1   **Modeling the decay of nitrite oxidizing bacteria under different reduction potential**

2   **conditions**

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21   **Highlights:**

22   A respirometric methodology was validated to measure NOB activity as OUR.

23   The NOB decay rate for anoxic conditions was 85% lower than for aerobic conditions.

24   The NOB decay rate for anaerobic conditions was 92% lower than for aerobic

25   conditions.

26   Simulating with proposed reduction factor renders up to 86% less effluent soluble N.

27

Abstract

Autotrophic growth and decay rates of ammonium and nitrite oxidizing bacteria (AOB and NOB, respectively) have a significant impact on the design and on the process performance of wastewater treatment systems where nitrification occurs. Literature data on the separate decay rates of AOB and NOB is scarce and inconsistent. In this study, batch experiments based on respirometric techniques were conducted to determine the NOB decay rates under different oxidation-reduction potential conditions, in order to widen the understanding of nitrite dynamics.

The decay rate measured under anoxic conditions was 85% lower than under aerobic conditions, whereas under anaerobic conditions the decay rate reduction was 92%. A design and simulation tool was used to assess the impact of applying these results in differentiated areas of an activated sludge system. Simulations show a greater impact for systems with a sludge retention time under 10 days, for which up to a 16-fold increase in NOB biomass concentration and up to 86% and 80% reductions in ammonium and nitrite concentrations in the effluent were calculated.

Therefore, this work demonstrates that considering different decay rates for autotrophic biomass under different ORP conditions avoids underestimating system performance and over dimensioning new activated sludge schemes.
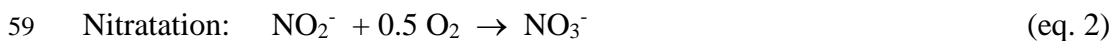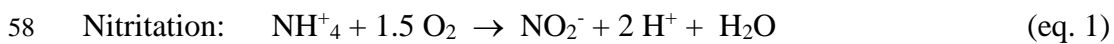
Keywords: ammonia oxidizing bacteria (AOB), decay rate, nitrite oxidizing bacteria (NOB), OUR, respirometry.

1. Introduction

The most used nitrogen removal strategy in wastewaters is the nitrification-denitrification process, through which ammonium is converted into nitrate (nitrification) and nitrate is converted to nitrogen gas (denitrification). Nitrification is a two-step process where two different groups of autotrophic bacteria take part: in a first step, ammonium oxidizing bacteria (AOB) convert ammonium into nitrite (eq. 1); in a second step, nitrite oxidizing bacteria (NOB) convert nitrite into nitrate (eq. 2). Denitrification is carried out by heterotrophic bacteria, which reduce nitrate and nitrite to nitrogen gas.

Nitritation: $NH^+_4 + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$ (eq. 1)

Nitratation: $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ (eq. 2)

For most municipal wastewater treatment systems under normal operating conditions, nitritation is the limiting step and there is practically no nitrite accumulation. Therefore, this component can be disregarded: traditionally, most activated sludge models include one-step nitrification in their structure (e.g. 1]). This simplification renders acceptable results for conventional operation of nitrification processes [2].

However, under certain conditions, nitrite peaks can be detected in activated sludge systems. They usually indicate a disturbance in the microbiological processes, which normally happens under unstable operation caused by a number of reasons: insufficient oxygen, low temperature, high temperature, low sludge retention time or presence of inhibitory compounds. Nitrite concentration in these cases needs to be controlled, since it can rise to toxic levels. One-step nitrification models cannot predict nor analyze these problems.

72   Besides the abovementioned cases, in the treatment of side streams and of industrial

73   wastewaters, processes where nitrite formation is specifically promoted are increasingly

74   used. In these processes (e.g. partial nitrification and anaerobic ammonium oxidation

75   processes) nitrite plays a fundamental role, and it becomes a key component to be

76   measured and controlled. For this task, models which account for the two-step

77   nitrification process have been developed or adapted in the last two decades [2-7].

78   These mathematical structures proposed by different authors to model nitrite show

79   discrepancies about some of the aspects involved, such as how denitrification occurs or

80   which nitrogen species are the active substrates. At the same time, significant variability

81   of model parameter values among different studies can be found. In those studies, some

82   of the adopted parameters were measured by the authors whereas some others were

83   assumed from different literature sources. A review on nitrite modeling in wastewater

84   treatment systems can be found in [2].

85   Growth and decay coefficients of autotrophic bacteria directly affect the performance of

86   nitrification, since they determine the amount of bacteria in the system and, as a result

87   overload and bacteria wash-out are phenomena that depend on them. Therefore, they are

88   the most important parameters affecting the design and operation of activated sludge

89   systems. Unlike growth, autotrophic decay is an uncertain process that has been seldom

90   studied. Still, Koch et al. [8] identified it in the set of sensitive parameters for ASM3.

91   The term decay represents the loss of bacterial activity, which includes maintenance,

92   lysis and predation, and is proportional to biomass loss. Specifically differentiated AOB

93   and NOB decay rates were not frequently measured in the studies referred above,

94   although some authors have developed and calibrated specific models for nitratation,

95   thus obtaining a decay coefficient for NOB [9].

96   It has been noted that NOB (and AOB) decay rates under anoxic conditions are smaller

97   than under aerobic conditions [10-13], although the range of observed decay rate

98   reduction ranges from 30 to 100% and therefore further research is needed to clarify this

99   phenomenon. Expanding the knowledge of the activity kinetics of the NOB and their

100   dependence on ORP conditions will allow for a better control of the nitrification process

101   and will help adapting the design of wastewater treatment plants for nitrification.

102   The aim of this work is to determine the NOB decay rate (b_{NOB}, d^{-1}) in different

103   reduction potential (ORP) conditions (aerobic, anoxic and anaerobic) by means of

104   laboratory batch experiments using respirometric techniques, which are simple and

105   reproducible. The studied biomass from which three different decay rates were obtained

106   was obtained from a pilot scale wastewater treatment plant. On the other hand,

107   simulations on an activated sludge system run with the software DESASS [14] were

108   used to assess the extent to which the use of differentiated NOB decay rates influence

109   the results (water quality) based on system parameters (mainly SRT and anoxic-

110   anaerobic-aerobic volumes) as compared to using an unified decay rate.

111

2. Material and methods

2.1 Setup descriptions

2.1.1 Pilot plant

115   NOB used in this study were obtained from a pilot plant which was located within the

116   full-scale WWTP "Conca del Carraixet" and treated its primary settler effluent. The

117   pilot plant had a modified University of Cape Town (UCT) scheme for both organic

118   matter and nutrient removal. Temperature was controlled, with a set point at 30 ˚C. The

119 average hydraulic retention time (HRT) and the SRT for the pilot plant were maintained

120 at 9.6 h and 7 days, respectively. The blower frequency was controlled to keep the

121 dissolved oxygen (DO) concentration in the aerated compartment around a desired set

122 point (2-2.5 $mgO_2 \cdot l^{-1}$). Figure 1 shows the layout of this process.

