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**Reference:**
Van Winckel Tim, Vlaeminck Siegfried, Al-Omari Ahmed, Bachmann Benjamin, Sturm Belinda, Wett Bernhard, Takács Imre, Bott Charles, Murthy Sudhir N., De Clippeleir Haydée.- Screen versus cyclone for improved capacity and robustness for sidestream and mainstream deammonification
Environmental Science: Water Research & Technology - ISSN 2053-1400 - 5:10(2019), p. 1769-1781
Full text (Publisher's DOI): https://doi.org/10.1039/C9EW00384C
To cite this reference: https://hdl.handle.net/10067/1625400151162165141

# Screen versus cyclone for improved capacity and robustness for sidestream and mainstream deammonification

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**Abstract**

Deammonification systems are being implemented as cost- and resource-efficient nitrogen removal processes. However, their complexity is a major hurdle towards successful transposition from side- to mainstream application. Merely out-selecting nitrite oxidizing bacteria (NOB) or retaining anammox bacteria (AnAOB) does not guarantee efficient mainstream deammonification. This paper presents for the first time the interactions and synergies between kinetic selection, through management of residual substrates, with physical selection through separation of solids retention times (SRT). This allowed the formulation of tangible operational recommendations for successful deammonification. Activity measurements were used to establish retention efficiencies ($\eta$) for AnAOB for full-scale cyclones and rotating drum screens installed at a sidestream and mainstream deammonification reactor (Strass, Austria). In sidestream, using a screen ($\eta = 91\%$) instead of cyclone ($\eta = 88\%$) may increase the capacity up to 29%. For mainstream, higher AnAOB retention efficiencies achieved by the screen ($\eta = 72\%$) compared to cyclone ($\eta = 42\%$) induced a prospected increase~~d~~ in capacity by 80-90%. In addition, the switch in combination with bioaugmentation from sidestream made the process less dependent on nitrite availability, thus aiding the outselection of NOB. This allowed for a more flexible (intermittent) aeration strategy and a reduced need for tight SRT control for NOB washout. A sensitivity analysis explored expected trends to provide possible operational windows for further calibration. In essence, characterization of the physical selectors at full-scale allowed a deeper understanding of operational windows of the process and quantification of capacity, ultimately leading to a more space and energy conservative process.

**Keywords:** *nitrification, denitrification, shortcut nitrogen removal, partial nitritation/anammox, anammox, energy self-sufficient*

## 1. Introduction

Deammonification has been the cornerstone for energy conservative nitrogen removal with the goal being to make wastewater treatment plants energy self-sufficient. Deammonification (partial nitritation/anammox) consists of partial nitritation of ammonium to nitrite through aerobic ammonia-oxidizing bacteria (AerAOB), followed by subsequent removal of the remaining ammonium in combination with the formed nitrite with the help of anoxic ammonium-oxidizing bacteria (AnAOB). The competition for nitrite between AnAOB and NOB is the key challenge in deammonification technologies.(1)

Microbial growth is managed by choosing substrate levels, which through the Monod relationship determine the overall growth kinetics, hence "kinetic selection" was coined to denote growth rate manipulation.(2-4) In the case of sidestream deammonification processes, high temperature(5), free ammonia (FA) inhibition(6) in combination with low dissolved oxygen (DO) levels are the predominant mechanisms to manage NOB growth kinetics.(7) The DEMON® process has been the most widely implemented sidestream deammonification process.(8, 9) DEMON utilizes a pH-driven aeration control at a low dissolved oxygen (DO) setpoint (0.3 mg $O_2$/L) to tightly control the nitrite availability in the reactor, while maintaining high residuals of ammonium and alkalinity.(2, 10)

Mainstream conditions do not allow for complete kinetic NOB outselection due to low FA concentrations. Multiple strategies have been proposed, for example bioaugmentation with desirable organisms (e.g. sidestream AnAOB and AerAOB), and/or out-selecting of others (e.g. NOB).(2-4) This way, a maximum growth rate differential between AerAOB and NOB is created to subsequently expose them to "physical selection", washing NOB out while retaining AerAOB.(11, 12) Key to such growth rate differential are tightly controlled levels of ammonium, nitrite and DO. A high ammonium residual (2-5 mg N/L) has been found to be paramount for NOB outcompetition in all process configurations, which can be managed with advanced control strategies like ammonia versus NOx (AvN).(3, 13)

In flocculent mainstream systems, NOB are controlled based on SRT where the higher maximum growth rate for AerAOB is exploited by reducing the SRT up to the point that NOB wash out.(14) However, AnAOB intrinsically have a low growth rate (0.06 – 0.21 $d^{-1}$)(15, 16), which counteracts the SRT control required to wash out NOB in mainstream applications. Suspended growth deammonification systems in sidestream conditions generally require a total SRT of 30-45 days(7, 17) for adequate AnAOB to be present in the system. Because AnAOB prefer to grow in granules, physical selection can exploit this difference in morphology. Physical selection can be achieved based on density with hydrocyclones(12), size with screens(11, 18) or critical settling velocity in granular technologies like ANAMMOX® and ELAN®.(19, 20) Cyclones and screens are external selectors, typically on the waste activated sludge (WAS) line. The dense or big fraction (‘retained’) is sent back to the reactor from the cyclone or screen respectively, while the light or small fraction (‘rejected’) is wasted. Cyclones and screens allow for direct management of two morphologies (granules and flocs), and it has been shown for deammonification systems that the retained fraction is the smallest in sludge mass, yet the highest in AnAOB activity, and the rejected fraction the highest in mass and NOB activity.(7, 14) Physical selectors therefore allow for a more direct management of the microbial conversions and could provide more operational flexibility than feasible in biofilm technologies.

Little is known however on how physicals selectors’ activity splits on the process’ performance and how these interact with kinetic selection in full-scale conditions. While Strass WWTP successfully achieved deammonification in the side- and mainstream lines with the help of physical selectors(9, 21), this success is not guaranteed, as it results from a complex interplay of several mechanisms. Achieving deammonification, especially in the water line, is only feasible when a balance is found between kinetic selection (NOB out-selection) and physical selection (AnAOB retention). In 2014, the Ejby Mølle wastewater treatment plant in Denmark installed cyclones on the RAS line of the BNR reactor with aim to increase settleability and achieve mainstream deammonification. This concept was also combined with bioaugmentation of AnAOB from the sidestream DEMON, similar to the Strass WWTP. However, both goals were challenging due to the long SRT (~ 30 days) applied, wastewater characterization and reactor conditions. No deammonification was observed despite AnAOB retention with the cyclones and bioaugmentation.(22, 23) Some minor improvements in settleability were achieved at lower SRT, while AnAOB contribution remained questionable.(22, 23) This shows that some core understanding of the process is still lacking, despite ample literature available. Solely applying a mechanism to retain AnAOB does not guarantee AnAOB activity. Mechanistic understanding of the impact of reactor conditions and physical selection parameters is needed to define a potential operational windows of success for real-life applications.

In essence, while ample literature is available on ideal conditions to grow and retain AnAOB or out-select NOB, no work has been done on the interactions, tradeoffs and potential synergies between kinetic and physical selection. This is important because, as exemplified above, just retaining AnAOB or out-selecting NOB might not be enough to achieve mainstream deammonification. This work relies on a straightforward and easy to apply model which combines steady-state measurements from full-scale physical selectors installed at Strass WWTP with ~~a~~ straightforward (steady-state) equations describing both selection types ~~to show~~ showing how overall and specific selection efficiencies impact both sidestream and mainstream deammonification technologies. Kinetic selection is approached through a minimum Monod function, whereas physical selection was calculated based on a modified sludge washout function. This study mechanistically shows the interactions, tradeoffs and potential synergies between kinetic and physical selection for a broad range of conditions. Sensitivity analysis is provided to explore expected trends when selection changes and to provide possible operational windows where further rigorous calibration and validation or expansion of the concept can be tested on. The resulting operational window is instrumental to formulating expectations and recommendations for full-scale realization of these deammonification concepts.

