# Effect of process parameters and operational mode on nitrous oxide emissions from a nitritation reactor treating reject wastewater

Maite Pijuan[a]*, Josep Torà[b], Adrián Rodríguez-Caballero[a], Elvira César[c], Julián Carrera[b], Julio Pérez[b].

[a] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, 17003, Girona (Spain).

[b] Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, Spain.

[c] Depuración de Aguas del Mediterráneo (DAM), Guglielmo Marconi, 11 piso 2º, despacho 19, Parque Tecnológico 46980 Paterna (Valencia).

* Corresponding author: email: mpijuan@icra.cat; phone: +34972183380; fax: +34972183248.

**ABSTRACT**

Nitrous oxide ($N_2O$) and methane emissions were monitored in a continuous granular airlift nitritation reactor from ammonium-rich wastewater (reject wastewater). $N_2O$ emissions were found to be dependent on dissolved oxygen (DO) concentration in the range of 1 to 4.5 mg $O_2$/L, increasing within this range when reducing the DO values. At higher DO concentrations, $N_2O$ emissions remained constant at 2.2% of the N oxidized to nitrite, suggesting two different mechanisms behind $N_2O$ production, one dependent and one independent of DO concentration. Changes on ammonium, nitrite, free ammonia and free nitrous acid concentrations did not have an effect on $N_2O$ emissions within the concentration range tested. When operating the reactor in a sequencing batch mode under high DO concentration (> 5 mg $O_2$/L), $N_2O$ emissions increased one order of magnitude

1

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

## 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 nitritation 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 nitritation 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 nitritation 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 nitritation 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 nitritation (50% conversion of $NH_4^+$ to $NO_2^-$) depending on the

65  subsequent denitritation 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 $N_2O$ mitigation strategy was conducted for the pilot plant and extrapolated to

71    real facilities.

72

## 2. Materials and methods

73

### 2.1. Pilot plant

74

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 30ºC 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 $Na_2CO_3$. 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    $(TAN=NH_4^+-N/L+NH_3-N/L)$ 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

4

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 $\pm$ 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 nitritation 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. $\pm$ 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 $726 \pm 50$ 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 $5\pm3$ % 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 $450 \pm 78$ mg N/L.

**2.3. Nitrous oxide and methane monitoring**

Off gas was collected continuously (at 0.5L/min) from the reactor headspace which was covered with a plastic bag and connected via a gas tube to a gas conditioning unit (series CSS, M&C Tech group). The gas outlet from the conditioning unit was connected to the multicomponent online gas analyser (VA-3000, Horiba, Japan) which provided an online measurement of $N_2O$ and $CH_4$ concentrations from the gas flow. Data were logged every 15 seconds for a period of 3-4 h for each of the monitoring tests.

2.3.1. Monitoring during continuous operation

Twenty-four monitoring tests (T1-T24) were conducted to assess the $N_2O$ and $CH_4$ emission dynamics from the reactor under different DO concentrations in the range of 1.1 to 7.7 mg $O_2$/L. The different DO concentrations are summarised in table 1 and were achieved by varying the air flow-rate from 11 to 100 L/min. The reactor operated under the conditions described for each of the tests (table 1) 24 h previous to the monitoring.

To explore the effect of $NH_4^+$, $NO_2^-$, free ammonia (FA) and free nitrous acid (FNA) concentration on $N_2O$ emission, six of these tests (T11-T16) were conducted under the same DO concentration (3.2-3.3 mg/L) but with different $NH_4^+$ concentration and pH, providing different FA, $NO_2^-$ and FNA concentrations (table 2).

2.3.2. Monitoring during SBR operation

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

**2.4. Calculations**

The total $N_2O$ and $CH_4$ emitted were calculated using the following equations:

162    N$_2$O emitted= $\sum(C_{N2O-Ngas} \times Q_{gas} \times \Delta t)$                    (1)

163    CH$_4$ emitted= $\sum(C_{CH4gas} \times Q_{gas} \times \Delta t)$

                                                                                    (2)

164    Where

165    $C_{N2O}$ (g N$_2$O-N/L) = C $_{N2O}$ (ppmv)*10$^{-6}$ * molar gas volume$^{-1}$ (0.0414 mol/L at 25ºC and

166    1atm)*28.

167    $C_{CH4}$ (g CH$_4$/L) = $C_{CH4}$ (ppmv)*10$^{-6}$ * molar gas volume$^{-1}$ (0.0414 at 25ºC and 1atm)*16.

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

169    $\Delta t$= time interval by which the off-gas N$_2$O concentration was recorded.

