Nitrous oxide abatement coupled with biopolymer production as a model GHG biorefinery for cost-effective climate change mitigation

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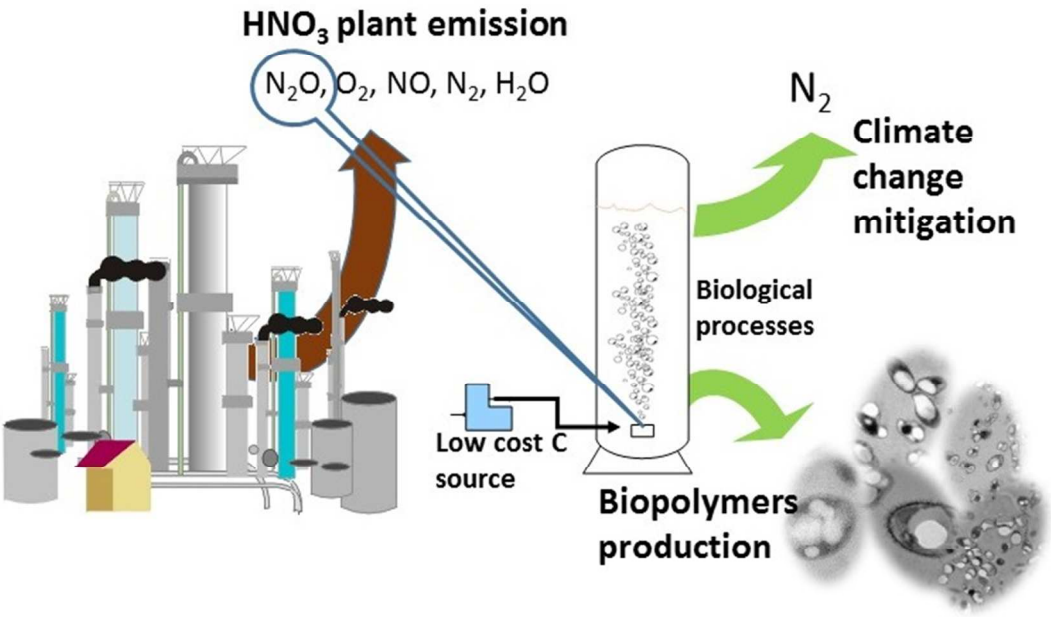
ABSTRACT

N_2O represents ~6% of the global greenhouse gas emission inventory and the most important O_3-depleting substance emitted in this 21st century. Despite its environmental relevance, little attention has been given to the development of cost-effective and environmentally friendly N_2O abatement methods. In this context, the potential of a bubble column (BCR) and an internal loop airlift (ALR) bioreactors of 2.3 L for the abatement of

N_2O from a nitric acid plant emission was systematically evaluated. The process was based on the biological reduction of N_2O by *Paracoccus denitrificans* using methanol as a carbon/electron source. Two nitrogen limiting strategies were also tested for the co-production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) coupled with N_2O reduction. High N_2O removal efficiencies (REs) (≈87%) together with a low PHBV cell accumulation were observed in both bioreactors in excess of nitrogen. However, PHBV contents of 38-64% were recorded under N limiting conditions along with N_2O-REs of ≈57% and ≈84% in the ALR and BCR, respectively. Fluorescence in-situ hybridization analyses showed that *P. denitrificans* was dominant (>50%) after 6 months of experimentation. The successful abatement of N_2O concomitant with PHBV accumulation confirmed the potential of integrating biorefinery concepts into biological gas treatment for a cost-effective GHG mitigation.

30 **TOC/Abstract Art**



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INTRODUCTION

The increasing public concern about global temperature rise and climate change has attracted the attention of politicians and the scientific community during the past decade. Nowadays, there is no doubt about the fact that these environmental problems are caused by the rapid accumulation of greenhouse gases (GHGs), whose concentrations are 45 % higher than those prevailing in the preindustrial era.[1] Nitrous oxide (N_2O), the third most important GHG with a global warming potential 300 times higher than that of CO_2 due to its high atmospheric persistence (150 years), accounts for 6.2 % of the total GHG emissions globally. N_2O is also one of the main sources of stratospheric NO_x and is considered the most important ozone depleting substance emitted in this 21st century.[2] Agriculture is the principal source of anthropogenic N_2O emissions, followed by chemical industry and waste management processes. The production of nitric and adipic acid are the major N_2O source in industry, whose global emissions reach up to 400 Kton of N_2O per year.[3] A typical waste gas from nitric acid production plants is characterized by 100-3500 ppm_v of NO_x, 300-3500 ppm_v of N_2O, 1-4 % of O_2 and 0.3-2 % of H_2O (in a N_2 matrix).[4]

Several physical-chemical technologies such as non-selective catalytic reduction (NSCR) or catalytic decomposition have been applied as end-of-the-pipe strategies for the treatment of N_2O emissions from industrial sources.[5] However, these technologies entail the consumption of a reducing agent such as hydrocarbons or ammonia and require the preheating of the tail gas for N_2O destruction, resulting in a considerable energy consumption since nitric acid waste gas is typically emitted at ambient temperature.[6] Moreover, the environmental sustainability of NSCR technologies can get also jeopardized

by fugitive emissions of CH_4 derived from an incomplete fuel combustion during the treatment of N_2O.[7]

