

Influence of the feeding regime on the start-up and operation of the autotrophic nitrogen removal process

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Abstract

The autotrophic nitrogen removal process (partial nitrification combined with the Anammox process) is a sustainable nitrogen removal technique for nitrogen-rich streams. A modelling and experimental study was performed to define optimal process conditions for the autotrophic nitrogen removal process. Special attention was given to the influence of feeding characteristics on the performance of both the partial nitrification reactor and the Anammox reactor. It was revealed that the feeding regime is an important factor in the successful start-up of the Anammox process. Nitrite concentration peaks at the beginning of a feeding period will lead to an unsuccessful start-up, while a slow input of nitrogen speeds up the process. Feeding regimes are less important in partial nitrification reactors since laboratory results show that slow or fast supply of influent does not influence the growth of ammonium oxidisers.

Keywords: Anammox, autotrophic nitrogen removal, modelling, optimisation, start-up analysis

Introduction

Nitrogen, which is generally in the form of ammonium or organic nitrogen, is removed by biological nitrification-denitrification in most modern wastewater treatment plants (WWTP). Benefits of the process are the high potential removal efficiency, high process stability and reliability, relatively easy process control, low area requirement and moderate cost (Tchobananoglous, 2003). As a first step, ammonium is converted to nitrate (nitrification). In a second step, nitrate is converted to nitrogen gas (denitrification).

A major stress factor on nitrogen removal is reject-water from sludge digesters, which is recycled back to the main WWTP. This reject-water can represent up to 25% of the total nitrogen load, but only 1 to 2% of the volumetric load (Janus and Van der Roest, 1997). Treating this return stream separately by nitrification-denitrification would become expensive and non-sustainable, as this treatment would require large oxygen consumption and the addition of a carbon source due to the high nitrogen concentration (up to 2 g·ℓ⁻¹ N) and unfavourable carbon-to-nitrogen (C/N⁻¹) ratio for denitrification (Henze et al., 2008). This would therefore result in high operational costs. Also, other streams have a high nitrogen and a low organic carbon content, such as anaerobic digester effluents, landfill leachate and industrial wastewaters (Wiesmann, 1994). Treating these streams with conventional nitrification-denitrification will lead to high operational costs and possibly also the emission of large amounts of greenhouse

gasses (Jetten et al., 1997; Kampschreur et al., 2009).

A more sustainable and cost-effective alternative to conventional nitrogen removal systems is the autotrophic conversion of ammonium to nitrogen gas, especially in cases where the carbon-to-nitrogen ratio is low and aeration capacity is limiting (Van Dongen et al., 2001). The first step, called 'partial nitrification', includes a conversion of the incoming ammonium to nitrite, obtaining a ammonium:nitrite ratio of 1:1 (Hellinga et al., 1998). This partial nitrification process can only be realised when the nitrite-oxidizing bacteria are inhibited, outcompeted or removed, while the ammonium oxidizers are retained due to a higher relative growth rate of ammonium oxidizers at higher temperature (> 25°C), oxygen limitation (0.3-0.5 mg·ℓ⁻¹ O₂) and higher pH (Van Hulle et al., 2010). The second process is the anaerobic oxidation of ammonium (Anammox) with nitrite as electron acceptor (Van De Graaf et al., 1996; Jetten et al., 1997). With this Anammox process, ammonium and nitrite are combined under anoxic conditions, without addition of an external carbon source, directly to nitrogen gas, with the production of a small amount of nitrate (Jetten et al., 1999).

To date, several pilot-scale and full-scale reactors are in operation (Van Hulle et al., 2010). However, a shortcoming in the application of the Anammox process is the slow growth rate, resulting in a very time-consuming experimental start-up (Hao et al., 2002; Strous et al., 1998; Van der Star et al., 2007).

