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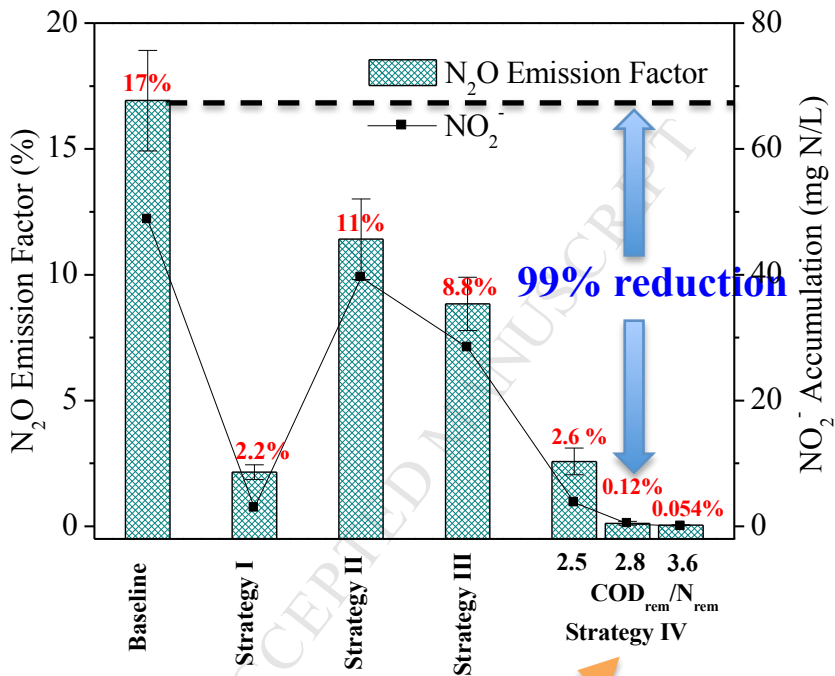
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Comparison of N$_2$O mitigation strategies
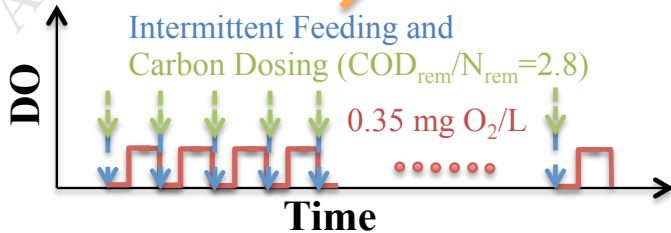
1   **Smart operation of nitritation/denitritation virtually abolishes nitrous oxide**

2   **emission during treatment of co-digested pig slurry centrate**

3

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14   **ABSTRACT:**

15   The implementation of nitritation/denitritation (Nit/DNit) as alternative to

16   nitrification/denitrification (N/DN) is driven by operational cost savings, e.g. 1.0-1.8

17   EUR/ton slurry treated. However, as for any biological nitrogen removal process,

18   Nit/DNit can emit the potent greenhouse gas nitrous oxide (N_2O). Challenges remain in

19   understanding formation mechanisms and in mitigating the emissions, particularly at a

20   low ratio of organic carbon consumption to nitrogen removal (COD_{rem}/N_{rem}). In this study,

21   the centrate (centrifuge supernatant) from anaerobic co-digestion of pig slurry was treated

22   in a sequencing batch reactor. The process removed approximately 100% of ammonium a

23   satisfactory nitrogen loading rate (0.4 g N/L/d), with minimum nitrite and nitrate in the

24   effluent. Substantial N_2O emission (around 17% of the ammonium nitrogen loading) was

25   observed at the baseline operational condition (dissolved oxygen, DO, levels averaged at

26   0.85 mg O_2/L; COD_{rem}/N_{rem} of 2.8) with ~68% of the total emission contributed by

27   nitritation. Emissions increased with higher nitrite accumulation and lower organic

28   carbon to nitrogen ratio. Yet, higher DO levels (~2.2 mg O_2/L) lowered the aerobic N_2O

29   emission and weakened the dependency on nitrite concentration, suggesting a shift in

30   N_2O production pathway. The most effective N_2O mitigation strategy combined

31   intermittent patterns of aeration, anoxic feeding and anoxic carbon dosage, decreasing

32   emission by over 99% (down to ~0.12% of the ammonium nitrogen loading). Without

33   anaerobic digestion, mitigated Nit/DNit decreases the operational carbon footprint with

34   about 80% compared to N/DN. With anaerobic digestion included, about 4 times more

35   carbon is sequestered. In conclusion, the low COD_{rem}/N_{rem} feature of Nit/DNit no longer

36   offsets its environmental sustainability provided the process is smartly operated.

37   Keywords: nitritation/denitritation; nitrous oxide; pig slurry; mitigation strategy; carbon

38   footprint

39

40   **1. Introduction**

41   Anaerobic digestion has been widely used for energy recovery from waste streams

42   (i.e. livestock waste) by producing a renewable energy source, methane. However, the

43   yielding ammonium-rich effluent low in biodegradable organic carbon requires

44   subsequent treatment. The ammonium (NH_4^+) oxidation to nitrite (NO_2^-) and its reduction

45   to nitrogen gas (N_2), termed as nitritation/denitritation (Nit/DNit), is one of the promising

46   technologies for N removal from N-rich waste streams and derivatives, due to economic

47 incentives. Nit/DNit saves 25% of the total oxygen demand and 40% of the carbon

48 demand, compared to conventional nitrification and denitrification (N/DN) (Fux et al.,

49 2006; Vlaeminck et al., 2012; Lackner et al., 2014; Schaubroeck et al., 2015; Chen et al.,

50 2016).

51   However, as any biological nitrogen removal process, a potent greenhouse gas

52 nitrous oxide (N_2O) could be emitted from Nit/DNit. N_2O is of significant environmental

53 concern since it not only has approximately 265-fold stronger global warming potential

54 than carbon dioxide (IPCC, 2013), but is also responsible for stratospheric ozone

55 depletion (Ravishankara et al., 2009). Previous studies suggested that N_2O emission

56 could be a significant or even major contributor (up to 80%) to the carbon footprint of

57 wastewater treatment plants (WWTPs) (Joss et al., 2009; Weissenbacher et al., 2010;

58 Wunderlin et al., 2012a; Desloover et al., 2012). During nitritation or nitrification, two

59 main pathways carried out by ammonia oxidizing bacteria (AOB) contribute to N_2O

60 production: (i) the sequential reductions from NO_2^- to nitric oxide (NO) and to N_2O as the

61 end product, termed as AOB denitrification pathway (Kampschreur et al., 2007; Kim et

62 al., 2010; Yu et al., 2010); (ii) N_2O as a side product during the incomplete oxidation of

63 hydroxylamine (NH_2OH) to NO_2^-, known as NH_2OH oxidation pathway (Chandran et al.,

64 2011; Stein, 2011). Dissolved oxygen (DO), nitrite, ammonia loading rate, pH, alkalinity,

65 etc. were reported to affect N_2O production by AOB (Tallec et al., 2006; Kampschreur et

66 al., 2007; Kim et al., 2010; Law et al., 2011; Wunderlin et al., 2012b; Peng et al., 2014;

67 Peng et al., 2015a; Peng et al., 2015b; Wang et al., 2017). Mathematical modelling and

68 isotopic techniques provided opportunities to further identify the N_2O production

69 pathways by AOB under varying DO and nitrite concentrations (Wunderlin et al., 2013;

70   Peng et al., 2015; Harris et al., 2015; Peng et al., 2016). Additionally, N_2O is an

71   obligatory intermediate of both denitritation and denitrification with N_2 being the end

72   product for most cases. However, some environmental factors such as DO (Zhu and

73   Chen, 2011; Wunderlin et al., 2012b), carbon source availability (Itokawa et al., 2001; Lu

74   and Chandran, 2010; Quan et al., 2012), free nitrous acid (Zhou et al., 2008), sulfide (Pan

75   et al., 2013), etc. could cause N_2O accumulation during denitritation or denitrification.

76   The N_2O emission factor, defined as the ratio between N_2O nitrogen emitted and the

77   influent nitrogen loading, displayed a large variation depending on operational conditions

78   and types of processes (Kampschreur et al., 2009; Law et al., 2012). Various levels of

79   N_2O emission were also reported in the partial nitrition process. High concentration of

80   nitrite (500 – 1000 mg N/L) has been shown to exert an inhibitory effect on N_2O

81   production from a nitrition reactor, leading to a substantially lower N_2O emission factor

82   (0.2–1.4%) as compared to those from conventional activated sludge systems (Law et al.,

83   2013). Lv et al. (2016) reported that the N_2O emission factor increased from 1.8% to

84   2.4% as DO decreased from 0.6 mg O_2/L to 0.35 mg O_2/L in a lab-scale nitrition

85   sequencing batch reactor (SBR). In full-scale nitrition reactors, the N_2O emission

86   factors were even higher, ranging from 1.7% to 6.6% (Kampschreur et al., 2008;

87   Desloover et al., 2011; Mampaey et al., 2016).

88   The lower investment cost and simpler process control drive the implementation of

89   single-stage Nit/DNit, despite of the potential for higher loading in the two-stage

90   approach. However, to date, there is still a lack of research focusing on N_2O production

91   from such a system treating real ammonium-rich wastewater. N_2O emission was

92   negligible (0.07–0.15%) in a pilot-scale continuous stirred tank reactor treating piggery

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93   wastewater (Rajagopal and Béline, 2011). In contrast, Scaglione et al. (2013) measured

94   much higher N_2O emission (3–24%) from a Nit/DNit reactor fed with liquid fraction of

95   digested agro-wastes and found that the ratio between biodegradable organic matter

96   (bCOD) and nitrogen (N) (COD/N) played an important role in determining N_2O

97   production. However, the absence of online and continuous monitoring in these two

98   studies brings difficulties to accurately identify the overall N_2O emission from the

99   reactors. Moreover, effective and efficient mitigation strategies for N_2O emission from

100   side-stream treatment, validated by experimental demonstration, are urgently in need due

101   to the potentially higher N_2O production than that from mainstream processes.

