 of $308 \pm 36 \text{ g m}^{-3} \text{ d}^{-1}$, while the ARL CO_2 production was $346 \pm 28 \text{ g m}^{-3} \text{ d}^{-1}$ (Figure 3A). The statistical analysis of the CO_2 production data showed significant differences between both bioreactors. The biomass concentration achieved under steady state after 30 days of operation remained very similar in both reactors at TSS concentrations of $867 \pm 109 \text{ mg L}^{-1}$ in the BCR and $874 \pm 88 \text{ mg L}^{-1}$ in the ALR (Figure 3B). No significant differences were observed between both TSS concentrations. The dissolved CH_3OH and TOC concentrations gradually increased up to day 30, when steady values were observed concomitant with the TSS concentration stabilization. Thereafter, the

concentrations of CH_3OH and TOC in the BCR remained stable at 1115 ± 99 and $390 \pm 38 \text{ mg L}^{-1}$, respectively. Similarly, the concentrations of CH_3OH and TOC in the ALR remained at 967 ± 96 and $348 \pm 28 \text{ mg L}^{-1}$, respectively (Figure 3C and 3D).

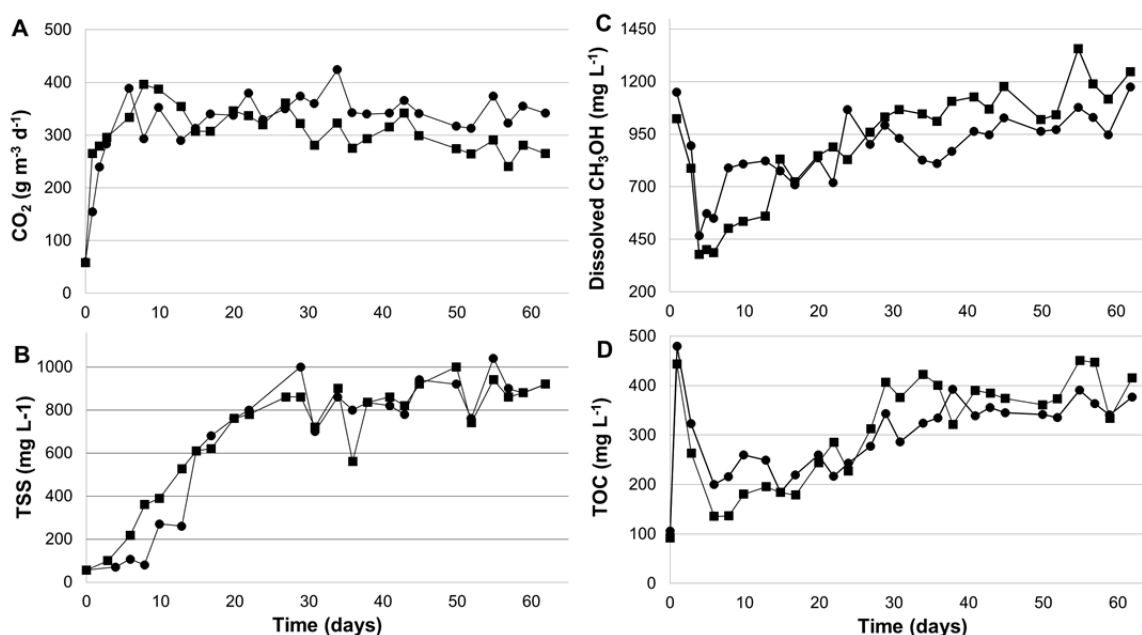


Figure 3: Time course of CO_2 production (A), total suspended solid concentration (B), dissolved CH_3OH concentration (C) and TOC concentration (D) in the ALR (circle) and BCR (square).

The higher N_2O RE and CO_2 production in the ALR under same biomass concentrations observed in both bioreactors confirmed the slightly better performance of the ALR over the BCR. Thus, the greater N_2O RE of the ALR can be attributed to the particular configuration of this bioreactor, which promoted the maintenance of anoxic conditions due to the liquid recirculation in the downcomer (non aerated). Others authors have previously proposed ALRs as a platform for the removal of contaminants in processes that require both aerobic and anoxic conditions. Thus, Dhamole et al. (2009) used a 42 L ALR for the simultaneous elimination of COD in the riser (aerated part) and the denitrification of nitrate in the downcomer (anoxic part). Similarly, Zhang and Wei (2013) used a 47 L airlift reactor for the successful development of simultaneous nitrification and denitrification treating synthetic wastewater.

The results here reported showed that biotechnologies may be an interesting alternative to physical-chemical technologies for the abatement of nitrous oxide, exhibiting similar N_2O REs and without the production of undesirable secondary pollutants. Furthermore, the biotechnologies here studied were characterized by the low energy consumption, simple configuration and operation. On other hand, the use of specific microorganisms capable of producing high added-value by-products such as biopolymers during the simultaneous abatement of pollutant may be an interesting approach to improve the cost-effectiveness of these technologies. The greatest limitation of the systems here proposed was the high gas EBRT (>15 min) required to obtain high elimination due the poor solubility of N_2O , which results in large volume reactors with the consequent increase in capital cost. Therefore, more studies devoted to optimize bioreactor design and operational strategies are necessary in order to overcome the N_2O mass transfer limitations typically encountered during the off-gas abatement of this GHG.

4. Conclusions

Bubble column and internal loop airlift bioreactors are considered low cost technologies due to their low power consumption and maintenance and therefore represent a promising platform for the biological abatement of N_2O . To the best of our knowledge, this is the first systematic study where two biotechnologies are analysed for the treatment of N_2O emissions from nitric acid plants. The study showed the high N_2O RE of both BCR and ALR along 62 days of operation. The latter presented a better performance for the elimination of N_2O likely due to its particular configuration, which allowed the maintenance of the anoxic conditions required for the complete reduction of N_2O to N_2 by *P. denitrificans*.

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