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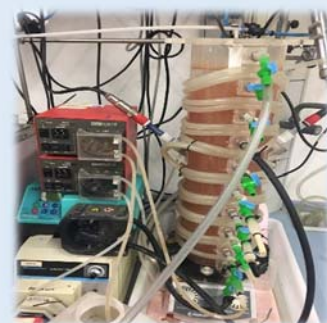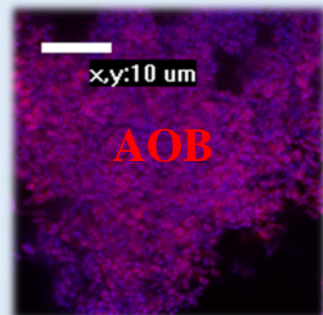Zhiyuan Bao, Anna Ribera-Guardia, Matteo Spinelli, Dezhi Sun, Maite Pijuan

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PN-SBR

AOB

x,y:10 um

Reject wastewater
1g/L NH$_4^+$-N → **Temperature**
30-25-20-15-10°C
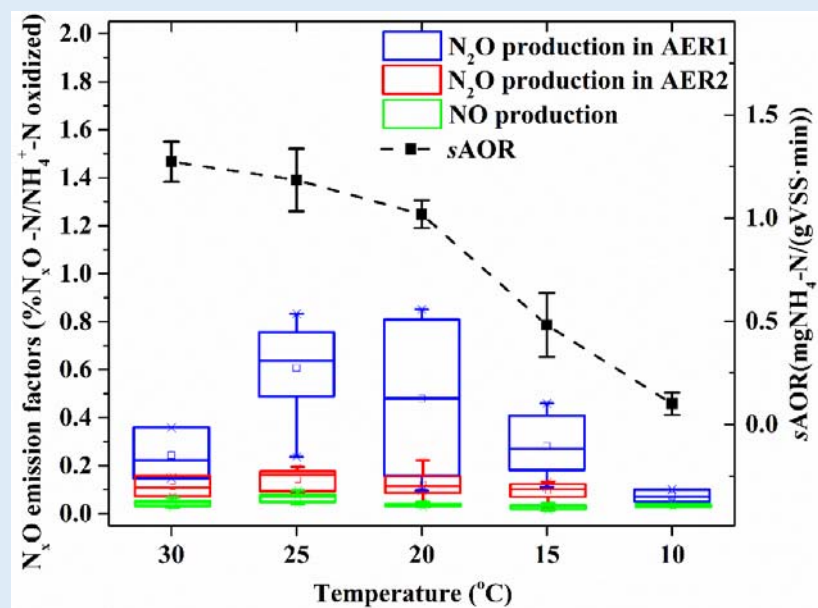
The effect of temperature shifts on N_2O and NO emissions from a partial nitritation

reactor treating reject wastewater

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Abstract

Temperature has a known effect on ammonia oxidizing bacteria (AOB) activities, reducing its ammonia oxidizing rate (AOR) when temperature is lowered. However, little is known concerning its effect on N_2O and NO emissions which are produced during ammonia oxidation having a greenhouse effect. To study this, an AOB enriched partial nitrification sequencing batch reactor (PN-SBR) was operated within a two step-wise feed under 5 different temperatures (30-25-20-15-10ºC). A decrease on the specific AOR (sAOR) was detected when decreasing the temperature. N_2O emissions were also affected by the temperature but only the ones produced during the first aeration of the cycle, when AOBs shifted from a period of low activity to a period of high activity. N_2O emission factors (%) detected during the second aerobic phase were similar among all temperatures tested and lower than the emissions detected during the first aerated phase. The average N_2O emission factor was in the range of 0.15 - 0.70% N_2O-N/NH_4^+-N oxidized in the first aeration phase and 0.14 - 0.15% N_2O-N/NH_4^+-N-oxidized in the second aeration phase at 10 to 30 ºC, respectively. On the other hand, NO emissions were very similar under all temperatures resulting in 0.03 - 0.06% of NH_4^+-N oxidized.

Keywords

1. Introduction

Fugitive nitrous oxide (N_2O) and nitric oxide (NO) emissions can occur during nitrification because of the activity of ammonia oxidizing bacteria (AOB) (Rodríguez-Caballero and Pijuan, 2013). N_2O is one of the most important greenhouse gas (GHG) having approximately 300 times stronger warming potential than that of CO_2. On the other hand, NO is another detrimental byproduct which is toxic for the activities of microbes and has harmful effects on the ozone layer (Wang et al., 2016). Both N_2O and NO are mainly produced and emitted during nitrification and can have a considerable effect on the carbon footprint from wastewater treatment systems (Sun et al., 2017; Spinelli et al., 2018). N_2O and NO can be formed via two pathways by AOB: (*i*) the hydroxylamine (NH_2OH) oxidation pathway (Tallec et al., 2006; Law et al., 2012b), and (*ii*) the nitrifier denitrification pathway (Shaw et al., 2006; Ishii et al., 2014; Massara et al., 2018). Significant amount of research has been conducted to unravel what are the factors affecting N_2O emissions. In this sense, nitrite concentration, pH and DO have been identified as key parameters affecting these emissions (Shaw et al., 2006; Pijuan et al., 2014; Rathnayake et al., 2015). However, lonely few studies have focused on the parameters affecting NO emissions and on the relationship existing between NO and N_2O (Ribera-Guardia and Pijuan, 2017). Rodriguez-Caballero and Pijuan (2013) observed that mitigation strategies for N_2O resulted in an increase of NO in a reactor enriched with AOB, which highlights the importance of monitoring both gases simultaneously.

