Assessing the influence of the carbon source on the abatement of industrial N_2O emissions coupled with the synthesis of added-value bioproducts

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Abstract

The continuous abatement of a synthetic N_2O emission from a nitric acid plant coupled with the simultaneously production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

(PHBV) copolymer and the coenzyme Q10 (CoQ_{10}) in a bubble column reactor (BCR) was tested using methanol, glycerol and a mixture of sodium acetate-acetic acid (Ac-HAc) as a carbon and electron donor source. The BCRs were inoculated with *Paracoccus denitrificans* and supplied with the carbon/electron donor at a loading rate of 139 g C m^{-3} d^{-1}. High N_2O removal efficiencies (81-91 %) were achieved, with glycerol supporting the highest abatement. The PHBV cell content ranged from 25 to 53 %, with highest accumulation in the culture obtained with methanol and Ac-HAc. However, the greatest PHBV productivities were observed in the BCRs operated with glycerol and Ac-HAc (21.7 and 33.5 g PHBV m^{-3} d^{-1}, respectively). Glycerol supply induced the highest molar ratio (23 %) of the homopolymer 3-hydroxyvalerate in the composition of PHBV. In addition, the specific cell content of CoQ_{10} ranged from 0.4 to 1 mg g^{-1}. This work constitutes, to the best of our knowledge, the first study combining N_2O abatement with the simultaneous production of multiple bioproducts, which pave the way to the development of greenhouse gas biorefineries for climate change mitigation.

Keywords

Bubble column, Climate change, CoQ_{10}, Nitric acid plants, Nitrous oxide, PHBV

35

1. Introduction

37 The reduction of greenhouse gas (GHG) emissions has emerged as one of the most
38 important environmental and economic challenges for the countries endorsing the recent
39 Paris Agreement (COP21), which aimed at maintaining below 1.5 ºC the global average
40 temperature increase compared to the levels of the pre-industrial era (UNFCCC, 2015). In
41 this context, the abatement of the emissions of nitrous oxide (N_2O), a potent GHG with a
42 global warming potential 298 times higher than that of CO_2 and a contribution to the global
43 GHG inventory of ~6.2 %, will be mandatory in any future national strategy to mitigate
44 climate change.

45 Anthropogenic N_2O in industry is mainly emitted during nitric and adipic acid production,
46 which is responsible for the emission of approximately 500,000 tons of N_2O per year
47 (Pérez-Ramírez et al., 2003). The control of these industrial emissions has been
48 traditionally conducted by physical/chemical technologies, which are characterized by the
49 utilization of costly catalysts (at high pressure and temperature) and reducing agents such
50 as ammonia or hydrocarbons. In addition, physical/chemical processes entail pernicious
51 environmental impacts such as secondary gas emissions, the generation of a toxic spent
52 catalyst and a high energy consumption (Environmental Protection Agency, 2010).
53 Conversely, biotechnologies based on the use of denitrifying bacteria capable of
54 heterotrophically reducing N_2O to N_2 have recently emerged as a low-cost and eco-friendly
55 alternative to conventional physical/chemical technologies (Frutos et al., 2015; Frutos et al.,
56 2016). These biotechnologies consist of a two-stage process where N_2O is firstly
57 transferred from the gas emission to an aqueous phase and thereafter reduced biologically

using an external carbon/electron donor source. The purchase of this carbon/electron donor entails an increase in the operating cost of biotechnologies devoted to N_2O abatement, particularly when high levels of oxygen are present in the N_2O-laden emission and therefore a high organic loading rate is required to maintain anoxic conditions.

The biological transformation of GHGs such as CH_4 or N_2O into added value biopolymers such as poly(3-hydroxybutyrate) (PHB) and the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) has emerged as a promising alternative to enhance the cost-competitiveness of biological off-gas treatment (Myung et al., 2016; Zúñiga et al., 2011; Frutos 2017). These biodegradable biopolymers, which are present as water insoluble inclusions, accumulate as energy reservoir in eubacteria and archaea under excess of carbon source and nutrient limitation (Rehm, 2003). PHBV exhibits similar characteristics to the fossil thermoplastics polypropylene and polyethylene in terms of molecular weight, tensile strength or even melting point (Khosravi-Darani et al., 2013). Furthermore, superior physical properties than PHB can be achieved when a high proportion of the homopolymer 3-hydroxyvalerate (PHV) is present in the PHBV copolymer (Khanna and Srivastava, 2005; Reddy et al., 2003). Likewise, the co-production during GHG treatment of the coenzyme Q10 (CoQ_{10}), with a market price of ~300 € kg^{-1}, could turn climate change mitigation into a profitable process (Wu and Tsai, 2013). CoQ_{10} is intensively used nowadays for the treatment of cancer and hypertension (Jeya et al., 2010), and as antiaging agent in cosmetics manufacture (Ernster and Dallner, 1995). Despite the economic and environmental advantages derived from the co-production of PHBV and CoQ_{10} coupled to N_2O abatement, the potential of this novel GHG abatement approach has been poorly explored.

The present study systematically evaluated the influence of the type of carbon source/electron donor (methanol, glycerol and acetate-acetic acid) on the abatement of N_2O from a nitric acid production plant coupled to the simultaneous production of PHBV and CoQ_{10} in a bubble column bioreactor using *Paracoccus denitrificans* as a model denitrifying microorganism.

2. Material and Methods

2.1. Chemicals and mineral salt medium

The mineral salt medium (MSM) used 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 0.26 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 pH of the MSM was adjusted to 7. All chemicals were purchased from PANREAC with purities >99%. PHBV (12 % of PHV on a molar basis, equal to ~14 % on a mass basis), benzoic acid, methanol (CH_3OH), glycerol ($C_3H_8O_3$), sodium acetate ($C_2H_3NaO_2$) and acetic acid (CH_3COOH) were obtained from Sigma-Aldrich® (Sigma-Aldrich, St. Louis, MO, USA). The 40 L calibrated gas cylinders of 50,000 ppm_v of N_2O in N_2 and 50 L industrial N_2 cylinders were purchased from Abelló Linde S.A. (Barcelona, Spain).