123

124 **2.1.2 Reactor 1**

125 *Reactor 1* consisted of an aerobic completely stirred tank reactor (CSTR) installed in the

126 laboratory, with a total volume of 10 L. Temperature was kept at 30 °C with the aid of a

127 thermostatic bath. Dissolved oxygen (DO) concentration in the tank was monitored with

128 a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET

129 WTW, Germany). An air blower was switched on when DO went below 2 mg O_2/L,

130 aerating the reactor through fine bubble diffusors installed at the bottom. The blower

131 switched off when DO reached 5 mg O_2/L.

132 **2.1.3 Reactors 2**

133 *Reactors 2Ae, 2Ax* and *2An* were used for achieving different ORP conditions in the

134 laboratory. Each had a working volume of 3 L. Their temperatures were controlled at 30

135 °C and the DO concentrations were monitored with a Cellox 325 electrode (WTW,

136 Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). *Reactor 2Ae* had

137 an aeration system analogous to that one described for *Reactor 1*. Nitrate was added to

138 *Reactor 2Ax* to sustain a concentration between 7 and 10 mg NO_3-N/L.

139 **2.1.4 Batch Reactor**

140 The *Batch Reactor*, with a volume of 300 mL, was used to examine the activity of the

141 NOB biomass present in the different reactors, by means of short specific respirometric

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142 experiments. This reactor was water jacketed for keeping temperature at 30 °C. An air

143 blower aerated the samples at the beginning of each specific respirometry. The DO

144 concentration was monitored like previously explained.

145 **2.2 Experimental procedure**

146 **2.2.1 Bringing biomass to endogenous conditions**

147 In the first place, the biomass from the pilot plant was brought to endogenous conditions

148 in *Reactor 1*. For this, the aeration control system described above was switched on and

149 biomass was therefore given enough oxygen (and time) to consume all possible

150 substrate. The periods when the blower was in off mode were used to determine the

151 Oxygen Uptake Rates (OURs), calculated (in Excel 2011) as the slope of the recorded

152 DO concentrations regression line. Endogenous conditions were achieved when the

153 OUR values remained practically constant, which happened after approximately 20 h.

154 After achieving endogenous conditions, the sludge in *Reactor 1* was split into reactors

155 *2Ae*, *2Ax* and *2An*, being the ORP the only difference among them, as previously

156 explained: in *Reactor 2Ae* the oxygen concentration remained over 2 mg O_2/L; in

157 *Reactor 2Ax* nitrate concentration was kept over 7 mg NO_3-N/L; *Reactor 2An* remained

158 under anaerobic conditions. There was no detectable nitrite in the reactors, and

159 therefore, in the absence of substrate, there was no NOB growth. NOB activity from all

160 three reactors was examined along the whole study, which lasted 160 hours, by

161 performing short respirometric studies as explained in section 2.2.3 below.

162 **2.2.2 Determination of NO_2 concentration required to achieve maximum growth**

163 **rate**

164 Prior to the respirometric studies, eight samples from the sludge under endogenous

165 conditions in *Reactor 1* were used for determination of the required nitrite concentration

166 to increase the growth rate up to its maximum. Each of these eight samples was

167 transferred to the *Batch Reactor* and kept under aeration before adding a certain nitrite

168 concentration ranging from 0.5 to 10 mg NO_2-$\cdot L^{-1}$. Each test was short enough (5 min)

169 to assume that biomass concentration in the reactor remained constant. Since (different)

170 substrate additions caused a (different) increase in the NOB activity, an increase in the

171 OUR could be measured each time (calculated as the difference between the measured

172 OUR before and after nitrite addition). The obtained values from the eight tests were

173 represented along a substrate concentration axis and thus the nitrite concentration

174 required to achieve the maximum growth rate could be determined. The affinity

175 constant for nitrite could also be obtained by minimizing the sum of the squared errors

176 between experimental and predicted data, which was done using the Solver function in

177 Microsoft Excel 2011.

178 **2.2.3 Respirometric studies**

179 Each respirometric study consisted in measuring the OUR increase of a specific

180 biomass sample due to nitrite oxidation by NOB. For this, the following steps were

181 followed: i) the sample was transferred to the *Batch Reactor*, where the initial OUR

182 (OUR_1) was first measured (corresponding to endogenous activity) as the linear

183 decrease in DO; ii) Nitrite from a 10 $g \cdot L^{-1}$ sodium nitrite solution was added manually

184 to the reactor in order to reach a concentration of at least 3 mg NO_2-$N \cdot L^{-1}$. The nitrite

185 concentration required for reaching maximum growth had been determined in a

186 previous experiment (see 2.2.2). The substrate pulse addition reactivated the NOB

187 biomass and was enough to reach maximum growth rate. iii) OUR was measured three

188 consecutive times after substrate addition, and the highest measured value was taken as

189   OUR$_2$; iv) The difference (OUR$_2$ - OUR$_1$) was calculated, which was, as expected,

190   always positive and corresponded to the oxygen uptake rate due to nitrite oxidation by

191   NOB v) the sample was disposed of.

192   In the case of *Reactor 2Ax* and *Reactor 2An* (see below) the sample had to be reaerated

193   before adding the nitrite, so that oxygen was available for substrate consumption. In all

194   cases pH was monitored to assure that it stayed between 7.5 and 8.5.

195   **2.3 Analytical methods**

196   Ammonium (NH$_4$-N), Phosphate (PO$_4$-P), Nitrite (NO$_2$-N) and nitrate (NO$_3$-N) were

197   determined by applying Standard Methods [15] (4500-NH3-G, 4500-P-F, 4500-NO2-B,

198   4500-NO3-H, respectively) in a Smartchem 200 automatic analyzer (Westco Scientific

199   Instruments, Westco).

200   **2.4 Simulations**

201   The software DESASS [14] was used to simulate a WWTP for biological nitrogen

202   removal. The mathematical model implemented in this software is the Biological

203   Nutrient Removal Model No.2 (BNRM2, [16]). In this study, the parameter values

204   proposed by Henze et al., [1] were used for heterotrophic bacteria. For ammonia

205   oxidizing and nitrite oxidizing bacteria the parameter values determined by Jimenez et

206   al., [17-18] and Jimenez [7] were used.

207   **3. Results**

208   **3.1 Determination of NO$_2$ concentration required to achieve maximum growth rate**

209   As explained before, each OUR was calculated with Excel 2011 as the slope of the DO

210   concentration regression line. R^2 was always above 0.97. An example can be seen in

211   figure 2. The different nitrite concentrations tested were plotted against the obtained

212   OUR increase due to their oxidation (specifically, against the percentage of this increase

213   over the maximum OUR measured). According to these results, the concentration

214   needed in further experiments to assure maximum growth rate of NOB was set to 3 mg

215   NO_2-N $\cdot L^{-1}$, since in that case 99.4% of the maximum OUR was already achieved

216   (figure 3).

217   Additionally, a Monod half saturation constant for nitrite (k_{NO}) of 0.378 mg NO_2-N$\cdot L^{-1}$

218   was calculated which properly reproduced the experimental data (figure 3), albeit with a

219   regression coefficient r^2=0.67. The decrease observed in the OUR when 10 mg NO_2-N

220   were added suggests a NOB inhibition by free nitrous acid (FNA) accumulation.