Partial nitrification (PN) conducted by AOB dominated systems is widely implemented for saving carbon and energy when treating reject wastewater and having highly feasible and efficient conversion of ammonia to nitrite (Ge et al., 2015). The

optimal temperature for PN-SBR is found around $30^{o}C$ favoring the dominantly growth of AOB as compared to that of nitrite oxidizing bacteria (NOB) (Hellinga et al., 1998). However, some studies have also indicated that partial nitritation can still be achieved under lower temperatures, with AOB dominating in the system (Guo et al., 2010).

Temperature acts as a key parameter which can severely affect ammonium oxidation rate (AOR) during PN (Weon et al., 2004; Kim et al., 2008; Guo et al., 2010). On the other hand, the differences caused by decreasing temperature can also affect many other aspects, such as AOB growth rate, enzymatic activities related to the nitrification process, the concentration of free ammonia (FA) and/or free nitrous acid (FNA) which can further affect the performance of PN (Gabarró et al., 2012), as well as the equilibrium solubilities of N_2O and NO.

Firstly, AOR was shown to have an exponentially relation with temperature in a PN-SBR treating domestic wastewater in a range of 5 to $35^{o}C$ (Guo et al., 2010). Moreover, AOR increased with the increased temperature in a range of 10 to 30 ℃ (Kim et al., 2008). There are some studies also suggesting that temperature may have an effect on N_2O production in PN. Recently, two different studies have tried to better understand its role on the N_2O emission in two laboratory nitrifying reactors treating low strength wastewater (Chen et al., 2016; Reino et al., 2017). Chen et al., (2016) found different emission factors when operating a simultaneous nitrification and denitrification SBR within a temperature range from 21℃ and 31 ℃ treating synthetic domestic wastewater, being the highest emission detected at higher temperature. In the same line, Reino et al., (2017) found different emission factors when operating a PN reactor under low strength of ammonia and nitrite at $10 ^{o}C$, $15 ^{o}C$ and $20^{o}C$. In their studies, the highest emission

84 factor corresponded to the highest temperature tested which also resulted in the highest

85 AOR. In another study, the effect of the temperature was assessed in a mix culture

86 performing both nitrification and denitrification and the largest N_2O was found at 15^oC

87 (Hu et al., 2011). Furthermore, in a full-scale nitrifying biofilter, N_2O emission was found

88 to be 2.1 times higher in winter than that in summer which shows an opposite trend of

89 N_2O compared to the temperature (Bollon et al., 2016). From these studies it is still

90 unclear if temperature can have a direct effect on emissions a part of the one that has on

91 AOR. Also, the effect of temperature on NO is unknown.

92   In this study, the effect of temperature shifts on N_2O and NO and their correlation

93 with AOR was studied by using an AOB dominated PN-SBR treating synthetic reject

94 wastewater. The reactor was exposed for 3 weeks at each of the 5 different temperatures

95 (30-25-20-15-10 ºC) tested and its performance in terms of emissions and chemical

96 changes was periodically monitored.

97

2. Materials and Methods

2.1. Configuration of the PN-SBR

100   A cylindrical SBR treating synthetic reject wastewater (1 g NH_4^+-N /L) was used to

101 develop PN system enriched with AOB. The wastewater characteristics were of a

102 typical reject wastewater and modified from Kuai and Verstraete (1998): 5.63 g/L of

103 NH_4HCO_3 for, 0.064 g/L of each KH_2PO_4 and K_2HPO_4 and trace element. The recipe of

104 trace element solution (g/L) is composed by: 1.25 EDTA, 0.55 $ZnSO_4 \cdot 7H_2O$, 0.4

105 $CoCl_2 \cdot 6H_2O$, 1.27 $MnCl_2 \cdot 4H_2O$, 0.40 $CuSO_4 \cdot 5H_2O$, 0.05 $Na_2Mo_4 \cdot 2H_2O$, 1.37 $CaCl_2 \cdot$

106 $2H_2O$, 1.25 $FeCl_3 \cdot 6H_2O$ and 44.40 $MgSO_4 \cdot 7H_2O$. The PN-SBR was inoculated with

107 mixed liquor sludge from a local domestic wastewater treatment plant (WWTP) located

108 at the municipality of Girona (Spain). Before starting the tests, the reactor was operated

109 for more than 2 years at 30ºC , mimicking the temperature conditions for treating reject

110 wastewater. AOB was highly enriched with more than 80% of the total abundance

111 which was quantified through fluorescence in situ hybridization (FISH) (Ribera-Guardia

112 and Pijuan, 2017).

113   The effect of temperature shifts on N_2O and NO emissions was assessed under five

114 different temperatures: 30ºC, which was the normal temperature, and four progressively

115 decreased temperatures as follows: 25ºC, 20ºC, 15ºC and 10ºC. The reactor was operated

116 for more than 3 weeks at each temperature.

117   The operational mode was set as: settling (132 min), decant (15 min, withdraw 2L

118 of supernatant), pre-aeration (15min), feed 1 (1 min 15 sec), aeration 1 (AER1), feed 2 (1

119 min 15 sec), aeration 2 (AER2), purge (0-2 min according to the MLVSS concentration).

