

The start-up of an anammox reactor as the second step for the treatment of ammonium rich refinery (IGCC) wastewater with high C_{org}/N ratio

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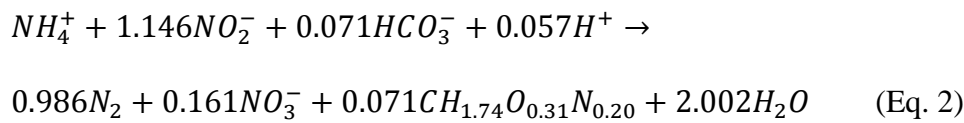
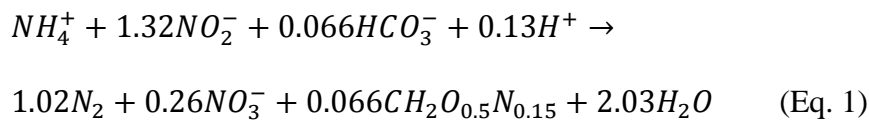
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Abstract. In this study, the refinery wastewater produced by the integrated gasification combined cycle (IGCC) and pre-treated in a lab-scale partial nitrification chemostat was fed to a granular anammox reactor, in order to evaluate its feasibility as the final treatment step. The IGCC wastewater was characterized by high $\text{NH}_4\text{-N}$ concentration ($540\pm 82 \text{ mg L}^{-1}$), high organic carbon to nitrogen ratio ($\text{C}_{\text{org}}/\text{N}$), and by the presence of toxic substances. A conservative exponential law was adopted to progressively replace the synthetic influent with the pre-treated IGCC wastewater, in order to avoid any stressful conditions which could hinder the process. An increase in specific anammox activity (SAA) up to $0.104 \text{ gNO}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$ was initially observed, suggesting that stimulation may occur if pre-treated IGCC wastewater dilution is sufficiently high. A system malfunction caused a worsening of process performance, which was partially restored: when only pre-treated IGCC wastewater was fed, the nitrogen removal efficiency and SAA were $71\pm 3\%$ and $0.045\pm 0.002 \text{ gNO}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$, respectively, and the removal of organic matter due to denitrification was negligible. As to physical/morphological properties of anammox granules, they did not change significantly during the whole experimental campaign. Results showed that the anammox process can be successfully applied to treat complex industrial wastewaters with high $\text{C}_{\text{org}}/\text{N}$ ratio, if a conservative start-up strategy is adopted and the preliminary partial nitrification step guarantees an efficient removal of readily degradable organic matter.

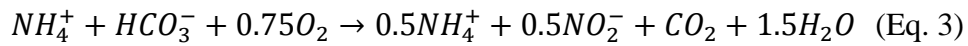
Keywords: anammox; nitrogen removal; industrial wastewater; integrated gasification combined cycle; organic carbon; petrochemical wastewater.

1 Introduction

The reduction of nitrogen discharge in water bodies (including freshwater, estuarine and coastal marine ecosystems) has become a priority, since high levels in water represent a threat for the environment, as well as for human health (Daverey et al., 2013; Camargo et al., 2006). Anaerobic ammonium oxidation (anammox) has been recognized as a cost-effective alternative for biological nitrogen removal, compared with conventional systems. In this process, ammonium is oxidized to dinitrogen gas using nitrite as the electron acceptor, with the formation of $\text{NO}_3\text{-N}$ as minor product: according to Strous et al. (1998), 1.32 moles of $\text{NO}_2\text{-N}$ are required for the oxidation of 1 mole of $\text{NH}_4\text{-N}$ (Eq. 1). Recently, Lotti et al. (2014a) proposed a slightly different anammox stoichiometry, whereas 1.146 moles of $\text{NO}_2\text{-N}$ are required per mole of $\text{NH}_4\text{-N}$ (Eq. 2).



Since nitrite is not usually present in wastewater, the anammox process requires a pre-treatment step based on partial nitrification, where around 50% of influent ammonium is converted into nitrite by ammonium oxidizing bacteria (AOB), under aerobic conditions (Eq. 3).



Nowadays, the combination of a partial nitrification step followed by anammox represents a reliable, cost-effective and energetically efficient alternative for the treatment of ammonium-rich wastewaters with a low content of readily degradable organic carbon (i.e., low C_{org}/N ratio) like sludge digester supernatant, landfill leachate and livestock manure digester liquor (Jenni et al., 2014; Scaglione et al., 2012; Yamamoto et al., 2011). Compared to conventional nitrification-denitrification process, the combination of partial nitrification and anammox requires less energy for oxygen supply (−60%), there is no need for external carbon sources (the process is completely autotrophic), surplus sludge production is reduced significantly (−90%) due to the extremely low growth rate of anammox bacteria, and CO_2 emissions are reduced by 85% (Van Loosdrecht, 2008; Lackner et al., 2014). **This widely accepted economical comparison makes the partial nitrification-anammox process an attractive solution even when the influent organic carbon is sufficient to sustain heterotrophic denitrification.** On the other hand, if the ammonium-rich wastewater is characterized also by a high C_{org}/N ratio, the partial nitrification-anammox process may lack in terms of nitrogen and organic matter removal: Mosquera-Corral et al. (2005) observed a 10% inhibition of AOB activity when acetate was fed to a partial nitrification reactor (the resulting C_{org}/N ratio was 0.3 gC/gN). As to the subsequent treatment step (anammox), the presence of significant amounts of readily degradable organic substrate fosters the development of heterotrophic denitrifiers, since anammox bacteria are not able to compete for nitrite due to their lower growth rate (Molinuevo et al., 2009; Van Hulle et al., 2010; Tang et al., 2010). Moreover, the heterotrophic growth leads to a higher sludge production, with a corresponding decrease

in the sludge retention time (SRT) due to the increasing sludge loss (Jenni et al., 2014), which is not favorable to anammox. Although the effects of readily degradable short chain fatty acids (VFAs) on anammox activity have been investigated, results are contradictory: acetate and formate were proved to inhibit anammox bacteria in continuously operated systems (Van de Graaf et al. 1996), while Kartal et al. (2007) showed that propionate, formate and acetate can be even co-metabolized by anammox biomass which use them as the electron donors, with nitrates as the electron acceptors. More specifically, Güven et al. (2005) stated that anammox bacteria have the organotrophic capability of oxidizing VFAs mostly to CO₂, using nitrates as electron acceptors, while forming ammonium with nitrites as intermediates. Such capability was then used by Winkler et al. (2012) to explain the nitrate consumption and the low sludge production observed in two partial nitrification-anammox reactors treating VFAs containing wastewater.

Especially when dealing with complex wastewaters produced by industries like, for instance, coking, petrochemical, waste management, steel manufacturing and coal gasification factories, the presence of specific compounds may also hinder AOB or anammox activity (Ramos et al., 2015): complete inhibition of AOB was observed by Dyreborg and Arvin (1995) when phenol or cresol were present at low concentrations (3.7 and 1.3 mg L⁻¹, respectively); in batch experiments, initial concentrations of benzene, toluene and *m*-xylene above 10 mgC L⁻¹ were shown to affect the ammonia oxidation pathway significantly, thus inducing a decrease in nitrification specific rates (Zepeda et al., 2006). Although anammox activity is hindered by toxic compounds like phenol, cyanides and thiocyanates at high concentrations, acclimation was shown to be achievable (Toh and Ashbolt, 2002). Ramos et al. (2015) observed that *p*-nitrophenol,

quinolone, *o*-cresole and *o*-chlorophenol, which are commonly present in complex industrial wastewaters, caused a reduction of specific anammox activity even at low concentrations (up to 25 mg L⁻¹).

So far, only few studies have focused on the application of the partial nitrification-anammox process to the treatment of complex industrial wastewaters characterized by high ammonium concentration, high content of readily degradable organic matter (i.e., high C_{org}/N ratio), and by the presence of toxic/inhibiting substances: the feasibility of partial nitrification as the preliminary treatment of refinery wastewater produced by the integrated gasification combined cycle (IGCC) and characterized by high amounts of ammonium and readily degradable formate (C_{org}/N ratio up to 1.1 gC/gN), as well as by the presence of toxic cyanides and phenols, has been recently investigated (Milia et al., 2016a). Based on those results, a granular anammox sequencing batch reactor (SBR) was started-up and fed with the pre-treated IGCC wastewater, in order to evaluate its feasibility as the final treatment step in a double stage system based on partial nitrification and anammox. The replacement of the synthetic influent with the pre-treated IGCC wastewater followed a conservative exponential law, in order to implement an operating strategy which would avoid stressful conditions for anammox biomass and promote its acclimation; reactor performance was monitored in terms of nitrogen removal rate (NRR), nitrogen removal efficiency (NRE), nitrite discharge rate (NitDR), specific anammox activity (SAA), total organic carbon (TOC) removal efficiency, granules physical and morphological properties, overall effluent quality. A comprehensive set of information concerning operating strategies and process performance was gathered, which can be useful for the treatment of similar wastewaters characterized by a high C_{org}/N ratio, as well as by the presence of toxic substances.