170    The emission factor for N$_2$O was calculated based on the total amount of N$_2$O emitted in

171    a particular time (equation 1) relative to the total NH$_4^+$ converted to NO$_2^-$ in that time (mg

172    N$_2$O-N/mg NH$_4^+$-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 nitritation).

175    To directly compare the emission factors reported in the literature for single-stage N

176    removal systems (nitritation and anammox in 1 single reactor) with the emissions

177    reported in this study and in other nitritation systems (Table 5), the following procedure

178    was applied: (i) the values reported for single-stage N-removal systems (normally given

179    in %N$_2$O/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 NO$_2^-$ (roughly following the

181    anammox stoichiometry); iii) all the N$_2$O emissions reported in these systems were

182    assumed to be produced during the nitritation process as anammox bacteria do not

183    produce N$_2$O (Kartal et al., 2010).   This procedure could be summarized with the

184    following equation:

8

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 nitritation 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±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 nitritation conditions for the majority of the monitoring period. However, the control

236     system applied also allowed its operation under partial nitritation 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% $N_2O$-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 nitritation

252     process, and was not linked to either stripping or other physical processes that might be

253     occurring in the reactor.

**3.3. Effect of DO concentration on N$_2$O emission**

The effect of different DO concentrations on N$_2$O emissions was tested by changing the aeration flow rate in the pilot plant. To compare the emissions under different DO concentrations, the N$_2$O emission factor was calculated as described in Materials and Methods section. Figure 3 presents the N$_2$O emission factor dependency on DO concentration.

The lowest N$_2$O emission factor was measured at DO concentrations of 4.5 mg O$_2$/L or higher (Figure 3, region b). At this range, 2.2 $\pm$ 0.4 % of the NH$_4^+$ nitrified was emitted as N$_2$O and was not dependent on the DO concentration. However, when reducing the DO concentration to levels lower than 4.5 mg O$_2$/L, the N$_2$O emission factor increased, reaching values around 6% of the NH$_4^+$ nitrified being emitted as N$_2$O (Figure 3, region a). Operating the reactor under partial or full nitritation conditions did not have an effect on N$_2$O emissions. Emissions detected under partial nitritation conditions matched the same profile as the emissions found when operating under full nitritation within the concentration DO range of 1.6-5.3 mg O$_2$/L (highlighted in figure 3 with empty circles and triangles).

Stripping conditions changed among the experiments, since changes on DO concentration in the reactor could only be achieved by changing the aeration flow rate (table 1). It can not be excluded that changes in stripping conditions might also have a direct effect on the N$_2$O emissions detected but changes in DO concentration seem to be the main driver to changes on N$_2$O as shown by the strong correlation depicted in figure 3 and the bigger dispersion observed when depicting the correlation between N$_2$O emission factor and air flow rate (figure SI.1)

**3.4. Effect of ammonium, free ammonia, nitrite and free nitrous acid on $N_2O$ emission.**

The effect of operating the pilot plant at different $NH_4^+$ and FA concentrations on $N_2O$ production was tested by varying the $NH_4^+$ concentration set-point in the control loop and the pH (Table 2). FNA concentration was calculated considering the amount of $NO_2^-$ present in the reactor and the pH and was also related to $N_2O$. Results are presented in figure 4.

The pilot plant operated under the same DO concentration (3.2-3.3 mg $O_2$/L) when these experiments were carried out to exclude the effect of DO on $N_2O$ emissions. The $N_2O$ emission factor remained constant at 4.4±0.3% of the $NH_4^+$ oxidized emitted as $N_2O$ despite of the wide range of $NH_4^+$, FA, $NO_2^-$ and FNA concentrations tested. Therefore it can be concluded that within the concentration range tested and with a DO concentration of 3.3 mg $O_2$/L, changes on $NH_4^+$, FA, $NO_2^-$ and FNA did not have an effect on $N_2O$ production. Interestingly, the $N_2O$ emission factor was independent of the performance of the granular reactor for achieving full nitritation (100% conversion of $NH_4^+$ to $NO_2^-$) or partial nitritation (50-75% conversion of $NH_4^+$ to $NO_2^-$).

**3.5. Emission dynamics of $N_2O$ and $CH_4$ during SBR operation**

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

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

300 (from 5.6 till 7.2 mg $O_2$/L approximately), a sharp decrease as soon as aeration stopped

301 during the settling phase (from 7.2 till 0.5 mg $O_2$/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 $O_2$/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 $NH_4^+$ 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. $NO_2^-$ 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 $NH_4^+$ concentration present in

312 the reject wastewater when the reactor operated in SBR mode.

313 Peaks of $CH_4$ and $N_2O$ were detected at the beginning of the cycle as soon as aeration

314 started. The $CH_4$ peak corresponded to the stripping of the soluble $CH_4$ 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 $CH_4$ emitted per wastewater treated (Figure SI.2). The $N_2O$ 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 $N_2O$ 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 $O_2$/L; 7.5-8 pH). However,

325     when considering the total $N_2O$ emitted per N oxidized, the emission factor obtained

326     during SBR operation was $19.3 \pm 7.5$ %, one order of magnitude higher than the $2.2 \pm 0.4$

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 $N_2O$ emitted during the cycle (Figure SI.3).

