

## Syntrophy of aerobic and anaerobic ammonia oxidisers

B. Wett, M. Hell, G. Nyhuis, T. Puempel, I. Takacs and S. Murthy

### ABSTRACT

Deammonification is known as an efficient and resource saving sidestream process option to remove the nitrogen load from sludge liquors. The transfer of the intermediate product nitrite between both syntrophic groups of organisms – aerobic and anaerobic ammonia oxidizers (AOB) – appears very sensitive to process conditions such as temperature, dissolved oxygen (DO) and operating nitrite level. Growth kinetics for aerobic and anaerobic AOBs differ by one order of magnitude and require an adequate selection of sludge retention time. This paper provides measurement- and model-based results on how selected sludge wasting impacts population dynamics in a suspended growth deammonification system. Anammox enrichment up to a doubled portion in mixed liquor solids can substantially improve process stability in difficult conditions. A case-study on low temperature operations outlines two possible strategies to balance syntrophic consumption of ammonium and nitrite.

**Key words** | anammox, AOB, cyclone, deammonification, DEMON, sidestream, sludge liquor

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### INTRODUCTION

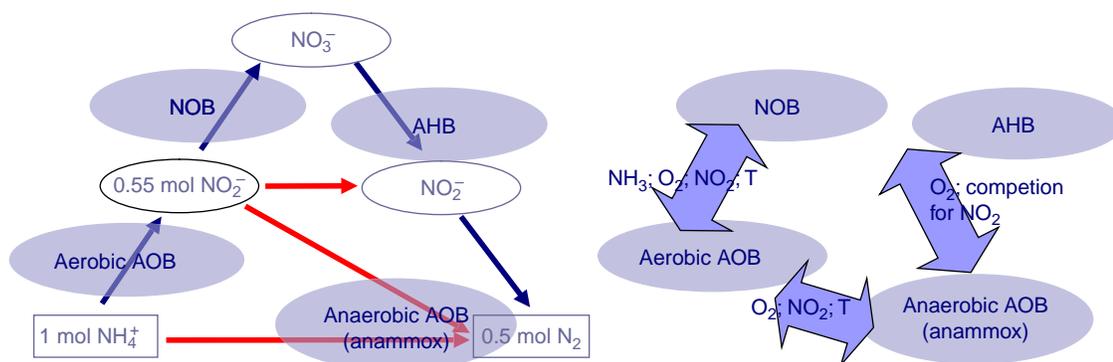
Single- and two-stage systems have been developed where aerobic and anaerobic ammonia oxidation processes are catalysed either by suspended or fixed biomass. A fast growing number of successful full-scale implementations contribute operational experience and build up confidence in these innovative technologies.

Basically four different microbial consortia are involved in nitrogen removal processes (Figure 1):

- One out of these – nitrite oxidisers (NOB) – need to be repressed by taking advantage of their higher oxygen half saturation concentration and different temperature sensitivity (Arrhenius coefficient) compared to aerobic ammonia oxidisers. Moreover free ammonia inhibition can out-compete NOB as long as sludge age does not

allow advanced acclimation to toxic ammonia levels (Turk & Mavinic 1989).

- Another group – anoxic heterotrophic biomass (AHB) – plays a minor role due to limited availability of organic carbon but can help to reduce residual nitrate (11% stoichiometric nitrate production from deammonification according to Strous *et al.* 1998). The main microbial players in deammonification process coexist in close syntrophy:
- Anaerobic AOB depend on aerobic AOB producing nitrite which serves as electron acceptor but turns into a toxic compound when exceeding a certain level. Strous *et al.* (1999) allude to the negative influence of nitrite concentration and exposure time on anammox



**Figure 1** | Functional groups of organisms catalysing nitrogen removal processes (left) and variables that impact population dynamics between these groups (right).

performance. Depending on size of biomass aggregates and acclimation period, the accumulated nitrite results in an irreversible toxic impact on anammox organisms. Considering e.g. suspended growth deammonification Fux (2003) observed an almost complete loss of anammox activity at an average nitrite level of 42 mg NO<sub>2</sub>-N/L within 11 days and Wett *et al.* (2007b) reported an acceleration of anammox decay rates starting from about 5 mg NO<sub>2</sub>-N/L. Therefore this impact should be addressed as toxicity (increased decay) and not inhibition (reduced growth). Anammox organisms have to compete with both NOB and AHB for nitrite as electron acceptor. Finally anaerobic organisms require elevated process temperature preferably in mesophilic range.