102   This work aims at a sustainable treatment of high nitrogenous wastewater with

103   minimized N_2O emission. To achieve this, a lab-scale SBR was operated treating the

104   centrate (centrifuge supernatant) from an anaerobic digester processing mainly pig slurry.

105   After obtaining stable performance of nitrification and denitrification via nitrite, N_2O

106   emission from the SBR was on-line monitored under different operational conditions (i.e.

107   DO level, the amount of external carbon, etc.). Based on the observations and data

108   analysis, several mitigation strategies were proposed and compared.

109

110   **2. Materials and methods**

111   2.1. Set up and operation of the Nit/DNit SBR

112   A SBR with a working volume of 4.5 L was operated in the laboratory seeded with

113   sludge from a WWTP treating the centrate of pig slurry in Izegem, Belgium. One cycle

114   consisted of anoxic feeding, aerating, anoxic mixing, settling, and decanting. In each 8-

115   hour cycle, the SBR was fed with 0.3 L of the centrate containing 2.0 ± 0.31 g N/L (n = 9)

5

116   of ammonium from an anaerobic digester processing mainly pig slurry (~84%),

117   supplemented with a co-substrate (~16% Ecofrit). The total Kjeldahl nitrogen (TKN) in

118   the influent was 3.0 ± 0.44 (n = 9) g N/L. The centrate also contained a high level of

119   solids and organic matter (the details refer to Table S1 in Supplementary Material). The

120   volume exchange rate (VER), the hydraulic retention time (HRT) and the targeted

121   nitrogen loading rate were ~6.7%, 5 days and 0.4 g N/L/d, respectively. Glycerin, a by-

122   product of vegetable oil production was supplied at the beginning of the second anoxic

123   phase as external carbon source. The pH in the reactor was controlled in the range of 7.5

124   – 8.0 by programmed logic controller (PLC) through dosing either 1 M $NaHCO_3$ or 1M

125   H_2SO_4. Compressed air was supplied to the reactor during aerobic phases. The DO

126   concentration was controlled in the range of $0 – 1.5$ mg O_2/L with a mass flow controller

127   (0 – 2 L/min STANDARD, Aalborg) and a proportional-integral-derivative controller.

128   The operational temperature of 35 °C was selected to represent the typical temperature of

129   mesophilic digestates. The reactor vessel was jacketed, and the temperature was

130   controlled with a circulating thermostatic water bath (14 L Heated PPO Bath, Thermo

131   Scientific). The solid retention time (SRT) was kept at ~15 days by manually wasting on

132   a daily basis. To monitor the system performance, mixed liquor samples were taken

133   periodically from influent, reactor and effluent for analysis of ammonia nitrogen, TKN,

134   nitrite, nitrate, COD, total suspended solids (TSS), and volatile suspended solid (VSS).

135   After stabilization, characterization of the biomass compositions was conducted using

136   fluorescence in situ hybridization (FISH, the results refer to Supplementary Material).

137   The reactor was initially loaded at 0.1 g N/L/day. Subsequently the ammonium

138   loading was stepwise increased by shortening the cycle time, while the VER was kept

139 constant at ~6.7%. Hence, the substrate concentrations would not vary, while the loading

140 rate increased. In particular, due to pulse feeding, the initial high ammonium nitrogen

141 concentration led to a high free ammonia (FA) concentration (around 9 mg NH_3-N/L at

142 pH of 7.8 and temperature of 35 °C), which had a negligible effect on AOB, but rather

143 inhibited nitrite oxidizing bacteria (NOB) (Vadivelu et al., 2007).

144

145 2.2. N_2O measuring campaign

146   To assess the effect of some key parameters on N_2O production, the Nit/DNit SBR

147 was operated under varying DO concentrations and with different amount of external

148 carbon supplement. The effect of nitrite on N_2O production in the system was

149 investigated in one additional set of experiments, where a certain amount of nitrite was

150 dosed into the parent SBR reactor at the beginning of the cycle. Each experiment lasted a

151 minimum of 5 days (one HRT). Mixed liquor samples were taken from the reactor at

152 different phases and from the effluent for NH_4^+, NO_2^-, NO_3^-, COD, TSS, and VSS

153 analysis. The detailed operational conditions are described in Table S2.

154   The stripped gas from the Nit/DNit reactor was analyzed on-line for N_2O

155 concentration through an Emerson Rosemount X-STREAM Gas Analyzer, preceded by a

156 condenser (4 °C). Data were logged every 5 s. The flow rate of the sampling pump in the

157 analyzer was constant at 100 L/h. The N_2O analyzer was calibrated periodically as per

158 manufacturer's instruction and no signal drift was detected.

159   A control run was conducted to verify the N_2O production by heterotrophs.

160 Ammonium and nitrite were completely depleted during aerobic phase and anoxic phase,

161 respectively. Subsequently, 75 mg N/L nitrite and 250 mg COD/L glycerin were added in

162 the beginning of aeration in the next cycle without feeding any centrate. The absence of

163 ammonium substrate would result in a minimum AOB activity. Hence, the on-line

164 measured N$_2$O emission was mostly attributed to the activity of heterotrophs.

165 Two sets of short-term experiments (Experiment I and Experiment II) were carried

166 out in the parent SBR reactor to reveal the effect of nitrite and DO levels on N$_2$O

167 emission rate (defined below). In Experiment I, the N$_2$O emission was on-line monitored

168 in three consecutive cycles with DO concentrations varying between 0.5 mg O$_2$/L and 1.0

169 mg O$_2$/L. Mixed liquor samples were taken hourly during the 6-hour aerobic phases for

170 analysis of nitrite and nitrate. The DO levels were in the range of 1.4 – 2.8 mg O$_2$/L

171 during the other three consecutive cycles in Experiment II. A moving window was used

172 to determine each N$_2$O emission rate and its corresponding nitrite and DO level.

173 The different evaluated mitigation strategies are described in Figure 1A. In brief,

174 N$_2$O was on-line monitored in different scenarios with varying DO levels, carbon

175 availability, modes of feeding, aeration, and carbon dosage (continuous or intermittent).

176 The continuous or intermittent modes were achieved by on/off control through a

177 programmed logical controller.

178

179 2.3. Calculations

180 The N$_2$O emission factor was calculated based on the following equation:

181 $$N2O_{ef} = \frac{N2O_{em}}{NH_4^+ \ loading} \times 100 \qquad (1)$$

182 Where $N2O_{ef}$ is the N$_2$O emission factor, %; $N2O_{em}$ is the mass of N$_2$O nitrogen

183 emitted over the complete reactor cycle, mg N$_2$O-N; $NH_4^+ \ loading$ is the mass of

184 ammonium nitrogen loading over the reactor cycle, mg NH$_4^+$-N.

185   The total N_2O emission factor is the sum of the N_2O emission factor under aerobic

186   phase (the aerobic N_2O emission factor) and N_2O emission factor under anoxic, settling

187   and decanting phases (the anoxic N_2O emission factor), which were calculated based on

188   the following:

189    $$N2O_{ef}^{ae} = \frac{N2O_{em}^{ae} - N2O_{em}^{ini}}{NH_4^+ \; loading} \times 100 \qquad (2)$$

190   Where $N2O_{ef}^{ae}$ is the aerobic N_2O emission factor, %; $N2O_{em}^{ae}$ is the mass of N_2O

191   nitrogen emitted during the aerobic phase, mg N_2O-N; $N2O_{em}^{ini}$ is the initial peak mass of

192   N_2O emission once aeration starts, caused by stripping of the N_2O accumulated in liquid

193   phase during the non-aerated phases (if applicable), mg N_2O-N.

194    $$N2O_{ef}^{an} = \frac{N2O_{em}^{an} + N2O_{em}^{ini}}{NH_4^+ \; loading} \times 100 \qquad (3)$$

195   Where $N2O_{e}^{an}$ is the anoxic N_2O emission factor, %; $N2O_{em}^{an}$ is the mass of N_2O

196   nitrogen emitted during the non-aerated phases, mg N_2O-N.

197   The stirring of the mixed liquor and the constant gas flow from the reactor

198   headspace into the pump of N_2O analyzer facilitates the mass transfer of N_2O from liquid

199   phase to gas phase, which results in N_2O emission during non-aerating period. The rapid

200   increasing N_2O emission upon the beginning of aeration is also due to mass transfer from

201   aqueous N_2O, accumulated during the anoxic period, to gas phase (if applicable).

202   The N_2O emission rate is calculated as follows:

203    $$N2O_{rate} = N2O_{conc} \times Q \qquad (4)$$

204   Where $N2O_{rate}$ is the N_2O emission rate, mg N/h; $N2O_{conc}$ is the N_2O

205   concentration measured in gas phase, mg N/L; Q is the air flow rate in the reactor, L/h.

