

1 **Effect of process parameters and operational mode on nitrous oxide**
2 **emissions from a nitrification reactor treating reject wastewater**

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13

14 **ABSTRACT**

15 Nitrous oxide (N₂O) and methane emissions were monitored in a continuous granular
16 airlift nitrification reactor from ammonium-rich wastewater (reject wastewater). N₂O
17 emissions were found to be dependent on dissolved oxygen (DO) concentration in the
18 range of 1 to 4.5 mg O₂/L, increasing within this range when reducing the DO values. At
19 higher DO concentrations, N₂O emissions remained constant at 2.2% of the N oxidized to
20 nitrite, suggesting two different mechanisms behind N₂O production, one dependent and
21 one independent of DO concentration. Changes on ammonium, nitrite, free ammonia and
22 free nitrous acid concentrations did not have an effect on N₂O emissions within the
23 concentration range tested. When operating the reactor in a sequencing batch mode under
24 high DO concentration (> 5 mg O₂/L), N₂O emissions increased one order of magnitude

25 reaching values of 19.3 ± 7.5 % of the N oxidized. Moreover, CH_4 emissions detected were
26 due to the stripping of the soluble CH_4 that remained dissolved in the reject wastewater
27 after anaerobic digestion. Finally, an economical and carbon footprint assessment of a
28 theoretical scaled up of the pilot plant was conducted.

29 *Keywords:* partial nitrification; reject wastewater; nitrous oxide emissions; continuous vs
30 discontinuous operation; economical analysis.

31

32 **1. Introduction**

33 Specific treatments for high ammonium (NH_4^+) streams such as reject wastewater
34 produced in the anaerobic digester sludge dewatering process have been implemented in
35 many wastewater treatment plants (WWTPs). Anaerobic digestion reject water is
36 characterized by its high NH_4^+ content (500-1500 mg N/L) and its treatment is normally
37 done via partial nitrification followed by denitrification (Hellinga et al., 1998; Mulder et
38 al., 2001) or the combination of partial nitrification with anammox (van Dongen et al.,
39 2001) to reduce the operational costs. In the last few years, several studies have reported
40 uncontrolled direct nitrous oxide (N_2O) emissions during this treatment, especially in the
41 nitrification reactor, where conversion of NH_4^+ to nitrite (NO_2^-) occurs due to the action of
42 ammonia oxidizing bacteria (AOB) (Kampschreur et al., 2008a; de Graaff et al., 2010;
43 Desloover et al., 2011; Law et al., 2012). In full-scale partial nitrification installations N_2O
44 values ranging from 1.7 to 6.6% of the nitrogen load have been measured, which
45 correspond to 3.4-11.2 % of the NH_4^+ -N oxidized emitted as N_2O since these systems
46 operate with partial conversion of NH_4^+ to NO_2^- (Kampschreur et al., 2008a; Desloover et
47 al., 2011). N_2O has a warming potential 265 times higher than that of CO_2 (IPCC 2013)

48 and in some cases might be responsible of the majority of the carbon footprint of the
49 plant, especially in those systems with high conversion of NH_4^+ to NO_2^- .

50 AOB are known to be net producers of N_2O which originates via two possible pathways:
51 a) oxidation of hydroxylamine (NH_2OH) which could be regulated by the concentration
52 of free ammonia (Stein 2011); b) the reduction of NO_2^- to N_2O in a process known as
53 nitrifier denitrification (Bock et al., 1995). Low dissolved oxygen (DO) concentration
54 levels, high NO_2^- concentrations and variation in influent NH_4^+ concentrations have been
55 identified to promote N_2O formation (Kampschreur et al., 2009a). To this end, effective
56 process control specifically devoted to keep desired set-points for the key parameters of
57 operation in nitrification reactors (i.e. DO concentration, NH_4^+ concentration, pH) should
58 be targeted.

59 The aim of this manuscript was to identify the DO, NH_4^+ and free ammonia concentration
60 thresholds that originated the lowest N_2O emissions in a nitrification reactor. The novel
61 control strategy applied in this reactor and described in Bartrolí et al. (2010) allowed the
62 flexibility of operating at a desired DO set-point without compromising the effectiveness
63 of the system. The control strategy also allowed to operate at full (100% conversion of
64 NH_4^+ to NO_2^-) or partial nitrification (50% conversion of NH_4^+ to NO_2^-) depending on the
65 subsequent denitrification step: either heterotrophic or autotrophic (anammox),
66 respectively. A comparison in terms of treatment performance and N_2O emissions
67 between continuous and discontinuous operation mode for the same reactor is also
68 presented and highlights the importance of considering greenhouse gas emissions when
69 implementing a technology. Finally, an economic and carbon footprint analysis of

70 applying a N₂O mitigation strategy was conducted for the pilot plant and extrapolated to
71 real facilities.

72

73 **2. Materials and methods**

74 **2.1. Pilot plant**

75 2.1.1. Continuous operation

76 The pilot plant consisted in a 150L granular airlift reactor with a height to diameter ratio
77 of 8.4. It was located in a municipal WWTP in Catalonia, Spain, and it was performing
78 full partial nitrification of reject wastewater produced in situ during the dewatering
79 process of the anaerobic digester sludge from the WWTP. The temperature of the reactor
80 was kept at 30°C by using an electric heating system and a temperature controller. The
81 pH was maintained at 7.5±0.2 through the addition of solid Na₂CO₃. DO concentration
82 was monitored with an online DO probe (LDO luminescence sensor, Hach-Lange,
83 Düsseldorf, Germany) and was maintained around the desired set-point (see table 1) by
84 changing the aeration flow-rate (from 11 to 100 L/min). The total ammonium nitrogen
85 (TAN=NH₄⁺-N/L+NH₃-N/L) concentration in the bulk liquid was monitored with an
86 online probe (NH₄D sc probe with a Cartrical cartridge, Hach Lange, Düsseldorf,
87 Germany).

88 The reactor was operated with a variation of the control strategy presented in Bartrolí et
89 al. (2010) during the period of monitoring. The variable measured for the control loop
90 was the TAN concentration whereas the manipulated variable was the wastewater inflow
91 rate fed to the reactor. The feedback control loop maintaining the TAN concentration in
92 the bulk liquid allows for a maximization of the treatment capacity at any time, because

93 the loading rate is as high as possible during the continuous operation of the reactor. The
94 influent wastewater was added in an on/off mode controlled by the concentration of NH_4^+
95 present in the bulk liquid. When the NH_4^+ concentration was lower than the set-point (40
96 mg NH_4^+ -N/L), the feeding pump was activated, until the NH_4^+ concentration was again
97 at the set-point value. With this strategy, NH_4^+ concentration was always kept between
98 the desired set-point ± 5 mg N/L and NO_2^- concentration depended on the concentration
99 of NH_4^+ in the influent, but always with an NH_4^+ to NO_2^- conversion higher than 92%,
100 except for the period where partial nitrification was applied (see figure 1). Nitrate (NO_3^-)
101 was hardly detected in the reactor at all times, presenting concentrations around 1-2 mg
102 N/L in the bulk liquid (see figure 1). The system was controlled and monitored through a
103 SCADA (supervisory control and data acquisition) program. For the control system, the
104 manipulated variable was the inflow rate of the reject water. DO concentration was
105 manipulated by changing the air flow-rate through the opening of the pneumatic valve
106 that could be regulated continuously (i.e. via a frequency modulated solenoid valve). The
107 air flow-rate was kept constant during each monitoring period to decrease the impact of
108 total aeration flow-rate on the estimation of N_2O and CH_4 emissions. The DO varied
109 during a monitoring period within a very small range (i.e. ± 0.1 of the desired set-point).
110 At the time of the study, the reactor had been working for more than 100 days under
111 stable operation (see figure 1) and had a nitrogen loading rate (NLR) of 0.85 g N/Ld, a
112 biomass concentration of 5 g MLVSS/L, and a mean granule size of 0.5 mm (Torà et al.,
113 2013). The hydraulic retention time (HRT) was maintained within the range of 0.4-0.6 d
114 and the sludge residence time (SRT) was kept at 50 d.

