

## Sidestream Deammonification Startup - Process Controls

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### ABSTRACT

In May 2015, Alexandria Renew Enterprises (AlexRenew), a 54 MGD advanced resource recovery facility, started up a centrate pretreatment (CPT) system. This system utilizes the sidestream deammonification process (DEMON<sup>®</sup>) to remove nitrogen in the centrate that results from dewatering anaerobically-digested sludge. During the startup period, the ammonia removal rate averaged 89% and TN removal averaged 77%. Major process control approaches were evaluated from various perspectives, including flow and solids distribution, microbial activities, particle size distribution etc. Molecular microbiology analyses were carried out to improve the understanding of microbial population transitions at different operating conditions.

Hydrocyclones are effective in retaining anammox bacteria within the system, with average capture of flow, TSS, aerobic and anammox fraction through hydrocyclones at 8%, 44%, 10% and 85%, respectively. Hydrocyclone operation also has a big impact on the granule size distribution. Smaller granules (125-250  $\mu\text{m}$  and 250-500  $\mu\text{m}$ ) are not captured as effectively as the larger granules (>500  $\mu\text{m}$ ). Short hydrocyclone operation may cause excessive growth of heterotrophic bacteria. Despite periodical disruptions from dirty centrate discharge, NOB is not very active as nitrate production ratio in the CPT system generally is close to the 11% stoichiometric nitrate production from deammonification reaction. This proves that the intermittent on/off aeration control is effective in suppressing NOBs.

**Key Words:** sidestream deammonification, DEMON, startup, process control, hydrocyclone

### INTRODUCTION

In wastewater treatment plants, anaerobic digesters produce a significant source of ammonia which contains up to 15-25% of the total nitrogen load to the plant. Dewatering operation on anaerobic digester sludge and supernatant recycles the highly-concentrated ammonia stream back to the liquid stream of the plant. Conventionally ammonia is removed to nitrite and nitrate via autotrophic nitrification and subsequently reduced to nitrogen gas through heterotrophic denitrification. This route demands a lot of energy and supplemental carbon. In contrast, it is cost effective to remove ammonia from dewatered centrate using combined partial nitrification/deammonification processes (sidestream deammonification). Sidestream deammonification processes removes ammonia by partially nitrifying ammonia to nitrite with anammox bacteria converting nitrite and remaining ammonia to nitrogen gas using nitrite as the electron acceptor. It provides approximately 63% reduction in oxygen, nearly 100% in supplemental carbon, 80% less biomass production and normally no additional alkalinity requirement.

In May 2015 Alexandria Renew Enterprises started up a sidestream DEMON® process. During startup phase, a number of challenges were encountered, including centrate quality, nitrite spike, micronutrients deficiency, equipment and probe control issues, etc. These challenges were gradually tackled and alleviated through operational and process control modification which facilitated microbial adaption to the operating environment. After one and a half years of operation, the process reached 100% centrate loading ( $0.5 \text{ kg N/m}^3/\text{d}$ ).

This paper focused on the process performance and impact of key process control measures including hydrocyclones, pH based on/off aeration etc. Major process control approaches were evaluated from various perspectives, including flow and solids distribution, microbial activities, particle size distribution etc. Molecular microbiology analyses were carried out to improve the understanding of microbial population transitions at different operating conditions. This paper details the startup and optimization experiences from process control perspective and provides additional process and operational learnings to the full-scale sidestream deammonification process.

## METHODOLOGY

### CPT Process Overview

The overall process flow diagram for the CPT facility is presented in Figure 1. Dewatering centrate containing a high concentration of ammonia is discharged into a sequencing batch reactor (SBR), where partial nitrification and deammonification take place. The reactor aeration is cycled on and off to create transient anoxia and the cycle time is controlled to maintain the pH in the reactor within a narrow band. During the air on period, the pH in the reactor drops as the nitrification process consumes alkalinity. Once the pH reaches a low level the aeration is turned off and the centrate, which is rich in ammonia and contains alkalinity, is added to raise the pH in the reactor. Once the pH reaches an upper limit or centrate feeding reaches preset time, the aeration turns back on.