Biological technologies have been shown to exhibit a high robustness, cost efficiency and environmental friendliness for the treatment of industrial off-gases containing malodorous and volatile organic compounds.[8] In spite of their inherent advantages, no biological process has ever been evaluated for the abatement of N_2O emissions from nitric and adipic acid plants.[9,10] This GHG is an obligate intermediate in the reduction of NO_3^- and NO_2^- to N_2, which to the best of our knowledge is the only known biochemical mechanism for N_2O removal. Thus, since nitric and adipic acid emissions are mainly composed of N_2O, N_2 and trace levels of O_2, denitrification appears as an attractive alternative for the abatement of N_2O when a cheap source of organic carbon and electron donor is available for the growth of heterotrophic bacteria.[9,10] In this context, the economic viability of this process can be significantly improved by coupling the abatement of N_2O via denitrification with the production of added value bioproducts such as polyhydroxyalkanoates (PHA) biopolymers. These bio-based chemicals, especially poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), share with conventional fossil-derived thermoplastics similar physical/chemical characteristics such as melting point, molecular weight and tensile strength.[11] PHAs also possess a rapid biodegradability in nature, which render them a perfect substitute of conventional fossil polymers. There are several denitrifying bacteria such as *Paracoccus denitrificans, Pseudomonas aeruginosa* and *Ralstonia eutropha*, capable of producing intracellular PHA as a carbon storage material in excess of organic carbon under nutrient limitation.[12,13] Therefore, an innovative GHG biorefinery could be engineered for the simultaneous abatement of N_2O and co-production

of PHAs in nitric and adipic acid plants in order to enhance the economic and environmental sustainability of N_2O abatement.

In this context, the potential of a bubble column (BCR) and an airlift (ALR) bioreactors for the treatment of a synthetic N_2O emission from nitric acid plant was compared. The strain *Paracoccus denitrificans* (DSM 413) was used as a model denitrifying bacterium in the co-production of the co-polyester PHBV using methanol as a carbon-energy source under nitrogen sufficiency and two different nitrogen limiting strategies.

MATERIALS AND METHODS

Chemicals and mineral salt medium

The mineral salt medium (MSM) used in the experimentation was composed of (g L^{-1}): $Na_2HPO_4 \cdot 12H_2O$ 6.16, KH_2PO_4 1.52, $MgSO_4 \cdot 7H_2O$ 0.2, $CaCl_2$ 0.02, NH_4Cl 1.5 and 10 mL L^{-1} of a trace element solution containing (g L^{-1}): EDTA 0.5, $FeSO_4 \cdot 7H_2O$ 0.2, $ZnSO_4 \cdot 7H_2O$ 0.01, $MnCl_2 \cdot 4H_2O$ 0.003, H_3BO_3 0.03, $CoCl_2 \cdot 6H_2O$ 0.02, $CuCl_2 \cdot 2H_2O$ 0.001, $NiCl_2 \cdot 6H_2O$ 0.002, $NaMoO_4 \cdot 2H_2O$ 0.003. The final pH of the MSM was 7. All reagents, including methanol, were purchased from PANREAC with a purity of >99 %. Benzoic acid (>99 %) and PHBV standards were obtained from Sigma-Aldrich® (Sigma-Aldrich, St. Louis, MO, USA). A 40 L calibrated gas cylinder of 50,000 ppm_v of N_2O in N_2 and 50 L industrial N_2 cylinder were purchased from Abelló Linde S.A. (Barcelona, Spain).

Microorganism cultivation

The methylotrophic strain *Paracoccus denitrificans* (DSM 413) was purchased from DSMZ (Braunschweig, Germany). The bacterium was cultivated in sterilized 1 L E-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.

Experimental set up

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 (ID = 5.5 cm, H = 29.5 cm) located at 4 cm from the bottom of the reactor, were inoculated with 0.5 L of *P. denitrificans* 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 synthetic nitric acid plant N_2O emission was obtained by mixing the calibrated mixture of N_2O (50,000 ppm_v), air from a compressor and pure N_2 using mass flow controllers (Aalborg, Denmark). The gas mixture resulted in BCR and ALR inlet N_2O gas concentrations of 3520 ± 290 and 3560 ± 300 ppm_v, respectively. The O_2 inlet gas concentration remained at 1.1 ± 0.1 % in each bioreactor. Both the BCR and ALR were supplied with an inlet gas flow rate of 137 ± 8 and 140 ± 10 mL min^{-1}, respectively, which corresponded to a gas empty bed residence time (EBRT) of ≈17 min. Pure methanol (CH_3OH) was injected in the gas line by means of a syringe pump in a sample port filled with fiberglass wool to facilitate solvent evaporation. The systems were operated in a controlled temperature room at 25 ºC. A detailed diagram of the experimental setup can be found in Figure S1 (supporting information).

