Response to the Independent Science Advisory Panel Workshop No. 3 Report

Questions to the Panel

The following questions were presented to the Panel, and are addressed in this technical letter report:

1. What is the Panel's evaluation of Metropolitan's recommendations for operating nitrification-only MBRs to meet targeted LRVs for the MBR process?
2. What is the Panel's evaluation of Metropolitan's recommendations for operating nitrification-only MBRs to meet basin plan objectives for the full process train?
3. What are the Panel's recommendations for proceeding with baseline testing?

Panel General Comments

The Metropolitan Water District of Southern California (Metropolitan) project team has extensively evaluated many unit processes, including the membrane bioreactor (MBR), for tertiary treatment. The team has obtained some useful results, but additional studies are necessary.

The Panel understands that the Regional Recycled Water Program is still in a study phase and no decision has been made regarding the optimal process configuration for full-scale advanced water treatment processes to treat either primary or secondary wastewater effluent from the Sanitation District of Los Angeles County's Joint Water Pollution Control Plant. Metropolitan has indicated that further fine-tuning and additional process evaluation will occur later in the program.

Metropolitan is proposing to proceed at this time with the nitrification-only operational mode, which deviates from the nitrification-denitrification (NdN) mode proposed in the current approved Testing and Monitoring Plan (TMP).

Treatment of non-nitrified secondary effluent in NdN mode at the demonstration facility has resulted in elevated nitrite levels in MBR filtrate which are anticipated to have adverse impacts on downstream reverse osmosis (RO) and ultraviolet/advanced oxidation process (UV/AOP) performance. While the Panel thinks that additional work to fine-tune the NdN process could adequately control nitrite, the Panel recommends that the development plan should be expanded to objectively consider and evaluate other plausible technologies (such as annamox) to manage nitrite as well as consideration of the other recommendations in the Panel memo.

One of the main goals of the approved TMP is to demonstrate pathogen removal through the MBR system. Metropolitan has noted that a pilot study in 2010–2012 to assess efficacy of a nitrification-only mode MBR-RO-UV/AOP treatment train on effluent from the plant showed that treated water quality goals could be achieved based on the draft groundwater recharge regulations available during the time of the pilot study and that a nitrification- only operation mode is anticipated to provide conservative conditions to assess pathogen removal through the MBR process.

The Panel is prepared to review the results of the pathogen removal evaluation for the nitrification-only mode MBR-RO-UV/AOP treatment train to demonstrate that the process can reliably meet treated water quality goals once Metropolitan develops the data.

Metropolitan's ultimate choice of treatment train will greatly affect the long-term operations and maintenance costs for the region's recycled water supply. The goal should be to select the best technologies for the best long-term operational and economic results and to find the best available technology. The process of selecting the treatment train will require the project team to learn about and benefit from other developing technologies and to conduct some bench-scale and pilot testing.

Response:

Metropolitan's Advanced Purification Center (APC), a 0.5-MGD demonstration facility, represents a critical component of its Regional Recycled Water Program (Program). The demonstration facility (Demo) is intended to directly address key regulatory and process challenges, allowing the project to move ahead with confidence. A few key objectives of the APC are as follows:

- Obtain pathogen credits for the MBR process to achieve overall log removal goal for the MBR-RO-AOP process train
- Assess the performance of RO when operating downstream of an MBR process with intact and breached membranes
- Demonstrate reliability (operational and water quality) of the MBR-RO-AOP process train and obtain regulatory approval of the train
- Assess nitrogen and boron removal across the process trains along with other key parameters described in the Testing and Monitoring Plan; the target limits for nitrate and boron are 3.4 mg/L-N and 0.5 mg/L, respectively in the plant effluent

The project team appreciates the Panel's concerns on the following topics and has therefore, included some clarifications:

- **Incomplete Nitrification**: It appears the Panel thinks the presence of nitrite in MBR filtrate was due to incomplete nitrification. The project team conducted bioreactor profiling (nitrogen speciation in aerobic, anoxic and membrane tanks) during the NdN optimization efforts and found that nitrite was present in the anoxic zone and not the aerobic zone. Data suggested that the presence of nitrite in MBR filtrate was due to incomplete denitrification and not incomplete nitrification. Additionally, Metropolitan has been operating the MBR process in N-only mode since early June 2020 and it has consistently achieved complete nitrification (ammonia + nitrite < 0.5 mg/L-N), further solidifying our findings from the NdN optimization efforts.
- **Use of Methanol as a Carbon Source for Denitrification**: JWPCP is a high-purity oxygen activated sludge (HPOAS) facility, and based on expected dose at a potential full-scale AWT facility, several hundred thousand gallons (175,000 – 280,000 gallons) of methanol would need to be stored on site. With the potential safety concern in mind, the APC was not designed to test or store methanol. However, bench-scale testing with methanol was conducted by Metropolitan and Sanitation Districts and showed positive results with respect to achieving

complete denitrification and meeting nitrate goals. Therefore, Metropolitan and Sanitation Districts will continue to evaluate use of methanol as a carbon source in future.

- **Further Optimization of the NdN Tertiary MBR Process**: Metropolitan plans to continue its efforts with Sanitation Districts to optimize the NdN Tertiary MBR process at bench- and pilot-scale while the demonstration-scale testing at APC is being conducted using N-only Tertiary MBR train.

- **Cost Comparison of "NdN Tertiary MBR + RO" and "N-only Tertiary MBR + Two-pass RO" Trains**: Metropolitan developed cost estimates for both trains during the conceptual planning phase and plans to refine those costs once additional data is available from the APC regarding operating parameters, chemical doses, membrane cleaning/replacement frequencies, etc. Please note that both trains achieved the nitrate goal in RO permeate at the APC with the primary difference being whether nitrate removal was primarily achieved by the MBR process (biological denitrification) or by RO (physical removal by membranes). However, the presence of nitrite in the NdN Tertiary MBR train posed several operational challenges for downstream process control and therefore, further evaluation would be needed if NdN Tertiary MBR were to be considered for the full-scale AWT facility.

- **Limited Experience with Two-pass RO**: The project team acknowledges the Panel's comment regarding limited experience with the two-pass RO in the municipal water reuse industry. The operations and water quality data obtained from the APC will aim to address that concern.

- **Alternative Biological Process Configuration**: The project team agrees that an alternative biological process such as mainstream anammox may provide cost savings (e.g. process aeration and carbon needs) when treating non-nitrified secondary effluent. In fact, Sanitation Districts has been exploring both the sidestream and mainstream process applications over the past ten years, including literature reviews, lab-scale and pilot-scale testing, and development of conceptual designs and preliminary cost estimates. Considering that the preliminary design of the full-scale AWT facility may begin in next several years, considerable knowledge gaps would need to be evaluated before mainstream anammox could be implemented at a large-scale including the following:

    - Downstream Membrane Performance: Impact of residual carbon and ammonia in the anammox effluent on downstream membrane performance (MBR and RO) is unknown.

    - Biodegradation of Chemicals of Emerging Concerns (CECs): The biological process plays a crucial role in potable reuse projects with respect to biodegradation of CECs and bulk organic matter; removal of CECs is not well documented with the mainstream anammox process.

    - Brine Toxicity: Any residual ammonia and/or nitrite in the mainstream anammox effluent will be rejected by the RO and therefore, will be present in the RO brine; toxicity impact of such stream needs to be assessed.

    - Impact on UV/AOP Performance: Nitrite is a critical scavenger of hydroxyl radicals in a UV/AOP system and therefore, presence of nitrite in anammox effluent (and subsequently RO permeate) may be detrimental to UV/AOP performance.

- Regulatory Approval: Although California Division of Drinking Water (DDW) has not granted any pathogen credits to the MBR process yet; the project team anticipates that to occur in the near future. If an alternative biological process was used in conjunction with MBR or in lieu of MBR, then additional data may need to be collected to obtain regulatory approval.

- Number and Scale of Installations: The largest mainstream anammox facility (15 MGD) in the US is being operated by Hampton Roads Sanitation Districts; it treats secondary effluent from an upstream biological process that produces effluent with low carbon and nitrogen concentrations. The downstream anammox process is really a polishing step rather than a true mainstream process that achieves majority of nitrogen removal. Limited experience, if any, exists in the industry in implementation of a mainstream anammox process.

Because the Program is multifaceted beyond the testing currently being conducted at the APC, the most critical objective at the current testing phase is demonstration of reliable treatment processes that meet the key project objectives. Thus, responses herein reflect the need to focus primarily on reliability, with efficiency as a secondary goal.

Question 1: What is the Panel's evaluation of Metropolitan's recommendations for operating nitrification-only MBRs to meet targeted LRVs for the MBR process?

Response – The Panel believes that insufficient information was presented during the workshop to draw firm conclusions about nitrification-only MBRs (there were only two slides on the nitrification-only MBR option). The Panel observes/recommends:

a. The procedure and assumptions for calculating the nitrite reduction rate should be more clearly stated (see Slide 34). The nitrite reduction rate was calculated from raw (concentration vs. time) data via adjacent points. The data interpretation was not well developed or justified. Nitrite is accumulating, yet is shown as an uptake rate (still positive, along with nitrate, which decreased with time).

Response: A presentation has been developed to further elaborate the procedures and assumptions for calculating the nitrate as well as the nitrite reduction rates presented previously. Please see "ISAP Workshop 3 – Clarification (specific rates calc v3a).pdf" in Attachment 1.

b. Additional cost-benefit analysis should be conducted to better characterize the carbon cost for NdN versus the cost to use two-pass RO and potentially higher membrane replacement frequency should nitrate rejection rates decrease over the life of the RO membranes.

Response: As part of the joint Metropolitan/Sanitation Districts' nitrogen management study, preliminary cost estimates were developed for both process trains, i.e. NdN Tertiary MBR + RO and N-only Tertiary MBR + Two-pass RO. However, operations data, especially chemical consumption, is now available from the APC and these costs can be updated. When doing so, the costs associated with higher membrane replacement frequency can also be accounted for. Metropolitan plans to update these costs in the future once demonstration testing of all potential process trains is complete.

c. Ancillary benefits for log reduction values (LRVs) may exist because of two-pass RO but were not identified.

Response: The data collected from the APC operation will document the log removal of both electrical conductivity (EC) and Total Organic Carbon (TOC) for both single and double pass modes of RO operation and allow for that comparison. There may be benefits to a two-pass RO in terms of LRV; however it will be difficult to demonstrate with online monitoring. Reverse osmosis is credited for pathogen removal based on online monitoring of TOC and conductivity and associated LRV. However, the first pass RO permeate is expected to have low concentrations of both TOC and conductivity and therefore, any further reduction by the second pass is expected to be minimal, at least from the standpoint of monitoring/analyzer capabilities. The two-pass RO would have to reduce permeate TOC or conductivity by a factor of 10 to improve overall LRV of RO by 1. Further, the LRV benefits from two-pass RO are anticipated to be relatively minor compared to the benefits of advanced online monitoring (e.g., strontium, sulfate, fluorescent dye) implemented on the first pass, which have been shown to increase LRV credits from ~2 to 4 in different research studies.

d. Additional industrial user identification and discharge characterization should be conducted to gain a better understanding of the potential organic nitrogen load entering and passing through the secondary treatment process at the wastewater treatment plants.

Response:

Sanitation Districts' Industrial Waste Pretreatment Program was established to implement source control measures through continued enforcement of permitting, monitoring, and inspection requirements. Part of the permitting process is to characterize the discharger's waste stream, assess potential harmful constituents, and impose pretreatment requirements and discharge limits to protect the Sanitation Districts' facilities and ensure compliance with NPDES permits and reuse limits. All data are stored in the industrial user database.

Recalcitrant dissolved organic nitrogen (rDON), the potential amount of organic N passing through the secondary treatment process, would be measured by analyzing effluent dissolved organic nitrogen (DON). At this time, rDON and DONs loads are not evaluated or considered for source control at industrial facilities.

As part of a high-level plan to facilitate nitrification-only MBR operation, the Industrial Waste and Research Sections collaborated on a literature review to develop a list of industrial operations that could produce a loading of rDON capable of shocking or interfering with the MBR nitrification process. This literature review also identified other nitrification inhibitors that could be common in industrial processes. The industrial user database has been reviewed to identify users (IUs) with operations that could potentially be DON sources (Table 1). In the event that rDON sampling at the Joint Water Pollution Control Plant (JWPCP) indicates there is significant interference with the nitrification process due to rDON, the IUs in Table 1 with the largest discharge (and therefore highest likelihood of having an impact) will be targeted for Sanitation Districts sampling of rDON, DON, and any other expected inhibitor in the laboratory analyses. Any sampling of targeted IUs for toxic organics would include 1,2,4-

triazole. These same targeted industries may also have rDON, DON or any other expected inhibitor added to their Self-Monitoring Requirements.

e. Industrial users may be sources of nitrifying inhibitors, but this has not been evaluated; it will need to be evaluated if the technology moves toward nitrification-only MBRs.

Response:

The project team agrees that nitrifier inhibition can adversely impact the performance of nitrification-only MBR. However, as nitrification-only MBR employs the same nitrifiers as those in NdN MBR, the team does not believe the former is more susceptible to nitrifier inhibition nor is additional consideration warranted. If there's literature arguing otherwise, please advise.

Note that in the past ten years, three pilot/demo-scale studies involving nitrification of JWPCP secondary effluent have been conducted: (1) tertiary nitrification-only MBR (2010-2012); (2) tertiary nitrification/denitrification BAF (2014-2016); and (3) the current study. Reliable nitrification was maintained in all three studies. As such, nitrifier inhibition does not appear to be a significant barrier to nitrifying JWPCP secondary effluent.

At this time, we are focusing on the potential for industrial interference. Known inhibitors that may be present in industrial wastewater include toxic organics, heavy metals, and large slug loads of ammonia. Any significant industrial user (SIU) with the potential for a batch (slug) discharge of any of these materials has been placed in the Sanitation Districts' Slug Discharge Control Program. This program requires that any SIU that has the potential for a batch discharge of any wastewater (including from spill containment areas), treated or otherwise, that has the potential to adversely impact the Sanitation Districts' collection system or treatment plant must complete, implement, and maintain a Slug Discharge Control Plan (SDCP). Elements of the SDCP include:

- Description of discharge practices, including non-routine discharges. Discharge of wastewater resulting from non-routine operations is prohibited unless prior approval is obtained.
- Description of stored chemicals and a tank schedule identifying location and contents of tanks.
- Procedures for promptly notifying the Sanitation Districts of slug discharges (defined as any discharge that would contribute to a violation or endanger Sanitation Districts' facilities).
- Procedures to prevent adverse impact from accidental spills.