2 Materials and methods

2.1 Granular anammox SBR

A glass SBR with a working volume of 2 litres (inner diameter, 13 cm; working height, 15.1 cm) was used to carry out the experiments. The reactor was operated in fed-batch mode with a 8-hour cycle (110-400 min mixed feeding, 70-360 min reaction, 5 min settling and 5 min effluent withdrawal). Mechanical mixing was provided by a marine impeller (110±5 rpm). At the beginning of each working cycle, inert N₂ gas was flushed for 5 minutes, in order to assure anaerobic conditions inside the SBR. The influent flowrate was set at 1.0 mL min⁻¹; the volumetric exchange ratio (i.e., the ratio between the influent volume and the total working volume) was progressively increased from 0.07 to 0.2, and the hydraulic retention time (HRT) correspondingly decreased from 4.7 to 1.7 days. Temperature was controlled at 35±0.5 °C by a water jacket and a thermostatic bath (HAAKE, mod. F3-K); the pH was maintained at 7.7±0.3 using 1M HCl and 1M NaOH. The SBR was completely covered with a black coat, in order to avoid any penetration of light which would hinder anammox activity (Van der Star et al., 2008). Process timing, control and monitoring were performed via NI CompactRio and LabView (v.10.0): a schematic representation of the system is shown in Figure 1. The reactor was inoculated with anammox granular biomass drawn from the Dokhaven-Sluisjesdijk wastewater treatment plant in Rotterdam (The Netherlands), and gently provided by Paques, B.V. Initial total suspended solids (TSS) and volatile suspended solids (VSS) concentrations were 5.2 and 3.7 g L⁻¹, respectively.

2.2 Influent composition and feeding strategy

The granular anammox SBR was started-up and fed with a synthetic influent at a constant nitrogen loading rate (NLR) of $0.125 \text{ gN L}^{-1} \text{ d}^{-1}$ (results not shown), which was subsequently increased up to the target value of $0.35 \text{ gN L}^{-1} \text{ d}^{-1}$. In order to avoid nitrite accumulation, the influent $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ molar ratio was set at 1.25 (the stoichiometric value for anammox is 1.32), with a resulting total nitrogen (TN) concentration of 585 mg L^{-1} . A detailed macro-composition of the synthetic influent is given in Table 1, and micro-nutrients were provided according to Van de Graaf et al. (1996).

The IGCC wastewater (i.e., the process water used for the purification of syngas which is produced by the integrated gasification combined cycle) was supplied by SARAS SpA refinery located in Sarroch (Italy): the IGCC uses a gasification system to convert heavy refining liquid residues into a synthesis gas (syngas) and produce steam. The syngas contains, among the others, CO , CO_2 , H_2 , H_2S , CH_4 , NH_3 , formic acid, ammonium formate and cyanides, therefore it must be purified before it can fuel a combustion turbine generator. The IGCC wastewater is currently treated at full scale by a combination of chemical, physical, and biological processes: a preliminary step is aimed at increasing water temperature and pH, then a steam stripping unit removes the ammonium before the wastewater can enter a trickling filter (where the organic matter is removed) and lamella settlers (for the removal of suspended solids). The effluent is subsequently sent to the equalization tank of the main activated sludge wastewater treatment plant, and mixed with other raw or pre-treated liquid streams.

The IGCC wastewater was collected immediately before the steam stripping unit, and pre-treated in a lab-scale SHARON (Single reactor for High activity Ammonium Removal Over Nitrite) reactor, as described in Milia et al. (2016a). The main characteristics of both raw and pre-treated IGCC wastewater are summarized in Table 2. The synthetic influent fed to the granular anammox SBR was progressively replaced by the pre-treated IGCC wastewater: **the experimental activity lasted 202 days in total**, and was divided into four Phases (Table 3).

In order to promote the acclimation of anammox biomass, a conservative exponential law (Eq. 4) proposed by Lopez et al. (2008) was used to increase the NLR during Phases I and II; an operating strategy based on the same exponential law was chosen to increase the share of pre-treated IGCC wastewater in the influent during Phase III:

$$NLR(t) = A \cdot \exp(f \cdot \mu_{max} \cdot t) \quad (\text{Eq. 4})$$

Where: A, pre-exponential factor, corresponding to the initial NLR ($\text{gN L}^{-1} \text{d}^{-1}$); f, safety factor (0.3); μ_{max} , maximum anammox specific growth rate (0.065 d^{-1}), as proposed by Strous et al. (1998); t, time (d).

The resulting evolution over time of the applied NLR and share of pre-treated IGCC wastewater in the influent is depicted in Figure 2.

The influent (synthetic, semi-synthetic, and 100% pre-treated IGCC wastewater) was always flushed for 30 minutes with inert N_2 gas and kept in collapsible bags in order to minimize oxygen diffusion into the granular anammox SBR during feeding.

2.3 Analytical methods

Ammonium (as $\text{NH}_4\text{-N}$), cyanides and phenols were determined according to Standard Methods (2005). Total organic carbon (TOC) was measured using a TOC analyser (SHIMADZU, mod. TOC-V CSN). The concentrations of $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and formate were determined on filtered samples ($0.45 \mu\text{m}$) by ion-cromatography, using a DIONEX chromatograph (mod. ICS-90) equipped with an Ion-PAC AS14A- $5\mu\text{m}$ column (DIONEX). All the analyses were performed in triplicate on both influent and effluent samples.

The nitrogen removal rate (NRR, i.e. the amount of $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ removed per litre of reactor per day), the nitrite discharge rate (NitDR, i.e. the amount of $\text{NO}_2\text{-N}$ discharged per litre of reactor per day), and the nitrogen removal efficiency (NRE, i.e. the actual removal of influent nitrogen, which takes into account the production of nitrate) were calculated as follows.

$$\text{NRR} = \frac{(\text{NO}_2\text{-N} + \text{NH}_4\text{-N})_{\text{inf}} - (\text{NO}_2\text{-N} + \text{NH}_4\text{-N})_{\text{eff}}}{1000} \cdot \frac{V_{\text{inf}} \cdot N_c}{(V_{\text{min}} + V_{\text{inf}})} \quad (\text{gN L}^{-1} \text{d}^{-1}) \quad (\text{Eq. 5})$$

$$\text{NitDR} = \frac{(\text{NO}_2\text{-N})_{\text{eff}}}{1000} \cdot \frac{V_{\text{inf}} \cdot N_c}{(V_{\text{min}} + V_{\text{inf}})} \quad (\text{gNO}_2\text{-N L}^{-1} \text{d}^{-1}) \quad (\text{Eq. 6})$$

$$\text{NRE} = \frac{\text{TN}_{\text{inf}} - \text{TN}_{\text{eff}}}{\text{TN}_{\text{inf}}} \cdot 100 \quad (\%) \quad (\text{Eq. 7})$$

Where: $(\text{NO}_2\text{-N})_{\text{inf}}$ and $(\text{NH}_4\text{-N})_{\text{inf}}$, influent $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentration, (mg L^{-1}); $(\text{NO}_2\text{-N})_{\text{eff}}$ and $(\text{NH}_4\text{-N})_{\text{eff}}$, effluent $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentration, (mg L^{-1}); $(\text{TN})_{\text{inf}}$

and $(\text{TN})_{\text{eff}}$, total nitrogen (as $\text{NH}_4\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) concentration in the influent and effluent, (mg L^{-1}); V_{inf} , influent volume, (L); N_c , number of cycles per day, (3 cycles/day); V_{min} , minimum working volume, (1.6 L).

The concentrations of TSS and VSS were determined according to Standard Methods (2005). Granule density was determined by dextran blue method, according to Beun et al. (1999).