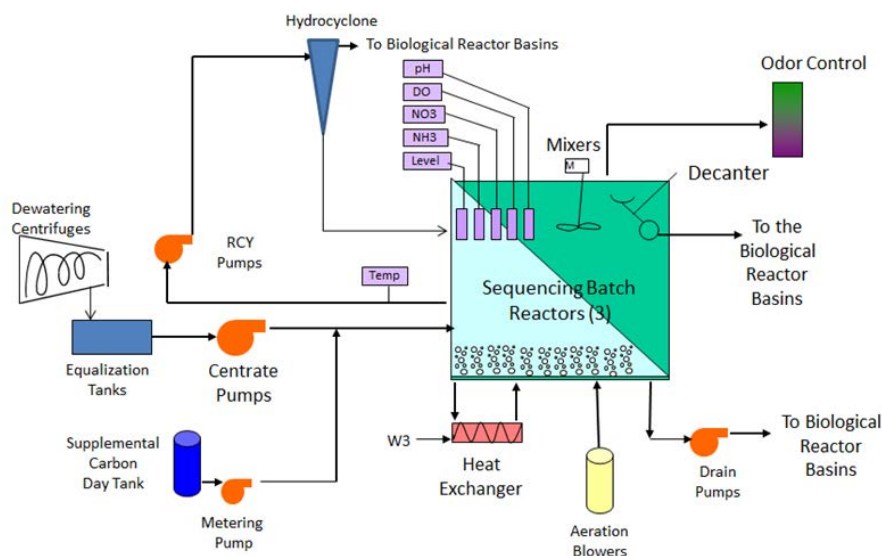


Figure 1: Process Flow Diagram for CPT Process

The reactor contents are pumped through hydrocyclones to separate anammox granules from the mixed liquor. Underflow from the hydrocyclones containing concentrated anammox granules is sent back to the reactor while overflow is discharged into the mainstream biological reactor basins (BRBs). Excess flow beyond the hydrocyclone overflow discharge is removed via a decanting step. Dissolved oxygen (DO), ammonia and nitrate are monitored using online analyzers. The CPT facility is equipped with heat exchangers to cool down the reactor should the need arise in summer. The target temperature in the reactor is 80-95 °F.

### **Sample Collection, DNA Extraction, qPCR**

Biomass from the CPT reactors were periodically collected and stored at -20°C until subsequent DNA extraction process. DNA was extracted using Qiacube with the DNeasy mini kit (Qiagen, CA) and extracted DNA was tested by Nanodrop Lite spectrophotometer (Thermo Scientific, WI) to ensure good DNA quality for the following molecular experiments. Quantitative polymerase chain reaction (qPCR) assays, using an iQ5 realtime PCR thermal cycler (BioRad Laboratories, Hercules, CA) with primers and Taqman probe, were performed to evaluate the microbial concentration for AOB, *Nitrospira* spp. related NOB, *Nitrobacter* spp. related NOB, and ANAMMOX. Standard curves for qPCR assays were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. Melting curve for each qPCR assay was analyzed to confirm primer specificity.

### **Particle Size Distribution**

Particle Size Distribution through sieving analyses is utilized to track the particle size variations during the operation. To obtain particle size distribution, uniformly mixed samples were spread onto a stacked sieve set of various mesh sizes, including 500 µm, 250 µm, 125 µm and 63 µm from top to bottom. Permeate is collected on a catch pan stacked at the bottom of the sieve set. Total suspended solids (TSS) and volatile suspended solids (VSS) analyses are performed on retentate at various mesh sizes and permeate. Fractions of retentate of different sizes and permeate are calculated and incorporated into particle size distribution profiles.

### **Anammox Activity**

Anammox activity is defined as the nitrogen removal rate, sum of  $\text{NH}_4\text{-N}$  and  $\text{NO}_2\text{-N}$ . Since mid-2016, anammox activities were periodically measured in batch tests to monitor the nitrogen removal performance of anammox bacteria in the CPT system. 6 L of well-mixed test solution was taken directly from CPT reactor which normally contains  $\text{NH}_4\text{-N}$  greater than 100 mg N/L.  $\text{NO}_2\text{-N}$  supplied as  $\text{NaNO}_2$  was spiked into the test solution to reach a target concentration of 20 mg  $\text{NO}_2\text{-N/L}$ . Consumption rates of  $\text{NH}_4\text{-N}$  and  $\text{NO}_2\text{-N}$  were calculated by linear regression of grab sample measurements of the batch tests. Sampling intervals varied between 5 to 20 minutes depending on the reaction rates.

## **RESULTS**

### **Nitrogen Removal Performance**

It took almost 1.5 years for the CPT system to reach and steadily process 100% centrate loading (0.5 kg N/m<sup>3</sup>/d). Average ammonia and total nitrogen (TN) removal ratio are 89% and 77% respectively and nitrate production ratio is 12% since process startup. Process influent properties and average concentration of major reactor constituents are summarized in Table 1.

Table 1: Influent and Effluent Characteristics

	Influent	Reactor/Effluent
Ammonia (mg N/L)	1200	130
sCOD (mg/L)	360	-
Alkalinity (mg/L)	4000	320
Nitrite (mg N/L)	-	5
Nitrate (mg N/L)	-	135
Loading (kg N/m <sup>3</sup> /d)	0.5 (100% centrate production)	

During initial process startup, a number of issues were identified, including poor centrate quality, lack of micronutrients, over-aeration, hydrocyclone operations, etc. Nitrite concentration control has been a major challenge during the reactor startup. After anammox bacteria was seeded to the reactor on 5/4/2015, within a few days ammonia oxidizing bacteria (AOB) and anammox bacteria quickly recovered their activities despite months of idle time during harvest, storage and shipment. Three weeks later it was discovered that high solids containing centrate generated during centrifuge startup/shutdown period caused inhibitory effect on both AOB and anammox as evidenced by lowered ammonia (below 85%) and TN (below 75%) removal ratios. Therefore, operational controls were implemented to divert dirty centrate away from the CPT system. However, 2 months' later reactor nitrite spiked higher and reactor performance turned worse even at ammonia loading below 0.15 kg N/m<sup>3</sup>/d which later found out to be lack of micronutrients. Since the end of July 2015, micronutrients were dosed into the reactor which allowed the reactor to quickly lower reactor nitrite concentration and increase ammonia loading. In early September 2015, reactor nitrite spiked again possibly due to over-aeration. As a result, ammonia loading was lowered again to stabilize nitrite. Since then ammonia loading ramp-up speed has been controlled very slow to allow the microbes to gradually adapt to any change in operation conditions. In November 2016, influent ammonia loading reached design target of 0.5 kg N/m<sup>3</sup>/d (100% centrate production) and can reliably process at this rate since then.

