Autotrophic nitrogen removal by a two-step SBR process applied to mixed agro-digestate

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1. Introduction

Anaerobic digestion (AD) can effectively treat livestock wastewater and produce renewable energy as biogas. The liquid fraction of the digested material is rich in ammonium and its disposal on agricultural soil is regulated by the European directive on nitrates (91/676/CEE). Therefore, N removal may be required in intensive breeding farms. This aspect has prompted attention toward advanced biological processes such as the nitritation–denitritation process (Scaglione et al., 2013), and the completely autotrophic process (partial nitritation and anammox) that are cost effective thanks to lower aeration and external carbon source requirements (Van Hulle et al., 2010), nevertheless N_2O emissions could be a critical issue (Kampschreur et al., 2009; Kong et al., 2013; Rodriguez-Caballero and Pijuan, 2013).

The anammox process was studied and applied since the 90s mainly for the treatment of the liquid fraction of municipal sludge digestate, of waste streams from the food industry (such as digested potato wastewater, and fish canning effluents, Van Hulle et al., 2010), and of leachate (Ruscalleda et al., 2010; Wang et al., 2010).

Recently, the physiology and kinetics of Brocadia Anammoxidans in a highly enriched cell suspension were explored finding a specific maximum growth rate of 0.21 d^{-1}, corresponding to a doubling time of 3.3 days at 30 °C (Lotti et al., 2014).

Lab tests on the treatment of livestock effluent with anam-mox bacteria have been performed in the USA (Vanotti et al., 2007; Magrí et al., 2012a), Korea (Dong and Tollner, 2003; Choi et al., 2004), Japan (Yamamoto et al., 2008), China (Zhang et al., 2013), and in Northern Europe (Molinuevo et al., 2009). In all these studies, the livestock wastewater was either diluted or intensive pretreatment was applied prior to the anammox process.

Hwang et al. (2005) tested a lab-scale combined SHARON-ANAMMOX process treating piggery wastewater with 40% dilution. In the anammox reactor, the volumetric nitrogen conversion rate (NCR) and the specific nitrogen removal rate (NRR) were

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0.72 kg N $(m^3_{reactor}\ d)^{-1}$ and 0.44 kg N $(kg\ VSS\ d)^{-1}$, respectively, at a nitrogen loading rate (NLR) of 1.36 kg N $(m^3_{reactor}\ d)^{-1}$.

Besides the huge variability in the quality and quantity, a relevant issue when applying the anammox process to piggery manure could be the excess of biodegradable organic carbon, as reported by Molinuevo et al. (2009). They treated the pig manure effluent after UASB-post-digestion and partial oxidation in a granular anammox reactor. After increasing the fraction of digestate blended with synthetic wastewater above 12% v/v (corresponding to 242 mg L^{-1} COD) denitrification was observed to become the dominant process. No details are given on the biodegradable fraction of the influent COD.

Yamamoto et al. (2008) tested a partial nitritation/anammox configuration (up-flow fixed bed column) to treat a piggery digestate. The liquid fraction of digestate was pre-treated by clari-flocculation and diluted. The anammox nitrogen removal rates decreased to 0.22 kg N $(m^3_{reactor}\ d)^{-1}$ corresponding to 10–20% of the NRR obtained with a synthetic influent. In a later experimenta-tion, Yamamoto et al. (2011), while treating piggery digestate, obtained a relatively high anammox nitrogen removal rate of 2.0 kg N $(m^3_{reactor}\ d)^{-1}$ under a NLR of 2.2 kg N $(m^3_{reactor}\ d)^{-1}$. How-ever, the partial nitritation effluent was filtered and diluted 7–10 times before being fed to the anammox reactor. Qiao et al. (2010) reported a combined lab-scale partial nitritation reactor and a gran-ular anammox reactor treating the liquid fraction of digested piggery waste. By diluting a minimum of 1.5 times the partially nitrified effluent, the NRR of the anammox reactor reached 3.1 kg N $(m^3_{reactor}\ d)^{-1}$ under a NLR of 4.1 kg N $(m^3_{reactor}\ d)^{-1}$.

A relevant issue when treating livestock wastewaters could be the presence of antibiotics or heavy metals. Fernandez et al.(2009) reported a decrease of 75% of the specific anammox activity when 20 mg L^{-1} of chloramphenicol were continuously fed to an anammox SBR. These authors also observed similar effects when 50 mg L^{-1} of tetracycline hydrochloride were continuously fed.

Lotti et al. (2012a) performed batch inhibition tests on copper, zinc and antibiotics. Increasing concentrations and prolonged exposure to copper and zinc led to a decreasing specific anammox activity (SAA). The authors concluded that these substances do not pose a real hazard to the application of the anammox process, since a lower specific activity can be handled by a higher biomass concentration in the reactor.

Most of the cited authors suggest that the anammox process can be applied to piggery wastewaters and much interest is posed on autotrophic nitrogen removal applied on manure digestate (Magrì et al., 2013). However, to the knowledge of the authors, no published data are available on the treatment of undiluted pig-gery effluent at pilot scale. Moreover, experimental data are lim-ited to piggery wastewaters and no results are available on the treatment of digestates with mixed composition (e.g. piggery and poultry manures and energy crops).