Specific anammox activity (SAA) was assessed as described in Lotti et al. (2014a), by considering the maximum nitrite removal rate. Both $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations were measured at the end of the anaerobic feeding, and proper amounts of a stock solution containing $\text{NH}_4\text{-N}$ (Stock A, $2 \text{ gNH}_4\text{-N L}^{-1}$) and $\text{NO}_2\text{-N}$ (Stock B, $2 \text{ gNO}_2\text{-N L}^{-1}$) were added in order to achieve an initial concentration of $25 \text{ mgNH}_4\text{-N L}^{-1}$ and $25 \text{ mgNO}_2\text{-N L}^{-1}$, respectively. Samples were taken at fixed intervals in order to determine $\text{NO}_2\text{-N}$ concentration profiles. Maximum specific removal rates were calculated as the ratio between the maximum removal rates (corresponding to the maximum slope of each concentration profile) and biomass concentration in the reactor.

Image analysis (IA) was performed on representative granules samples: each sample (60 mL) was put into a Petri dish on a light background, and pictures were taken in b/w mode via a high resolution digital camera placed onto a horizontal holder. Image-Pro Plus v.6 was used to determine granule size (*mean diameter*, calculated as the average length of diameters measured at 2 degree intervals and passing through granule centroid). More than 200 granules were considered for each sample.

3. Results and discussion

3.1 NRR, NitDR and NRE

The applied NLR was gradually increased from 0.125 to 0.35 gN L⁻¹ d⁻¹ (days 0-62, Phases I and II). During Phase I, the observed NH₄-N and NO₂-N removal efficiencies were 61±12 and 100%, respectively; since NH₄-N removal efficiencies initially showed a decreasing trend, the increase in the applied NLR was prudentially stopped at 0.22 gN L⁻¹ d⁻¹ (days 26-38). As soon as process performance stabilised, the NLR was increased up to the target level (Phase II) and anammox showed a positive response, indicating progressive biomass acclimation to higher nitrogen loads (Figure 3).

Consistently, the NRR got progressively closer to the applied NLR, and the NitDR was always negligible (Figure 4), confirming good process performance.

From day 63, the pre-treated IGCC wastewater was added to the synthetic influent in a 10/100 v/v ratio, which was progressively increased over time up to 100/100 (Phase III). During Phase III-A (days 63-153), the observed NH₄-N and NO₂-N removal efficiencies remained high (96±7 and 98±2%, respectively), despite the increasing share of pre-treated IGCC wastewater (10→66%). The NRR was close to the applied NLR (the NRR/NLR ratio was 0.97±0.02), and the NitDR remained very low (5·10⁻³ gN L⁻¹ d⁻¹), indicating excellent process performance. The observed NRE showed an increasing trend till day 100, then it stabilised at 85±4%. On day 154 (Phase III-B), a system malfunction determined a sudden increase in the applied NLR from 0.35 to 0.47 gN L⁻¹ d⁻¹ (+35%). Although no negative effects on process performance were observed immediately after system malfunction, a slight decrease in NRR was observed from day

160, together with a corresponding increase in NitDR (Figure 4): such behaviour suggested a delayed response of anammox biomass to external stressful events. Process performance was restored only partially, since NRR and NitDR got more stable from day 173 till the end of the experimental activity (Phase IV, 0.28 ± 0.02 and 0.023 ± 0.004 $\text{gN L}^{-1} \text{d}^{-1}$, respectively), but they did not get back to the values observed before the nitrogen shock load. Although the nitrogen removal efficiency (NRE) decreased after system malfunction (from $85\pm 4\%$ to $71\pm 3\%$), process performance was recovered at least in terms of stability, and results were still comparable with others previously reported in literature (Table 4): Huang et al. (2014) observed up to 86% NRE in an anammox CSTR treating synthetic wastewater at various NLR ($0.1\text{-}1.2$ $\text{gN L}^{-1} \text{d}^{-1}$); Alavi et al. (2011) reported a total nitrogen removal efficiency of 92.4% in an upflow anaerobic bioreactor (UABR) treating ammonium-rich wastewater produced by an ammonia plant, at similar applied NLR (0.45 $\text{gN L}^{-1} \text{d}^{-1}$); Lin et al. (2014) observed a total nitrogen removal efficiency of 62-67% in a one stage anammox hybrid biofilm-carrier reactor (HBCR) treating petrochemical wastewater (NLR, $0.11\text{-}0.31$ $\text{gN L}^{-1} \text{d}^{-1}$). A temporary decrease in NRE (not considering $\text{NO}_3\text{-N}$) was observed by Tomar and Gupta (2016) in an anammox hybrid reactor fed with synthetic wastewater (influent $\text{NH}_4\text{-N}$ concentration, 250 mg L^{-1} ; NLR, 0.5 $\text{gN L}^{-1} \text{d}^{-1}$) after a 4-hour exposure to 1.5-, 2.5-, and 3.5-fold increase in nitrogen concentration (substrate shock load) and flow rate (hydraulic shock load), both of them leading to the sudden increase in NLR. The simultaneous increase in NRR was also observed, and related to the resulting higher substrate availability: since specific anammox activity tests were not carried out in order to evaluate biomass inhibition, it is possible that overall anammox response was more

likely caused by the nitrogen overload, rather than by actual inhibition of anammox biomass. Complete recovery of process performance was achieved within 24 hours.

Conversely, both NRR and NRE decreased in this study, suggesting that actual inhibition of anammox activity occurred, as confirmed also by the decrease in the observed SAA, which will be discussed in details. Such a different behaviour may be ascribed to the different time exposure to the higher NLR (6 cycles, 400 min/cycle), as well as to the different influent used in this study (semi-synthetic): in particular, the sudden increase in NLR was accompanied by the simultaneous increase in pre-treated IGCC wastewater share (from 66 to 75%), which likely played a role in the inhibition of anammox activity.

Consistently with NRR, NitDR and NRE, also $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ removal efficiencies decreased after the nitrogen shock load (from $98.9 \pm 1.0\%$ and $96.5 \pm 2.1\%$ to $84.4 \pm 3.9\%$ and $87.3 \pm 2.2\%$, respectively). Nevertheless, such values were still comparable with other results previously reported in literature (Table 4) for anammox systems treating real wastewater: Tang et al. (2011) observed similar $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ removal efficiencies (84-85% and 95-97%, respectively) in two anammox upflow anaerobic sludge blanket reactors treating pharmaceutical wastewater ($\text{NH}_4\text{-N}$, 123-257 mg L^{-1} ; $\text{NO}_2\text{-N}$, 133-264 mg L^{-1} ; $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ molar ratio, 1.0-1.4). In the same study, an operating strategy based on sequential biocatalyst addition (i.e., the addition of anammox granules) was adopted in order to prevent process failure and maintain long-term process stability: $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ removal efficiencies observed after sequential biocatalyst addition were 80-86% and 93-95%, respectively. Yamamoto et al. (2011) reported a 79.2% and 89.7% removal efficiency for $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$, respectively, in

an anammox reactor treating livestock manure digester liquor ($\text{NH}_4\text{-N}$, $130\pm 15 \text{ mg L}^{-1}$; $\text{NO}_2\text{-N}$, $117\pm 6 \text{ mg L}^{-1}$).

3.2 Mass balances and stoichiometry

Although a stable influent $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ molar ratio of 1.25 ± 0.18 and 1.25 ± 0.08 was applied during Phases I and II, respectively, the $\text{NO}_2\text{-N}_{\text{removed}}/\text{NH}_4\text{-N}_{\text{removed}}$ molar ratio was highly fluctuating (consistently with non-steady state conditions in terms of NLR), and mostly higher than the stoichiometric value of 1.32, indicating that nitrite consumption was greater than expected (Fig. 5). Also the observed nitrate production was fluctuating and higher than the expected stoichiometric value (Fig. 5): Du et al. (2015) related the excess of nitrate production in an anammox SBR treating synthetic wastewater to unpredicted oxygen leakage into the reactor (during feeding or sampling), which could lead to the oxidization of $\text{NO}_2\text{-N}$ to $\text{NO}_3\text{-N}$. Moreover, Li et al. (2009) reported that aerobic autotrophic bacteria coexisted with anammox in oxygen-limited environments, and were able to oxidise $\text{NH}_4\text{-N}$ although they accounted for a small part of the total biomass. In this study, the presence of oxygen in the granular anammox SBR can be reasonably excluded as a possible cause of $\text{NH}_4\text{-N}$ oxidation to $\text{NO}_3\text{-N}$, since the reactor was flushed with nitrogen at the beginning of each cycle and after each sampling, and the influent was also flushed before connecting it to the feeding system. Although oxygen measurements never showed the presence of dissolved oxygen in the reactor, specific tests were carried out as described for SAA assessments, but without $\text{NH}_4\text{-N}$, in order to investigate the possible occurrence of non-anammox related $\text{NO}_2\text{-N}$ conversion to $\text{NO}_3\text{-N}$: results showed that $\text{NO}_2\text{-N}$ depletion and $\text{NO}_3\text{-N}$ formation were

