INFLUENCE OF HYDRAULIC CONDITIONS ON THE START-UP AND OPERATION OF THE AUTOTROPHIC NITROGEN REMOVAL PROCESS

Helge Vandeweyer[a], Stijn W.H. Van Hulle[a], Wim Audenaert[a], Annick Monballiu[b], Boudewijn Meesschaert[b] and Ann Dumoulin[a]

[a]EnBiChem Research Group, Department of Industrial Engineering and Technology, University College West Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium. (Email: Helge.Vandeweyer@howest.be)
[b]Chemistry Department, Catholic University College of Bruges–Ostend, Zeedijk 101, B-8400 Ostend, Belgium

Abstract The autotrophic nitrogen removal process (partial nitritation combined with the Anammox process) is a new and sustainable nitrogen removal technique for nitrogen rich streams. A modeling study was performed to define optimal process conditions on two reactor configurations: a single oxygen limited partial nitritation reactor and a single Anammox reactor and to investigate the influence of feeding characteristics on the performance of the Anammox reactor. The simulations revealed that the feeding regime is an important factor in the successful startup of Anammox reactors. Nitrite concentration peaks in the beginning of a feeding period will lead to an unsuccessful start-up while a slow input of nitrogen fastens up the process. Feeding regimes are less important in partial nitritation reactors since lab results show that slow or fast supply of influent does not influence the growth of partial nitrifiers.

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). 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 this 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 [1]. 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 because of the high nitrogen concentrations (up to 2 g N L^{-1}) and the unfavorable carbon-to-nitrogen (C N^{-1}) ratio for denitrification [2], resulting in high operational costs.

A more sustainable and cost-effective alternative of conventional nitrogen removal systems is the autotrophic conversion of ammonium to nitrogen gas, especially in cases where the aeration capacity is limiting [3]. The first step, called ‘partial nitritation’, includes a transformation of the incoming ammonium to nitrite obtaining a NH_4^+-N:NO_2^--N ratio of 1:1 [4].

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

This partial nitritation process can only be realized 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 O_2 L^{-1}) and higher pH [4, 5, 6, 7]. The second process is the anaerobic oxidation of ammonium (ANAMMOX) with nitrite as electron acceptor [8, 9]. The Anammox bacteria convert ammonium and nitrite under anoxic conditions without addition of an external carbon source directly to nitrogen gas with the production of a small amount of nitrate [10].

$$1NH_3 + 1{,}32NO_2^- + H^+ \rightarrow 1{,}02N_2 + 0{,}26NO_3^- + 2H_2O$$

A shortcoming in the application of the Anammox process is the slow growth rate (μ^{max}_{AN} = 0.08 d^{-1} [12, 13]) resulting in a very time consuming experimental start-up [11, 12, 13, 14].

In view of the very time consuming experimental start-up [11, 14], a model based analysis was performed to define optimal process conditions (temperature, oxygen supply, pH and biomass retention) on two reactor configurations: a single oxygen limited partial nitritation reactor and a single Anammox reactor. The simulation results were discussed in detail in a previous publication [15]. A summary is depicted in Table 1.

Table 1: Optimal conditions for the autotrophic nitrogen removal process as determined by a modeling study [15]

	Partial nitritation	Anammox
Temperature (°C)	30 – 40	30 – 40
pH	7 – 8	7 – 8.5
Biomass withdrawal (%)	< 4	< 2.5
O_2 concentration (g m^{-3})	0.04 – 0.06	0

One important factor not considered in the table are the feeding characteristics of the reactor. De Clippeleir et al. [16] already stated that a low substrate shock (a low volumetric exchange ratio) resulted in a faster start-up of an oxygen limited autotrophic nitrification/denitrification (OLAND) SBR reactor. In this contribution it will be demonstrated that applying the conditions in Table 1 does not always lead to a successful start-up and that especially the feeding regime in SBR type reactors plays a role in the successful start up of Anammox reactors. Starting from the optimal conditions, the effect of feeding characteristics on the performance of the Anammox reactor and the partial nitritation reactor are tested on lab-scale and/or modeling basis.

METHODS

Reactor systems

2 partial nitritation reactors are operated on a different feeding regime to observe the effect on their operation, i.e. the production of a NH_4^+-N : NO_2^--N ratio of 1. The 'fast fill' reactors are operated with a feed period of 1 minute every cycle while the feed period of the 'slow fill' reactors occurs continuously. This kind of feeding (slow and fast filling) is also used for 2 sequencing batch Anammox reactors. A fast fill sequencing batch reactor (SBR) with a feed period of 1 minute every cycle is operated to observe the removal of NO_2^--N and NH_4^+-N. The feed period of the slow fill reactor occurs continuously.

