The effect of urban landfill leachate characteristics on the coexistence of anammox bacteria and heterotrophic denitrifiers

M. Ruscalleda, S. Puig, X. Mora, H. López, R. Ganigué, M. D. Balaguer and J. Colprim

ABSTRACT

Heterotrophic denitrification coexists with the anammox process contributing to N removal owing to the biodegradable organic matter supply from urban landfill leachate and the decay of microorganisms. Both biomasses consumed nitrite increasing the nitrite requirements of the system. The aim of this paper is the study of the causes which induce the system to decrease nitrogen removal efficiency. In this study, urban landfill leachate has been treated in an anammox Sequencing Batch Reactor (SBR) for 360 days. The anammox reactor treated on average 0.24 kgN m^{-3} d^{-1} obtaining nitrogen removal efficiencies up to 89%. The results demonstrated that i) a suitable influent nitrite to ammonium molar ratio is a crucial factor to avoid troubles in the anammox reactor performance; ii) an excess of nitrite implied nitrite accumulation in the reactor; iii) a lower nitrite supply than the necessary for the system could force a loss of specific anammox activity due to nitrite competition with denitrifiers. These results pointed out the importance of the previous partial-nitritation process control in order to obtain a correct influent nitrite to ammonium molar ratio for the anammox reactor. In addition, sudden variation of the leachate characteristics must be avoided.

Key words | anammox process, biodegradable organic matter, heterotrophic denitrification, nitrite supply, urban landfill leachate

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INTRODUCTION

One of the most problematic aspects in landfill sites is the treatment of leachates produced by water infiltration and waste degradation. Urban landfill leachates are characterized by significant contaminant strength, with high diversity of contaminant compounds; furthermore their composition changes according to their age. In this sense, their maturity implies a gain in nitrogen ammonium content, as well as organic matter becoming mainly refractory (Horan *et al.* 1997). Consequently, the low N/COD fraction of urban landfill leachates is difficult to treat by conventional nitrification-denitrification process and requires the development of more economically and environmentally sustainable alternatives.

Focusing on highly nitrogen-loaded wastewater, the anaerobic ammonium-oxidation (anammox) process represents one of the most cost-effective alternatives. It is based on the oxidation of ammonium to nitrogen gas under anoxic conditions by chemolithotrophic bacteria (anammox bacteria) which uses nitrite as the final electron acceptor (Jetten *et al.* 1999). This process removes about 89% of the nitrogen from wastewater to dinitrogen gas, producing 11% of waste nitrogen as nitrate (Equation (1); Strous *et al.* 1998).

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$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{2.5}N_{0.15} + 2.03H_2O \qquad (1)$$

To treat high ammonium content wastewater by anammox process, it is necessary to couple to the anammox reactor a previous step where ammonium is partially oxidized to nitrite by ammonium oxidizing bacteria in aerobic conditions (Hellinga *et al.* 1998; Ganigué *et al.* 2007). The aim of these processes is to obtain a suitable influent wastewater for the next anammox process, with a nitrite to ammonium molar ratio close to 1.32.

As presented in Equation (1), nitrite is the electron acceptor in the anammox reaction. Thus, enough must be supplied to avoid substrate limitations. However, it becomes inhibitory at relatively low concentrations, between 50–150 mg N L^{-1} (Strous *et al.* 1999; Dapena-Mora *et al.* 2007). Thus, the control of the nitrite supply is an important aspect during the operation of anammox reactors, particularly for the enrichment period (Van der Star *et al.* 2007).

Previous studies presented the coexistence of anammox with heterotrophic denitrification process due to the presence of low-biodegradability organic matter in the system (Ruscalleda *et al.* 2008). The organic matter content is partially supplied by biomass wash-out from a previous partial nitritation process (Hwang *et al.* 2005). Therefore, the presence of heterotrophic denitrifiers in an anammox reactor influences the process efficiency and modifies the nitrite/nitrate to ammonium molar ratios. In this way, the heterotrophic nitrite reduction process increases the removed nitrite to ammonium molar ratio up to 1.32 (Yamamoto *et al.* 2008). Thus, nitrite is removed through both processes. Several studies reported the loss of anammox activity as a result of the development of heterotrophic denitrifiers when enough biodegradable organic matter was available (Chamchoi *et al.* 2008; Molinuevo *et al.* 2009).

Heterotrophic denitrification coexists with anammox process contributing to N removal through the presence of biodegradable organic matter in the urban landfill leachate. Both biomasses consumed nitrite increasing the nitrite requirements of the system. The aim of this paper is the study of the causes which induce the system to decrease nitrogen removal efficiency, directing attention to the variations of the nitrite supply in the system by the nitrite to ammonium molar ratio and the characteristics of the influent landfill leachate.