221   Values of k_{NO} found in the literature range from 0.008 mg NO_2-N$\cdot L^{-1}$ [19] to 3 mg NO_2-

222   N$\cdot L^{-1}$ [20]. Jiménez et al. [17] obtained a similar value of 0.26 NO_2-N$\cdot L^{-1}$ when

223   working with sludge obtained in the same pilot plant used in this study. Nitrite affinity,

224   and in general, substrate affinity, can vary among bacterial cultures due to biomass floc

225   morphology, nitrifier enrichment [21], conditioning processes and depending on the

226   history of the sludge (process characteristics in origin, wastewater strength, etc).

227   **3.2 Respirometric studies from biomass under aerobic, anoxic and anaerobic**

228   **conditions**

229   *Reactor 2Ae* maintained a culture of NOB under endogenous conditions and under

230   constant oxygen supply. Seven samples were taken and underwent the respirometric test

231   described in the *Batch Reactor*. The results, plotted in figure 4a, were seven points

232   representing the OUR (that is, the increase in oxygen uptake due to the supplemented

233   nitrite oxidation, calculated as the difference between the OUR before and after nitrite

234   addition) along the 160 hours of the experiment. As expected, the measured OURs

235   decreased with time up to a 100% decrease. Regression coefficients were always above

236   0.96.

237   The same procedure was applied to data obtained from *Reactor 2Ax* and *Reactor 2An*.

238   In *Reactor 2Ax* a reduction in the OUR of 40% was observed after 160h (figure 4b),

239   whereas in *Reactor 2An* this decrease was only of 20% (figure 4c). In all cases,

240   measured ammonium and phosphate concentrations increased with time during the

241   operation of the reactors (due to hydrolysis processes), confirming the limitation of

242   activity of nitrifiers due to the absence of DO.

243   Assuming that the measured OUR is directly proportional to the amount of NOB

244   biomass in the culture (measured as mg $COD \cdot L^{-1}$):

245   $OUR = k \cdot NOB$ (3)

246   And considering that the NOB decay function follows a first order kinetics with a decay

247   constant b (d^{-1}), in agreement with general literature (elsewhere):

248   $dNOB/dt = -b \cdot NOB$ (4)

249   Then equation (5) can be obtained after integration of equation (4) and combination

250   with equation (3):

251   $OUR = OUR_0 \cdot \exp(-b \cdot t)$ (5)

252   Equation 5 can be used to reproduce the obtained data. A decay constant was obtained

253   for each case (using the Solver function of Microsoft Excel 2011) which minimized the

254   error between experimental and predicted data (table 1).

255   The obtained aerobic decay rate is in the high end of the range found in literature (0.15

256   d^{-1} in [12]; 0.22-0.28 d^{-1} in [22]; 0.5 d^{-1} in [23], which could be partially explained by

257   the high temperature used in this study. On the other hand, it has been demonstrated that

258   decay rates are different in different systems [24] and therefore the origin, history and

259   degree of physiological adaptation of the culture can, at least partly, explain the

260   differences in literature data as well. Martinage and Paul [25], for instance, reported

261   increases of autotrophic decay rates from double to 4.5 times higher following changes

262   in the wastewater fed to the analyzed activated sludge system. It also has to be taken

263   into account that the design of the experiments used for the determination of the decay

264   coefficient could also be an important factor affecting the reported decay rates.

265   Obtained data in the current work showed that nitrite oxidizing bacteria of the studied

266   system present very low decay rates under anoxic conditions (85% smaller than aerobic)

267   as well as under anaerobic conditions (92% smaller than aerobic). These reduction

268   factors are among the highest values reported, in the range of those authors who found

269   reductions between 50 and 100% [12, 22, 23].

270   The exact reason for this decrease is not well known. It could be hypothesized that

271   maintenance or endogenous respiration, which involve consumption of external and

272   cell-internal substrate respectively, cannot take place under anoxic and anaerobic

273   conditions, since NOB are not able to utilize their own substrate due to the lack of

274   oxygen. Therefore, when again available, more substrate (and therefore oxygen) is

275   consumed (feast-famine phenomenon, [11]) and thus a higher activity than expected is

276   observed. This would explain that the measured OURs along the experiment do not

277   decay as fast as in aerobic conditions.

278   Another factor which could play a role in the decrease of the anoxic and anaerobic

279   decay constants could be predation by other microorganisms, which causes bacterial

280 losses and therefore decay in biomass activity. Predators, mainly protozoa, which are

281 present to a greater or lesser extent in activated sludge systems, are predominantly

282 obligate aerobes and therefore their impact in biomass loss is stronger under aerobic

283 conditions. Martinage and Paul [25] reported that 50% of their observed decrease in

284 autotrophic anoxic decay rate with respect to aerobic decay rate was explained by

285 grazers.

286 Salem et al. [24] performed respirometric studies which lasted between 3 and 14 days,

287 and obtained, for NOB in a nitrifier-enriched culture, anoxic decay rates which were

288 lower than the anaerobic rates -although the difference was very small (0.06 d^{-1} versus

289 0.07 d^{-1}). These rates were only 25% and 12.5% lower than the aerobic decay rates. In

290 an activated sludge system, the same authors found an aerobic decay rate of 0.21 d^{-1},

291 with anoxic decay rate 43% lower and anaerobic decay rate 71% lower. In view of the

292 results obtained in this study and the previous works on the matter, it seems reasonable

293 to conclude that: i) decay of NOB under aerobic conditions is confirmed to be higher

294 than under anoxic and anaerobic conditions *for the same culture* and ii) the origin, state

295 and history of the culture will influence both the aerobic decay rate and the decrease of

296 decay rate under anoxic/anaerobic conditions. It can be noted that the results of

297 fluorescence in situ hybridization (FISH) technique showed that NOB population in all

298 reactors was mainly composed of *Nitrospira* (data not shown).

299

300 **3.3 Simulations**

301 Two sets of simulations have been carried out in order to numerically assess the effect

302 of considering different lysis rates for different environmental conditions present in an

303 activated sludge system. A Modified Ludzack Ettinger scheme with an anoxic reactor

304 volume being one third of the total volume was simulated in the software DESASS,

305   using the mathematical model and parameters detailed in section 2.4, except for the

306   lysis rate of autotrophs under anoxic conditions, which was reduced by 85%. Although

307   the reduction factor of AOB decay rate under anoxic or anaerobic conditions has not

308   been determined, it has been assumed that the decay rate of AOB is affected by the

309   same reduction factor as for NOB since a decrease in the decay rate has also been

310   observed for AOB [24] . The influent wastewater pattern proposed in the Benchmark

311   Simulation Model n.1 [26] was used in this work. Therefore, the proposed MLE-scheme

312   WWTP was designed to handle an influent flow of 18,446 $m^3 \cdot d^{-1}$. The simulated

313   scheme is shown in the supplementary material and the concentrations of the main

314   pollutants are shown in Table 2.

315   In the first two sets of simulations settling and biological processes taking place in the

316   secondary settler were not considered. Thus, the secondary settler was simulated as a

317   pure concentrator. In these simulations the performance of the MLE-scheme for

318   nitrogen removal at different sludge retention times (SRT) (ranging from 7 to 22 days)

319   was evaluated considering the same lysis rate in all the environmental conditions (1st

320   set) and considering a reduction factor of 85% for lysis rate under anoxic conditions (2nd

321   set).