- Aerobic AOB represent the only functional group of microorganisms consuming ammonia as an energy source for their metabolism, without competitors, irrespective of process conditions. Reflecting growth kinetics aerobic AOB appear less vulnerable – both substrate inhibition by free ammonia and product inhibition by nitrite or nitrous acid, respectively, are less significant compared to toxicity impacts on NOB and anammox (Sin *et al.* 2008).

## APPROACH

Due to a significant difference in growth rates (typical  $\mu_{\max}$  parameter values for AOB are about 10 times higher than for anammox; Sin *et al.* 2008) a corresponding difference in sludge retention time (SRT) would be perfect for a balanced

biomass composition. In biofilm systems (Rosenwinkel & Cornelius 2005) a kind of natural SRT selection happens by stratification of the biofilm structure. Depending on oxygen diffusion anaerobic AOB settle at inner biofilm layers while aerobic AOBs prefer outer layers well supplied with oxygen. The outer layers on rotating contactor discs or moving bed media experience higher shear stress and more aggressive erosion than the inner more protected ones. Thus the anaerobic AOB tend to achieve a higher SRT without specific control actions. Obviously anammox organisms at the inner biofilm layer require higher concentrations of ammonia and nitrite in the bulk liquid to overcome diffusion limitations. On the one hand diffusion protects sensitive anammox biomass from toxic nitrite levels and biofilm systems are shown to be tolerant against operational failures (e.g. over-aeration). On the other hand higher bulk concentrations mean higher effluent values – a drawback of diffusion driven biomass separation.

In suspended growth deammonification systems different types of biomass aggregates showing different properties are mixed. Typical activated sludge flocs are built up of all kinds of particles including biomass of high biological diversity. Embedded in this floc structure there are small granules of higher density and the characteristic red colour containing a more homogeneous biomass (Innerebner *et al.* 2007). Control measures can try to make use of this difference in biomass structure. For the DEMON process (Wett 2007a) a device has been developed and patented in order to separate the two sludge fractions. The waste activated sludge is fed to a cyclone (Figure 3) from where the overflow is wasted and the underflow is recycled to the SBR. This paper provides measurement- and model-based

results on how selected sludge wasting impacts population dynamics in a suspended growth deammonification system.