115 2.1.2. Sequencing Batch Reactor operation

116 Towards the end of the study, the reactor was switched from continuous to sequencing
117 batch reactor mode (SBR) for a period of 2 weeks. The reactor volume was decreased,
118 resulting in 100 L after the feeding phase. The cycle time consisted in: 6 min feeding,
119 where 50L of wastewater was fed into the reactor; an aerobic phase, with the length being
120 controlled by the control strategy explained above and based on the TAN concentration; a
121 settling phase which varied between 7 and 30 min depending on the cycle; and 2 min
122 decanting phase where 50L of treated wastewater was discharged. The aeration was kept
123 constant at 100 L/min during the reaction phase which provided a DO concentration
124 range between 5.7 and 7.2 in all cycles tested. Solid Na_2CO_3 was added when the pH
125 reached values lower than 7.5 in the reactor bulk liquid. HRT and SRT were maintained
126 in the same range than in the continuous operation.

127 **2.2. Wastewater characteristics**

128 The reject water produced in the WWTP where the pilot plant was located was stored into
129 two tanks of 1000L at room temperature, connected alternatively to the reactor inflow
130 pump. During the study under continuous operation the composition of the reject water
131 was within the following concentrations: TAN 726 ± 50 mg N/L, total organic carbon
132 (TOC) 240 – 696 mg C/L, total inorganic carbon (TIC) 358 – 723 mg C/L, total nitrite
133 nitrogen (TNN= NO_2^- -N + HNO_2 -N) 2 – 7 mg N/L, NO_3^- 0 mg N/L, MLSS 122 – 239
134 mg/L, MLVSS 100 – 206 mg/L; pH 8.1 – 8.8. The percentage of biodegradable organic
135 matter in the reject water was determined as only 5 ± 3 % of the total TOC following the
136 methodology described in Suárez-Ojeda et al. (2007). Due to some changes in the
137 operation of the WWTP anaerobic digester, the concentration of TAN decreased in the
138 reject wastewater when the reactor operated in SBR mode resulting in 450 ± 78 mg N/L.

139 **2.3. Nitrous oxide and methane monitoring**

140 Off gas was collected continuously (at 0.5L/min) from the reactor headspace which was
141 covered with a plastic bag and connected via a gas tube to a gas conditioning unit (series
142 CSS, M&C Tech group). The gas outlet from the conditioning unit was connected to the
143 multicomponent online gas analyser (VA-3000, Horiba, Japan) which provided an online
144 measurement of N₂O and CH₄ concentrations from the gas flow. Data were logged every
145 15 seconds for a period of 3-4 h for each of the monitoring tests.

146 **2.3.1. Monitoring during continuous operation**

147 Twenty-four monitoring tests (T1-T24) were conducted to assess the N₂O and CH₄
148 emission dynamics from the reactor under different DO concentrations in the range of 1.1
149 to 7.7 mg O₂/L. The different DO concentrations are summarised in table 1 and were
150 achieved by varying the air flow-rate from 11 to 100 L/min. The reactor operated under
151 the conditions described for each of the tests (table 1) 24 h previous to the monitoring.

152 To explore the effect of NH₄⁺, NO₂⁻, free ammonia (FA) and free nitrous acid (FNA)
153 concentration on N₂O emission, six of these tests (T11-T16) were conducted under the
154 same DO concentration (3.2-3.3 mg/L) but with different NH₄⁺ concentration and pH,
155 providing different FA, NO₂⁻ and FNA concentrations (table 2).

156 **2.3.2. Monitoring during SBR operation**

157 Five cycles (TC1-TC5) were monitored when the reactor was operated in SBR mode. The
158 conditions of each cycle monitored are described in table 3. These conditions were
159 applied in the SBR at least 24 h before the monitoring was conducted.

160 **2.4. Calculations**

161 The total N₂O and CH₄ emitted were calculated using the following equations:

162
$$N_2O \text{ emitted} = \sum (C_{N_2O-N_{gas}} \times Q_{gas} \times \Delta t) \quad (1)$$

163
$$CH_4 \text{ emitted} = \sum (C_{CH_4_{gas}} \times Q_{gas} \times \Delta t) \quad (2)$$

164 Where

165 C_{N_2O} (g N₂O-N/L) = C_{N₂O} (ppmv)*10⁻⁶ * molar gas volume⁻¹ (0.0414 mol/L at 25°C and
166 1atm)*28.

167 C_{CH_4} (g CH₄/L) = C_{CH₄} (ppmv)*10⁻⁶ * molar gas volume⁻¹ (0.0414 at 25°C and 1atm)*16.

168 Q_{gas}= the gas flow rate of the aeration (L/min).

169 Δt= time interval by which the off-gas N₂O concentration was recorded.

170 The emission factor for N₂O was calculated based on the total amount of N₂O emitted in
171 a particular time (equation 1) relative to the total NH₄⁺ converted to NO₂⁻ in that time (mg
172 N₂O-N/mg NH₄⁺-N). This way of calculating the emission factor is very important to
173 compare the emission factors when the reactor is oxidizing only a certain fraction of the
174 ammonium load (e.g. either full or partial nitrification).