The main objective of this experimentation was to study the applicability of the fully autotrophic N removal process to the undiluted liquid fraction of a digestate coming from the anaerobic digestion of mixed agro-wastes, mainly made of piggery and poultry manure.

Results of the following experimental phases will be presented and discussed:

- Application of the partial nitritation process (PARNIT) at pilot-scale using a 650 L sequencing batch reactor to the treatment of a real supernatant from the AD of animal and agricultural wastes (378 days of experimentation);
- Treatment of the effluent of the PARNIT process at decreasing dilution ratios with water, up to undiluted waste-water, in a lab-scale ANAMMOX reactor (3 L sequencing batch reactor, 334 days of experimentation).

2. Methods

2.1. Origin of the supernatant

The supernatant used in this experimentation came from a piggery farm in Northern Italy (Lombardy Region, province of Cremona) breeding up to 20.000 pigs. At the hosting farm, a full scale wastewater treatment plant (WWTP: fine screening, flotation, and a conventional predenitrification/nitrification, secondary set-tling) and a full scale anaerobic digester fed on a mixture of the floated piggery solids from the WWTP, poultry manure and energy crops (maize or wheat), were in operation followed by solid/liquid separation by centrifugation. The supernatant from the solid/liquid separation step (centrifuge, Pieralisi, FP600 2RS/M) was normally returned to the WWTP. The pilot plant was fed on this supernatant that had highly variable characteristics (Table 1) because of seasonal variations in the piggery waste production, and in the quality and quantity of the digested co-substrates.

2.2. Reactors characteristics, operation and monitoring plan

2.2.1. Pilot scale partial nitritation reactor (PARNIT reactor)

The pilot plant was made of a buffer tank with a total volume of 1.8 m^3 and a sequencing batch reactor (SBR) with a maximum volume of 0.8 m^3 and an actual reaction volume of 0.65 m^3. Details on the reactor can be found in Scaglione et al. (2013).

As for the operational plan, the SBR was inoculated with activated sludge taken from the WWTP and it was previously operated in nitritation–denitritation (DENO2) mode for 1 year, as reported in Scaglione et al. (2013). The transition from the DENO2 mode to the PARNIT mode was conducted in 8 days by modifying the REACT phase within each cycle, i.e. by the progressive elimination of the denitrification phases. At the end of this transition phase, the SBR performed the following 6-hour cycle: 1 h aerobic FILL and REACT, 3 h aerobic REACT, 1 h SETTLE + DRAW, 1 h IDLE.

The ammonium oxidation efficiency was controlled by regulating the influent alkalinity with HCl dosage to obtain the optimal NH_4-N to alkalinity molar ratio of 1:1.

As for the monitoring plan, the following analyses were performed: nitrogen compounds, alkalinity, COD, TSS in the influent and effluent were measured twice a week; mixed liquor total and volatile suspended solids (MLTSS and MLVSS) were measured weekly; BOD_5 and BOD_{20} in the influent and effluent were measured every 3–4 weeks; maximum specific nitritation activity of

Table 1
Characteristics of the supernatant used to feed the PARNIT reactor.

Parameter	Unit	Average ± st. dev.
TKN	mg/L	1590 ± 10%
NH_4-N	mg/L	1220 ± 16%
CODsol	mg/L	2350 ± 50%
BOD_5sol	mg/L	500 ± 48%
BOD_{20} sol	mg/L	620 ± 37%
pH	–	8.1 ± 1%
Conductivity	(mS/cm)	14.7 ± 8%
VSS	mg/L	274 ± 45%
TSS	mg/L	326 ± 54%
Alkalinity	$mgCaCO_3/L$	6330 ± 11%
NH_4-N/alkalinity	mol/mol	0.70 ± 13%
SO_4^{2-}	mg/L	76 ± 43%
Cl^-	mg/L	1996 ± 2%
PO_4^{3-}	mg/L	8.9 ± 50%
F^-	mg/L	<0.1
Ca^{2+}	mg/L	203 ± 23%
Mg^{2+}	mg/L	93 ± 36%
K^+	mg/L	985 ± 35%
Na^+	mg/L	706 ± 36%

the SBR activated sludge was assessed once a month according to the procedure described in Scaglione et al. (2013).

2.2.2. Lab-scale anammox reactor

The anammox reactor was a 3 L jacketed and thermostated (34–35 °C) lab-scale SBR (Fig. 1). It was equipped with time-controlled feeding/discharging pumps, mechanical mixing (100 rpm), a pH control unit (HCl and $NaHCO_3$ solutions dosage) to maintain the pH between 7.0 and 8.0, and two temporized valves. One valve was placed in the gas flushing line (95% N_2 and 5% CO_2) and the other in the off-gas line to release the off-gas accumulated during each cycle. The reactor operated in a SBR mode with a 12 h cycle (9 h FILL, 2.5 h REACT, 10 min SETTLE and 20 min DRAW). Before the settling phase the mixed liquor was flushed at a flow rate of approximately 0.5 LPM for 2 min to improve granules settleability (removing nitrogen trapped in the interstitial spaces of granules), to assure anoxic conditions (removing traces of dissolved oxygen) and to generate overpressure in the headspace to avoid vacuum following the DRAW phase.