## METHODS

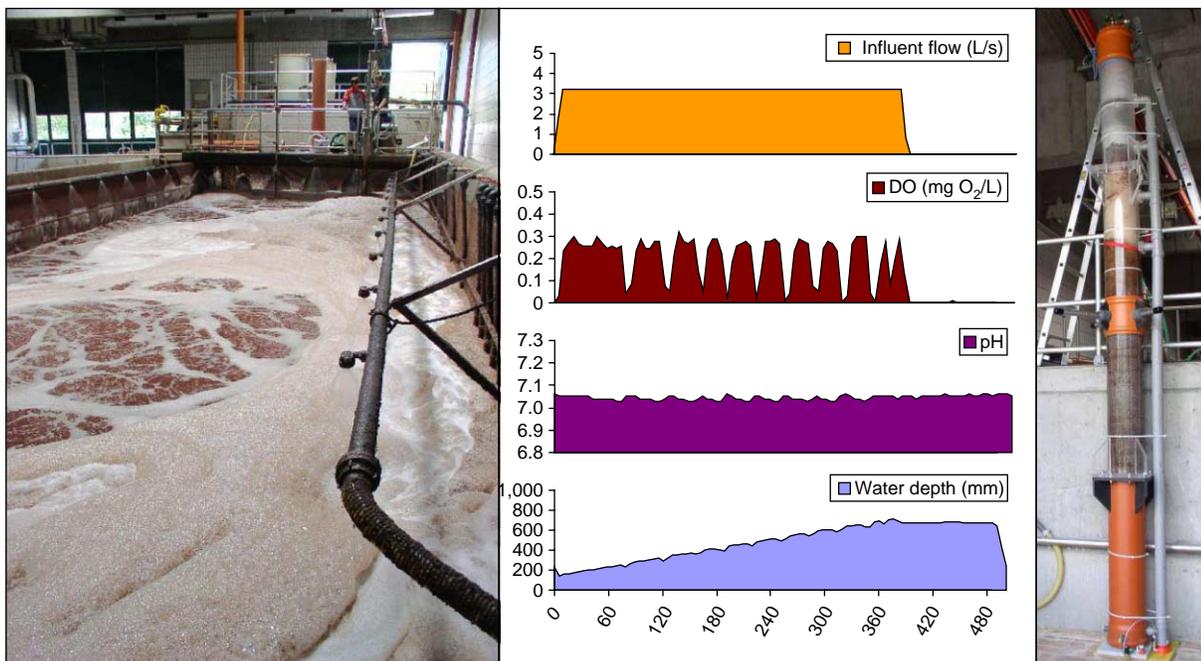
### DEMON full-scale implementation at WWTP Strass

In 2004 deammonification has been implemented at the WWTP Strass, Austria, in an SBR tank (Figure 2) with a maximum volume of 500 m<sup>3</sup> and at loading rates up to 340 kg of ammonia nitrogen per day (Wett 2007a). The aeration system is activated within a very tight pH-bandwidth of only 0.01 pH unit. Due to oxygen input nitrification runs at a higher rate than anaerobic ammonia oxidation and H<sup>+</sup> production drives the pH-value to the lower set-point and aeration stops. While dissolved oxygen is depleted all the nitrite that has been accumulated during the aeration interval is used for oxidizing ammonia. In the course of this biochemical process some alkalinity is recovered and additionally alkaline rejection water is fed continuously to the reactor until the pH-value reaches the upper set-point and aeration is switched on again. In spring

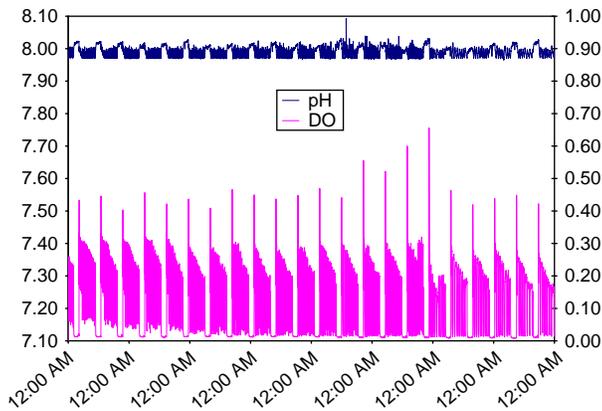
2009 a cyclone has been installed as a handle to control selected SRT.

### Pilot system for landfill leachate treatment at low operating temperature

The pilot reactor for continuous kinetic experiments (Figure 2) has been installed at the Solid Waste Treatment Center Lustenau, Austria, in order to optimize process control for biological treatment of landfill leachate (average concentrations: ammonia 441 mg N/L; COD 1,258 mg/L; alkalinity 65 mmol/L). The pipe-shaped plexi-glass SBR with a diameter of 0.25 m and minimum volume of 150 L is operated at a hydraulic residence time (HRT) of 1.5 days and mixed liquor solids concentration (MLSS) of 7.5 g/L. Tall reactor geometry was selected in order to achieve a realistic representation of aeration performance and gas-stripping effects. The bottom of the reactor is covered with a fine bubble membrane diffuser and the flow of pressurized air is continuously metered. On-line probes for pH (WTW: SensoLyt SEA) and DO (WTW: TO700IQ) measurements are installed and connected to the programmable logic



**Figure 2** | Photograph of the full-scale DEMON-system Strass (left; 500 m<sup>3</sup> maximum volume), the pilot system for landfill leachate treatment (right; 200 L volume) and typical process variables during 1 SBR cycle (flowrate, DO, pH and water table; middle).