MATERIALS AND METHODS

Experimental set-up

The study was carried out in a 2 L glass cylindrical (BIOSTAT® B-PLUS, Sartorius) operated as SBR. The experimental period comprises 360 days of operation, treating urban landfill leachate. During the whole study, pH, Oxidation-Reduction Potential (ORP), DO and temperature (*T*) were monitored online.

The 8-hour cycle was defined in Ruscalleda *et al.* (2008). The minimum working volume was set at 1.5 L, with a fill volume of 1.05 L day^{-1}. Temperature was maintained at 36.5 ± 0.3°C by a water jacket and a mechanical stirrer was equipped. A PID controller acting on the acid pump regulated the hydrochloric acid (0.1 M) dosage to maintain the pH value at a set-point of 7.3 ± 0.05. No gas was supplied to keep the reactor under anoxic conditions. In contrast, the SBR was completely sealed and the gas production was recovered in a 20 L expansion vessel. The reactor was seeded with granular anammox biomass coming from the parent reactor (López *et al.* 2008). PCR amplification of the inoculum showed that 98% of biomass was *Candidatus* Brocadia anammoxidans.

Influent media

The influent urban landfill leachate used came from a previous Partial Nitritation SBR (PN-SBR) (Ganigué *et al.* 2008) and was diluted to achieve the desired nitrogen loading rate (NLR). The main characteristics are collected in Table 1.

Analytical procedures

Influent and effluent ammonium, nitrite, nitrate and Total COD analyses were performed during the whole

Table 1 | Composition of the influent landfill leachate

	Total COD	TOC	IC	N-ammonium	N-nitrite	N-nitrate
Units	$mgO_2\,L^{-1}$	$mgC\,L^{-1}$	$mgC\,L^{-1}$	$mgN\,L^{-1}$	$mgN\,L^{-1}$	$mgN\,L^{-1}$
Range	111.0–786.0	25.4–510.5	50.2–179.2	68.9–374.3	99.4–485.6	8.9–70.0
Mean	364.5	137.7	131.2	182.2	253.0	21.1
Stand. Dev.	138.8	78.7	35.6	74.6	97.9	6.3

experimental period. Ammonium (N-NH_4^+, 4500-NH_3-B-C), nitrites (N-NO_2^-, 4110B) and nitrates (N-NO_3^-, 4110B), Total Organic Carbon (TOC), Inorganic Carbon (IC) and Chemical Oxygen Demand (COD) were all measured according to *Standard Methods* (APHA 2005). Biochemical oxygen demand (BOD) was measured according to Euro Norm EN 1899-1/1998.

RESULTS AND DISCUSSION

Process performance

The experimental period covers 360 days of the anammox SBR operation. During this period, the nitrogen loading rate (NLR; calculated as ammonium plus nitrite) was increased progressively meanwhile the nitrogen removal rate (NRR; ammonium plus nitrite) was maintained. Figure 1 presents the evolution of the NLR with the respective nitrogen discharge rate (NDR), as well as the efficiency of the system in terms of nitrogen removal during the whole study.

The maximum and minimum values of NLR achieved during the study were 0.49 and 0.10 $kgN\,m^{-3}\,d^{-1}$, respectively (average of 0.24 ± 0.10 $kgN\,m^{-3}\,d^{-1}$). Concerning the effluent quality, the average nitrogen removal efficiency was $89.2 \pm 6.6\%$ (calculated taking into account the nitrate increment in the system for the anammox reaction). Consequently, the average NDR was set at 0.01 ± 0.02 kgN $m^{-3}\,d^{-}1$. A maximum discharge of 0.18 $kgN\,m^{-3}\,d^{-1}$ was achieved on day 350, when the SBR was treating 0.4 $kgN\,m^{-3}\,d^{-1}$ and was affected by a depletion of anammox activity due to inhibition by nitrite. The average effluent N-NH_4^+, N-NO_2^- and N-NO_3^- were $8.7 \pm 15.3\,mg\,L^{-1}$, $8.7 \pm 16.9\,mg\,L^{-1}$ and $49.7 \pm 14.0\,mg\,L^{-1}$, respectively.