For each SIU, the assigned field inspector must evaluate the potential impact to the sewer system or treatment plant for each pollutant of concern. These pollutants include but are not limited to alkalies or alkaline substances, acids, oils, foam generating wastes, highly colored wastes, pesticides, high chemical oxygen demand (COD) wastes, high total solids wastes, organic solvents, metals, ammonia, sulfides, and toxins. For each pollutant, the inspector assigns a risk factor of either low, medium, or high. The database for SDCPs can be searched and filtered for specific pollutants, or risk factors (e.g., all SIUs with high risk of an organic solvents slug discharge). This program allows the Sanitation Districts to know where potential nitrification inhibitors may be used and stored, and what the risks of harmful discharges are. It also requires SIUs to notify the Sanitation Districts immediately if a slug discharge occurs.

Per the Panel's suggestion, the industrial user database has been reviewed to identify IUs required to maintain an SDCP based on operations that could potentially be sources of nitrifier inhibitors. These identified IUs are shown in Table 2.

f. The Panel assumes that the LRV sampling plan is still consistent with the plan the project team previously provided to the Panel. The LRV plan and approach are appropriate. The Panel agrees that all LRVs should be adjusted based on matrix spike information, which we assume is being done for each sample.

Response: Yes, each field sample for pathogen analysis is spiked with control organisms to calculate the recovery for each sample. Samples for *Cryptosporidium* and *Giardia* analysis are spiked with ColorSeed oocysts and cysts, while virus cell culture samples are spiked with cell culture infectious murine norovirus.

g. Does the project team anticipate a difference in the removal of protozoans under aerobic versus anaerobic conditions and, if yes, should the LRV study plan take this into account?

Response: Our approach is to assess the LRV across the MBR process (i.e. bioreactor + membrane as one system) and therefore, we are collecting screened secondary effluent (feed to the MBR from JWPCP) and MBR filtrate samples to quantify the LRVs across the MBR. Irrespective of the bioreactor configuration (N-only vs NdN tertiary MBR), the pathogen concentrations are expected to be similar in screened secondary effluent and MBR filtrate (almost complete removal expected with intact membranes) and therefore, we anticipate minimal difference, if any, in LRVs between these two configurations.

h. Given the performance observed in this round of testing, it is premature to remove mainstream anammox from consideration without more information.

    i. The Panel suggests considering mainstream anammox plus RO. For example, the Hampton Roads Sanitation District (HRSD) has developed, demonstrated and is moving forward with design of an AvN (ammonia-oxidizing bacteria, or AOB, versus nitrite-oxidizing bacteria, or NOB) mainstream anammox process.

    ii. AvN uses established sensor control strategies to select for AOB, repress NOB, and enhance anammox performance. Consequently, mainstream anammox should be objectively investigated as part of this stage of project development. It has progressed from a few years ago.

    iii. A visit to plants and/or other facilities with mainstream anammox experience (for example Hampton Roads or DC Water) is warranted to develop confidence in the status of the technology, a better understanding about the approach, and potential partnership with these leading utilities. They are very willing to share their experience with Metropolitan about application and scale-up of their technologies. These two utilities collaborated to create the utility-utility partnership concept that is now LIFT.

    iv. The annamox process warrants a deeper evaluation and a side-by-side cost comparison with the other options. The process might work even better in California's climate (warmer water temperatures).

Response:

The Sanitation Districts share the Panel's enthusiasm for Anammox-based technologies. While the current demonstration project is focusing on nitrification-only and NdN MBR, anammox-based technologies are still being considered for the JWPCP. For the past ten years, we have been actively exploring potential applications of such technologies at Sanitation Districts facilities, including both sidestream and mainstream deammonification. On the latter, a pilot-scale evaluation of Veolia/Kruger's IFAS ANITA Mox technology was conducted at the JWPCP in 2017/2018. The results indicated that the technology is promising for the JWPCP, though major knowledge gaps exist due to its lack of full-scale implementations elsewhere near the scale of interest, and its unknown efficacy as pretreatment upstream of AWT. If interested, additional details of the Sanitation Districts' work in this area can be found in Attachment 2.

The Sanitation Districts intend to continue monitoring development in Anammox-based technologies. Recently Sanitation Districts and Metropolitan staff engaged HRSD (Dr. Charles Bott) on this subject and learned more about the PdN/A (Partial Denitrification Anammox)-based approach for implementing mainstream deammonification. Evaluation of this approach may be considered at the JWPCP.

Metropolitan acknowledges the considerable research that has been conducted by the Sanitation Districts as well as other applications currently implemented elsewhere and understands that additional evaluation and testing is warranted. The project team plans to re-assess the applicability of this process at a later time.

i. Recent research on nutrient removal is included in three attachments to this report:

    i. Presentation by Haydee De Clippeleir at IWA Nutrient Removal conference in November 2018 (Attachment 3). This includes an explanation of Partial Nitrification Anammox (PNA) and Partial Denitrification Anammox (PdNA) pathways through the nitrogen cycle that form the basis of deammonification strategies (anammox treatment).

    ii. Paper by Tri Le, a PhD student who conducted his work at DC Water (Attachment 4). The paper discusses the use of NO3-N residual as a control parameter.

    iii. Poster by Priyanka Ali, who completed her masters at DCW last year, showing she could get a similar result with primary sludge fermentation vs. acetate as the carbon source (Attachment 5).

Response:

Thank you for sharing the information. Sanitation Districts have been tracking the development and application of Anammox-based treatment technologies for the last ten years. The development of PdNA is particularly exciting as it offers another route to take advantage of Anammox without needing to suppress NOB, which can be very challenging. Coupling PdNA with an in-plant source of carbon (e.g., primary sludge fermentate as described by Ali et al.) is one way to further reduce the O&M cost associated with nitrogen removal.

Metropolitan appreciates the references shared. The project team plans to re-assess the applicability of the anammox-based processes at a later time.

Question 2: What is the Panel's evaluation of Metropolitan's recommendations for operating nitrification-only MBRs to meet basin plan objectives for the full process train?

Response – The Panel believes more detailed justification should be given for the recommendation that the tertiary MBR should be for nitrification only rather than NdN.

The Panel observes/recommends the following:

a. Conduct an economic comparison (capital and O&M costs) between NdN plus RO, nitrification only and two-pass RO, and mainstream anammox plus RO.

Response: An economic comparison will be conducted once Phase II secondary MBR testing is completed, and results will be shared with the Panel.

b. If all the nitrogen is converted to nitrate, the nitrification-only MBR would have ~50 mg NO3-N/L. Please show projections for nitrate in the effluent of the first- and second-pass RO. Please show staging of RO arrays.

Response:

Based on the influent/secondary effluent TKN concentrations (45-50 mg/L-N), the effluent nitrate concentration in the MBR filtrate, when operating in nitrification-only mode, is expected to range from 39-44 mg/L-N. Assuming 90% rejection of nitrate by the RO membranes, nitrate concentration in the permeate of the first and second pass RO is expected to be 3.9-4.2 and 0.39-0.42 mg/L-N, respectively. Using these concentrations, approximately 40-60% of the first pass RO permeate may need to be treated with second pass RO to safely meet the Orange County groundwater basin nitrate goal of 3.4 (mg/L-N). The RO system at the APC has been operating a two-pass configuration since June 29, 2020 and the final (first and second pass blended) permeate nitrate concentration has been approximately 2 mg/L-N, with approximately 30% of the first pass permeate being retreated with the second pass.

The RO system at the APC has total of 16 vessels, 13 of them are for the first pass and remaining three for the second pass. Each vessel is equipped with seven 8" RO elements. Both first and second pass have two-stage configuration with 9:4 and 2:1 array for the first and second pass, respectively.

c. The replacement frequency for RO modules may be more frequent to achieve nitrate treatment targets. These costs should be considered.

Response: Agreed, operations and water quality data from the APC will be utilized to determine suitable replacement frequency for the full-scale AWT facility. Costs for more frequent replacement will be accounted for when developing cost estimates.

d. The ancillary benefits for two-pass RO, beyond nitrate removal, should be identified (for example, impacts on boron).

Response: Acknowledged. A two-pass RO system provides additional boron and other contaminant removal. Another potential ancillary benefit for two-pass RO is its enhanced ability to remove boron with pH adjustment. It is expected that by operating the second pass RO at a higher pH, speciation of boron would shift from boric acid (H_3BO_3) to borate ($B(OH_4)^-$); the latter would be more easily rejected by RO. These potential benefits will be identified.

e. Secondary impacts of two-stage RO were only briefly identified (for example, brine composition and impact on brine disposal, plus how this affects recent and planned brine toxicity testing).

Response:

If the system is switched to nitrification only (with or without 2nd pass RO), all the nitrogen in the brine should be in the form of nitrate, which is less toxic than either ammonia or nitrite. However, at the expected nitrate levels in the brine, there could be potential toxicity to aquatic life.

Ultimately, we have determined that the test plan provides sufficient testing to allow us to evaluate the toxicity of the brine to a variety of different marine species using acute and chronic tests. Toxicity is very difficult, if not impossible, to predict with complex mixtures such as brine. Synergistic effects between toxicants could yield higher than expected levels of toxicity, while antagonistic effects could yield lower than expected levels of toxicity. Performing toxicity testing on the brine is the only way to gauge the level of toxicity (and take into account dilution effects) and to determine what the most sensitive species will be. Brine disposal options will be evaluated based on the level of toxicity observed.

f. It is important to establish an ammonia goal after nitrification-only MBR and determine how the system will be operated to assure complete nitrification. The factors influencing complete nitrification should be understood as well as the risks if complete nitrification is not achieved.

Response:

It should be noted that presence of nitrite in the MBR filtrate at the APC has been due to incomplete denitrification and not incomplete nitrification; nitrogen profiling in the bioreactor confirmed this. When testing the NdN configuration, complete nitrification was achieved for most of the test period with filtrate ammonia concentration of less than 0.5 mg/L-N (treatment goal) for majority of time. An online ammonia analyzer monitors the ammonia concentration in the MBR filtrate every 20 minutes.

During nitrification-only mode, our goal is to maintain combined ammonia and nitrite concentrations in MBR filtrate below 1 mg/L-N. Since the onset of nitrification-only testing, the combined ammonia and nitrite concentrations in MBR filtrate have stayed below 0.5 mg/L-N.

i. Without nitrification for 100 percent of the day, the residual ammonia could make it difficult to dose chlorine accurately and to maintain constant chloramine residuals in the water flowing onto the RO membrane.

Response: With relatively low and stable MBR filtrate ammonia concentration, chloramine dosing control for RO pretreatment has been much easier for the nitrification-only mode.

ii. Another risk is producing off-specification water that does not meet the nitrate goal.

Response: When treating N-only MBR filtrate with a two-pass RO system, the nitrate concentration in the RO permeate has been approximately 2 mg/L-N, well below our goal of 3.4 mg/L-N.

iii. Critical Control Points should be defined for off-specification water.

Response: We will define the critical control points for off-spec water as we collect additional data.

g. The Panel would like to receive copies of the WEFTEC 2019 paper (ref 6) and the other conference paper from 2018 (ref 5).

Response: The requested papers are attached (Attachment 3 and 4, respectively).

h. The Panel is curious to see how the hypothesis about predation going down with an N-only system pans out and encourages the collection of quantitative data. Nitrifiers are quite vulnerable to predation—more so than heterotrophs, since ammonia- oxidizers often are detached during perturbations and predators are more successful with detached cells. The paper, A comparative analysis of drinking water employing metagenomics, is attached (Attachment 6) for reference.

Response:

The project team acknowledges that this is an interesting research area. Once LRV studies are completed, Metropolitan and the Sanitation Districts will consider a broad range of research topics for future projects. Samples are being collected periodically throughout APC testing and frozen for future analysis such as metagenomic profiling. We expect to see *Acanthamoeba* spp. and other potentially predatory protozoa in wastewater samples, but assessing their impact on the nitrifying community would likely require a dedicated study.

The current test plan approved by the Panel focused on pathogen removal through the overall MBR process and did not include additional effort to establish the LRV of the biology alone. While a longer SRT would concentrate more of the pathogens that were retained by the membrane, a longer SRT also provides for a more diverse community and longer time for the pathogens to persist outside of a host. Two other important factors are the HRT and MLSS concentration. Due to the complexities, the project team will at this time remain focused on the execution of the test plan for demonstrating the LRVs through the entire MBR process.

Question 3: What are the Panel's recommendations for proceeding with baseline testing?

Response - The Panel observes/recommends the following:

a. While it is understandable why the team only operated for approximately one solids retention time (SRT) under the nine different NdN conditions evaluated, it is possible that with a 15-day SRT, the nitrifiers simply did not have ample time to acclimate. Typical minimums before starting a study is three SRTs. The Panel feels that NdN has not been truly ruled out yet.

Response: We acknowledge the need to test each condition for at least three SRTs. However, it should be noted that complete nitrification was observed in the aerobic zone for nearly the entire study and the project team is confident that the nitrifiers were appropriately acclimated.

b. The current conclusion that denitrification is not possible at the proposed scale of operation may be premature and difficult to explain to regulators.

Response: We plan to continue exploring and optimizing the denitrification process on demonstration facility influent (JWPCP secondary effluent) on a bench/pilot-scale and potentially test it again at the APC once the issues with incomplete denitrification are identified. From a regulatory standpoint, at this time meeting the product water quality goals is viewed as more important than the process train that was utilized to achieve those goals.

c. The proposed fiber cutting/integrity test approach should still be valid for NdN-MBR or nitrification-only MBR.

   i. The working assumption is that sufficient water can be filtered that will not result in an unreasonably sized pellet for examination.

   ii. Does the microbial research team believe there may be a problem with filtering enough water with a two- to three-order reduction of flow through the MBR after cutting fibers?