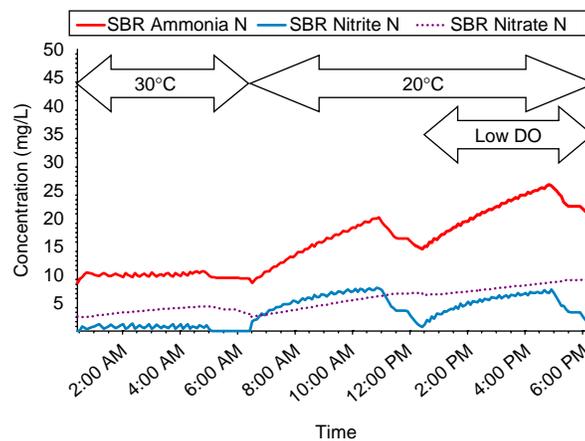
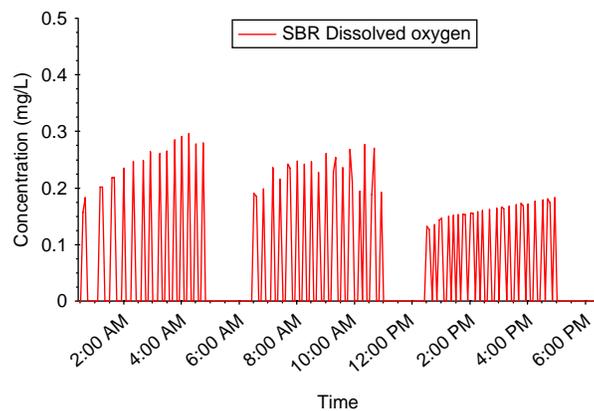


**Figure 3** | One week pH on-line monitoring at high level around 8.0 (due to high alkalinity in landfill leachate) and corresponding DO-profile at intermittent aeration.

control (Siemens Logo). Water temperature was kept stable between 29 and 30°C by controlling a heating element and was then lowered to expected winter operating conditions around 20°C. For monitoring purpose (outside the control loops) ammonia and nitrate (WTW: VARiON 700Plus IO) is measured on-line and a pressure meter detects water level. All the collected data is transmitted to allow remote operation monitoring and control.

### Deammonification modelling

Subsequent to experimentation, kinetic results have been converted into a mathematical model using the process simulator BioWin. The BioWin<sup>®</sup> ASDM model



**Figure 4** | Simulated profiles of inorganic nitrogen compounds indicate high sensitivity to a temperature drop from 30 to 20°C (right) and only limited impact of a lower DO level (left).

(Jones *et al.* 2007) contains all required processes in a whole plant model that occur within the DEMON system:

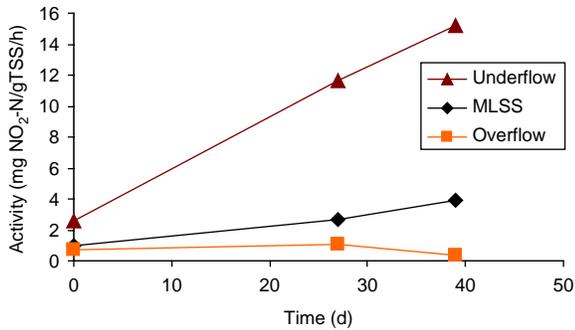
- Two-step nitrification, including temperature, DO and pH effects
- Anaerobic ammonia oxidation including nitrite toxicity
- pH calculation based on equilibrium chemistry
- Gas exchange, ammonia- and CO<sub>2</sub> stripping
- An SBR reactor element to host the sidestream process
- The BioWin Controller is used to implement the DEMON pH strategy for control of aeration and DO level.