330     **3.6. Economical assessment of $N_2O$ mitigation**

331     From the results presented, operating the reactor in a continuous mode at high aeration

332     rates (DO concentration $> 4.5$ mg $O_2$/L) can be postulated as an effective approach to

333     mitigate $N_2O$ 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 $N_2O$

344     emission factors have been applied to a scaled up theoretical nitritation 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 $N_2O$ emissions resulted in a

350     43% lower carbon footprint. Remarkably, at low DO concentration $N_2O$ 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 nitritation of reject wastewater in view of the costs analysis.

360

361     **4. Discussion**

362     **4.1. Possible $N_2O$ production pathways affecting $N_2O$ emissions under different DO**

363     **concentrations**

364     DO is considered an important parameter affecting $N_2O$ emissions, with lower DO

365     concentrations increasing $N_2O$ emissions (Kampschreur et al., 2009a). However, it is still

366     unclear if a DO concentration threshold to minimise $N_2O$ emissions can be established for

367     nitrifying systems since different $N_2O$ emission factors have been reported at different

368     DO concentrations (Table 5).

369    It is accepted that two different $N_2O$ 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 $N_2O$ production in AOB is still unclear.

372    Sutka et al. (2006) used stable nitrogen isotopes to conclude that the $NH_2OH$ oxidation

373    pathway contributed to $N_2O$ 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 $N_2O$ production

376    pathways were identified using site-specific isotope composition of $N_2O$ in real time. In

377    their nitrification tests, there was always a combination of the two pathways that

378    produced the $N_2O$ detected, except for the cases where only $NO_2^-$ 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 $N_2O$ 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 $N_2O$ 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 $N_2O$ 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 $N_2O$ 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 $N_2O$ peak detected. Several studies have inferred in the effect

416    that periods of anoxia can have on $N_2O$ production from nitrifying cultures. Kampschreur

417    et al. (2008b) reported the effect of oxygen limitation during $NH_4^+$ oxidation in a

418    nitrifying lab-scale system. They observed an immediate $N_2O$ 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 $N_2O$ 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 $N_2O$ emitted from a nitritation lab-scale system treating

424    reject wastewater originated during settling and depended on the presence of $NH_4^+$ and

425    $NO_2^-$.

426    Another explanation for the higher $N_2O$ production when operating in SBR mode could

427    be the sudden variations on $NH_4^+$ and $NO_2^-$ concentrations experienced during the cycle,

428    more pronounced than in continuous operation. Dynamic process conditions can enhance

429    $N_2O$ production. Kampscheur 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 $NO_2^-$ concentrations which gave an increase on $N_2O$ production.

432    Recently, Law et al. (2013) reported a relationship between the specific $N_2O$ production

433    rate and a gradual $NO_2^-$ accumulation in a partial nitritation culture treating synthetic

434    reject wastewater. Interestingly, $NO_2^-$ had a suppressive effect on $N_2O$ production when

435    increasing the concentration from 50 to 500 mg N/L. At higher $NO_2^-$ concentrations, $N_2O$

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 nitritation of reject wastewater treatment

439  needs further evaluation due to the higher $N_2O$ 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 $NH_4^+$ 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 $N_2O$ emission from these systems which could

447  have a big impact on the overall carbon footprint of the plant. Currently, $N_2O$ emission

448  values from single stage systems are still scarce and rather variable (see table 5). An $N_2O$

449  emission factor of 1.67% of the N removed was reported in a full-scale single stage

450  nitritation-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, nitritation and

452  autotrophic denitritation 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 $N_2O$ emissions when $NO_2^-$ 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 $N_2O$ emissions in single stage nitritation-

458  anammox systems.

459  On the other hand, emissions from partial nitritation 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 $N_2O$. Our findings indicate that

462   changes in the $NH_4^+$, $NO_2^-$, FNA or FA concentrations have no impact on $N_2O$ emissions

463   during nitritation within the common operational ranges. Therefore, emissions detected in

464   two stage nitritation-anammox systems are expected to be similar to those found in one-

465   stage nitritation-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 $N_2O$ 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 $NO_2^-$ in single nitritation-anmmox reactors would lead

470   to an increased emission factor (Kampschreur et al., 2009b). More full-scale $N_2O$

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 nitritation step similar to the one

479   presented here, will only increase slightly the $N_2O$ emissions if adequate DO

480   concentration is maintained in the bulk liquid. This strategy may be an alternative to be

481   considered since nitritation has been successfully tested at low temperatures (Jemaat et

482   al., 2013).

483

## 5. Conclusions

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

- DO concentration can be used as a control parameter to minimise $N_2O$ emissions. Increasing the DO to 4.5 mg $O_2$/L resulted in a decrease on $N_2O$ emissions from 6 to 2.2%. However, a further increase on DO did not result in an additional reduction, suggesting the involvement of two different mechanisms responsible for $N_2O$ production.