175 To directly compare the emission factors reported in the literature for single-stage N
176 removal systems (nitrification and anammox in 1 single reactor) with the emissions
177 reported in this study and in other nitrification systems (Table 5), the following procedure
178 was applied: (i) the values reported for single-stage N-removal systems (normally given
179 in %N₂O/N-load) were used to calculate emission factor as percent of N removed; (ii) a
180 50% of the N removed was considered to be oxidized to NO₂⁻ (roughly following the
181 anammox stoichiometry); (iii) all the N₂O emissions reported in these systems were
182 assumed to be produced during the nitrification process as anammox bacteria do not
183 produce N₂O (Kartal et al., 2010). This procedure could be summarized with the
184 following equation:

185 $\%N_2O$ emitted (per NH_4^+ oxidized to NO_2^-) = 2* ($\%N_2O$ emitted per N removed) (3)

186 **2.5. Economical assessment of N_2O mitigation**

187 To conduct an economic and carbon footprint analysis of implementing a N_2O mitigation
188 strategy we considered a WWTP of ca. 140000 p.e. and dimensioned a nitrification reactor
189 to treat the reject water coming from the dewatering process of the anaerobic digester
190 sludge (reactor volume of ca. $100m^3$ treating ca. 160 kg N/d). Two different scenarios
191 were taken into consideration: (i) low DO concentration (1.5 mg O_2/L) and (ii) high DO
192 concentration (4.5 mg O_2/L). Aeration flow rates were estimated for both scenarios
193 scaling up the values required for the pilot reactor (i.e. 1400 and 2000 m^3/d respectively).
194 For convenience, aeration efficiency was assumed to be equivalent to that in the pilot
195 reactor, and the aeration flow rates were scaled up as proportional to reactor volume. The
196 same N_2O emission factors determined with the pilot installation for each one of the
197 scenarios (~ 6% and 2% respectively) were assumed for the full scale installation. To
198 estimate the energy consumption associated to aeration, a pressure difference of 1.2 bar
199 was assumed, accounting for both the reactor height and pressure drop (i.e. effective
200 height of water column of 12 m). The energy requirements of a displacement screw
201 blower at each one of the air flow rates (1400 and 2000 m^3/d) were estimated as 47 and
202 71 kWh for each scenario, respectively. To this end, the indications of the manufacturer
203 on efficiency of the equipment related to the particular compressed flow were followed
204 although it has to be taken into account that these efficiencies often incorporate an over
205 estimation.

206 To estimate the carbon footprint, the following equivalences were assumed: 1 kg of N_2O
207 = 265 CO_2 equivalents (IPCC 2013) and 1kWh = 0.544 CO_2 equivalents (UKWIR 2008).

208 For the economical assessment with the carbon taxes, the two scenarios chosen (low and
209 high carbon tax) were based on the values expected to be implemented in Europe (SBS,
210 2012).

211 **2.6. Chemical and microbial analysis**

212 TAN was analyzed using a continuous flow analyzer based on potentiometric
213 determination of ammonia. TNN and NO_3^- were measured with ionic chromatography
214 using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler
215 AS40. TIC and TOC were measured with an OI Analytical TOC Analyzer (Model
216 1020A) equipped with a non-dispersive infrared (NDIR). Mixed liquor suspended solids
217 (MLSS), and volatile MLSS (MLVSS) were determined according to standard methods
218 (APHA, 1995). A Malvern Mastersizer 2000 instrument was used to measure the granule
219 size and size distribution. N_2O and CH_4 analysis were performed by a commercial
220 infrared analyzer (VA-3000, Horiba, Japan). Fluorescence in situ hybridization (FISH)
221 was performed to quantify the amount of AOB and NOB microorganisms present in the
222 reactor. Full details about the procedure can be found in the supplementary information
223 section.

224

225 **3. Results**

226 **3.1. Nitrogen transformations in the granular airlift reactor**

227 At the time of the study, the granular airlift reactor had been operating in continuous
228 mode for more than 100 days, achieving partial nitrification from reject wastewater as
229 shown in figure 1. More details about the reactor start-up and stabilization can be found
230 in Torà et al. (2013). At the time of the experiments, the mean size of the granular sludge

231 was 0.5 mm and the microbial composition consisted of $70\pm 10\%$ AOB and $<1\%$ NOB.
232 The strong oxygen limiting conditions even at high DO concentrations were assured due
233 to the great excess of NH_4^+ in the bulk liquid (Bartrolí et al., 2010). NO_3^- was always at
234 very low values (< 1.5 mg N/L) during the period of the study. The pilot plant operated at
235 full nitrification conditions for the majority of the monitoring period. However, the control
236 system applied also allowed its operation under partial nitrification conditions as shown in
237 figure 1 (days 255-280).

238 **3.2. Emission dynamics of N_2O and CH_4 during continuous operation**

239 An example of the N_2O and CH_4 emission dynamics from the reactor under stable
240 operation is represented in figure 2. N_2O levels in the off-gas oscillated within 40 and 85
241 ppmv when the reactor was operated with an aeration flow of 50 L/min, increasing the
242 concentration a few minutes after each addition of wastewater (Figure 2). The minor
243 oscillations produced by the on-off action of the NH_4^+ concentration control loop resulted
244 in slight NH_4^+ and NO_2^- concentration disturbances which seem to have an effect on the
245 N_2O emissions (Figure 2A&B). Taking into account these emissions and the NH_4^+
246 transformed to NO_2^- , the N_2O emission factor during this particular period of monitoring
247 was 2.4% $\text{N}_2\text{O-N/oxidized-N}$.

248 Regarding CH_4 , the emissions detected in our system came from the stripping of the
249 soluble CH_4 that remained dissolved in the reject wastewater after anaerobic digestion
250 and were not produced in the reactor. These emissions are clearly uncoupled from the
251 N_2O emissions (see Figure 2B), showing that the N_2O variation was due to the nitrification
252 process, and was not linked to either stripping or other physical processes that might be
253 occurring in the reactor.

254 **3.3. Effect of DO concentration on N₂O emission**

255 The effect of different DO concentrations on N₂O emissions was tested by changing the
256 aeration flow rate in the pilot plant. To compare the emissions under different DO
257 concentrations, the N₂O emission factor was calculated as described in Materials and
258 Methods section. Figure 3 presents the N₂O emission factor dependency on DO
259 concentration.

260 The lowest N₂O emission factor was measured at DO concentrations of 4.5 mg O₂/L or
261 higher (Figure 3, region b). At this range, 2.2 ± 0.4 % of the NH₄⁺ nitrified was emitted as
262 N₂O and was not dependent on the DO concentration. However, when reducing the DO
263 concentration to levels lower than 4.5 mg O₂/L, the N₂O emission factor increased,
264 reaching values around 6% of the NH₄⁺ nitrified being emitted as N₂O (Figure 3, region
265 a). Operating the reactor under partial or full nitrification conditions did not have an effect
266 on N₂O emissions. Emissions detected under partial nitrification conditions matched the
267 same profile as the emissions found when operating under full nitrification within the
268 concentration DO range of 1.6-5.3 mg O₂/L (highlighted in figure 3 with empty circles
269 and triangles).

270 Stripping conditions changed among the experiments, since changes on DO concentration
271 in the reactor could only be achieved by changing the aeration flow rate (table 1). It can
272 not be excluded that changes in stripping conditions might also have a direct effect on the
273 N₂O emissions detected but changes in DO concentration seem to be the main driver to
274 changes on N₂O as shown by the strong correlation depicted in figure 3 and the bigger
275 dispersion observed when depicting the correlation between N₂O emission factor and air
276 flow rate (figure SI.1)

277 **3.4. Effect of ammonium, free ammonia, nitrite and free nitrous acid on N₂O**
278 **emission.**

279 The effect of operating the pilot plant at different NH₄⁺ and FA concentrations on N₂O
280 production was tested by varying the NH₄⁺ concentration set-point in the control loop and
281 the pH (Table 2). FNA concentration was calculated considering the amount of NO₂⁻
282 present in the reactor and the pH and was also related to N₂O. Results are presented in
283 figure 4.