Response:

The MBR filtrate flow will be the same for all challenge conditions (after membrane fibers have been cut) and it will be maintained at approximately 0.25 MGD for each MBR system. At the third Panel meeting, the presentation on the challenge conditions did explain how cut fibers will undergo a natural "healing" process and we anticipate that the flow rate of mixed liquor that is able to pass through the opening created by cut fibers would reduce with time. While natural fiber "healing" is anticipated to improve water quality, the system should stabilize after two to three days and the project team does not anticipate any significant issues with filtering the MBR filtrate after fiber cutting. As noted during the third Panel meeting, frequent communication between the field team and laboratory is necessary to ensure that filtration volumes are optimized for pellet volume; this will allow the team to manage the analytical burden while providing the desired enhanced limits of detection.

d. Continuous in-line nitrogen sensors (all species) will be installed soon, and it is imperative they collect MBR influent data on nitrate, nitrite, and ammonia to understand how to design and operate the MBR. It is possible that diurnal changes or industrial inputs may be leading to large variations in nitrogen species concentrations.

Response: We acknowledge the need to monitor nitrogen species in demonstration facility influent (JWPCP secondary effluent). Sampling conducted in 2016 showed that JWPCP effluent ammonia concentration exhibited a clear diurnal pattern, but effluent nitrite+nitrate concentration was consistently below the detection limit (0.2 mgN/L; see Figure 1 below). For the current study, an online ammonia analyzer has been installed to monitor secondary effluent whereas grab samples are being collected and analyzed for nitrate and nitrite.

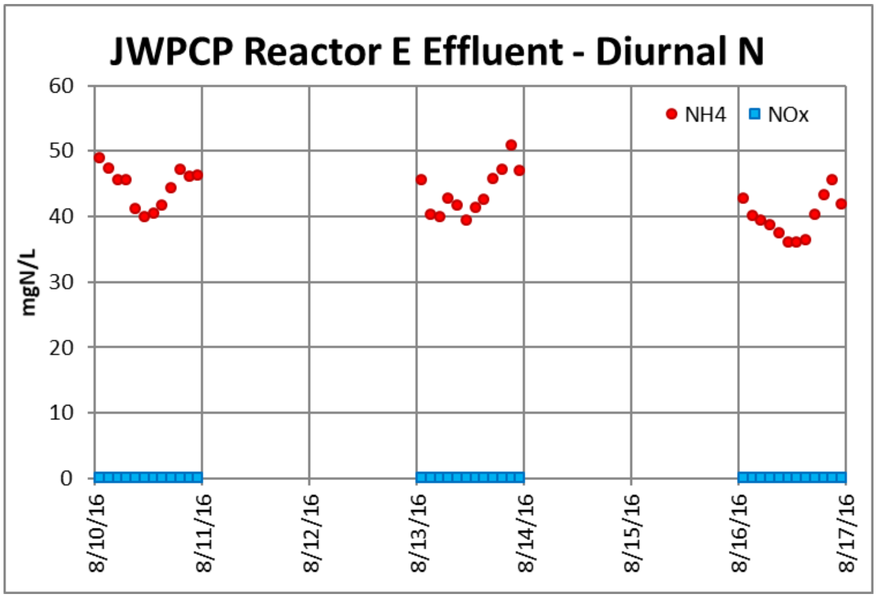


Figure 1. JWPCP Effluent Ammonia, Nitrate, and Nitrate Concentration

e. No data on MBR effluent dissolved organic carbon (DOC) were presented. How did DOC vary with the nine different NdN operational configurations? Is there anything to learn about the NdN process from the MBR effluent DOC data?

Response:

Considering the membrane pore size in MBR (0.04 µm), the DOC concentration in MBR filtrate is expected to be same as TOC, as long as the membranes are intact. The median MBR filtrate TOC concentration observed during the NdN operation was 8.7 mg/L whereas that for N-only operation (when no supplemental carbon is added) was 8.08 mg/L, suggesting that most of the added carbon was consumed in the bioreactor for most of the time. However, the TOC concentration in the MBR filtrate varied from 5.8 to 11.3 mg/L during the NdN testing so the project team acknowledges the need to understand this trend. MBR filtrate TOC data is available for each test condition and will be compiled and presented to the panel at a later date.

f. Projections of nitrate (and nitrite) rejection by RO should be documented, based upon membrane life observed at other facilities.

   i. It appears that 80 percent would be the minimum nitrate rejection that would be acceptable.

   ii. Will nitrate rejection be the likely controlling factor influencing RO membrane replacement frequency? If not, what factor is likely?

Response:

As requested, Figure 2 shows the nitrate rejection from the Groundwater Replenishment System operated by the Orange County Water District. This figure indicates that the RO rejection of nitrate will be between 87 and 91% for the first five years of operation and that nitrate rejection does decline with time in service, approaching 80% after 7 years of operation.

The 80% nitrate rejection that has been used for planning is based on RO manufacturer's models and is conservative. The nitrate data observed at the APC facility will be used for future planning and design as this information becomes available.

Based on experience at other operating AWTFs, the rejection of nitrate declines before other important parameters, such as TOC or TDS. While nitrate is likely to be an important factor in determining membrane replacement, boron rejection is even more sensitive to oxidant exposure (time in service) and depending on the outcomes with source control and the ultimate permitting requirements, boron could be more sensitive and require membrane replacements prior to loss in nitrate rejection performance occurring.

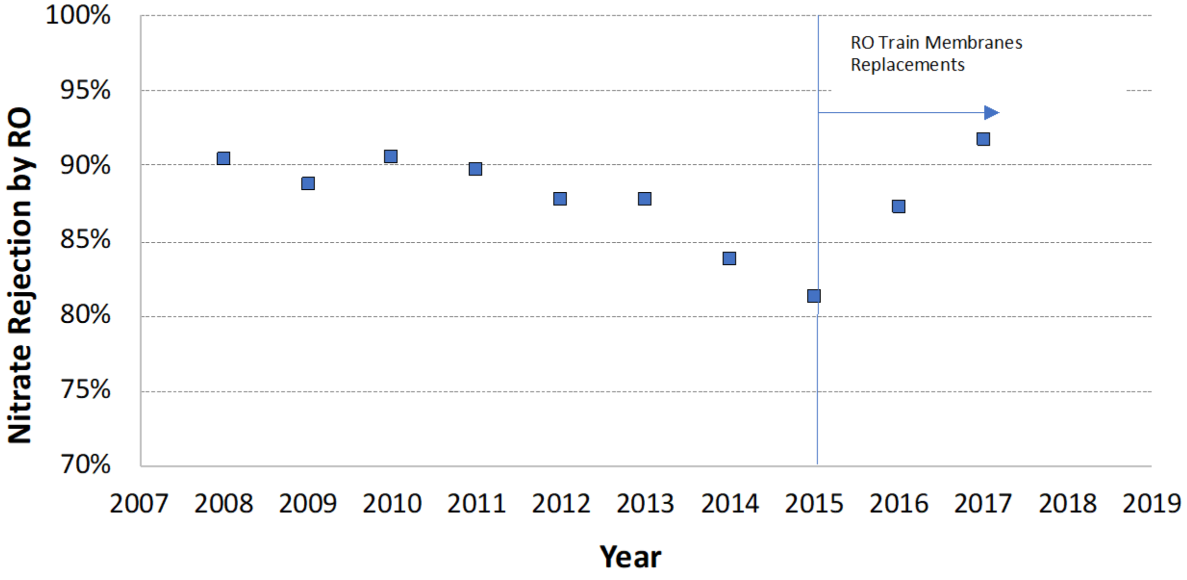


Figure 2. Nitrate Rejection from the Groundwater Replenishment System

g. It was not clear why two-pass RO was under consideration. Was it as a backstop in case nitrate or nitrite was not being sufficiently removed by NdN or other processes?

  i. The extra expense of two-pass RO should not be necessary with proper choice of earlier steps in the treatment train.

  ii. There was no documented evidence presented that two-pass RO has been operated at scale for nitrate removal. It was applied for seawater desalination before the World Health Organization revised its boron guideline to 2.4 mg/L, which made it unnecessary.

iii. Very detailed work has been done on tertiary MBR.

iv. Nitrogen reduction is occurring but it appears to be sensitive to several conditions.

v. Indications of need for additional phosphate are not favorable.

vi. Before additional work on tertiary MBR is undertaken, the upcoming secondary bench-scale work should be conducted as soon as possible, because it might provide different results, it might not require additional phosphate, and the carbon requirements might be less.

Response: Biological denitrification of secondary effluent is reliant upon supplemental carbon source, which comes at a cost and supply reliability concern. At full scale, several thousand gallons (25,000-40,000 gallons per day) of carbon is expected to be consumed on a daily basis for biological denitrification to meet the product water goals. Using RO instead to remove nitrate alleviates the supply reliability concern and therefore it was considered as a potential option during the conceptual planning studies for the full-scale AWT facility. The APC facility was also configured with two-pass RO to allow testing this alternative. We acknowledge the fact that limited experience, if any, exists on operating the two-pass RO systems on wastewater. The project team also plans to continue bench/pilot-scale studies on NdN configuration to optimize process performance and potentially reduce chemical consumption.

h. Modifications of the primary/secondary treatment process may be an option for NdN.

Response: Agreed. In previous efforts evaluating nitrogen removal options for the JWPCP, converting the existing HPOAS process to Secondary MBR was identified as a possible option. Secondary MBR is scheduled to be tested during the next phase of the demonstration testing. In addition, Sanitation Districts has procured a consultant to help develop more detailed design and cost associated with retrofitting the existing HPOAS to Secondary MBR. The latter is anticipated to be completed in 2021. As such, either modifying a portion of the JWPCP's to secondary MBR or adding a new secondary MBR facility is being investigated along with other treatment options.

i. Concern about the flammability of methyl alcohol as a candidate carbon source seems to be misplaced.

i. Methanol has many commercial applications. It is flammable, but it is also highly water soluble.

ii. The worldwide methanol production capacity is about 36 billion gallons, so it is readily available at relatively low cost because it is produced by hydrogenation of carbon monoxide. If the concern is with storage and transporting the volumes required, that is certainly manageable as has been demonstrated by its multitude of applications as solvent and feed stock.

Response: Acknowledged. Currently the demonstration facility is not equipped to use methanol. The use of MicroC at the demonstration facility provided a carbon source during the testing phase that would avoid these requirements and associated safety concerns. While preliminary bench-scale studies have shown that methanol could be used effectively for denitrification and dilution could address flammability concerns, the project team is still exploring other carbon sources for denitrification given the outstanding safety concerns for handling methanol.

iii. Nitrite is readily converted to volatile nitrogen oxides or ammonia under appropriate conditions, if necessary.

iv. It is reduced by sulfur dioxide to NO and N_2O.

v. It is reduced by hydrogen sulfide to ammonia.

vi. Basin plan water quality objectives have been presented as a single value and the plant effluent data for comparison against the basin plan objectives have as well. Would it be more appropriate to characterize both values on a statistical basis, if possible?

Response: Statistical analyses can be provided as additional data is collected during the test plan.

vii. What minimum nitrate removal is projected after RO membranes begin to age (80 percent nitrate rejection)?

Response: As noted above, 80% or higher rejection is expected for less than 5-year old membranes but data collected from the APC will confirm this.

viii. What pressures would membranes operate under?

Response: Depending on target flux and recovery, the feed water pressure for the first pass of the RO system typically ranges from 110-150 psi when operating in a single-pass configuration and 150-175 psi when operating in a two-pass configuration. The feed water pressure to the second pass of the RO system typically ranges from 50-70 psi.

ix. If all the nitrogen is converted to nitrate, the nitrification-only MBR would have ~50 mg NO_3-N/L. Please show projections for nitrate in effluent of first- and second-pass RO. Please show staging of RO arrays.

Response: See response to question 2.b.

x. It was stated the nitrite goal after the nitrification-only MBR would be less than 0.2 mg NO_2-N/L. Is this correct?

Response: The nitrite goal for the MBR filtrate at the APC will be 0.5 mg/L-N.

xi. How will you prepare or protect against upset events (nitrification is notoriously vulnerable to toxic upset)?

Response:

A multi-tier strategy can be employed to protect the nitrification system against toxic upsets:

(1) The nitrifying MBR can be designed and operated with a sufficient margin in HRT to allow complete nitrification even during a toxic upset event;

(2) The nitrifying MBR can be designed and operated with a sufficient margin in SRT to ensure a toxic upset event would not result in nitrifier washout;

(3) Wastewater processes upstream of the MBR (e.g., primary treatment, secondary treatment)

can act as additional barriers by pretreating potential nitrifier inhibitors in the influent wastewater;

(4) Deploy online sensors indicative of potential nitrifying inhibitors at the JWPCP headworks and in the collection system. Note that to Sanitation Districts' knowledge, such sensors are not yet proven and/or not commercially available. Sanitation Districts will continue to monitor and facilitate the development of such sensors.

xii. What are the known risks from upstream industries?

Response: See response to 1, e. above. To the extent that we know/understand the typical/general inhibitors to the nitrification MBR process, staff will incorporate candidate industries into the sampling study discussed in 1, d. above.

xiii. What will the utility do if there is a toxic load coming at them?

Response: To the extent that the Industrial Waste Section is involved in the initial notification of a potentially harmful discharge, that staff will promptly contact appropriate collection system and operations staff, both Sanitation Districts and Metropolitan. JWPCP and Metropolitan operations staff will then make operational decisions regarding how best to manage the "toxic load" to minimize the impact to treatment and reuse operations. Ultimately, this operational response must be part of the high-level plan referred to in the next sub-section below.

xiv. A high-level plan should at least be part of a nitrification-only recommendation.

Response:

The high-level plan (specific to a nitrification-only system) is envisioned to include:

- Literature review for better understanding of nitrification inhibition and rDON and DON sources.
- Review of OCSD reuse plan and response to nitrification inhibitors.
- Sampling effort to determine DON concentration – and nitrogen speciation – in effluent at JWPCP and Long Beach Water Reclamation Plant (as a control with minimal industrial waste stream). Sampling should consist of:
  - rDON (and speciation) sampling once per month over the next 12 months.
  - Quarterly diurnal effluent sampling every 2 hours over several days that includes weekends.
- Continue to pursue "early alert" monitoring opportunities for various locations in the collection system. Online measurements with real time reporting would be the goal.
- Comprehensive operational contingency plan for potentially harmful slug discharges, including consideration of diversions and segregation of flows where possible.

- Continuing to explore source control options when technologically and economically feasible.
- Outreach that includes letters to permittees to discuss advances in water recycling and the Sanitation Districts' role in potable reuse. This outreach should identify critical pollutants that we must control in the nitrification-only reuse environment.

xv. Will DOC be different for nitrification-only MBR compared to NdN-MBR? If so, what impacts on RO fouling would be expected?