322   In the last two sets of simulations settling and biological processes taking place in the

323   secondary settler were considered. The settling processes model [27] integrated in the

324   BNRM2 consists in a one-dimensional model based on both the solids flux concept and

325   the conservation of mass. The settling velocity is obtained by using the model proposed

326   by [28] for the flocculated and hindered settling. The settling velocity in the lower

327   layers is corrected by a compression function similar to the one proposed by [29]. This

328   model is linked to the biological model in order to consider the biological processes

329   taking place in the ten layers in which secondary settlers were divided. As it was done

330   previously, in these simulations the effect of sludge retention time on nitrogen removal

331   was evaluated considering the same lysis rate in all the environmental conditions (3rd

332   set) and considering a reduction factor of 85% for lysis rate under anoxic conditions (4th

333   set). A summary of the simulation conditions for each set are shown in Table 3.

334   Considering the reduction of anoxic and anaerobic decay rates showed to have a greater

335   impact on NOB than on AOB biomass concentration. This impact was more relevant

336   when biological reactions were taken into account in the settler, since the settler acts as

337   a non-aerated reactor. NOB washout was predicted at a SRT of 7 days when the same

338   decay rate was used in all the ORP conditions. On the contrary, when a reduction factor

339   for the lysis rate was considered, the simulation predicted NOB proliferation.

340   Figures 5a and 5b show the effect of considering a decay rate reduction factor on the

341   effluent ammonium concentrations at different simulated SRT. When a decay rate

342   reduction factor was introduced the ammonium levels in the effluent were lower than

343   the respective values in the sets where the same decay rate was used for all ORP

344   conditions. This impact is more relevant at lower SRT, and when considering biological

345   processes in the secondary settler. The same trend can be observed for the nitrite levels

346   in the effluent (figures 6a and 6b). For complete nitrification in the calculated example,

347   a SRT of at least 10 days would be chosen based on simulations without a decay rate

348   reduction factor. On the contrary, results obtained when a decay rate reduction factor is

349   used indicate that a SRT of 8 days would suffice. Designing the system with an SRT of

350   10 days instead of 8 would lead to an excess reactor volume of 25% (for the same

351   suspended solids concentration).

352   These sets of simulations demonstrate that using the same decay rate under all ORP

353   conditions can have as a consequence that the performance of an existing system is

15

354 underrated when its operational conditions are simulated with tools such as the one

355 presented here, or that an activated sludge system is greatly over-dimensioned in its

356 design phase.

357 It has to be noted, however, that in a full-scale WWTP bacteria and predators change

358 their environmental conditions more frequently, and therefore biomass might not

359 behave exactly like in the laboratory reactors where anoxic and anaerobic conditions

360 were maintained for several days. Further research is needed to validate the calibration

361 methodology and the obtained results with data from full-scale WWTPs.

362 **4. Conclusions**

363 A fast and reproducible methodology was proposed to measure NOB activity in the

364 form of oxygen uptake rate. This respirometric method was applied to a UCT bacterial

365 sludge and an important reduction was observed for anoxic and anaerobic decay rates,

366 compared to those under aerobic conditions. Simulations of an Activated Sludge System

367 were performed taking this fact into account. Differences in the ammonium and nitrite

368 levels in the effluent obtained could translate in a 25% smaller designed reactor volume.

369 This procedure avoids underestimating the performance of an existing system or the

370 over dimensioning of a new Activated Sludge scheme.

371 E-supplementary data of this work can be found in online version of the paper.

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460

Figure legends

462 Figure 1: Layout of the modified UCT pilot plant origin of the NOB culture.

463 Figure 2: OUR calculation as the slope of the DO concentration vs. t regression line.

464 Figure 3: Dots: experimental OUR values (% over maximum OUR) plotted versus
465 concentration of added nitrite. Line: predicted values with $k_{NO} = 0.378$ mg NO_2-N·L^{-1}.

466 Figure 4: Dots: experimental data. Line: model calculation. a: OUR evolution under
467 aerobic conditions; b: OUR evolution under anoxic conditions; c: OUR evolution under
468 anaerobic conditions.

469 Figure 5: Ammonium concentration in effluent in a) Sets 1 and 2 and b) Sets 3 and 4,
470 using same and smaller decay rate and for each SRT simulated.

471 Figure 6: Nitrite concentration in effluent in a) Sets 1 and 2 and b) Sets 3 and 4, using
472 same and smaller decay rate and for each SRT simulated.

473 Figure S1: Screenshot from the software interface showing the simulated scheme and
474 settler effluent characteristics.

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476

Table 1: obtained decay constants for NOB in different OPR conditions

Decay constant b (d^{-1})		
Aerobic conditions	**Anoxic conditions**	**Anaerobic conditions**
0.528	0.078	0.040

477

478   Table 2: Concentrations of the main pollutants in the influent to the simulated WWTP

Parameter	Value	Unit
TSS	200	mg TSS $\cdot$ L^{-1}
VSS	160	mg VSS $\cdot$ L^{-1}
T-COD	381	mg COD $\cdot$ L^{-1}
S-COD	99.5	mg COD $\cdot$ L^{-1}
T-BOD20	300	mg COD $\cdot$ L^{-1}
S-BOD20	69.5	mg COD $\cdot$ L^{-1}
VFA	10	mg COD $\cdot$ L^{-1}
TN	50	mg N $\cdot$ L^{-1}
NH$_4$-N	31.5	mg N $\cdot$ L^{-1}
TP	6.9	mg P $\cdot$ L^{-1}
PO$_4$-P	5	mg P $\cdot$ L^{-1}
Alkalinity	350	mg CaCO$_3$ $\cdot$ L^{-1}
pH	7	

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Table 3: Simulation conditions for each simulation set performed