The reactor was inoculated with granular sludge from the upper part of the lower compartment of the full-scale anammox reactor of Dokhaven–Sluisjesdijk WWTP in Rotterdam, the Netherlands (Van der Star et al., 2007). The reactor contains granular anammox sludge and treats reject water after partial nitritation in a SHARON reactor. The inoculum was confirmed to consist of a "*Brocadia*" enrichment by *fluorescence in situ hybridization* (FISH), the sludge having been hybridized with AMX-820 and not with KST-157 oligonucleotide probes (Lotti et al., 2014). The initial biomass concentration was 2.3 g TSS L^{-1} and 79% VSS/TSS.

The following analyses were performed for monitoring the process: N compounds, COD, TSS in the influent and effluent: twice a week; MLTSS and MLVSS: monthly; BOD_5 and BOD_{20} in the influent and effluent: every 3–4 weeks; maximum specific anammox activity (NRRmax) of the SBR biomass: once or twice a week.

The NRRmax was measured as follows: the SBR was set in the batch mode, then a spike of a known amount of nitrous and ammoniacal nitrogen at a stoichiometric ratio of 1:1.3 was performed. Then, 3 or 4 more samples were taken at regular intervals of 10–30 min, and the concentrations of nitrous and ammonium nitrogen were measured. The NRRmax was obtained from the linear regression slope.

2.3. Analytical methods

Commercial photochemical test kits (Hach Lange GmbH, Dusseldorf, Germany), were used for ammonium, nitrite, nitrate and COD measurements on 0.45 μm filtered samples. Total Kjeldahl Nitrogen (TKN), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Biochemical Oxygen Demand (BOD), alkalinity (by the potentiometric method) were all measured according to the APHA Standard Methods for the Examination of Water and Wastewater. Conductivity was measured with multimeter dual 3420 (WTW, DE). Soluble components were analyzed without replicates, since representative samples were easily obtained, while VSS and TSS were measured in duplicate.

Metals were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with a ICPMS model 7700X (Agilent Technologies, USA) according to the US-EPA method 200.8 EMMC version revision 5.4 (1994).

Particle size distribution was measured with the CILAS 1180 analyzer which allows the measurement of particles between 0.04 and 2.500 μm. The fine particles were measured by the Fraunhofer diffraction method, while the coarse particles were measured with a real-time Fast Fourier Transform of the image obtained by a CCD camera, equipped with a digital processing unit.

2.4. Nitrous oxide off-gas analyses

To assess the N_2O concentration in the off-gas, six to ten gas samplings were taken manually during the SBR cycles using 1 or 2 L Cali-5-BondTM gas-bags. The off-gas grab samples were analyzed for N_2O concentration by gas chromatography (MICROGC 3000, Agilent, USA) using a PLOT-Q column (8 m × 0.32 mm, temperature 60 °C and pressure 15 psi) and Helium as gas-carrier. Then, the N_2O emission (kg N d^{-1}) was calculated by integrating the product of the off-gas flow rate with the measured partial

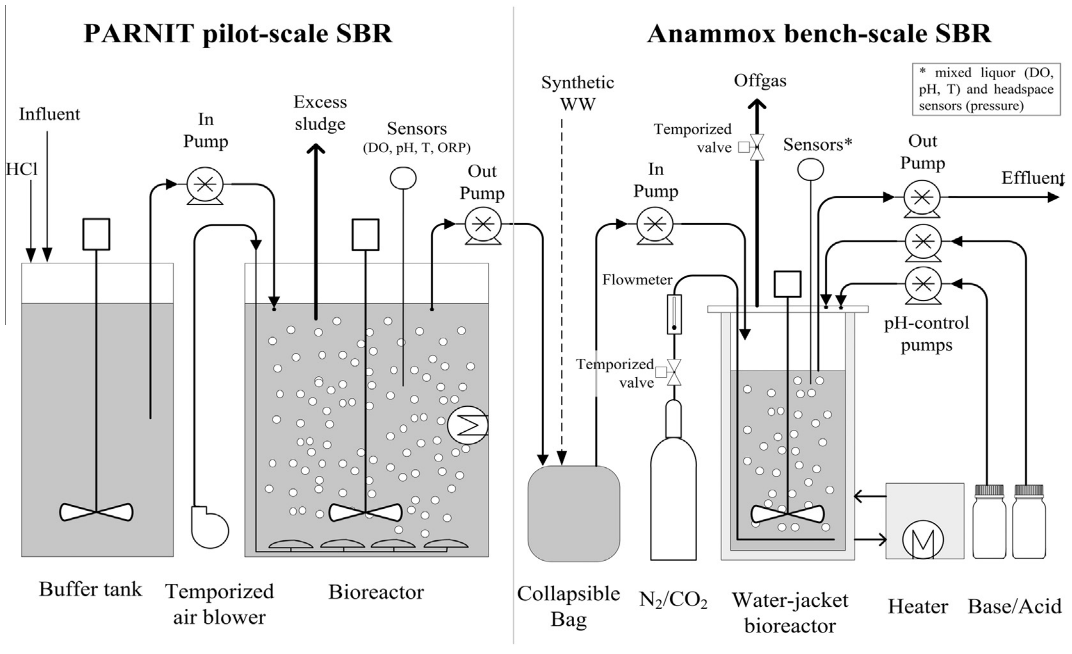

Fig. 1. Scheme of the partial nitritation and anammox pilot plants.