284 The pilot plant operated under the same DO concentration (3.2-3.3 mg O₂/L) when these
285 experiments were carried out to exclude the effect of DO on N₂O emissions. The N₂O
286 emission factor remained constant at 4.4±0.3% of the NH₄⁺ oxidized emitted as N₂O
287 despite of the wide range of NH₄⁺, FA, NO₂⁻ and FNA concentrations tested. Therefore it
288 can be concluded that within the concentration range tested and with a DO concentration
289 of 3.3 mg O₂/L, changes on NH₄⁺, FA, NO₂⁻ and FNA did not have an effect on N₂O
290 production. Interestingly, the N₂O emission factor was independent of the performance of
291 the granular reactor for achieving full nitrification (100% conversion of NH₄⁺ to NO₂⁻) or
292 partial nitrification (50-75% conversion of NH₄⁺ to NO₂⁻).

293 **3.5. Emission dynamics of N₂O and CH₄ during SBR operation**

294 The pilot plant was shifted from continuous to SBR operation mode to study if this
295 operation had an effect on the overall nitrogen transformations in the plant. For a period
296 of two weeks, five different cycle studies (Table 3) were monitored and one of them is
297 presented in figure 5.

298 During SBR operation an aeration flow rate of 100L/min was applied. DO concentration
299 followed the same pattern in all the cycles: a slow increase during the reaction phase

300 (from 5.6 till 7.2 mg O₂/L approximately), a sharp decrease as soon as aeration stopped
301 during the settling phase (from 7.2 till 0.5 mg O₂/L in the cycle with the longest settling
302 time tested, 30 min) and a sharp increase as soon as aeration started during the feeding
303 (reaching 5.2-5.5 mg O₂/L in the first minute of aeration). pH increased at the beginning
304 of the cycle to values close to 8 due to the alkalinity provided by the wastewater. After
305 that, and due to the nitrification reaction, pH decreased until 7.5, when pH control started.
306 The cycle length was controlled by the NH₄⁺ set-point applied in the control loop. As
307 soon as the concentration reached values lower than 40 mg N/L, aeration stopped and
308 settling started. NO₂⁻ concentrations in the bulk liquid were comprised between 190
309 (beginning of the cycle) and 370 mg N/L (end of the aeration phase) at all times during
310 SBR operation. These concentrations were lower than those measured in the bulk liquor
311 during continuous monitoring. This was due to the lower NH₄⁺ concentration present in
312 the reject wastewater when the reactor operated in SBR mode.

313 Peaks of CH₄ and N₂O were detected at the beginning of the cycle as soon as aeration
314 started. The CH₄ peak corresponded to the stripping of the soluble CH₄ present in the
315 reject wastewater. Indeed, this peak was 10 times higher than the peaks detected under
316 continuous operation but this was because 10 times more wastewater was added as a
317 pulse under SBR operation. No difference was found when considering the total amount
318 of CH₄ emitted per wastewater treated (Figure SI.2). The N₂O profile also displayed a
319 peak at the beginning of the aeration phase reaching concentrations of 1000 ppmv during
320 the first 10 min of the cycle. After that the N₂O concentration decreased until values
321 around 100 ppmv towards the end of the aerobic phase. This concentration from the end
322 of aeration was very similar to the concentrations measured during the monitoring

323 conducted under continuous operation at similar operational conditions (Tests T18, T22,
324 T24 performed at 100L/min of aeration flow rate; 4.4-6.7 mg O₂/L; 7.5-8 pH). However,
325 when considering the total N₂O emitted per N oxidized, the emission factor obtained
326 during SBR operation was 19.3 ± 7.5 %, one order of magnitude higher than the 2.2 ± 0.4
327 % found under continuous operation at the same DO concentration range. The application
328 of different settling times during SBR operation mode did not show an apparent
329 correlation with the N₂O emitted during the cycle (Figure SI.3).

330 **3.6. Economical assessment of N₂O mitigation**

331 From the results presented, operating the reactor in a continuous mode at high aeration
332 rates (DO concentration > 4.5 mg O₂/L) can be postulated as an effective approach to
333 mitigate N₂O emissions. However, from the practical point of view, this can be seen as an
334 expensive operational strategy, since it involves higher electricity consumption. In fact
335 the main operating costs of such an installation have been related to the electricity used to
336 aerate the reactor (Carrera et al., 2010). If lowering greenhouse gas emissions during
337 wastewater treatment is not associated with an economical incentive it will be difficult for
338 the industry to implement mitigation strategies that imply increasing operational costs.
339 However, many governments are starting to implement a price tag or carbon tax on
340 pollution to discourage industry from emitting greenhouse gases in an attempt to control
341 global warming. In this sense, a more elaborated assessment has been performed taking
342 into account three different scenarios: (i) without carbon tax; (ii) applying a low carbon
343 tax; (iii) applying a high carbon tax (Table 4). To conduct such analysis the obtained N₂O
344 emission factors have been applied to a scaled up theoretical nitrification system treating
345 the reject wastewater from a 140,000 p.e. WWTP. It has to be taken into account that

346 theoretical calculations incorporate some assumptions such as same stripping efficiency
347 for the pilot plant as for the full-scale theoretical installation, which might differ from the
348 reality.

349 In continuous mode, operating at a higher DO to mitigate N₂O emissions resulted in a
350 43% lower carbon footprint. Remarkably, at low DO concentration N₂O emissions were
351 estimated to account for 81% of the total carbon footprint. The analysis shows that
352 operating with the lowest carbon emission will only be economically feasible in the high
353 carbon tax scenario.

354 A similar analysis was conducted with the emission factors found for the SBR operation.
355 For convenience, nitrogen loading rate and aeration needs were considered equivalent to
356 those determined for the continuous mode of operation. The carbon footprint increased
357 almost three times even when comparing with the low DO scenario in the continuous
358 mode. In case of application of carbon taxes, the SBR technology would not be a good
359 choice for the nitrification of reject wastewater in view of the costs analysis.

360

361 **4. Discussion**

362 **4.1. Possible N₂O production pathways affecting N₂O emissions under different DO** 363 **concentrations**

364 DO is considered an important parameter affecting N₂O emissions, with lower DO
365 concentrations increasing N₂O emissions (Kampschreur et al., 2009a). However, it is still
366 unclear if a DO concentration threshold to minimise N₂O emissions can be established for
367 nitrifying systems since different N₂O emission factors have been reported at different
368 DO concentrations (Table 5).