Chemical analysis

The concentration of ammonium-N, nitrite-N and nitrate-N were measured colorimetrically according to standard methods [17].

PARTIAL NITRITATION REACTOR

Fast fill partial nitritation reactor

The partial nitritation reactor filled with 2 liter nitrifying and denitrifying sludge of the municipal WWTP of Harelbeke (www.aquafin.be) was initially fed with 0.5 liter of synthetic influent containing 1 g NH_4Cl N L^{-1} and an equimolair amount of $NaHCO_3$ reaching a working volume of 2.5 liter. Each day 0.5 liter is drawn out of the solution after a biomass settling period of 0.5 hour followed by a filling period with 0.5 liter influent resulting in a HRT of 5 days and a loading rate of 0.2 g N $(L*day)^{-1}$. After 44 days the loading rate is increased to 0.4 g N $(L*day)^{-1}$ by increasing the volume influent and effluent to 1 liter decreasing the HRT to 3 days. The reactor conditions are the same as the optimal conditions found by simulation (Table 1) except for the oxygen concentration. The O_2 concentrations simulated by the model (0.04 – 0.06 ppm O_2) was lower than those set in the lab reactor (0.18 – 0.5 ppm O_2) as the simulation model assumes perfect mixing, while in practice floc formation will induce an O_2 concentration gradient and consequentially a higher O_2 bulk concentration. After 100 days, 2 liter of this sludge is transferred to a bigger reactor of 20 liter. In this reactor also 8 liter of nitrifying and denitrifying sludge of the WWTP of Harelbeke was added. This reactor is daily fed with 10 liter 1 g NH_4Cl N L^{-1} under the same operational conditions as in the smaller reactor except that the HRT decreases to 2 days.

Slow fill partial nitritation reactor

After 104 days, a membrane is placed in the 20 liter reactor. The reactor was now fed continuously with a flow rate of 10 liter a day. As such the HRT reached a value of 2 days.

ANAMMOX REACTOR

Modeling of the Anammox reactor

For the simulations a previously developed model [13] was used and Haldane kinetics were introduced to describe the dependence of the growth rate on nitrite with K = 0.3 and K_I = 200.

$$f\left(NO_2^-\right)=\frac{NO_2^-}{\frac{\left(NO_2^-\right)^2}{K_I}+K+NO_2^-}$$

The extended model was implemented in the software program WEST® (www.mostforwater.com).

Start-up of a fast fill Anammox reactor

A SBR reactor with working volume of 3 liter filled with 1 liter nitrifying and denitrifying sludge and 1 liter anaerobic digester sludge of the WWTP of Harelbeke was initially fed with 1 liter of synthetic influent containing $(NH_4)_2SO_4$, $NaNO_2$ and $KHCO_3$ (Table 2). The reactor conditions are the same as the optimal conditions found by simulation (Table 1). The biomass is able to settle during 0.5 hour before 1 liter is withdrawn out of the reactor. After feeding with 1 liter reaching a HRT of 3 days and before addition of $KHCO_3$, the lab reactor is flushed with N_2 to achieve anoxic conditions.

Start-up of a slow filling Anammox reactor

The SBR reactor with working volume of 1.2 liter filled with 200 ml nitrifying and denitrifying sludge, 200 ml anaerobic digester sludge of the WWTP of Harelbeke, 100 ml of sludge from the partial nitritation reactor and 100 ml heterotrophic sludge. 4 cycles were performed each day. One complete cycle consisted of a feeding period of 5 hours in which the reactor is filled with 300 ml synthetic influent containing 28 mg N L^{-1} $(NH_4)_2SO_4$, 28 mg N L^{-1} $NaNO_2$ and $KHCO_3$ at a flow rate of 1 ml min^{-1} (Table 2). At start, 140 mg N L^{-1} $NaNO_3$ is added in the influent to guarantee that not all nitrite is removed by denitrifying bacteria so that nitrite can be taken up by Anammox bacteria. This nitrate concentration is decreased in the influent after 83 days to 70 mg N L^{-1} since less denitrifying activity is measured. After this feeding period, the sludge is able to settle for 50 minutes followed by a decanting period of 5 minutes in which 25 % of the volume is removed (300 ml). At the beginning of each cycle, 900 ml of liquid was present in the reactor and at the end of the filling period, 1.2 liter was present. The hydraulic retention time was thus 1 day. The reactor conditions are the same as the optimal conditions found by simulation (Table 1). To achieve anoxic conditions, the reactor and the influent is hold under a 5 % CO_2 / 95 % N_2 environment by gas bags.