Operational conditions

Three operational strategies, corresponding to Stages I, II, and III, were evaluated in both bioreactors under different MSM nitrogen concentrations in order to assess the feasibility of a simultaneous N_2O removal and PHBV cell accumulation. During the first 43 days of

operation (Stage I) the bioreactors were maintained under nitrogen sufficiency by supplying MSM with 396 mg N L^{-1} and 124 g C m^{-3} d^{-1} of CH_3OH. During Stage I, 300 mL of the cultivation broth was replaced by fresh MSM three times per week, which resulted in a dilution rate of~0.056 d^{-1} and an N inlet load of 22.1 g N m^{-3} d^{-1}. Stage II (days 44 to 127) was devised to promote the accumulation of intracellular PHBV at a CH_3OH inlet load of 93 g C m^{-3} d^{-1}, which guaranteed carbon availability. The N concentration in the MSM was reduced to 34 mg N L^{-1} during Stage II, with 300 mL of fresh MSM being replaced every two days. This resulted in a N inlet load of 2.2 g N m^{-3} d^{-1}, a dilution rate of 0.065 d^{-1} and nitrogen fast:famine cycles of 1d:1d. In Stage III (days 128 to 179), the nitrogen concentration in the MSM was increased to 68 mg N L^{-1} while decreasing the frequency of MSM replacement (300 mL) from two to four days at a CH_3OH inlet load of 108 g C m^{-3} d^{-1}. The dilution rate and N inlet load during Stage III was 0.033 d^{-1} and 2.2 g N m^{-3} d^{-1}. A mass transfer test was carried out according to Cantera and coworkers[14] at the end of Stages II and III by increasing the N_2O inlet concentration from ≈3527 to ≈9058 in order to elucidate the limiting factor during N_2O reduction to N_2 under the experimental conditions evaluated.

Sampling and analytical procedures

The gas phase monitoring procedure entailed the periodical measurement of N_2O, CO_2 and O_2 gas concentrations at both inlet and outlet bioreactors sampling ports. The monitoring of the liquid phase involved the withdrawal of 300 mL of cultivation broth from each bioreactor in order to determine the dissolved total organic carbon (TOC), total nitrogen (TN), CH_3OH, TSS and PHBV concentrations. The dissolved oxygen concentration was measured in-situ. In addition, 20 mL of the cultivation broth was centrifuged, wash with

distilled water and dried at 105 ºC for 24 h for the measurement of C, N, H and S cell content at the end of each experimental conditions.

The N_2O and CO_2/O_2 gas concentrations were measured by GC-ECD and GC-TCD according to Frutos et al.[9] and Lopez et al.,[15] respectively. TOC and TN concentrations were measured using a TOC-VCSH analyzer (Shimadzu, Tokyo, Japan) coupled with a total nitrogen chemiluminescence detection module (TNM-1, Shimadzu, Japan). Dissolved CH_3OH concentration was determined in a GC-FID (Bruker 3900, Palo Alto, USA) equipped with a SupelcoWax (15 m × 0.25 mm × 0.25 µm) capillary column. GC-FID injector and detector temperatures were maintained at 200 and 250 ºC, respectively. Nitrogen was used as the carrier gas at 1 mL min^{-1} while H_2 and air flows were fixed at 30 and 300 mL min^{-1}, respectively. N_2 was also used as the make-up gas at 25 mL min^{-1}. The determination of TSS concentration was performed according to standard methods[16]. The dissolved oxygen concentration was measured with a handheld OXI 330i oximeter (WTW, Germany) while pH was periodically monitored using a pH/mV/°C meter (pH 510 Eutech Instruments, Nijkerk, the Netherlands). To quantify the PHBV concentration, 2 mL of the cultivation broth were centrifuged at 9000 rpm for 15 min and the biomass pellet obtained was processed according to Zuñiga and coworkers,[17] using chloroform as extraction solvent. The PHBV extracted was measured by GC-MS (Agilent Technologies: GC System 7820A MSD 5977E, Santa Clara, USA) equipped with a DB-wax column (30 m × 250 µm × 0.25 µm) with detector and injector temperatures of 250 ºC and a split ratio of 1:10. The oven temperature was initially maintained at 40 ºC for 5 min, increased at 10 ºC min^{-1} up to 200 ºC and maintained at this temperature for 5 min. The PHBV cell content was

normalized as %PHBV= (g PHBV/g TSS) × 100. The analysis of C, N, H and S biomass content was conducted using a LECO CHNS-932 elemental analyzer.

Electron microscopy analysis

Cultivation broth samples of 1 mL were drawn from the bioreactors at the end of Stage III and centrifuged at 4000 rpm and 4 ºC for 5 min. Subsequent biomass pellets conditioning was carried out according to Bozzola.[18] The samples were then cut in thin slices by a microtome and contrasted according to Wendlandt and coworkers.[19] A TEM JEOL JEM-1011 electron microscope (Teknolab, Indonesia) equipped with an ES1000W Erlangshen CCD camera (Gatan, Germany) was used for the analysis.