negligible ($< 1.8 \text{ mgN L}^{-1} \text{ h}^{-1}$), and definitely too low to justify the fluctuations of nitrite removal and nitrate production above the stoichiometric levels. For these reasons, such fluctuations were related to the overall unsteady behaviour of the granular anammox SBR, exposed to the increasing NLR: as a confirmation, under steady NLR conditions (Phase III, days 63-172) both the $\text{NO}_2\text{-N}_{\text{removed}}/\text{NH}_4\text{-N}_{\text{removed}}$ and $\text{NO}_3\text{-N}_{\text{produced}}/\text{NH}_4\text{-N}_{\text{removed}}$ molar ratios got progressively closer to the stoichiometric values, indicating the occurrence of stable anammox activity. In particular, the average $\text{NO}_2\text{-N}_{\text{removed}}/\text{NH}_4\text{-N}_{\text{removed}}$ molar ratio was 1.30 ± 0.11 , and the observed 0.22 ± 0.05 moles of $\text{NO}_3\text{-N}$ produced per mole of $\text{NH}_4\text{-N}$ removed were in good agreement with the stoichiometric values of 0.26 and 0.16 $\text{molNO}_3\text{-N/molNH}_4\text{-N}$ proposed by Strous et al. (1998) and Lotti et al. (2014a), respectively (Fig. 5).

Maximum process stability was observed during days 100-154 (Phase III-A), when the average $\text{NO}_2\text{-N}_{\text{removed}}/\text{NH}_4\text{-N}_{\text{removed}}$ and $\text{NO}_3\text{-N}_{\text{produced}}/\text{NH}_4\text{-N}_{\text{removed}}$ molar ratios were comparable to other results previously reported in literature for both synthetic and real wastewaters (Table 4). Coherently with the reduced NRR and NRE, a slight decrease in both $\text{NO}_2\text{-N}_{\text{removed}}/\text{NH}_4\text{-N}_{\text{removed}}$ and $\text{NO}_3\text{-N}_{\text{produced}}/\text{NH}_4\text{-N}_{\text{removed}}$ molar ratios was observed during Phases III-B and IV, after system malfunction.

3.3 Specific anammox activity

Specific anammox activity was assessed during anammox SBR operation at constant NLR ($0.35 \text{ gN L}^{-1} \text{ d}^{-1}$, Phases II, III and IV). As shown in Fig. 6, the specific $\text{NO}_2\text{-N}$ removal rates increased as the share of pre-treated IGCC wastewater in the influent was increased up to 35%. Such behaviour suggests that some of the substances contained in

the pre-treated IGCC wastewater may stimulate anammox activity, despite the presence of possibly inhibiting compounds, at least if the share of pre-treated IGCC wastewater in the influent is low enough (i.e., dilution is high). As previously reported, low concentrations of organic matter did not cause negative impacts (Güven et al., 2005; Rusalleda et al., 2008), and even increased anammox activity (Dapena Mora et al., 2007). Kartal et al. (2007) observed that volatile fatty acids like propionate, formate and acetate can be co-metabolized by some anammox species which use them as the electron donors, with nitrates as the electron acceptors; in batch conditions, Dapena-Mora et al. (2007) showed that acetate can enhance SAA (approx. +15%) if its concentration is below 10 mM, above which the inhibiting effect prevails. In this study, the strong enhancement of SAA due to the oxidation of organic substrates by anammox bacteria is unlikely, since the observed TOC removal was negligible. Other substances have also been recognised as stimulators for anammox activity (if below the inhibition threshold concentrations), namely inorganic salts (Fernández et al., 2008), sulphides and thiosulfate (Van de Graaf et al., 1996), although results are often contradictory due to the different operating conditions and anammox species involved in the experiments (Jin et al., 2012).

Given the complex composition of the IGCC wastewater, the identification of the substance (or mix of substances) responsible for the increase in anammox activity was out of the aim of this study, and it would require a specific investigation. However, such stimulating effect decreased and eventually ceased as the share of pre-treated IGCC wastewater in the influent was increased to 45%: a steady specific anammox activity of $0.102 \pm 0.002 \text{ gNO}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$ was observed, and corresponded to the highest values recorded during the whole experimental campaign (Fig. 6). Under such conditions, a

similar assessment was carried out using also $\text{NH}_4\text{-N}$ as the target compound, and specific removal rates were $0.078 \pm 0.002 \text{ gNH}_4\text{-N gVSS}^{-1} \text{ d}^{-1}$ on average (the corresponding total nitrogen specific removal rate was calculated as equal to $0.180 \pm 0.003 \text{ gN}_{\text{tot}} \text{ gVSS}^{-1} \text{ d}^{-1}$). Although such values are lower than those achieved in anammox systems fed with synthetic wastewater (Dapena-Mora et al., 2004; Puyol et al., 2013), they are comparable with other results concerning real wastewater treatment (Qiao et al., 2010; Strous et al., 1997), as summarized in Table 4.

A significant decrease in SAA (down to $0.025 \text{ gNO}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$) was observed during Phase III-B, confirming that the unexpected sudden increase in NLR and share of pre-treated IGCC wastewater (day 154) had a strong inhibiting effect on anammox biomass. As shown in Fig. 6, the recovery of SAA was only partial: although the system malfunction had a key role in sudden SAA drop, it is likely that SAA would gradually decrease even if it had not occurred, due to the further increase in IGCC wastewater share in the influent. At the end of Phase IV, the observed SAA activity was $0.045 \pm 0.002 \text{ gNO}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$.

3.4 Removal of organic matter

As the share of pre-treated IGCC wastewater in the influent was increased, the heterotrophic denitrification was negligible (although formate was completely removed, the TOC removal efficiency was always below 4%), indicating that no significant competition between anammox bacteria and heterotrophic denitrifiers occurred. Rusalleda et al. (2008) reported that anammox activity is not hindered by denitrifiers in a granular anammox SBR treating urban landfill leachate, if readily degradable organic

matter is previously removed (e.g., in the partial nitrification stage). In this study, the organic matter actually available for denitrification was low due to the high efficacy of the preliminary treatment step (SHARON), which removed most of the readily degradable organic matter, especially formates (> 95%) (Milia et al., 2016a). As to the removal of toxic substances, cyanides were not detected in the anammox effluent, likely due to the combination of biotic and abiotic removal processes.

3.5 Anammox granules properties

In previous studies, stressful operating conditions were shown to affect both physical and morphological properties of granular aggregates. For instance, prolonged shear stress induced by high mechanical mixing and gas flow rate led to changes in size and density in granular anammox sludge systems (Arrojo et al., 2008, 2006). Milia et al. (2016b) observed the increase in density and the corresponding decrease in size of aerobic granular aggregates exposed to toxic 2,4,6-trichlorophenol (given the same concentration of organic growth substrate). In particular, the increasing density of granular aggregates is aimed at minimizing diffusive penetration of toxic substances within granules, so that biomass in the inner layers is protected against the potential inhibiting effects (Jiang et al., 2002).

In this study, granules *mean diameter* did not change significantly throughout the experimental activity (636 ± 20 μm), although a shift of granule size distribution toward smallest size fraction was observed (Figure 7) as the share of pre-treated IGCC wastewater in the influent was increased.

Coherently, the increasing share of pre-treated IGCC wastewater had no significant effects on granules density, which was always high ($60.6 \pm 4.5 \text{ gVSS L}_{\text{gran}}^{-1}$) and comparable to other granular anammox (Tang et al., 2011; Arrojo et al., 2006) and aerobic granular sludge systems (Milia et al., 2016b). Such behaviour can be likely ascribed to the conservative feeding strategy applied during start-up, which avoided any stressful condition for granular anammox biomass. Both granules size and density were not affected by the malfunction occurred on day 154: the preservation of granular structure likely facilitated the restoration of overall process performance.