2.2. Experimental set-up and operational conditions

The influence of the type of carbon source/electron donor (methanol, glycerol and acetate-acetic acid) on the abatement of N_2O and co-production of PHBV and CoQ_{10} was assessed in three independent experiments in a 2.5 L glass bubble column bioreactor (BCR) treating

a synthetic nitric acid plant emission. In each experimental run, the BCR was inoculated with 1 L of fresh *Paracoccus denitrificans* culture (Frutos et al., 2016) and filled up with MSM to a working volume of 2.3 L (Figure 1). The synthetic nitric acid plant emission, prepared by mixing the 50000 ppm_v N_2O standard with pure N_2 and air, was composed of 3560 ± 360 ppm_v of N_2O, 1.1 ± 0.1 % of O_2 and 98.5 % of N_2. The synthetic N_2O emission was fed at the bottom of the BCR via a gas diffuser (2 µm) at a flow rate of 137 ± 7 mL min^{-1}, thus resulting in a gas empty bed residence time (EBRT) of ~17 min. Aliquots of 300 mL of cultivation broth were exchanged every 4 days with fresh MSM. This strategy imposed a series of 1 day - 3 days nitrogen feast-famine cycles under excess of carbon source that promoted biopolymer accumulation. The experiments were conducted at 25 ºC in a temperature-controlled room.

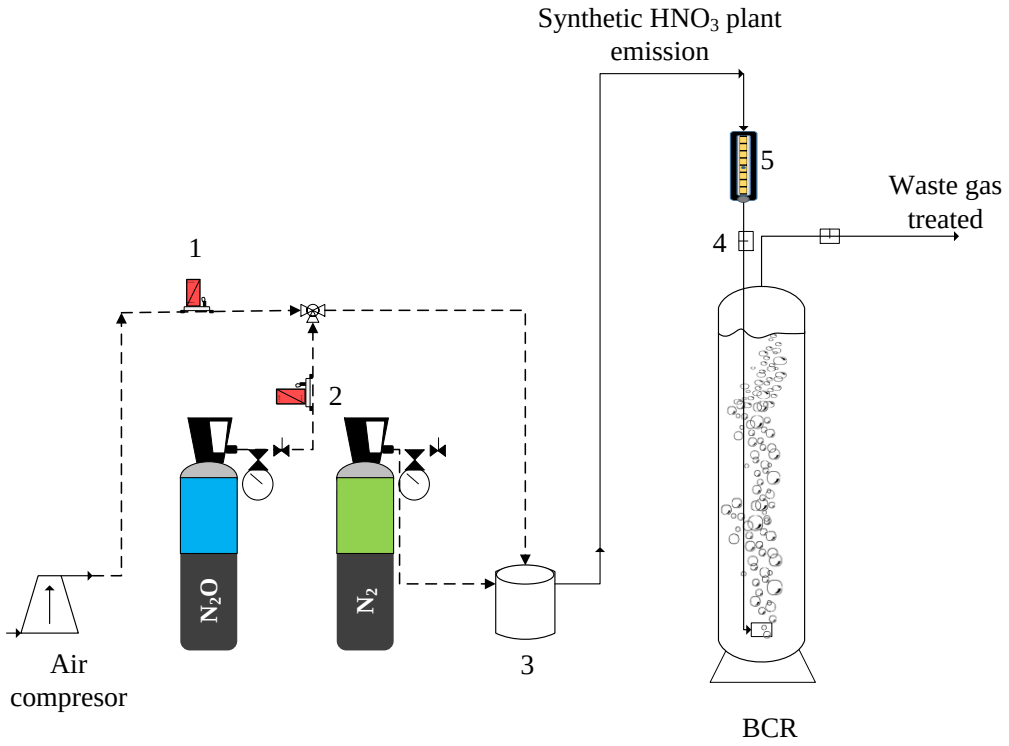


Figure 1. Scheme of the bubble column reactor. 1) Air mass flow controller, 2) N_2O mass flow controller, 3) Gas mixing chamber, 4) Gas sampling port and 5) Rotameter.

The experiment using CH_3OH as a carbon source/electron donor (BCR-A) was carried out for 65 days at a carbon loading rate of 139 g C m^{-3} d^{-1}. The experimental run using glycerol (BCR-B) was initially operated at a carbon loading rate of 139 g C m^{-3} d^{-1} for 40 days, which was increased to 209 g C m^{-3} d^{-1} for the last 25 days of operation to prevent carbon limitation in the process. The experiment with acetate (BCR-C) was performed with a 95%/5 % (Cmol/Cmol) mixture of sodium acetate/acetic acid (Ac-HAc; pH 6.5) at a carbon loading rate of 139 g C m^{-3} d^{-1} for 75 days. BCR-C was operated without pH control for the first 34 days and at a pH of 7 afterwards via daily addition of HCl (37 %).

The determination of the gas concentrations of N_2O, CO_2 and O_2 was daily conducted by GC-ECD and GC-TCD according to Frutos et al. (2016). The gas concentration of CH_3OH was determined by GC-FID every 4 days before MSM exchange. The total organic carbon (TOC), total nitrogen (TN) and inorganic carbon (IC) concentrations were measured every 4 days from the withdrawn cultivation broth. Similarly, the aqueous concentration of CH_3OH, volatile fatty acid (VFA) and glycerol in the cultivation broth was determined by GC-FID and HPLC-IR, respectively. Liquid samples of 40 mL were drawn for the measurement of the concentrations of total suspended solid (TSS) and PHBV every 4 days, while 10 mL were used for CoQ_{10} determination at the end of each BCR operation. pH was determined every 4 days in each bioreactor, but daily measured during BCR-C operation with pH control.