Fluorescence in situ hybridization (FISH) analysis

Aliquots of 250 uL of the cultivation broth from both bioreactors at the end of each operational stage were fixed in 4 % (w/v) paraformaldehyde for 3 h, washed three times with phosphate-buffered saline (PBS) and then preserved in alcohol 96 % (v/v). Aliquots of 10 µL of samples were placed on glass microscope slides and dehydrated with ethanol at 50 %, 80 % and 96 % (v/v). The probes used were EUB338 I-II-FITC (for general bacteria)[20,21] and PAR651-Fam (specific for the genus *Paracoccus*).[22] Hybridization was carried out at 46 ºC using formamide at 40 % .[23] For quantitative FISH analysis, 16 images were randomly acquired from each well on the slides using a Leica DM4000B microscope (Leica Microsystems, Wetzlar, Germany). The relative bio-volumes of the specific genus *Paracoccus* from the total bacteria (EUB338 I-II) were calculated using the commercial software DAIME and split into individual color channels before image segmentation.[24]

RESULTS AND DISCUSSION

Influence of nitrogen supplementation on N_2O abatement

The two bioreactors exhibited a low and stable dissolved oxygen (DO) concentration during the entire experimentation (0.07 ± 0.1 mg L^{-1} in both bioreactors). Similarly, a stable pH of 6.8 ± 0.2 was recorded in both systems along the three operational stages. The N_2O REs reached a steady state 10 days after the startup of the bioreactors. Hence, steady state N_2O REs of 87 ± 3 % were reached during Stage I in the BCR with inlet and outlet N_2O concentrations of 3380 ± 340 and 440 ± 74 ppm_v, respectively (Figure 1A). Similarly, the ALR supported steady state REs of 88 ± 2 % with inlet and outlet N_2O concentrations of 3610 ± 340 and 420 ± 69 ppm_v, respectively (Figure 1B).

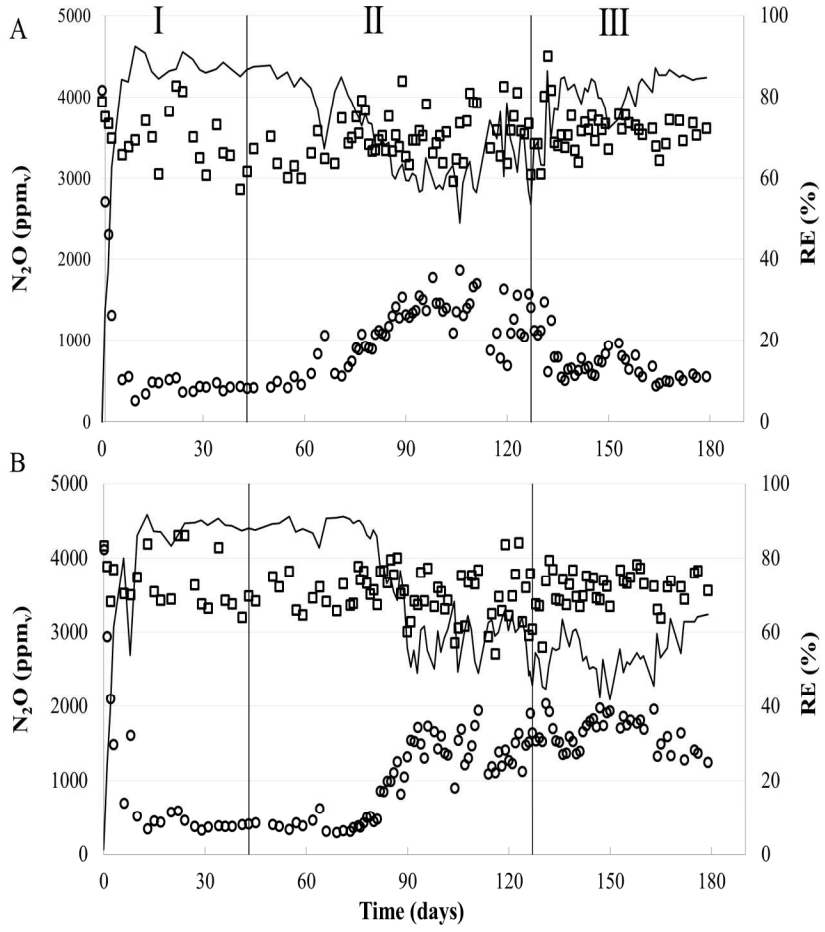

Figure 1. Time course of the inlet (□) and outlet (○) N_2O gas concentrations and removal efficiency (solid line) in the BCR (A) and ALR (B). Vertical lines indicate the different operation stages.

The CO_2 produced from the oxidation of CH_3OH during Stage I was correlated with the removal of N_2O, resulting in comparable CO_2 production rates of 85 ± 8 g C m^{-3} d^{-1} and 91 ± 8 g C m^{-3} d^{-1} in the BCR and ALR, respectively (Figure 2A). Biomass concentration, measured as TSS, reached stable values of 853 ± 76 and 856 ± 90 mg L^{-1} in BCR and ALR, respectively, after 20 days of operation (Figure 2B).