In view of the very long start-up time, a model-based analysis was performed to define optimal process conditions (temperature, oxygen supply, pH and biomass retention) for both an oxygen-limited partial nitrification reactor and an Anammox reactor. The simulation results were discussed in detail in a previous publication (Veys et al., 2010), and a summary is provided in Table 1.

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	Partial nitrification	Anammox
Temperature (°C)	30 - 40	30 - 40
pH	7 - 8	7 - 8.5
Minimal sludge age (d)	25	40
O ₂ concentration (mg·l ⁻¹ O ₂)	0.04 - 0.06	0

One important factor not considered in the previous study is the feeding characteristics of the reactor. De Clippeleir et al. (2009) stated that a low substrate shock (a low volumetric exchange ratio) resulted in a faster start-up of an oxygen-limited autotrophic nitrogen removal sequencing batch reactor (SBR). Van Hulle et al. (2005) stated that a dedicated start-up for a partial nitrification reactor is necessary.

In this study, it is demonstrated that applying the conditions in Table 1 does not always lead to a successful start-up. The feeding regime plays an important role in the successful start-up of Anammox reactors. As such this paper focuses on this feeding regime and aims at exploring the related problems and/or bottlenecks in view of the establishment of a start-up protocol.

Methods

Reactor systems

Partial nitrification reactor

The partial nitrification reactor filled with 2 l of nitrifying and denitrifying sludge of the Harelbeke municipal WWTP (www.aquafin.be) was initially fed with 0.5 l of synthetic influent (prepared with tap water) containing 1 g·l⁻¹ NH₄Cl-N and an equimolar amount of NaHCO₃, reaching a working volume of 2.5 l. Each day, 0.5 l of effluent was drawn out of the reactor after a biomass settling period of 30 min, followed by a filling period with 0.5 l of influent. This results in a hydraulic residence time (HRT) of 5 d and a loading rate of 0.2 g·l⁻¹·d⁻¹ N. After 44 d the loading rate was increased to 0.4 g·l⁻¹·d⁻¹ N by increasing the influent and effluent volume to 1 l. As such, the HRT was decreased to 3 d. The reactor conditions are the same as the optimal conditions found by the simulation study of Veys et al. (2010) (Table 1), with the exception of O₂ concentration. The O₂ concentration simulated by the model (0.04-0.06 mg·l⁻¹ O₂) was lower than the applied set point in the laboratory reactor (0.2-0.5 mg·l⁻¹ O₂), as the simulation model assumes perfect mixing. However, in practice, floc formation will induce an oxygen concentration gradient and, consequentially, a higher oxygen bulk concentration.

After 100 days of operation, 2 l of the sludge was transferred to a 20 l reactor. In this reactor, 8 l of nitrifying and denitrifying sludge of the Harelbeke municipal WWTP was also added. This reactor was fed on a daily basis with 10 l of synthetic influent containing 1 g·l⁻¹ NH₄Cl-N. The same operational conditions as for the smaller reactor were applied, except that the HRT was decreased to 2 d.

After 104 d of operation, a microfiltration membrane (pore size of 0.45 µm) was placed in the 20 l reactor, so that it was transformed to a membrane bioreactor (MBR). The reactor was fed continuously with a flow rate of 10 l·day⁻¹, reaching a HRT of 2 d. All other conditions were kept constant in the reactor.

Anammox reactor

Two Anammox reactors were operated on a different feeding regime to observe the effect on their operation, i.e., on the removal of ammonium and nitrite. The 'fast-fill' SBR reactor was operated with a feed period of 1 min·d⁻¹, while the 'slow-fill' reactor was fed continuously.