### Effect of Hydrocyclone on Flow and Microbial Activity and Solids Capture

The DEMON<sup>®</sup> process utilizes hydrocyclones to retain anammox granules within the reactor and waste excess or unwanted species including ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and heterotrophs out of the system. The performance of the hydrocyclones is critical to the success of the DEMON<sup>®</sup> process. Evaluations were carried out to measure the capture efficiencies of flow, total suspended solids (TSS), aerobic and anammox activities through the hydrocyclones. Samples periodically taken from the reactor mixed liquor, hydrocyclone overflow and underflow were analyzed for TSS and oxygen uptake rates. Flow diversion in the hydrocyclones was determined through mass and flow balance. Figure 2 summarizes the effect of hydrocyclones on the capture of flow, solids and aerobic and anammox activities. As seen in the figure, an average of 8% of flow was captured by the underflow of

hydrocyclones and 92% was discharged out of the system through hydrocyclone overflow. An average of 44% of TSS was captured in underflow and 56% ended in overflow. About 10% of aerobic activity was retained in the underflow and the rest is discharged along with the overflow. Around 85% of the anammox activity primarily stayed in the underflow and 15% went out with the overflow. These results confirmed that hydrocyclones are generally effective in retaining anammox within the system and a lot of aerobic microbial fractions were discharged out which should contain great amount of AOBs.

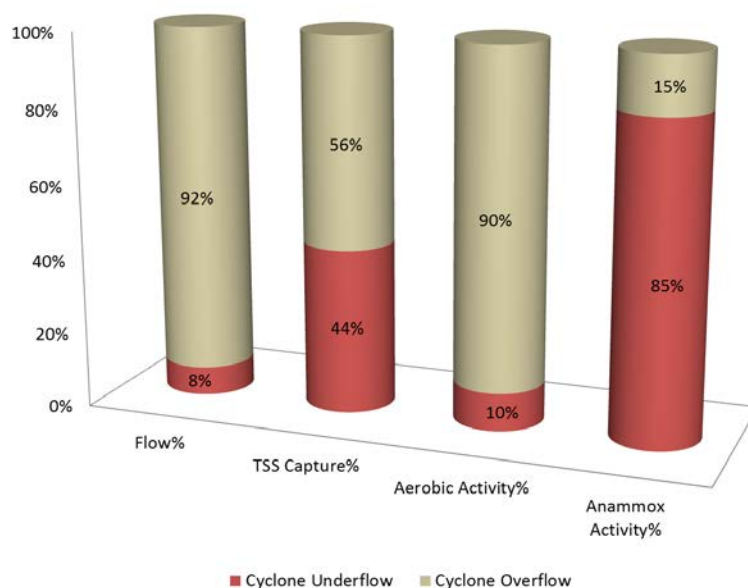


Figure 2: Hydrocyclone Capture Efficiency on Flow, TSS, and Microbial Activities

### Effect of Hydrocyclone on Particle Size Distribution

Impact of the hydrocyclone on the particle size distribution was also evaluated. Figure 3 showed the distribution of particle sizes between hydrocyclone overflow and underflow in a sample dated on 5/9/17. 83% of the solids in hydrocyclone overflow are less than 125  $\mu\text{m}$  (68% at 63-125  $\mu\text{m}$  and 15% less than 63  $\mu\text{m}$ ) representing mostly flocculent sludge, while 91% of the solids in hydrocyclone underflow are greater than 500  $\mu\text{m}$  representing mostly big granules. This demonstrated effective capture of large granules (>500  $\mu\text{m}$ ) by hydrocyclones underflow and flocculent sludge by hydrocyclone overflow. However, smaller granules (125-250  $\mu\text{m}$  and 250-500  $\mu\text{m}$ ) do not seem to be captured as effectively as the larger granules (>500  $\mu\text{m}$ ). One possible reason for the low separation efficiency on smaller particles is low cyclone operating pressure, slightly above 15 psi, as opposed to design required 23.5 psi due to trash accumulation in the CPT reactor. Therefore if hydrocyclone pressure cannot be controlled well for some reason, larger granules are desired in the system to avoid being washed out.