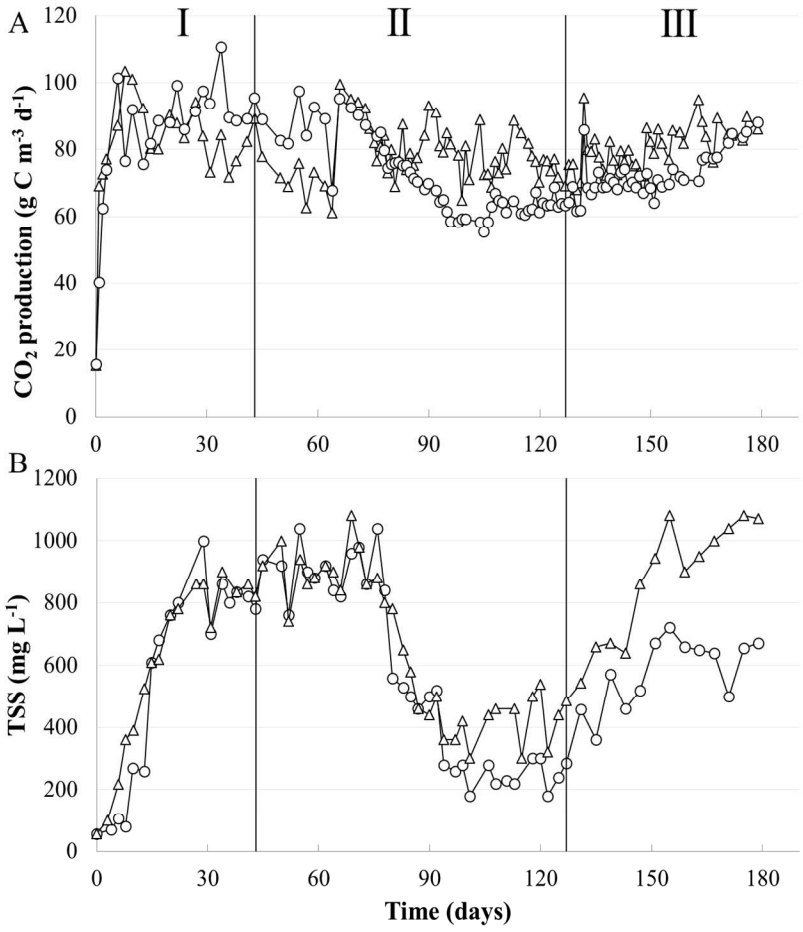

Figure 2. Time course of CO_2 production rates (A) and TSS concentrations (B) in the BCR (Δ) and ALR (○). Vertical lines indicate the different operation stages.

The decrease in N supply rate from day 44 (Stage II) in order to achieve 1d:1d nitrogen fast-famine cycles resulted in a progressive reduction in the N concentration down to a complete depletion by day 66 in both bioreactors (Figure 3). Nitrogen depletion entailed a gradual deterioration in N_2O REs down to steady state values of 62 ± 7 % in BCR and 58 ± 6 % in ALR (Figure 1A). This significant decrease in N_2O REs was correlated to a concomitant reduction in biomass concentration as a result of the limited N availability. In this context, the TSS concentration decreased gradually to steady values of 422 ± 76 in the

BCR and of 285 ± 99 mg L^{-1} in the ALR from day 94 (Figure 2B). Surprisingly, the microbial population in the ALR was more impacted by N deprivation than that present in the BCR. This result suggested that the hydrodynamics of the ALR configuration might entail a harmful stress to microbial growth. In this sense, the internal draft tube of the ALR may have avoided a proper liquid mixing, which ultimately resulted in a poor nutrient distribution and a lower biomass growth compared to the BCR. This phenomenon was previously observed by Wong and co-workers[25] during the operation of two ALRs with different draft tube lengths (35 and 50 cm) and a BCR for *Chlorella vulgaris* cultivation. The authors recorded higher biomass concentrations in the BCR and in the ALR with the shorter draft tube as a result of a better liquid mixing (i.e. nutrients distribution) compared to the ALR with the longer draft tube. The reduction in biomass concentration and N_2O RE resulted in a concomitant decrease in the CO_2 production rate in the ALR (63 ± 3 g C m^{-3} d^{-1}) compared to CO_2 production rates of 78 ± 7 g C m^{-3} d^{-1} in the BCR (Figure 2A). A mass transfer test was conducted at this point to assess the limiting factor in N_2O removal during Stage II. An increase in the N_2O inlet load by a factor of 2.4 ± 0.2 did not result in a concomitant increase in CO_2 production rate and N_2O elimination capacity (Figure S2). Hence, this tests confirmed that both bioreactors were limited by microbial activity due to the low biomass concentration supported by the limited N supply imposed.