2.3. Analytical procedures

137 The concentration of TOC, TN and IC was measured in a TOC-VCSH analyzer (Shimadzu,
138 Tokyo, Japan) coupled with a TN chemiluminescence detection module (TNM-1,
139 Shimadzu, Japan). The TSS concentration was determined according to Standard methods
140 (APHA, 2005).

141 The GC-FID (Bruker 3900, Palo Alto, USA) used for CH_3OH determination was equipped
142 with a SupelcoWax (15 m × 0.25 mm × 0.25 μm) capillary column. The injector and
143 detector temperatures were maintained at 200 and 250 ºC, respectively. Nitrogen was used
144 as the carrier gas at 1 mL min^{-1} and make-up gas at 25 mL min^{-1}, while the flowrates of H_2
145 and air were set at 30 and 300 mL min^{-1}, respectively. Glycerol was determined in a Waters
146 e2695 HPLC (Massachusetts, USA) equipped with a Waters 2414 refractive index detector
147 using a Bio-Rad HPX-87H column at 50 °C and a 5 mM H_2SO_4 aqueous eluent at 0.6 mL
148 min^{-1}. The concentration of dissolved VFA was determined by GC-FID following the
149 procedure indicated in Alcántara et al. (2015). The analysis of PHBV involved the
150 centrifugation of 2 mL of cultivation broth at 9000 rpm for 15 min and the processing of
151 the biomass pellet according to Zúñiga et al. (2011). Then, the PHBV was extracted for 4 h
152 at 100 ºC using 2 mL of chloroform and quantified (using external standards) by GC-MS in
153 a GC System 161 7820A MSD 5977E (Agilent Technologies, Santa Clara, USA) equipped
154 with a DB-wax capillary column (30 m × 250 μm × 0.25 μm). The temperatures of the
155 detector and injector were set at 250 ºC, with a split ratio of 1:10. The oven temperature
156 was initially maintained at 40 ºC for 5 min, increased at 10 ºC min^{-1} up to 200 ºC and
157 maintained for 5 min. The PHBV cell content was normalized as %PHBV= (g PHBV/g
158 TSS) × 100. The PHV molar ratio of the PHBV copolymer was estimated based on the

159 concentration of each homopolymer and the weight/molar ratio of the standard as follow
160 %PHV= (PHV/PHBV)×100×(12/14).

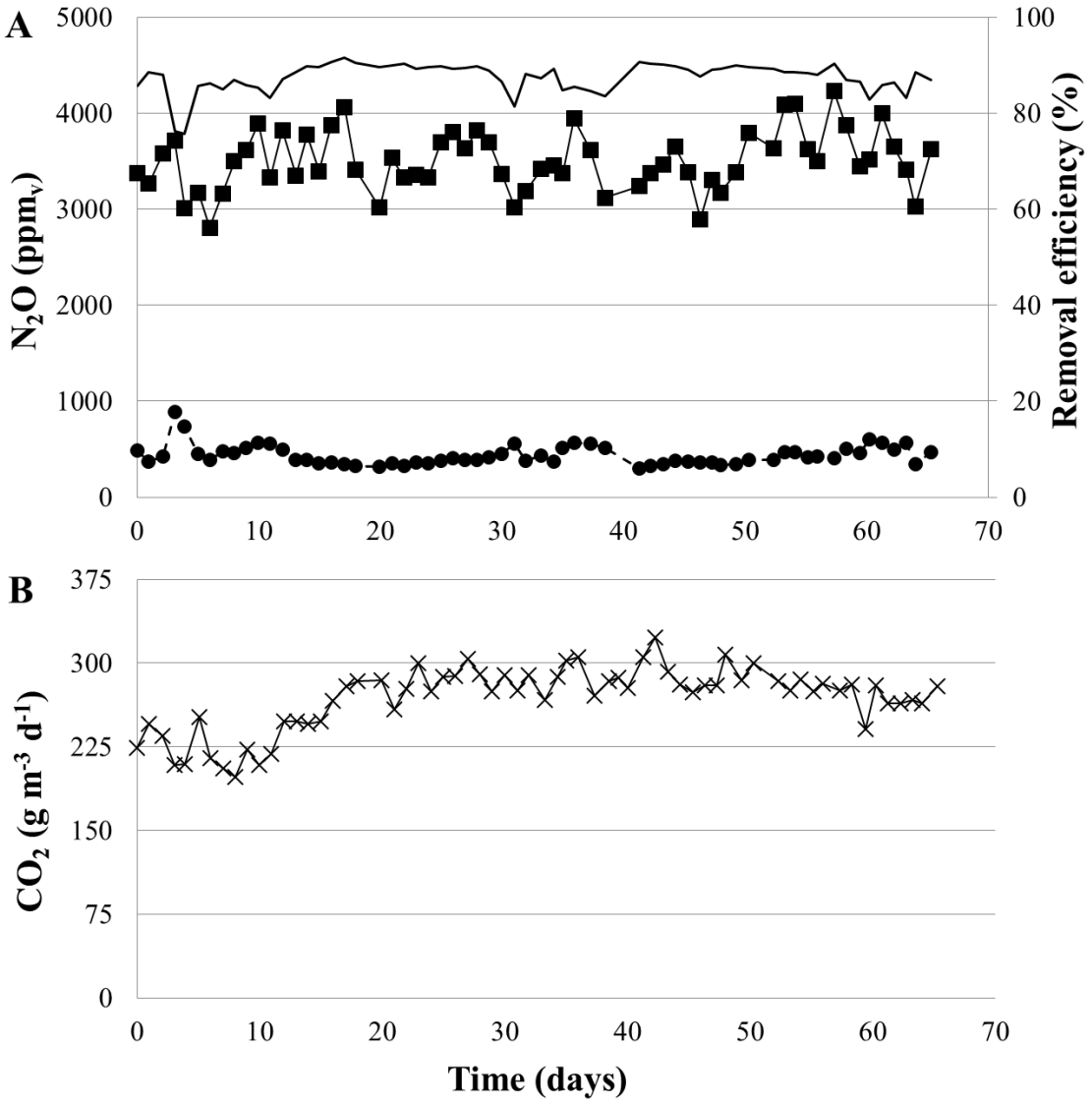
161 Similarly, the analysis of CoQ_{10} involved the centrifugation of 10 mL of cultivation broth
162 and the lysis of the biomass pellets by addition of 0.5 mL of CelLytic™, vortexing and
163 incubation in an ultrasonic bath for 30 min. CoQ_{10} was then extracted in a water bath with
164 2.5 mL of a propanol/hexane solution (3:5 v:v) at 40 ºC (30 min). Finally, the organic phase
165 was filtered (0.2 µm) and transferred to a 1 mL vial for analysis by HPLC-UV. The Waters
166 e2695 HPLC was equipped with a Waters symmetry C18 column (3.5 µm × 3 mm × 100
167 mm) using a methanol/hexane (83:17 v:v) mobile phase at a flow rate of 0.6 mL min^{-1}. A
168 UV detector (UV 2487) was used for CoQ_{10} determination at a wavelength of 275 nm. The
169 final CoQ_{10} concentration was correlated to the TSS concentration and expressed as
170 milligram of CoQ_{10} per gram of biomass (mg g^{-1}).