Response: MBR filtrate DOC is expected to be either same or lower when operating in N-only mode compared to NdN mode. As a result, RO membranes may see improved performance.

xvi. No data on MBR effluent DOC was presented.

Response: See response to question 3.e.

xvii. How did this vary with the nine different NdN operational configurations?

Response: See response to question 3.e.

xviii. Can we learn anything about the NdN process from the MBR effluent DOC data?

Response: See response to question 3.e.

xix. Projections of nitrate (and nitrite) rejection by RO should be documented, based upon membrane life observed at other facilities.

Response: See response to question 3.f.

j. Will nitrate rejection be the likely controlling factor influencing RO membrane replacement frequency? If not, what factor is likely?

Response: See response to question 3.f.

k. The Panel has included a reference regarding recent work on metagenomics for your information.

Response: The microbiology team is closely following developments in applying metagenomic and related techniques to a variety of environmental samples, including recycled water. We are also familiar with the CosmosID platform. Metropolitan's microbiology team has previously used nucleic acid-based whole community profiling on pre-RO treated drinking water and biofouled RO membranes and so we are aware of the utility of these tools. Although beyond the scope and resources of the immediate APC testing project, detailed community analysis and comparisons may be incorporated into future research at the APC.

References

Branch, A. and Le-Clech, P. (2015) *National Validation Guidelines for Water Recycling: Membrane Bioreactors*. Australian Water Recycling Center of Excellence.

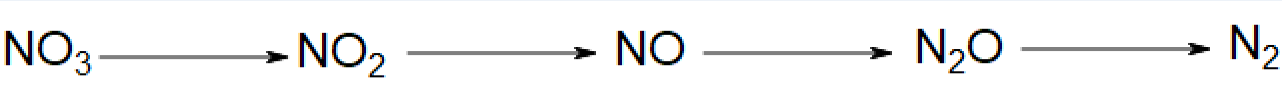
Attachment 1 – Calculation of Specific Nitrate and Nitrite Reduction Rates

Attachment 1

Calculation of Specific Nitrate and Nitrite Reduction Rates

Denitrification and SDNR

- Denitrification



$$NO_3 \longrightarrow NO_2 \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

- SDNR (Specific Denitrification Rate)
  - Denitrification rate normalized by the quantity of biomass in the assay
  - In the unit of mgN/g-VSS/hr
  - Commonly used metric for designing and evaluating the performance of denitrification systems

Typical and Adapted SDNRs

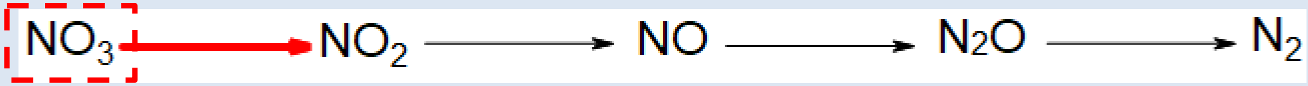
- Typically, SDNR is calculated based on the rate of disappearance of the substrate (NO_3).

- This approach does not distinguish between the rates of the individual steps. Typically, such distinction is not needed, as there's usually minimal accumulation of the intermediates (i.e., NO_2, NO, N_2O).

- For this project, due to observation of NO_2 accumulation during denitrification, it was thought that being able to differentiate the rates of the first two steps may provide additional insight.

Specific Nitrate Reduction Rate

- Step of interest (red arrow) and species tracked (red box)



- Calculation

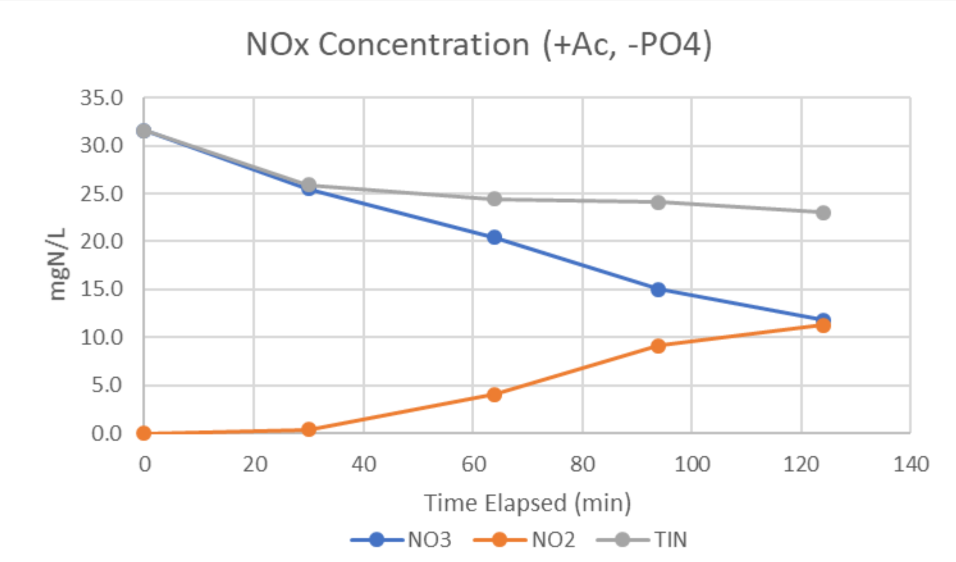
$$\frac{NO_3\ consumed}{time\ elapsed * Biomass} = -\frac{[NO_3]_{t_2} - [NO_3]_{t_1}}{(t_2 - t_1)[MLVSS]V}$$

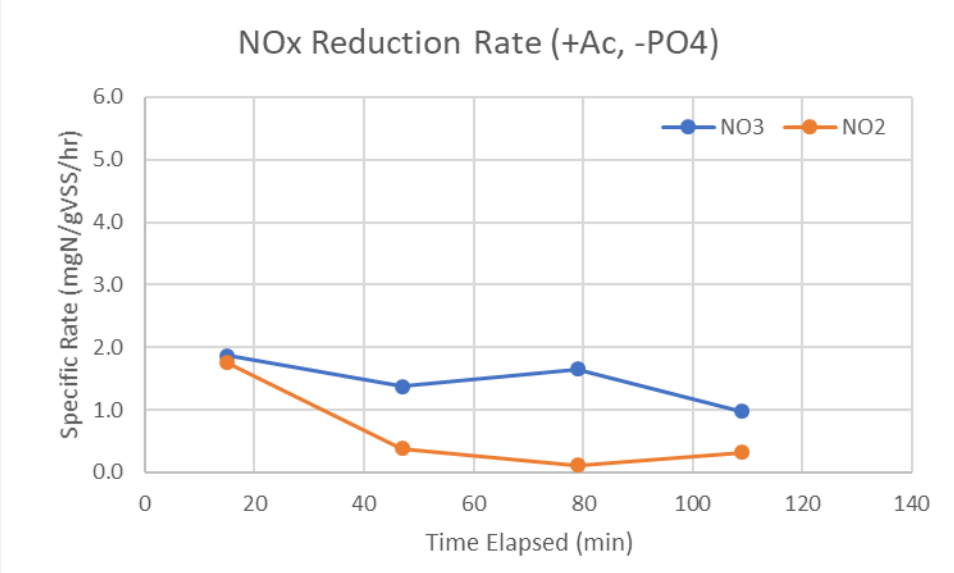
where $[NO_3]_t$ is the NO_3 concentration at time t
[MLVSS] is the mixed liquor volatile suspended solids concentration
V is the volume of the batch reactor

- Assumptions
  - Nitrate reduction is the only sink for NO_3
  - No reverse reaction (i.e., re-oxidation of NO_2 to NO_3)
  - No other sources of NO_3
  - No significant change in MLVSS during the test

Specific Nitrate Reduction Rate: Example

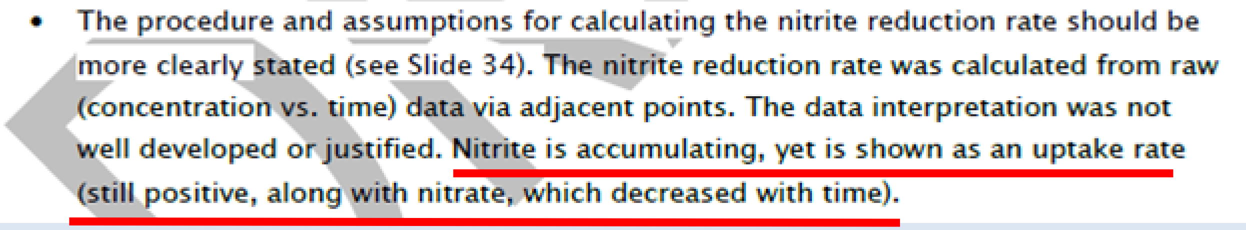
- Left chart
  - Blue line shows the nitrate concentration over time.
  - For each pair of points, the slope represents the average specific reduction rate during that period. A steeper slope corresponds to a higher rate.
- Right chart
  - Blue line shows the specific nitrate reduction rate over time





Specific Nitrite Reduction Rate

- Panel Comment



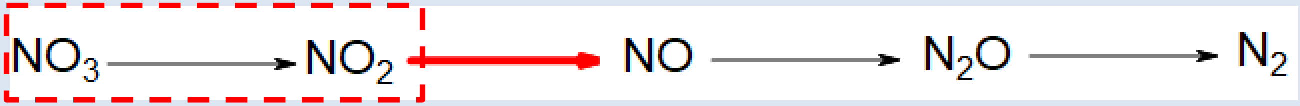
- The procedure and assumptions for calculating the nitrite reduction rate should be more clearly stated (see Slide 34). The nitrite reduction rate was calculated from raw (concentration vs. time) data via adjacent points. The data interpretation was not well developed or justified. Nitrite is accumulating, yet is shown as an uptake rate (still positive, along with nitrate, which decreased with time).

- Response

1. Tracking changes in nitrite concentration alone would not allow estimating nitrite reduction rate. This is because nitrite concentration is affected by both nitrate reduction ($NO_3 \rightarrow NO_2$) and nitrite reduction ($NO_2 \rightarrow \rightarrow N_2$). Nitrite is produced by the former and consumed by the latter.
2. On Slide 34 of the ISAP Workshop #3 presentation, nitrite accumulated due to nitrate reduction occurring at a higher rate than nitrite reduction. Note the rates of both processes are positive, but the former is higher than the latter.
3. One way to estimate nitrite reduction rate is by tracking changes in TIN (i.e. NO_2+NO_3 as NH4 is negligible in our case). The rationale is that, as nitrate reduction does not alter TIN, changes in TIN would then reflect only nitrite reduction which can and was used to estimate the nitrite reduction rate.

Specific Nitrite Reduction Rate

- Step of interest (red arrow) and species tracked (red box)



- Calculation

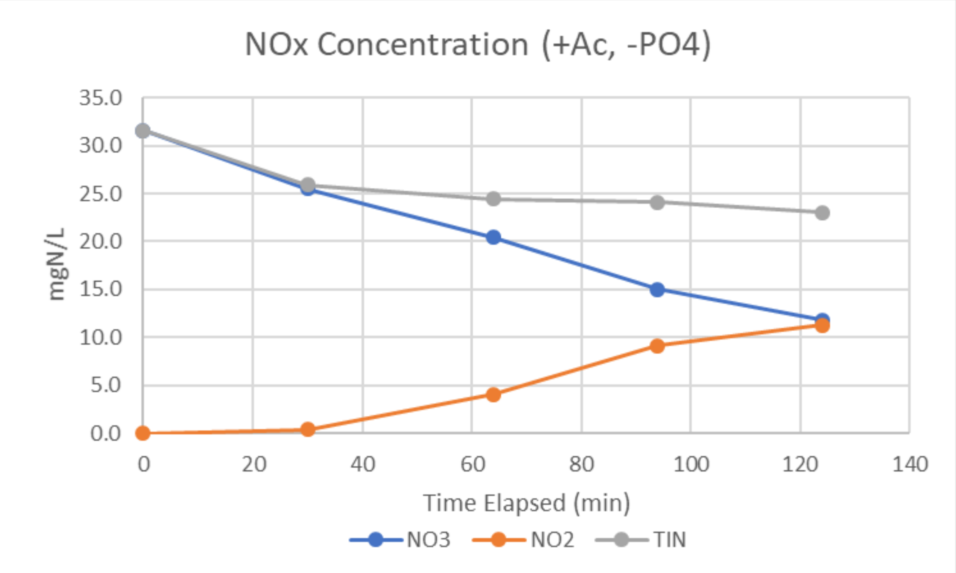
$$-\frac{TIN\ consumed}{time\ elapsed * Biomass} = -\frac{[TIN]_{t_2} - [TIN]_{t_1}}{(t_2 - t_1)[MLVSS]V}$$

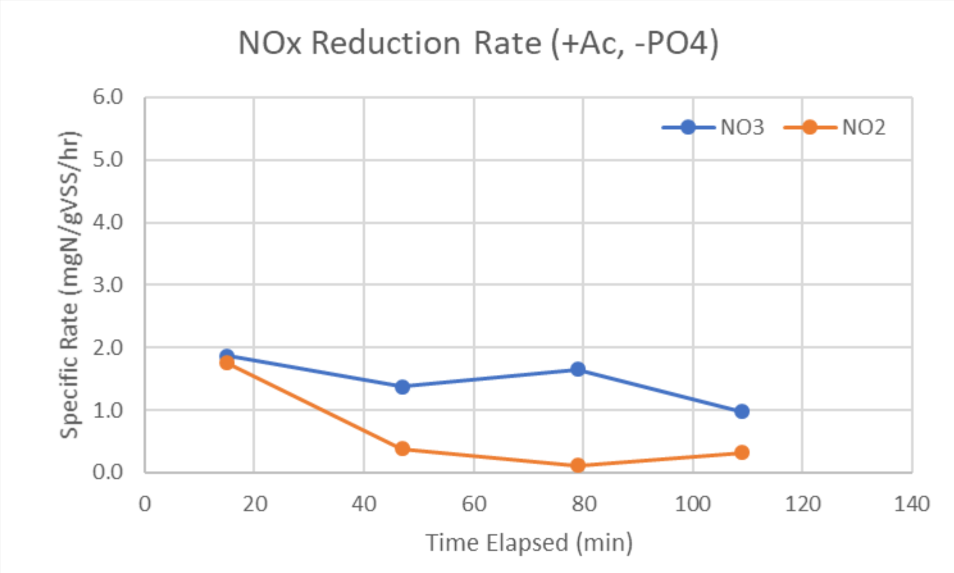
where [TIN]$_t$ is the sum of NO_2 and NO_3 at time t
[MLVSS] is the mixed liquor volatile suspended solids concentration
V is the volume of the batch reactor

- Assumptions
  - Nitrite reduction the only sink for TIN
  - No re-oxidation of NO or N_2O to TIN
  - No other sources of TIN
  - No significant changes in MLVSS during the test

Specific Nitrite Reduction Rate: Example

- Left chart
  - Gray line shows the TIN concentration over time.
- Right chart
  - Orange line shows the specific nitrite reduction rate over time.
  - Higher initial rate is expected as TIN concentration exhibited a steeper slope between 0 and 30 mins. Very low rate is expected from 30 min onward as TIN concentration stayed flat in this time period.