concentration of N_2O. With regards to the PARNIT reactor the N_2O emission was calculated only for the aerobic phase of the SBR cycle assuming an off-gas flow rate equal to the air flow rate provided by the aeration system. Differently, for the anammox bioreactor, the off-gas flow rate was calculated from overpressure data.

2.5. Statistical analyses

The software Minitab® 16.2.4 (from Minitab Inc.) was used to perform linear regression of ammonium and nitrite data in the NRRmax determination. Together with the best fitting value, its standard error was also retrieved.

The average removal efficiency was computed by means of a conventional spread sheet; both the mean value and its coefficient of variation were computed per each experimental period.

3. Results and discussion

The chemical composition of the anaerobic digester supernatant is shown in Table 1.

The characteristics varied notably during the course of the experimentation. Average BOD concentration values are quite high as well as the BOD_5 to BOD_{20} ratio (around 80%), indicating an incomplete anaerobic degradation upstream. Significant changes in the concentration of total and volatile suspended solids (TSS and VSS) were also observed. Nevertheless, the average value of the suspended solids proves the good separation efficiency guaranteed by the centrifuge. The ammonia, TKN, and alkalinity contents are also variable. In this regard, it is noteworthy that the NH_4-N/alkalinity molar ratio is 0.7 which is slightly lower than the ideal value of 1. A lower value is indicative of the presence of other sources of alkalinity besides ammoniacal nitrogen, which can be produced by anaerobic removal of sulfates or the dosage of iron oxides or hydroxides in the digester for the precipitation of hydrogen sulfide. The conductivity of the digestate was quite high but within the range of other reported experiences for complete autotrophic process (Van Hulle et al., 2010) and pH was around 8.

The metals content concentration (as mg L^{-1}) was measured once (day 264) providing the following values: Al 2.1, Sb < 0.02, As < 0.02, Ba < 0.02, B 0.92, Cd < 0.003, Cr tot < 0.02, Cu 0.9, Fe 1.42, Mn 0.4, Hg < 0.001, Mb 0.05, Ni 0.05, Pb < 0.01, Se 0.042, Sn < 0.02, Zn 1.42. Zinc and Copper concentrations were less than half of the IC50 concentration for anammox bacteria (3.9 mg L^{-1} for zinc and 1.9 mg L^{-1} for copper) reported by Lotti et al. (2012a).

3.1. Partial nitritation reactor

During the course of the experimentation, the pilot plant was operated according to the following operational conditions: the temperature was kept at 30 °C, the HRT was 2 d, the OD in the range 0.5–1 mg L^{-1} and the NLR (calculated including the idle phase) between 0.5 and 0.7 g N $(L\ d)^{-1}$. Regarding the total sludge retention time (SRT), the value was 20 days. However, its control has been particularly difficult because of the impossibility of measuring on site the daily fluctuations of suspended solids concentrations in the effluent and in the SBR reactor. This parameter has then varied significantly (between 10 and 50 days) during the course of the experimentation, but the variability of the SRT did not imply a significant impact on the efficiency of the process since process control was performed by adjusting the influent alkalinity to ammonium ratio, as explained hereafter.

The ammonium nitrogen to alkalinity ratio was 0.7 mol NH_4^+/mol Alk, lower than the optimal ratio of 1 mol/mol that allows the nitritation process to proceed up to 50% ammonium oxidation without external control (Ganigué et al., 2007), as all the alkalinity would be consumed once half the ammonium is oxidized to nitrite. Under the pilot plant operating conditions, a kinetic-based control strategy would require a careful sludge age control, but this option was practically not feasible, because of the difficulty in operating at constant SRT. Therefore, it has been necessary to correct the ammonium to alkalinity molar ratio from 0.7 to 1 mol/mol by dosing HCl in the influent buffer tank. As expected, during the aerobic phase of the SBR cycle, the pH value decreased continuously until a final value of 5.9–6.5 was achieved. At this point, the nitritation process was inhibited and no relevant ammonium oxidation was further observed.

In Fig. 2 nitrogen compounds in the influent and effluent streams (2a) and NO_2/NH_4 ratio in the effluent (2b) are reported. As expected, the influent ammonium is partially oxidized to nitrite, while the nitrate concentration always remained below 17 mg N L^{-1} with an average value of 13 ± 4 mg N L^{-1}, proving that the applied operational conditions allowed for a stable suppression of NOB (Nitrite Oxidizing Bacteria). After few days from the start-up, the average value of the nitrite to ammonium molar ratio in the effluent was 1.25 ± 0.25 mol/mol with few very high (up to 4.9) or very low (down to 0.19) values obtained during unusual transient operational conditions. Therefore, the PARNIT effluent can be considered as suitable to be fed to the anammox reactor.