## Materials & Methods

### *2.1 Model development*

Growth rates ($\mu_{AerAOB}$, $\mu_{NOB}$ and $\mu_{AnAOB}$) were estimated using minimum Monod equations corrected for decay and based on work of Stewart et al. (Eq. 1)(4):

$$\mu_{\text{organism}} = \mu_{\text{max,organism}} * f_{aer} * \min\left(\frac{S_1}{K_{S_1} + S_1}, \dots, \frac{S_n}{K_{S_n} + S_n}\right) - f_{aer} * b_{aer} - (1 - f_{aer}) * b_{an} \tag{1}$$

where $\mu_{\text{max,organism}}$ is the maximum growth rate of AerAOB, NOB or AnAOB ($d^{-1}$), $f_{aer}$ the aerobic fraction (percentage of reactor's volume that is aerated) (-), $S_n$ the concentration of substrate $n$ (mg/L; $NH_4$-N and DO for AerAOB, $NO_2$-N and DO for NOB, and $NH_4$-N and $NO_2$-N for AnAOB), $K_{S_n}$ the associated half-saturation constant (mg/L) and $b$ the decay rate ($d^{-1}$). Note that for AnAOB, the factor $f_{aer}$ was replaced by the anoxic fraction $(1 - f_{aer})$ and an anoxic decay coefficient was used. In addition, decay was only accounted for in the respective zones where growth occurred.

The washout rate of AerAOB, NOB or AnAOB ($1/SRT_{organism}$) is given by the sludge mass that is removed by sludge wasting independent of the growth rate, thus inversely proportional to the SRT (24). The external selector induced a split in biomass into a retained and rejected fraction. The retained fraction is sent back the WAS line, while the rejected fraction is wasted. The rejection mass split $f_{M,rejected}$ (%) of the external selection was defined as (Eq. 2):

$$f_{M,rejected} = \frac{X_{rejected} * Q_{rejected}}{X_{rejected} * Q_{rejected} + X_{retained} * Q_{retained}} = \frac{X_{rejected} * Q_{rejected}}{Q_{selector} * X_{selector}} \tag{2}$$

Where $X$ (kg TSS/$m^3$) is the sludge concentration and $Q$ ($m^3$/d) the flow rate of the respective fraction. The waste flow $Q_{selector}$ ($m^3$/d) from the reactor with volume $V$ ($m^3$) to the external selector will therefore have to increase depending on $f_{M,rejected}$ (%) to reach a similar SRT (d) at a certain recycle ratio (Eq. 3).

$$SRT_{system} = \frac{X_{reactor} * V}{X_{rejected} * Q_{rejected}} = \frac{X_{reactor} * V}{f_{M,rejected} * Q_{selector} * X_{selector}} = \frac{V}{(1 + R) * f_{M,rejected} * Q_{selector}} \tag{3}$$

No impact of effluent suspended solids on washout was considered. Schematic of different streams can be found in Supplemental A.

To calculate the washout rate for a specific target group of organisms (AerAOB, NOB or AnAOB), an activity balance was calculated over the external selector, which determined the activity retention efficiency $\eta$ (%). Activity retention efficiency was defined as the percentage of volumetric activity ($r_V$ , kg N/m$^3$/d) measured in the retained fraction of the external selector compared to the total volumetric activity coming in the selector (Eq. 4).

$$\eta_{organism} = \frac{r_{V,organism,retained}}{r_{V,organism,retained} + r_{V,organism,rejected}} \tag{4}$$

The retention efficiency (Eq. 4) can be inserted in the modified SRT equation (Eq. 3) to calculate the organism specific washout rate (Eq. 5):

$$\frac{1}{SRT_{organism}} = \frac{(1 - \eta_{organism})}{SRT_{system}} \tag{5}$$

The presence or absence of an organism is ultimately determined by the balance between growth of the organism and pressure applied by the washout rate, thus a net growth rate ($\mu_{net}$) can be calculated by subtracting Eq. 5 from Eq. 1.

## 2.2 <u>Determination of capacity</u>

Capacity in sidestream systems was defined as the maximum load that can be treated while retaining a 90% $NH_4^+$-N removal efficiency, which can be calculated based on the total inventory of AnAOB (Eq. 6).

$$r_{V,AnAOB} = \mu_{net,AnAOB}\left(\frac{SRT_{AnAOB}}{HRT}\right)\left(\frac{(S_o - S_{out})}{1 + \mathrm{b}_{AnAOB} * SRT_{AnAOB}}\right) \tag{6}$$

Full derivation can be found in Supplemental B. As sidestream systems are more granular in nature, capacity was not considered to be limited by sludge loading rates to the clarifiers. In mainstream, this assumption is invalid, thus the increase in capacity was approximated by the percentual difference in total SRT required.

### 2.3 *Fraction of deammonification in mainstream and minimum required AnAOB growth rate*

In mainstream deammonification, complete deammonification cannot always be achieved, therefore the degree of deammonification $f_{deam}$ (% total inorganic nitrogen, TIN) was introduced. First, a deammonification rate (in g TIN removed/d) was calculated based on an assumed $f_{deam}$ and the total daily TIN removal calculated by the product of the influent TIN concentration $S_{TIN,in}$ (g N/m$^3$), influent flow $Q_{in}$ (m$^3$/d), and removal efficiency (%) (Eq. 7):

$$r_{deam} = f_{deam} * Q_{in} * S_{\mathrm{TIN},in} * \left(1 - \frac{S_{TIN,out}}{S_{TIN,in}}\right) \tag{7}$$

The AnAOB rate (g $NH_4^+$-N/d) is calculated based on the deammonification rate, corrected for the TIN to $NH_4^+$-N conversion based on the stoichiometry of AnAOB (16) (Eq. 8). The NOB rate (**k**g TIN-N/d) was obtained as the TIN conversion rate that did not go through deammonification (Eq. 9), whereas the AerAOB rate (**k**g $NH_4^+$-N/d) was calculated as the converted TIN load that did not go to AnAOB (Eq. 10).

$$r_{AnAOB} = r_{deam} * \frac{1}{1 + 1.32} \tag{8}$$

$$r_{NOB} = Q_{in} * S_{TIN} - r_{deam} \tag{9}$$

$$r_{AerAOB} = Q_{in} * S_{TIN} - r_{AnAOB} \tag{10}$$

Note that only autotrophic metabolisms were considered to limit the number of organisms competing for nitrite. This further allowed the simulation of a “worst-case scenario” where NOB only need to compete with AnAOB for nitrite. Nitrate production and subsequent heterotrophic N removal was not considered and will require COD (present or dosed) to be removed. The AerAOB/NOB ratio was subsequently determined by dividing Eq. 10 by Eq. 9.

Last, a criterion for sufficient AnAOB growth was determined based on the calculated AnAOB rate This total rate (in kg $NH_4^+$-N/d) can be modified to a volumetric rate (in kg $NH_4^+$-N/m$^3$/d) which can subsequently be inserted into Eq. 6.

$$\mu_{min,AnAOB} = \frac{\left(1 - \frac{S_{TIN,out}}{S_{TIN,in}}\right)}{\left(\frac{SRT_{AnAOB}}{1 + SRT_{AnAOB} * b_{AnAOB}}\right)} \tag{11}$$

Full proof of Eq 11. can be found in Supplemental C.