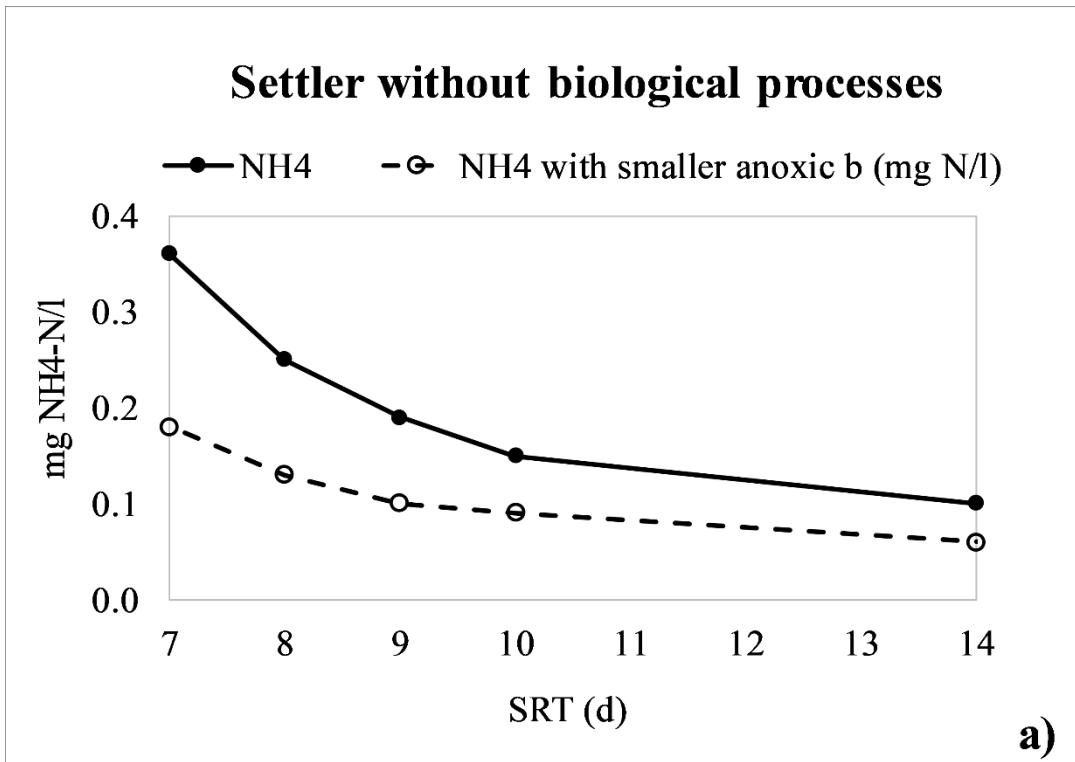| Set	Biological processes considered in Settler	b_{anox}/b_{aer}
1st	No	1.0
2nd	No	0.15
3rd	Yes	1.0
4th	Yes	0.15

483
484   Fig 1

485
486   Fig 2
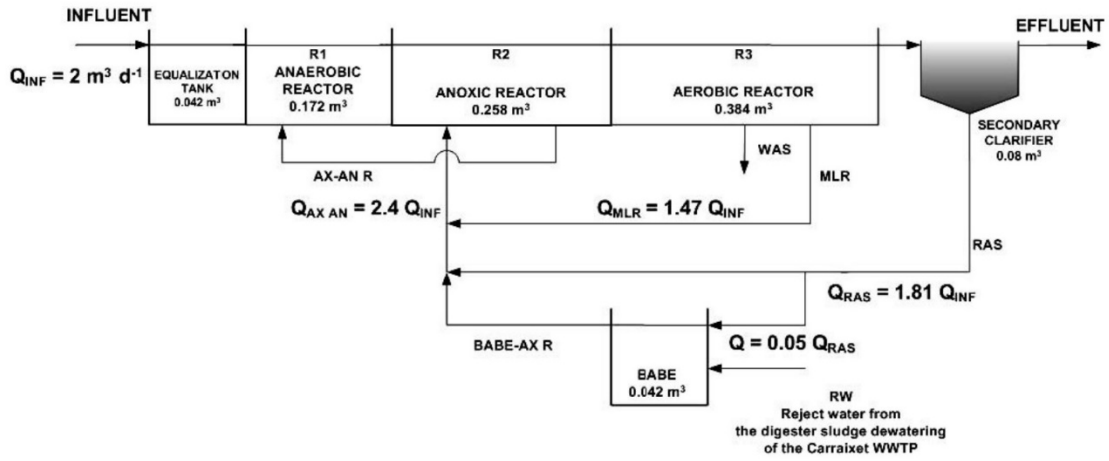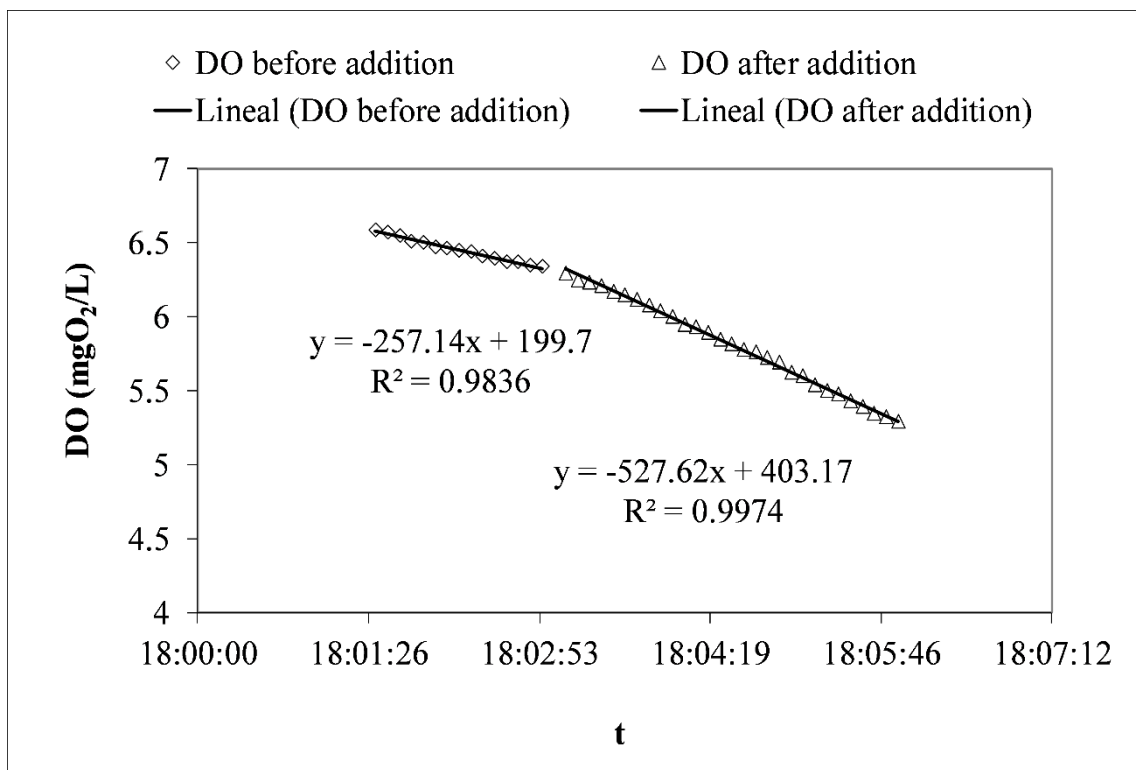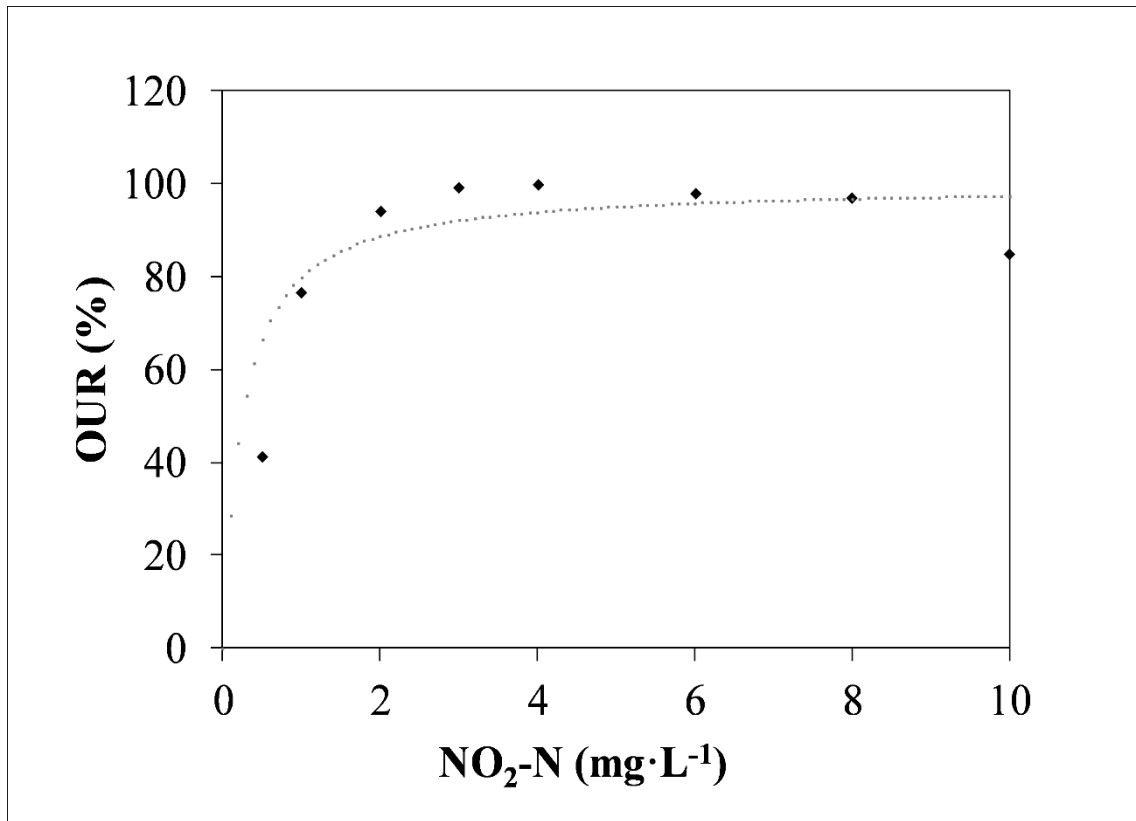
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490   Fig 3