The fast-fill SBR reactor with a working volume of 3 l was inoculated with 1 l of nitrifying and denitrifying sludge, and 1 l of anaerobic digester sludge of the Harelbeke WWTP. On a daily basis, 1 l of synthetic influent containing (NH₄)₂SO₄, NaNO₂ and other minor components (Table 2) was fed into the reactor. The reactor conditions were the same as the optimal conditions found by the simulation study of Veys et al. (2010) (Table 1). The biomass was able to settle for 30 min before 1 l was withdrawn from the reactor. As such, a HRT of 3 d was reached. In order to achieve anoxic conditions, the reactor was flushed with N₂ gas. After this flushing, KHCO₃ was added to the reactor in an equimolar amount compared to ammonium. A continuously operating slow-fill reactor was inoculated with 600 ml of sludge of 2 different WWTP: the Harelbeke municipal WWTP and a landfill leachate WWTP. The top of the reactor was packed with polyurethane foam to ensure biomass retention. After start-up, it was estimated that about 10% of the biomass was present in this foam. The reactor was initially fed with a synthetic solution (prepared with tap water) containing 100 mg·l⁻¹ N (NH₄)₂SO₄, 40 mg·l⁻¹ N NaNO₂, 0.5 g·l⁻¹ KHCO₃, and some other minor components (see Table 2), at a flow rate of 1.7 ml·min⁻¹ to reach a HRT of 10 h. No N₂ flushing was applied in this reactor. During operation the influent concentration was gradually increased. The reactor conditions were the same as the optimal conditions found by the simulation study of Veys et al. (2010) (Table 1).

Component	Concentration (g·l⁻¹)
NaH ₂ PO ₄ ·2H ₂ O	0.029
CaCl ₂	0.226
MgSO ₄ ·7H ₂ O	0.2
FeSO ₄ ·7H ₂ O	0.021
EDTA·2H ₂ O	0.0076
Trace elements	1.25 ml·l ⁻¹

Effluent suspended solid concentration was not measured on a regular basis, but for both the fast-fill and the slow-fill reactor a clear effluent without turbidity was obtained. We therefore consider the loss of biomass in the effluent to be very low.

Chemical analysis

The concentration of ammonium-N, nitrite-N and nitrate-N was measured colourimetrically according to Standard Methods (1998).

Modeling and simulation of the Anammox reactor

For the simulations, a previously-developed model (Wyffels et al., 2004; Dapena-Mora et al., 2004, Veys et al., 2010) was extended with Haldane kinetics to describe the dependence of

the Anammox growth rate on nitrite:

$$f(NO_2^-) = \frac{[NO_2^-]}{\frac{[NO_2^-]^2}{K_I} + K_{NO_2^-} + [NO_2^-]}$$

where:

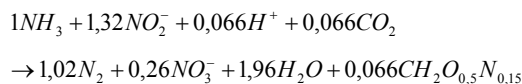
$$K_{NO_2^-} = 0.3 \text{ mg}\cdot\ell^{-1} \text{ N and}$$

$$K_I = 200 \text{ mg}\cdot\ell^{-1} \text{ N}$$

As such the expression for the growth rate of the Anammox organisms is presented in the equation below. Monod kinetics were used to describe the dependency of the growth rate of Anammox on ammonium. An additional inhibition term was used to describe an eventual inhibition of the Anammox organisms by oxygen.

$$\mu = \mu_{AN}^{\max} \frac{[NH_4^+]}{K_{NH_4^+} + [NH_4^+]} \frac{[NO_2^-]}{\frac{[NO_2^-]^2}{K_I} + K_{NO_2^-} + [NO_2^-]} \frac{K_{O_2}}{K_{O_2} + [O_2]}$$

The ammonium and nitrite concentration values are expressed in $\text{mg}\cdot\ell^{-1} \text{ N}$, the oxygen concentration is expressed in $\text{mg}\cdot\ell^{-1} \text{ O}_2$ and the concentration of Anammox biomass is expressed in $\text{mg}\cdot\ell^{-1} \text{ COD}$. The maximum specific growth rate of the Anammox biomass (μ_{AN}) was derived from Strous et al. (1998) and was set to a value of 0.08 d^{-1} at 35°C . The affinity constant for ammonium ($K_{NH_4^+}$) was set to $0.3 \text{ mg}\cdot\ell^{-1} \text{ N}$, while the inhibition constant for oxygen was set to $0.01 \text{ mg}\cdot\ell^{-1} \text{ O}_2$. The stoichiometry of the Anammox process was based on the reaction stoichiometry put forward by Strous et al. (1998), as implemented by Dapena-Mora et al. (2004):



The extended model as well as the SBR reactor configuration discussed below were implemented in the simulation program WEST® (Vanhooren et al., 2003).