Biomass concentration inside the anammox reactor did not change significantly during the whole experimental campaign (VSS $3.8 \pm 0.2 \text{ g L}^{-1}$; VSS/TSS $73 \pm 3\%$), consistently with the other granules physical and morphological properties. Due to their high compactness and density, anammox granules were easily retained in the reactor and the effluent quality was excellent in terms of solids content ($\text{TSS} < 20 \text{ mg L}^{-1}$), suggesting that no additional settling system would be required.

3.6 Some practical considerations

Some of the main issues concerning anammox enrichment include slow growth rate and low biomass yield, which can lead to a start-up longer than 4 months if inoculation from non-enriched cultures (e.g., sewage and digester sludge) is used (Suneethi et al., 2014). As reported by Van der Star et al. (2007), the start-up period will be considerably shortened if the biomass produced by other existing anammox reactors can be used for the inoculation of new ones, requiring only 3–5 anammox doubling times to achieve full operation. The first full-scale anammox reactor was started-up in Rotterdam (The

Netherlands) using anammox biomass collected from a 5 m³ enrichment reactor located in Balk (The Netherlands), in order to accelerate the start-up (Van der Star et al., 2007).

In the case of complex industrial wastewaters, the low growth rate combined with the sensitivity of microorganisms to inhibitory concentrations of some compounds can significantly hinder the start-up of the anammox process (Suneethi et al., 2014). As to IGCC wastewater, the preliminary partial nitrification step was able to remove most of the organic substrates and toxic substances (Milia et al., 2016a), therefore the start-up of an anammox reactor should not be hindered at full-scale; however, the presence of an anammox enrichment reactor, or the use of enriched seeds from already existing full-scale anammox reactors are a viable and recommendable operating strategy to accelerate process start-up.

A relatively high working temperature (30-35 °C) is required for the optimal operation of partial nitrification and anammox reactors (Van Hulle et al., 2010), therefore the cost-effectiveness of the entire process may be compromised if heating is required. As to the IGCC wastewater, since its chemical pre-treatment (before entering the steam stripping unit) requires the increase in temperature up to 80-90 °C, the excess heat will be even recovered by heat exchange units, in the prospect of pilot or full scale application.

As to reactor configuration, a SBR with mechanical mixing is probably the simplest and easy-to-handle solution at lab-scale. In order to reduce costs at full-scale, the required turbulence could be obtained in different ways, e.g. by using the nitrogen gas produced by the anammox process in a gas lift system, as described by Van der Star et al. (2007) for the first full-scale anammox reactor.

Although such basic considerations about process start-up, reactor configuration and operating conditions are promising and encourage its scale-up, a comprehensive

evaluation of potential issues concerning anammox application at full-scale should be better based on pilot-plant experiences and deserves a specific investigation.

4. Conclusions

The feasibility of the anammox process as the final step for the treatment of ammonium-rich IGCC wastewater with high C_{org}/N ratio was assessed in this study. The results showed that:

- reactor start-up can be successfully achieved by gradually increasing the amount of real wastewater in the influent: the choice of a conservative exponential law avoided the occurrence of stressful conditions for granular anammox biomass, favoring its acclimation;
- despite the presence of possibly inhibiting compounds, the pre-treated IGCC wastewater stimulated anammox activity, at least as its share in the influent was low enough;
- although the system was shown to be sensitive to unexpected system malfunctions during reactor start-up, process performance recovered fairly well and was still comparable with other results reported in literature, especially in terms of NRR, NitDR and NRE;
- granules physical and morphological properties did not change significantly throughout the experimental activity, and were not negatively affected by system malfunctions;
- in order to avoid any competition between anammox bacteria and heterotrophic denitrifiers, the preliminary partial nitrification step must guarantee an efficient

removal of the organic matter, so that mostly slowly or not biodegradable carbon sources are left in the influent to the anammox reactor.

Although focusing on IGCC wastewater, the information achieved in this study can be extended also to the treatment of similar ammonium-rich wastewaters characterized by a high C_{org}/N ratio, and containing toxic substances.