206

207   The ratio between consumed COD and consumed nitrogen was termed COD_{rem}/N_{rem}.

208   The removed nitrogen was calculated as the difference between influent and effluent

209   ammonium nitrogen concentrations. Accurately determining the consumed COD was

210   difficult in this study considering the high COD background levels (refer to Figure 3A).

211   As the parameter is of great practical relevance, its value was estimated based on two

212   assumptions: I) the external carbon from glycerin (added in the anoxic phase) is readily

213   biodegradable, all of which will be consumed anoxically; II) the removed nitrogen during

214   the aerobic phase is due to simultaneous nitritation and denitritation, utilizing internal

215   COD, based on the theoretical COD_{rem}/N_{rem} ratio of 2.3. So the total converted COD is

216   the sum of internal and external COD consumption. Given these assumptions, the

217   obtained values should be interpreted with care.

218   In addition, to investigate the effect of carbon availability on N_2O production from

219   anoxic denitrification, COD_{an}/N_{an} is defined as the ratio between the mass of COD

220   derived from glycerin dosed in the anoxic phase, and the sum of nitrite and nitrate

221   nitrogen at the beginning of anoxic phase.

222

223   2.4. Chemical analysis

224   NH_4^+, TKN, TSS, VSS and biochemical oxygen demand (BOD_5) were measured

225   according to standard methods (APHA, 1998). COD was measured by photometric

226   methods using Nanocolor test tubes (Machereye-Nagel, Germany). Nitrite and nitrate

227   were determined on a 761 Compact Ion Chromatograph (Metrohm, Switzerland)

228   equipped with a conductivity detector.

229

230   **3. Results**

231   3.1. Nit/DNit in SBR

232   The system performance of the SBR is shown in Figure 2. The details for the

233   operational conditions are shown in Table S2 in Supplementary Information. It should be

234   noted over days 89 – 108, the DO level was elevated to 2.2 mg O_2/L to evaluate its

235   impact on 1) NOB suppression, due to the fact that *Nitrospira* is preferably out-selected

236   at high DO, while *Nitrobacter* is preferably suppressed at low DO (Regmi et al., 2014;

237   Blackburne et al., 2008); 2) nitrite reduction, with varying external COD dosage; 3) N_2O

238   emission, since DO level has been shown as an important factor shifting N_2O pathways

239   and thus leading to varying N_2O emissions (Peng et al., 2014). Within the first 30 days,

240   ammonium loading rate increased from ~0.1 g N/L/day to ~0.4 g N/L/day, while the

241   corresponding cycle time decreased from 32 hours to 8 hours (Figure 2A). Aeration

242   failure led to incomplete ammonium oxidation on day 35. Hence, the ammonium loading

243   rate was lowered to help the microbial activity to recover. The reactor was running with

244   full capacity since day 60 at a NH_4^+ loading rate in the range of 0.3 – 0.4 g N/L/day. TKN

245   loading rate displayed a similar trend and was stable in the range of 0.4 – 0.55 g N/L/day.

246   The removal efficiencies for ammonium and TKN were almost 100% and 80%,

247   respectively for most of the testing period (Figure 2A). At steady state, ~40% of nitrogen

248   was removed during aerobic phase. Based on the assumption above, the COD_{rem}/N_{rem} is

249   estimated at 2.8 with ~33% of internal and ~67% of external carbon consumed by

250   heterotrophic denitrifiers.

251   With some seldom exceptions, the effluent ammonium, nitrite and nitrate were

252   depleted shortly after the start-up (Figure 2B). As mentioned above, there was

253   ammonium accumulation up to ~770 mg N/L in the effluent due to aeration failure. The

11

254   subsequent nitrite accumulation up to ~78 mg N/L was due to the fact that the high FA

255   concentration negatively affected the activity of AOB, NOB and denitrifiers. During

256   Period II, III and IV from day 89 to day 108 (Table S2), the average DO level in the

257   Nit/DNit reactor was changed from ~0.85 mg O_2/L to ~2.2 mg O_2/L to evaluate N_2O

258   response under varying conditions (refer to Figure 4). The buildup of nitrite and nitrate in

259   the effluent during these periods suggested that the higher DO concentrations recovered

260   NOB activity (Figure 2B). Given the insufficient carbon (COD_{rem}/N_{rem} around 2.8) for

261   complete denitrification, nitrate and nitrite started to accumulate in the effluent. The

262   decrease of DO to 0.5 mg O_2/L in the following period (Period V, Table S2) successfully

263   suppressed the NOB activity again. Throughout the testing period, effluent concentrations

264   of NH_4^+, NO_2^- and NO_3^- were mostly below 20 mg N/L, indicating a good system

265   performance (Figure 2B). The TKN concentrations in the effluent varied between 430 mg

266   N/L and 810 mg N/L during the 160 days (Figure 2B).

267   The nitrate and nitrite levels at the end of the aerobic phase were used as indicators

268   for nitrate formation and nitrite removal (Figure 2C). At the beginning, nitrite and nitrate

269   concentrations were around 170 mg N/L and 80 mg N/L, respectively, both of which

270   decreased rapidly within the first five days. In the 160-day reactor operation, the nitrate

271   was kept at a low level, indicating a good performance of nitritation. Concomitantly, the

272   nitrite profile displayed a decreasing trend, indicating an improving performance of

273   denitritation under aerobic conditions. At the higher DO concentrations from day 89 to

274   day 108 (Period II, II and IV, Table S2), nitrate became the dominant nitrogen substrate

275   over nitrite, indicating a transformation from the nitritation to complete nitrification. By

276   lowering the DO set point, the Nit/DNit system was back to normal.

277   Figure 2D shows the solid content in the SBR. At the beginning, the reactor

278   contained ~ 3 g VSS/L, followed by a rapid increase up to ~14 g VSS/L due to the high

279   level of solids in the influent as shown in Table S1. The effluent VSS changed

280   correspondingly. After reaching the steady state, the VSS concentrations in the reactor

281   and in the effluent were in the ranges of 10 g/L – 14 g/L and 7 – 10 g/L, respectively. The

282   VSS/TSS was relatively stable, which was between 0.5 and 0.6 over the test period. Due

283   to the high solids concentration in the pig slurry, the VSS concentration in the SBR (10-

284   14 g/L) was much higher than the typical VSS concentration in activated sludge plants

285   (3-5 g/L). SRT was controlled at a constant level (around 15 days) by daily manual

286   sludge wasting, taking the effluent loss of suspended solids into consideration. Despite of

287   the high solids concentration in the influent, washout of AOB activity was not observed

288   during operation, and the system performance performed at satisfactory removal rate,

289   efficiency and stability, as shown in Figure 2.

290

291   3.2. N_2O emission from baseline operation

292   As an example, Figure 3 presents gas-phase N_2O concentrations along with N, DO,

293   pH, and COD in one SBR cycle under baseline conditions (Table S2). Similar trends

294   were observed in the profiles of other cycles with the same operational conditions. Time

295   0 corresponded to the beginning of the aerobic phase. The total COD (tCOD) and soluble

296   COD (sCOD) were ~24.5 g COD/L and ~5.5 g COD/L, respectively (Figure 3A). Due to

297   the high background COD concentrations in the reactor, the consumed COD by aerobic

298   growth of heterotrophs and anoxic growth of denitrifiers could not be accurately

299   determined by the photometric method. The initial ~120 mg N/L of NH_4^+ was depleted

13

300　within 6 hours (the end of the aerobic phase), resulting in an ammonia oxidation rate of

301　~19.4 mg N/L/hour (Figure 3B). The NO_2^- concentration kept increasing over time,

302　peaked at ~57 mg N/L during the aerobic phase and was completely denitritated during

303　the anoxic phase. NO_3^- was below 2 mg N/L during the entire cycle time. DO varied

304　between 0 and 1.5 mg O_2/L, while pH was around 7.7 for most of the time, but rapidly

305　increased to ~7.9 after feeding of centrate (Figure 3C). N_2O concentration in ppm_v was

306　simultaneously monitored by the gas analyzer under the conditions of 1 atm and 22 °C.

307　As shown in Figure 3D, N_2O gradually increased from 0 to ~150 ppm_v during the aerobic

308　phase and further elevated up to 280 ppm_v during the anoxic phase. The N_2O emission

309　factor was ~15.6% for the whole cycle and the aerobic N_2O emission factor was around

310　9.5%.

311

312　3.3. N_2O emission corresponding to varying NO_2^-, DO, and COD_{an}/N_{an} levels

313　　　The N_2O emission factors obtained from daily analysis of baseline and Period I – V

314　(Table S2) were correlated to nitrite accumulation (Figure 4). When the reactor was

315　operated at DO concentrations below 1.5 mg O_2/L (Figure 4A), both the total and aerobic

316　N_2O emission factors were linearly dependent on nitrite accumulation (R^2 = 0.76).

317　However, at DO levels above 1.5 mg O_2/L (Figure 4B), the increase of nitrite

318　concentration from ~33 mg N/L to ~188 mg N/L had negligible impact on the total and

319　aerobic N_2O emission factors. The corresponding R^2 was only 0.3. With similar nitrite

320　concentration, lower N_2O emission was observed at high DO levels (Figure 4B) as

321　compared to lower levels (Figure 4A). At a low DO concentration, around 81% of N_2O

322　was emitted during the aerobic phase (Figure 3). However, at a high DO level only ~11%

14

323   of the   measured N_2O was emitted during the aerobic phase, which suggests

324   incomplete denitrification as dominant mechanism of N_2O production (Figure 4B).