The increase in N concentration by a factor of 2 along with the reduction in the dilution rate from 0.065 d^{-1} to 0.033 d^{-1} by day 127 (Stage III) supported an increase in biomass concentration to 1017 ± 71 mg TSS L^{-1} and 646 ± 64 mg TSS L^{-1} in the BCR and ALR, respectively. This entailed a concomitant increase of N_2O removal in the BCR up to steady REs of 84 ± 3 % but similar N_2O-REs of 57 ± 7 % were recorded in the ALR during Stage

III (Figure 1, Figure 2). Biomass concentration did support an increase in the CO_2 production rates up to 85 ± 5 and 78 ± 6 g C m^{-3} d^{-1} in the BCR and ALR, respectively. In this context, the mass transfer test carried out at the end of Stage III revealed that both systems were limited by N_2O mass transfer. Thus, the increase in N_2O inlet load by a factor of 2.8 ± 0.1 promoted a rapid increase in the N_2O elimination capacity by a factor of 2.4 ± 0.1 in both reactors (Figures S3). Mass transfer limitations have been previously identified as the limiting step in a bioscrubber treating N_2O laden air emissions from wastewater treatment plants, where a gas EBRT of 40 min was needed in the adsorption column in order to obtain a satisfactory N_2O RE of 92 %.[10]

To the best of the authors' knowledge, this study constitutes the first biological process devoted to the treatment of N_2O emissions originated from a nitric or adipic acid production plants and one of the pioneering works on the development of GHG biorefineries. Bubble column and internal loop airlift bioreactors have been consistently proven as low cost alternative technologies for the treatment of wastewaters and off-gases.[26–29] These bioreactor configurations are pneumatically agitated, resulting in low energy consumptions. Moreover, their simple construction (with no moving parts) and high gas-liquid mass transfer rates constitute also key advantages over their biological counterpart.[30–32] In our particular study, N_2O-REs of 80-90 % were consistently achieved concomitantly with the co-production of added value biopolymers (see section below), which were comparable with the N_2O abatement efficiencies of conventional physical/chemical technologies such as NSCR[7]. However, the gas EBRT (≈17 min) required to obtain high REs in the two bioreactor configurations evaluated would entail high bioreactor volumes.

PHBV accumulation during N_2O abatement

A low PHBV cell content was recorded during Stage I (1.9 ± 1.3 % in the BCR and 2.6 ± 1.3 % in the ALR) under TN concentrations in the cultivation broth of 238 ± 38 and 238 ± 40 mg N L^{-1} in the BCR and ALR, respectively. The dissolved CH_3OH concentrations in the BCR and ALR also remained constant during Stage I at 395 ± 20 and 367 ± 39 mg C L^{-1}, respectively.

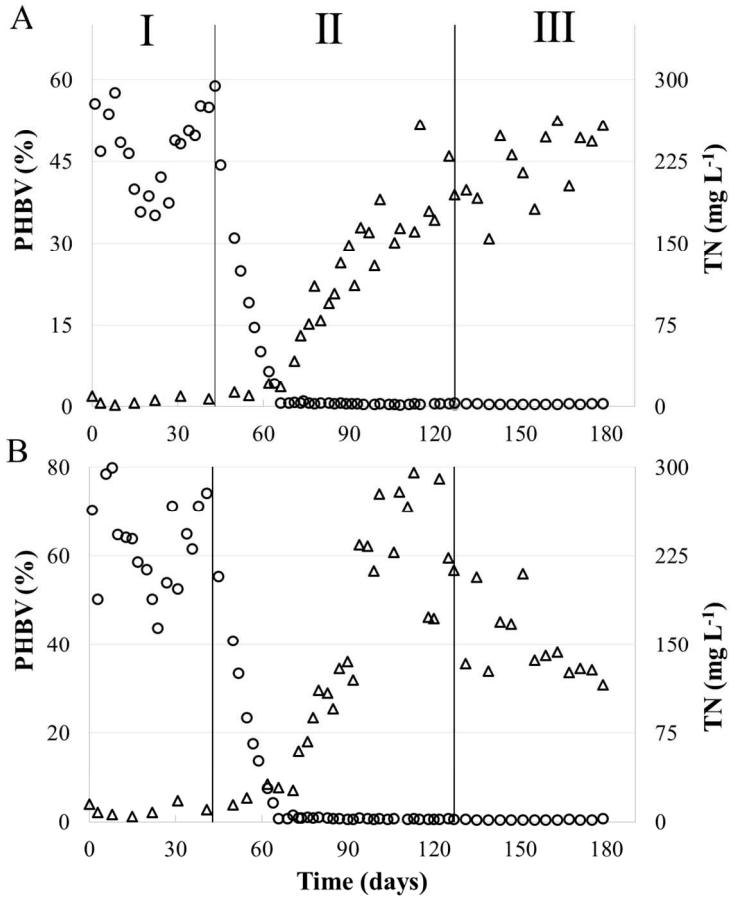

Figure 3. Time course of the PHBV cell content (Δ) and TN concentrations (○) in the BCR (A) and the ALR (B). Vertical lines indicate the different operation stages.