Nitrite was accumulated in the system up to 50 mg N-NO_2^- L^{-1} (i.e. 105, 262 and 349). The nitrite inhibitory effect on anammox microorganism growth was proportional with the nitrite concentration (Dapena-Mora *et al.* 2007) and, both parameters have a retroactive effect. To avoid this loop, the NLR was decreased when nitrite accumulation was detected in the reactor. In this sense, two strategies were applied to handle the inhibition periods. To recover the system efficiency after day 105, the NLR was reduced to the last level of stable operation. As can be seen in Figure 1, the nitrogen removal efficiency was recovered after two days. However, effluent concentrations of nitrite and ammonium increased during the next days and consequently, the NLR was forced to decrease. This fact concludes with a long period until the NLR recovered an uptrend. To deal with the next two anammox activity inhibition events, the reactor was operated using a different strategy. The NRR was quantified and the NLR applied was according to the calculated nitrogen removal capacity (0.15 $kgN\,m^{-3}\,d^{-1}$, on day 270). At this point, the NLR was quickly increased during the next days.

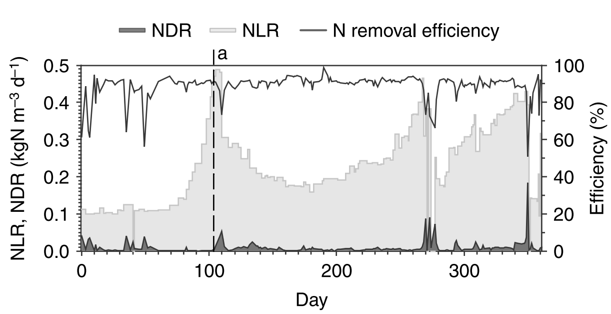

Figure 1 | Process performance in terms of Nitrogen Loading Rate (NLR) and Nitrogen Discharge Rate (NDR), with the corresponding nitrogen removal efficiency. (a) New leachate.

Heterotrophic denitrification influence on anammox SBR performance

According to the anammox reaction (Equation (1)), around 10% of the total nitrogen (TN) of the influent should be converted to N-NO_3^-. In this sense, the N-NO_3^- effluent concentration was in close relationship with the NLR. Nevertheless, the effluent nitrate content represents a lower fraction with respect to the influent TN. On average, the effluent nitrate concentration corresponded to $6.6 \pm 2.5\%$ of the leachate nitrogen content (ammonium plus nitrite). This fact was reported in previous studies (Ruscalleda *et al.* 2008) and was attributed to the presence of heterotrophic denitrifying activity in the anammox SBR.

Nitrite and nitrate removal via heterotrophic denitrification are associated with organic matter consumption. The biodegradable organic matter supply for denitrification could come from two sources: i) the influent leachate and ii) the endogenous decay which concludes with soluble COD release. A small fraction of the influent TOC was removed in the system (between 1.1 and 31.5% with an average of $15.5 \pm 6.0\%$) due to exogenous heterotrophic denitrification processes. The influent leachate BOD_5/COD ratio ranged between 0.02 and 0.12. Taking into account the total COD consumption, the stoichiometric nitrate denitrification ($2.86\,mgCOD\,mg^{-1}\,NO_3^-$) was calculated. The results obtained reported an average nitrate removal of $15.6 \pm 8.8\,mg\,N - NO_3^-\,L^{-1}$, while the average experimental nitrate denitrified was $20.1 \pm 12.5\,mg\,N\text{-}NO_3^-\,L^{-1}$. Moreover, a supplementary COD consumption must be considered due to nitrite denitrification. Thus, the endogenous denitrification had a significant contribution on the nitrogen removal of this system.

Therefore, the reactor was mainly filled with a nitrite to ammonium molar ratio over 1.32 (more than anammox requirements) in order to avoid competition for nitrite between anammox bacteria and denitrifiers and optimize the nitrogen removal capacity of the system. The average influent $NO_2^-:NH_4^+$ molar ratio was 1.40 ± 0.19. The consumption of ammonium and nitrite by anammox route and the nitrite/ nitrate removed by heterotrophic denitrifiers were quantified according to Ruscalleda *et al.* (2008) and the difference between the $NO_2^-:NH_4^+$ ratio necessary for the system and the influent ratio was defined as nitrite excess ratio.

Figure 2 presents the evolution of the nitrite excess ratio, which can be compared with the experimental results of effluent nitrite concentration and the leachate $NO_2^-:NH_4^+$ molar ratio. A strongly relationship between the nitrite effluent concentration and nitrite excess ratio can be seen. When a surplus of nitrite was supplied to the anammox SBR, the effluent nitrite concentration increased. However, any clear correspondence between the influent nitrite to ammonium molar ratio with the nitrite excess or the effluent concentration can be established. As was expected, there are some days in which an influent $NO_2^-:NH_4^+$ molar ratio up to 1.32 concluded with a nitrite accumulation as an excess of substrate which was not removed by the system.