From the available data on the influent and effluent COD concentrations (COD_{IN} and COD_{OUT}, respectively), the COD removal efficiency (η_{COD}) was calculated as follows:

$$\eta_{COD} = (COD_{IN} - COD_{OUT})/(COD_{IN})$$

The removal efficiency of COD averaged as low as 42 ± 18%, which suggests the presence of recalcitrant organics that could not be fully oxidized under the PARNIT operational conditions. The almost complete removal of biodegradable organics is confirmed by BOD_5 data, as it reduced from 500 ± 238 mg L^{-1} in the

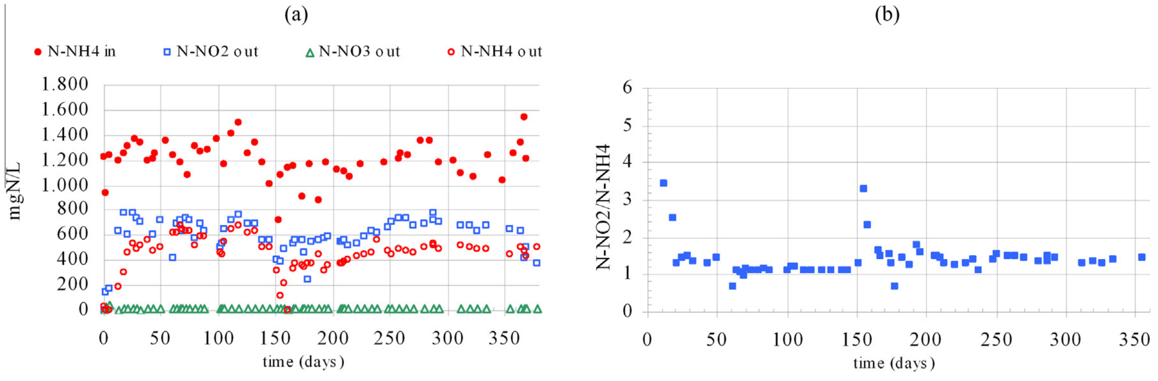

Fig. 2. Concentrations of nitrogen compounds in influent and effluent streams of the PARNIT reactor (a) and NO_2/NH_4 ratio in the effluent (b).

influent to 25 ± 15 mg L^{-1} in the effluent, corresponding to a 95% removal. This is a crucial issue, since the presence of high concentrations of biodegradable organic matter would favor the development of heterotrophic bacteria at the expenses of anammox bacteria in the subsequent anammox reactor (Molinuevo et al., 2009).

The nitrogen removal efficiency (η_N) was estimated as follows:

$$\eta_N = (TKN_{IN} - N_{OUT})/(TKN_{IN})$$

where TKN_{IN} = TKN concentration in the influent, N_{OUT} = sum of the ammoniacal (accounting on average for the 93% of the effluent TKN), nitrous and nitric nitrogen concentrations. An average value of 0.09 ± 0.16 was obtained, confirming that the PARNIT reactor did not contribute to a substantial nitrogen removal for the lack of a denitrification phase.

An average contribution of the nitrogen uptake for heterotrophic growth was estimated from the average COD reduction as follows:

$$\Delta N_{growth} = (COD_{IN} - COD_{OUT}) \times Y_{obs} \times i_{XB}$$

where i_{XB} is the nitrogen content in the heterotrophic bacteria cell (0.086 g N g^{-1} COD, after Henze et al., 2000) and Y_{obs} is the observed growth yield, estimated as:

$$Y_{obs} = Y/((1 + b' \times SRT))$$

where b' is the biomass decay constant corrected for cryptic growth, computed as follows:

$$b' = b \times [1 - (1 - f) \times Y]$$

f = inert fraction in the heterotrophic biomass (0.1 g COD/g COD, after Henze et al., 2000); Y = heterotrophic biomass yield (0.63 g COD/g COD, after Henze et al., 2000); b = heterotrophic decay constant; a value of 1.76 d^{-1} was computed for "b" by using the conventional value (0.62 d^{-1} at 20 °C, after Henze et al., 2000) and by taking into account the temperature correction factor θ of 1.11 (Van Hulle, 2005).

As for the SRT, the lower (10 d) value was used to compute the corresponding maximum value of the nitrogen uptake for heterotrophic growth, which resulted to be 7 mg N L^{-1} equals to 0.044% of the influent TKN. Nitrogen uptake is therefore almost irrelevant and the observed 9% nitrogen removal should be attributed to denitrification likely occurring during the quite long IDLE phase.

Disregarding the first 20 days of operation, it was possible to continuously obtain a NO_2/NH_4 molar ratio in the effluent of 1.30 ± 0.22 mol/mol, suitable for the anammox process.