N was completely depleted by day 66 in Stage II, which promoted the gradual increase in the PHBV cell content in both bioreactors (Figure 3). The nitrogen supply strategy

evaluated during Stage II resulted in 24 hour of nitrogen sufficiency after MSM renewal followed by 24 h under nitrogen limitation, where PHBV synthesis and accumulation was likely to occur. N-limitation induced a steady state PHBV cell content of 38 ± 7 % in the BCR under TN and dissolved CH_3OH concentrations of 2.6 ± 0.5 mg N L^{-1} (Figure 3A) and 177 ± 28 mg C L^{-1}, respectively. The PHBV cell content recorded in the ALR was significantly higher than in the BCR, with average values of 64 ± 11 % (Figure 3B) under steady TN concentrations of 2.8 ± 0.6 mg N L^{-1} and dissolved CH_3OH concentrations of 368 ± 39 mg C L^{-1}. The transmission electron micrographs depicted in Figure 4 confirmed the accumulation of PHBV as granules inside bacteria with a cell diameter ranging from 0.5 to 1 µm, which matched the cell size of *P. denitrificans*.[33]

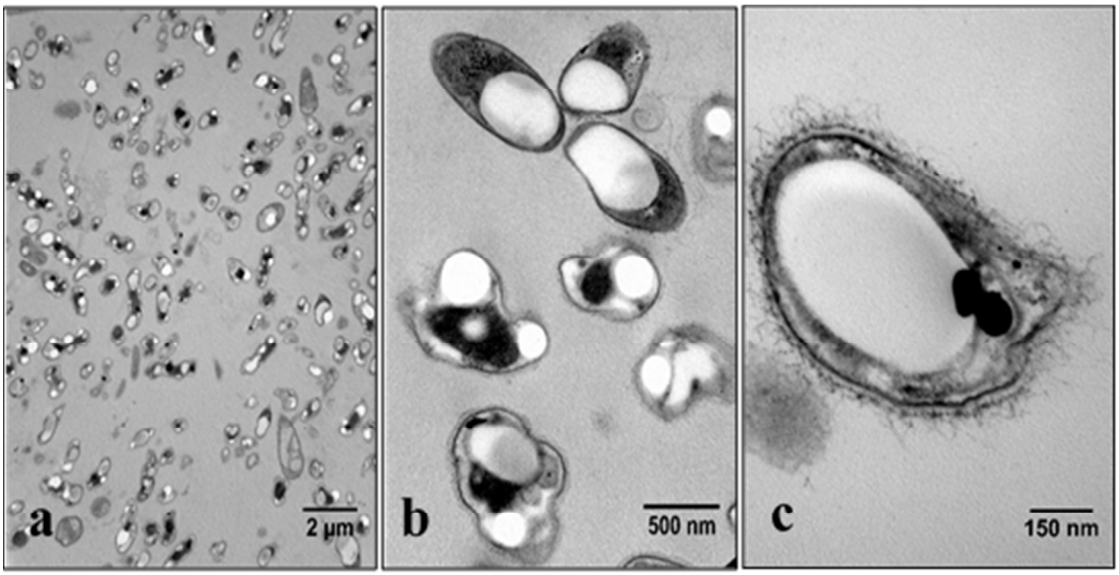

Figure 4. Transmission electron micrographs of cells containing PHBV in the BCR (a, c) and the ALR (b). Samples were drawn at the end of Stage III.

Process operation at a reduced dilution rate of 0.033 d^{-1} under similar N loads as Stage II mediated microbial cultivation with N sufficiency for 24 h followed by 3 days of N

deprivation in Stage III. These operational conditions promoted an enhanced PHBV cell content in the BCR of 47 ± 5 %, under steady TN and dissolved CH_3OH concentrations of 2.3 ± 0.3 mg N L^{-1} and 134 ± 23 mg C L^{-1}, respectively. However, the PHBV cell content recorded in the ALR decreased to 40 ± 8 % in spite of the comparable TN concentration (1.9 ± 0.4 mg N L^{-1}) and dissolved CH_3OH concentrations (373 ± 72 mg C L^{-1}). The highest PHBV yield (0.44 ± 0.2 gPHBV g^{-1}_{CH3OH}) was observed in the ALR in Stage II, decreasing to average values of 0.22 ± 0.03 gPHBV g^{-1}_{CH3OH} during Stage III. The BCR supported comparable production yields of 0.17 ± 0.05 gPHBV g^{-1}_{CH3OH} and 0.22 ± 0.03 gPHBV g^{-1}_{CH3OH} in Stages II and III, respectively. The yields obtained were in agreement with previously reported PHBV yields ranging from 0.06 to 0.4 gPHBV g^{-1}_{CH3OH} using methanol as the carbon source.[34–37]

The GC-MS analysis of the copolymer PHBV showed a small share of 3-hydroxyvalerate (PHV) regardless of the operational conditions evaluated. PHV/PHBV molar ratios of 2.5 ± 0.9 % and 2.9 ± 1.6 % were recorded at Stage I in the BCR and the ALR, respectively. When the bioreactors were subjected to nutrient limitation during Stages II and III, this ratio decreased to 0.46 ± 0.2 and 0.29 ± 0.1 in the BCR, and to 0.35 ± 0.1 and 0.32 ± 0.2 % in the ALR, respectively. Several authors have recorded similar results using methanol as the sole carbon and energy source under different nutrient limitation strategies. In this context, Ueda et al.[38] did not detect PHV in the PHBV copolymer accumulated in *P. denitrificans* when CH_3OH was used as the sole substrate (0.3% v/v). However, the PHV molar fraction increased up to 87 % when n-amyl alcohol (0.25 % v/v) was supplied together with CH_3OH (0.3 % v/v) . Similarly, Yamane et al.[12] explored the role of the type of alcohols (methanol, ethanol, n-propanol, n-butanol and n-pentanol) at a concentration of

0.1 % (v/v) on the PHBV cell content in *P. denitrificans* under N limiting conditions. The results revealed no PHV accumulation when CH_3OH was the sole carbon source, which suggested that CH_3OH is not the most suitable carbon source when a high share of PHV is desired.