### *2.4 Strass WWTP and physical selectors*

The strass wastewater treatment plant is a two-stage wastewater treatment facility (A/B configuration), treating 250,000 people equivalents.(21) Produced sludge was anaerobically codigested with food waste, and the filtrate was treated with a DEMON reactor (500 m$^3$).(9) In 2007, cyclones were installed in the DEMON reactors, operating at 10 m$^3$/h and 2 bar inlet pressure. In 2015, the cyclones were replaced by a rotating drum screen with a 52 µm screen size. The "B-stage" mainstream deammonification reactor had cyclones installed in 2011, operating at 20 m$^3$/h and 1.8 bar inlet pressure. The cyclone was replaced with a rotating drum screen in 2015 with a 250 µm screen size.

### *2.5 Activity tests*

Specific activity tests were performed on full-scale samples taken from the rejected and retained streams for the screens and cyclones after at least 6 months of operation of these selectors to determine the AnAOB retention efficiencies. Four tests were done in total, two from sidestream sludge (cyclone and screen) and two from the mainstream reactor (cyclone and screen), to determine the selection efficiencies. Activity tests were performed according to Wett *el al.*(25) and Sabine Marie et al.(26). Reactors were operating under steady state conditions at the time of sampling. Fresh sludge was put in a closed vessel and controlled at 20°C. Both ammonium and nitrite were spiked to 25 mg N/L. The sludge was aerated for 15 minutes prior the test to remove any COD present. Next the sludge was purged with $N_2$ gas to ensure anoxic

(DO = 0 mg/L) conditions, where after liquid samples were taken every 10 minutes for 1 hour and analyzed for ammonium and NOx. pH was controlled when necessary. The AnAOB activity was derived from the data using linear regression, fitting the linear part of the activity test. The stoichiometry of ammonium and nitrite removal was checked to be close to theoretical value of 1.32 confirming AnAOB activity rates rather than denitrification.

Ammonium determination is based on derivatization with o-phthaldialdehyde/N-acetyl-cysteine (OPA/NAC) and fluorescence measurement of the formed isoindols.[27] Nitrite and nitrate were quantified by ion pair chromatography with n-octylamine as the pairing reagent on a C18 HPLC column and UV-detection at 210 nm according to Doblander and Lackner.[28] TSS was measured according to the standard methods.(29)

As a proxy for the AnAOB abundance and hence activity, heme c protein measurements were performed based on the method by Sabine Marie *et al.*(26) First, 1.5 mL sludge was centrifuged for 3 minutes at 5000 rpm and the supernatant was discarded. The pellet was incubated at 100°C with 1.5 mL concentrated NaOH for 2 minutes. The mixture was centrifuged again at 5000 rpm for 3 min. After centrifugation, 100 μ L Na-dithionite was added and absorbance was measured at 535, 550, 570 nm. The reduced heme compound showed it sharpest peak at 550 nm. Calibration was performed with the 1-heme cytochrome c from horse heart. Heme c protein levels in biomass were found to be strongly positively correlated with sludge-specific AnAOB rates.(26)

### *2.6 Bioaugmentation of sidestream AerAOB and AnAOB into the mainstream system*

The full-scale mainstream deammonification reactor was bioaugmented with sidestream sludge. The bioaugmentation rate was calculated as a percentage of the organism's maximum growth rate for this simulation exercise. A bioaugmentation rate of 25% and 17% was assumed for AerAOB and AnAOB, given that 25% of the sidestream reactor's volume gets seeded into mainstream on a weekly basis based on operation data from Strass and former pilot work(14). Sidestream AerAOB have been observed to lose some of their activity when introduced in the mainstream reactor. A review on bioaugmentation of autotropic nitrifiers by Parker and Wanner (30) concluded that temperature shock was a major culprit in loss in AerAOB activity. Wett, Jimenez (31) estimated that 30-50% of the community is active depending on the ammonium residual, while Head and Oleszkiewicz (32) determined that AerAOB lost 58% activity when a temperature shock of 10°C was induced. Note that bioaugmentation is an exchange of mass, hence the specific activity of the seeded AerAOB will always be greater than prior to bioaugmentation.(31, 33) For this reason, AerAOB bioaugmentation was assumed to be 50% efficient, reducing the AerAOB bioaugmentation rate to 12.5%. No loss in activity for AnAOB was assumed, as no studies quantifying the activity loss of AnAOB from bioaugmentation from sidestream to mainstream are published to the authors' knowledge. The bioaugmentation increased the maximum growth rate for AerAOB with 12% (from 0.9 to 1.01 $d^{-1}$) and for AnAOB with 17% (from 0.100 to 0.117 $d^{-1}$). All scenarios were bioaugmented unless otherwise stated.

### 2.7 Model implementation and kinetic parameters

The model output was calculated with Microsoft Excel. The model was thereafter exported to R to allow for 2 or more independent variables to be varied at the same time. Steady-state was assumed for all calculations and model outputs.

Maximum growth rates, half saturation constants, and yields for AerAOB and NOB were taken from the calibrated model in Al-Omari, Wett (34), and can be found in supplemental D. The half saturation indices for AnAOB were modified to 0.5 mg N/L for both ammonium and nitrite based on experimental data (data not shown). Kinetic parameters were considered equal for sidestream and mainstream with exception of $K_o$, which was 0.4 and 0.14 mg $O_2$/L for AerAOB and NOB respectively for mainstream. The $K_o$ values for AerAOB and NOB under sidestream conditions were 0.25 and 0.5 mg $O_2$/L, respectively.

## 3. Results & Discussion

### *3.1 Sidestream deammonification*

At Strass WWTP in Austria, the deammonification (DEMON) process was used to treat sidestream water high in ammonium and was operated at a low DO setpoint based on pH (0.3 mg $O_2$/L) (9). NOB were metabolically out-selected (i.e. net growth rate was 0 $d^{-1}$) because of aeration control used in DEMON, represented by as a low anoxic fraction (33%), high free ammonia (1.33 mg N/L), and high temperature (30 °C). This was achieved with the higher $K_O$ for NOB than AerAOB within the model (0.5 vs. 0.25 mg $O_2$/L) as confirmed by a pervious study by Al-Omari, Wett (34) Therefore, only the growth rate for AerAOB and AnAOB were shown in figure 1A. The favorable conditions within the sidestream reactor, i.e. 100 mg $NH_4$-N/L residual ammonium allowed for high growth rates for AnAOB (0.032 $d^{-1}$), leading to a high retention potential for AnAOB (Figure 1B).

#### 3.1.1 Impact of cyclones

Cyclones installed on the sidestream achieved a rejection mass split of 80%. Based on steady-state activity balance performed at full-scale, an 88% retention efficiency was obtained for AnAOB (Table 1). The cyclones were replaced with rotating drum screen with 52 µm screen size (270 mesh) in 2015 and a 70% rejection mass split and obtained a steady-state retention efficiency for AnAOB of 91%. While the enrichment of AnAOB was larger for the cyclone (30x) than for the screen (24x), the screen achieved a higher overall retention efficiency. The screen's smaller rejection mass split meant that more sludge was returned to the reactor, resulting in more AnAOB mass retained. Visually, the retained streams of screens and sieves contained larger aggregates than the rejected flows (Figure F1-F2). The selective retention of AnAOB decreased their washout pressure (Figure 1A), thus increasing their net growth rate (Figure 1B). ~~At~~ Given a total SRT of 30 days, which is the typical operating SRT for a DEMON system(9), ~~The~~ the effective AnAOB-specific total SRT increased from 30 days without external selector to 313 and 334 days for the cyclone and screen respectively. This led to a total capacity of 1.04 kg N/$m^3$/d (cyclone) and 1.16

kg N/m$^3$/d (screen) for cyclone and screen respectively given a 30-day total system SRT, 2 day HRT, an incoming ammonium concentration of 1000 mg N/L, and a 90% N-removal efficiency (Figure 1C).