Results

Experimental results of the partial nitritation reactor

Two weeks after start-up, a successful operation of the partial nitritation reactor was noticed, i.e., production of an ammonium:nitrite ratio of 1:1 (Fig. 1). Since in both reactors only small amounts of nitrate were observed in the effluent, nitrite-oxidizing bacteria are not present in high concentrations. Both the SBR and the MBR reactor can produce an Anammox suited effluent. As such, it can be seen that both a discontinuous and a continuous feeding regime give excellent results in view of coupling with an Anammox reactor. Hence, feeding regime is not important for the partial nitritation reactor and start-up can be accomplished by slow or fast feeding. Some researchers even state that a discontinuous operation is beneficial, as it imposes more stress on the nitrite-oxidising organisms. Hyungseok et al. (1999), for example, reported that nitrate formation can effectively be prevented by frequent switching between oxic and anoxic phases. Therefore, the aeration is switched off before all the ammonium is consumed and before nitrite can be further converted to nitrate. A continuation of the aeration phases in a SBR lowered the stress on nitrite oxidisers, resulting in an increase of nitrate (Katsogiannis et al., 2003; Blackburne et al., 2008).

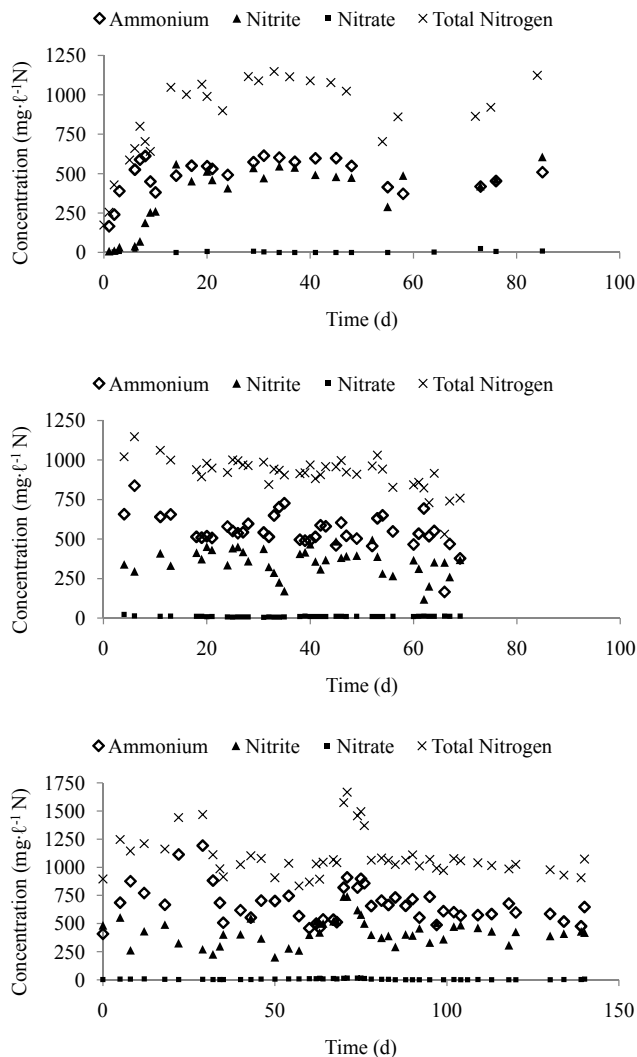


Figure 1

Overview of the N concentration in the effluent of a fast-fill partial nitritation reactor with a volume of 3 ℓ (top), a fast-fill partial nitritation reactor with a volume of 20 ℓ (middle) and a slow-fill partial nitritation reactor with a volume of 20 ℓ (bottom)