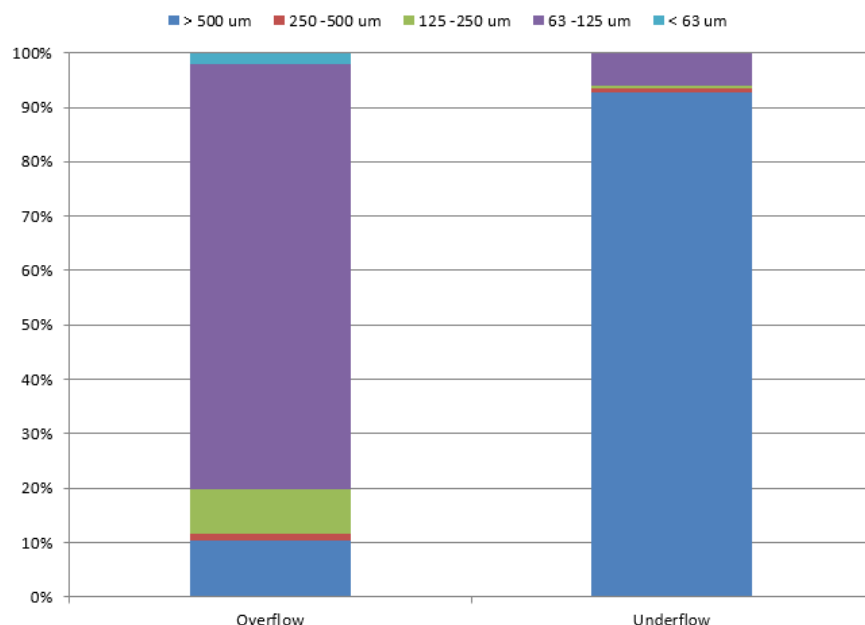


Figure 3: Particle Size Distribution of CPT Hydrocyclone Overflow and Underflow

Particle size distribution in the mixed liquor of CPT reactor was also monitored during the operation (Figure 4). It is discovered that hydrocyclone operation has a big impact on the granule size of the reactor solids. Hydrocyclone is utilized to retain anammox and waste unwanted flocculent sludge. The longer hydrocyclone operates; the more CPT reactor mixed liquor is cycled through the hydrocyclones and the more hydrocyclone overflow is wasted out of the system. Figure 5 below shows the profile of hydrocyclone operation hour setpoint and hydrocyclone overflow daily waste flow. Due to CPT reactor trash accumulation, hydrocyclone operation pressure was not maintained well, therefore hydrocyclone operation hours are not linearly correlated with the hydrocyclone daily waste overflow. Between April and October of 2016, hydrocyclone operation hours remained constant 360 mins per batch. Fractions of granule sizes of 500  $\mu\text{m}$  and above, 250-500  $\mu\text{m}$ , and 125-250  $\mu\text{m}$  average 7%, 32% and 17% respectively. Later hydrocyclone operation hour was reduced to evaluate its impact on granule size (Figure 5). As noticed in Figure 4, granules grew bigger with average fractions changed to 46%, 27% and 8% for granule sizes of 500  $\mu\text{m}$  and above, 250-500  $\mu\text{m}$ , and 125-250  $\mu\text{m}$  respectively. This confirms that hydrocyclone operation affects granule size and larger granule tends to grow with shorter hydrocyclone operation hours.