- Continuous operation is preferred to SBR for partial nitrification systems. SBR operation resulted in a substantial increase on $N_2O$ emissions when compared to those obtained in continuous mode.

- $N_2O$ emissions would dominate the total carbon footprint in a hypothetical scale-up of the reactor studied. Operating at minimal $N_2O$ emission would only be economically feasible if a carbon tax on emissions is implemented.

506 course of the research. M. Pijuan also acknowledges the Ramon y Cajal research
507 fellowship (RYC-2009-04959) provided by the Spanish Government.

508

509 **Supporting information available**

510 **References**

511 Anderson, I., Poth, M., Homstead, J., Burdige, D. 1993. A comparison of NO and N2O
512 production by the autotrophic nitrifier Nitrosomonas europaea and the heterotrophic
513 nitrifier Alcaligenes faecalis. Applied Environmental Microbiology 59(11), 3525-3533.

514 Anthonisen, A., Loehr, R., Prakasam, T., Shinath, E. 1976. Inhibition of nitrification by
515 ammonia and nitrous acid. Journal of Water Pollution Control Federation 48, 835-852.

516 APHA. 1995. Standard methods for the examination of water and wastewater. 19th ed.
517 American Public Health Association. Washington, DC.

518 Bartrolí, A., Pérez, J., Carrera, J. 2010. Applying ratio control in a continuous granular
519 reactor to achieve full nitritation under stable operating conditions. Environmental
520 Science and Technology 44, 8930-8935.

521 Bock, E., Schmidt, I., Stuven, R., Zart D. 1995. Nitrogen loss caused by denitrifying
522 Nitrosomonas cells using ammoinium or hydrogen as electron-donors and nitrite as
523 electron-acceptor. Archives in Microbiology 163, 16-20.

524 Carrera, J., Bartrolí, A., Isanta, E., Jubany, I., Torà, J. A., Baeza, J. A., Pérez, J. 2010.
525 Advanced technology for the biological nitrogen removal of the reject water from the
526 sludge dewatering systems. In Reduction, Modification and Valorisation of Sludge

527  (REMOVALS); Fabregat, A., Bengoa, C., Font, J., Stüber, F., Eds.; IWA Publishing:

528  London, 101-114.

529  Castro-Barros, C., Daelman, M., Mampaey K., van Loosdrecht, M., Volcke E. 2013.

530  Dynamics of N2O emission from partial nitritation-anammox in a full-scale granular

531  sludge reactor. Proceedings from the 9th International Conference on Biofilm reactors,

532  28-31th of May, Paris.

533  De Graaff, M.S., Zeeman, G., Temmik, H., van Loosdrecht, M. 2010. Long term partial

534  nitritation of anaerobically treated black water and the emission of nitrous oxide. Water

535  Research 44, 2171-2178.

536  Desloover, J., de Clippeleir, H., Boeckx, P., du Laing, G., Colsen, J., Verstraete, W.,

537  Vlaeminck, SE. 2011. Floc-based sequential partial nitritation and anammox at full scale

538  with contrasting N2O emissions. Water Research 45, 2811-2821.

539  Hellinga, C., Schellen, A., Mulder, J., van Loosdrecht, M., Heijnen, J. 1998. The

540  SHARON process: an innovative method for nitrogen removal from ammonium-rich

541  wastewater. Water Science and Technology 37(9), 135-142.

542  IPCC, 2013. The final draft Report, dated 7 June 2013, of the Working Group I

543  contribution to the IPCC 5th Assessment Report "Climate Change 2013: The Physical

544  Science Basis". www.climatechange2013.org/report/review-drafts/

545  Jaroszynski, L.W., Oleszkiewicz, J.A. 2011. Autotrophic ammonium removal from reject

546  water: Partial nitrification and Anammox in one-reactor versus two-reactor systems.

547  Environmental Technology  32, 289–294.

548    Jemaat, Z.; Bartrolí, A.; Isanta, E.; Carrera, J.; Suárez-Ojeda, M.E.; Pérez, J. 2013.

549    Closed-loop control of ammonium concentration in nitritation: Convenient for reactor

550    operation but also for modeling. Bioresource Technology 128, 655-663.

551    Joss, A., Salzgeber, D., Eugster, J., Konig, R., Rottermann, K., Burger, S., Fabijan, P.,

552    Leumann, S., Mohn, J., Siegrist, H. 2009. Full-scale nitrogen removal from digester

553    liquid with partial nitrition and anammox in one SBR. Environmental Science and

554    Technology 43, 5301-5306.

555    Kampschreur, M.J., van der Star, W., Wielders, H., Mulder, J.W., Jetten, M., van

556    Loosdrecht, M. 2008a. Dynamics of nitric oxide and nitrous oxide emission during full-

557    scale reject water treatment. Water Research 42, 812-826.