As for the mixed liquor total suspended solids (MLTSS), their concentration varied in the course of the experimentation within the interval 2–6 g L^{-1}, while the ratio between volatile and total suspended solid remained between 0.89 and 0.95. The MLTSS variation was due to both the variation in the SRT and in the influent organic load.

The specific nitrification rate (SNR) of the mixed liquor was measured every second week. During the initial 100 days, a constant increase in SNR was observed from 10 to 15 g N (kg MLVSS $h)^{-1}$, typical of the previous operating conditions (pro-cess DENO2, Scaglione et al., 2013) up to 30 g N (kg MLVSS $h)^{-1}$. Later on, the SNR remained relatively stable around a mean value of 26 ± 9.3 g N (kg MLVSS $h)^{-1}$. The high specific nitritation activity is indicative of an enrichment of nitrifying bacteria in the mixed liquor.

3.2. Anammox lab reactor

The anammox reactor was operated with a HRT of 2 d and an average NLR of 0.5–0.6 g N (L $d)^{-1}$. The feed was initially made of a mineral medium (Scaglione et al., 2012), then the PARNIT effluent was blended at increasing percentage (10%, 25%, 33%, 50%, 70%, 100%), while adjusting the nitrogen loading and the NH_4/NO_2 molar ratio (kept at 1.1 to work under nitrite-limiting conditions and increased to 1.3 from day 228) by adding $NaNO_2$ and NH_4Cl. Since, the PARNIT effluent had a very low alkalinity, $NaHCO_3$ was added to a final concentration of 1 g L^{-1}. The reactor was operated for 350 days with a 40 days stop during summer, while the bio-mass was kept at 4 °C. The average MLSS concentrations was 2–3 g TSS L^{-1} with 88% VSS/TSS but this data are to be considered as a rough estimation of real values because it was difficult to take a representative biomass sample. As a consequence, the SRT was not controlled but was estimated to be in the range 30–50 d during the experimentation.

In Fig. 3a, the NRRmax is plotted together with the percentage of PARNIT effluent in the feed. During the initial start-up and restart after the first break, the activity increased from values in the range 1–2 kg N (m^3 $d)^{-1}$ up to values in the range 3–4 kg N (m^3 $d)^{-1}$ in about 30 days, suggesting an increase of the active bio-mass. In contrast, after each increase in the percentage of PARNIT effluent in the feed, the activity decreased, suggesting that the real wastewater had an inhibitory effect on the anammox bacteria.

A minimum value of 0.14 kg N (m^3 $d)^{-1}$ was achieved during the first days of operation at 75% and 100% PARNIT effluent. In these low-activity phases, temporary but significant increase in nitrite concentration in the effluent was observed. After 30 days of operation with undiluted PARNIT effluent, the anammox activity began to recover and continued rising until the end of the experi-mentation, achieving values greater than 1 kg N (m^3 $d)^{-1}$. The trend observed in the last 2 months of operation at 100% PARNIT effluent suggests the ability of anammox bacteria to adapt to inhib-itory substances that may be present in the agricultural digestate.

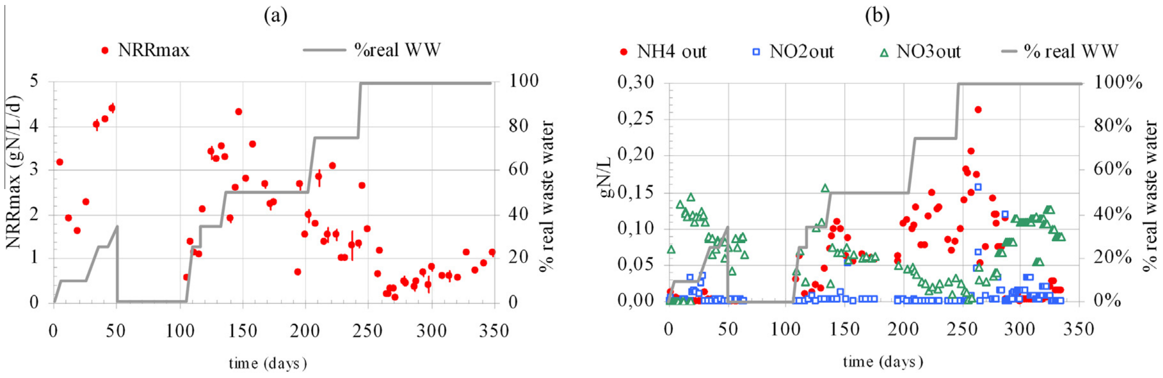

Fig. 3. Maximum nitrogen removal rate (NRR_{max}, error bars refer to the standard error of slope estimates) measured in the anammox reactor during the experimentation (a) and concentration of the nitrogen species in anammox effluent (b). The continuous line represents the % of PARNIT effluent (real WW) blended.

Table 2
Removal efficiencies in the anammox SBR compared to the average fraction of PARNIT effluent blended in the feed.