### 3.1.2 Switch and impact of rotating drum screen

The screen's small edge in AnAOB retention efficiency (3%) increased the treatment capacity of the DEMON reactor with 12%. This allowed for a more intensified operation at a smaller footprint. Alternatively, the SRT could be dropped from 30 days to 22.6 days to match the screen's AnAOB-specific SRT with the cyclone's while still providing the same 90% removal efficiency at similar loads. The excess biomass can be seeded to a mainstream reactor for enhanced mainstream deammonification, without sacrificing filtrate treatment efficiency. The washout SRT for AerAOB was calculated to be 18 days (Figure 1B), thus preemptive measures should be taken if one wants to retain a healthy AerAOB rate and avoid excess washout. In addition, to manage the mass load to the screens, lamella clarifiers, which select of critical settling velocity, were installed upstream to the screen to minimize the number of flocs sent to the latter. Flocs are compressible and therefore limit the effectiveness of the screen on AnAOB retention. A longer retention time on the screen would be required for the same retention efficiency, limiting the mass load that can be applied.

### 3.1.3 Implications of enhanced AnAOB retention

Some filtrate streams originating from thermally hydrolyzed (THP) sludge like at the Blue Plains Advanced Wastewater treatment plant in Washington, DC, may have inhibitory compounds in the matrix that limit AnAOB growth.(35) For this reason, more AnAOB retention would be increasingly important to safeguard the DEMON's performance when inhibitory compounds are present. For this reason, a screen might be advantageous over a cyclone because of the increased AnAOB retention it provides. Zhang, De Clippeleir (35) were able to successfully operate a sidestream SBR with THP filtrate at similar loading rates to conventional anaerobic digestion filtrate when AnAOB were selectively retained with a screen and DO was increased to 1 mg $O_2$/L to offset colloid-induced mass transfer limitations. However, with no THP at Strass WWTP, the extent of overcoming inhibition was not testable.

Rotating drum screens are, unlike hydrocyclones, not dependent on a specific (constant) flow to achieve the desired separation. The separation is achieved gravitationally and controlled by the liquid level rather than nozzle pressure. This makes screens more energy conservative (<0.001 kWh/$m^3$) than cyclones (0.01-0.1 kWh/$m^3$). The ability to operate at differential flows allowed DEMON to operate as a continuous flow system rather than as a sequencing batch reactor (SBR). The continuous DEMON reactor eliminated the need for a settling and decanting phase, saving one hour out of a typical six-hour SBR cycle, thus lowering the HRT by 17%. This effectively increased the DEMON system's capacity by an additional 17% over the SBR with screen installed, netting a total of 29% over a traditional DEMON reactor with cyclones. The ability to operate at a range of flows which the screen provides offers great perspective for practice as it makes the DEMON process more versatile and robust.

The capacity increase that was achieved with implementation of the continuous DEMON reactor was tested with a stress test and presented in Figure 2. The loading rate was ramped up from 1 to 1.4 kg N/$m^3$/d in a 21-day period, where after no more filtrate was available to increase the load further. Note that the average filtrate concentration was 1860 ± 50 mg $NH_4^+$-N/L, significantly higher than typical filtrate (~1000 mg $NH_4^+$-N/L), because of co-digestion of food waste in the anaerobic digesters. During the ramp-up, both ammonium and TIN removal percentages remained stable at 94 ± 1% and 89 ± 1%, respectively. The theoretically calculated maximum load for the Strass sidestream reactor, given the increased loads due to food waste codigestion, was 2.8 kg N/$m^3$/d, which was a magnitude greater than the loading rate applied (0.5 – 1 kg N/$m^3$/d) in practice for filtrate treatment technologies. During the ramp-up test, the concentration of the filtrate remained the same, and the increase in loading was achieved by gradually increasing the flow from 216 to 311 $m^3$/d, resulting in an HRT decrease from 1.85 to 1.3 days. This shorter HRT was not incorporated in the capacity calculation Eq. 6., which assumed a design HRT of 2 days. Filtrate concentration generally does not change much, given a stable anaerobic digestion performance. An increase in loading will therefore typically be accompanied by a decrease in HRT. As capacity negatively correlated with HRT based on Eq. 6, the true capacity will be lower than the theoretically calculated value based on the initial design. In addition, DEMON reactors operating in SBR mode will have additional loading constraints when HRT, which is managed with volume exchange ratios, is pushed too short. Enough time for settling is required as the sludge bed needs to be settled sufficiently during decant phase. This potentially puts potential constraints on the MLSS levels in the reactor. Further practical tests will be required to pinpoint what the limiting factor in DEMON installations will be. Despite these hurdles, switching from cyclone to continuous screen operation should achieve an overall 29% net capacity increase.

### *3.2 Mainstream deammonification*

#### 3.2.1 NOB outselection

In mainstream deammonification systems, NOB are not fully kinetically outcompeted and thus need to be considered. Full deammonification may not be realistic given the low substrate concentrations and impact of available carbon for denitrifies.(36) In addition, no AerAOB/NOB activity ratios have been reported above 2-2.5(13, 36), indicating that complete NOB outselection might not be feasible. A more realistic approach was to assume an in-situ observed AerAOB/NOB activity rate ratio, which correlates with a percentage of deammonification in the reactor. Han, Vlaeminck (14) showed that mainstream deammonification was achieved at an AerAOB/NOB ratio of 2. This optimal ratio was adapted within model to reflect a threshold for adequate NOB outselection. Given the operational conditions of the mainstream biological nutrient removal reactor at Blue Plains AWTP (N load = 34065 kg N/$m^3$/d, influent TN = 30 mg N/L, and TIN removal = 92%), a 68% deammonification contribution was found to correspond to the previously determined optimal AerAOB/NOB ratio of 2 (Figure 3D). In addition, heterotrophic denitrifiers were not considered to allow for the worst-case scenario where nitrite not used by AnAOB will be consumed by NOB.

Increasing the ammonium or DO concentration~~s~~ was beneficial towards kinetically outcompeting NOB independent of the SRT strategy applied, because the AerAOB/NOB ratio increased (Figure 3A/B).

High ammonium residuals lowered the dependency of the AerAOB/NOB ratio on low nitrite availability in the aerobic zone. Operation at ammonium residuals greater than 1.5 mg N/L at a DO of 1.5 mg $O_2$/L allowed for an AerAOB/NOB ratio greater than 2 at nitrite residuals of 0.5-0.75 mg N/L (Figure 3A). Similarly, operation at a high DO setpoint (> 1.5 mg $O_2$/L) is beneficial when an ammonium residual of 2 mg N/L was maintained, because of the decreased dependency on tight nitrite management (Figure 3B). High ammonium has been widely cited in literature to be imperative for mainstream deammonification.(34, 37, 38) This study further confirms the that high DO is required for flocculent deammonification systems as postulated by Regmi, Miller (38)

The main goal of kinetic selection was to create a gap in washout SRT between AerAOB and NOB that can be exploited by sludge wasting. Figure 3E shows the maximum aerobic SRT (AerSRT) that can be applied to ensure an AerAOB/NOB ratio of 2 in function of the nitrite residual in the aerobic zone for three different ammonium residuals. The higher the maximum AerSRT, the bigger the eligible AerSRT range. At 0.75 mg $NO_2^-$-N/L residual, the maximum SRT was 4, 6, and 10 for 0.5, 1, and 2 mg $NH_4^+$-N/L respectively. This decreased to 2, 3, and 4 at 2 mg $NO_2^-$-N/L for the same respective ammonium residuals. This maximum AerSRT increased with decreasing nitrite concentration in the aerobic zone. However, the impact of ammonium residual became more significant at lower nitrite concentrations, stressing the importance of managing AerAOB growth.