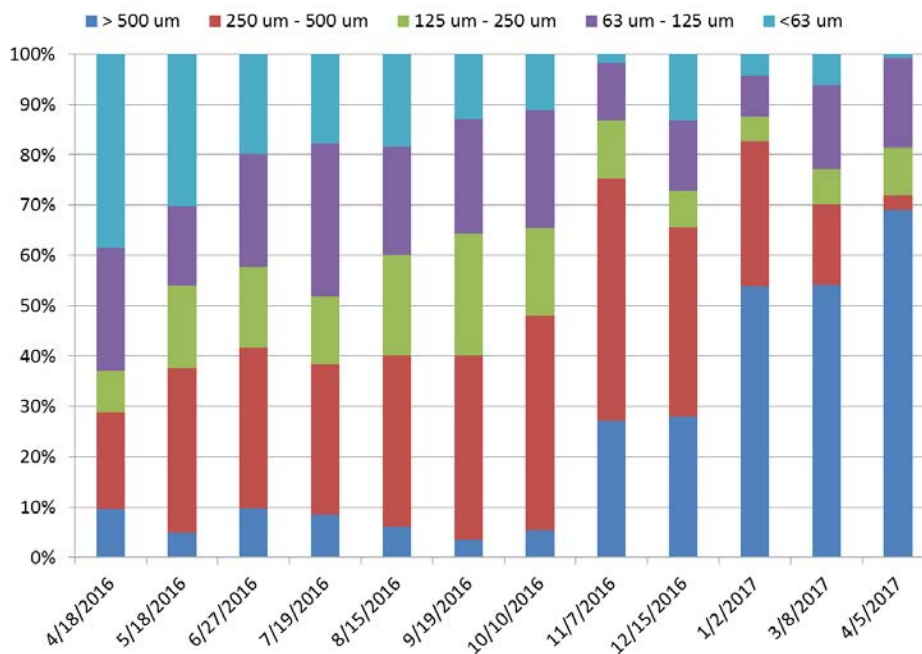


Figure 4: Particle Size Distribution of CPT Reactor Mixed Liquor

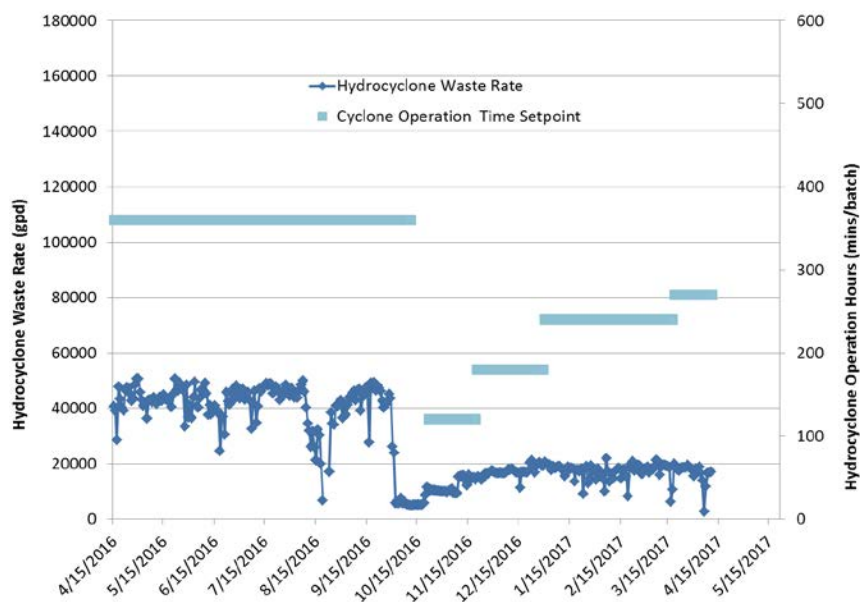
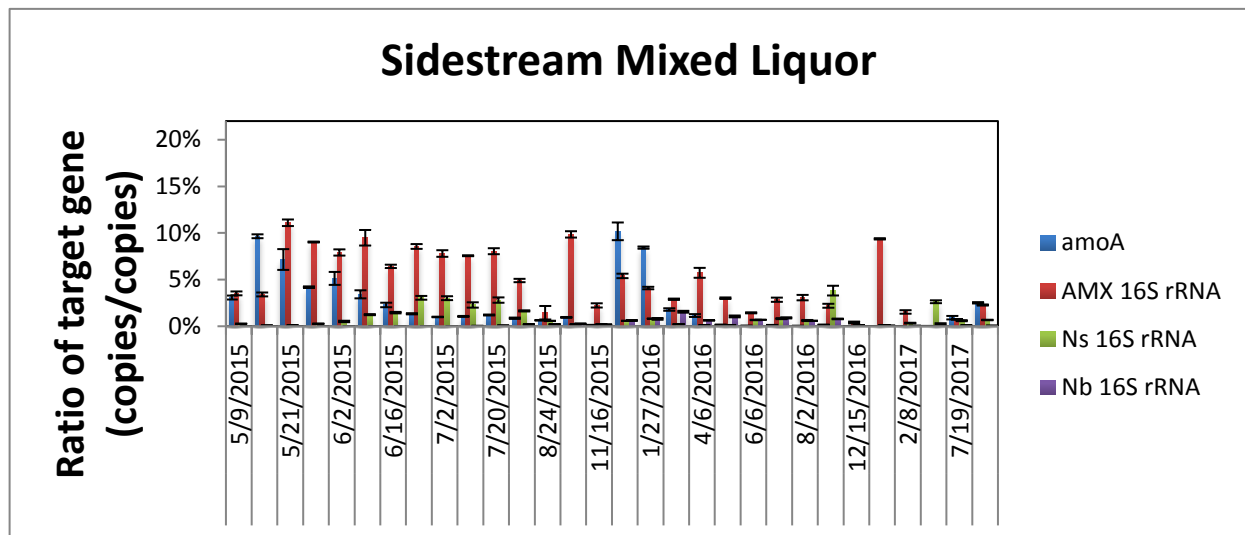