558    Kampschreur, M., Tan, N., Kleerebezem, R., Picioreanu, C., Jetten, M., van Loosdrecht,

559    M. 2008b. Effect of dynamic process conditions on nitrogen oxides emission from a

560    nitrifying culture. Environmental Science and Technology 42, 429-435.

561    Kampschreur, M., Temmink, H., Kleerebezem, R., Jetten, M., van Loosdrecht, M. 2009a.

562    Nitrous oxide emission during wastewater treatment. Water Research 43, 4093-4103.

563    Kampschreur, M., Poldermans, R., Kleerebezem, R., van der Star, W., Haarhuis, R.,

564    Abma, W., Jetten, M., van Loosdrecht, M. 2009b. Emission of nitrous oxide and nitric

565    oxide from a full-scale single-stage nitritation-anammox reactor. Water Science and

566    Technology 60(12), 3211-3217.

567    Kartal, B., Tan, N., van de Biezen, E., Kampschreur, M., van Loosdrecht, M., Jetten, S.

568    2010. Effect of nitric oxide on anammox bacteria. Applied and Environmental

569    Microbiology 76(18), 6304-6360.

570 Law, Y., Lant, P., Yuan, Z. 2011. The effect of pH on $N_2O$ production under aerobic

571 conditions in a partial nitritation system. Water Research 45, 5934-5944.

572 Law, Y., Ni, B.J., Lant, P., Yuan, Z. 2012. $N_2O$ production rate of an enriched ammonia-

573 oxidizing bacteria culture exponentially correlates to its ammonia oxidation rate. Water

574 Research 46(10), 3409-3419.

575 Law, Y., Lant, P., Yuan, Z. 2013. The cofounding effect of nitrite on $N_2O$ production by

576 an enriched ammonia-oxidising culture. Environmental Science and Technology 47,

577 7186-7194.

578 Mulder, J., van Loosdrecht, M., Hellinga, C., van Kempen, R. 2001. Full-scale

579 application of the SHARON process for the treatment of rejection water of digested

580 sludge dewatering. Water Science and Technology, 43(11), 127-134.

581 Pijuan, M., Werner, U., Yuan, Z. 2009. Effect of long term anaerobic and intermittent

582 anaerobic/aerobic starvation on aerobic granules. Water Research 43, 3622-3632.

583 Rodriguez-Caballero, A., Pijuan, M. 2013. $N_2O$ and NO Emissions from a Partial

584 Nitrification Sequencing Batch Reactor: Exploring Dynamics, Sources and Minimization

585 Mechanisms. Water Research 47, 3131-3140.

586 SBS. Review of carbon taxes around the world. Special Broadcasting Service (SBS).

587 Australian public broadcasting network. Published on 21st August 2012.

588 http://www.sbs.com.au/news/article/1492651/Factbox-Carbon-.

589 Schreiber, F., Wunderlin, P., Udert, K., Wells, G. 2012. Nitric oxide and nitrous oxide

590 turnover in natural and engineered microbial communities: biological pathways, chemical

591 reactions, and novel technologies. Frontiers in Microbiology 3, article 372, 1-24.

592     Stein, L. Surveying $N_2O$-producing pathways in bacteria. 2011. In Methods of

593     Enzymology Klotz MG. Ed. Academic press, 131-152.

594     Suárez-Ojeda, M.E., Guisasola, A., Baeza, J.A., Fabregat, A., Stüber, F., Fortuny, A.,

595     Font, A., Carrera, J. 2007. Integrated catalytic wet air oxidation and aerobic biological

596     treatment in a municipal WWTP of a high-strength o-cresol wastewater, Chemosphere

597     66, 2096-2105.

598     Sutka, R., Ostrom, N., Ostrom, P., Breznak, H., Gandhi, H., Pitt, A. et al. 2006.

599     Distinguishing nitrous oxide production from nitrification and denitrification on the basis

600     of isotopomer abundances. Applied and Environmental Microbiology 72, 638-644.

601     Tallec, G., Garnier, J., Billen, G., Gousailles, M. 2006. Nitrous oxide emissions from

602     secondary activated sludge in nitrifying conditions of urban wastewater treatment plants:

603     Effect of oxygenation level. Water Research 40, 2972-2980.

604     Torà, J., Bartrolí, A., Moliné, E., Carrera, J., Perez, J. 2013. Efficient and automated start-

605     up of a pilot granular reactor for full nitration of reject water. Chemical Engineering

606     Journal 226, 319-325.

607     UKWIR, 2008. Carbon Accounting in the UK Water Industry: Operational Emissions.

608     Report Reference No. 08/CL/01/5. UK Water Industry Research Limited, London.

609     Van Dongen, U., Jetten, M., van Loosdrecht, M. 2001. The SHARON-Anammox process

610     for the treatment of ammonium rich wastewater. Water Science and Technology 44(1),

611     153-160.