The best kinetic strategy for deammonification was to shift the focus from creating conditions that hampered NOB growth to creating an environment that favored AerAOB growth. Ammonium and DO are easy to control in a deammonification system with control strategies like ammonium-based aeration control (ABAC)(39) or ammonium vs NOx (AvN) control.(3, 13) Smart design of the aeration control, like more rapid intermittent aeration (in time or space) as opposed to longer periods, might allow for better management of nitrite.(37)

### 3.2.2 AnAOB retention

Next to NOB outselection, AnAOB activity is crucial for the success of mainstream deammonification. The AnAOB in the system should be able to cope with the ammonium loading rate they receive based on the deammonification fraction determined above. This can be approximated by requiring a minimal AnAOB net growth rate in the system to meet a certain TIN removal percentage (Figure 3F), which is dependent on the AnAOB-specific anoxic SRT (AnSRT). The latter was assumed to be 30 days, which is considered the design operational SRT for many sidestream deammonification systems, thus a relevant target for the AnSRT under mainstream conditions. The minimum net growth rate for AnAOB to maintain a 94% TIN removal was 0.04 $d^{-1}$, based on the conditions found at Blue Plains AWTP (see section 3.2.1) (Figure 3F).

The physical selection of AnAOB with screen and cyclone was significantly less efficient in mainstream compared to sidestream deammonification (Table 1). Furthermore, the difference in retention efficiency between screen and cyclone was much more pronounced (72 vs 42% respectively). The lower retention efficiencies were most likely the result of a mainstream system being a less ideal environment for AnAOB growth. Mainstream would have a higher percentage of flocs relative to granules, leading to a difference in overall sludge characteristics. Picture of mainstream sludge passed through the screen can be found in Figure F3. In addition, larger nozzle size and screen pore size (250 μm) were required to deal with larger debris found in the mainstream reactor and reduce maintenance. Sidestream, having lower flow rates and less debris, allowed for the installation of a smaller pore size as the risk for clogging was lower. Increasing the retention efficiency or changing the mass split of the external selectors would require changing the selector’s specifications, such as decreasing the screen’s pore size or installing a smaller nozzle on the cyclone. However, this would also induce challenges in maintenance because more pressure is applied on these selectors. The competitive edge of the screen is dependent on the AnAOB growth within the system, which was limited by nitrite availability. Indeed, as nitrite availability decreased in the reactor, the difference in minimum AnSRT for AnAOB between screen and cyclone increased, indicating that the retention rather than growth was more dominant (Table 2).

Growth of AnAOB was equally dependent on the ammonium and nitrite levels in the anoxic zone, meaning that the lowest substrate determined the growth rate. Given the 30-day AnAOB-specific AnSRT, a minimum ammonium or nitrite in the anoxic zone of 0.83 mg N/L would be required to meet the 70% deammonification minimum as determined above (Figure 3C). While higher nitrite residuals would benefit AnAOB growth, they hampered NOB outselection. Maximizing the specific retention of AnAOB (and therefore maximizing its specific SRT) should be prioritized to offset the reduced growth rate. Without any form of AnAOB retention mechanism, the minimum required AnSRT for AnAOB was 48 days for an average nitrite residual of 0.75 mg N/L (Figure 4A). While this nitrite residual was ideal for NOB outselection (Figure 4B), the anoxic SRT was too high to be practical. When the nitrite residual was increased, the required SRT became more manageable (35 and 22.5 days for 1 and 2 mg NO2-N/L respectively, Figure 4C/E), but potential for NOB outselection was sacrificed. Physical selectors would therefore be crucial in mainstream application to make simultaneous AnAOB retention and NOB outselection possible. While only two selector types with associated AnAOB activity retentions have been performed within this paper, Figure 4A/C/E presents the full sensitivity of the required SRT over the entire range of AnAOB retention efficiencies. This allows plants to narrow down the operational window based on their measurements, thus assessing the feasibility of mainstream deammonification ~~to be calculated~~ for different AnAOB retention efficiencies. Activity measurement would be most suitable as they reflect the actual capability of AnAOB mediated N removal, rather than the mere presence of the organism. Future studies future studies should further detail separation efficiency, backed up with molecular characterization (qPCR) and more heme measurement, as both have been found to correlate very well with AnAOB abundance (26).

In addition, more research is needed to optimize the effect of screen size/operation of cyclone on AnAOB retention at certain mixed liquor concentrations. It is known that microbial (sub)communities preferentially grow in small or large flocs depending on the type of organism or operational condition. The migration dynamics of some species, if any, would affect retention and should be investigated in the future.

In addition, new installations should be encouraged to acquire retention efficiencies to finetune the framework. Finally, plants are encouraged to transfer the concept to their needs and model calibration capabilities (40), possibly incorporating more complex model structures to increase the accuracy of predictions.

Bioaugmentation of sidestream sludge (AerAOB + AnAOB) into mainstream further increased the feasibility as it significantly reduces the minimum total SRT (80, 55, and 36% for a 0.75, 1, and 2 mg $NO_2$-N/L residual respectively), thus if the plant has a DEMON sidestream facility, bioaugmentation into the mainstream reactor should be a priority to aid mainstream deammonification as this is a typically low-cost capital investment (Table 2). However, bioaugmentation is not a sole recipe for success as it does not per se lead to successful deammonification.(23) The full non-bioaugmented scenario can be found in Supplemental E. The higher retention efficiency obtained by the screen also directly translated into a higher AnAOB biomass fraction in the reactor. Given the total SRT reported in Table 2, screen would have 1.8-1.9x the AnAOB biomass in the reactor if both the cyclone and screen scenario would operate at similar SRT. Alternatively, this meant that the screen allowed operation at total SRTs 1.8-1.9x lower than the cyclone, while having the performance. This shows that, like sidestream, switching from a cyclone to screen reduces the footprint of the mainstream reactor by 80-90% based on the increase in total SRT, thus intensifying the process by the same amount.

At a nitrite residual of 0.75 mg N/L, the minimum anoxic SRT to achieve 70% deammonification dropped from 28 to 13 days when the cyclone was swapped out with a screen. Once more nitrite was introduced into the system, the required minimum anoxic SRT dropped further as the net AnAOB growth rate increased (Table 2). Increased nitrite residuals also enhanced NOB growth, requiring a more precise and aggressive aerobic SRT control. Maximizing the retention efficiency of AnAOB therefore ensures less dependency on stringent intermittent aeration control for nitrite management as it allows for operation at lower nitrite residuals. Screen allowed for the most flexible operation. The efficacy of the external selector is also further influenced by the growth of AnAOB. With increasing nitrite residual, the impact of AnAOB retention decreased as indicated by the decreasing slope in Figure 4A to 4E. In addition, the operational SRT range in Table 2 was increasingly narrow the more AnAOB growth was assumed. This means that capacity limited systems with limited growth will benefit most from the effect of an external selector. Systems with adequate capacity will be able to more loosely control their nitrite residuals.