Figure 5: Hydrocyclone Operation Hours and Overflow Waste Rates

### Microbial Population Dynamics

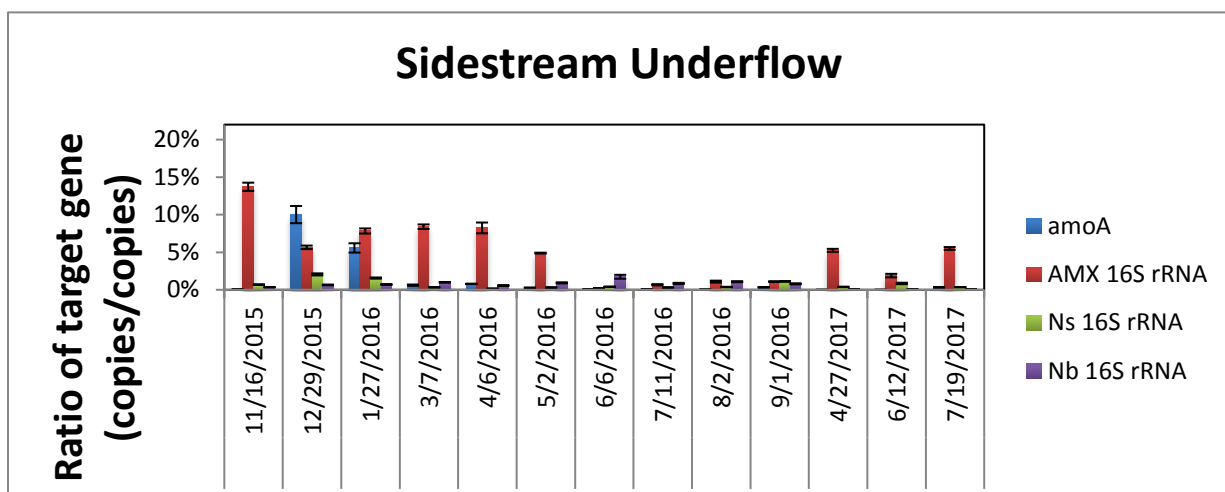
qPCR analyses were conducted to quantify individual members of functional bacterial groups (AOB, anammox bacteria and NOB) during the DEMON® startup phase. As shown in Figure

6a below, Anammox bacteria was the dominant bacteria in sidestream mixed liquor. During initial reactor startup, AOB was present in high fraction and NOB was low. After June 2015, AOB decreased to less than half of the initial fraction along with quick accumulation of *Nitrospira* spp. This coincided with the period of dirty centrate entering the reactor and micronutrient deficiencies, which explained higher nitrite and nitrate production in this period and possibly impaired activity of anammox due to competition from NOBs. After micronutrient addition, the sample on 9/29/15 showed low NOB presence. Limited fractions of *Nitrospira* spp. was observed in hydrocyclone underflow (Figure 6b), which could explain the existence of *Nitrospira* spp. in mixed liquor. It is not clear though what role the micronutrients played in flushing out NOBs because the AOB fraction did not change after micronutrients addition and anammox population continuously increased. In early 2016, AOB gained back its population, but decreased again in March and April and reduced to very low amount in summer 2016. It should be noted that starting April 2016, hydrocyclone overflow was reduced therefore CPT reactor MLSS increased greatly and contained more flocculent sludge. It is suspected that low AOB amount was resulted from the competition of NOBs as seen from persistent existence of both *Nitrospira* spp. and *Nitrobacter* spp. The fraction of anammox also decreased compared to 2015, which may be a result of the competition from increased presence of heterotrophs along with the increased flocculent sludge.

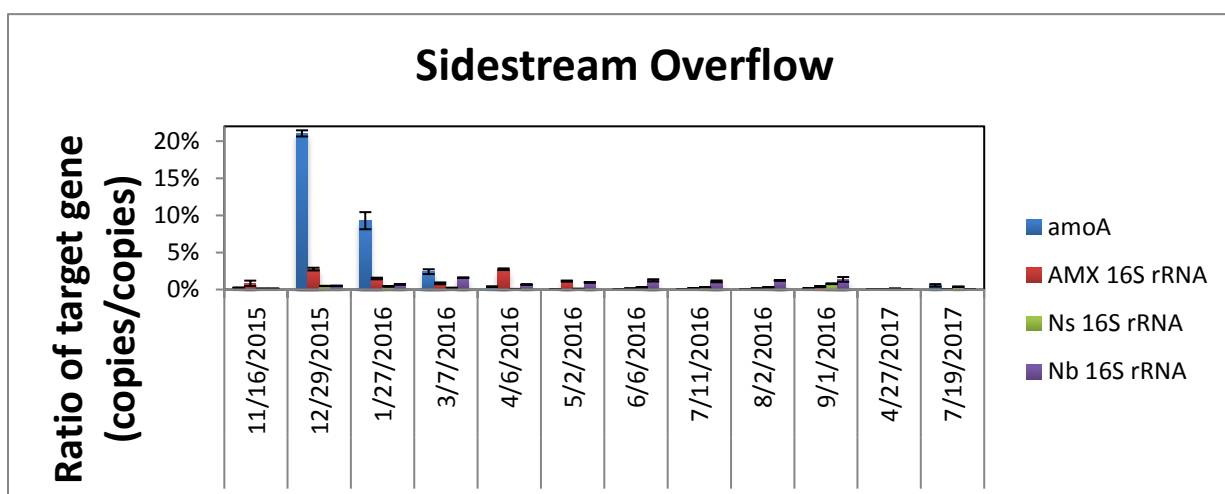


a)





b)



c)

Figure 6: Relative concentrations of AOB, NOB and AMX in a) mixed liquor, b) underflow, c) overflow. Results represent average and standard deviation of triplicate measurements.