Simulation results of the Anammox reactor

Based on the optimal conditions for the Anammox process (Table 1), the effect of feeding characteristics on the performance of the Anammox reactor was tested by simulating the nitrogen gas production under different conditions. A first simulation run was performed with an Anammox SBR that was operated according to the start-up strategy of Dapena-Mora et al. (2004). A 6-h cycle and a volume exchange ratio of 25% were applied. Within the 6-h cycle, 5.5 h (330 min) were used for filling and reaction, 20 min were used for settling and the biomass was withdrawn for 10 min. A second simulation was run with a 1-min feed period and a period of 329 min of reaction without feeding. Again, 20 min were used for settling and the biomass was withdrawn for 10 min. Figure 2 (top) gives an overview of the nitrogen concentration in the influent used to simulate the performance of these 2 Anammox SBR reactors.

In Fig. 2 (bottom) the performance in terms of nitrogen gas production is demonstrated. The nitrogen gas production

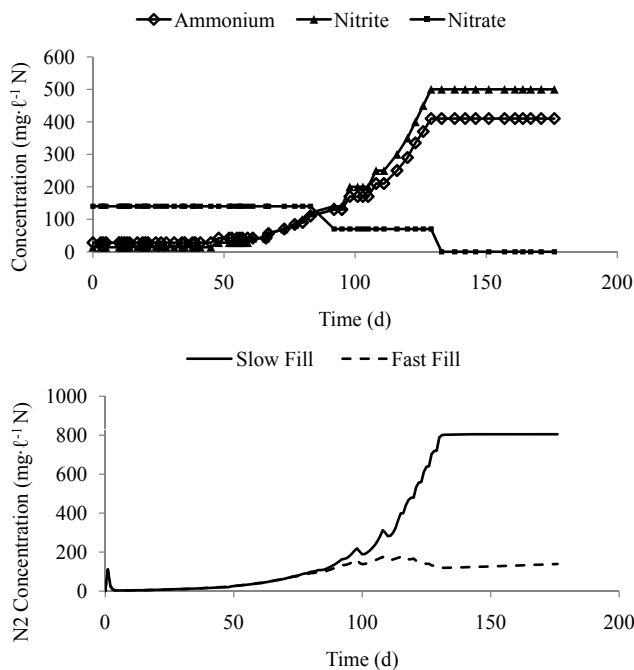


Figure 2

The influent nitrogen concentration for simulation of the Anammox SBR reactor (top) and the simulated nitrogen gas production of a slow-fill and a fast-fill Anammox reactor (bottom)

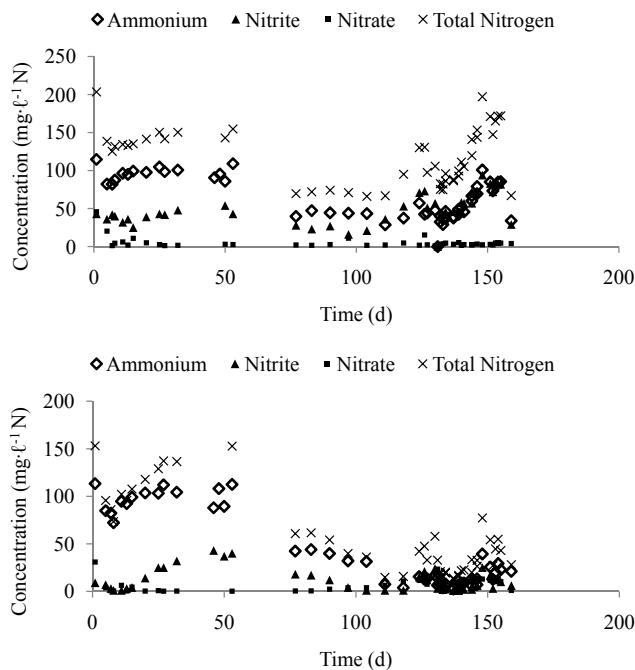


Figure 4

Nitrogen concentration values in the influent (top) and the effluent (bottom) of the continuous slow-fed Anammox reactor