171 # 3. Results and Discussion

172 ## 3.1. Process performance with methanol

173 The use of methanol as a carbon source/electron donor supported average removal
174 efficiencies (REs) of 87 ± 3 % along the entire operational period (Figure 2A). Steady CO_2
175 production rates of 283 ± 15 g m^{-3} d^{-1} were recorded from day 20 despite N_2O-REs
176 remained constant from day 5 onward (Figure 2B). This stabilization in CO_2 production
177 occurred concomitantly with the stabilization in biomass concentration, which averaged
178 1086 ± 80 mg L^{-1} (Table 1). In this context, the specific N_2O elimination capacity supported
179 by methanol under biomass steady state concentration was 0.46 ± 0.05 gN_2O $gTSS^{-1}$ d^{-1}. No

180 significant variation was however observed in the pH of cultivation broth, which remained
181 constant at 6.8 ± 0.1 along the 65 days of operation.

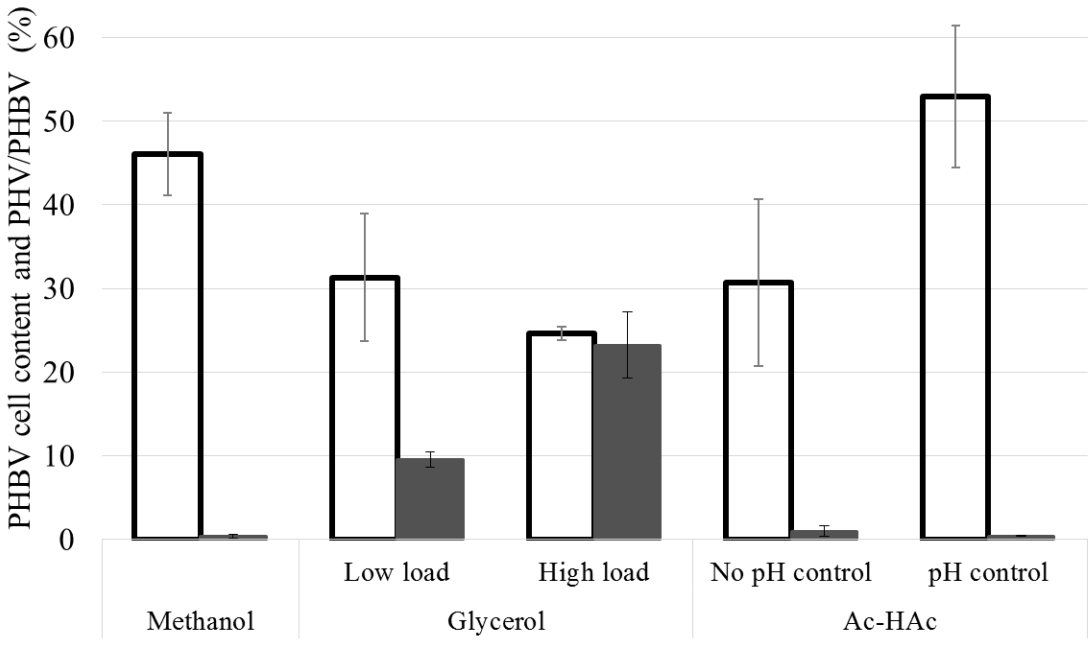


182

183 **Figure 2.** Time course of the A) inlet (■) and outlet (●) N_2O concentrations and removal
184 efficiency (solid line), and B) CO_2 production rates in the BCR supplied with methanol.

185 The ratio of CO_2 produced per CH_3OH consumed averaged 0.83 ± 0.18 gC gC^{-1} under
186 steady state conditions, which indicates that most of the carbon supplied as methanol was

used for energy production purposes. Likewise, the ratio of CO_2 produced per N_2O reduced to N_2 accounted for 0.54 ± 0.07 gCO_2 gN_2O^{-1}. Part of the CH_3OH supplied accumulated as dissolved TOC in the culture broth (356 ± 54 mg L^{-1}, which represented ~ 29 % of the methanol fed to the BCR-A), while the remaining methanol resulted in the formation of biomass and accumulation of PHBV to a cell content of 46 ± 5 % under nitrogen limitation (Figure 3). Nitrogen limitation was characterized by steady state TN concentrations of 3.2 ± 0.4 mg L^{-1} (Table 1). The PHBV cell content here recorded was higher than that reported by Yamane et al., (1996) using CH_3OH as the carbon source during *P. denitrificans* growth under nitrogen limitation and aerobic conditions (13.3 %). Our study revealed a PHV molar ratio of 0.38 ± 0.25 % in the PHBV copolymer, which agreed with the results reported in previous works using CH_3OH as a substrate (Ueda et al., 1992; Yamane et al., 1996). The PHBV productivity in BCR-A under steady state conditions averaged 16.3 gPHBV m^{-3} d^{-1}.