Table 2: Composition of the synthetic wastewater of the Anammox reactor used in this study according to Dapena-Mora et al. [13] (expressed in g L^{-1})

	Synthetic wastewater fast fill	Synthetic wastewater slow fill
$(NH_4)_2SO_4$	0.132 (0.028 g N L^{-1})	0.165 (0.035 g N L^{-1})
$NaNO_2$	0.138 (0.028 g N L^{-1})	0.1725 (0.035 g N L^{-1})
$NaNO_3$	-	0.850 (0.140 g N L^{-1})/ 0.425 (0.070 g N L^{-1})
$KHCO_3$	1.25	1.25
$NaH_2PO_4.\ 2H_2O$	0.029	0.029
$CaCl_2$	0.226	0.226
$MgSO_4.7H_2O$	0.2	0.2
$FeSO_4.7H_2O$	0.021	0.021
$EDTA.2H_2O$	0.0076	0.0076
Trace elements	1.25 mL L^{-1}	1.25 mL L^{-1}

RESULTS

Results of the partial nitritation reactor

After 2 weeks a successful operation of the 'fast fill' partial nitritation reactor is noticed, i.e. the production of a NH_4^+-N : NO_2^--N ratio of 1:1 (Figure 1a, 1b). The same ratio of 1:1 is observed in the 'slow fill' partial nitritation reactor (Figure 1c). Since in both reactors only small amounts of nitrate are observed in the effluent, nitrite oxidizing bacteria are not present in high concentration. It can be concluded that discontinuous and continuous feedings give both excellent results meaning that the feeding regime is not important for the partial nitritation reactor and start-up can be accomplished by slow or by fast feeding.

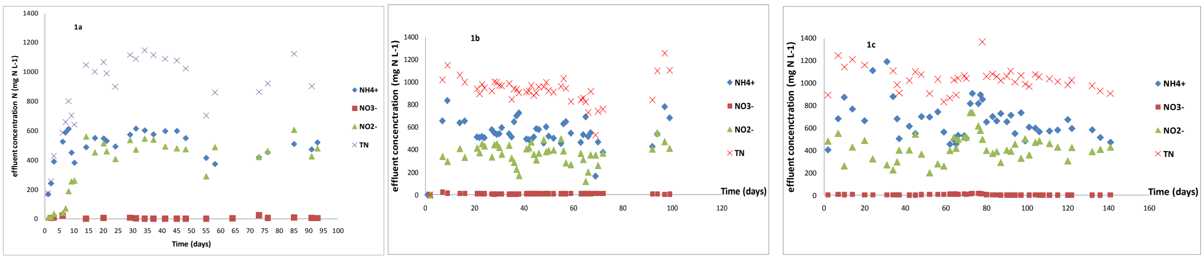

Figure 1: Overview of the N concentration in the effluent of a fast fill partial nitritation reactor of 3 liter (a), a fast fill partial nitritation reactor of 20 liter (b) and a slow fill partial nitritation reactor of 20 liter (c)

Modeling results of the Anammox reactor

When the ideal conditions of the Anammox reactor (Table 1) were known, the effect of feeding characteristics was tested on the performance of the Anammox reactor by simulating the nitrogen gas production. 2 Anammox SBR reactors are operated according to the start-up strategy of Dapena-Mora et al. [13] with a 6 hour cycle and a volume exchange ratio of 25 %. Figure 2a gives an overview of the N concentration in the influent used to simulate the performance of these 2 Anammox SBR reactors. The only difference is the feed period: for the first reactor this period lasts 5.5 h while for the second reactor the feed period lasts only 1 minute.

Figure 2b states that a slow fill reactor has a better performance than a fast fill reactor. A short feeding period causes a temporary nitrite peak leading to an unsuccessful start-up of the fast fill reactor. This can be seen by the fact that the N_2 gas production does not increase over time in the fast fill reactor although the N in the influent increases.