Attachment 2 – Summary of LACSD Experience in Anammox-Based Technologies

Attachment 2: Summary of LACSD Experience in Anammox-Based Technologies

For the past ten years, LACSD have been actively exploring potential applications of such technologies at Districts facilities. Initial efforts focused on sidestream applications which are considered more mature. Activities included literature review, lab-scale and pilot-scale testing, and development of conceptual designs and preliminary cost estimates. Staff also visited pilot and full-scale facilities (DEMON at the Egan WRP; ANITA Mox at the North Durham WRF), presented related work at industry conferences (Liu et al., 2014), and engaged subject matter experts in the industry, including those at HRSD, MWRD Denver, and MWRD Chicago to learn from their experience.

On mainstream deammonification, LACSD's experience began in 2014/2015 while conducting a pilot-scale test of biological aerated/anoxic filters (BAFs) for treating JWPCP secondary effluent. The pilot system consisted of two upflow filters in series with the first filter aerated for nitrification and the second filter anoxic for denitrification using acetic acid as the carbon source (Figure 2).

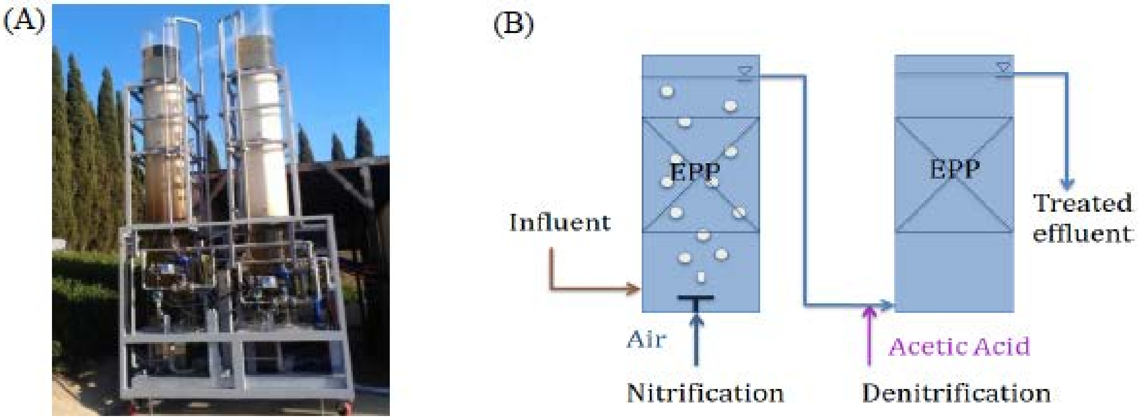


Figure 1. (A) 2-stage NdN BAF Pilot; (B) Process flow diagram of the NdN BAF Pilot System

During the testing, substantial (25~30%) nitrogen loss unexpectedly developed in the nitrification filter after six months of operation (Park et al., 2017). Quantitative PCR using biomass recovered from the column confirmed the presence of anammox bacteria. Anammox activity in the column further improved with NO_2 supplementation, suggesting the system was limited by ammonia oxidation. Overall the results showed promise in removing nitrogen from JWPCP secondary effluent using mainstream deammonification. Notably, anammox activity emerged and was successfully maintained without seeding with imported anammox bacteria, similar to observations at the Changi WRP in Singapore (Cao et al., 2017).

Following the serendipitous discovery of anammox activity during the aforementioned BAF testing, LACSD partnered with Veolia/Kruger to pilot test the latter's IFAS-based ANITA Mox technology for mainstream deammonification at the JWPCP (Liu et al., 2018; Krikorian et al., 2019). During the initial 8 months of testing, the pilot was operated at ambient temperature and achieved median ammonia and TN removal efficiencies of 90% and 71%, respectively (Figures 3 and 4). Median ammonia and TN removal rates were 0.96 and 0.97 $g/m^2/d$, respectively.

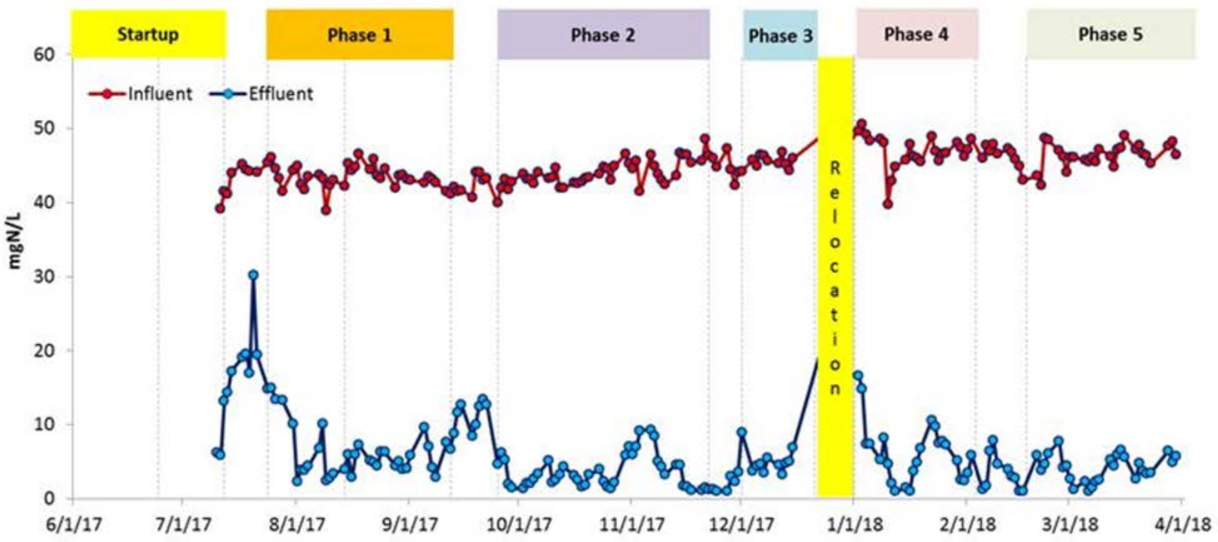


Figure 2. JWPCP Mainstream ANITA Mox Pilot Testing – Influent and Effluent NH_4 during Ambient Temperature Testing

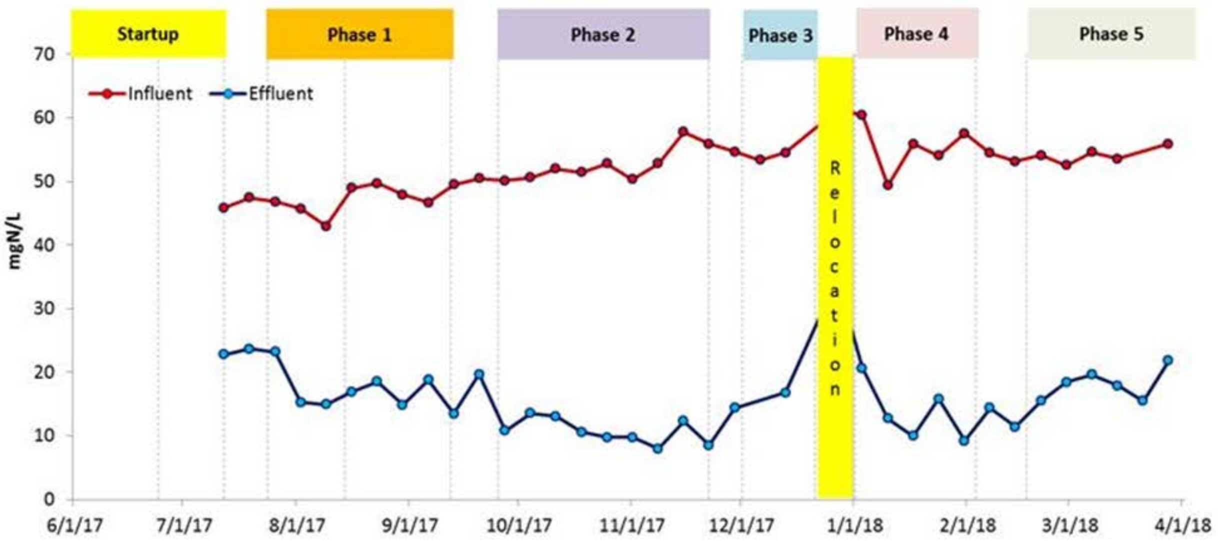


Figure 3. JWPCP Mainstream ANITA Mox Pilot Testing – Influent and Effluent TN during Ambient Temperature Testing

During ambient temperature testing, poor sludge settling characteristics were observed. While this issue can be addressed by increasing the size of clarifiers, it would lead to higher capital cost and footprint requirements. To explore alternative solutions, optimization was conducted to assess if sludge settling can be improved by increasing the feed C/N and/or adding an anoxic selector. While the effort improved sludge settling, it did not result in an improvement in the reactor's nitrogen removal rate, though the changes initiated and maintained EBPR concurrent with deammonification in the system.

To explore the process' range of applications, the study also evaluated the impact of temperature on process performance. Process temperature ranging from 14 to 24°C was evaluated by chilling the feed water. As expected, process performance exhibited strong sensitivity to temperature with an apparent

Arrhenius coefficient of 1.1, suggesting the process was limited by biological activity rather than mass transfer (Figure 5).

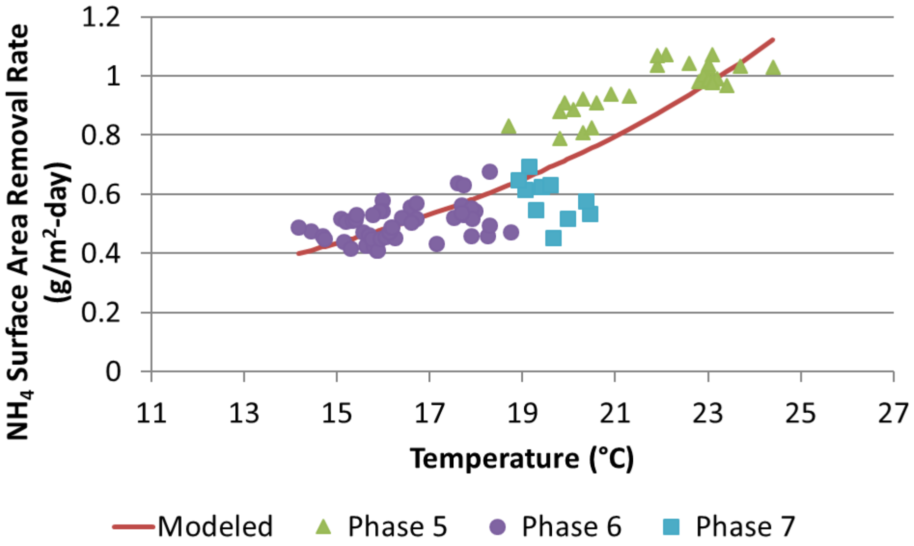


Figure 4. JWPCP Mainstream ANITA Mox Pilot Testing –NH_4 Surface Removal Rate vs. Process Temperature

Last but not least, stability of anammox activity was of interest due to the key organism's low growth rate. To this end, process stability was evaluated from a short-term and a longer-term perspective. To assess short-term process stability, the system was subjected to five simulated process outage/upset scenarios, including under/over aeration, under/over feeding, and reactor solids loss. For the first four scenarios, system performance was moderately impacted and recovered within 8 hours after the outage/upset conditions were removed (Table 1). For the scenario with substantial reactor solids loss, extended recovery time was observed, but the same would also be expected for typical NdN systems.

Table 1. JWPCP Mainstream ANITA Mox Pilot Testing – Summary of Short-Term Process Stability Testing

		ΔP* (%)		$T_{recovery}$ (hr)	
Scenario	Test Conditions	NH_4	TIN	NH_4	TIN
Under aeration	**DO = 0.5 mg/L**	**-14**	**-20**	**8**	**8**
Over aeration	**DO = 2.0 mg/L**	**9**	**-11**	**0**	**8**
Underfeed	**Effluent NH_4 < 1 mg-N/L**	**-16**	**-18**	**8**	**8**
Overfeed	**Effluent NH_4 > 10 mg-N/L**	**-3**	**-18**	**0**	**8**
Solids loss	**MLSS reduced to 1000 mg/L**	**-25**	**-23**	**>144**	**>144**

ΔP*: Change in surface removal rate; $T_{recovery}$: Recovery time

To assess longer-term process stability, reactor media were periodically removed and tested *ex-situ* for anammox activity. During ambient temperature testing, media anammox activity remained stable

(Figure 6). The same was observed during reduced temperature testing, except for a sharp reduction during the transition from ambient to reduced temperature operation.