120 Pre-aeration phase was only implemented when monitoring N_2O and NO emissions, in

121 order to remove the dissolved N_2O and NO produced in the settling phase. During the

122 feed phases (both feed 1 and feed 2), 1L of synthetic influent was pumped into the reactor

123 respectively resulting in an 8L working volume after feed 2. Aeration phases at 30ºC and

124 25ºC were set at 105 min and 103 min in AER1 and AER2 respectively. However, AOR

125 dropped with the decrease of the temperature at 20ºC, 15ºC and 10ºC. Thus, to maintain a

126 certain ammonia oxidation and an equally HRT, the aeration time was extended and

127 settling time was correspondingly reduced. The aeration time was fixed at 120 min, 180

128 min and 300 min at $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively. For the cycles conducted at 15ºC,

129 the cycle time was extended to 8h to allow a complete oxidation of ammonium. In the

130 case of the cycles conducted at 10ºC, only the first feed was supplied and the cycle was

131 also extended to 8h.

132   During the pre-aeration and the two aeration phases, DO was automatically

133 controlled within the range of 1.5-2.0 mg O_2/L by adding air or nitrogen gas at 5L/min.

134 The air or N_2 flow was supplied through the bottom of the SBR. When the DO was higher

135 than 2.0 mg/L, air stopped and N_2 was switched on to keep the DO within the selected

136 range and maintain a constant flux at 5.0L of gas /min. pH was also controlled by PLC

137 when its value was below 7.0 by adding $NaHCO_3$ (1M). The temperature of the reactor

138 was controlled at each desired set-point by using a water jacket.

139

140 **2.2. Analytical methods and gaseous measurements**

141   Two cycle studies per week were conducted resulting in 6 cycle studies carried out at

142 each temperature. Samples for the analysis NH_4^+-N, NO_2^--N and NO_3^--N were taken

143 during the cycle studies and filtered with 0.22μm Millipore filters. No NO_3^--N was

144 detected in any of the aeration phases ($< 1\%$) and in any of the temperatures tested.

145 Samples were analyzed using ion chromatography (ICS5000, DIONEX). Mixed liquor

146 suspended solids (MLSS) and volatile MLSS (MLVSS) were determined as described in

147 standard methods (APHA, 2005).

148   N_2O and NO emissions were measured using commercially available online gas

149 analyzers Servomex 4900 and ECO physics CLD60, respectively. Off gas from the

150 headspace of SBR was collected continuously and passed through a condensation unit

151 before reaching the online analyzer. The two analyzers were installed in series and data

152 was logged with a 5 seconds interval. The lower detection limit for the N_2O and NO

153 analyzer was 0.1 ppmv and 0.01 ppmv respectively.

154

155 **2.3. Calculation of gaseous productions**

156   Specific ammonia oxidation rate (*s*AOR) was calculated as shown in eq.1. The

157   relationship between *s*AOR and temperature can be described in the simplified

158   Arrhenius equation (Sözen et al., 1996) as eq.2:

$$sAOR = dC_{NH_4^+-consumed}/(dt \times MLVSS) \qquad \text{(eq.1)}$$

$$r_T = r_{T293} \cdot \theta^{(T-293)} \qquad \text{(eq.2)}$$

161   where total consumed ammonia concentration ($C_{NH_4^+-consumed}$), solids concentration

162   (MLVSS) and aeration time (*d*t is time interval, min), the r_T is reaction rate (i.e.

163   maximum *s*AOR, determined by the slope of ammonia concentration along with the

164   aeration time) at the temperature T (°K) and θ is the temperature coefficient. The

165   ammonia oxidation reaction presented a zero-order reaction kinetics after the feed was

166   added into the reactor. Mixed liquor samples were taken at 0, 10 min,1/4 (25min at 30 °C

167   and 25°C, 30min at 20°C, 45min at 15 °C and 75min at 10 °C), 1/2 (50min at 30 °C and

168   25°C, 60min at 20°C, 90min at 15 °C and 150min at 10 °C), 3/4 (75min at 30 °C and 25°C,

169   90min at 20°C, 135min at 15 °C and 225min at 10 °C) and end of aeration time in each

170   aeration phase. The *s*AOR could be determined by the absolute value of linear fitting

171   (ammonia concentration and time stand for 'y' and 'x' respectively).

172   The equations used to calculate the emission of N$_2$O (eq.3) and NO (eq.4) are detailed

173   below.

$$C_{N_2O} = 28(g/mol) \cdot 10^{-6} \cdot C_{N_2O}(\text{ppmv})/RT \qquad \text{(eq.3)}$$

$$C_{NO} = 14(g/mol) \cdot 10^{-6} \cdot C_{NO}(\text{ppmv})/RT \qquad \text{(eq.4)}$$

176   where $\frac{1}{RT}$ is the N$_2$O or NO molar volume being 0.0431 at 10 °C, 0.0423 at 15°C,

177   0.0416 at 20°C, 0.0409 at 25°C, 0.0402 at 30°C).

178   The emission factor (EF) of both N$_2$O and NO was calculated as described in eq.5 and

179   eq 6.

180
$$EF_{N_2O} = \sum_{t_0}^{ti} C_{N_xO}\, Q_{gas}\, dt\, /m_{NH_4^+} \times 100\% \qquad \text{(eq.5)}$$

181
$$EF_{NO} = \sum_{t_0}^{ti} C_{NO}\, Q_{gas}\, dt\, /m_{NH_4^+} \times 100\% \qquad \text{(eq.6)}$$

182

183 Where t_i is aeration time at each temperature (detailed in section 2.1), t_0 is the time

184 while the aeration starts in both AER1 (pre-aeration time used to remove dissolve gases

185 produced during settling and not considered in the calculations) and AER2. Q_{gas} is the

186 gas flow rate (5.0 L/min) and $m_{NH_4^+}$ is the total ammonia oxidized in each aeration

187 phase $m_{NH_4^+} = (C_0 - C_t)V_i$, while C_0 and C_t are the NH_4^+-N concentration at the

188 beginning and end of each aeration phase. V_i is the working volume at each aeration

189 phase (AER1=7L and AER2=8L).