612    Van Hulle, S., van de Weyer, H., Meesschaert, B., Vanrolleghem, P., Dejans, P.,

613    Dumoulin, A. 2010. Engineering aspects and practical application of autotrophic nitrogen

614    removal from nitrogen rich streams. Chemical Engineering Journal 162, 1–20.

615    Winkler, M., Kleerebezem, R., van Loosdrecht, M.C.M. 2012. Integration of Anammox

616    into the aerobic granular sludge process for main stream wastewater treatment at ambient

617    temperatures. Water Research 46, 136-144.

618    Wunderlin, P., Lehmann, M., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L., Mohn,

619    J. 2013. Isotope signatures of $N_2O$ in a mixed microbial population system: constraints on

620    $N_2O$ production pathways in wastewater treatment. Environmental Science and

621    Technology 47(3), 1339-1348.

622    Yu, R., Kampschreur, M., van Loosdrecht, M., Chandran, K. 2010. Mechanisms and

623    specific directionality of autotrophic nitrous oxide and nitric oxide generation during

624    transient anoxia. Environmental Science and Technology 44, 1313-1319.

625

626

627

628

629

630

631

632

633

634

654

655

656

657

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 $NH_4^+$, 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 $O_2$/L; high DO: 4.5 mg $O_2$/L

| Data | Low DO (Continuous) | High DO | | Units |
|---|---|---|---|---|
|  |  | Continuous | SBR |  |
| Annual energy requirements for aeration | 408,303 | 625,421 | | kWh year$^{-1}$ |
| Annual $N_2O$ emissions | 3.5 | 1.2 | 11.1 | Tn $N_2O$ year$^{-1}$ |
| Equivalent $CO_2$ emissions for aeration | 222 | 340 | | Tn $CO_2$ eq year$^{-1}$ |
| Annual $N_2O$ emissions ($CO_2$ eq.) | 927 | 310 | 2941 | Tn $CO_2$ eq year$^{-1}$ |
| **Annual carbon footprint** | **1,149** | **650** | **3282** | **Tn $CO_2$ 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 $CO_2$ eq / kWh; 265 kg $CO_2$ eq / kg
673 $N_2O$. [a]Low carbon tax: 0.004 euro / kg $CO_2$; [b]High carbon tax: 0.03 euro / kg $CO_2$. 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-$N_2O$/N-oxidized) |
|---|---|---|---|
| Anaerobically digested industrial WW (Desloover 2011) | Partial nitritation+anammox (2-stage). Full-scale. (Floccular sludge) | 0.4-1.0 | 8.1-11.2*[a] *emissions from nitritation 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 nitritation+anammox (1-stage). SBR full-scale. (type of sludge not described) | <0.5 | 0.8[a] |
| Reject WW (Kampschreur 2008a) | Partial nitritation + anammox (2-stage). Full-scale. (Floccular sludge) | 2.5 | 3.4* *emissions from nitritation reactor |
| Reject WW (Kampschreur 2009b) | Partial nitritation+ anammox (1-stage). Full-scale. (Granular sludge) | 5 | 2.5[a,b] |
| Reject WW (Castro-Barros 2013) | Partial nitritation+anammox (1-stage). Full-scale. (Granular sludge) | N.R. | 4.0[a,b] |
| Reject WW (Law 2011) | Partial nitritation 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 nitritation 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 %$N_2O$/N load and the $NH_4^+$
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.
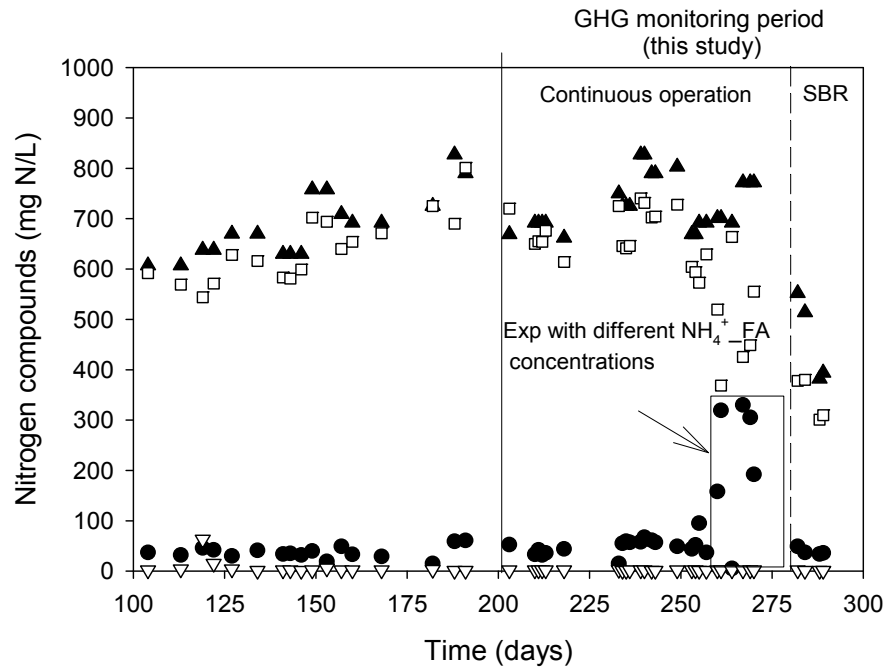682
683