200 **Figure 3.** Specific PHBV cell content (white column) and the homopolymer (PHV) molar
201 ratio (gray column) of the cultures supplied with the three carbon sources evaluated.

202 Despite no particular strategy was implemented to boost CoQ_{10} accumulation in the
203 microbial culture, a specific cell content of 0.9 mg g^{-1} (associated to a productivity of 32
204 mg m^{-3} d^{-1}) was recorded in BCR-A (Table 1). Similar results were reported by Yoshida et
205 al., (1998) assessing the performance of 34 bacterial strains previously characterized as
206 CoQ_{10} producers. These authors found CoQ_{10} specific cell contents of 0.86 mg g^{-1} in
207 *Paracoccus denitrificans* strain (ATCC19367) under aerobic conditions using cane
208 molasses as a carbon source at a concentration of 5 %. Likewise, a study assessing the
209 effect of O_2 levels on the production of CoQ_{10} in *P. denitrificans* CCM 982 showed that the
210 highest yield (1.2 mg g^{-1}) was obtained at an oxygen concentration of 2.5 %, with a
211 decrease in the coenzyme content to 0.43 mg g^{-1} when the O_2 levels increased to 21 %
212 (Kaplan et al., 1993). Recently, the CoQ_{10} biosynthesis capacity of a mutant strain of *P.*
213 *denitrificans* (P-87) has been evaluated using the precursor parahydroxy benzoic acid
214 (Tokdar et al., 2014). This study revealed a maximum specific cell content of 1.63 mg g^{-1},
215 which was only 1.8-fold higher than that obtained in our study with no particular
216 optimization strategy.

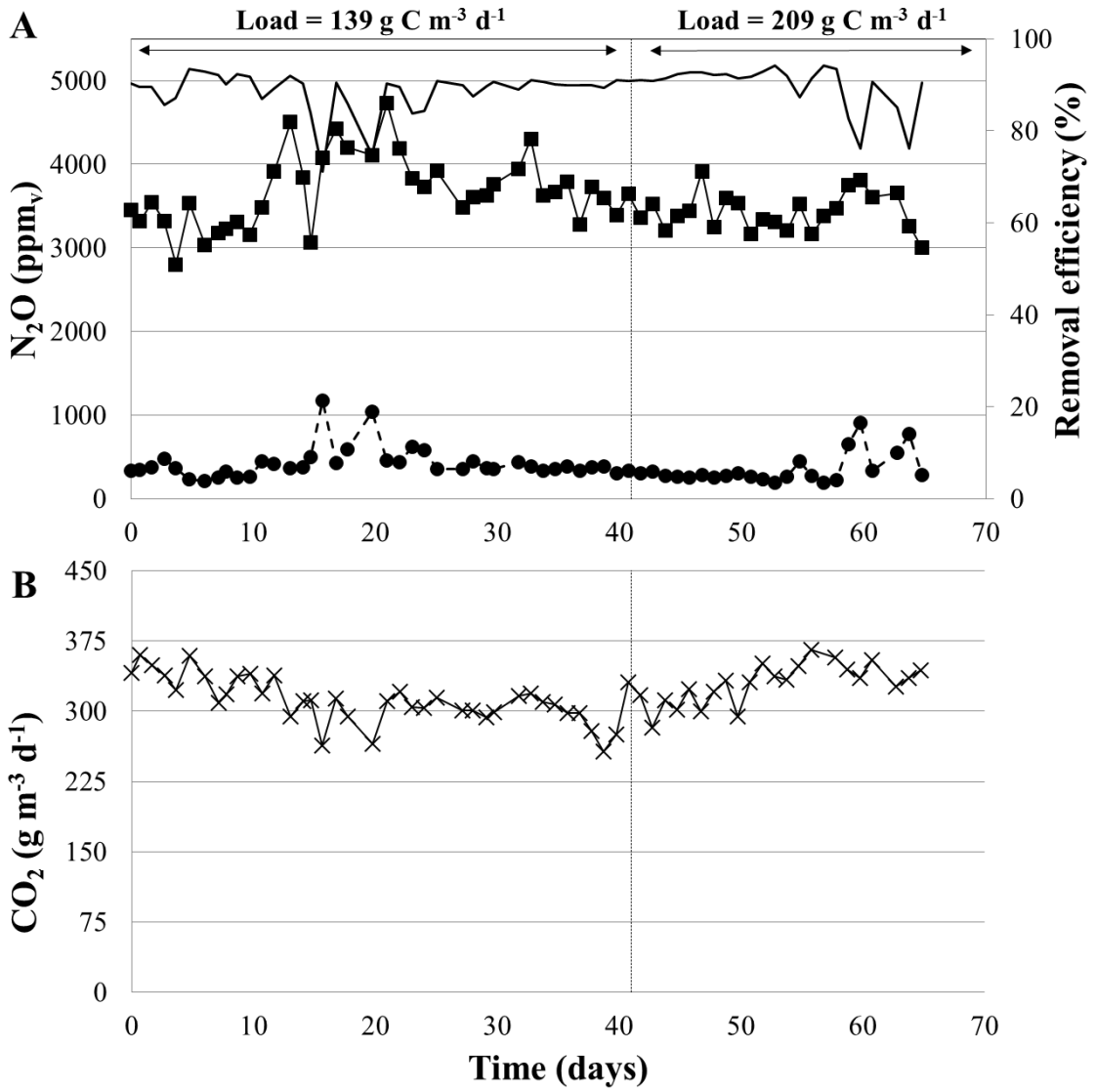
217 **Table 1**. Summary of the main process performance parameters during steady state for the
218 three carbon source/electron donors evaluated in this study

Bioreactors		**TSS (mg L^{-1})**	**TOC (mg L^{-1})**	**TN (mg L^{-1})**	**CoQ10 (mg $gTSS^{-1}$)**	**CoQ10 productivity (mg m^{-3} d^{-1})**
BCR-A	-	1086 ± 80	356 ± 54	3.2 ± 0.4	0.9	32
BCR-B	Low load	1638 ± 76	75 ± 9	4.2 ± 0.5	-	-
	High load	2707 ± 85	331 ± 61	4.9 ± 0.5	0.4	33