Nevertheless, it was unexpected that periods with an influent ratio above the stoichiometric for anammox (i.e. days 3–8, 141–188 or 301–349) were related to a relatively high nitrite concentration in the effluent. In this way, effluent levels up to $50\,mg\,L^{-1}$ (days 82, 109, 268 or 348) were achieved and corresponded with a sudden decrease of the influent nitrite to ammonium molar ratio under 1.32. In such days, the nitrite excess grew due to a loss of biomass activity (inhibited by nitrite).

The processes' coexistence could be the cause of the incomplete nitrite removal despite being the limiting substrate. Anammox and heterotrophic bacteria coexist in equilibrium according to the system characteristics and the availability of substrates (ammonium, nitrite and biodegradable organic matter). Thus, a sudden reduction of the leachate nitrite content could induce the competence for nitrite. If the available biodegradable organic matter for denitrifiers was not decreased, heterotrophic denitrification was favored, concluding with a lack of nitrite for anammox bacteria and troubling their activity. A loss of anammox activity could conclude with a nitrite accumulation in the system. However, further research is necessary to clarify these dynamics in the anammox reactor.

The effect of the nitrite competition could be more harmful for process performance than a short-term relatively high nitrite accumulation. In this sense, effluent nitrite concentrations of 36.5 and $50.8\,mgN\,L^{-1}$ were achieved on days 293 and 294, respectively, with no ammonium content in the effluent (under detection limits), owing to a leachate $NO_2^-:NH_4^+$ ratio about 1.94. Despite the high nitrite concentration in the reactor, the NLR was maintained