325   N_2O emission rates from two sets of short-term experiments (Experiment I and

326   Experiment II, as described above) were plotted against varying nitrite accumulations and

327   DO set points in Figure S1. The results further confirmed the strong dependency of N_2O

328   emission rate on nitrite accumulation ($R^2 = 0.93$) at low DO levels (0 - 1.0 mg O_2/L) and

329   on DO concentration ($R^2 = 0.93$) at high DO levels (1.4 - 2.8 mg O_2/L).

330   As shown in Figure S2 the anoxic emission factor was strongly dependent on

331   COD_{an}/N_{an}. As COD_{an}/N_{an} increased from ~1.4 to ~14, anoxic N_2O emission factor

332   decreased substantially. The highest anoxic emission factor of ~21% occurred at

333   COD_{an}/N_{an} of ~1.9, whilst the lowest (~0.41%) was observed at the highest COD_{an}/N_{an} of

334   ~14.

335

336   **4. Discussion**

337   4.1. Single-stage Nit/DNit process can be a significant contributor to N_2O emission

338   during wastewater treatment

339   In this study, the single-stage Nit/DNit process was successfully implemented in a

340   bench-scale SBR treating centrate from pig slurry co-digestion. Suppression of NOB was

341   achieved using a combination of low dissolved oxygen levels (average 0.85 mg O_2/L),

342   high temperature (35ºC), and relatively high initial FA concentration (around 9 mg NH_3-

343   N/L). After stabilization, the system was able to remove approximately 100% of

344   ammonium (~2 g N/L in the influent) and 80% of TKN (~3 g N/L in the influent) with

345   minimum nitrite and nitrate in the effluent, at a reasonably high nitrogen loading rate of

346   ~0.4 g N/L/d.

347   This work fills the gaps with regard to accurate identification of N_2O emission from

348   the single-stage Nit/DNit system. Under baseline operational conditions, about 17% of

349   ammonium nitrogen load was emitted as N_2O gas from the Nit/DNit SBR, which could

350   be a significant source of carbon footprint if implemented in full scale (Rodriguez-

351   Caballero et al., 2015). This value is consistent with the reported emission levels on

352   similar waste streams. Scaglione et al. (2013) reported N_2O emission factors of 2–20% in

353   off-gas samples taken from a pilot Nit/DNit SBR treating the liquid fraction of agro-

354   digestate.

355   However, N_2O emission from our system is substantially higher than the reported

356   values (0.2–2.4%) in lab-scale partial nitritation SBRs fed with synthetic wastewater in

357   the absence of COD, which may be attributed to three reasons (Law et al., 2013; Lv et al.,

358   2016). First of all, heterotrophic denitritation may contribute to N_2O production during

359   anoxic phase. Further data analysis indicated that ~26% of the total N_2O emission was

360   from anoxic denitritation during baseline operation. Secondly, heterotrophic denitritation

361   may also contribute to N_2O production during the aerobic phase. According to the mass

362   balance, about 40% of influent ammonium ended as N_2 gas, demonstrating the

363   simultaneous nitritation and denitritation in the presence of oxygen. The denitritated

364   nitrogen had the opportunity to be transformed to N_2O gas. To identify the contribution of

365   aerobic denitritation to N_2O production, an additional test was performed in SBR

366   mimicking the conditions of aerobic phase in baseline operation, but in the absence of

367   ammonium and with initial addition of nitrite and glycerin. As shown in Figure S3, the

368   DO level in the reactor was controlled between 0 – 1.0 mg O_2/L. The nitrite level was

16

369   initially around 75 mg N/L and decreased gradually to 34 mg N/L at the end of 5-h

370   aerating period. On the other hand, N_2O concentration mostly fluctuated between 2.5

371   ppm_v and 10 ppm_v, suggesting a minimum contribution by aerobic heterotrophic

372   denitritation (~6% of the total aerobically emitted N_2O under baseline conditions).

373   Finally, the varying nitrite levels would exert diverse or even opposite impacts on

374   N_2O production. Law et al. (2013) found that high nitrite accumulation (500 – 1000 mg

375   N/L) in partial nitration SBR could lower N_2O production via AOB denitrification

376   pathway. In our reactor, the build up of nitrite was within the range of 30 – 60 mg N/L,

377   which was far below the inhibition threshold (Law et al., 2013). Moreover, the relatively

378   high pH (averaged at 7.7) also eliminated the possibility of free nitrous acid inhibition. In

379   essence, the moderate nitrite accumulation may play a key role in triggering N_2O

380   production by AOB. Peng et al. (2015a) revealed that N_2O emission factor increased from

381   ~6% to 13% as nitrite concentration increased from 3 mg N/L to 50 mg N/L at DO level

382   of 0.85 mg O_2/L in a enriched nitrifying culture.

383

384   4.2. Metabolic pathways of N_2O production during nitration and denitritation

385   As shown in Figure 4A, N_2O emission factor increased with the increase of nitrite at

386   DO concentrations below 1.5 mg O_2/L. AOB denitrification pathway was activated under

387   oxygen limiting condition and promoted by nitrite accumulation (Tallec et al., 2006;

388   Kampschreur et al., 2007; Wunderlin et al., 2012b; Peng et al., 2014). The stimulated

389   N_2O emission at higher nitrite level was possibly related to a promoted expression of

390   nirK gene or increased activity of NO_2^- reductase with increasing substrate concentration

391   (Beaumont et al., 2004). Thus, at lower DO (<1.5 mg O_2/L), AOB denitrification was

17

392   possibly the major contributor to N_2O production by nitritation.

393   However, both AOB denitrification and NH_2OH oxidation pathways made

394   contribution to N_2O emission from the Nit/DNit system at higher DO levels. On one hand,

395   the N_2O emission factors were not correlated to nitrite concentrations any more at DO

396   above 1.5 mg O_2/L (Figure 4B). The increase of DO concentrations completely altered

397   the dependency of N_2O production on nitrite accumulation. It is likely that the higher DO

398   triggered the occurrence of NH_2OH oxidation pathway since the observation here was in

399   line with the prediction by a two-pathway N_2O model showing that the increase of nitrite

400   had no effect on N_2O production via NH_2OH oxidation pathway (Peng et al., 2015a). Ni

401   et al. (2014) and Chandran et al. (2011) also reported that an elevated DO concentration

402   favored NH_2OH oxidation pathway. On the other hand, at similar nitrite conditions, a

403   lower N_2O emission factor was observed at high DO levels (>1.5 mg O_2/L) compared to

404   that at lower DO levels (<1.5 mg O_2/L) (Figure 4). This observation was further validated

405   by the results from two additional experiments, where the N_2O emission rates were

406   correlated to varying nitrite and DO levels (Figure S1). An elevated DO level decreased

407   the importance of AOB denitrification pathway in both mixed culture (Peng et al., 2015a)

408   and pure AOB cultures (Poth and Focht, 1985; Remde and Conrad, 1990). Hence, these

409   observations together confirmed that N_2O emission at higher DO (>1.5 mg O_2/L) resulted

410   from a combination of AOB denitrification pathway and NH_2OH oxidation pathway. The

411   two important compounds, oxygen and nitrite, competed for electrons generated by

412   NH_2OH oxidation, leading to the varying contributions of the two known pathways

413   (Anderson et al., 1993; Peng et al., 2015a).

414   N_2O production by nitritation served to be the major contributor in this Nit/DNit

415   system in comparison to N_2O production by denitritation. The contribution of nitritation

416   including both AOB denitrification and NH_2OH oxidation pathways accounted for ~68%

417   of total N_2O emission under baseline conditions. In contrast, with aid of isotopic

418   techniques and denitrification functional gene transcriptome analysis, Ishii et al. (2014)

419   identified that heterotrophic denitrification was the main contributor to N_2O production in

420   a partial nitritation aerobic granule reactor, fed with ammonium and acetate. The

421   divergence is possibly due to the fact that the granules create extensive anoxic layers,

422   where denitrification occur in the absence of oxygen, as opposed to the mild gradients

423   expected in the flocculent sludge used in this study. With the addition of nitrite (Period I

424   in Table S2), it was seen that the total, aerobic and anoxic N_2O emission factors in the

425   Nit/DNit reactor were all substantially promoted (Figure 4A). As nitrite increased from

426   ~20 to ~130 mg N/L, the contribution of anoxic denitritation to the total N_2O emission

427   increased from ~10% to ~53%, accompanied by a decrease of the contribution of aerobic

428   nitritation (Figure 4A). The increased build up of nitrite led to a lower COD_{an}/N_{an} ratio

429   and the resulting carbon limitation yielded higher N_2O production from denitritation

430   (Itokawa et al., 2001; Lu and Chandran, 2010; Scaglione et al., 2013).

431

432   4.3. Minimizing N_2O from the Nit/DNit reactor

433   Animal waste, the biggest wasted nitrogen mass flow through our economies, was

434   used as a model in this work (Coppens et al., 2016). Comparing to some full-scale partial

435   nitritation reactors (Kampschreur et al., 2008; Desloover et al., 2011; Mampaey et al.,

436   2016), the Nit/DNit SBR in this work generated much more N_2O (~17%) in baseline

437   operation (Figure 1B). The nitrite produced by AOB will be simultaneously consumed by

438 denitrifiers at aerobic phase in the Nit/DNit system. A much lower nitrite (30 – 60 mg

439 N/L) than that in partial nitration leads to N_2O spikes, which has been also found in lab-

440 scale (Peng et al., 2015a) and full-scale studies (Kampschreur et al., 2009). Consequently,

441 further minimizing the nitrite accumulation would be an effective way to limit N_2O

442 production.