### Anammox Activities

The anammox activity test was periodically carried out in batch reactors using fresh biomass withdrawn from the CPT reactor. As seen in Figure 7, anammox activities in the CPT reactor increase over time. Our measured anammox activities varied increased from approximately 300 to 1400 mg N/L/d between 1 to 2 years of operation. Mass specific anammox activities increase from 6 to 12 mg N/g VSS/hr. The average observed growth rate ( $\mu_{obs}$ ) of anammox is estimated from the change of anammox activities to be 0.0058 d<sup>-1</sup>, and maximum specific growth rate is 0.02 d<sup>-1</sup> using linear regression model with logarithmic transformation.

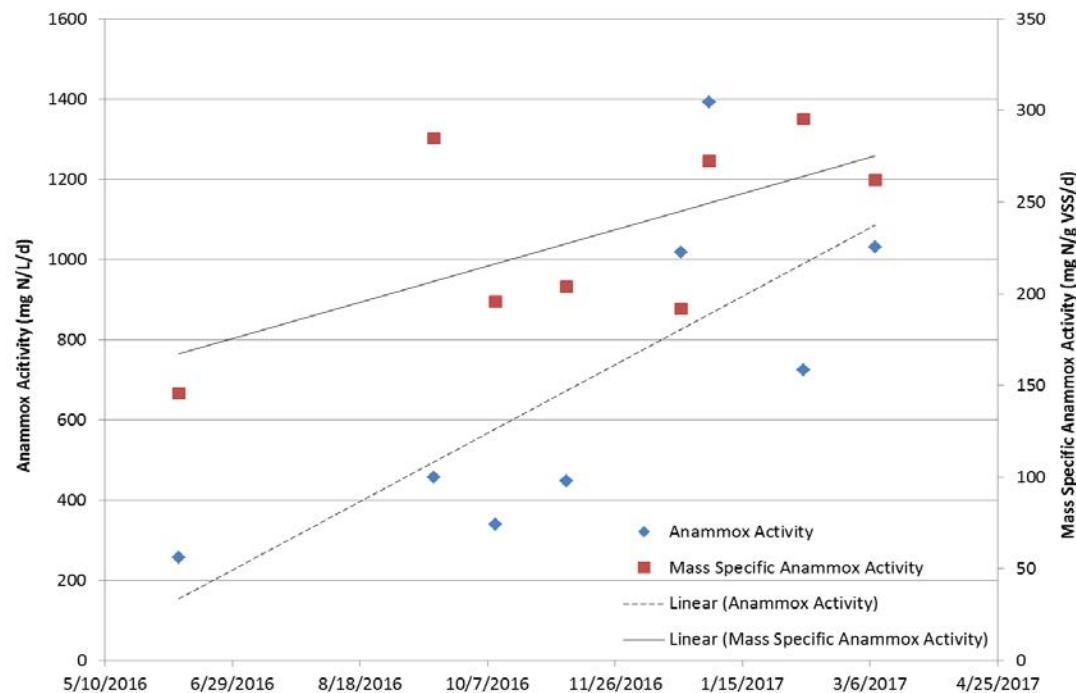


Figure 7: Anammox activities in CPT

## DISCUSSION

### Startup and long-term operation

Two major process control measures, hydrocyclone and intermittent on/off aeration control, are utilized in the CPT DEMON<sup>®</sup> process to grow and maintain desired microbial population in the system, AOB and anammox. Hydrocyclone is effective in retaining anammox granules in the system and hydrocyclone operation also affects granule size distribution. These findings are in general agreement with limited literature report. Strass wastewater treatment plant (WWTP) reported a 44 times higher measured activity in the cyclone underflow than in the overflow after 40 days of operation hydrocyclone operation. Biowin modeling simulations indicated an increase of anammox fraction from 8% to 15% in MLSS and AOB portion remained constant at 5% due to the operation of hydrocyclones (Wett et al., 2010a). Larger granules are beneficial for anammox to compete with NOBs as it shields anammox better from oxygen penetration compared to small granules (Vlaeminck et al., 2010). However *Nitrospira* spp. were also reported commonly co-existence with anammox bacteria in both granular and biofilm systems. Under low dissolved oxygen ( $< 0.5$  mg- $O_2$ /L) and long SRT (40 days) condition, *Nitrospira* spp. were more competitive than *Nitrobacter* spp. (Liu and Wang, 2013).

In our CPT system, as hydrocyclone operation hours decreased, granules grew bigger with better ammonia and TN removal and CPT reactor nitrite concentration control became easier. After hydrocyclone operation reduction, decrease of anammox bacteria was observed along with

increase of MLSS in sidestream. Less wasted sludge resulted in more organic matter in sidestream, which could enhance the accumulation of heterotrophs. However, hydrocyclone operation hours cannot be decreased extensively because reduced hydrocyclone overflow waste would lead to increased SRT for flocculent sludge and may cause excessive growth of unwanted competing species.