The cyclone is modelled as a separate element in the sludge waste stream where all fractions of solids are reduced to a specified portion of the initial value. For sake of simplicity only two selectivity parameters are defined – one for compounds predominantly being wasted and one for enriched compounds predominantly being recycled.

## RESULTS AND DISCUSSION

### Case-study – population dynamics at low temperature operation

Reject water from sludge treatment and leachate of a covered landfill show similar properties in terms of C/N ratio. A significant difference concerns available alkalinity: Landfills can provide additional sources of alkalinity besides released ammonia. Without alkalinity limitation



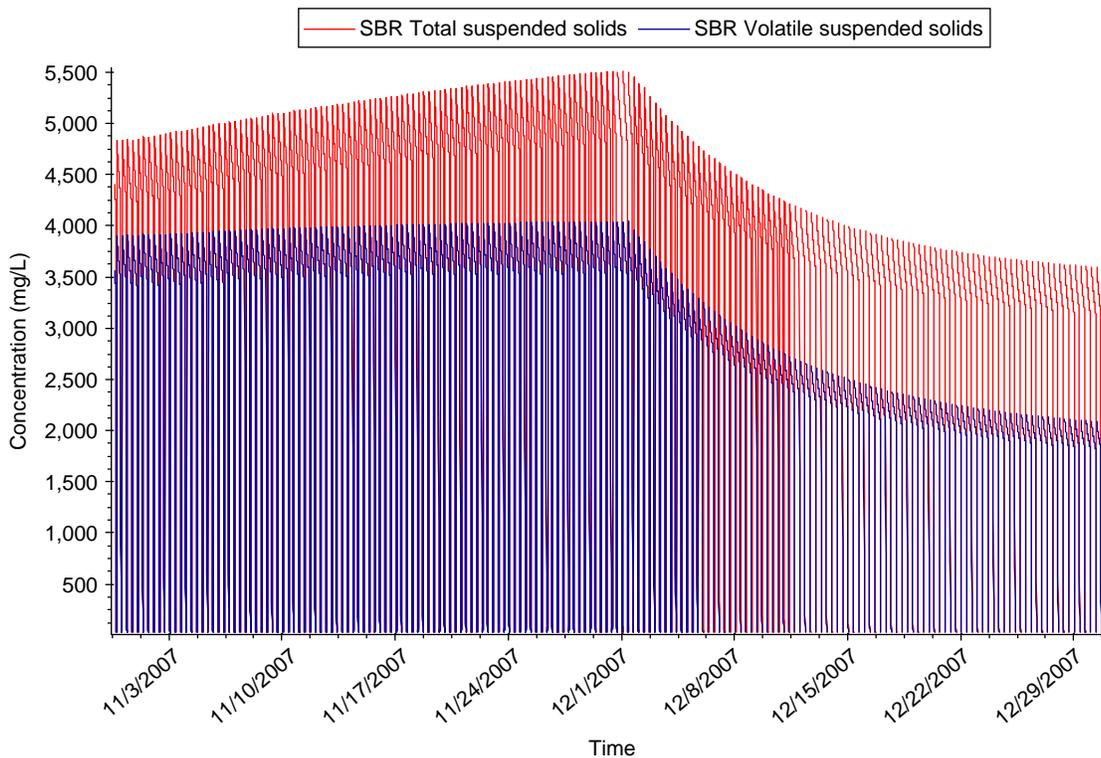
**Figure 5** | Development of anaerobic AOB (anammox) activity after process restart comparing mixed liquor, overflow and underflow of the cyclone used in the DEMON process for biomass separation.

almost complete ammonia conversion is feasible and set-points of pH-control need to be set to a higher level – to 8.0 in current case study (Figure 3).