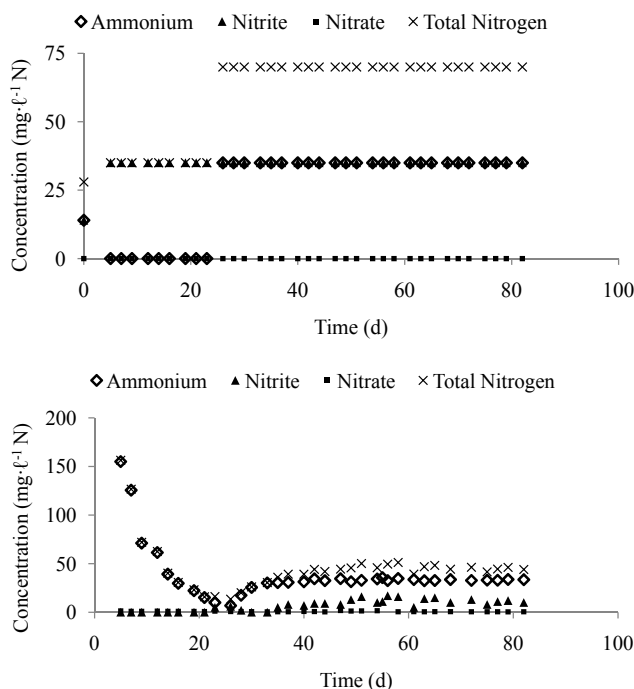


Figure 3

Nitrogen concentration values in the influent (top) and in the effluent (bottom) of a fast fill Anammox reactor

does not increase over time in the fast-fill reactor, although the nitrogen concentration in the influent increases. This can be explained by the fact that a short feeding period causes a temporary nitrite peak leading to Anammox inhibition and, consequently, an unsuccessful start-up of the fast-fill reactor.

Experimental results for the Anammox reactor

When operating the fast-fill Anammox reactors, initially bacterial decay occurs causing high ammonium concentration and organic carbon in the effluent. The ammonium concentration was therefore reduced in the influent until the ammonium concentration decreased to lower values, while the nitrite concentration was maintained at $35 \text{ mg}\cdot\text{l}^{-1} \text{ N}$. After 25 d, the ammonium concentration in the influent was also increased to $35 \text{ mg}\cdot\text{l}^{-1} \text{ N}$. Figure 3 shows that the ammonium concentration in the influent and effluent are the same, while the nitrite concentration in the effluent is lower than in the influent. It can be concluded that denitrifying bacteria used the organic carbon derived from bacterial decay to convert the incoming nitrite to nitrogen gas by denitrification. Since ammonium was not removed, it can be stated that Anammox bacteria are not active in this laboratory reactor. A possible explanation could be the feeding characteristics of this reactor. These tests were performed several times and every time similar results were obtained.

In the slow-fill reactor, initial bacterial decay also occurs, leading to high concentrations of organic carbon. This organic carbon will also be used to denitrify the incoming nitrite, while the ammonium concentration stays equal to that of the influent. The bacterial community stabilises after 30 d and, since no organic carbon is added to the influent, less denitrification will occur and so less nitrite will be removed. After 60 d of operation, nitrogen removal was very small. Fux (2003) showed in a long-term experiment that maintaining a nitrite concentration of $40 \text{ mg}\cdot\text{l}^{-1} \text{ N}$ over several days led to the irreversible inactivation of the Anammox organisms. To ensure that no inhibition can occur, the influent nitrogen concentration was reduced ($20 \text{ mg}\cdot\text{l}^{-1} \text{ NO}_2\text{-N}$ and $50 \text{ mg}\cdot\text{l}^{-1} \text{ NH}_4\text{-N}$). After 30 d of operation the removal of ammonia and nitrite was observed to be increasing (Figs. 4 and 5). This gives a first indication of Anammox activity. This nitrogen removal was accompanied with production of a small amount of nitrate, which can also be attributed