443 Based on previous observations, high DO concentration was able to reduce aerobic

444 N_2O emission (Figure 4) and high glycerin supplement led to lower anoxic N_2O emission

445 (Figure S2). By combining supplement of sufficient carbon (COD_{rem}/N_{rem} of 6.5) and

446 application of elevated DO level (averaged at ~2.2 mg O_2/L) in Strategy I, the N_2O

447 emission factor was down to ~2.2% (Figure 1B). However, these implemented conditions

448 reactivated NOB (Figure 2) and thus lost the cost savings brought by the Nit/DNit system

449 concerning the lower oxygen and carbon demand. Additionally, more energy would be

450 consumed to maintain DO at high levels and carbon was excessively wasted in this

451 scenario. Hence, the subsequent scenarios were all investigated at low DO levels

452 (averaged at 0.35 mg O_2/L) and low COD_{rem}/N_{rem} ratio (around 2.8).

453 Different aeration patterns were previously reported to deliver varying N_2O

454 dynamics in WWTPs (Rodriguez-Caballero et al., 2015). N_2O emission was reduced by

455 replacing continuous aeration with intermittent aeration in lab-scale reactors treating

456 swine wastewater (Osada et al., 1995; Béline and Martinez, 2002). Implementation of

457 short aeration periods during intermittent aeration was found to significantly minimize

458 N_2O emission in both lab-scale (Béline and Martinez, 2002) and full-scale investigations

459 (Rodriguez-Caballero et al., 2015). In Strategy II, we applied intermittent aeration instead

460 of continuous aeration during aerobic phase with aeration to non-aeration ratio ranging

461   from 0.43 to 2.33. The overall N_2O emission (~11%) was reduced by ~35% compared to

462   baseline operation (~17%) (Figure 1B) and the varying lengths of aerating period showed

463   a negligible impact on N_2O emission. The carbon loss during aerating period may cause

464   carbon limitation during non-aeration, resulting in a nitrite build-up of ~40 mg N/L in

465   Strategy II. To further utilize the bCOD in the wastewater, anoxic intermittent feeding

466   (Strategy III) was implemented, leading to a decreased N_2O emission by 20% compared

467   to Strategy II (Figure 1B).

468   The optimal scenario with minimum N_2O emission factor of 0.12% was obtained by

469   applying intermittent patterns of aeration, anoxic feeding and anoxic carbon dosing in

470   Strategy IV. The consistently changing trend of nitrite accumulation against N_2O

471   emission factor in Figure 1B demonstrated that the minimized N_2O was attributed to

472   promoted consumption of nitrite or even N_2O itself by heterotrophic denitrifiers. Figure

473   S4 presents the liquid-phase N and DO along with gas-phase N_2O in the Nit/DNit SBR

474   with Strategy IV applied. The concentrations of NH_4^+, NO_2^- and NO_3^- were all below 1

475   mg N/L during the entire cycle. The intermittent aeration created alternate 7-min aerobic

476   phase and 3-min anoxic phase. The N_2O peak of ~6 ppm_v occurred upon the start of the

477   aeration. We also identified the optimal COD_{rem}/N_{rem} as 2.8 in Strategy IV. The lower

478   COD_{rem}/N_{rem} ratio (2.5) substantially triggered N_2O emission (~2.6%), while higher

479   COD_{rem}/N_{rem} of 3.6 (a cost factor) slightly reduced N_2O emission (~0.054%) (Figure 1B).

480   In partial nitritation/anammox, several operational conditions are similar to

481   nitritation/denitritation, and both processes strive for NOB suppression and minimum

482   N_2O emission. A study by Wang et al. (2016) revealed that sludge treatment based on

483   free nitrous acid (FNA) exposure can mitigate N_2O emission from a partial nitritation

484   system. Hence, future work may focus on combining optimal aeration and feeding

485   regimes with FNA-based sludge treatment, and reinforce mitigation of N_2O emissions in

486   a range of shortcut nitrogen removal applications.

487

488   4.4 Impact extrapolation

489   Animal slurry processing can avoid severe environmental problems related to

490   intensive livestock production to a considerable extent, yet comes at a cost, and should

491   not generate high alternative emissions (Pintucci et al., 2016). Based on a desktop

492   extrapolation study, selected impact categories of direct economic and environmental

493   relevance were evaluated: (i) key operational cost categories and (ii) carbon gas footprint,

494   based on direct and indirect sources and sinks. The considered pig slurry treatment lines

495   consisted of a centrifuge (solid/liquid separation) followed by a biological nitrogen

496   removal stage. Furthermore, the effect of an anaerobic (co-) digestion stage prior to the

497   centrifuge was evaluated. Nitrogen treatment scenarios compared the baseline scenario of

498   N/DN, at a typical N_2O emission (0.75% of the TN loading) (Lemmens, et al. 2007) and

499   with external methanol addition (fossil C source), to the Nit/DNit process, at worst and

500   best N_2O emission (11-0.080% of the N loading) and, if necessary, with external glycerol

501   addition (biogenic C-source). Further calculation assumptions are detailed in Tables

502   S3&S4.

503   Table S3 displays the operational cost differences of the scenarios. Applying the

504   Nit/DNit process lowers the requirements for external carbon and aeration so that the

505   operational cost falls by 1.07-2.07 EUR/m^3 of centrate, and hence 0.952-1.84 EUR/m^3 of

506   raw slurry. For the treatment line without digestion, a global processing rate of about 15

507   EUR/m^3 can be assumed (Lemmens, et al. 2007). The Nit/DNit scenario hence yields an

508   overall saving potential of about 12% of the total treatment cost.

509   The operational carbon footprint of the full treatment lines is compared in Table S4.

510   And the key findings are highlighted in Figure 5. To exemplify the potential negative

511   impact of improper Nit/DNit operation, a scenario with the highest measured N_2O

512   emission (11.3% of total N loading) was included in the comparison. In the absence of

513   anaerobic digestion (AD), this worst case Nit/DNit (Baseline, Figure 1A) increases the

514   carbon footprint with about a factor 8, while the mitigated scenario (Strategy IV, Figure

515   1A) can lower the footprint with 83%, both compared to the N/DN baseline scenario

516   (Figure 5). Including AD, the net carbon sequestration in the mitigated Nit/DNit scenario

517   is approximately 4 times better than in the N/DN process (Figure 5). The high N_2O

518   emission Nit/DNit scenario, in contrast, strongly outweighs the carbon sequestration

519   potential of AD. The typical carbon footprint from animal slurry management is 35 - 132

520   kg CO_2/m^3 (including collection, transportation, storage and land application), depending

521   on the practice and farm size (Aguirre-Villegas and Larson, 2016). Maloperation of a

522   nitrogen removal stage can strongly aggravate this, as shown from the worst-case

523   Nit/DNit scenarios (102 - 187 kg CO_2/m^3 of raw slurry, derived from 115 - 210 kg

524   CO_2/m^3 centrate). The slurry processing by anaerobic digestion and Nit/DNit on the other

525   hand could allow for a considerable overall reduction of 17-64% (best case; -22.5 kg

526   CO_2/m^3 of slurry, from -25.3 kg CO_2/m^3 of centrate).

527   Production of renewable energy is one of the objectives of including AD in a

528   treatment line for animal waste slurry. To have a sustainable environmental impact, the

529   carbon footprint of such installation should be below the one of current electricity

530   generation, i.e. 0.276 kg CO_2 emitted/kWh$_{el}$ produced (EU-28 in 2014). However, the

531   operational carbon footprint of the improper Nit/DNit operation (worst case) yields a CO_2

532   footprint that is more than 5 times higher. In order to restrict this impact to 10-50% of the

533   one of conventional electricity generation, the maximum allowed N_2O emissions for

534   Nit/DNit and N/DN are respectively 2.3-3.2% and 1.5-2.3% of the TN loading. The

535   operational footprint of the best-case Nit/DNit AD scenario (0.01 kg CO_2/kWh$_{el}$)

536   illustrates that proper nitrogen removal can enable a sustainably sound solution in

537   comparison to the lowest footprint of other renewable power sources like wind (0.01-0.12

538   kg CO_2/kWh$_{el}$) and photovoltaics (0.05-0.25 kg CO_2/kWh$_{el}$) (Bhat and Prakash, 2009).

539   Retrofitting an existing N/DN system to a Nit/DNit process is fairly simple,

540   including adjustment of a periodic feeding strategy, alternate aerobic and anoxic periods,

541   DO control at relatively low setpoint and a periodic dosage of external carbon (no need

542   for slurry, only for digestate). We believe that our findings on single-stage Nit/DNit

543   systems treating co-digested pig slurry centrate could be extrapolated to the treatment of

544   other high nitrogenous wastewater (i.e. landfill leachates, industrial effluent, etc.) and

545   potentially serves as a guideline for operation aiming at minimum carbon footprint.

546

547   **5. Conclusion**

548   A SBR reactor performing Nit/DNit was operated to treat centrate of pig slurry co-

549   digestion with a nitrogen loading rate of ~0.4 g N/L/d. Approximately 100% of

550   ammonium and 80% of TKN were removed with minimum effluent nitrite and nitrate.