% PARNIT effluent blended	NLR (kg N m^{-3} d^{-1})	NO_2/NH_4 fed (mol/mol)	N removal efficiency (%)	$\Delta NO_2^-/\Delta NH_4^+$ (mol/mol)	$-\Delta NO_3^-/\Delta NH_4^+$ (mol/mol)
20	0.56 ± 0.09	1.14 ± 0.05	90.5 ± 2.7	1.14 ± 0.05	0.169 ± 0.06
33	0.56 ± 0.01	1.00 ± 0	93.5 ± 4.7	1.01 ± 0.02	0.127 ± 0.099
50	0.55 ± 0	1.08 ± 0.04	87.7 ± 3.5	1.25 ± 0.09	0.119 ± 0.062
75	0.55 ± 0	1.10 ± 0	88.0 ± 2.6	1.38 ± 0.08	0.043 ± 0.027
100	0.49 ± 0.11	1.28 ± 0.03	92.8 ± 4.6	1.35 ± 0.20	0.076 ± 0.060

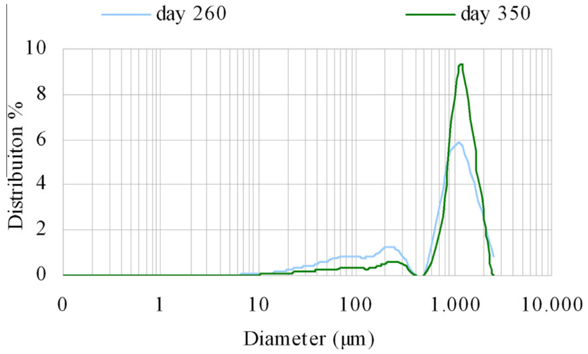

Fig. 4. Particle size distribution of granular biomass inside the reactor sampled at day 260 and day 350.

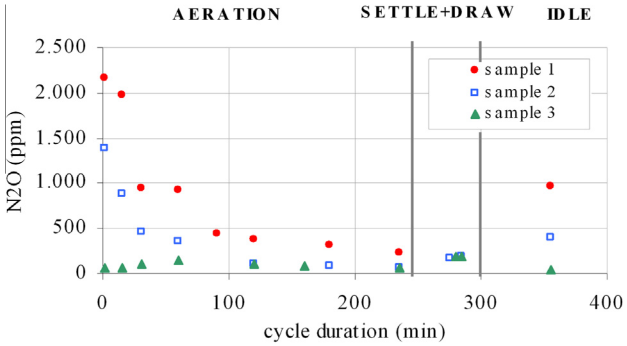

Fig. 5. N_2O concentration in the PARNIT off-gas.

In Fig. 3b, the effluent soluble nitrogen concentrations during the course of the experimentation are plotted. This values were used to compute the anammox removal efficiency as well as the stoichiometric ratios of nitrate formation and nitrite consumption per mol of ammonium removed. Overall, the average N removal efficiency was 91 ± 10% with an average nitrite removal efficiency higher than 97%, the NO_2-$N_{removed}$/NH_4-$N_{removed}$ molar ratio was 1.28 ± 14% while the NO_3-$N_{produced}$/NH_4-$N_{removed}$ molar ratio was 0.10 ± 72%. In Table 2, these parameters are reported as a function of the influent characteristics. One can see that the removal efficiency remained high and close to the theoretical value corresponding to the anammox reaction stoichiometry (89%), but slightly higher suggesting the occurance of a concomitant heterotrophic denitrification made possible by the residual organics in the PARNIT effluent. This high nitrogen removal efficiency is in agreement with the NRRmax of the anammox reactor which was largely higher then the applied nitrogen load with the only exception of the last phase when 100% of PARNIT effluent was used as feed. Denitrification on residual organics is also suggested by the nitrate to ammonium molar ratios that were generally lower than the stoichiometric values of 0.26 and especially in the last period with 75% and 100% real wastewater.

For the characterization of the granular biomass contained in the SBR reactor, two particle size measures were carried out at a distance of 3 months (day 260 and day 350, undiluted feeding) as reported in Fig. 4. Both samples have a bimodal distribution, with a mode for granules of small diameter between 0 and 500 μm and the another between 500 and 2500 μm for bigger granules. For both samples, the highest frequencies are related to particles with diameter between 500 and 2500 μm. It can be clearly seen that the sample collected at day 350 shows less dispersion in the mean value with respect to the sample taken 3 months before. In particular, the sample collected at day 350 has the highest relative frequency for a narrow diameter range between 1100 and 1200 μm and the sample collected at day 260 shows similar relative frequencies for a wider range between 800 and 1500 μm. This experimental evidence shows that the hydrodynamic conditions in the SBR were quite favorable to maintain a range of particle sizes of about 1000 μm and also allow a further increase in the size of granules thus making the anammox biomass able to cope with a potential higher presence of inhibitory agents, as observed by Lotti et al. (2012b) for nitrite inhibition.

In Table 3, the results of this study are compared with the main literature studies about the application of the anammox process to the treatment of the liquid fraction of piggery manure digestate

Table 3
Comparison among different literature studies on anammox process treating liquid fraction of piggery digestate.