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Glossary

Anammox: anaerobic ammonium oxidation

AOB: ammonium oxidizing bacteria

CSTR: complete stirred-tank reactor

C_{org}/N : organic carbon to nitrogen ratio

HBCR: hybrid biofilm-carrier reactor

HRT: hydraulic retention time

IGCC: integrated gasification combined cycle

MBR: membrane bioreactor

NitDR: nitrite discharge rate

NLR: nitrogen loading rate

NRE: nitrogen removal efficiency

NRR: nitrogen removal rate

SAA: specific anammox activity

SBR: sequencing batch reactor

SHARON: Single reactor for High activity Ammonium Removal Over Nitrite

SRT: sludge retention time

TN: total nitrogen

TOC: total organic carbon

TSS: total suspended solids

UABR: upflow anaerobic bioreactor

UASB: upflow anaerobic sludge blanket

VFA: volatile fatty acids

VSS: volatile suspended solids

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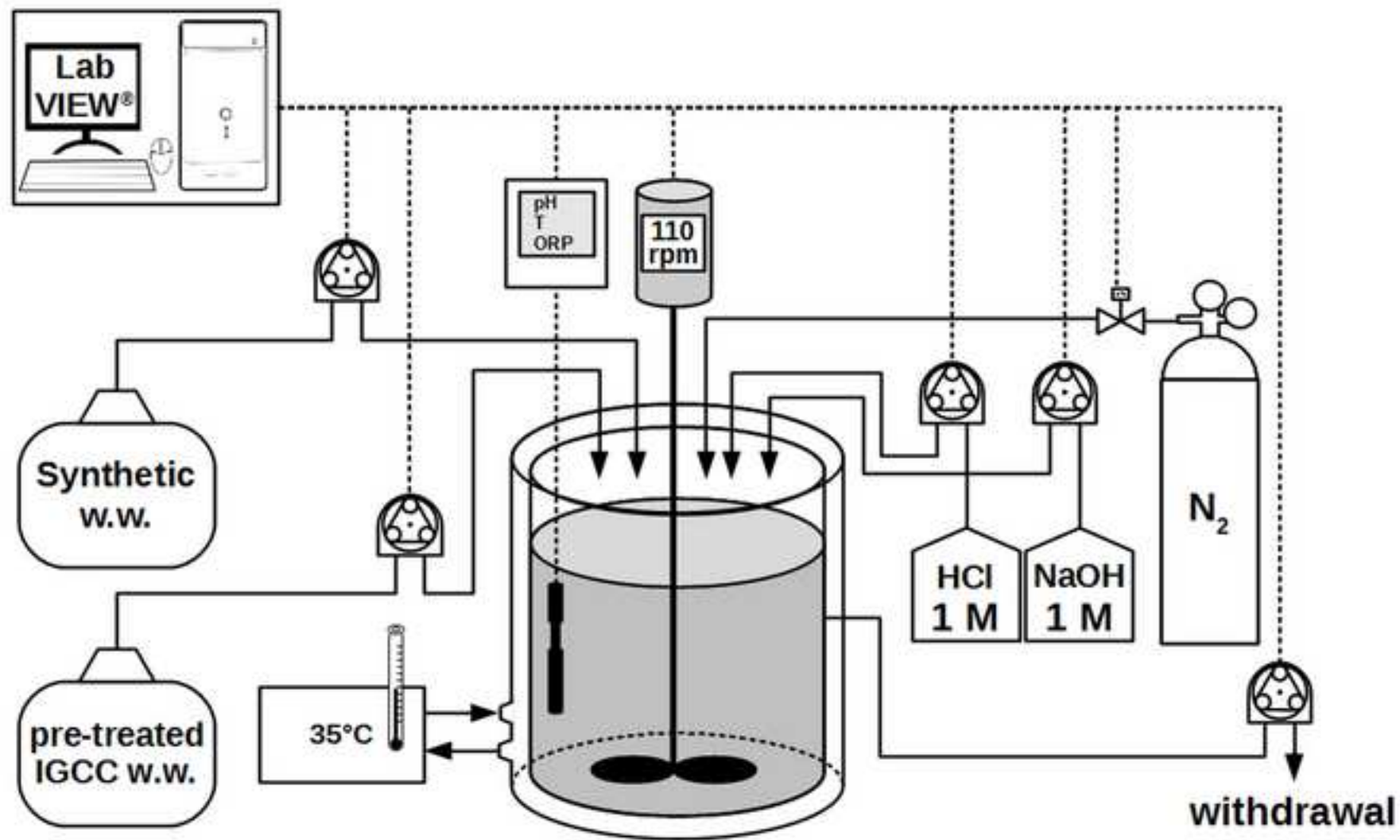
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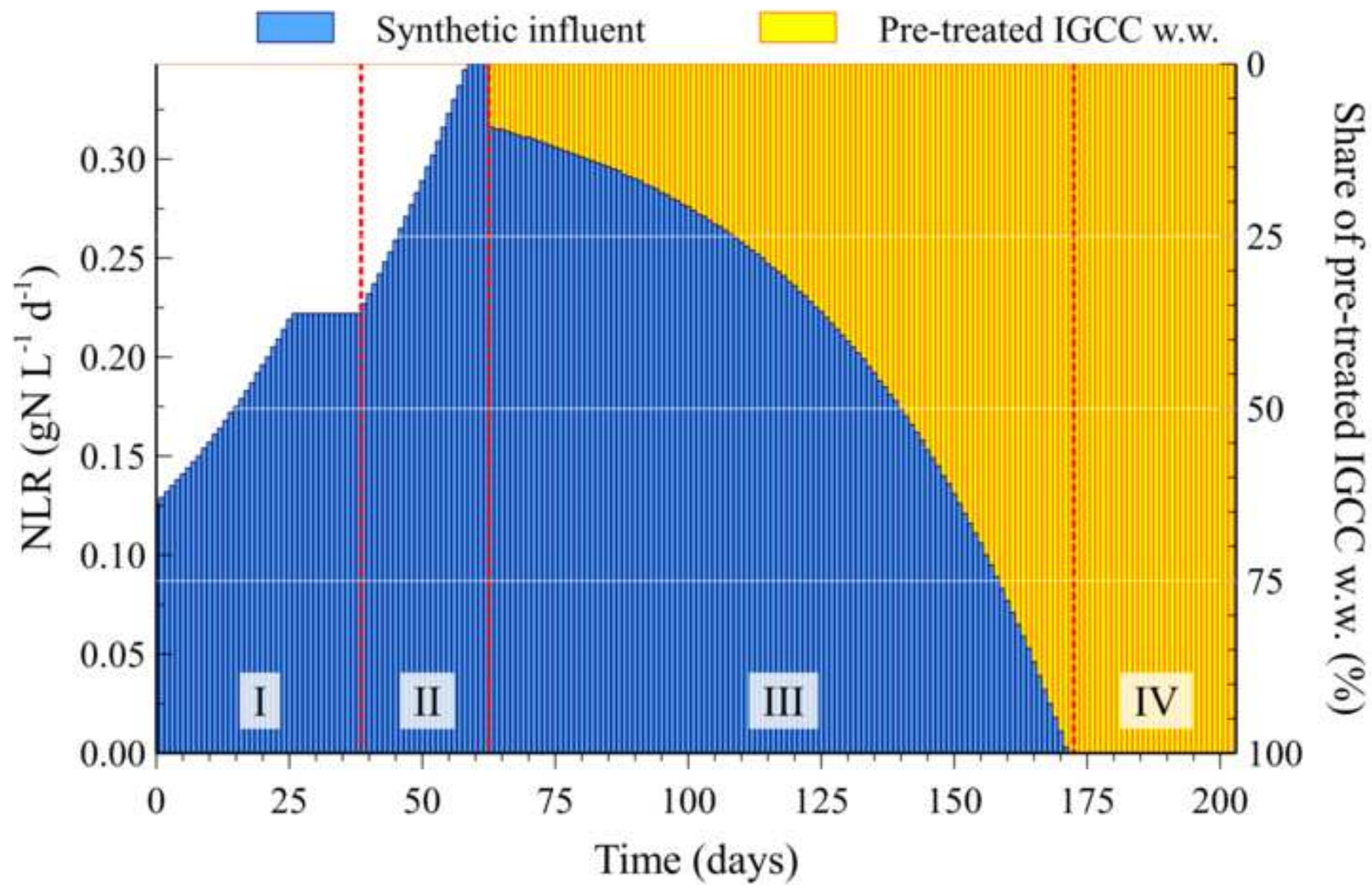
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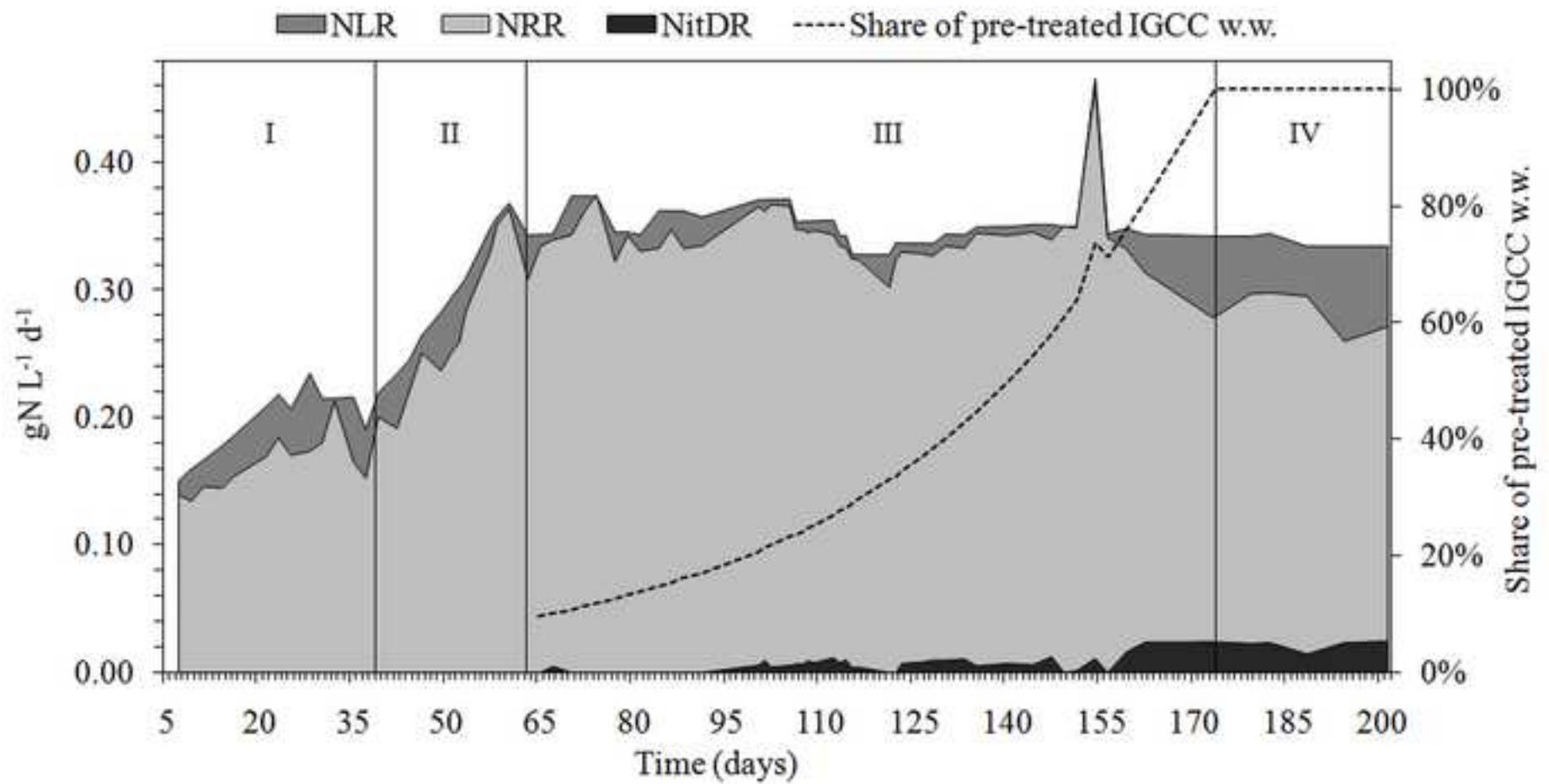
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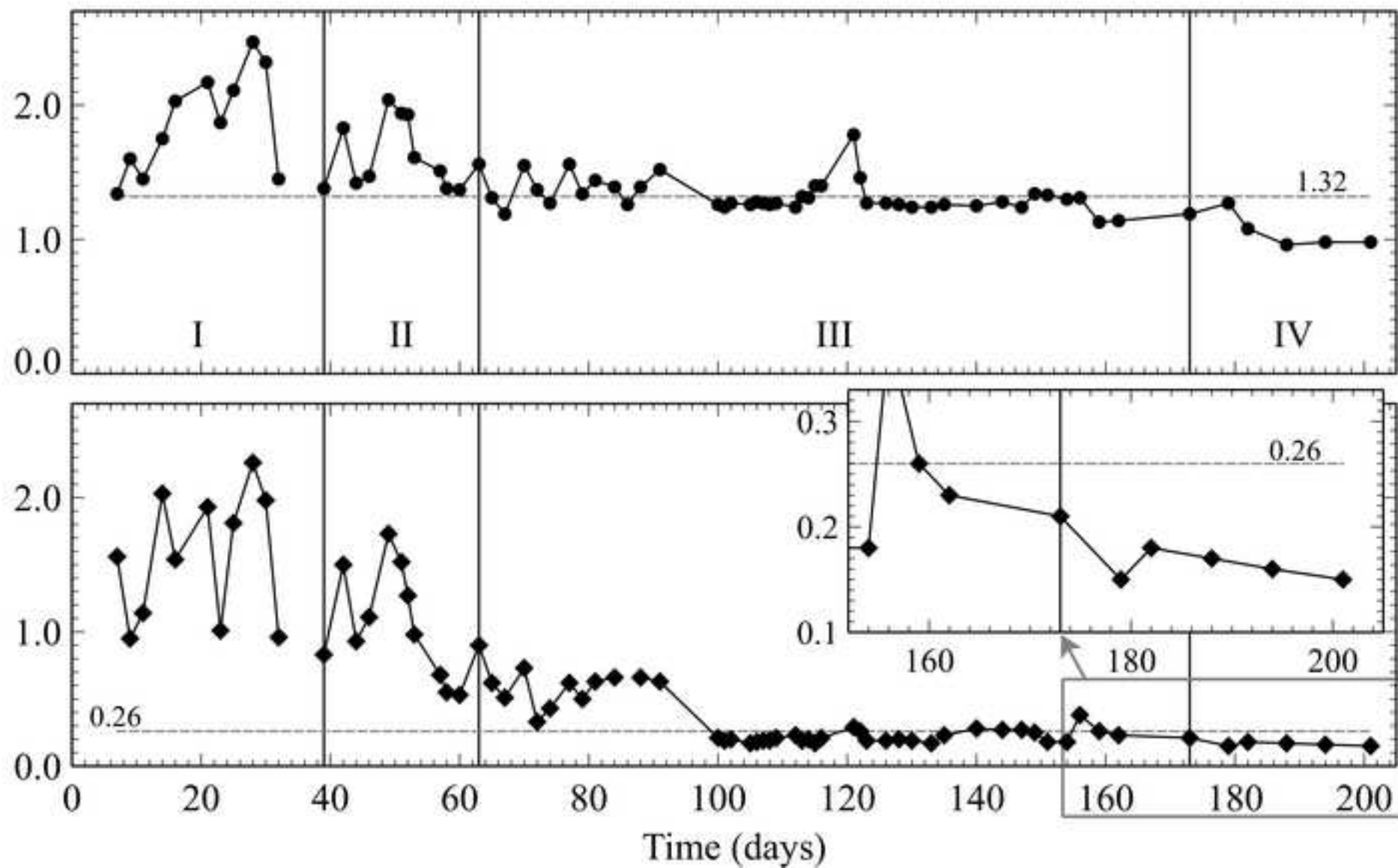
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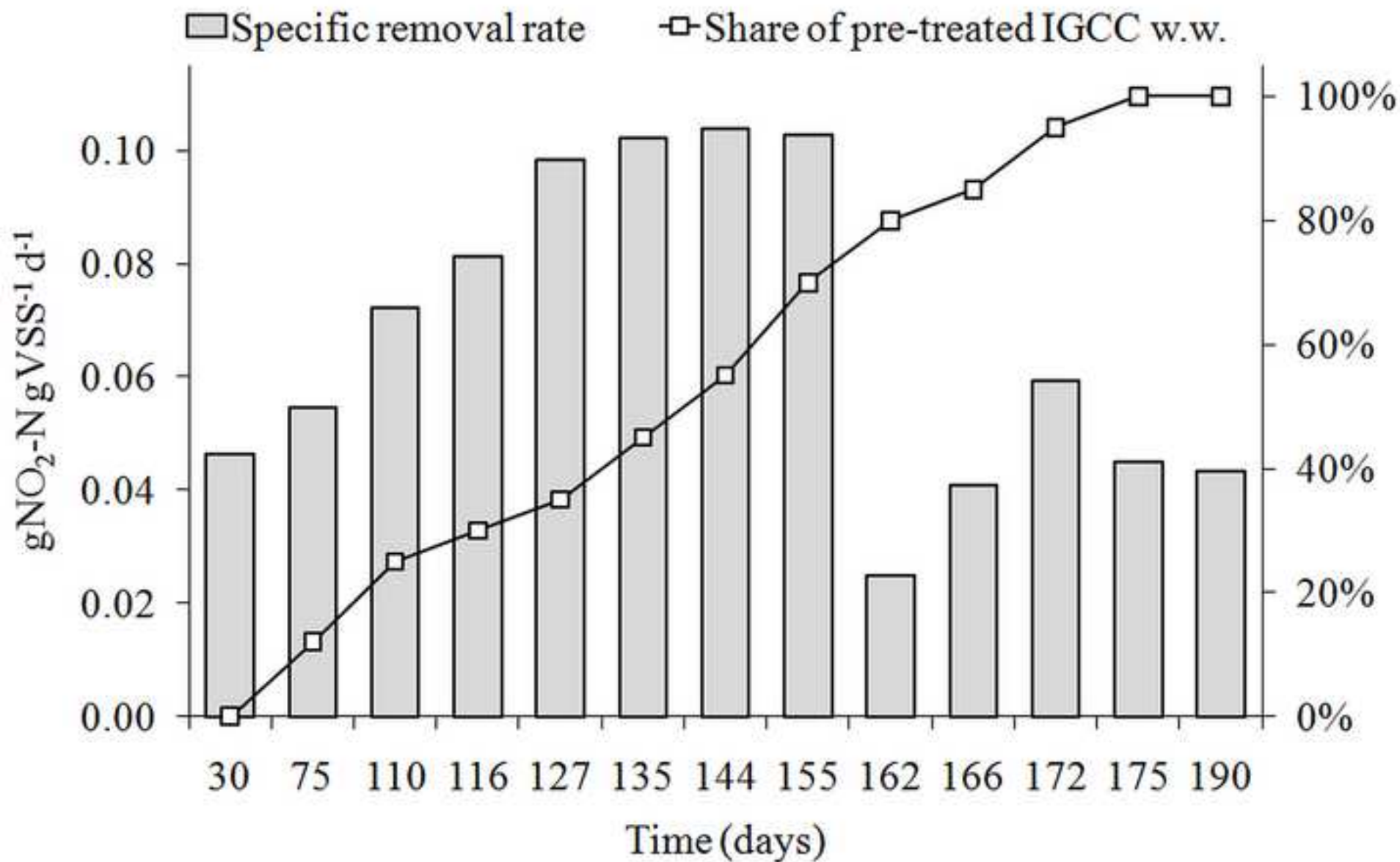
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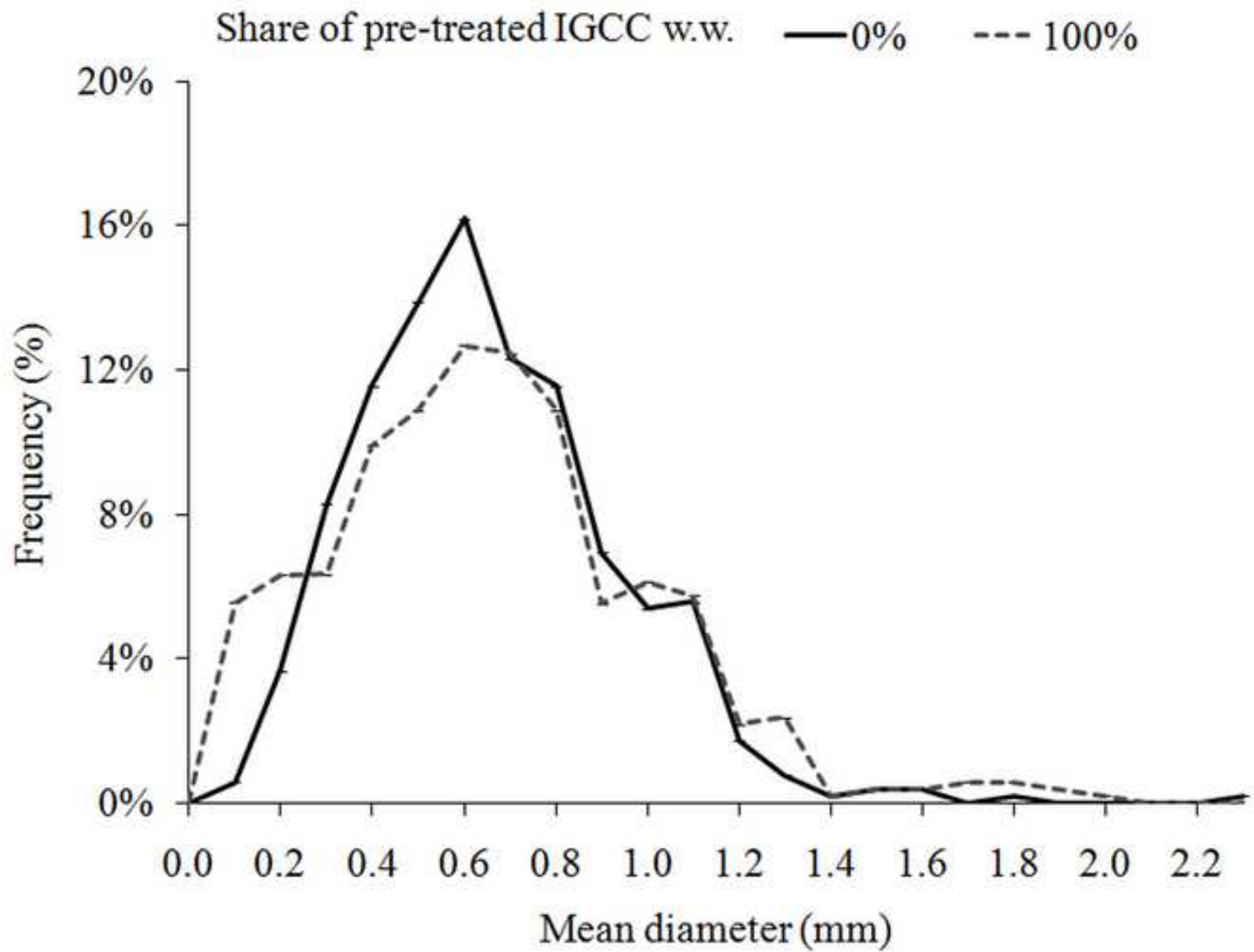