BCR-C	No pH control	525 ± 119	55 ± 29	4.3 ± 1.0	1	18
	pH control	1940 ± 106	62 ± 4	4.5 ± 0.5	0.5	30

3.2. Process performance with glycerol

The use of glycerol at a loading rate of 139 g C m^{-3} d^{-1} in BCR-B supported a N_2O-RE of 89 ± 2 % (Figure 4A), which was similar to the removal efficiency supported by CH_3OH at a similar C loading rate. Glycerol, which was a more favorable substrate than CH_3OH from an energy viewpoint, promoted higher steady state biomass concentration (1638 ± 76 mg L^{-1}) than that attained with methanol after 10 days of bioreactor operation (Table 1). This higher biomass concentration resulted in greater steady state CO_2 productions of up to 306 ± 8 g m^{-3} d^{-1} (Figure 4B).



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228 **Figure 4.** Time course of the A) inlet (■) and outlet (●) N_2O concentrations and removal
229 efficiency (solid line), and B) CO_2 production rates in the BCR supplied with glycerol.
230 Two-way arrows indicate the carbon loading rates applied.

231 Process operation at a low glycerol loading rate resulted in a similar CO_2 produced per N_2O
232 consumed ratio (0.59 ± 0.08 gCO_2 gN_2O^{-1}) to that recorded in BCR-A, but a lower specific
233 CO_2 production yield (0.58 ± 0.04 gC gC^{-1}). This lower respiration yield revealed that more
234 carbon was devoted to the synthesis of new microbial cells compared to the use of

methanol as a carbon source/electron donor. Likewise, the specific N_2O removal capacity of the culture decreased to 0.33 ± 0.03 $gN_2O\ gTSS^{-1}\ d^{-1}$ mediated by the higher biomass concentration and the limited mass transfer of N_2O from the gas emission. Indeed, the fact that similar N_2O removal efficiencies were recorded at higher *P. denitrificans* concentrations clearly showed that the process was limited by the mass transport of N_2O from the gas to the liquid phase rather than by biological activity. The low water solubility of N_2O (H= 1.6 at 25 ºC (Sander, 2014)) resulted in a poor concentration gradient from the gas to liquid phase, thus limiting the N_2O elimination capacity of the bioreactor. On the other hand, the dissolved TOC concentration in the cultivation broth stabilized at 75 ± 9 mg L^{-1} from day 10 to 40 (Table 1). Surprisingly, the analysis of the cultivation broth by HPLC revealed that this TOC did not correspond to residual glycerol and was likely due to the carbon released from cell lysis (cell debris). In this scenario of glycerol limitation and high biomass concentration, a PHBV cell content of 31 ± 8 % was recorded under nitrogen limitation (TN concentrations of 4.2 ± 0.5 mg L^{-1}). Despite PHBV accumulation under glycerol limitation was slightly lower than in BCR-A, a remarkable increase in the content of the PHV homopolymer was recorded (with a PHV/PHBV ratio of 10 ± 1 % on a molar basis) in the first 40 days of BCR-B operation (Figure 3). In addition, the productivity of PHBV was slightly higher (16.7 $gPHBV\ m^{-3}\ d^{-1}$) than that recorded in BCR-A.

The increase in glycerol loading rate to 209 g C $m^{-3}\ d^{-1}$ resulted in a slight increase in the N_2O-RE to 91 ± 3 %, with a concomitant increase in the CO_2 production rates to 342 ± 15 g $m^{-3}\ d^{-1}$ (Figure 4). Likewise, biomass achieved stable concentrations of 2707 ± 85 mg L^{-1} mediated by the higher glycerol loading rate (Table 1). These high biomass concentrations resulted in a reduction in the specific N_2O removal capacity to 0.17 ± 0.03 $gN_2O\ gTSS^{-1}\ d^{-1}$,