369 It is accepted that two different N₂O production pathways exist during nitrification: i) the
370 nitrifier denitrification pathway and ii) the hydroxylamine oxidation pathway. Which one
371 of these pathways is the main responsible for the N₂O production in AOB is still unclear.
372 Sutka et al. (2006) used stable nitrogen isotopes to conclude that the NH₂OH oxidation
373 pathway contributed to N₂O production mainly at high DO concentrations whereas the
374 nitrifier denitrification pathway was more active at low DO concentrations. Recently,
375 Wunderlin et al. (2013) carried out a series of batch tests where the N₂O production
376 pathways were identified using site-specific isotope composition of N₂O in real time. In
377 their nitrification tests, there was always a combination of the two pathways that
378 produced the N₂O detected, except for the cases where only NO₂⁻ was added, in which
379 only the nitrifier denitrification pathway was active. Our results suggest the presence of at
380 least two different predominant pathways for N₂O production: one linked to the DO
381 concentration, probably the nitrifier denitrification pathway (as can be observed in region
382 “a” from Figure 3) and another one that would not depend on it (as can be observed in
383 region “b” from Figure 3). The fact that N₂O dependency on DO concentrations starts at a
384 DO concentration relatively high (4 mg/L), could be due to the pilot plant operating with
385 aerobic granular sludge. In aerobic granules, DO is consumed very fast by the
386 microorganisms present in the outer layers of the granules (Pijuan et al., 2009), thus
387 creating micro-aerobic or anoxic conditions in the inside of the granules which could
388 favour the denitrification pathway in AOBs resulting in N₂O formation, even at relatively
389 high DO concentrations in the bulk liquid.

390 It can not be excluded either the possibility that a fraction of the N₂O detected is
391 produced by chemical reactions. High ammonia oxidation rates may lead to high

392 concentrations of NH_2OH which has been demonstrated to react with NO_2^- or FNA to
393 form N_2O . As suggested by Schreiber et al. (2012) chemical N_2O production in systems
394 dealing with high-strength N wastewater could be important. Our results show how N_2O
395 emissions can be reduced by increasing the DO concentration in the bulk liquid, but this
396 reduction has a minimum value (the baseline in region “b”, as defined in Figure 3), that
397 seems to be unalterable when DO concentration is further increased.

398 Interestingly our results also show that operating the reactor under different NO_2^-
399 concentrations (from 368 till 740 mg N/L) or different FNA concentrations (from 0.006
400 till 0.065) did not affect the N_2O emissions. This could be due to the fact that the AOB of
401 this study were adapted to high concentrations of NO_2^- (500-750 mg N/L). Previous
402 studies have reported an increase on N_2O emissions when increasing NO_2^- concentrations
403 in a pure culture of *Nitrosomonas europaea* (Anderson et al., 1993) and several mixed
404 nitrifying systems (Kampchreur et al., 2008b; Tallec et al., 2006). These differences
405 could be related to the fact that different AOB strains possess different adaptation
406 strategies to high NO_2^- environments and therefore it is possible that the same NO_2^-
407 concentration triggers different N_2O production depending on the adaptation of AOB at
408 that particular environment.

409 **4.2. Continuous versus discontinuous operation**

410 One of the key differences between continuous and SBR operation mode is the presence
411 of a settling phase in the last one. During settling, aeration stops, and DO sharply
412 decreases (Figure 5), reaching DO levels < 1 mg O_2/L , which could be even lower at the
413 bottom of the reactor where all the biomass concentrates. These conditions might trigger
414 N_2O formation, which would be stripped from the bulk liquid in the subsequent aeration

415 phase, originating the high N₂O peak detected. Several studies have inferred in the effect
416 that periods of anoxia can have on N₂O production from nitrifying cultures. Kampschreur
417 et al. (2008b) reported the effect of oxygen limitation during NH₄⁺ oxidation in a
418 nitrifying lab-scale system. They observed an immediate N₂O increase when air was
419 replaced by nitrogen gas attributed to the activation of the denitrification pathway in
420 AOBs. On the other hand, Yu et al. (2010) only observed N₂O production in a pure
421 culture of *Nitrosomonas europaea* during the recovery from a 48h period of anoxia, as
422 soon as aeration started. Recently, Rodriguez-Caballero & Pijuan (2013) demonstrated
423 that the majority (60-80%) of the N₂O emitted from a nitritation lab-scale system treating
424 reject wastewater originated during settling and depended on the presence of NH₄⁺ and
425 NO₂⁻.

426 Another explanation for the higher N₂O production when operating in SBR mode could
427 be the sudden variations on NH₄⁺ and NO₂⁻ concentrations experienced during the cycle,
428 more pronounced than in continuous operation. Dynamic process conditions can enhance
429 N₂O production. Kampschreur et al. (2008b) studied the effect of dynamic process
430 conditions on nitrogen oxides in a nitrifying culture. They subject the culture to a
431 stepwise increase on NO₂⁻ concentrations which gave an increase on N₂O production.
432 Recently, Law et al. (2013) reported a relationship between the specific N₂O production
433 rate and a gradual NO₂⁻ accumulation in a partial nitritation culture treating synthetic
434 reject wastewater. Interestingly, NO₂⁻ had a suppressive effect on N₂O production when
435 increasing the concentration from 50 to 500 mg N/L. At higher NO₂⁻ concentrations, N₂O
436 production remained constant. It is clear that more research on a fundamental level is
437 needed to clarify these hypotheses.

438 At this stage, the use of SBR technology for nitrification of reject wastewater treatment
439 needs further evaluation due to the higher N₂O emissions it presents compared with
440 continuous operation.

441 **4.3. Impact of these results in the selection of single- or two-stage N removal**

442 Currently, the treatment of high and low-strength NH₄⁺ wastewater can be carried out by
443 single- or two-stage autotrophic N removal systems. The choice of one or another may
444 depend on several factors such as: reactor volume, loading rate, process stability,
445 economical issues, etc. (van Hulle et al., 2010; Jaroszynski et al., 2011). Another factor
446 that is increasingly gaining attention is the N₂O emission from these systems which could
447 have a big impact on the overall carbon footprint of the plant. Currently, N₂O emission
448 values from single stage systems are still scarce and rather variable (see table 5). An N₂O
449 emission factor of 1.67% of the N removed was reported in a full-scale single stage
450 nitrification-anammox reactor located in the Netherlands which corresponds to 2.5% of the
451 N oxidized to nitrite (Table 5, Kampschreur et al., 2009b). In this reactor, nitrification and
452 autotrophic denitrification was occurring in the same tank. Another monitoring performed
453 recently in the same plant reported an emission factor of 2.1% of the N removed which
454 corresponds to 4.0% of the N converted to nitrite (Castro-Barros et al., 2013).
455 Interestingly, they detected a peak on N₂O emissions when NO₂⁻ accumulated in the
456 reactor, during periods of low anammox activity, highlighting the need for efficient
457 process control to avoid a sudden increase on N₂O emissions in single stage nitrification-
458 anammox systems.

459 On the other hand, emissions from partial nitrification systems from lab and full-scale
460 reactors have been also reported in the literature (Table 5). In these cases, emissions vary

461 from 0.8 to 11.2 % of the N oxidized being emitted as N₂O. Our findings indicate that
462 changes in the NH₄⁺, NO₂⁻, FNA or FA concentrations have no impact on N₂O emissions
463 during nitrification within the common operational ranges. Therefore, emissions detected in
464 two stage nitrification-anammox systems are expected to be similar to those found in one-
465 stage nitrification-anammox reactors for the treatment of reject wastewater. A possible
466 advantage for the two-stage systems is that changes in the concentration of soluble N
467 compounds would not have a big effect on the N₂O emissions probably due to the
468 adaptation of the biomass at these N concentrations while the current data seems to
469 suggest that small accumulation of NO₂⁻ in single nitrification-anammox reactors would lead
470 to an increased emission factor (Kampschreur et al., 2009b). More full-scale N₂O
471 monitoring campaigns are needed for systems treating high strength N wastewater to
472 clarify the treatment technology that provides lower emissions.