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Evolution of NOB activity in Reactor An

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496   Figs 4a, 4b y 4c
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Settler without biological processes

a)

Settler with biological processes

b)

Figs 5a y 5b

Settler without biological processes
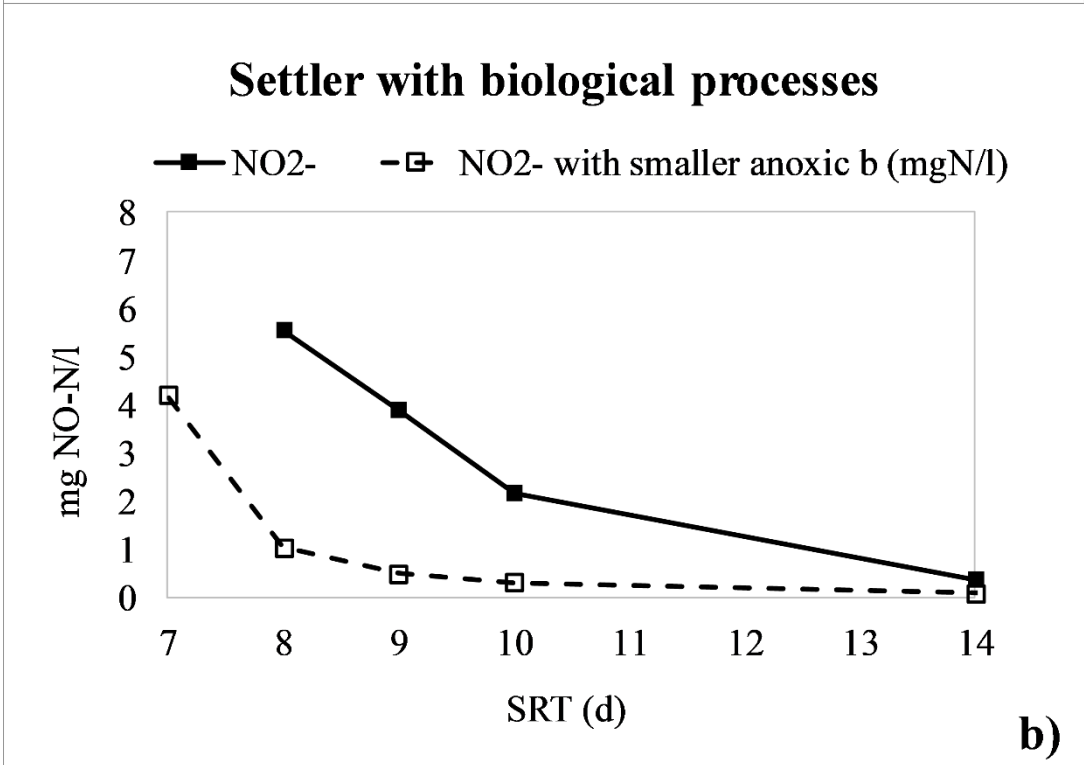
a)