551   Under baseline conditions, around 17% of the nitrogen loading was emitted as N_2O. The

552   aerobic nitritation and anoxic denitritation contributed to ~68% and ~26%, respectively

553 with the remaining from aerobic denitritation. At low DO concentration (<1.5 mg O_2/L),

554 N_2O emission was linearly dependent on nitrite accumulation, suggesting that AOB

555 denitrification pathway dominated. At elevated DO level (>1.5 mg O_2/L), nitrite had

556 negligible effect on N_2O production, while further increase of DO concentration inhibited

557 total N_2O emission, indicating the coexistence of AOB denitrification and NH_2OH

558 oxidation pathways. Effective N_2O mitigation was achieved by combining the

559 intermittent patterns of aeration, anoxic feeding, and anoxic carbon dosage, where the

560 N_2O emission factor was significantly reduced to ~0.12%. Based on desktop

561 extrapolation study, Nit/DNit, compared to conventional nitrification/denitrification, can

562 lower the operational costs with around 1.0-1.8 EUR/m^3. Without anaerobic digestion,

563 mitigated Nit/DNit decreases the operational carbon footprint with about 80% compared

564 to N/DN. With anaerobic digestion included, about 4 times more carbon is sequestered.

565 Our findings have important implications for sustainable treatment of high nitrogenous

566 wastewater through smart operation.

567

575

References:

Aguirre-Villegas, H.A., Larson, R.A., 2016. Evaluating greenhouse gas emissions from dairy manure management practices using survey data and lifecycle tools. *Journal of Cleaner Production*, 2017, 143, 169-179.

Anderson, I.C., Poth, M., Homstead, J., Burdige, D., 1993. A comparison of NO and N_2O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*. *Applied and Environmental Microbiology* 59, 3525-3533.

APHA, 1998. Standard Methods for Examination of Water and Wastewater. *Twentieth Ed. American Public Health Association, Washington, DC.*

Beaumont, H.J., Lens, S.I., Reijnders, W.N., Westerhoff, H.V., van Spanning, R.J., 2004. Expression of nitrite reductase in *Nitrosomonas europaea* involves NsrR, a novel nitrite-sensitive transcription repressor. *Molecular Microbiology* 54, 148-158.

Béline, F., Martinez, J., 2002. Nitrogen transformations during biological aerobic treatment of pig slurry: effect of intermittent aeration on nitrous oxide emissions. *Bioresource Technology* 83, 225-228.

Bhat, I.K., Prakash, R., 2009. LCA of renewable energy for electricity generation systemsa review. *Renewable and Sustainable Energy Reviews* 13, 1067-1073.

Blackburne, R., Yuan, Z., Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. *Biodegradation* 19, 303-312.

Chandran, K., Stein, L.Y., Klotz, M.G., van Loosdrecht, M.C.M., 2011. Nitrous oxide production by lithotrophic ammonia-oxidizing bacteria and implications for

599   engineered nitrogen-removal systems. *Biochemical Society Transactions* 39, 1832-

600   1837.

601   Chen, H., Liu, Y., Ni, B.-J., Wang, Q., Wang, D., Zhang, C., Li, X., Zeng, G., 2016. Full-

602   scale evaluation of aerobic/extended-idle regime inducing biological phosphorus

603   removal and its integration with intermittent sand filter to treat domestic sewage

604   discharged from highway rest area *Biochemical Engineering Journal* 113, 114-122.

605   Coppens, J., Meers, E., Boon, N., Buysse, J., Vlaeminck, S.E., 2016. Follow the N and P

606   road: High-resolution nutrient flow analysis of the Flanders region as precursor for

607   sustainable resource management. *Resources, Conservation and Recycling* 115, 9-

608   21.

609   Desloover, J., De Clippeleir, H., Boeckx, P., Du Laing, G., Colsen, J., Verstraete, W.,

610   Vlaeminck, S.E., 2011. Floc-based sequential partial nitration and anammox at full

611   scale with contrasting N_2O emissions. *Water Research* 45, 2811-2821.

612   Fux, C., Velten, S., Carozzi, V., Solley, D., Keller, J., 2006. Efficient and stable

613   nitritation and denitritation of ammonium-rich sludge dewatering liquor using an

614   SBR with continuous loading. *Water Research* 40, 2765-2775.

615   Harris, E., Joss, A., Emmenegger, L., Kipf, M., Wolf, B., Mohn, J., Wunderlin, P., 2015.

616   Isotopic evidence for nitrous oxide production pathways in a partial nitritation-

617   anammox reactor. *Water Research* 83, 258-270.

618   IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working

619   Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate

620   Change.

621   Ishii, S., Song, Y., Rathnayake, L., Tumendelger, A., Satoh, H., Toyoda, S., Yoshida, N.,

622 Okabe, S., 2014. Identification of key nitrous oxide production pathways in aerobic

623 partial nitrifying granules. *Environmental Microbiology* 16, 3168-3180.

624 Itokawa, H., Hanaki, K., Matsuo, T., 2001. Nitrous oxide production in high-loading

625 biological nitrogen removal process under low COD/N ratio condition. *Water

626 Research* 35, 657-664.

627 Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P.,

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

629 liquid with partial nitritation and anammox in one SBR. *Environmental Science &

630 Technology* 43, 5301-5306.

631 Kampschreur, M.J., Poldermans, R., Kleerebezem, R., Van Der Star, W.R.L., Haarhuis,

632 R., Abma, W.R., Jetten, M.S.M., Van Loosdrecht, M.C.M., 2009. Emission of

633 nitrous oxide and nitric oxide from a full-scale single-stage nitritation-anammox

634 reactor. *Water Science and Technology* 60, 3211-3217.

635 Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picioreanu, C., Jetten, M.S.M.,

636 Loosdrecht, M.C.M., 2007. Effect of dynamic process conditions on nitrogen oxides

637 emission from a nitrifying culture. *Environmental Science & Technology* 42, 429-

638 435.

639 Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., Van Loosdrecht, M.,

640 2009. Nitrous oxide emission during wastewater treatment. *Water Research* 43,

641 4093-4103.

642 Kampschreur, M.J., van der Star, W.R.L., Wielders, H.A., Mulder, J.W., Jetten, M.S.M.,

643 van Loosdrecht, M., 2008. Dynamics of nitric oxide and nitrous oxide emission

644 during full-scale reject water treatment. *Water Research* 42, 812-826.

645   Kim, S.W., Miyahara, M., Fushinobu, S., Wakagi, T., Shoun, H., 2010. Nitrous oxide

646   emission from nitrifying activated sludge dependent on denitrification by ammonia-

647   oxidizing bacteria. *Bioresource Technology* 101, 3958-3963.

648   Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.,

649   2014. Full-scale partial nitritation/anammox experiences--an application survey.

650   *Water Research* 55, 292-303.

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

652   conditions in a partial nitrition system. *Water Research* 45, 5934-5944.

653   Law, Y., Lant, P., Yuan, Z., 2013. The confounding effect of nitrite on N_2O production

654   by an enriched ammonia-oxidizing culture. *Environmental Science & Technology*

655   47, 7186-7194.

656   Law, Y., Ye, L., Pan, Y., Yuan, Z., 2012. Nitrous oxide emissions from wastewater

657   treatment processes. *Philosophical Transactions of the Royal Society B-Biological*

658   *Sciences* 367, 1265-1277.

659   Lemmens, B.; Ceulemans, J.; Elslander, H.; Vanassche, S.; Brauns en, E.; Vrancken, K.

660   Beste Beschikbare Technieken (BBT) voor mestverwerking, 2007. From

661   https://emis.vito.be/sites/emis.vito.be/files/pages/migrated/bbt_mestverwerking.pdf

662   Lu, H., Chandran, K., 2010. Factors promoting emissions of nitrous oxide and nitric

663   oxide from denitrifying sequencing batch reactors operated with methanol and

664   ethanol as electron donors. *Biotechnology and Bioengineering* 106, 390-398.

665   Lv, Y., Ju, K., Sun, T., Wang, L., Miao, R., Liu, T., Wang, X., 2016. Effect of the

666   dissolved oxygen concentration on the N_2O emission from an autotrophic partial

667   nitritation reactor treating high-ammonium wastewater. *International*

668   *Biodeterioration & Biodegradation* 114, 209-215.

669   Mampaey, K.E., De Kreuk, M.K., van Dongen, U.G., van Loosdrecht, M.C., Volcke, E.I.,

670   2016. Identifying N_2O formation and emissions from a full-scale partial nitritation

671   reactor. *Water Research* 88, 575-585.

672   Ni, B.J., Peng, L., Law, Y., Guo, J., Yuan, Z., 2014. Modeling of nitrous oxide

673   production by autotrophic ammonia-oxidizing bacteria with multiple production

674   pathways. *Environmental Science & Technology* 48, 3916-3924.

675   Osada, T., Kuroda, K., Yonaga, M., 1995. Reducing nitrous oxide gas emissions from

676   fill-and-draw type activated sludge process. *Water Research* 29, 1607-1608.

677   Pan, Y., Ye, L., Yuan, Z., 2013. Effect of H_2S on N_2O reduction and accumulation during

678   denitrification by methanol utilizing denitrifiers. *Environmental Science &*

679   *Technology* 47, 8408-8415.

680   Peng, L., Ni, B., Ye, L., Yuan, Z., 2015. Selection of mathematical models for N_2O

681   production by ammonia oxidizing bacteria under varying dissolved oxygen and

682   nitrite concentrations. *Chemical Engineering Journal* 281, 661-668.

683   Peng, L., Ni, B.J., Erler, D., Ye, L., Yuan, Z., 2014. The effect of dissolved oxygen on

684   N_2O production by ammonia-oxidizing bacteria in an enriched nitrifying sludge.

685   *Water Research* 66, 12-21.

686   Peng, L., Ni, B.J., Ye, L., Yuan, Z., 2015a. The combined effect of dissolved oxygen and

687   nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying

688   sludge. *Water Research* 73, 29-36.