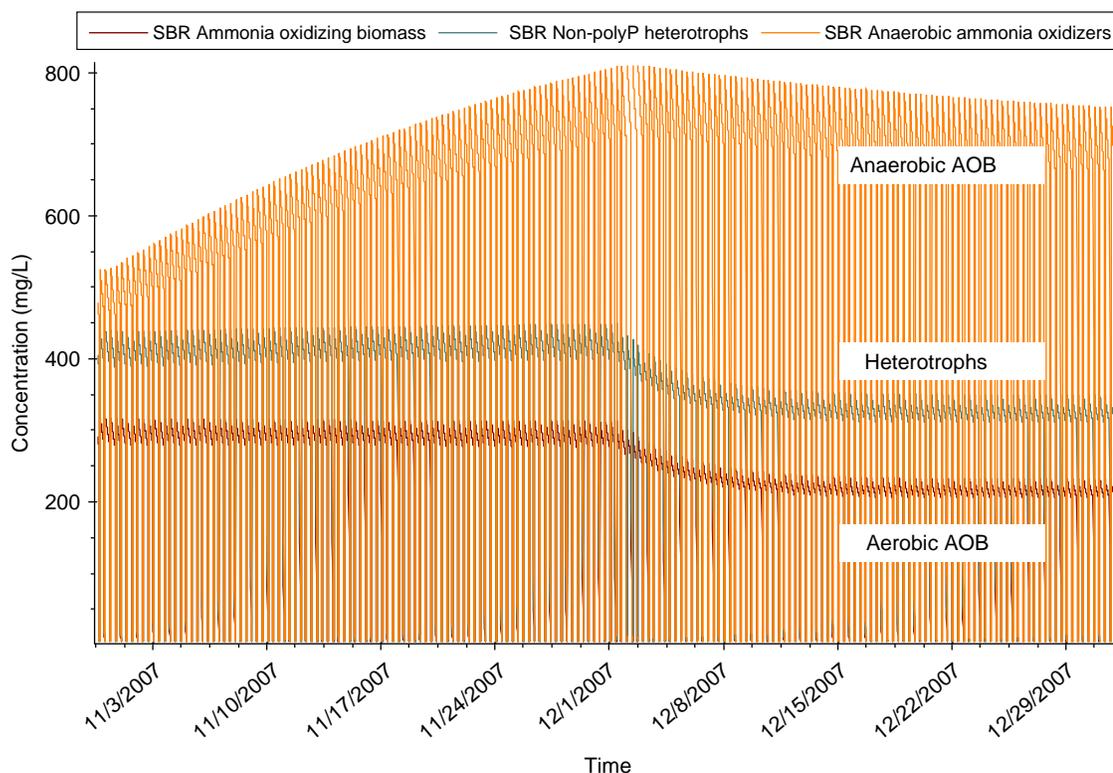
Sudden temperature drop from 30 to 20°C caused rapid nitrite accumulation. Corresponding to process temperature drop by 10°C the oxygen uptake rate (OUR) decreased by 50% and the aeration rate had to be reduced in order to maintain DO levels below 0.3 mg/L. At stepwise decrease of operating temperature the biomass would adapt to new

process conditions. Due to slower life cycles at lower temperature the simulated anammox biomass tends to decrease at same mixed liquor solids concentration while SRT is still sufficient for complete establishment of aerobic AOB. Therefore temperature driven population dynamics during an adaptation period cannot help to reconcile syntrophic balance of aerobic and anaerobic AOB.

Figure 4 presents simulation results from an instantaneous drop of temperature from one operation cycle to



**Figure 6** | Dynamic TSS- and VSS profiles during a 1 month simulation period after implementation of the cyclone model (same wastage flow) followed by 1 month simulation at a 3-fold wastage flow.



**Figure 7** | Simulated shift in AOB populations after application of biomass separation in order to select appropriate SRT each for anaerobic and aerobic AOB, respectively.

the next. The BioWin default value for maximum anammox growth rate ( $0.1 \times 1.1^{(T-20)}$  per day) has been used; the half inhibition constant for DO has been calibrated to 0.2 mg/L. The temperature drop caused a misbalance between nitrite production and nitrite consumption rates and consequently increased vulnerability of operational stability. The DO level had to be reduced from 0.3 to 0.2 mg/L for a partial compensation of this misbalance – a control strategy that has been confirmed by the experimental process behaviour. An alternative approach aims at selection of different SRTs for aerobic and anaerobic biomass.