Refs.	Reactor (volume)	T (°C)	HRT (d)	Influent N concentrations (mg N L^{-1})	Influent real wastewater % (v/v)	NLR (kg N m^{-3} d^{-1})	NRR (kg N m^{-3} d^{-1})	Pretreatment
Hwang et al. (2005)	Up-flow sludge bed reactor (1 L)	35	2.5	NH_4^+-N: 213 ± 32 NO_2^--N: 323 ± 34	40	1.36	0.72	Not reported
Yamamoto et al. (2008)	Up-flow fixed bed reactor (2.85 L)	35	0.5	NH_4^+-N: 76 ± 4 NO_2^--N: 111 ± 4	25	0.39	0.22	Clari-flocculation (cationic polymer)
Yamamoto et al. (2011)	Up-flow glass column reactor (3 L)	30	0.125	NH_4^+-N: 117 ± 6 NO_2^--N: 130 ± 15	10–15	2.2	2.0	Filtration (polyester non-woven sheet)
Qiao et al. (2010)	Granular reactor – gel carrier (0.73 L)	30	0.2	NH_4^+-N: 213 ± 94 NO_2^--N: 212 ± 94	50	4.1	3.12	No pretreatment
Magrí et al. (2012b)	Immobilized gel carriers (1.4 L)	33	0.23–0.45	NH_4^+-N: 150 NO_2^--N: 150	18	0.67–1.29	0.4	Clari-flocculation and partial nitritation
This study	Granular SBR (3 L)	34	2	NH_4^+-N: 558 ± 78 NO_2^--N: 655 ± 130	25–100	0.49–0.61	0.4–0.6	Centrifuge and partial nitritation

Table 4
Relevant data on N_2O emission tests from the PARNIT and anammox processes.

Reactor	Campaign	NLR (kg N m^{-3} d^{-1})	NRR (kg N m^{-3} d^{-1})	NRR/NLR	PARNIT effluent (%)	N_2O emission (% NLR)
PARNIT	1 (day 46)	0.59	–	–	–	15
	2 (day 214)	0.55	–	–	–	7
	3 (day 299)*	0.41	–	–	–	3
Anammox	1 (day 153)	0.55	0.45	82%	50%	0.08
	2 (day 251)	0.61	0.55	90%	100%	0.19

* Aerated idle was applied.

after a partial nitritation step. All literature studies deal with reactors working with diluted wastewater (from 2 to 10 times), and with influent ammonium and nitrite concentrations 3 times lower than those presented in this study. Some authors pretreated the influent for solids removal by means of clari-flocculation (Yamamoto et al., 2008) or filtration (Yamamoto et al., 2011), which was considered the best option. Others (e.g. Qiao et al., 2010) worked under extremely low HRT (0.2 d) to reduce the potential inhibitory effect of high concentration of slowly biode-gradable organics on anammox activity.

3.3. N_2O emissions

In addition to the above described monitoring, the N_2O concentration in the off-gas were monitored during three cycles (day 46, day 214, day 299) for the PARNIT reactor and during two cycles (day 153 and 251) for the anammox reactor in order to assess the relevance of N_2O emissions.

Fig. 5 shows the N_2O concentration in the off-gas of the PARNIT bioreactor, while Table 4 reports N_2O emission from both PARNIT and anammox reactors.

In the first two sampling campaigns N_2O emission resulted as high as 15% and 7% of the nitrogen load. These results are within the large ranges reported in the literature review by Wang et al.(2014), where N_2O emission factors range from 2.2% to 19.3% of the NH_4-N oxidized in nitritation reactors treating real digested liquor.

According to Foley et al. (2010) and Zhang et al. (2012), N_2O is predominantly emitted during the aerobic phase of an SBR cycle, due to air stripping, but, according to Desloover et al. (2012), it is mainly produced during the anoxic phases and then stripped out in the following aeration phase. Anoxic conditions favor N_2O emis-sion (Gabarró et al., 2014) since N_2O is the last intermediate in the denitrification pathway, and high nitrite concentrations and low COD/N ratios may lead to high N_2O production (Desloover et al., 2012). Actually, these conditions were systematically achieved during the anoxic idle phase during sampling campaigns 1 and 2. According to these indications, in the third sampling campaign the idle phase was aerated, to reduce undesired and incomplete heterotrophic denitrification. This strategy was successful in reducing the N_2O emission factor down to 3% of the nitrogen loaded, as denitrification was prevented by aerating the mixed liquor.

With regards to the anammox bioreactor, N_2O emission was negligible ($\leqslant$0.2% of NLR on average during a complete cycle), confirming that anammox bacteria are not directly involved in N_2O production (Desloover et al., 2012).

4. Conclusions

The following main conclusions can be drawn after more than 300 day of experimentation:

- Despite the high variability of the liquid fraction of the co-digested piggery poultry manure, the SBR performed the partial nitritation process successfully and the effluent was suitable to be fed to the anammox reactor;
- Maximum anammox activity monitoring in the SBR suggested that biomass rapidly got acclimated to the undiluted wastewater;
- In the long term, the anammox SBR proved successful in treating undiluted supernatant, with N removal efficiencies higher than 90% at applied NLR of up to 0.6 kg N $(m^3\ d)^{-1}$.

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