689   Peng, L., Ni, B.J., Ye, L., Yuan, Z., 2015b. N_2O production by ammonia oxidizing

690   bacteria in an enriched nitrifying sludge linearly depends on inorganic carbon

concentration. *Water Research* 74, 58-66.

Peng, L., Sun, J., Liu, Y., Dai, X., Ni, B.J., 2016. Nitrous oxide production in co- versus counter-diffusion nitrifying biofilms. *Scientific Reports* 6, 28880: DOI: 10.1038/srep28880.

Pintucci, C., Carballa, M., Varga, S., Sarli, J., Peng, L., Bousek, J., Pedizzi, C., Ruscalleda, M., Tarragó, E., Prat, D., 2016. The ManureEcoMine pilot installation: advanced integration of technologies for the management of organics and nutrients in livestock waste. Water Science and Technology, 75(5-6):1281-1293

Poth, M., Focht, D.D., 1985. [15]N kinetic analysis of N_2O production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. *Applied and Environmental Microbiology* 49, 1134-1141.

Quan, X., Zhang, M., Lawlor, P.G., Yang, Z., Zhan, X., 2012. Nitrous oxide emission and nutrient removal in aerobic granular sludge sequencing batch reactors. *Water Research* 46, 4981-4990.

Rajagopal, R., Béline, F., 2011. Nitrogen removal via nitrite pathway and the related nitrous oxide emission during piggery wastewater treatment. *Bioresource Technology* 102, 4042-4046.

Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326, 123-125.

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2014. Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. *Water Research* 57, 162-171.

714   Remde, A., Conrad, R., 1990. Production of nitric oxide in *Nitrosomonas europaea* by

715   reduction of nitrite. *Archives of Microbiology* 154, 187-191.

716   Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M., Pijuan, M., 2015.

717   Minimizing N_2O emissions and carbon footprint on a full-scale activated sludge

718   sequencing batch reactor. *Water Research* 71, 1-10.

719   Scaglione, D., Tornotti, G., Teli, A., Lorenzoni, L., Ficara, E., Canziani, R., Malpei, F.,

720   2013. Nitrification denitrification via nitrite in a pilot-scale SBR treating the liquid

721   fraction of co-digested piggery/poultry manure and agro-wastes. *Chemical*

722   *Engineering Journal* 228, 935-943.

723   Schaubroeck, T., De Clippeleir, H., Weissenbacher, N., Dewulf, J., Boeckx, P.,

724   Vlaeminck, S.E., Wett, B., 2015. Environmental sustainability of an energy self-

725   sufficient sewage treatment plant: improvements through DEMON and co-digestion.

726   *Water Research* 74, 166-179.

727   Stein, L.Y., 2011. Surveying N_2O-producing pathways in bacteria. *Methods in*

728   *Enzymology* 486, 131-152.

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

730   secondary activated sludge in nitrifying conditions of urban wastewater treatment

731   plants: Effect of oxygenation level. *Water Research* 40, 2972-2980.

732   Vadivelu, V.M., Keller, J., Yuan, Z., 2007. Effect of free ammonia on the respiration and

733   growth processes of an enriched *Nitrobacter* culture. *Water Research* 41, 826-834.

734   Vlaeminck, S.E., De Clippeleir, H., Verstraete, W., 2012. Microbial resource

735   management of one-stage partial nitritation/anammox. *Microbial Biotechnology* 5,

736   433-448.

737   Wang, D., Wang, Y., Liu, Y., Ngo, H.H., Lian, Y., Zhao, J., Chen, F., Yang, Q., Zeng,

738   G., Li, X., 2017. Is denitrifying anaerobic methane oxidation-centered technologies

739   a solution for the sustainable operation of wastewater treatment Plants? *Bioresource*

740   *Technology* 234, 456-465.

741   Wang, D., Wang, Q., Laloo, A.E., Yuan, Z., 2016. Reducing N_2O Emission from a

742   Domestic-Strength Nitrifying Culture by Free Nitrous Acid-Based Sludge

743   Treatment. *Environmental Science & Technology* 50, 7425-7433.

744   Weissenbacher, N., Takacs, I., Murthy, S., Fuerhacker, M., Wett, B., 2010. Gaseous

745   nitrogen and carbon emissions from a full-scale deammonification plant. *Water*

746   *Environment Research : A Research Publication of the Water Environment*

747   *Federation* 82, 169-175.

748   Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B.L., Joss, A., Emmenegger, L.,

749   Mohn, J., 2013. Isotope signatures of N_2O in a mixed microbial population system:

750   constraints on N2O producing pathways in wastewater treatment. *Environmental*

751   *Science & Technology* 47, 1339-1348.

752   Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L., Siegrist, H., 2012a. Mechanisms of

753   N_2O production in biological wastewater treatment under nitrifying and denitrifying

754   conditions. *Water Research* 46, 1027-1037.

755   Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L., Siegrist, H., 2012b. Mechanisms of

756   N_2O production in biological wastewater treatment under nitrifying and denitrifying

757   conditions. *Water Research* 46, 1027-1037.

758   Yu, R., Kampschreur, M.J., Loosdrecht, M.C.M., Chandran, K., 2010. Mechanisms and

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

760   transient anoxia. *Environmental Science & Technology* 44, 1313-1319.

761   Zhou, Y., Pijuan, M., Zeng, R.J., Yuan, Z., 2008. Free nitrous acid inhibition on nitrous

762   oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge.

763   *Environmental Science & Technology* 42, 8260-8265.

764   Zhu, X., Chen, Y., 2011. Reduction of N_2O and NO generation in anaerobic-aerobic (low

765   dissolved oxygen) biological wastewater treatment process by using sludge alkaline