190

191 **2.4. Microbial analysis**

192 Fluorescence in situ hybridization (FISH) was performed at the end of the

193 experiment as detailed in Nielsen et al. (2009) using Cyt5-labelled EUBmix and

194 Cyt3-labelled AOBmix for labeling all bacteria and AOBs respectively, AOBmix

195 contains equal amounts of Nso1225, NEU and NmV probes. Samples were visualized

196 using Nikon CS1 confocal laser-scanning microscope (CLSM, Plan-Apochromat 63 x oil

197 (NA1.4) objective). Thirty images were selected and quantified using the software

198 DAIME (Daims et al., 2006).

199

200 **2.5. Statistical analysis**

201 The statistical analysis was conducted with the results obtained with all the

202 temperatures except for the 30ºC. This temperature was excluded from the analysis

203 because the reactor had been operated under this temperature for more than 2 years and

204 the biomass adaptation under this temperature was different than the adaptation under the

205 other temperatures. The averaged emission factors at each temperature were processed in

206 statistical analysis by using IBM SPSS Statistics 21.

207

208 3. Results

209 3.1 The performance of PN-SBR at different temperatures

210 After each aerobic phase, the reactor achieved more than 90% of ammonia

211 conversion to nitrite (except for the 10ºC experiment where ammonia accumulated). In all

212 temperatures, nitrate concentration was negligible which indicated that the nitritation

213 performance of the system was not disturbed by decreasing the temperature. The nitrogen

214 transformations as well as the AOR measured during the operational period at different

215 temperatures are shown in Fig. S1.

216 MLVSS was measured throughout the study, having an average concentration of 1.5

217 g/L. According to eq.2, the maximum sAOR was calculated for each temperature and

218 results are plotted in Fig. 1.

219

220 Fig.1

221

222 A relatively high sAOR was achieved in the AOB-enriched SBR at temperatures of

223 30, 25 and 20ºC. Decreasing the temperature at 15ºC induced a 50% decrease in the

224 sAOR which was further decreased to almost negligible levels when the temperature was

225 decreased to 10ºC. The relationship between sAOR and temperature was found to be

226 exponential which is consistent with Guo et al., (2010). However, in this case, two

227 different correlations were distinguished, one for temperatures of 30- 20ºC and another

228 one for the lowest temperatures tested (15- 10ºC). This is in agreement with Weon et al.

229 (2004) who also found a transition on the $sAOR$ occurring in the range of 17-12ºC.

230   AOB abundance was also detected through FISH technique and the result indicated

231 that the volume fraction of AOB of the bacterial community was 96.7% with congruency

232 of 96.0%. A micrograph taken from one of the FISH sample preparations is shown in Fig.

233 S2. FISH results are comparable to the previous study of Ribera Guardia and Pijuan

234 (2017) for a similar SBR operating at 30ºC. Also, Lee et al. (2011), showed in their study

235 that AOB concentration and community composition did not change when shifting the

236 temperature from 22 to 32 ^{o}C.

237

238 **3.2 N$_2$O and NO emission at different temperatures**

239 3.2.1 N$_2$O and NO emission profiles

240   The cycle started with a 15 min pre-aeration phase (not shown in the graphs

241 presented), where air/N$_2$ was supplied to remove the dissolved N$_2$O and NO that was

242 produced during the settling phase as was previously demonstrated by

243 Rodriguez-Caballero et al., (2013). At the end of this 15 min, when ammonium was

244 always undetectable, the first feed started. Fig. 2 shows the N$_2$O and NO emission rates as

245 well as the ammonium concentration profiles from the AER1 (feed 1 is also included) and

246 the AER2 (feed 2 is also included).

247

248   Fig.2

249

11

250 N$_2$O started to be produced as soon as ammonium entered the reactor (Fig. 2a). In all

251 the cycles except for the one conducted at 10ºC, emissions during AER1 were higher than

252 those from AER2. Also, they were produced during the first 20-40 minutes of the aeration

253 period in the form of a peak despite ammonium being present during almost all the

254 aeration phase (Fig. 2c). This N$_2$O peak emission pattern is also reported in other studies.

255 Kong et al (2013) studied a PN for treating high strength ammonium wastewater at 32°C

256 and found that the majority of the N$_2$O was detected as a peak at the beginning of the

257 aeration phase and stabilized in a lower level during the rest of the aeration phase. Ju et al,

258 (2015) also reported an initial N$_2$O peak but in that case it seemed to be linked to the AOR

259 that also decreased together with the N$_2$O production rate. However, in our study,

260 ammonia oxidation was constant during all the aeration period (Fig. 2c), indicating that

261 N$_2$O emissions are not directly linked to AOR. Our hypothesis is that N$_2$O emissions are

262 linked to the supply of feed into the reactor. When the first feed enters the reactor, the

263 system has been without ammonium for more 120 min (considering the settling time and

264 the pre-aeration phase). The sudden increase of ammonium with the first feed, implies a

265 change on AOB activity, moving from a low activity period (without ammonia) to a high

266 activity period. This change in activity seems to be responsible for the emissions detected

267 at the beginning of AER1. On the other hand, emissions from AER2 are much lower

268 despite also having a supply of ammonium with the entrance of feed-2 into the reactor.