### Measured impacts of cyclone operation at DEMON Strass

The re-start from a status of low nitrogen turn-over up to full capacity of about 300 kg NH<sub>4</sub>-N/d of the DEMON-plant in Strass, Austria, was supported by the new cyclone device. Initially the selectivity of the cyclone was rather limited (Figure 5) while the solids concentration in the reactor was maintained below 2 g/L. During this operation

period the biomass selectivity (meaning the separation efficiency of anammox mass into the cyclone underflow) improved faster than the actual activity of the mixed liquor. After 40 days when the measured activity in the cyclone underflow was 44 times higher than in the overflow sufficient anaerobic AOB have been accumulated to be used as a seed for the start-up of the DEMON-plant in Apeldoorn, Netherlands.

### Model based investigation of SRT-selection

The question – how much anammox biomass can be held in the system in steady state compared to aerobic AOB – is

**Table 1** | Selected sludge retention time (SRT) due to simulated cyclone operation and corresponding shares of aerobic and anaerobic ammonia oxidisers in MLSS

	Without cyclone		With cyclone	
	Share in solids (%)	SRT (d)	Share in solids (%)	SRT (d)
Aerobic AOB	5	28	5	9
Anaerobic AOB	8	28	15	53

crucial for the robustness of the system and has been investigated numerically. The BioWin<sup>®</sup> ASDM model with 2-step nitrification and anaerobic ammonia oxidation was used to calculate the biomass composition in the reactor at steady state. Then the 2 selectivity parameters of the cyclone-model have been calibrated to solids flux- and anammox activity measurements (99% of AOB, NOB, AHB and endogenous products in the waste stream and 84% of anammox biomass and inorganic matter in the recycled stream) and the dynamic transition to a new steady biomass composition with selected SRT has been simulated. Selection of a higher share of inorganic matter and anammox in the cyclone underflow caused VSS reduction (Figure 6) and anammox enrichment in the reactor (Figure 7).

Due to the recycled underflow total solids in the reactor increased and the anammox concentration profile showing a steep slope during the 1 month simulation run. Then a threefold increase of the wastage rate was assumed resulting in a sharp decrease in solids down to 3.5 g/L but anammox biomass was maintained at a high level. The calculated steady state anammox portion increased from initially 8 to 15% of total solids while the aerobic AOB portion remained constant at 5% (Table 1). The final SRT for anaerobic AOB was almost 6 times higher than for aerobic AOB.

Investigation of impacts of the cyclone on process robustness at low temperature operation will require detailed monitoring and frequent activity tests during the cold season. Last winter two DEMON plants in Switzerland have been equipped with a cyclone. The deammonification system in Thun – where an uncovered sidestream reactor is operated – has experienced temperatures of 20°C without loss in operational stability (Nyhuis 2009).

## CONCLUSIONS

Long retention time for slowly growing anaerobic AOB and short acclimation periods for aerobic AOB seem to be contradicting control targets. This dilemma can be solved by measures separating these functional groups of ammonia oxidisers.

- Cyclone making use of centrifugal forces to select appropriate SRT for each AOB population

- Gas-lift for gravimetric granule separation (Van Dongen *et al.* 2001)
- Biofilm showing an uncontrolled biomass separation driven by diffusion limitation

Substantially higher accumulated mass of anaerobic AOB in the system compensates for slower kinetics of these organisms compared to aerobic ones. Model results indicate doubling of the anammox to aerobic AOB mass ratio. Selective sludge wasting at sidestream treatment systems can be used to tackle the following process engineering goals.

- Low temperature operations: Anammox enrichment to overcome temperature sensitivity of anaerobic organisms
- Stable repression of NOB: Accumulated anammox compete for generated nitrite
- Process robustness against disturbance: Over-capacity of anaerobic AOB improves tolerance for operational failures like over-aeration
- High COD- or solids loads in the influent flow: Insignificant impact on anammox retention time due to selected sludge wasting
- Savings in volume requirement: Same selected SRT for anaerobic AOB at lower mass of total solids or reactor volume, respectively

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