766   fermentation liquid. *Environmental Science & Technology* 45, 2137-2143.
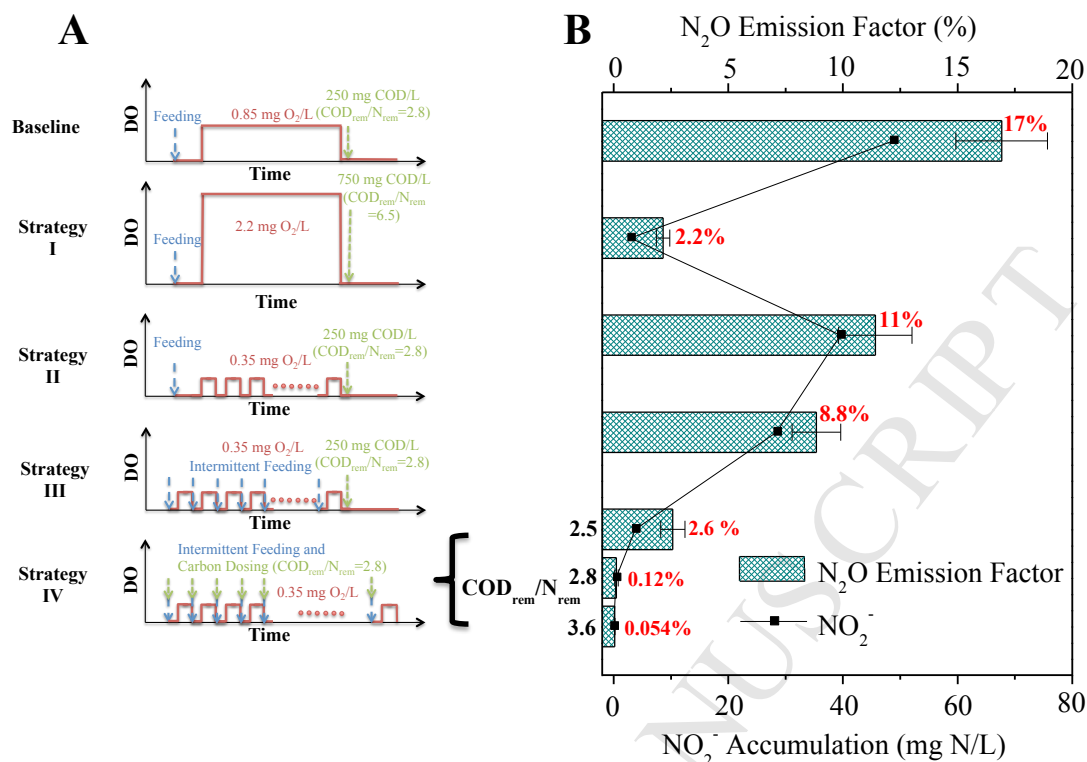
Figure 1. The impact of different mitigation strategies on total N_2O emission from the SBR. (A: Schematic representation of different mitigation experiments; B: N_2O emission from different mitigation strategies, Strategy IV was investigated under three COD_{rem}/N_{rem} levels as shown above.).
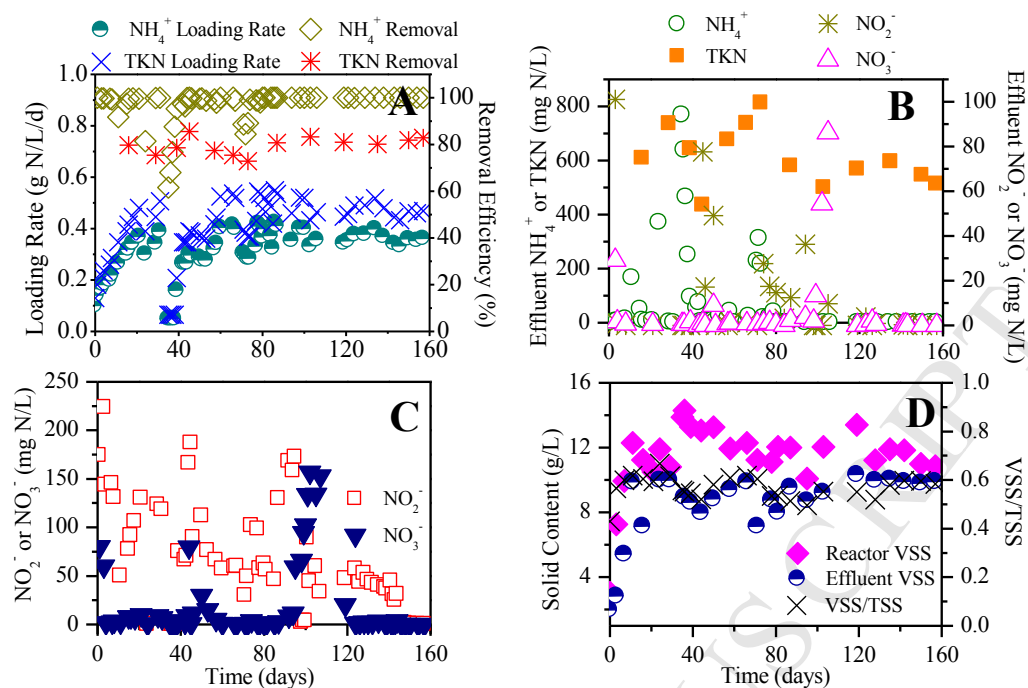
Figure 2. The system performance of Nit/DNit SBR treating manure centrate, operated with DO range of 0 - 1.5 mg O_2/L and pH range of 7.5 - 8.0. (A: loading rates and removal efficiencies of NH_4^+ and TKN; B: effluent TKN, NH_4^+, NO_2^- and NO_3^-; C: NO_2^- and NO_3^- accumulations at the end of aerobic phase; D: solid content in the reactor and the effluent).
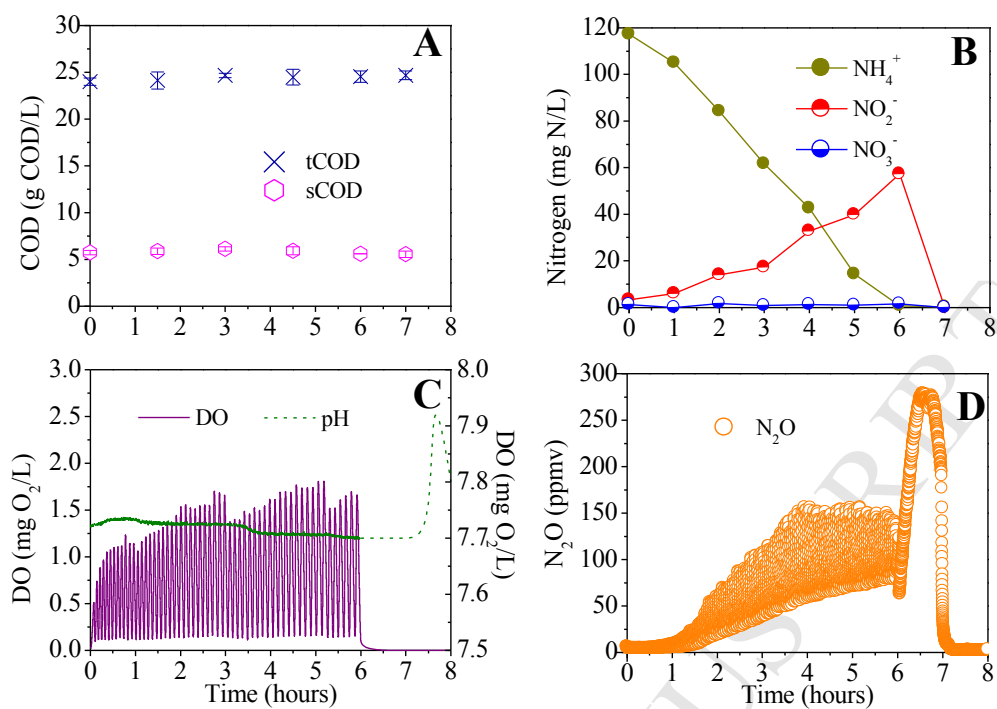
Figure 3. Profiles of (A) tCOD and sCOD; (B) NH_4^+, NO_2^- and NO_3^-; (C) DO and pH along with (D) gaseous N_2O in one SBR cycle under baseline conditions.
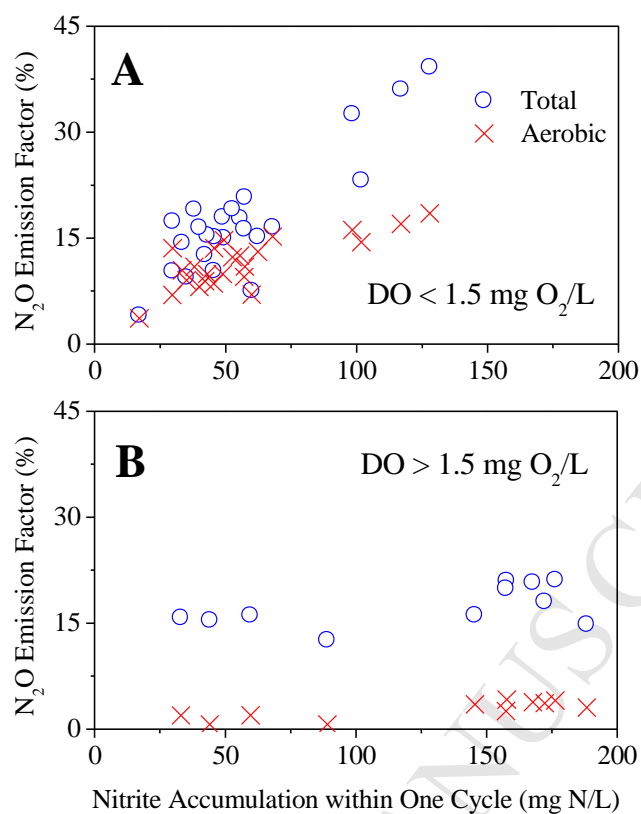
Figure 4. Relationship between N_2O emission factor (total and aerobic) and nitrite accumulation at varying DO concentrations (A: DO levels below 1.5 mg O_2/L; B: DO levels above 1.5 mg O_2/L).
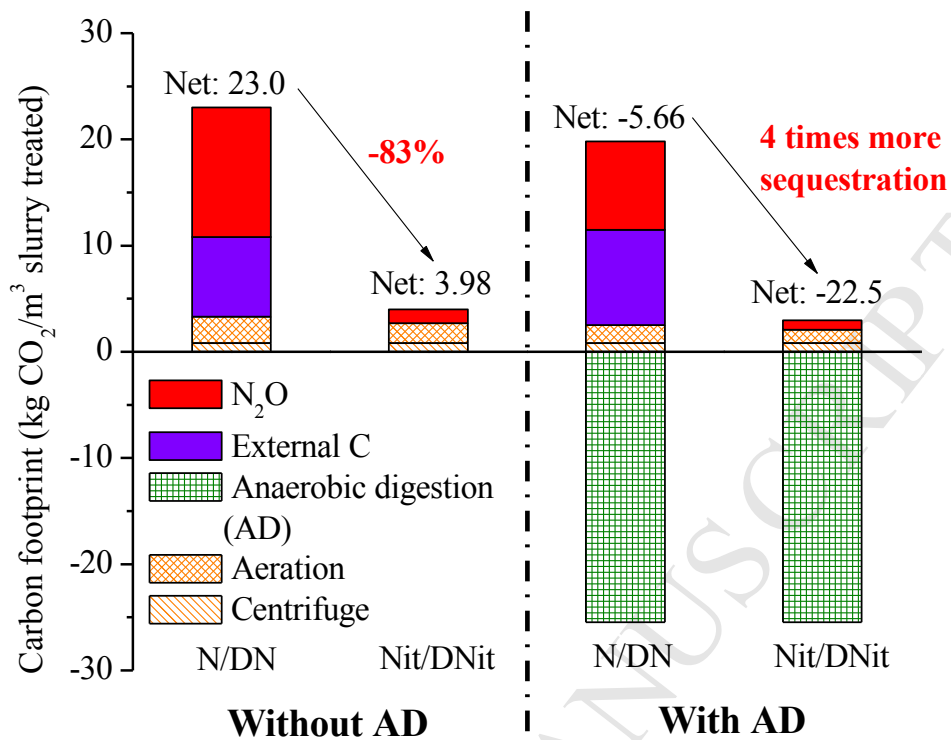
Figure 5. Extrapolated operational carbon footprint of pig slurry processing lines (without or with anaerobic digestion, AD): Comparison between nitrification/denitrification (N/DN) and nitritation/denitritation (Nit/DNit), expressed per m^3 of slurry treated, in the respective emission scenarios of 0.0075 and 0.0008 kg N_2O-N/kg N loaded.

Highlights

- Nit/DNit can lower operational costs with around 1-1.8 EUR/m^3, compared to N/DN.
- Improper Nit/DNit operation gave a very high N_2O emission: 17% of the NH_4^+-N load.
- Nitritation was the major N_2O source, stimulated by high nitrite and low DO levels.
- Synergy between aeration, feeding and carbon dosage mitigated the emission by 99%.
- The low COD/N requirement for Nit/DNit no longer offsets ecological sustainability.