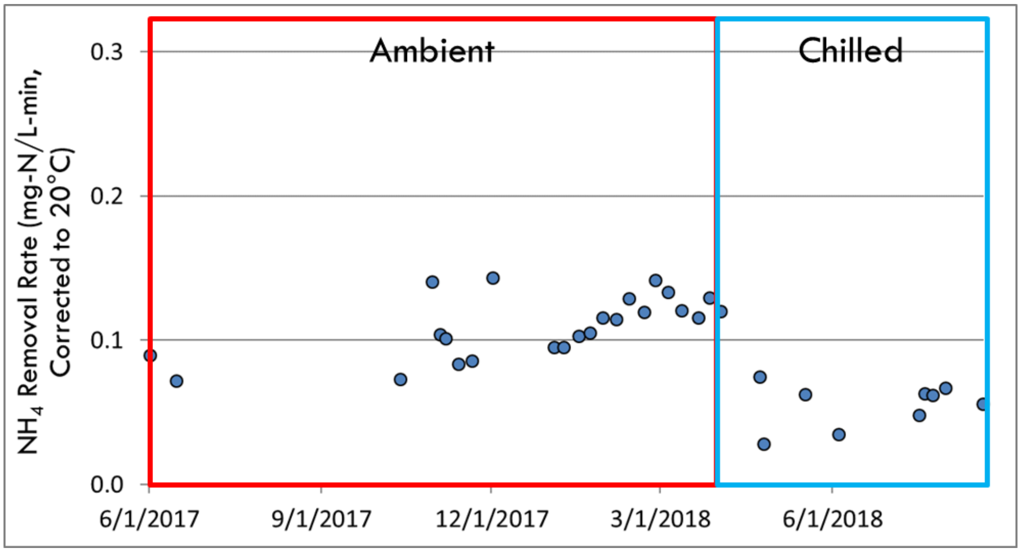


Figure 5. JWPCP Mainstream ANITA Mox Pilot Testing – Summary of Ex-Situ Anammox Activity Testing

In summary, LACSD have invested considerable efforts into exploring potential applications of anammox processes at Districts facilities. On potential applications of mainstream deammonification at the JWPCP, pilot testing results to-date suggest the technology is promising. That said, the following limitations should be noted for IFAS ANITA Mox: (1) achieving effluent TN substantially below 15 mgN/L can be challenging and will need additional research; (2) poor sludge settling characteristics should be considered in the design; and (3) gaps in operational knowledge likely exist as there are currently no known large scale (>20 MGD) full-scale implementation of this technology for mainstream deammonification.

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Attachment 3 – Mainstream ANITA Mox Pilot Testing at the Joint Water Pollution Control Plant

Mainstream ANITA Mox Pilot Testing at the Joint Water Pollution Control Plant

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ABSTRACT

The IFAS (Integrated Fixed-film Activated Sludge) variant of Kruger's ANITA Mox process was tested in a ten-month pilot study at the Joint Water Pollution Control Plant (JWPCP) for mainstream nitrogen removal via deammonification. The system was used to treat a mixture of non-nitrified secondary effluent (SE) from the facility's High Purity Oxygen Activated Sludge (HPOAS) and primary effluent (PE). Effects of feed C/N on process performance were evaluated by adjusting the proportion of SE to PE fed to the system. Over the course of the study, the system exhibited median NH_4 and TN removal efficiencies of 90% and 71%, and NH_4 and TN surface removal rates of 1.29 g/m^2-d and 0.97 g/m^2-d, at a median process temperature of 25°C. Higher feed C/N correlated with higher TN removal efficiency but lower TN removal rate and poorer sludge settling characteristics. Installation of a selector and switching to the use of fresh instead of stored PE improved sludge settling and effluent quality, and initiated and maintained enhanced biological phosphorus removal (EBPR). Routine media biomass density and Anammox activity testing conducted over the course of the study indicated that anammox activity on the media remained stable under the mainstream conditions tested.

KEYWORDS

Mainstream Deammonification, Anammox, Nitrogen Removal

INTRODUCTION

The Sanitation Districts of Los Angeles County operate the Joint Water Pollution Control Plant (JWPCP), an ocean-discharge wastewater treatment facility with a capacity of 400 MGD located in Carson, CA. The facility employs the High Purity Oxygen Activated Sludge (HPOAS) process with short solids retention time (SRT) for secondary treatment, and consequently does not nitrify nor remove nitrogen from the wastewater. While nitrogen removal is not currently required for the JWPCP, future regulations and reuse requirements may make nitrogen removal necessary. When such a need arises, the implementation of a nitrogen removal process is expected to be costly. In exploring opportunities to reduce cost, the utilization of different biochemical pathways to remove nitrogen was assessed.

Typically, the removal of nitrogen from wastewater is achieved via the nitrification/denitrification (NDN) pathway, which involves nitrification by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), followed by denitrification by denitrifiers (Figure 1, red and blue arrows). This pathway requires the input of oxygen and Biological Oxygen Demand (BOD) (4.57 gO_2/gN and 4~6 gBOD/gN), which can translate into substantial power and chemical costs, particularly for tertiary treatment configurations where the required BOD would be supplied from external resources. An alternative is employing the deammonification pathway (Figure 1, green arrows). Based on stoichiometry, this pathway allows for the reduction of aeration and BOD up to 63% and 100%, respectively.

The application of deammonification has faced numerous challenges, including: (1) maintenance of the critical anaerobic ammonia-oxidizing bacteria (AnAOB), which exhibits very low growth rate (doubling time > 10 days); and (2) suppression of competing organisms/pathways (e.g., NOB, denitrifier). Initial efforts targeted the treatment of centrate/filtrate (aka "sidestream deammonification"), the characteristics of which (e.g., relatively warm temperature, high ammonia concentration) helped alleviate these challenges. Several technologies, based on different reactor designs, have since been commercialized, including World Water Works' DEMON, Veolia/Kruger's ANITA Mox, Paques' ANAMMOX, and Degremont's ClearGreen. With more than 200 full-scale installations world-wide, sidestream deammonification is generally considered mature (Bowden and Stensel 2015).

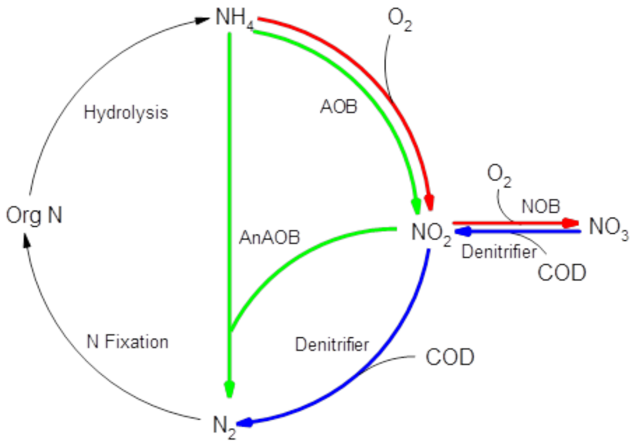

Figure 1 The Nitrogen Cycle. Red: Nitrification Pathway; Blue: Denitrification Pathway; Green: Deammonification Pathway.

The success of commercializing sidestream deammonification has motivated the industry to explore the potential of applying the technology to treat the main process stream (aka "mainstream deammonification", or MD). However due to the mainstream's lower temperature and ammonia concentration as compared to the sidestream, early attempts encountered difficulty in maintaining MD and consequently nitrogen removal performance was poor and unreliable. Some techniques have since been developed to improve MD reliability, such as AnAOB seeding (e.g., from a sidestream deammonification reactor), selective AnAOB retention (e.g., via hydrocyclone) and NOB suppression (e.g., transient anoxia). While these developments have improved MD, the technology is still considered "emerging". Only a very limited number of full-scale implementations currently exists: Strass, Austria (DEMON; Wett et al., 2015); Alexandria, VA (DEMON; 2017); and Changi, Singapore (incidental MD in step-feed NDN; Cao et al., 2015). In addition, several successful pilot-scale tests have been reported: Rotterdam, Netherlands (ANAMMOX; Lotti et al., 2015); Paris, France (ANITA Mox; Thomson et al., 2016); and Toulon, France (ANITA Mox; Thomson et al., 2016).

MD offers a unique upgrade path for the JWPCP where the effluent nitrogen can be removed in a tertiary process without carbon addition. This combination would exert no impact on existing treatment processes during construction and operation, while minimizing the operating cost of nitrogen removal. ANITA Mox also has the ability to handle primary effluent (PE) as feed, which can be used to relieve loading on the existing HPOAS and even enable an additional option to retrofit the existing HPOAS for nitrogen removal. This study evaluated the ANITA

Mox process for treating both JWPCP secondary and primary effluents, and extended prior work in MD research.

METHODOLOGY

Pilot System Description

Mainstream ANITA Mox is an Integrated Fixed-film Activated Sludge (IFAS) process. Figure 2 shows a flow schematic of the pilot system. The system contained the following major components: (1) Feed Tanks and Pumps; (2) Reactor; (3) Clarifier with sludge return; and (4) Selector. Each component, including instrumentation and control, is described in more detail below.

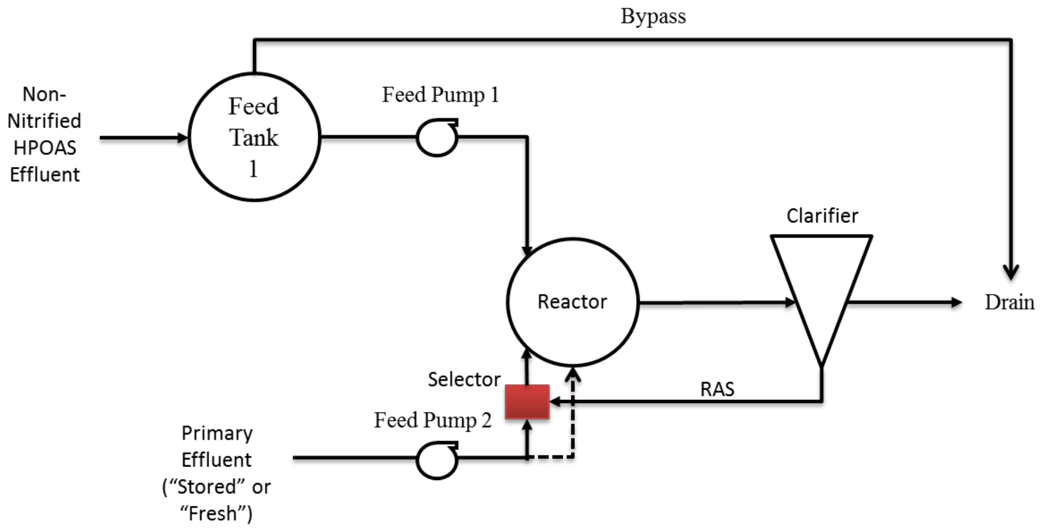

Figure 2 Pilot System Flow Schematic

Feed Tanks and Pumps

The system included feed tanks and pumps to allow feeding of two streams independently. Feed Tank 1 consisted of a cylindrical tank (ID= 3'; h=4'2") with a typical working volume of 194 gallons (SWD = 3'8") and served as a break tank for non-nitrified HPOAS effluent. During the first half of the study, two interconnected cylindrical tanks with a combined working volume of 20,000 gallons served as storage for PE, which was transported as needed using tanker trucks. It was later discovered that such storage of PE significantly altered its characteristics. About mid-way through the study, the pilot system was moved to a location with direct access to both primary and HPOAS effluent. Therefore, for the second half of the study, PE was directly pumped from the plant's PE channel. Source waters for the feed tanks are described in more details under the "Feed Source" subsection. Two progressive cavity pumps (Moyno 300-series; max flow ~ 10 gpm) were installed downstream of the feed tanks/channel to pump the SE/PE into the reactor. A mechanical mixer (Leeson; 1750 rpm; ½ hp) was installed and operated in Feed Tank 1, while a pump-driven recirculation system was installed and operated in the PE storage tanks prior to relocation of the pilot system.

Reactor and Seeded Media

The reactor is a 1,030 gallon stainless steel cylindrical vessel (ID=5’; h=7’) with a typical working volume of 701 gallons (SWD = 5’6”). A schematic/photo of the reactor/clarifier (described in the next sub-section) can be found in Appendix A. During startup, approximately 295 gallons of seeded AnoxKaldnes K5 media (Figure 3; protected surface area = 800 m^2/m^3), equivalent to a media fill of 37% by volume, was transferred into the reactor. The seeded media originated from a full-scale sidestream reactor at the South Durham Wastewater Treatment Plant (Durham, NC). The suspended growth sludge was not seeded, but instead grown on-site during the start-up phase. Media retention was achieved by screens installed on the tank outlets. Process air was provided by a mobile air compressor (Atlas Copco; 37 acfm @ 175 psi) with moisture knockout by two air chillers (Atlas Copco FX3 and FX5). Process air flow was regulated by two inline air mass flow controllers (Alicat; MC500SLPM) and distributed within the reactor via a medium bubble aeration grid located at the bottom of the reactor. The reactor was also equipped with a mechanical mixer (Nord; 1720 rpm; ½ hp) that was used to facilitate mixing during non-aerated periods.

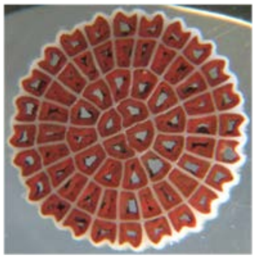

Figure 3 AnoxKaldnes K5 media

Clarifier

A lamella clarifier, with a working volume of 480 gallons, was installed downstream of the reactor. Figure 4 shows an oblique view of the clarifier with its four distinct zones: (1) influent distribution zone; (2) lamella settling zone; (3) sludge collection box; and (4) effluent collection box. The lamella settling zone consisted of 20 lamella plates with approximate total plate surface area of 80 ft^2 and volume of 85 gallons. Two air blast grids were also installed: one at the top of the lamella plates and another between the lamella plates and the sludge collection box. The air blast grids were activated periodically to scour clarifier surfaces to minimize blockage of the lamella plates.

Sludge was recycled by pumping from the sludge collection box back to the reactor, via a progressive cavity pump (Moyno 300-series; maximum capacity ~ 5 gpm). The system was equipped with sludge wasting capability both from the mixed liquor and the returned activated sludge (RAS), though typically the former was used for better control of the sludge wasting rate.

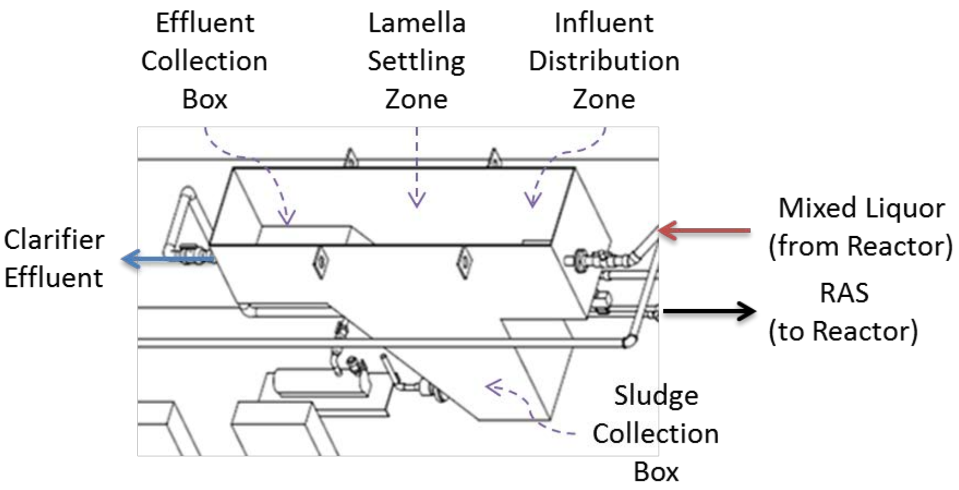

Figure 4 Oblique View of the Clarifier and its Four Zones (indicated by Purple arrows)

Selector

A selector was installed approximately mid-way through the study to help address sludge settling issues. The selector tank consisted of a cylindrical tank (ID= 3'; h=4'2") with a typical working volume of 150 gallons (SWD = 3'8"). Mixing of the selector tank contents was achieved by using a mechanical mixer (Leeson; 1750 rpm; ½ hp).