**Figure1**



**Figure 1.** Nitrogen transformations in the granular airlift reactor before and during the GHG monitoring period. ● $NH_4^+$ in the reactor; □ $NO_2^-$ in the reactor; ∇ $NO_3^-$ in the reactor; ▲ $NH_4^+$ in the wastewater.
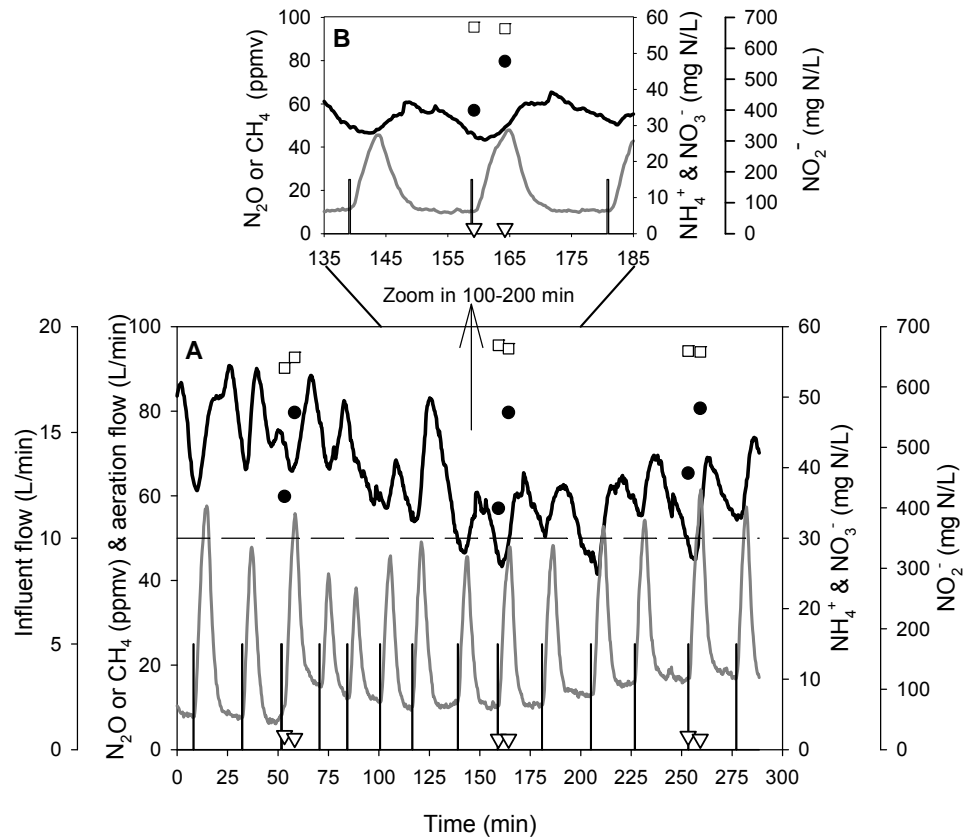
**Figure2_revised**



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

**Figure3**
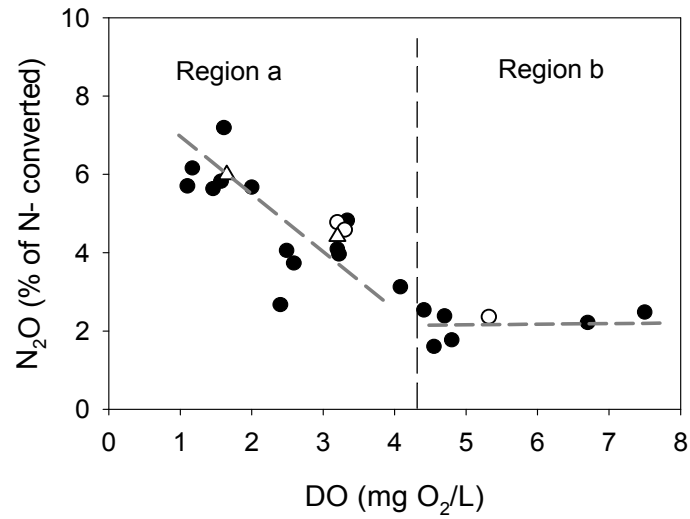


**Figure 3.** Correlation between the $N_2O$ emission factor and the DO concentration in the reactor: ● Operation under full nitritation conditions (>90% $NH_4^+$ oxidation to $NO_2^-$); ○ Operation under partial nitritation conditions (70%-75% $NH_4^+$ oxidation to $NO_2^-$); Δ Operation under partial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