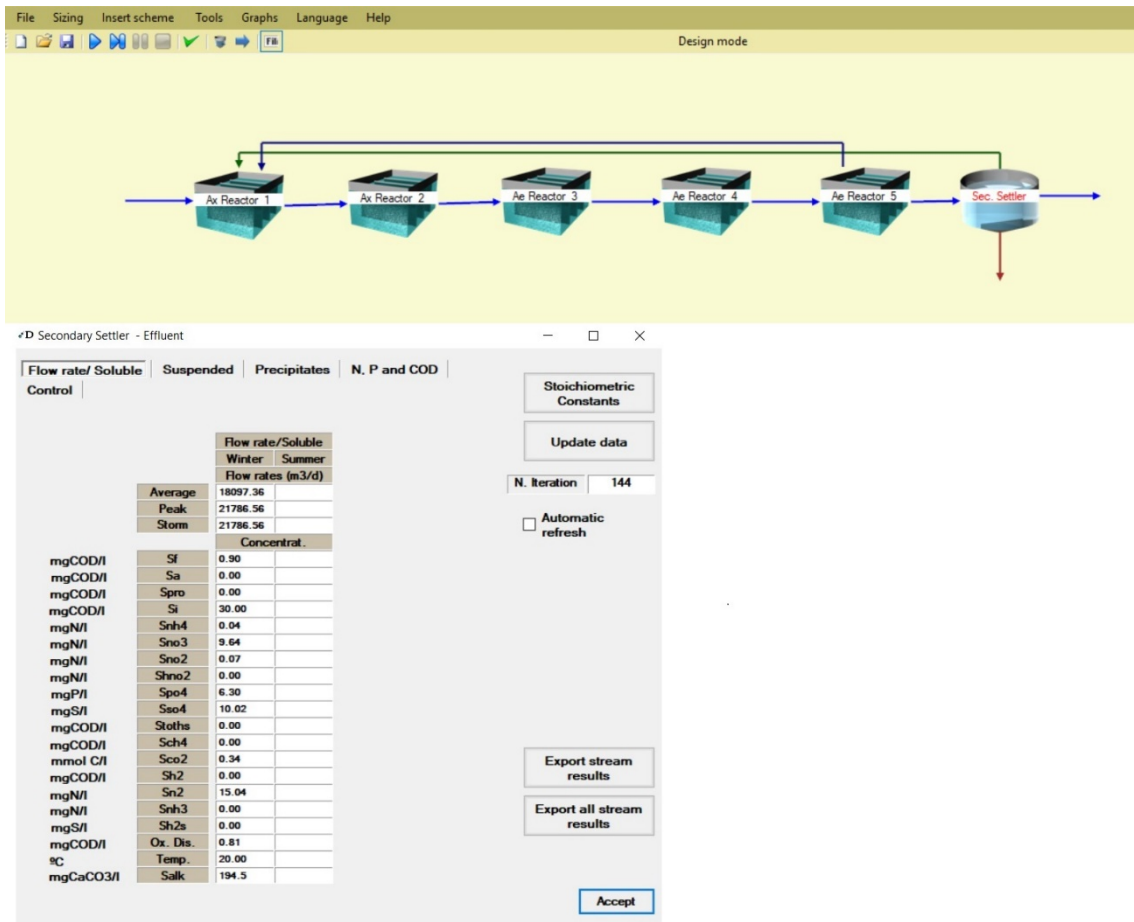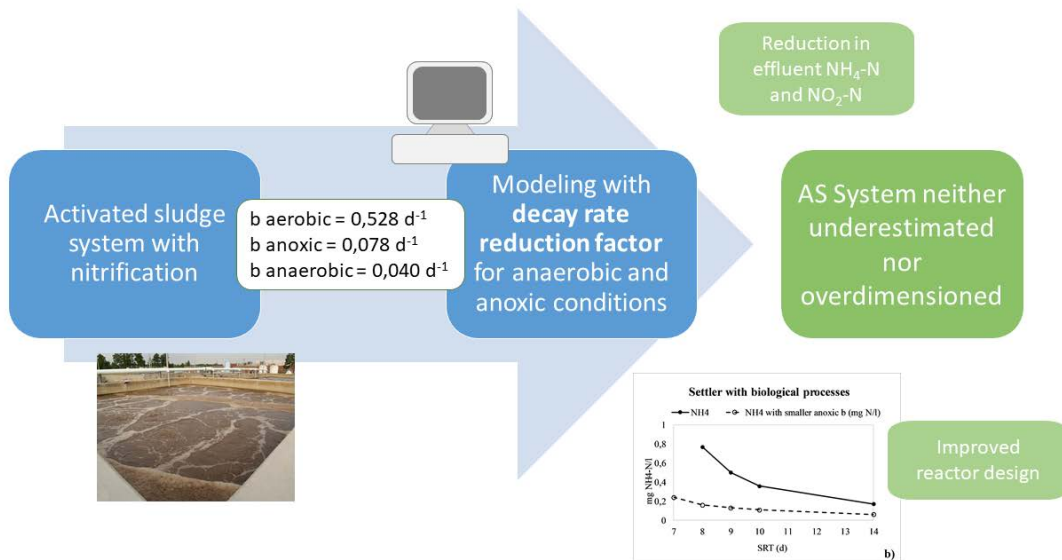**Settler with biological processes**
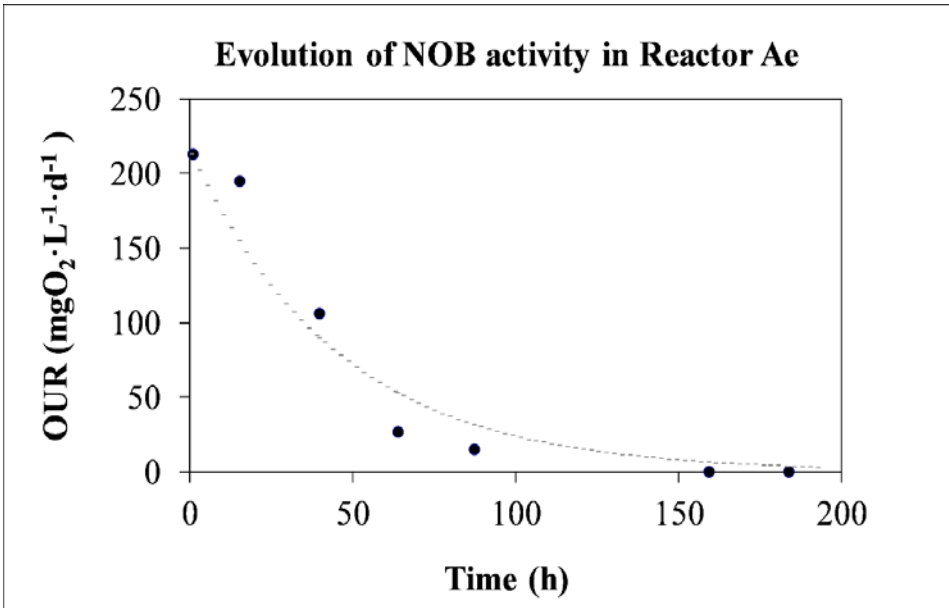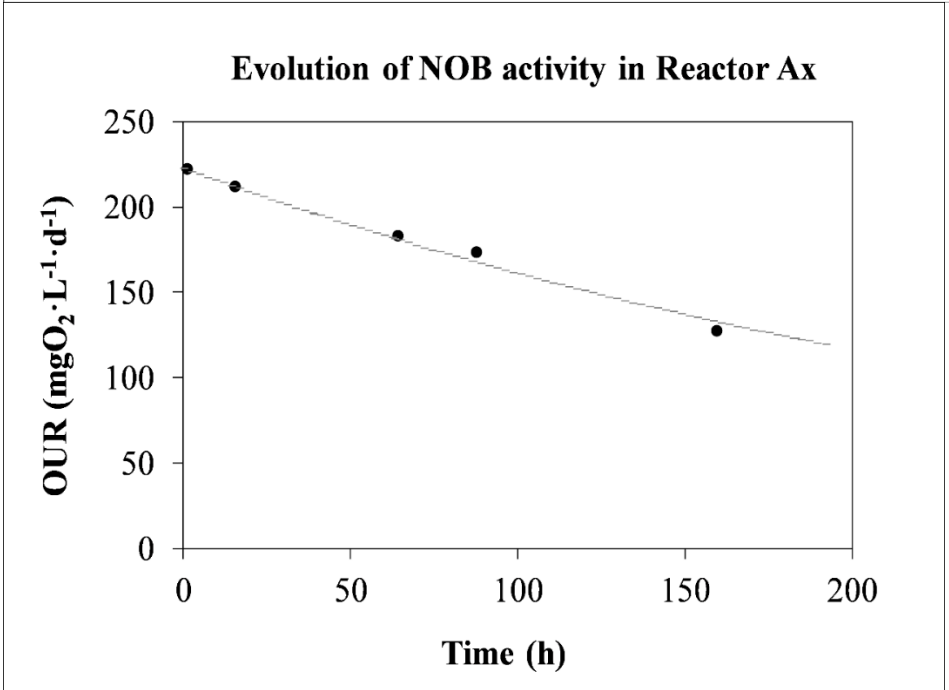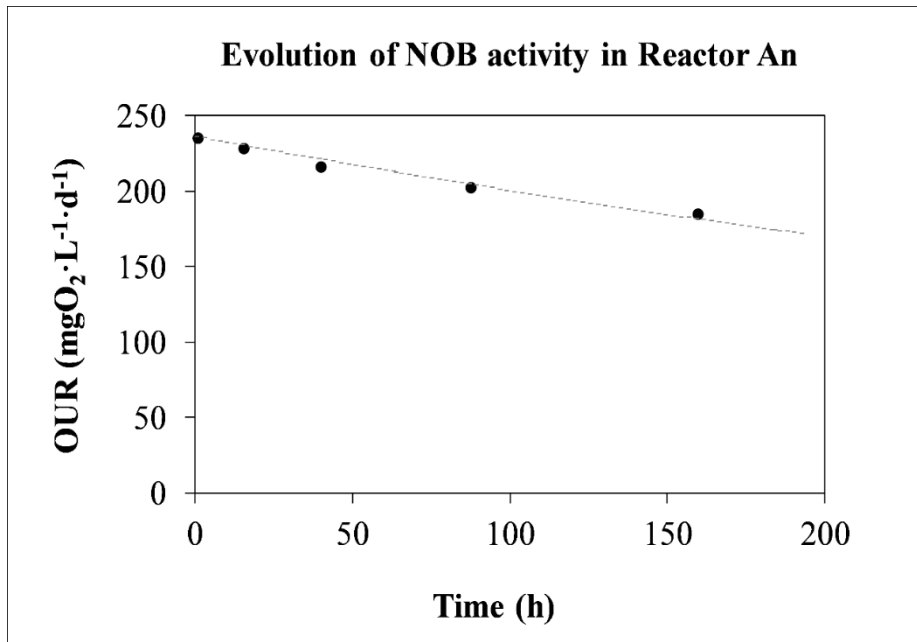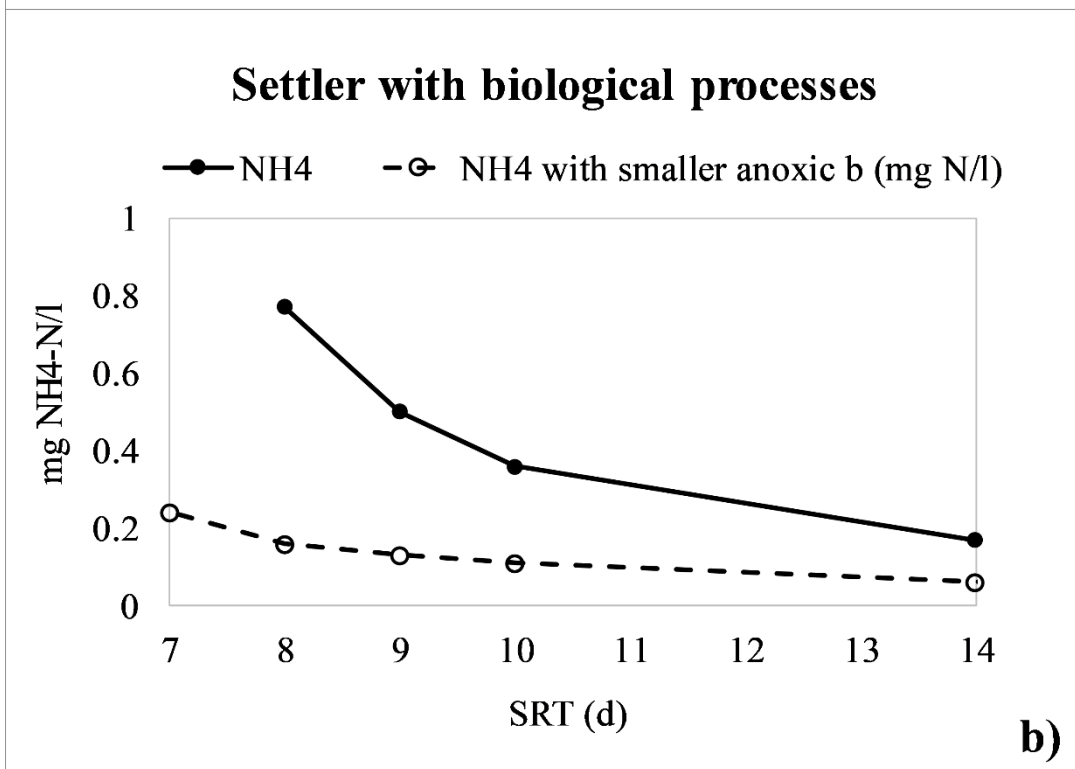