Anammox growth rates have been reported in a range of 0.05-0.2 d<sup>-1</sup> (Strous et al., 1999). Compared to these reported values, the estimated anammox growth rate in our CPT system is low. The anammox activities measured in our CPT system are also low compared to some activity data reported from Strass WWTP, an anammox activity of 15.9 mgN/gTSS/hr prior to hydrocyclone operation and 28.6 mgN/gTSS/hr after hydrocyclone operation (Wett et al., 2010b). One possible reason for the slow growth of anammox is the competition from heterotrophic denitrifiers due to poorly-controlled centrate quality. Frequent interruption of centrifuge operation brought dirty centrate containing high solids/heterotrophic bacteria to the CPT reactor. Excess carbon associated with the solids also fostered the growth of heterotrophic denitrifiers. Although heterotrophic denitrifiers are not wanted due to their competition with anammox, their presence can help reduce the nitrite and nitrate concentration in the reactor and increase TN removal. As seen in Figure 5, as hydrocyclone operation hours reduced in April 2016, hydrocyclone overflow decreased which caused the increase of reactor mixed liquor TSS immediately. Concurrently CPT reactor nitrite concentration reliably dropped below 5 mg/L despite process upset periods. These observations matched with the decreased anammox fraction observed in the period, which is believed to be associated with increased presence of heterotrophs.

### **Anammox activity and TIN removal efficiency**

Even though percentage of anammox bacteria in mix liquor decreased after less sludge was wasted (Figure 6a), total nitrogen removals were still over 80% and higher than that of previous period. It was mainly contributed by enhanced anammox activities (Figure 7). Mass specific anammox activities went up during sidestream start up, and meanwhile MLSS increased significantly, which led to the high removal of total nitrogen.

Reduced anammox bacteria were monitored in the overflow, showing small size granular biomass in the overflow contained limited amount of anammox bacteria. Thus anammox bacteria mainly stayed in larger size granule and were recycled into system. On the other hand, decreased anammox bacteria fractions were observed in underflow until April 2017, which was probably caused by the overgrowth of heterotrophic bacteria.

### **NOB Discussion**

Although present in significant amount in the system, NOB is not very active as nitrate production ratio in the CPT system generally is close to the 11% stoichiometric nitrate production from deammonification reaction. This proves that the intermittent on/off aeration control is effective in suppressing NOBs. As an intermediate of nitrification, hydroxylamine is accumulated during intermittent aeration condition, which could inhibit *Nitrospira spp.* It was reported that among three different size of granular sludge (<200 µm, 200 µm - 400 µm and >400 µm), *Nitrospira spp.* had a higher fraction in moderate-sized (200 µm - 400 µm) granular sludge and a lowest fraction in large-sized granular sludge. In this case, *Nitrospira spp.* fraction declined since July 2015, showing the successful out-selection by recycling larger granule size back into sidestream (Figure 6a). However limited *Nitrospira spp.* were still

detected in mixed liquor. There are two main sources contributing, one is the recycled sludge in underflow contained *Nitrospira spp.* (Figure 6b), and the other one is the self-growing of *Nitrospira spp.* in sidestream, which can be partly demonstrated by observed *Nitrospira spp.* in overflow (Figure 6c).

## CONCLUSION

Major control approaches for the CPT (sidestream deammonification) process include pH based on/off aeration control and retainage of anammox bacteria through hydrocyclones. One and a half years of operation proves that these control measures are effective in achieving their design objectives, retaining anammox bacteria and suppressing NOBs. Hydrocyclones achieved average capture of flow, TSS, aerobic and anammox fraction at 8%, 44%, 10% and 85%, respectively despite poorly controlled operating pressure. Hydrocyclone operation also has a big impact on the granule size distribution. Bigger granules tend to grow at shorter hydrocyclone operation hours. Despite periodical disruptions from dirty centrate discharge, NOB is not very active as nitrate production ratio in the CPT system generally is close to the 11% stoichiometric nitrate production from deammonification reaction. This proves that the intermittent on/off aeration control is effective in suppressing NOBs. qPCR analyses showed decreased anammox and AOB population during periods of poor centrate qualities and reduced flocculent sludge waste due to competitions from heterotrophic bacteria and NOBs. However, their microbial activities were higher therefore nitrogen removals were not negatively affected.

## REFERENCE

- Liu, G., Wang, J., 2013. Long-Term Low DO Enriches and Shifts Nitrifier Community in Activated Sludge. *Environ. Sci. Technol.* 47, 5109–5117.
- Strous, M., Kuenen, J.G., Jetten, M.S.M., 1999. Key Physiology of Anaerobic Ammonium Oxidation. *Appl. Environ. Microbiol.* 65, 3248–3250.
- Vlaeminck, S.E., Terada, A., Smets, B.F., De Clippeleir, H., Schaubroeck, T., Bolca, S., Demeestere, L., Mast, J., Boon, N., Carballa, M., Verstraete, W., 2010. Aggregate Size and Architecture Determine Microbial Activity Balance for One-Stage Partial Nitrification and Anammox. *Appl. Environ. Microbiol.* 76, 900–909.
- Wett, B., Hell, M., Nyhuis, G., Puempel, T., Takacs, I., Murthy, S., 2010a. Syntrophy of aerobic and anaerobic ammonia oxidisers. *Water Sci. Technol.* 61, 1915.
- Wett, B., Nyhuis, G., Takács, I., Murthy, S., 2010b. Development of enhanced deammonification selector. *Proc. Water Environ. Fed.* 2010, 5917–5926.