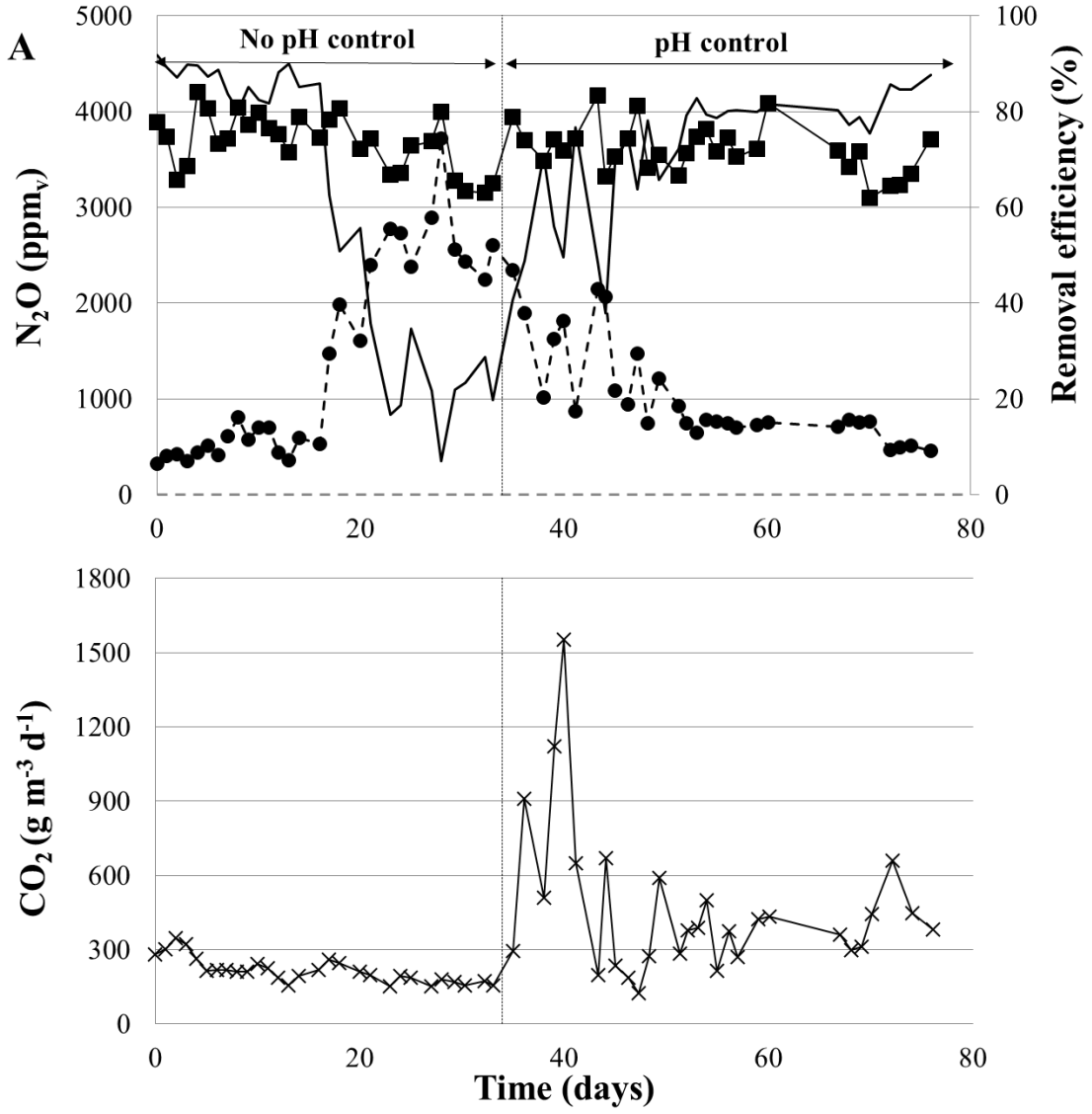
258 which supported the above-hypothesized N_2O mass transfer limitation. On the other hand,
259 the ratio of CO_2 produced per glycerol consumed decreased to 0.47 ± 0.05 gC gC^{-1}, which
260 confirmed that a higher proportion of the carbon present in glycerol was routed to the
261 production of cell material. In this sense, Rittman and McCarty (2012) described that
262 microorganisms use a larger proportion of the carbon present in the substrate for cell
263 maintenance when certain growth factors are limiting and viceversa. Glycerol has recently
264 emerged as a cost-competitive substrate for the production of added-value bioproducts and
265 represents an alternative to support the biological removal of N_2O as a result of the
266 decreasing market price (approx. 0.12 $ per kg of crude glycerol) mediated by the
267 increasing biodiesel market. Hence, the production of one liter of biodiesel generates ~125
268 gram of glycerol (Yang et al., 2012), which is currently handled as a residue. Despite the
269 fact that a higher glycerol loading rate entailed an increase in the dissolved TOC
270 concentration in the system (331 ± 61 mg L^{-1}), the microbial culture did not accumulate a
271 higher content of PHBV (25 ± 1 %) (Figure 3). However, the higher glycerol loading did
272 result in an increase in the PHV/PHBV ratio, which achieved stable values of 23 ± 4 %
273 (Figure 3). In this context, the physical-chemical properties of the PHBV copolymer
274 enhance with the increase in the proportion of the homopolymer PHV (Bonartsev et al.,
275 2007). Higher PHV/PHBV ratios confer greater copolymer characteristics such as a lower
276 crystallinity and melting point, which expand the range of potential uses of PHBV
277 (Eschenlauer et al., 1996). Typically, the production of PHBV with a high PHV monomer
278 ratio has involved the use as precursors of costly co-substrates such as propionic acid,
279 valeric acid, n-pentanol or other fatty acids (Shozui et al., 2010; Steinbüchel, 2001). Indeed,
280 propionic acid is the most commonly used precursor in the commercial production of
281 Biopol (PHBV) using glucose as the main carbon source. Finally, it must be highlighted

282 that despite the fact that no precursors were used to promote PHV accumulation, the
283 analysis of the cultivation broth by GC-FID revealed the presence of volatile fatty acids (in
284 particular propionic acid), which might have been produced from the anaerobic
285 fermentation of glycerol in the BCR-B thus contributing to the synthesis of PHV. Process
286 operation with glycerol also resulted in a lower CoQ_{10} content of 0.4 mg g^{-1} but a higher
287 productivity (33 g m^{-3} d^{-1}) compared to BCR-A as a result of the higher biomass
288 productivity (Table 1). Bule and Singhal (2010) reported a specific cell content of 6.1 mg
289 CoQ_{10} per gram of biomass in *Pseudomonas diminuta* using glycerol as a carbon source
290 and an optimized operation by switching the aeration flow and agitation rates for the
291 enhancement of coenzyme production.

292 **3.3. Process performance with acetic acid**

293 Process operation with Ac-HAc entailed a stable and high N_2O RE (~90 %) and a rapid
294 increase in the pH of the cultivation broth up to 9.3 by day 12 and (Figure 5A). This
295 increase in pH induced floc formation, which avoided a proper suspension of the culture
296 broth and resulted in a sharp reduction in N_2O-RE from day 16 (Figure 5A). Therefore, the
297 liquid broth was continuously recycled from the bottom to the top of the BCR-C by a
298 peristaltic pump at a flow rate of 200 mL min^{-1}. The pH under steady state remained at 9.4
299 ± 0.2, resulting in low biomass concentrations (525 ± 119 mg L^{-1}), N_2O REs (20 ± 7 %) and
300 CO_2 production rates (181 ± 28 g m^{-3} d^{-1}) (Figure 5). This high pH increased the capacity of
301 the cultivation to accumulate HCO_3^- and CO_3^{2-}, thus contributing to underestimate the CO_2
302 stripped out from the bioreactor. This phenomenon was confirmed by the high inorganic
303 carbon concentrations recorded in the cultivation broth (~520 mg C L^{-1}) compared to that
304 recorded in BCR-A and B (~1.2 and ~0.5 mg C L^{-1}, respectively). On the other hand, the