b)
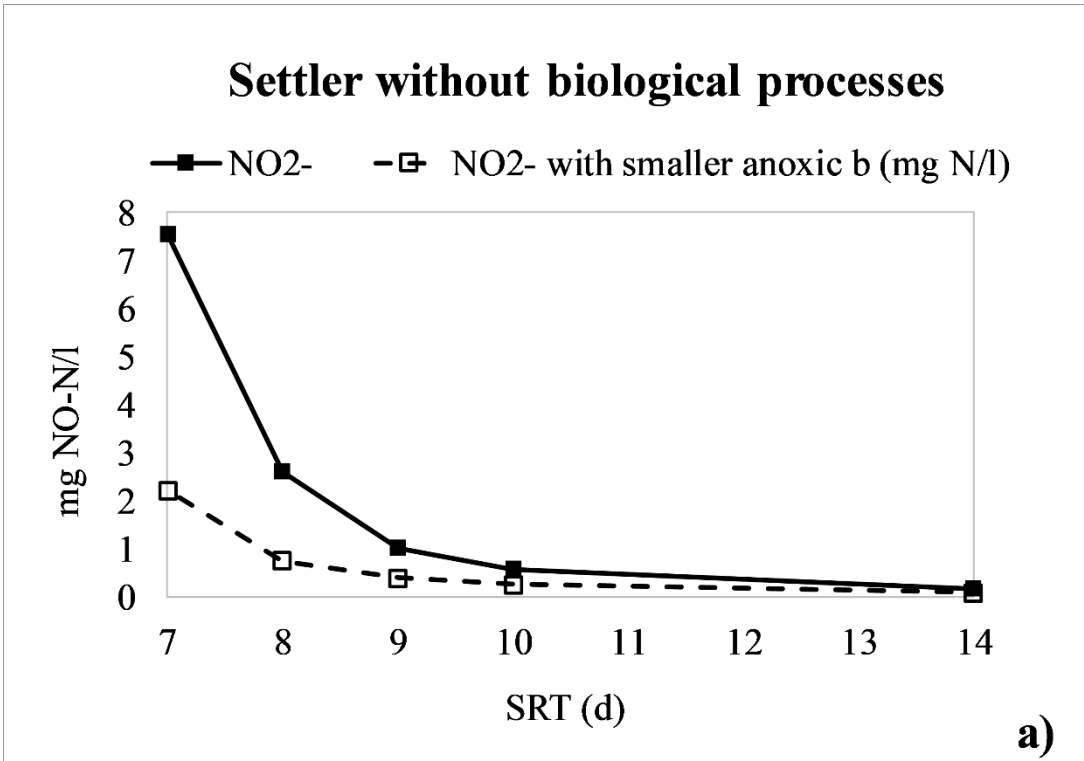
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505
506   Figs 6a y 6b

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508
509   Fig S1

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512   Graphical abstract