Instrumentation and Control

A Programmable Logic Controller (PLC) with a customized Human Machine Interface (HMI) maintained the system within the prescribed parameters and set points. Various online analyzers were installed (Table 1) to provide the PLC real-time data to facilitate process control.

Table 1 Summary of Online Analyzers Installed

Make	Model	Location	Parameter(s)	Range
Hach	AISE	Feed Tank 1	NH_4	0.2 ~ 1000 mgN/L
Hach	LDO2	Reactor	DO Temperature	0 ~ 20 mg/L 0 ~ 50°C
Hach	pHD	Reactor	pH Temperature	0 ~ 14 0 ~ 50°C
Hach	ANISE	Reactor	NH_4 NO_x	0.2 ~ 1000 mgN/L 0.1 ~ 100 mgN/L

Feed Sources

Two feed sources were available to the system: (1) HPOAS Effluent (unchlorinated); and (2) primary effluent ("Stored" and "Fresh"). Characteristics of the feeds are summarized in Table 2:

*Table 2 Feed Characteristics**

Parameter	Unit	HPOAS Effluent	Primary Effluent ("Stored")	Primary Effluent ("Fresh")
COD	mg/L	47.0	218	571
sCOD	mg/L	41.3	86.8	217
TKN	mgN/L	43.6	57.7	68.4
NH_4	mgN/L	41.2	47.4	51.4
NO_x	mgN/L	0.82	0.1	0.1
SOP	mgP/L	0.35	3.0	4.71
TSS	mg/L	6.6	114	249

*Median values observed over the course of the study

The need for PE for this study stems from Kruger's prior experience in operating mainstream ANITA Mox: while MD does not require BOD to remove nitrogen, in Kruger's particular implementation, system capacity can be reduced when the influent biodegradable COD is excessively low ($bsCOD/NH_4\text{-}N < 0.5$). Under such conditions, the developed suspended growth sludge would exhibit poor settling characteristics, consequently limiting the biomass inventory and system capacity.

Operational Strategies for NOB Control

NOB was controlled by a two-prong approach: aeration program and SRT control. Aeration to the reactor was intermittent and adjusted according to effluent ammonia and NO_x concentrations. Aerobic SRT was typically targeted at three days, though this criterion was relaxed when the system was judged to have insufficient biomass inventory.

Sampling and Analysis

Samples were collected and analyzed routinely to assess system performance and to aid operational decisions. Figure 5 shows the five sampling locations for this study: (1) Feed 1 (SE); (2) Feed 2 (PE); (3) Reactor / Mixed Liquor; (4) Clarifier Effluent; and (5) RAS. Table 3 summarizes the sampling schedule, analytical requirements and methods. Composite samples were collected on an hourly basis using composite samplers built in-house. Grab sampling and field analyses were performed daily.

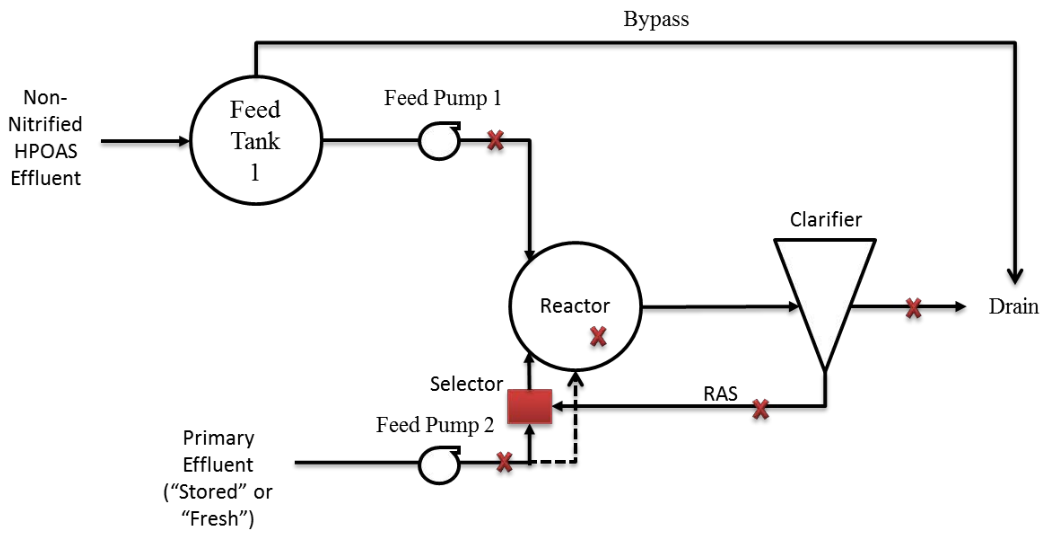

Figure 5 Pilot System Flow Schematic and Sampling Locations (Indicated by Red X's)

Table 3 Sampling Schedule and Required Analyses

Category	Analysis	Sampling Location						Method
		Feed 1	Feed 2	Effluent	Mixed Liquor	RAS	Media	
Oxygen Demands	COD	C-W2	C-W2	C-W2				SM 5220D
	sCOD	C-W2	C-W2	C-W2				SM 5220D
Solids	TSS	C-W2	C-W2	C-D	G-D	G-D		SM 2540D
	VSS				G-W2	G-W2		SM 2540E
	Biomass Density						G-W	Proprietary
Nitrogen	TKN	C-W	C-W	C-W				EPA 351.2
	NH_4	C-D G-D	C-D G-D	C-D G-D				SM 4500NH3G Hach TNT832
	NO_x	C-W	C-W	C-D				SM 4500NO3F
	NO_2	G-D	G-D	G-D				Hach TNT840
	NO_3	G-D	G-D	G-D				Hach TNT835
Phosphorus	Ortho-P	G-W	G-W	G-W				EPA 365.1 Hach TNT843
Activity	Anammox Activity Assay						G-W	Proprietary

C: 24h composites; G: Grabs; D: Daily; W2: Twice per week; W: Once per week

Biomass density testing was based on a protocol provided by Kruger. It involved measuring the TSS attached on a sample of media, followed by normalization by the media's protected surface area. Anammox activity assay was also based on a protocol provided by Kruger. It involved sampling 200 pieces of media, and incubating the media in 2 L of SE at room temperature spiked with $NaNO_2$ to a target NO_2 concentration of ~30 mgN/L. This mixture was stirred by a mechanical mixer with the intention to mix but not to aerate the content. Liquid samples were periodically collected from this mixture and measured for ammonia, nitrite and nitrate. Temperature of the mixture was also recorded at the time of each sampling. The data collected were used to calculate the rates of consumption (e.g., NH_4 and NO_2) corrected to 20°C.

Test Phases

The study consisted of five test phases. Each phase evaluated a particular combination of carbon source and target feed C/N (defined as the soluble biodegradable COD to ammonia-nitrogen ratio, or $bsCOD/NH_4$-N). Interest in feed C/N stemmed from previous reports that this factor can have a significant impact on MD performance (Du et al., 2014). Table 4 summarizes the key operating parameters for the test phases conducted.

Table 4 Test Phases

Phase	Date Range	Carbon Supplement	$Q_{PE}:Q_{SE}$	Target Feed C/N
1	7/24/17 – 9/11/17	Primary effluent (stored)	1:3	0.9
2	9/25/17 – 11/21/17	Primary effluent (stored)	2:1	2.2
3	12/1/17 – 12/15/17	Primary effluent (stored)	1:1	1.7
4	1/1/18 – 2/2/18	Primary effluent (fresh)	1:1	1.7
5	2/17/18 – 3/30/18	Primary effluent (fresh)	1:3	0.9

RESULTS

(1) Treatment Performance

The pilot system's influent and effluent ammonia concentrations throughout the study are shown in Figure 6; similarly, the total nitrogen (TN) concentrations are shown in Figure 7. Over the entire study, median influent and effluent ammonia concentrations were 44.9 mgN/L and 4.6 mgN/L, respectively, corresponding to an ammonia removal efficiency of 90%. Median influent and effluent TN concentrations were 52.7 mgN/L and 15.1 mgN/L, respectively, corresponding to a TN removal efficiency of 71%.

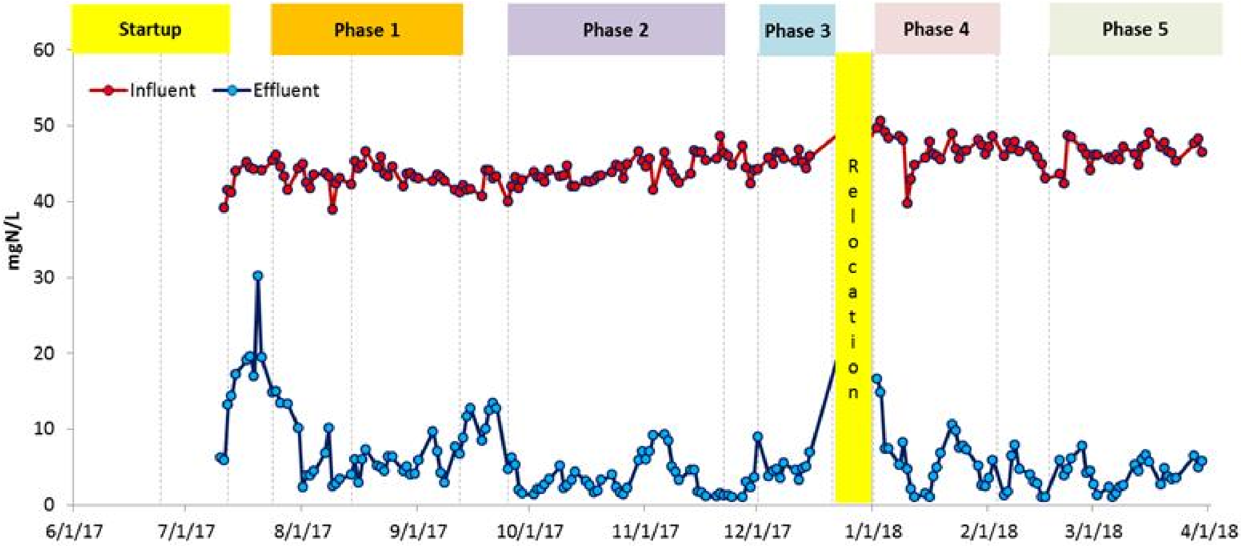

Figure 6 Influent and Effluent NH_4 Concentrations

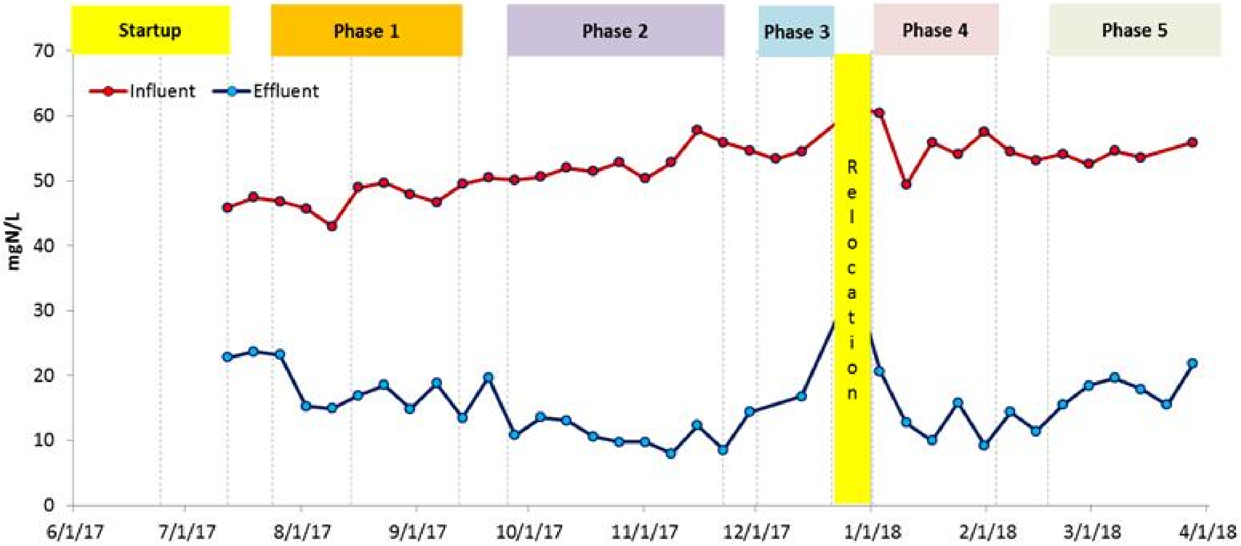

Figure 7 Influent and Effluent TN Concentrations

The pilot system's surface ammonia loading and removal rates throughout the study are shown in Figure 8; similarly the surface TN loading and removal rates are shown in Figure 9. Over the entire study, median surface ammonia loading and removal rates were 1.09 and 0.96 g/m^2-d; median surface TN loading and removal rates were 1.29 and 0.97 g/m^2-d. Additional operational and performance parameters are summarized in Table 5.