473 Single-stage N-removal systems operating at low temperatures and at low nitrogen
474 loading rates have been demonstrated as feasible, but several challenges may well
475 difficult the final implementation. These challenges include mainly the outcompetition of
476 anammox by NOB and conventional heterotrophic denitrifiers (Winkler et al., 2012).
477 These limitations would not be present in a two-stage N-removal system, and the
478 operation of such a system in continuous mode using a nitrification step similar to the one
479 presented here, will only increase slightly the N₂O emissions if adequate DO
480 concentration is maintained in the bulk liquid. This strategy may be an alternative to be
481 considered since nitrification has been successfully tested at low temperatures (Jemaat et
482 al., 2013).

483

484 **5. Conclusions**

485 Nitrous oxide emissions were monitored in a nitrification airlift reactor treating reject
486 wastewater. The main findings of this study are listed as follow:

- 487 • DO concentration can be used as a control parameter to minimise N₂O emissions.
488 Increasing the DO to 4.5 mg O₂/L resulted in a decrease on N₂O emissions from 6
489 to 2.2%. However, a further increase on DO did not result in an additional
490 reduction, suggesting the involvement of two different mechanisms responsible
491 for N₂O production.
- 492 • Continuous operation is preferred to SBR for partial nitrification systems. SBR
493 operation resulted in a substantial increase on N₂O emissions when compared to
494 those obtained in continuous mode.
- 495 • N₂O emissions would dominate the total carbon footprint in a hypothetical scale-
496 up of the reactor studied. Operating at minimal N₂O emission would only be
497 economically feasible if a carbon tax on emissions is implemented.

498

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508

509 **Supporting information available**

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635 **LIST OF FIGURES**

636 **Figure 1.** Nitrogen transformations in the granular airlift reactor before and during the
637 GHG monitoring period. ● NH_4^+ in the reactor; □ NO_2^- in the reactor; ▽ NO_3^- in the
638 reactor; ▲ NH_4^+ in the wastewater.

639 **Figure 2. A-** On-line N_2O and CH_4 emissions from the reactor during continuous
640 operation at DO 4.7 mg O_2/L and pH 7.5. N_2O (Black line); CH_4 (grey line); influent flow
641 (thin black line); aeration flow (dashed line); NH_4^+ (●); NO_3^- (▽); NO_2^- (□). **B-** Zoom in
642 from figure A (135-185 min).

643 **Figure 3.** Correlation between the N_2O emission factor and the DO concentration in the
644 reactor: ● Operation under full nitrification conditions (>90% NH_4^+ oxidation to NO_2^-); ○
645 Operation under partial nitrification conditions (70%-75% NH_4^+ oxidation to NO_2^-); Δ
646 Operation under partial nitrification conditions (50%-55% NH_4^+ oxidation to NO_2^-).

647 **Figure 4.** Correlation between the N_2O emission factor and ammonium /nitrite (A), free
648 ammonia (B) and free nitrous acid (C) concentrations. ●- Ammonium, FA & FNA; □-
649 Nitrite.

650 **Figure 5.** Cycle study profile of the pilot plant operating in SBR mode with a settling
651 time of 7 min and an aeration flow of 100 L/min. A- pH (grey line), DO (black line),
652 ammonium (discontinuous line) and nitrite (□) concentrations; B- N_2O (black line) and
653 CH_4 (grey line) emission profiles; aeration flow (discontinuous line).

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658 **TABLES**

659 **Table 1.** Experimental tests conducted under continuous operation at different dissolved
 660 oxygen concentrations.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
DO (mg O ₂ /L)	1.1	1.2	1.5	1.6	1.6	1.7	2.0	2.4	2.5	2.6	3.2	3.2
Air flow (L/min)	13	11	14	37	11	11	13	50	11	24	13	18
pH	7.5	7.6	7.7	7.7	7.6	8.1	7.6	7.5	7.6	7.6	7.6	7.7
	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
DO (mg O ₂ /L)	3.2	3.2	3.3	3.3	4.1	4.4	4.6	4.7	4.8	5.3	6.7	7.5
Air flow (L/min)	16	15	100	27	50	100	50	50	65	100	100	50
pH	8.1	7.6	8	7.7	8.4	7.7	7.6	7.5	7.6	8.0	7.5	7.2

661

662 **Table 2.** Experimental tests conducted under continuous operation at different NH₄⁺, FA
 663 and FNA concentrations.

	T11	T12	T13	T14	T15	T16
NH ₄ ⁺ (mg N/L)	37.5	158	319	5.3	192	58.6
NO ₂ ⁻ (mg/L)	629.1	519.4	368.4	663.5	554.9	740.0
FA (mg N/L)	1.2	2.0	29.4	0.2	14.3	2.3
FNA (mg N/L)	0.031	0.065	0.006	0.033	0.011	0.029
pH	7.6	7.2	8.1	7.6	8	7.7
DO (mg O ₂ /L)	3.2	3.2	3.2	3.2	3.3	3.3

664 FA and FNA concentrations were calculated according to Anthonissen et al. (1976).

665

666

667 **Table 3.** Experimental tests conducted under SBR operation.

	TC1	TC2	TC3	TC4	TC5
DO (mg O ₂ /L)	5.7-7.2	6.7-7.0	6.15-6.40	5.6-7.2	5.6-6.6
Air flow (L/min)	100	100	100	100	100
pH	8.4-7.4	8.4-7.6	8.5-7.6	8.2-7.4	8.4-7.4
Settling time (min)	7	15	15	23	30

668

669 **Table 4.** Annual carbon footprint and cost analysis calculated with and without carbon
 670 taxes, considering two different DO scenarios in an installation treating the reject water
 671 of a WWTP of 140,000 p.e. Low DO: 1.5 mg O₂/L; high DO: 4.5 mg O₂/L

Data	Low DO (Continuous)	High DO		Units
		Continuous	SBR	
Annual energy requirements for aeration	408,303	625,421		kWh year ⁻¹
Annual N ₂ O emissions	3.5	1.2	11.1	Tn N ₂ O year ⁻¹
Equivalent CO ₂ emissions for aeration	222	340		Tn CO ₂ eq year ⁻¹
Annual N ₂ O emissions (CO ₂ eq.)	927	310	2941	Tn CO ₂ eq year ⁻¹
Annual carbon footprint	1,149	650	3282	Tn CO₂ eq year⁻¹
Annual cost associated to energy requirements for aeration	34.7	53.2		k€ year ⁻¹
Annual cost associated to CO ₂ emissions (Low carbon tax ^a)	4.6	2.6	13.1	k€ year ⁻¹
Annual cost associated to CO ₂ emissions (High carbon tax ^b)	34.5	19.5	98.5	k€ year ⁻¹
Total annual costs (without carbon tax)	34.7	53.2		k€ year⁻¹
Total annual costs (low carbon tax)	39.3	55.8	66.3	k€ year⁻¹
Total annual costs (high carbon tax)	69.2	72.7	151.6	k€ year⁻¹

672 Factors used for calculations: 0.085 euro / kWh; 0.544 kg CO₂ eq / kWh; 265 kg CO₂ eq / kg
 673 N₂O. ^aLow carbon tax: 0.004 euro / kg CO₂; ^bHigh carbon tax: 0.03 euro / kg CO₂. For details of
 674 each scenario, associated calculations and references see section 2.5 of the Materials & Methods.