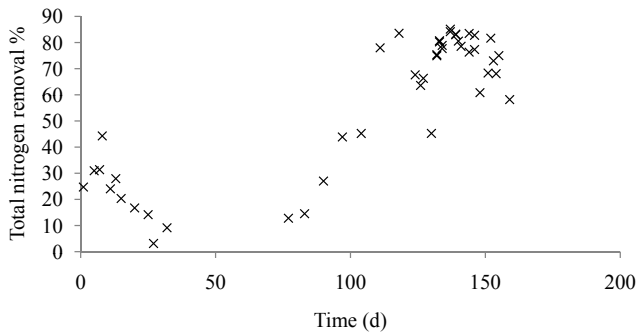


Figure 5
Nitrogen removal in the continuous slow-fed Anammox reactor

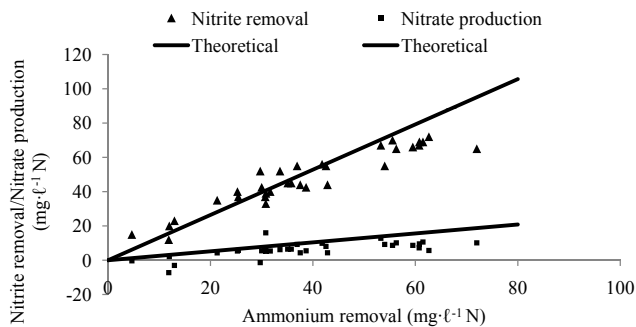


Figure 6
Removal of nitrite and production of nitrate as function of ammonium removal: experimental and theoretical values.

to the Anammox process, as 26% of the removed ammonium is converted to nitrate (Strous et al., 1998).

After about 100 d of operation, an exponential increase in nitrogen removal was detected. Because of this increasing removal the influent ammonium and nitrite concentration values were increased. After about 130 d of operation the sludge colour changed to red, which is the typical colour of Anammox biomass (Van de Graaf et al., 1996).

In order to prove the existence of Anammox organisms in the reactor some stoichiometric and kinetic parameters have been calculated from the experimental data and compared with typical Anammox values that are reported in the literature. First, the obtained doubling time was calculated based on the exponential increase of the nitrogen removal and the increase in influent total nitrogen concentration. This resulted in a doubling time of, respectively, 10 d and 19 d, which is very similar to reported values in literature (Stous et al., 1998). The somewhat higher value obtained when using influent total nitrogen concentration can be explained by the fact that the influent concentration was not continuously increased, because of operational problems such as pump failure. Other typical values that were calculated were the nitrite:ammonium consumption ratio and the nitrate:ammonium production ratio. Theoretically, these ratios should be 1.32:1 and 0.26:1 (Strous et al., 1998). These ratios were calculated from Day 55 (see Fig. 5) onwards and are depicted in Fig. 6. It can be seen that the experimentally obtained ratios are very close to the theoretical ratios. As such, it can be concluded that Anammox is present in the slow-fill reactor and that the start-up is successful.

Conclusions

In this paper the effect of feeding characteristics on the performance of the Anammox and partial nitrification reactor are discussed. For the partial nitrification reactor, the feeding regime is not important and start-up can be accomplished by slow or fast feeding. This is not the case for the Anammox reactor. Fast feeding leads to a high nitrite peak at the beginning of the feeding resulting in a slow start-up. Simulation results show that a slow filling period is needed to achieve good Anammox activity. The simulation results were confirmed by experimental laboratory-scale data.

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