### 3.2.3 Excess NOB retention risk

The main function of physical selectors is to retain granular AnAOB. However, some AerAOB and NOB are inadvertently retained due to inefficiencies in the separation step. As long as NOB and AerAOB were retained in a similar way, the NOB outselection strategy was still driven by aeration strategy and aerobic SRT control as discussed in 3.2.1 and 3.2.2 (Figure 4). If more NOB were retained compared to AerAOB, the washout pressure on NOB decreased, counteracting the internal nitrite management. Figure 4B/D/F shows the operational SRT zone where the AerAOB/NOB ratio is equal to or exceeds 2 assuming an AerAOB retention efficiency of 30%. Higher NOB retention efficiencies led to an increased demand for tight SRT control as the operational window decreased. Furthermore, if NOB were retained twice as efficiently as AerAOB, no shortcut nitrogen removal would be possible as the aerobic SRT dropped below 2 days. According to the findings of Han, Vlaeminck (14), a 30% NOB retention efficiency was deemed the maximum allowable before performance started to deteriorate.

NOB have been reported to stick or migrate to the AnAOB granule's surface when sufficient washout pressure was supplied (14), linking the AnAOB retention with NOB retention. This could further be managed by operating at slightly higher SRT to avoid migration to the biofilm or apply a harsher shear on the granules in the external selector, which might reduce the AnAOB retention efficiency. AnAOB retention was still key as this also allowed operation at lower nitrite residual, thus aiding the kinetic outselection of NOB rather than a pure SRT driven one.

## 4. Conclusions

In conclusion, the balance between kinetic and physical selection is key to both sidestream and mainstream deammonification technologies. This study allowed to make the following conclusions:

- Screens had superior AnAOB retention over cyclones, this led to a 29% increase in treatment capacity for sidestream and 80-90% increase for mainstream deammonification.
- Superior retention with screens was more emphasized in mainstream compared to sidestream application due to the lower growth rates under these conditions with AnAOB retention efficiencies of 42 and 72% for the cyclone and screen, respectively.
- Maximization of AnAOB retention directly enhanced the success for mainstream deammonification as it decreased its dependency on nitrite residuals.
- Selective NOB retention compared to AerAOB retention decreases the chance for NOB out-selection when using external selectors and increased the importance of tight aerobic SRT control.
- Overall, this paper shows that operation and choice of external selector directly determine the operational strategy and footprint needed to achieve mainstream deammonification. The higher the AnAOB retention and NOB out-selection via the physical selector, the lower the need for tight aeration control.

**Acknowledgments**

This work was supported by the Water Environment and Research Foundation [grant number U1R14] and the National Science Foundation GOALI [grant number 1512667].

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## Figures and Tables

**Table 1.** AnAOB maximum activity (batch tests, 20°C), abundance (heme), and mass rejection efficiencies performed on rejected and retained fractions of the screens and cyclones installed on the full-scale sidestream and mainstream deammonification reactors at the wastewater treatment plant in Strass, Austria.

| ***Sidestream deammonification*** | | **Cyclone** | | **Screen** | |
|---|---|---|---|---|---|
| *Rejected* | **Specific AnAOB value** | 0.5 | *mg $NH_4^+$-N/g VSS/h* | 5 | *mAU/g TSS* |
| | **Mass split** | 80% | | 70% | |
| | **Volumetric AnAOB value** | 0.4 | *mg N/L/h* | 3.5 | *mAU* |
| *Retained* | **Specific activity** | 15 | *mg N/g VSS/h* | 122 | *mAU/g TSS* |
| | **Mass split** | 20% | | 30% | |
| | **Volumetric activity** | 3 | *mg N/L/h* | 82 | *mAu* |
| | **AnAOB enrichment** | 30x | | 24x | |
| | **AnAOB retention efficiency** | 88% | | 91% | |
| ***Mainstream deammonification*** | | **Cyclone** | | **Screen** | |
| *Rejected* | **Specific activity** | 5.5 | *mAu/g TSS* | 4 | *mAU/g TSS* |
| | **Mass split** | 80% | | 70% | |
| | **Volumetric activity** | 4.4 | *mAu* | 2.8 | *mAU* |
| *Retained* | **Specific activity** | 16 | *mAu/g TSS* | 24.5 | *mAU/g TSS* |
| | **Mass split** | 20% | | 30% | |
| | **Volumetric activity** | 3.2 | *mAu* | 7.35 | *mAU* |
| | **AnAOB enrichment** | 2.9x | | 6.1x | |
| | **AnAOB retention efficiency** | 42% | | 72% | |

**Table 2.** SRT required for a successful mainstream deammonification system given the imposed criteria of an AerAOB/NOB ratio > 2, an AnAOB net growth rate of >0.04 $d^{-1}$, at 20°C. The AerAOB and NOB retention efficiencies were considered equal at 30%.

| $NO_2^-$ (mg N/L) | AerSRT (d) | | Minimum AnSRT (d) | | Minimum total SRT(d) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Cyclone* | *Screen* | *Cyclone* | | *Screen* | |
| | min | max | | | min | max | min | max |
| **No bioaugmentation from sidestream** | | | | | | | | |
| **0.75** | 2.8 | 4.8 | 54.9 | 26.5 | 57.7 | 59.7 | 60.5 | 64.5 |
| **1** | 2.4 | 3.3 | 33.6 | 16.2 | 22.7 | 24.3 | 12.2 | 13.8 |
| **2** | 1.8 | 2 | 18.9 | 9.1 | 15 | 15.5 | 8.1 | 8.6 |
| **With bioaugmentation from sidestream** | | | | | | | | |
| **0.75** | 2.8 | 6.4 | 27.9 | 13.5 | 30.7 | 34.3 | 16.3 | 19.9 |
| **1** | 2.4 | 4 | 20.3 | 9.8 | 22.7 | 24.3 | 12.2 | 13.8 |
| **2** | 1.8 | 2.3 | 13.2 | 6.3 | 15 | 15.5 | 8.1 | 8.6 |