675

676 **Table 5.** Emission factors reported in the literature and in this study for the treatment of
 677 high strength nitrogen wastewater.

Wastewater (reference)	Process type	DO concentration (mg O ₂ /L)	Emission factor (%N-N ₂ O/N-oxidized)
Anaerobically digested industrial WW (Desloover 2011)	Partial nitrification+anammox (2-stage). Full-scale. (Floccular sludge)	0.4-1.0	8.1-11.2* ^a *emissions from nitrification reactor
Concentrated black water (de Graaff 2010)	Partial nitrification in continuous reactor. Lab-scale. (Floccular sludge)	4.1-4.2	3.2 ^a
Reject WW (Joss 2009)	Partial nitrification+anammox (1-stage). SBR full-scale. (type of sludge not described)	<0.5	0.8 ^a
Reject WW (Kampschreur 2008a)	Partial nitrification + anammox (2-stage). Full-scale. (Floccular sludge)	2.5	3.4* *emissions from nitrification reactor
Reject WW (Kampschreur 2009b)	Partial nitrification+ anammox (1-stage). Full-scale. (Granular sludge)	5	2.5 ^{a,b}
Reject WW (Castro-Barros 2013)	Partial nitrification+anammox (1-stage). Full-scale. (Granular sludge)	N.R.	4.0 ^{a,b}
Reject WW (Law 2011)	Partial nitrification Lab-scale SBR. (Floccular sludge)	0.5-0.8	1.0
Reject WW (Rodriguez-Caballero 2013)	Partial nitrification Lab-scale SBR. (Floccular sludge)	0.8-1.5	0.8
Reject WW (this study)	Full or Partial Nitrification Pilot-scale granular airlift. (Granular sludge)	4.5-7.5	2.2
Reject WW (this study)	Full or Partial nitrification Pilot-scale granular airlift. (Granular sludge)	1.1-4.5	6.1-2.2

678 N.R. Not reported; ^a values calculated taken into account the %N₂O/N load and the NH₄⁺
 679 converted to nitrite reported. In the case of one-stage systems a 50% conversion of the
 680 ammonium removed to nitrite has been assumed as reported in materials and methods section
 681 (equation 3). ^b Values reported for the same reactor under at two different monitoring campaigns.
 682
 683

Figure1

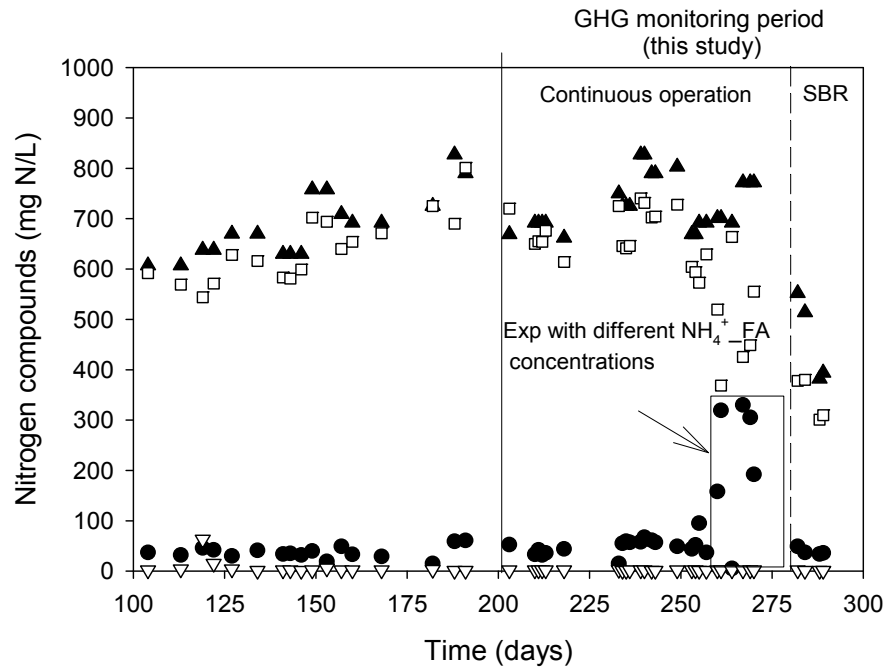


Figure 1. Nitrogen transformations in the granular airlift reactor before and during the GHG monitoring period. ● NH₄⁺ in the reactor; □ NO₂⁻ in the reactor; ▽ NO₃⁻ in the reactor; ▲ NH₄⁺ in the wastewater.

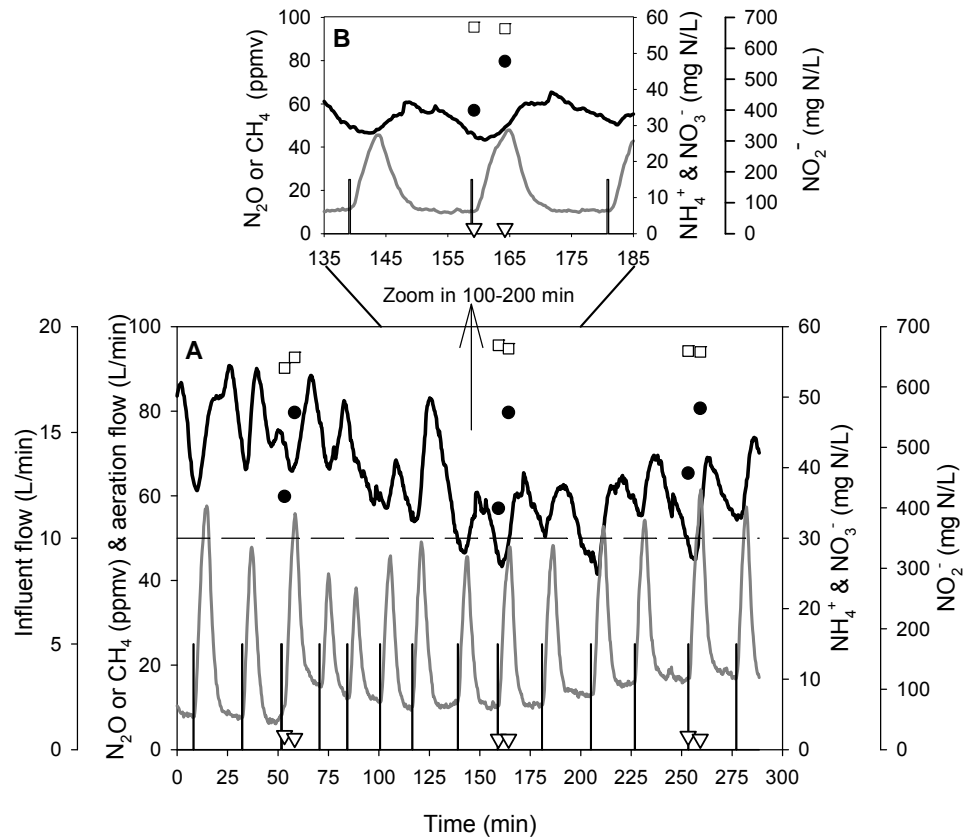


Figure 2. A- On-line N₂O and CH₄ emissions from the reactor during continuous operation at DO 4.7 mg O₂/L and pH 7.5. N₂O (Black line); CH₄ (grey line); influent flow (thin black line); aeration flow (dashed line); NH₄⁺ (●); NO₃⁻ (▽); NO₂⁻ (□). B- Zoom in from figure A (135-185 min).