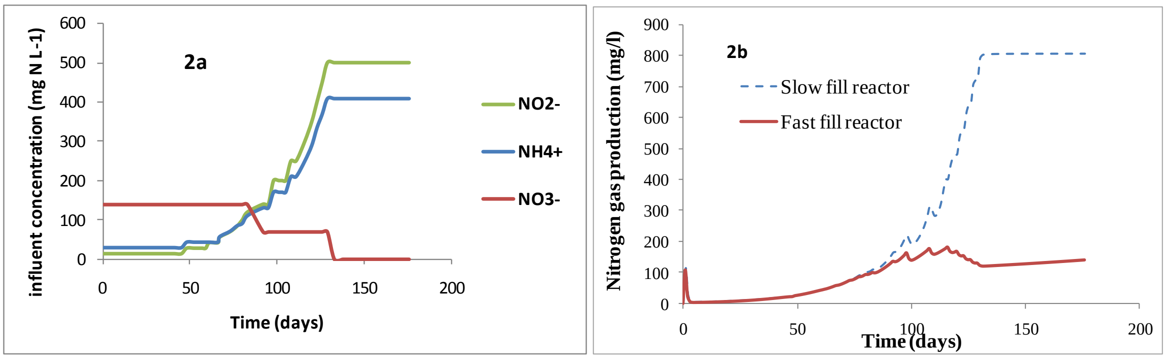

Figure 2: The N concentration in the influent for the modeling of a Anammox SBR reactor (a) and the simulated nitrogen gas production of a slow fill and a fast fill Anammox reactor (b)

Results of the Anammox reactor

Initially, bacterial decay occurs causing high ammonium concentration and organic carbon in the effluent of the fast fill reactor. The ammonium concentration was therefore omitted in the influent until the ammonium concentration decreased to lower values while the nitrite concentration was set to 35 mg N L^{-1}. After 25 days, the ammonium concentration in the influent was also increased to 35 mg N L^{-1}. 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 lab reactor. A possible explanation could be the feeding characteristics of this reactor.

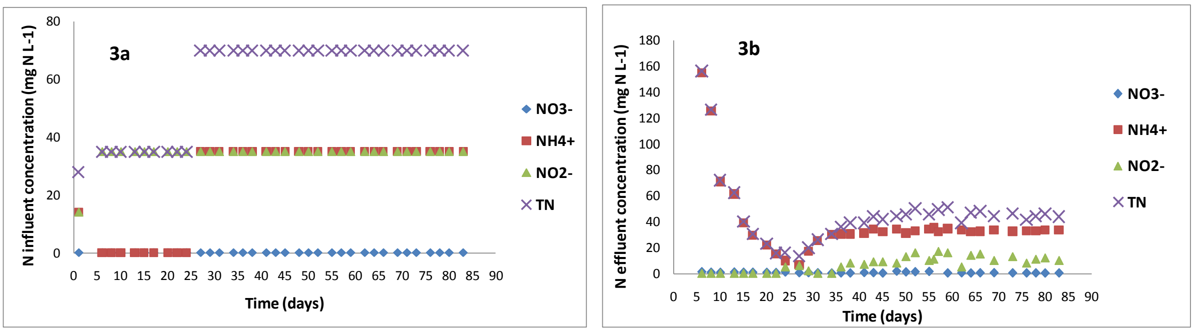

Figure 3: N concentration in the influent (a) and in the effluent (b) of a fast fill Anammox reactor

On a regular basis the ammonium-N, nitrite-N and nitrate-N concentration of the effluent of the slow filling reactor are determined colorimetrically. These results are used to detect the removal of ammonium and nitrite in the influent. Anammox activity is observed if the NO_2^--N : NH_4^+-N removal ratio is 1.32:1. In the beginning, bacterial decay occurs resulting in a higher concentration ammonium in the effluent than in the influent. Bacterial decay also leads to an increase of organic carbon so denitrifying bacteria will use this organic carbon to convert nitrite and nitrate to nitrogen gas, resulting in a decrease of nitrite. The strong increase of ammonium and the decrease in nitrite concentration lead to a negative NO_2^--N:NH_4^+-N removal ratio (Figure 4). After 85 days, NH_4^+ and NO_2^- concentrations both decrease giving a positive NO_2^--N:NH_4^+-N removal ratio. From that moment the NO_2^--N:NH_4^+-N removal ratio increase and lays on the line of the expected exponential removal ratio. The removal of ammonium and nitrite could be the result of Anammox activity but it could also be coming from nitrifying activity. Further research is therefore needed, although it was already demonstrated by modeling that a slow operational regime produce better results than a fast feeding regime.