**Figure4**
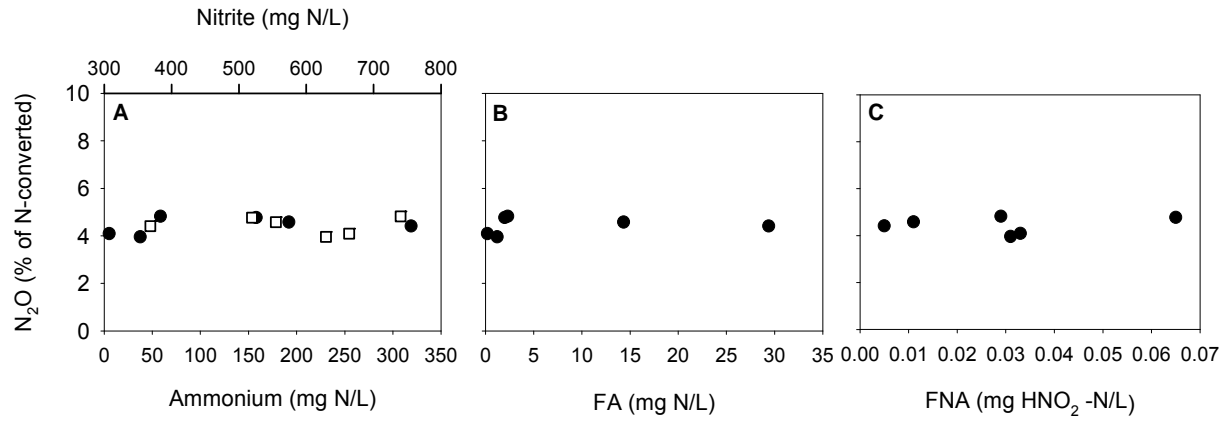


**Figure 4.** Correlation between the $N_2O$ emission factor and ammonium /nitrite (A), free ammonia (B) and free nitrous acid (C) concentrations. ●- Ammonium, FA & FNA; □- Nitrite.
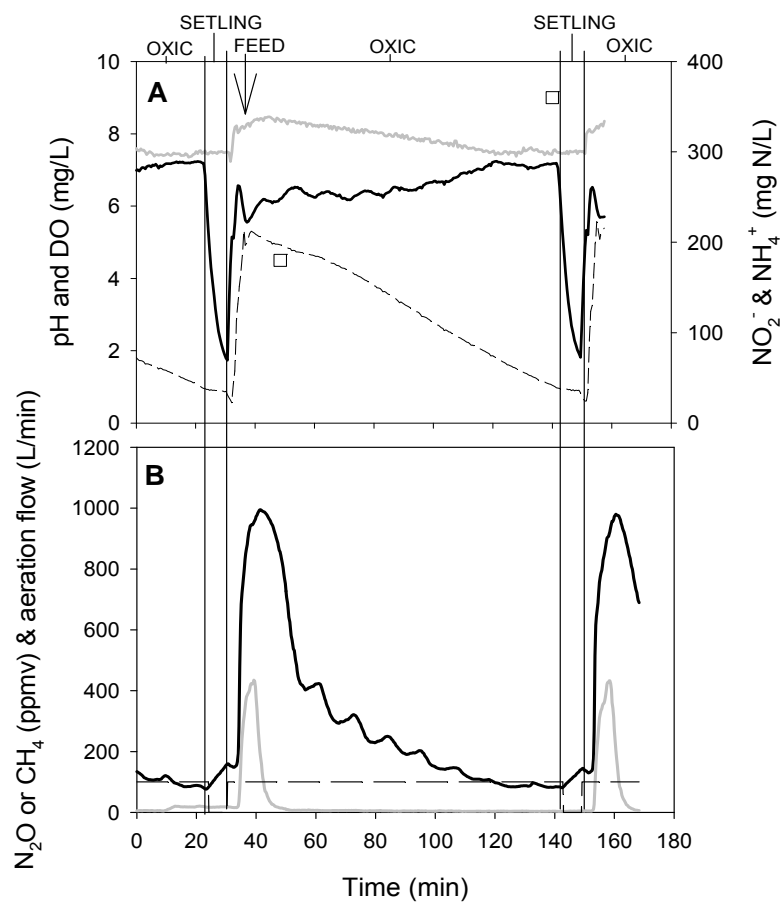
Figure5



**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 (□) concentrations; B- $N_2O$ (black line) and $CH_4$ (grey line) emission profiles; aeration flow (discontinuous line).