Figure3

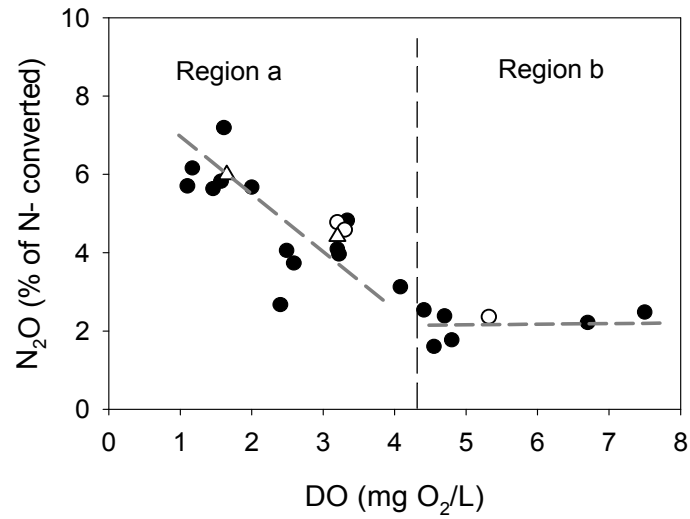


Figure 3. Correlation between the N₂O emission factor and the DO concentration in the reactor: ● Operation under full nitrification conditions (>90% NH₄⁺ oxidation to NO₂⁻); ○ Operation under partial nitrification conditions (70%-75% NH₄⁺ oxidation to NO₂⁻); Δ Operation under partial nitrification conditions (50%-55% NH₄⁺ oxidation to NO₂⁻).

Figure4

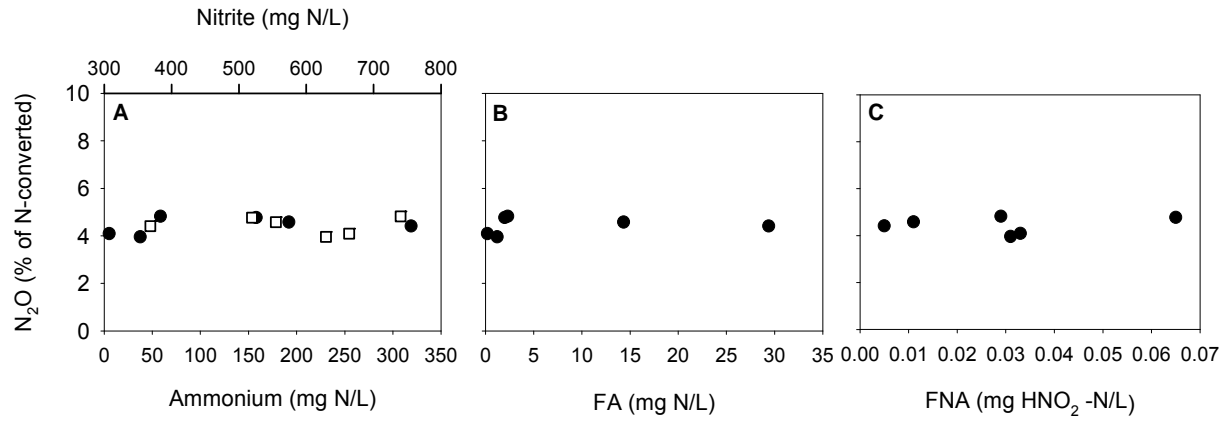


Figure 4. Correlation between the N_2O emission factor and ammonium /nitrite (A), free ammonia (B) and free nitrous acid (C) concentrations. ●- Ammonium, FA & FNA; □- Nitrite.

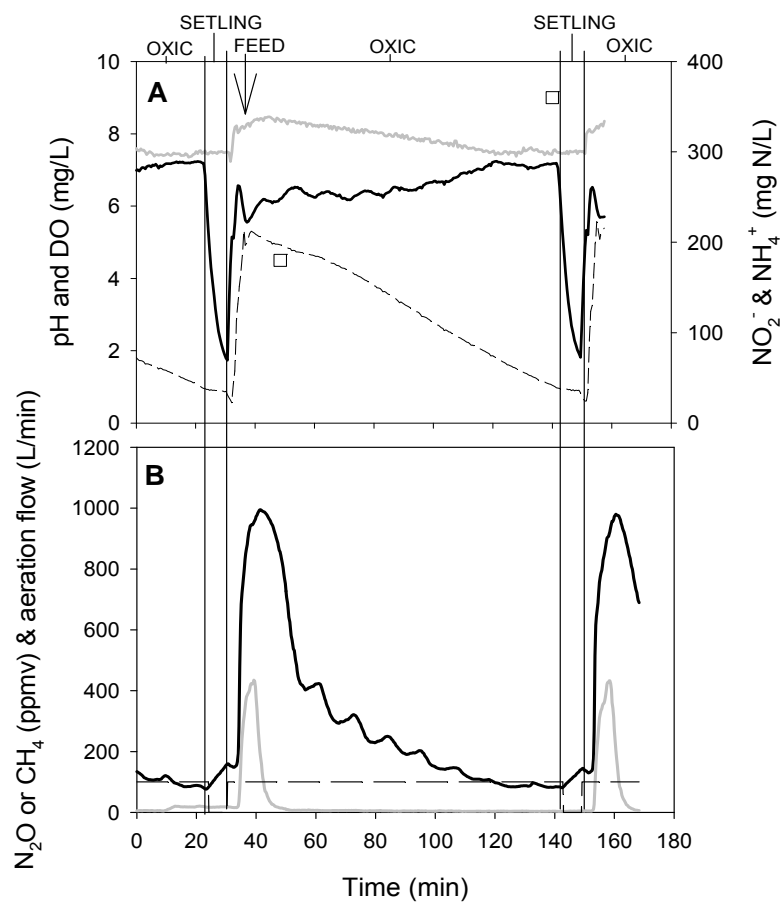


Figure 5. Cycle study profile of the pilot plant operating in SBR mode with a settling time of 7 min and an aeration flow of 100 L/min. A- pH (grey line), DO (black line), ammonium (discontinuous line) and nitrite (\square) concentrations; B- N_2O (black line) and CH_4 (grey line) emission profiles; aeration flow (discontinuous line).

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