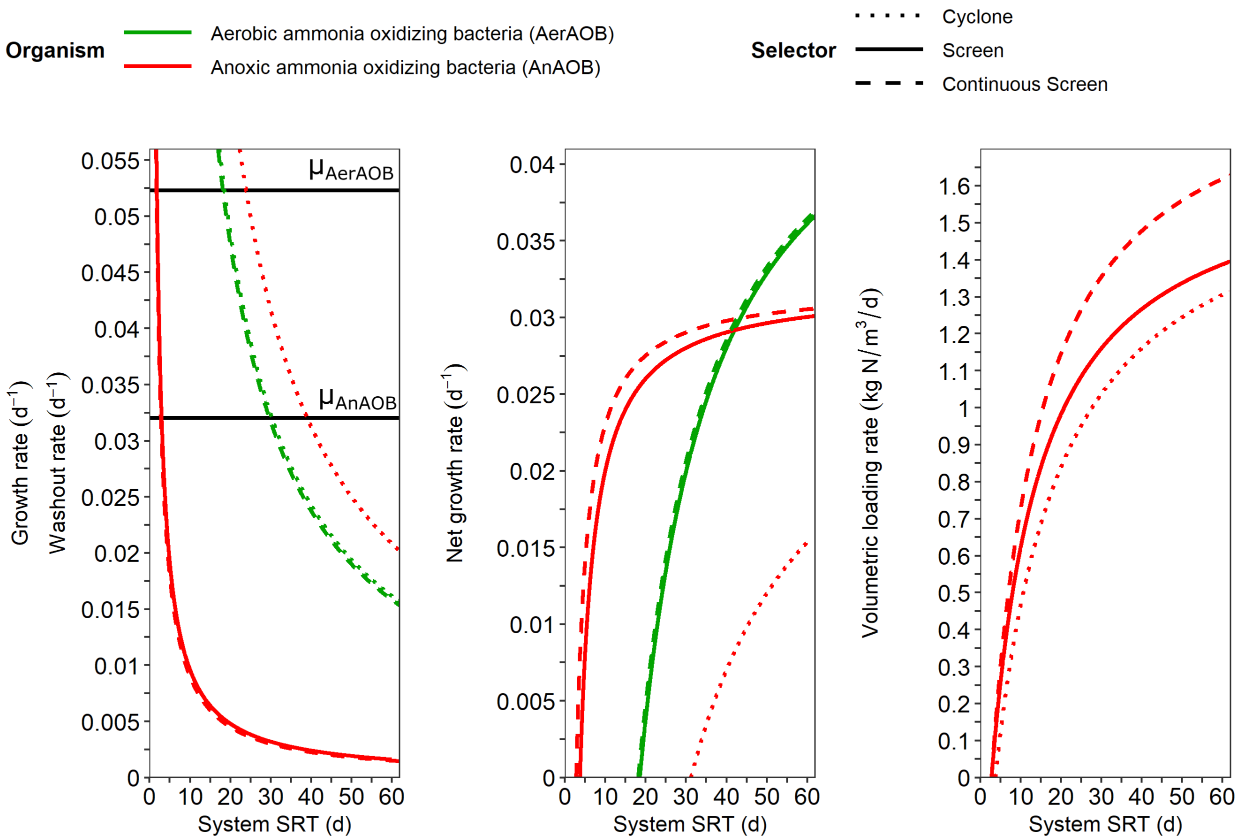


**Figure 1.** **(A)** Growth and washout rate of AerAOB and AnAOB under sidestream conditions ($NH_4^+$ = 100 mg N/L, $NO_2^-$ = 1 mg N/L, DO = 0.3 mg $O_2$/L) with cyclones ($f_{M,rejected}$ = 0.8; $\eta_{AnAOB}$ = 88%) and screen ($f_{M,rejected}$ = 0.7; $\eta_{AnAOB}$ = 91%). NOB were metabolically outselected (negative growth rate). **(B)** Selection efficiency achieved at given growth and outselection rates. **(C)** Volumetric N removal rate by AnAOB in sidestream deammonification with and without external selector based on a 2 day HRT, an incoming ammonium concentration of 1000 mg N/L, and a 90% N-removal rate

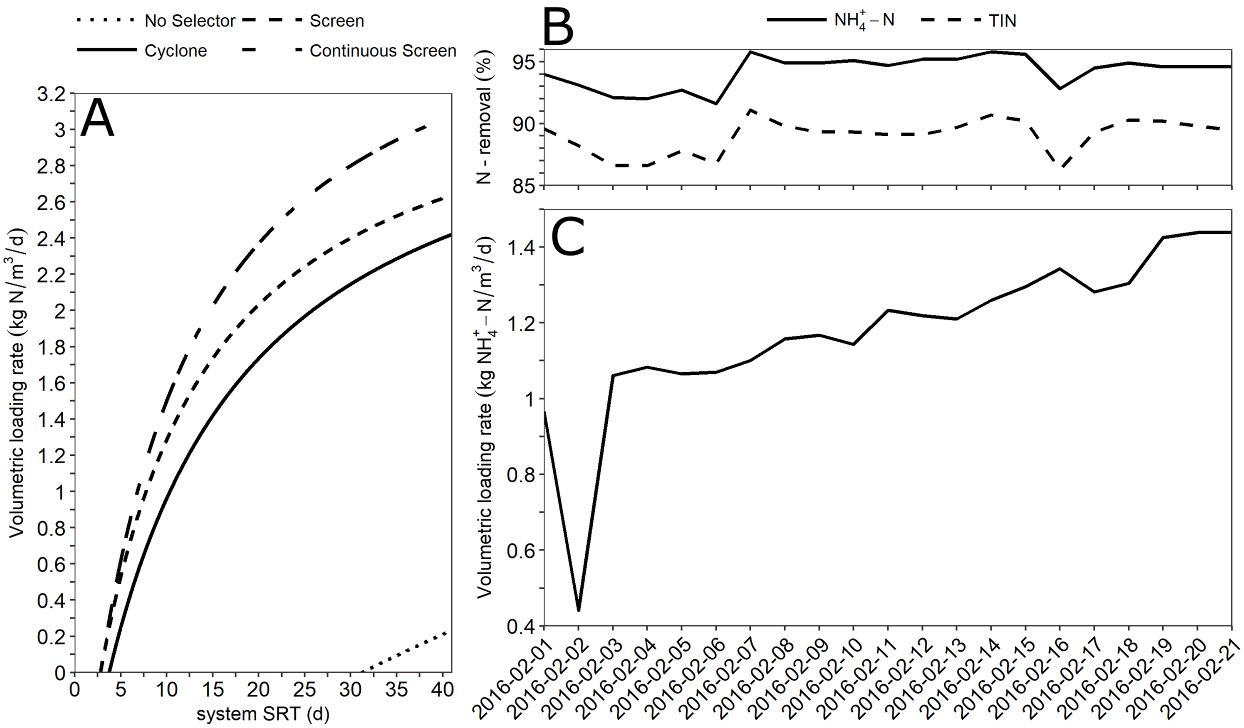


**Figure 2. (A)** Stress test performed on continuous sidestream DEMON reactor with screen installed at the wastewater treatment plant in Strass, Austria to evaluate its maximum capacity. (B) The ammonium and TIN removal percentage during the ramp-up. **(C)** The loading rate over a three-week period achieved by increasing flow rate (average influent $NH_4^+$ was 1859 ± 53 mg N/L).