269 The difference here is that ammonium is present in the reactor for almost all the first

270 aeration, so AOBs are active most of the time before the second feed enters the reactor.

271 Therefore, when ammonium is supplied during feed-2, the change on AOB activity might

272 be not as significant as in AER1, which could explain the lower emissions detected during

273 this period. In order to validate this hypothesis, a test was conducted in the reactor when

12

274   operating at 30ºC, where pulses of ammonium were supplied when ammonium was

275   depleted (Fig. 3a) and when ammonium was still present in the reactor (Fig. 3b).

276

277   Fig.3

278

279   The N_2O production rate during the first minutes after a pulse of ammonia was added

280   was higher in the tests where ammonia was added every 120 min, when there was not

281   remaining ammonia in the reactor (Fig. 3a).

282   Transient conditions have been already shown to enhance N_2O production in AOBs.

283   Yu et al. (2010) suggested that N_2O could be stimulated during the recovery from anoxic

284   to aerobic conditions, especially when a sudden shift from a low to a high AOB activity

285   occurred. The trigger of N_2O peak emission in AER1 might correlate with the situation of

286   microbial activity during the transition of low to high activity as well as enzymatic

287   activities which can also be affected by temperature.

288   On the other hand, NO emissions occurred while ammonium was present in the

289   reactor and seem to be linked to its oxidation (Wang et al., 2016). The emission profile

290   was very similar between AER1 and AER2. Higher emissions were found at higher

291   temperature tests but those were linked to the higher AOR.

292   N_2O and NO emissions from both aeration phases of the cycle were calculated under

293   each temperature and are shown in Fig. 4. N_2O emission during AER1 was always

294   significantly higher than in AER2 (Fig. 4a). After each decrease of temperature, N_2O

295   emission was firstly reduced to a lower level (lower than the average N_2O emission) but

296   increased afterwards in the cases of temperature 25 and 20ºC which indicated that an

297   adaptation period was needed and this period could last several days after a decrease of

298   temperature. However, the maximum sAOR (Fig. 1) did not change significantly during

299   the cycles conducted under the same temperature, suggesting that emissions were not

300   only linked to AOR but possibly to the adaptation to the new temperature applied.

301   Interestingly, the increase on N_2O emissions was not observed at 15ºC and 10ºC,

302   probably because the ammonium oxidation rate at these temperatures was already very

303   low (Hu et al., 2011).

304

305   Fig.4

306

307   NO emission during temperature decreasing tests is shown in Fig. 4b. Emissions

308   were lower than those of N_2O and no significant differences were observed between

309   AER1 and AER2. The highest NO emission rate was found when the reactor was

310   operating at 30 and 25 ºC, however NO decreased when operated at 25 and 20 ºC. After

311   operating under T10 for several weeks, the maximum AOR was very low, which lead

312   the lowest NO emission rate.

313

314   3.2.3 N_2O and NO emission factors

315   In order to compare the emissions obtained among the experiments and between the

316   two aeration periods, the N_2O and NO emission factors (EF) were calculated to figure out

317   the productive capacities of N_2O-N and NO-N during total NH_4^+-N oxidation. Table 1

318   presents a summary with the average emission factors obtained from all the cycle studies

319   conducted under each temperature.

320

321   Table 1

322

323   Interestingly, temperature seems to have an effect on emissions occurring during

324   AER1. Except for emissions found at 30ºC (which was the reactor normal operational

325   temperature), the highest N_2O emission factor was found at 25ºC decreasing afterwards

326   with each decrease of temperature.

327   However, no effect was observed in emissions from AER2, which were very similar

328   among all the temperatures. A possible explanation for this behavior could be that a

329   decrease of temperature has an effect during the activation of the metabolism of AOB that

330   causes and increase on emissions. However, this increase is not observed at 15ºC or 10ºC,

331   probably because the ammonium oxidation rate at these temperatures is already very low,

332   and the change from low to high activity when ammonium becomes available is

333   minimized by the low temperature.

334   Interestingly, the NO emissions detected during the whole cycle are not affected by

335   temperature. It is still unclear how AOB produce NO and the correlation with N_2O

336   emissions. Indeed, a recent study already shows a different behavior between N_2O and

337   NO emissions in a similar AOB culture (Ribera-Guardia and Pijuan, 2017). NO seems to

338   be more related to the oxidation of ammonium while N_2O could be more related to

339   changes on biomass activity driven by the presence of ammonium.

340

341   **4. Discussion**

342   **4.1 Effect of temperature on nitrification**

343   Temperature plays a key role on the activity of microbes and a clear example is that

344   oxidation of NH_4^+ is clearly affected by its changes (Fig. 2c). The bacterial activity

345   decreases with a decrease in temperature and nitrification is very limited when

346   wastewater temperature is below 10°C (Kim et al., 2006). The highest sAOR is

347   normally found at around 30 ºC as shown in studies such as the one of Randall and Buth

348   (1984), that followed nitrification activity within the range of 5 to 35°C. They found the

349   maximum AOR occurring at approximately 30°C and nitrification rate was reduced to

350   50% at 12°C. In our experiments the max sAOR dropped more than half when operating

351   at 15 and 10ºC (Fig. 1, yellow line), suggesting that the activity of different nitrifying

352   microbial communities can be differently affected by temperature.