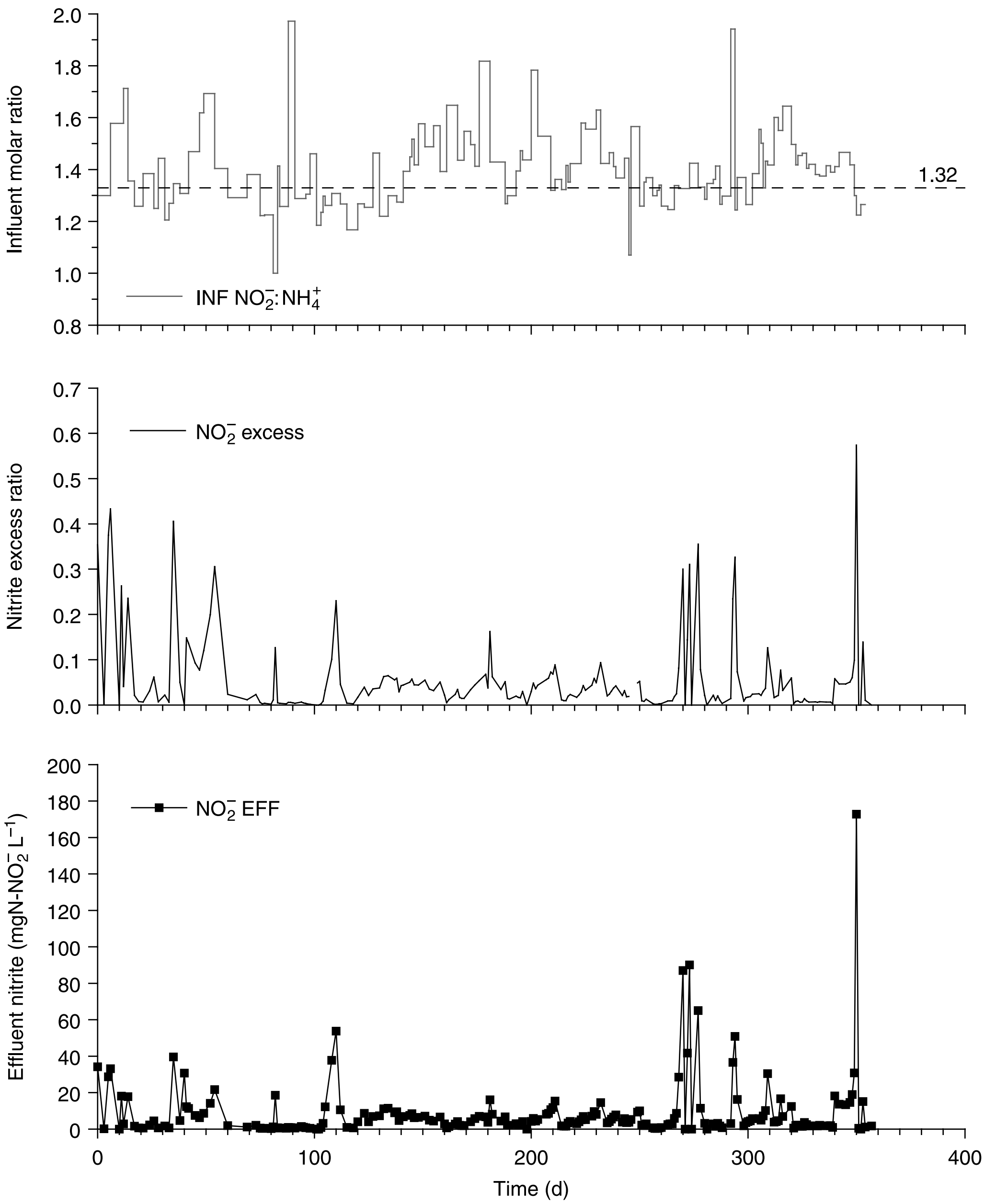

Figure 2 | The influent nitrite to ammonium molar ratio contrasted with the nitrogen nitrate effluent concentration and the corresponding nitrite excess.

when the leachate was corrected for a suitable influent ratio, and the effluent concentration decreased immediately.

Effect of drastic variations of the influent leachate characteristics

The urban landfill leachate was not treated *in situ* in the landfill site and it was received periodically for this study. Since the leachate characteristics suffered variations mainly due to the climatology, the wastewater composition was not homogenous during the study.

In this sense, an important negative effect on the anammox SBR efficiency can be observed in Figure 1 after day 103, when the influent leachate was changed (point a). The new leachate had a lower TN content (1,153 mg $N\text{-}NH_4^+ L^{-1}$ and 1523.3 mg $N\text{-}NO_2^- L^{-1}$) than previously

Table 2 | Comparison of the two leachates (Old and New) used on day 103, after dilution and correction

	Conductivity $mS\,cm^{-1}$	tCOD $mgO_2\,L^{-1}$	tBOD $mgO_2\,L^{-1}$	TOC $mgC\,L^{-1}$	IC $mgC\,L^{-1}$	Alkalinity $mg\,HCO_3^-\,L^{-1}$	Volume L*
Old	10.16	1,000	50.7	185.3	317.8	644	1.529
New	17.01	1,760	14.1	510.5	612.2	590	3.614

*Volume of leachate from the PN-SBR process used to prepare the influent leachate (10 L).

(2256.2 mgN-NH_4^+ L^{-1} and 965.2 mgN-NO_2^- L^{-1}). Thus, a lower dilution of the leachate was applied before feeding the SBR, which concludes in a higher concentrated leachate matrix in the mixed liquor of the anammox SBR. The characteristics of the old and new leachates just before and after day 103 are shown in Table 2. As a result of the lower dilution, all analyzed parameters increased with the new leachate with respect to the old one, except the BOD_5. One of the main consequences was an increase of the dissolved salts, which was revealed by the high conductivity.

Thus, the new leachate presented a conductivity 67.4% higher with respect to the old one. This sudden change in the environmental conditions could affect the microbial populations due to an increase of the osmotic pressure on the media (Dapena-Mora *et al.* 2007). The system needs a long period, up to 60 days, to recover an effluent production with a NDR close to 0.01 kgN m^{-1} d^{-1}. The response of the process to the change of leachate suggested that the system should be acclimated gradually to new leachate characteristics. In this sense, Kartal *et al.* (2006) successfully adapted anammox bacteria from fresh water to high salinity wastewater increasing gradually the salt concentration, proving the possibility of gradually adapting anammox biomass to different conditions.

Despite the lower BOD content of the new leachate, both COD and TOC increased with the change. In this sense, the average heterotrophic contribution on N removal was 7.6 ± 7.0% during the first 90 days of the study, while it grew to 15.0 ± 5.4% during the next 100 days. This increment on heterotrophic contribution would be related to an increment of the decay rates due to the new media and the consequent strengthening of the endogenous denitrification. Thus, a sudden change on the environmental conditions forced the system to reach a new equilibrium state.

CONCLUSIONS

The coexistence of anammox and heterotrophic denitrification processes determines the anammox SBR performance and nitrite availability is an important factor regulating the competition between bacterial populations. In this sense, the control of the influent $NO_2^-:NH_4^+$ molar ratio has been identified as a crucial aspect to avoid nitrite accumulation and a consequent anammox activity inhibition. Thus, the previous partial nitritation plays an important role in the anammox reactor performance.

Biomass can be adapted to different leachate characteristics. However, sudden changes in the environmental conditions could affect the activity of the microorganisms. In this sense, fast variation in the conductivity of landfill leachate must be avoided, although the biomass can be adapted to new conditions.

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