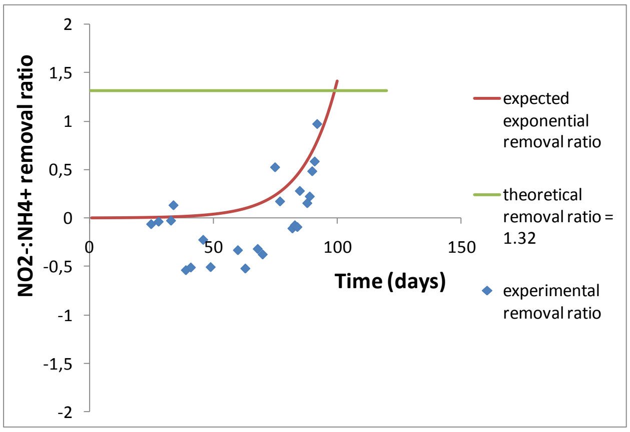

Figure 4: The theoretical, experimental and expected experimental NO_2^- -N : NH_4^+-N removal ratio of a slow fill Anammox reactor

CONCLUSIONS

In this paper the effect of feeding characteristics on the performance of the Anammox and partial nitritation reactor are discussed. For the partial nitritation reactor, the feeding regime is not important and start-up can be accomplished by slow or by fast feeding. This is not the case for the Anammox reactor. A fast feeding leads to a high nitrite peak in 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 are tested by experimental data, although further research is necessary.

LITERATURE

[1] Janus H.M. & van der Roest H.F. (1997). Don't reject the idea of treating reject water. *Water Science and Technology,* **35,** 27-34.

[2] Henze M., van Loosdrecht M., Ekama G. & Brdjanovic D. (2008). Biological Wastewater Treatment: Principles, and Design. *IWA Publishing*, 511p.

[3] van Dongen U., Jetten M.S.M. & van Loosdrecht M.C.M. (2001). The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Water Science and Technology,* **44,** 153-160.

[4] Hellinga C., Schellen A., Mulder J.W., van Loosdrecht, M.C.M. & Heijnen J.J. (1998). The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology,* **37,** 135-142.

[5] Wyffels S., Boeckx P., Pynaert K., Zhang D., Van Cleemput O., Chen G. & Verstraete W. (2004a). Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification denitrification process. *Water Science and Technology,* **49,** 57-64.

[6] Wyffels S., Van Hulle S.W.H., Boeckx P., Volcke E.I.P., Van Cleemput O., Vanrolleghem P.A. & Verstraete W. (2004b). Modeling and simulation of oxygen-limited partial nitritation in a membrane-assisted bioreactor (MBR). *Biotechnology and Bioengineering,* **86,** 531-542.

[7] Anthonisen A.C., Loehr R.C., Prakasam T.B.S. & Srinath E.G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *Journal of Water Pollution Control Federation,* **48**, 835-852

[8] van de Graaf A.A., De Bruijn P., Robertson L.A., Jetten M.S.M. & Kuenen J.G. (1996). Autotrophic growth of anaerobic ammonium-oxidizing microorganisms in a fluidized bed reactor. *Microbiology,* **142,** 2187-2196.

[9] Jetten M.S.M., Horn S.J. & van Loosdrecht M.C.M. (1997). Towards a more sustainable municipal wastewater treatment system. *Water Science and Technology,* **35,** 171-180.

[10] Jetten M.S.M., Strous M., van de Pas-Schoonen K.T., Schalk J., van Dongen U.G.J.M., Van De Graaf A.A., Logemann S., Muyzer G., van Loosdrecht M.C.M. & Kuenen J.G. (1999). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews*, **22**, 421-437.

[11] Hao X.D., Heijnen J.J. & van Loosdrecht M.C.M. (2002). Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process. *Biotech. and Bioeng.*, **77**, 266-277.

[12] Strous M., Heijnen J.J., Kuenen J.G. & Jetten M.S.M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology*, **50**, 589-596.

[13] Dapena-Mora A., Van Hulle S.W.H., Campos J.L., Mendez R., Vanrolleghem P.A. & Jetten M. (2004). Enrichment of Anammox biomass from municipal activated sludge: experimental and results. *Journal of Chemical Technology and Biotechnology,* **79,** 1421-1428.

[14] van der Star W.R.L., Abma W.R., Blommers D., Mulder J.W., Tokutomi T., Strous M., Picioreanu C. & Van Loosdrecht M.C.M. (2007). Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research,* **41,** 4149-4163.

[15] Vandeweyer H.J.P., Monballiu A., Meesschaert B.D. and Van Hulle S.W.H. (2009). Performance Analysis and Optimization of Autotrophic Nitrogen Removal in different Reactor configurations: A modeling Study. In: *Proceedings IWA YWP Benelux Congres,* 1-2 October 2009.

[16] De Clippeleir H., Vlaeminck S.E., Carballa M. and Verstraete W. (2009). A low volumetric exchange ratio allows high autotrophic nitrogen removal in a sequencing batch reactor. *Bioresource Technology*, **100**, 5010-5015.

[17] APHA, Standard Methods for the Examination of Water and Wastewater, 20^{th} ed., American Public Health Association, Washington, DC, 1998.