353   Temperature can also affect the competition among different microbial groups as it

354   is the case between AOB and NOB. It is well known that AOB has a faster growth rate

355   than NOB at temperatures around 30ºC favoring the implementation of the nitrite

356   pathway when working under this temperature (Hellinga et al., 1998). Also, temperature

357   is an important factor that regulates the equilibrium between ammonium and free

358   ammonia (FA), the true substrate for AOB (Suzuki et al., 1974). The higher the

359   temperature, the more concentration of FA.

360   Several studies have assessed the effect of decreasing temperature on partial

361   nitrification systems. Guo et al. (2010) found that both the rate of nitrite accumulation

362   and the oxidation of NH_4^+ decreased with the decreasing temperature but partial

363   nitrification was still predominant at low temperatures in a long term operation.

364   Lochematter et al. (2014) also maintained the NO_2^- pathway in a reactor at 15ºC by

365   using intermittent aeration. Similarly, in our study no deterioration of the nitrite

366   pathway was found in any of the temperatures tested and nitrate was never detected.

367   This is probably due to the high abundance of AOB in the microbial community that

368   was enriched by operating the reactor at 30ºC for a long period of time and with a high

369   strength wastewater.

370

4.2 Temperature effect on N_2O and NO emissions

Unravelling the true effect of temperature on N_2O and NO emissions by AOB is challenging because it has a direct effect on AOR which has been demonstrated to have a relationship with N_2O emissions. The relationship between different AOR (not caused by temperature shifts) and NO and N_2O emissions have been reported in literature, and found to be linear (Ribera-Guardia and Pijuan, 2017) and exponential (Law et al., 2012a) respectively. In our study, the temperature presents a significant correlation with $sAOR$ in both aeration phases ($p<0.05$) respectively (see the Pearson correlation in Table S1, N refers the numbers of total cycle studies, no AER2 was set at 10^oC). When analyzing the relationship between temperature and overall cycle emissions, no correlation was found. However when correlation between temperature and N_2O was assessed for each aeration phase, a significant correlation was found in AER1 ($p<0.05$), decreasing emissions when temperature decreased. (in the range of 25 to 10ºC). There was no significant correlation between NO and temperature and the emission factors were much lower than those of N_2O emission.

Chen et al., (2016) reported N_2O emission factor of 8.07% at 31ºC which was 2 times higher than the one found at 21ºC. Reino et al. (2017) further hypothesised that different kinetic dependency of the ammonia monooxygenase (AMO) enzyme and the hydroxylamine oxidoreductase (HAO) enzyme were affected by shifting temperature within the range of 10 to 20ºC.

They suggested that the limiting step for ammonia oxidation could have changed from AMO to HAO when the temperature was under 20^oC which might have promoted

393   the accumulation of hydroxylamine and further triggered N_2O emission resulting in $3.7 \pm$

394   0.5% at 20ºC while $1.5 \pm 0.3\%$ N_2O/TN was detected at 10ºC.

395   It also has to be taken into account that temperature shifts can alter the equilibrium

396   of ammonia and free ammonia (FA) concentration in the system which have an effect

397   on the performance of PN (Shi et al., 2011). Also, N_2O solubility increases while

398   temperature decreases within the range of 15 to 30ºC (Weiss and Price, 1980). However,

399   we believe these parameters did not affect our results. The air/N_2 flow rate was set as

400   5L/min in our system which most likely striped most of the N_2O and NO from the liquid

401   phase. Also, after each feed, the FA was 11.22 and 3.98 mg FA/L (average NH_4^+-N

402   concentration was 120mg/L and pH 8) when operating at 30 and 15ºC. However, no

403   significant difference was found in EFN_2O in AER2 (Table 1) when considering the

404   N_2O and NO emission of TN oxidized, which suggests that this range of FA

405   concentrations do not have an impact on the emissions found.

406   The emissions detected in our study have been fully attributed to the metabolism of

407   AOB. However, a previous study found a considerable increase of N_2O emissions when

408   treating real reject wastewater as compared to the ones found with synthetic reject

409   wastewater (Wang et al., 2014). These increased N_2O emissions were attributed to

410   heterotrophic denitrification which was very active when the reactor operated at DO

411   concentrations of 0.5 mg/L. Real reject wastewater can have a small fraction of

412   biodegradable COD allowing the development of an active denitrifying community. How

413   temperature affects emissions from this community remains unknown and more research

414   is needed to fully clarify the role of temperature in mixed microbial communities.

415

416   **5. Conclusions**

A laboratory scale PN-SBR enriched with AOB was used to explore the N_2O and NO emissions under different temperatures (from 30ºC to 10ºC). The main conclusions obtained are:

a) N_2O emissions were related to the transient conditions from a low activity period (with no ammonium) to a high activity period (when ammonium was supplied in the reactor) and these transient conditions were more marked in the first aeration phase.

b) Under all the temperatures tested, N_2O production rate in the first aeration phase was higher than the one found in the second aeration phase, despite the same amount of ammonium being oxidized in each phase.

c) Temperature has an effect on the N_2O emissions from the first aerated phase in the range of 25 to 10ºC, resulting in higher N_2O being produced at higher temperatures. On the other hand, NO is not affected by temperature.

d) Ammonium oxidation was not correlated with N_2O production as opposite to NO that was produced when ammonium was being oxidized.