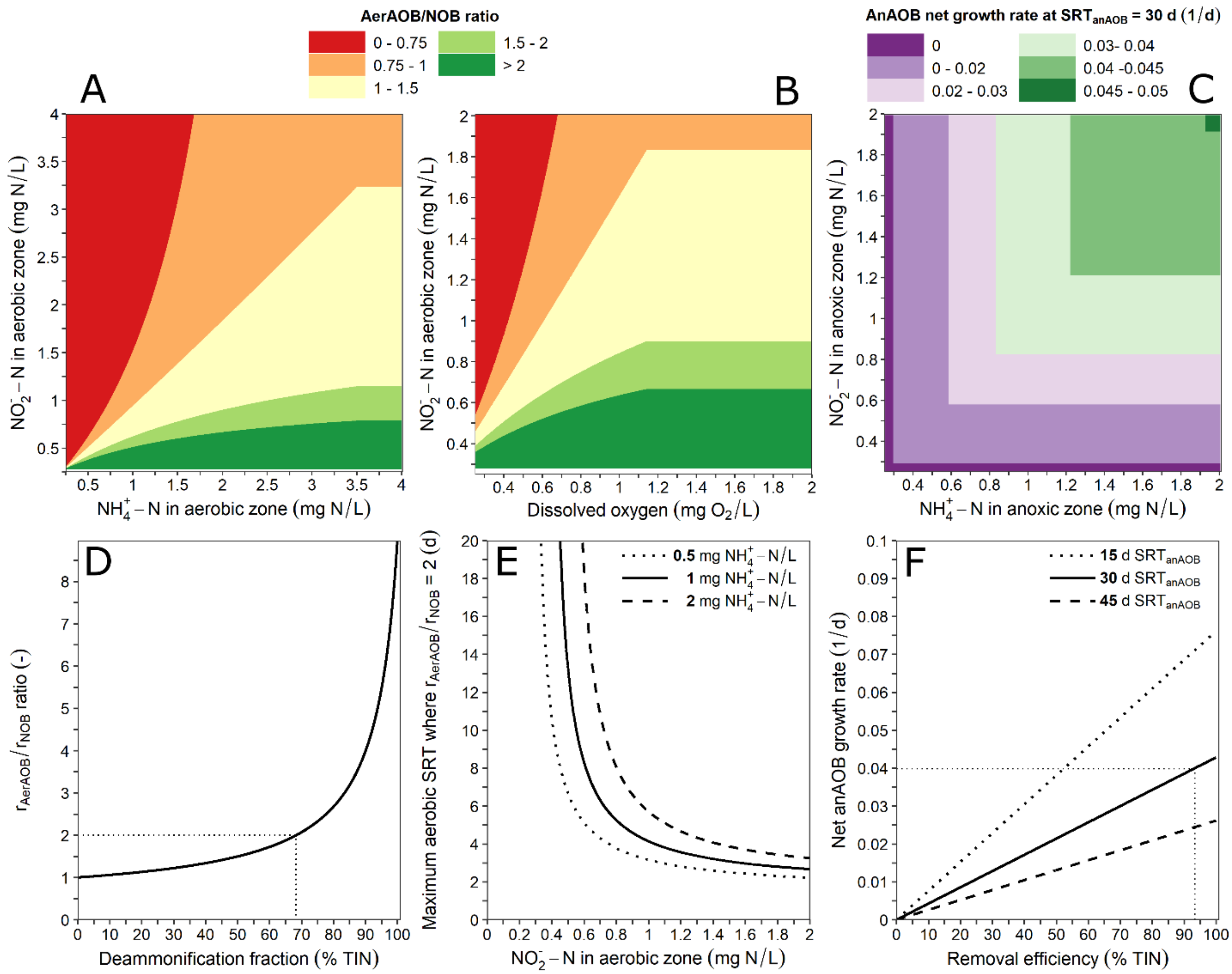


**Figure 3. (A/B)** Ratio of intrinsic AerAOB over NOB removal rates as a function of the average concentrations in the reactor's aerobic zones of ammonium and nitrite (A; DO = 1.5 mg $O_2$/L) and DO and nitrite (B; ammonium = 2 mg N/L). **(C)** The net growth rate of AnAOB given an AnAOB-specific SRT of 30 days. **(D)** Relationship between the percentage of TIN removed through deammonification and the AerAOB/NOB rates ratio in the system. **(E)** Minimum net AnAOB growth rate required for adequate deammonification given a certain TIN removal for three different AnAOB specific SRT. **(F)** Maximum aerobic SRT where the ratio of AerAOB over NOB removal rates equaled 2 in function of the average nitrite and ammonium in the aerobic zone.

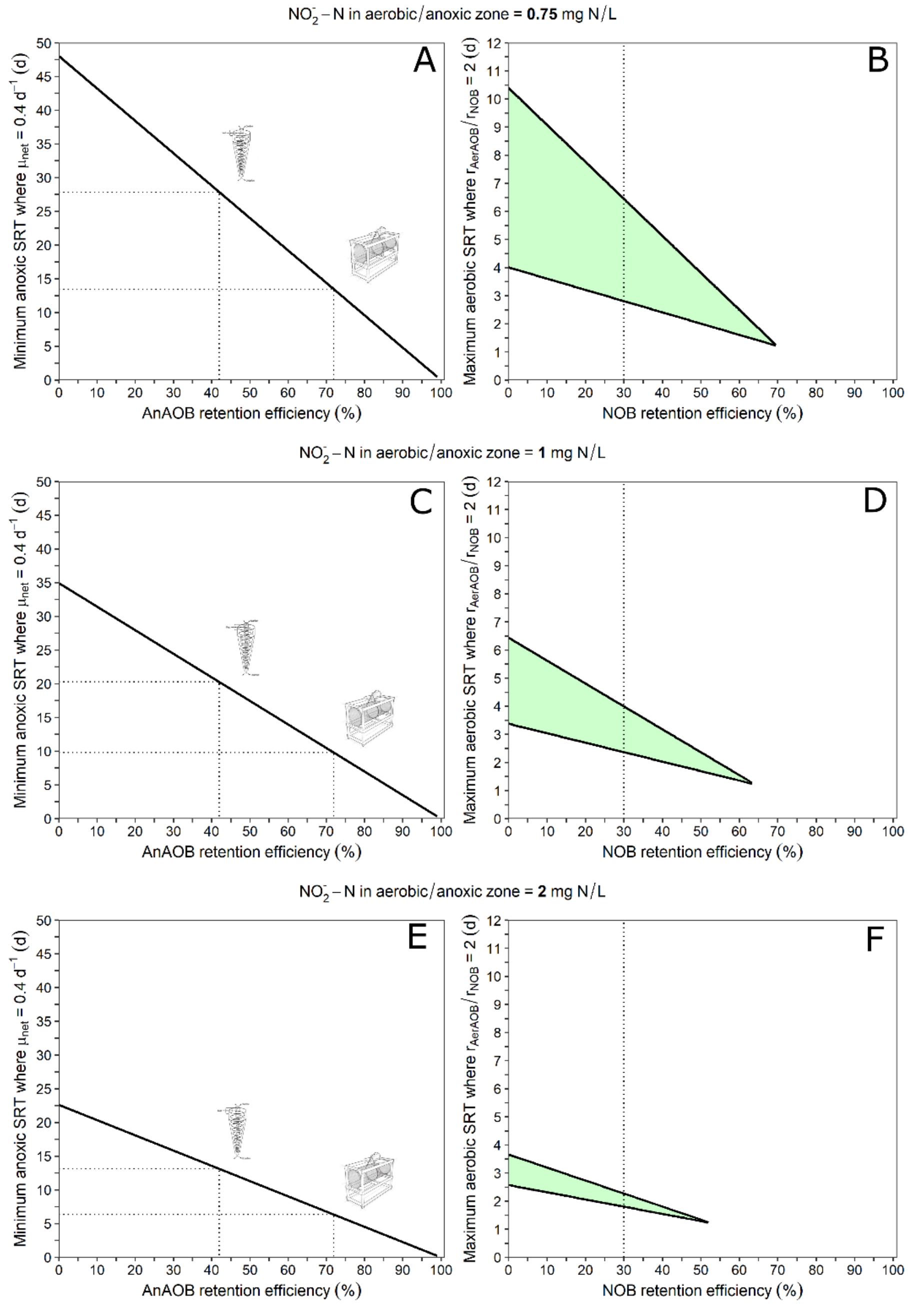


**Figure 4. (A/C/E)** Minimum anoxic SRT required to meet the minimum 0.04 $d^{-1}$ AnAOB net growth rate criterion in function of the AnAOB retention efficiency for an average nitrite residual of 0.75 **(A)**, 1 **(C)**, and 2 **(E)** mg N/L in the anoxic zone. **(B/D/F)** The spread of aerobic SRT where can be operated given an AerAOB/NOB ratio above or equal 2 as a function of the NOB retention efficiency for an average nitrite residual of 0.75 **(B)**, 1 **(D)**, and 2 **(F)** mg N/L in the anoxic zone. The upper boundary of the zone was given by the aerobic SRT where the rate ratio is 2, while the lower boundary is given by the washout SRT of NOB.