The analysis of the elemental cell composition (C, H, S, and N) carried out at the end of Stage I showed C and N cell contents of ≈44 and ≈11 %, respectively (Table S1), which represented a C/N ratio of ≈4. This value was in agreement with the typical elemental composition for bacterial cells.[39] However, a significant reduction in N cell content was observed in the biomass from both bioreactors as a result of cell adaptation when N limiting strategies were implemented in Stages II and III. Thus, the C/N ratio recorded in the ALR and the BCR under nitrogen limitation increased to values ranging from 6.1 to 8.2. The likely decrease in protein cell content due to the limited N uptake also entailed a decrease in the S content of the microbial communities present in both bioreactors. A variation in the C cell content was not observed in spite of the accumulation of the biopolymer likely due to the similar elemental composition (C, H and O) of PHBV and biomass.

FISH analysis of the microbial population structure

The FISH analysis revealed the variation of the abundance of the *P. denitrificans* along the entire operational period (Table S2). Both bioreactors showed a *P. denitrificans* abundance higher than 90 % by the end of Stage I (Figure 5, Table S2). At the end of Stage II (day 120), the abundance of the inoculated strain in the BCR and ALR slightly decreased to 88 % and 86 % (Table S2). These results confirmed that *P. denitrificans* was capable of growing and dominating the microbial culture under anoxic conditions using CH_3OH as the

sole carbon/energy source and N_2O as electron acceptor. By the end of the experimentation (day 180), *P. denitrificans* remained dominant in both bioreactors (abundances > 50 %) (Figures 5c and 5f). In this context, the presence of others microbial strains capable of accumulating biopolymers may explain the maintenance of the PHBV cell content observed in Stage III despite the decrease in *P. denitrificans* abundance.

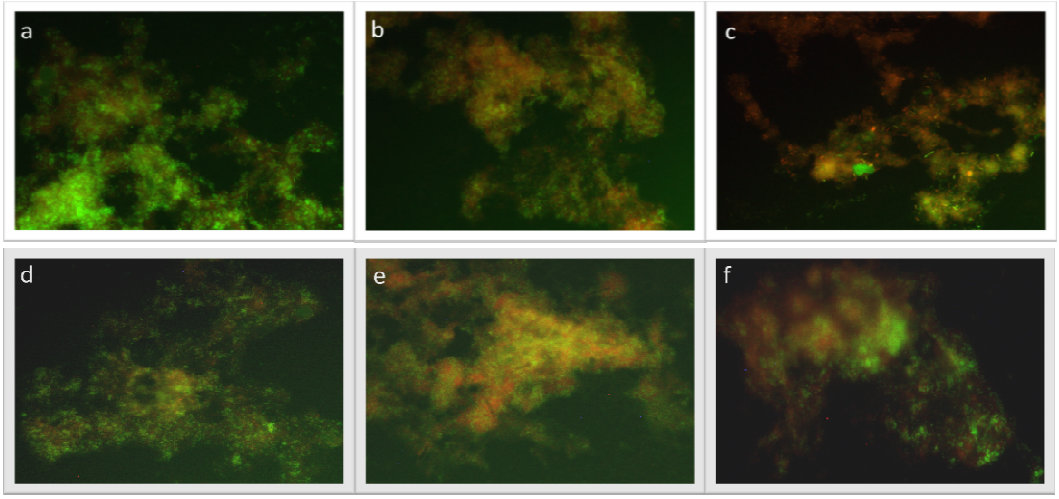

Figure 5. FISH micrographs of the microbial culture present at the end of the three operational stages evaluated in the ALR (a-c) and BCR (d-f). PAR651-fam (green) appears yellow due to a double hybridization with the EUB338 I-II- FITC probes (red).

In summary, this work demonstrated the feasibility of the combined biological abatement of N_2O from industrial emissions and co-production of PHBV. High N_2O-REs were recorded in spite of process operation under nitrogen limiting conditions. The nitrogen limiting strategies assessed in this study resulted in a high accumulation of PHBV by *P. denitrificans* using methanol and N_2O as the carbon/energy source and the electron acceptor, respectively. This study reports the first cost-efficient and environmentally

friendly bioprocess for the active abatement of N_2O using a waste-to-value biorefinery approach.

ASSOCIATED CONTENT

Supporting Information

A file with additional information is available free of charge via internet at http://pubs.acs.org. This file includes a schematic diagram of the operational set-up depicted in Figure S1 as well as additional data obtained from the mass transfer tests conducted at the end of Stages II (Figure S2) and III (Figure S3). Furthermore, the results from the analysis of the elemental composition (Table S1) and FISH analysis (Table S2) of the biomass are included in the file.

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Notes

The authors declare no competing financial interest.

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