Acknowledgements

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550

551

575 **Table 1.** N_2O and NO emission factor (EF N_2O-N or EF NO-N/ NH_4^+-N oxidized, %)

| Temperature		30	25	20	15	10
N_2O	Aer1	0.30 ± 0.10	0.70 ± 0.21	0.62 ± 0.43	0.43 ± 0.15	0.15 ± 0.00
	Aer2	0.14 ± 0.04	0.15 ± 0.05	0.15 ± 0.05	0.14 ± 0.03	
NO	Aer1	0.04 ± 0.02	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	*
	Aer2	0.06 ± 0.02	0.06 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	

576 Below detection limit

577

578

579

580

581
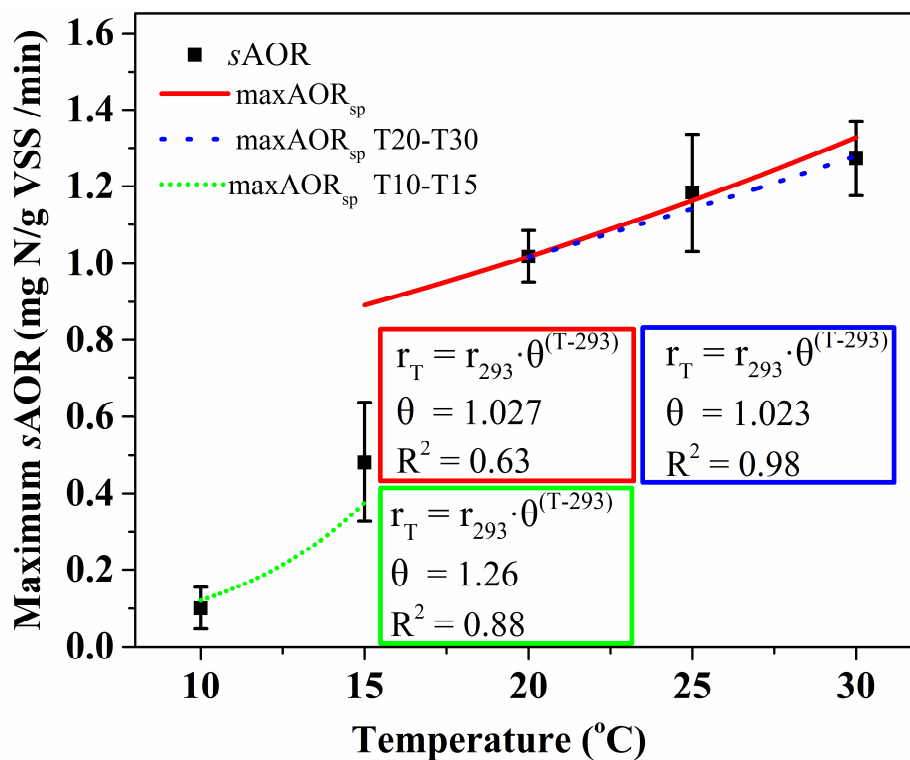
Fig. 1. Maximum *s*AOR obtained under each temperature tested. Plotted values are *s*AOR from all the cycle studies including AER1 and AER2 conducted under each temperature and are represented with the standard deviation.

585
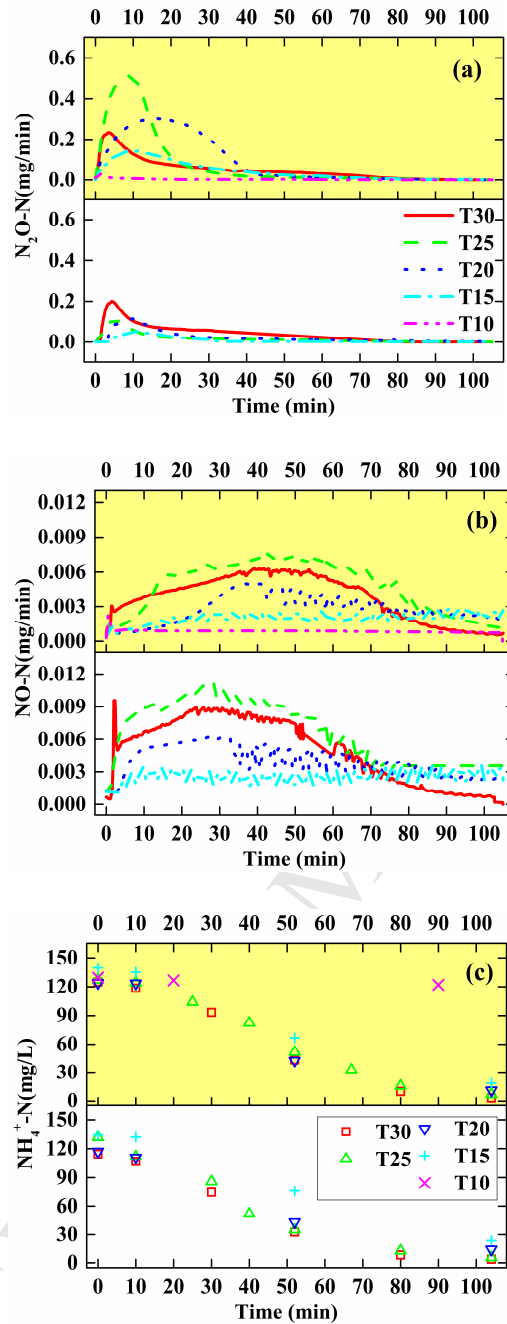
**Fig. 2.** N_2O production rate (a), NO production rate (b) and NH_4^+-N concentration (c) obtained during AER1 (yellow background) and AER2 (white background) from the last cycle study obtained under each temperature. Results from temperature 10°C are only plotted during aeration 1 since the cycle configuration did not have AER2.
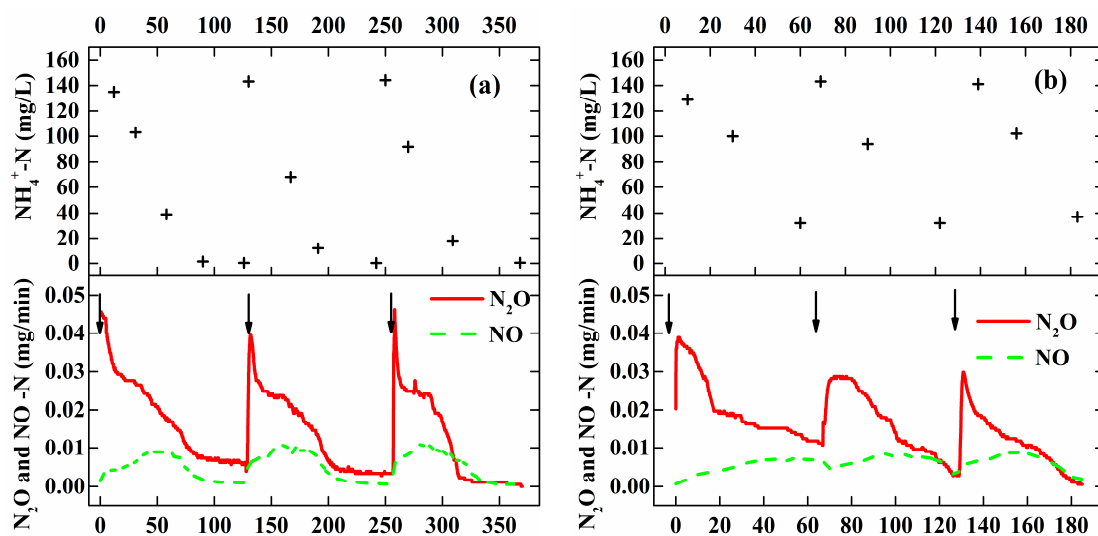
591   **Fig. 3.** N$_2$O and NO emission profiles and ammonia concentrations in two tests were