Table 1. Analytical composition of the synthetic influent

Compound	Concentration
NO ₂ -N (as NaNO ₂)	325 mg L ⁻¹
NH ₄ -N (as NH ₄ HCO ₃)	260 mg L ⁻¹
NaHCO ₃	500 mg L ⁻¹
MgSO ₄ ·7H ₂ O	200 mg L ⁻¹
KH ₂ PO ₄	6.25 mg L ⁻¹
CaCl ₂ ·2H ₂ O	300 mg L ⁻¹
micro-nutrients solution	10 mL L ⁻¹

Table 2. Main characteristics of raw and pre-treated IGCC wastewater

Type	NH ₄ -N mg L ⁻¹	NO ₂ -N mg L ⁻¹	TOC mg L ⁻¹	C _{org} /N	Formates mg L ⁻¹	Cyanides mg L ⁻¹	Phenols mg L ⁻¹
IGCC	540±82	-	444±99	up to 1.1	1,498±100	10.4±1	1.2±0.8
pre-treated IGCC (SHARON effluent)	272±70	326±62	101±40	0.18±0.05	< 10	< 0.1	n.d.

n.d.: not detected

Table 3. Organization of the experimental activity

Phase	Days (d)	Duration (d)	TN _{inf} (mg L ⁻¹)	NLR (gN L ⁻¹ d ⁻¹)	Pre-treated IGCC wastewater (%)	HRT (d)
I	0 – 38	39	585	0.125 → 0.22	0	4.7 → 2.7
II	39 – 62	24	585	0.22 → 0.35	0	2.7 → 1.7
III*	A	63-153	586±22	0.35±0.01	10 → 66	1.7
	B	154-172	579±5	0.35±0.01	66 → 99	1.7
IV	173 – 202	30	580±19	0.34±0.01	100	1.7

*on day 154, a prolonged malfunction of the feeding pump caused a sudden increase of the pre-treated IGCC wastewater share in the influent, as well as of the applied NLR (from 0.35 to 0.47 gN L⁻¹ d⁻¹, +35%).

Table 4. Comparison with other lab-scale experimental studies on anammox systems described in literature

Reference	Reactor	Wastewater	NLR	NRR	NRE	Specific NO ₂ -N removal rate	Specific NH ₄ -N removal rate	Specific TN removal rate	Stoichiometric ratios
			gNL ⁻¹ d ⁻¹	gNL ⁻¹ d ⁻¹	%	gNO ₂ -N gVSS ⁻¹ d ⁻¹	gNH ₄ -N gVSS ⁻¹ d ⁻¹	gN gVSS ⁻¹ d ⁻¹	
This study	SBR	Mixed synthetic and pre-treated PN refinery w.w.	0.35 ± 0.01	0.34 ± 0.01	86±4	0.102±0.002	0.078±0.002	0.18	1 : 1.30 : 0.21
Strous et al. (1997)	Fluidized bed	Synthetic	-	-	-	0.10	0.08	0.18	-
		Sludge digestion effluent (municipal WWTP)	-	-	NH ₄ -N = 82 NO ₂ -N = 99	0.08	0.07	0.15	-
Fux et al. (2002)	Pilot-scale SBR	Pre-treated PN activated sludge digestion supernatant / synth.	2.5	2.4	90 ÷ 95	-	-	0.3 gN gTSS ⁻¹ d ⁻¹	1 : 1.38 : 0.32
Dapena-Mora et al. (2004)	SBR	Synthetic	0.7	-	78	-	-	0.5 gN gTSS ⁻¹ d ⁻¹	1 : 1.11 : 0.20
Qiao et al. (2010)	Continuous stirred granular anammox reactor	Mixed pre-treated PN and raw livestock manure digester liquor	3.73	2.60	70 (calculated)	0.16	0.12	-	1 : 1.20 : 0.22
Alavi et al. (2011)	UABR	Ammonia plant effluent	0.45 (max 2.0)	-	92.4 (83.6)	-	-	-	-
Tang et al. (2011)	UASB	Pre-treated PN pharmaceutical w.w.	-	-	NH ₄ -N = 85.2 NO ₂ -N = 97.0	0.5 (calculated)	0.4 (calculated)	0.9 (calculated)	1 : 1.31 : 0.06
Yamamoto et al. (2011)	UASB	Pre-treated PN livestock manure digester liquor	2.2	2.0	NH ₄ -N = 79.2 NO ₂ -N = 89.7 TN = 71.0	-	-	-	-
Scaglione et al. (2012)	SBR	Landfill leachate	0.62	-	73÷93 (NH ₄ + NO ₂)	-	-	SAA = 0.32 gN ₂ gVSS ⁻¹ d ⁻¹	-
Puyol et al. (2013)	Expanded Granular Sludge Bed	Synthetic	3.36	-	-	0.30	-	-	-
	MBR	Synthetic	0.35	-	-	0.29	-	-	-
Tang et al. (2013)	SBR	Synthetic	0.71 (calculated)	0.69±0.03	86±3	-	0.49	-	1 : 1.33 : 0.28
Huang et al. (2014)	CSTR– Marine ANAMMOX	Synthetic	0.08 ÷ 1.20	-	86	-	0.95	-	1 : 1.22 : 0.22
Lotti et al. (2014a)	MBR	Synthetic	1.00	-	80÷85	2.66 (calculated)	-	-	1 : 1.22 : 0.20
Lotti et al. (2014b)	Granular Sludge Fluidized Bed	Pre-treated municipal w.w.	0.60÷1.80	-	-	-	-	0.08 (10°C) ÷ 0.50 (20°C)	-
Dosta et al. (2015)	SBR	Pre-treated reject water from anaerobic digestion of municipal sewage sludge	0.1÷0.2	-	88 (NH ₄ -N = 92)	-	-	SAA = 0.11÷0.27 gN ₂ gVSS ⁻¹ d ⁻¹	1 : 1.25 : 0.14