305 dissolved TOC concentration in the cultivation broth remained at 55 ± 29 mg C L^{-1} despite
306 the pH-hindered biomass growth, which suggested that a significant fraction of the acetic
307 acid was stripped out from the reactor. In this context, a fair estimation of the specific CO_2
308 yield was not possible in the absence of pH control. The specific N_2O removal remained
309 low at 0.17 ± 0.07 gN_2O $gTSS^{-1}$ d^{-1}, similar to that observed in BCR-B under high glycerol
310 loading rates. Process operation with acetic acid and no pH control was characterized by a
311 steady PHBV cell content of 31 ± 10 % promoted by the low dissolved TN concentration
312 (4.3 ± 1 mg L^{-1}) (Figure 3). The PHBV productivity of the BCR-C was very low (5.3
313 gPHBV m^{-3} d^{-1}) due to the low biomass concentration. In addition, the PHV/PHBV ratio
314 was ~1 %, which was slightly higher than that achieved during process operation with
315 CH_3OH, but much lower than that recorded using glycerol. Surprisingly, the highest
316 specific CoQ_{10} cell content (1 mg g^{-1}) was recorded under this steady state without pH
317 control.



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319 **Figure 5.** Time course of the A) inlet (■) and outlet (●) N_2O concentrations and removal
320 efficiency (solid line), and B) CO_2 production rates in the BCR supplied with Ac-HAc.
321 Two-way arrows indicate the period with and without pH control.

322 The pH of the BCR-C was daily reduced to ~7 from day 35 onward by the addition of HCl,
323 which promoted an increase in biomass concentration up to steady state values of 1940 ±
324 106 mg L^{-1} (Table 1). High and unstable CO_2 concentrations were observed following pH
325 control (Figure 5B). Thereafter, a stable CO_2 production of 361 ± 77 g m^{-3} d^{-1} was recorded

from day X. The increase in TSS concentration in BCR-C promoted an increase in N_2O-REs up to 81 ± 3 % (Figure 5A), which resulted in specific N_2O elimination capacities of 0.23 ± 0.03 $gN_2O\ gTSS^{-1}\ d^{-1}$. These two parameters were lower than those recorded in BCR-A and BCR-B likely due to the poor availability of the dissolved electron donor for N_2O reduction as confirmed by the low dissolved TOC concentration (62 ± 4 mg L^{-1}) (Table 1). pH control resulted in a limited stripping of acetic acid as confirmed by GC-FID analyses. The estimated specific CO_2 production yield accounted for 0.74 ± 0.1 gC gC^{-1}, and pointed out that a large fraction of the carbon input was dedicated to the energy production for cell growth and maintenance.

The steady state PHBV cell content obtained during BCR-C operation under pH control averaged 53 ± 8 % (Figure 3). The biomass concentration increase mediated by pH control resulted in an enhanced biopolymer productivity of up to 33.5 gPHBV $m^{-3}\ d^{-1}$, which constitutes the highest recorded in this work. However, the homopolymer PHV represented only 0.4 ± 0.1 % of the PHBV copolymer, a value comparable to that observed in the culture of BCR-A (Figure 3). Lemos et al., (1998) assessed the production of PHBV in a phosphate-accumulating bacterial culture using acetate, propionate and butyrate. The highest PHBV cell content of ~17.5 % (with a PHV/PHBV ratio of 24.75 %) was obtained with acetate as the sole carbon source. Similarly, Jiang et al., (2011) reported a PHB accumulation > 80 % using acetate in a fed-batch culture dominated by *Plasticicumulans acidivorans*. Similarly to our results, acetate supported a low PHV synthesis, while propionate supplementation increased the PHV/PHBV ratio to 89 %. Finally, the decrease in the specific cell content of CoQ_{10} to 0.5 mg g^{-1} when implementing pH control was counterbalanced by the increase in biomass which led to a CoQ_{10} productivity of 30 mg m^{-3}

349 d^{-1} (Table 1). A higher CoQ_{10} specific cell content could be achieved in this study via
350 supplementation of a precursor of the coenzyme.

351 The N_2O REs recorded in the three experimental runs (81 to 91 %) are comparable to those
352 supported by physical-chemical technologies (Environmental Protection Agency, 2010).
353 Although the implementation of this biotechnology at full scale still requires additional
354 research efforts for a complete understanding and optimization, the fundamental results
355 herein obtained demonstrated the potential production of added-value bio-products coupled
356 to the continuous abatement of N_2O from industrial emissions. At this point, it must be
357 stressed that the portfolio of biotechnologies capable to abate industrial N_2O emissions is
358 very limited (Frutos et al., 2016), this study representing a step forward in the development
359 of greenhouse gas biorefineries.

360 ## 4. Conclusions

361 This study aimed at evaluating the feasibility of coupling the abatement of industrial
362 emissions of N_2O to the co-production of commodities (PHBV) and high added value bio-
363 products (CoQ_{10}) using different carbon sources/electron donors. High N_2O removal
364 efficiency were achieved in the BCRs evaluated. However, further enhancement in N_2O
365 removal and biomass productivity was limited by the mass transport of this poorly water
366 soluble gas pollutant from the emission to the cultivation broth, regardless of the carbon
367 source. Methanol and glycerol supported similar PHBV productivities under the same
368 carbon loading rates, while the operation with acetate as a carbon source doubled its
369 productivity. Glycerol supported the highest content of PHV in the composition of the
370 PHBV copolymer likely due to the formation of propionic acid from glycerol fermentation.

371 To the best of our knowledge, this study reported for the first time the co-production of the
372 co-enzyme CoQ_{10} and the biopolymer PHBV by *P. denitrificans* during N_2O abatement,
373 which paves the way to the development of GHG biorefineries as a cost-competitive tool
374 for climate change mitigation.

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