592   different pulses of ammonia where applied at different time intervals: a) Every 120 min

593   and b) every 60 min.

594

595
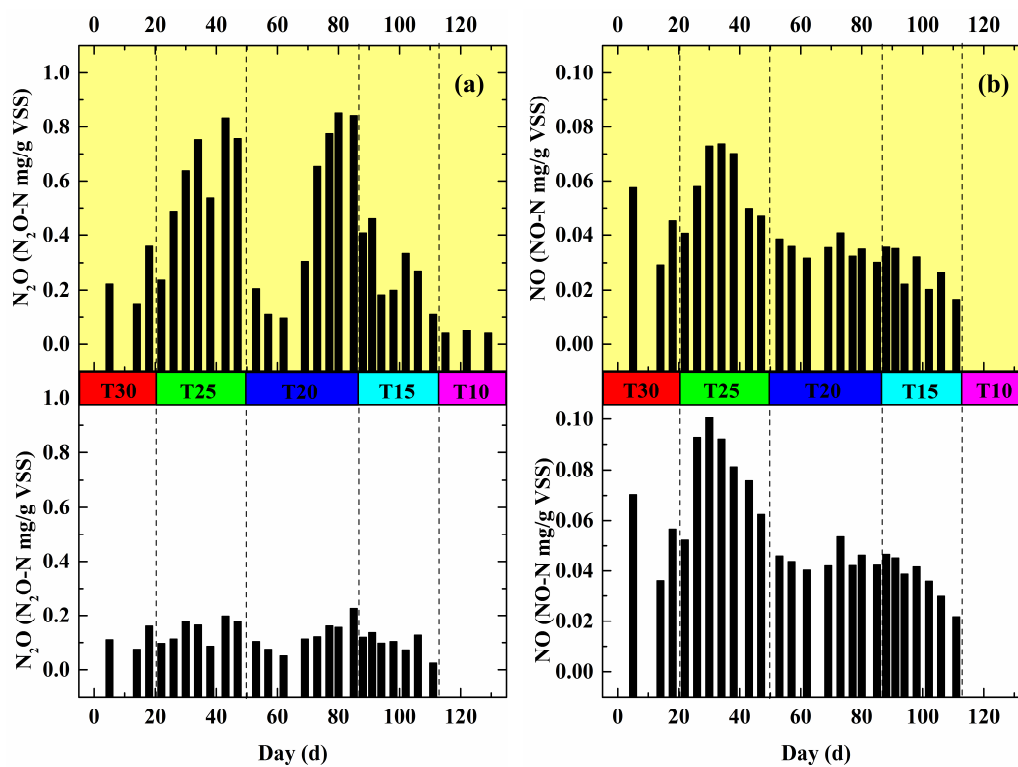
Fig. 4. Total specific N₂O (a) and NO (b) emission in the different cycle studies performed under each temperature. AER1 and AER2 are shown in yellow and white background respectively.

599

- N_2O and NO were measured in PN-SBR in the range of 30-10°C.

- N_2O peak emissions were related to the transition from low to high activity.

- N_2O emissions were affected by temperature in the first aeration.

- NO emissions were not affected by temperature.