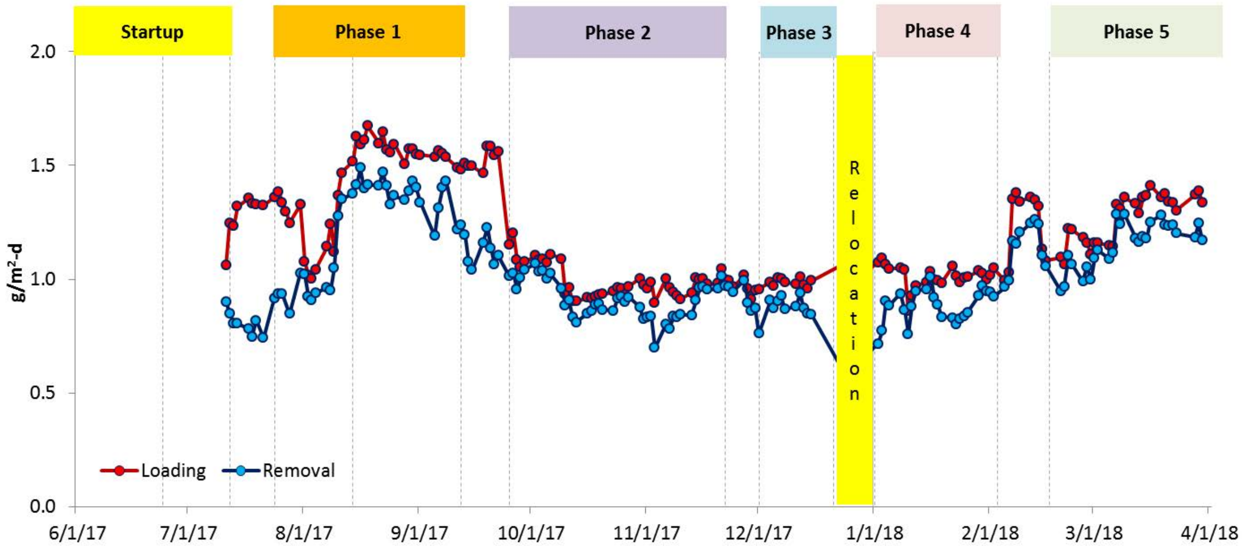

Figure 8 NH_4 Loading and Removal Rates

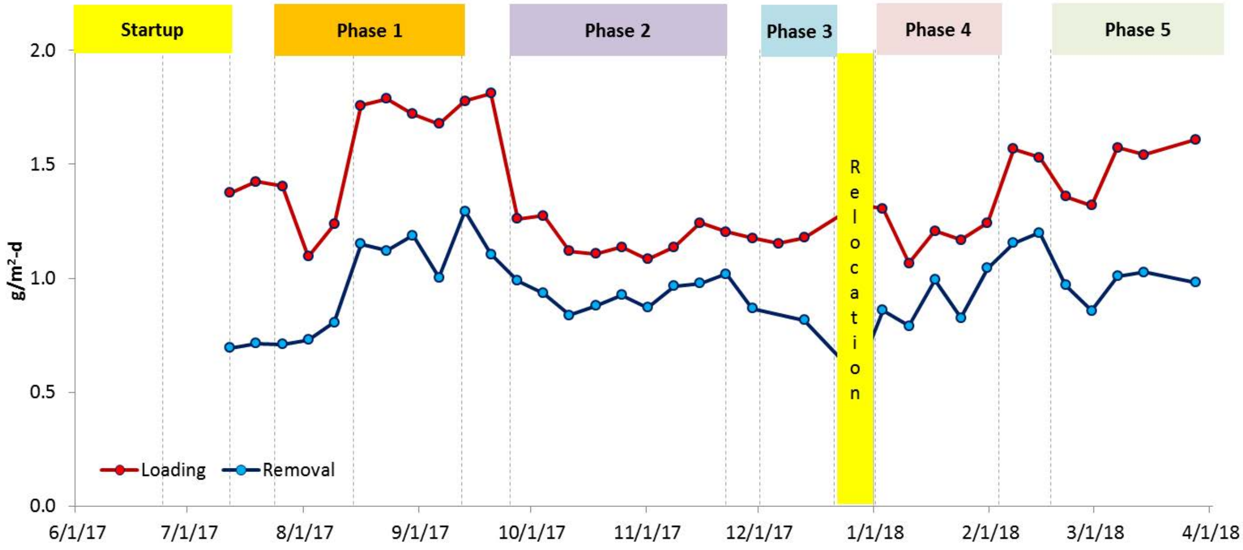

Figure 9 TN Loading and Removal Rates

Table 5 Median Operational and Performance Parameters

Category	Parameter	Unit	Value
General	Duration	days	262
	Temperature	°C	25
	MLSS	mg/L	2,035
Influent	NH_4	mgN/L	44.9
	TN	mgN/L	52.7
	tCOD/NH_4-N		3.8
	bsCOD/NH_4-N		1.1
Effluent	NH_4	mgN/L	4.6
	TN	mgN/L	15.1
Removal Efficiency	NH_4	%	90
	TN	%	71
Removal Rate	NH_4	g/m^2-d	0.96
	TN	g/m^2-d	0.97
Others	NO_3-N_{prod} / NH_4-N_{rem}	%	15.0

Compared with a similar pilot test previously conducted in Paris, France (Thomson et al., 2016), this study encountered higher process temperatures and different influent characteristics, notably a lower carbon to nitrogen ratio. Performance-wise, while this study observed similar NH_4 removal performance (e.g., removal rate and efficiency) as reported by Thomson et al. (2016), TN removal performance and NOB proliferation indicator (i.e., NO_3-N_{prod} / NH_4-N_{rem}) were worse.

(2) Effects of Feed C/N

Impact of feed C/N on treatment performance was evaluated, as it has been reported that the introduction of organics into a deammonification system can promote denitrification in conjunction with deammonification, potentially resulting in higher nitrogen removal efficiency (Du et al., 2014). Manipulation of the feed C/N was achieved by adjusting the ratio of HPOAS effluent and primary effluent in the feed. However during phases 1 through 3, the observed feed C/N was typically lower than the target, and the discrepancy increased as the study progressed (Table 6). It was subsequently discovered that storage of PE resulted in COD loss, presumably due to biodegradation within the storage tanks. To address this issue, the pilot system was relocated between phases 3 and 4 to a new location with access to fresh PE. After the relocation, the observed feed C/N tracked the target much better (Table 6).

Table 6 Target vs. Observed Feed C/N by Test Phase

Phase	Carbon supplement	Q_{PE}:Q_{SE}	Feed C/N	
			Target	Observed
1	Primary effluent (stored)	1:3	0.9	0.6
2	Primary effluent (stored)	2:1	2.2	1.4
3	Primary effluent (stored)	1:1	1.7	0.5
4	Primary effluent (fresh)	1:1	1.7	2.0
5	Primary effluent (fresh)	1:3	0.9	1.1

Impact of feed C/N on treatment performance was assessed by plotting each phase's median feed C/N against corresponding median values of various performance metrics. For example in Figure 10, the median feed C/N for each phase was plotted against the corresponding median TN removal efficiency. The positive correlation between these two parameters suggests that higher feed C/N may result in higher TN removal efficiency. Such correlation is consistent with Du et al. (2014), which proposed that higher feed C/N can promote denitrification, and consequently can contribute to the system's overall nitrogen removal efficiency.

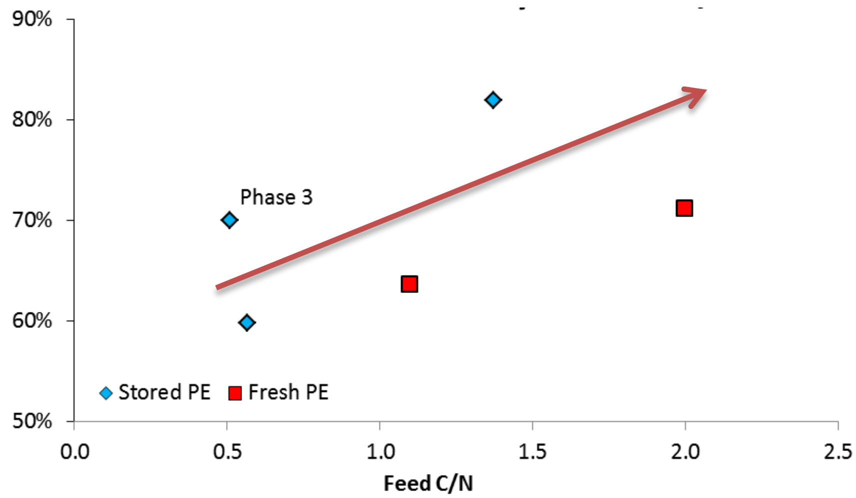

Figure 10 TN Removal Efficiency vs. Feed C/N

In Figure 11, the median feed C/N for each phase was plotted against the corresponding median TN removal rate. The negative correlation between these two parameters suggests that higher feed C/N may result in lower TN removal rate. Note that the point for phase 3 did not fit this trend but was ignored as the phase included a very limited dataset (n=1). The observed correlation may be attributed to higher feed C/N promoting greater heterotrophic activity, which would compete with AOB for oxygen and compete with AnAOB for nitrite, potentially leading to a reduction in the system's TN removal rate.

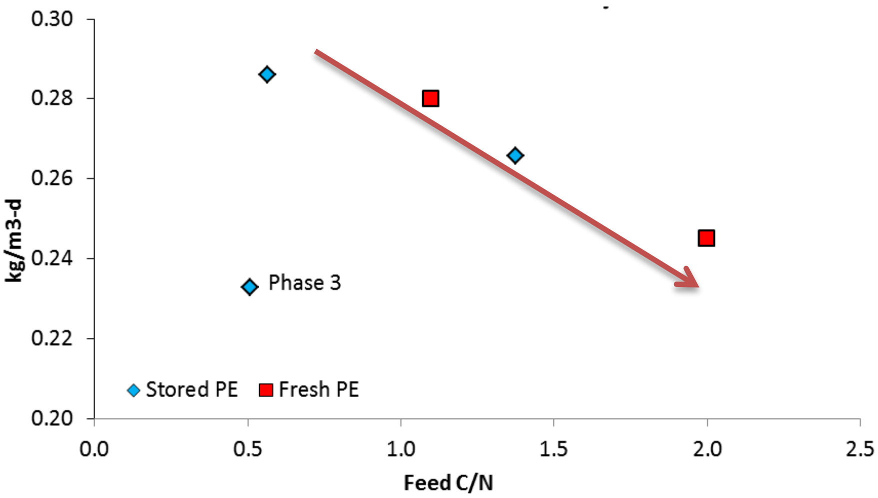

Figure 11 TN Removal Rate vs. Feed C/N

In Figure 12, the median feed C/N for each phase was plotted against the corresponding median mixed liquor SVI. The positive correlation between these two parameters suggests that higher feed C/N may result in higher mixed liquor SVI suggesting poorer settling. Such correlation may have significant design and operational implications, as higher C/N operation may require substantially higher clarifier volume and may be more subject to operational risk with respect to sludge settling.

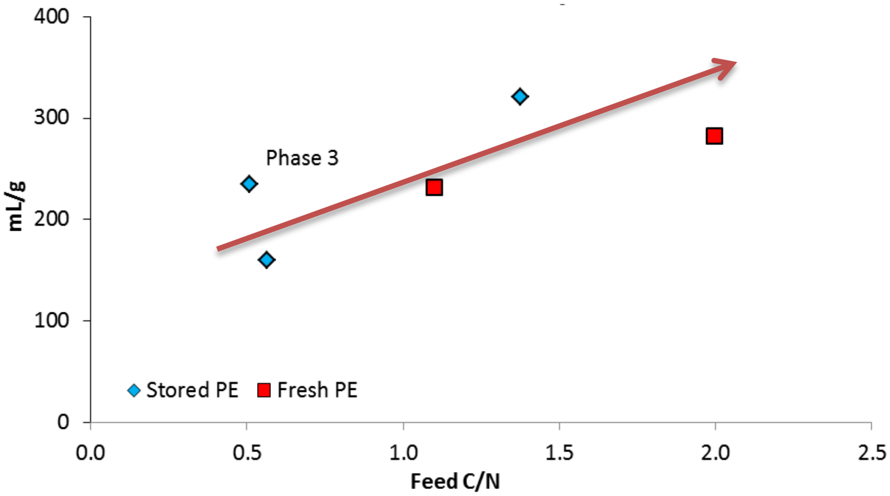

Figure 12 Mixed Liquor SVI vs. Feed C/N

(3) Operational Issues: Sludge Settling

As poor settling sludge would adversely impact the process capacity and economics, efforts were made to investigate potential causes and solutions to this issue. Figures 13 and 14 show the mixed liquor SVI and effluent TSS concentrations from startup through phase 4. As indicated previously, high SVI and effluent TSS concentrations correlated with high feed C/N operation (e.g., Phase 2), particularly when stored PE was utilized. Improvement in sludge settling correlated with installation of a selector (Figure 13). Additional improvement, based on reduction in effluent TSS, correlated with switch from stored to fresh PE (Figure 14).

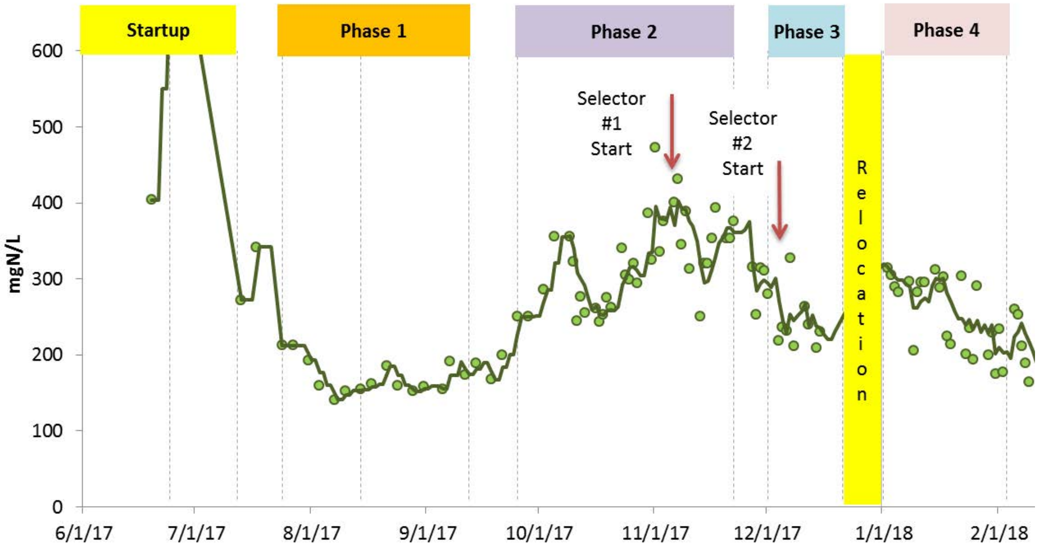

Figure 13 Mixed Liquor SVI. Selector #1 and #2 refer to two different selector designs tested.

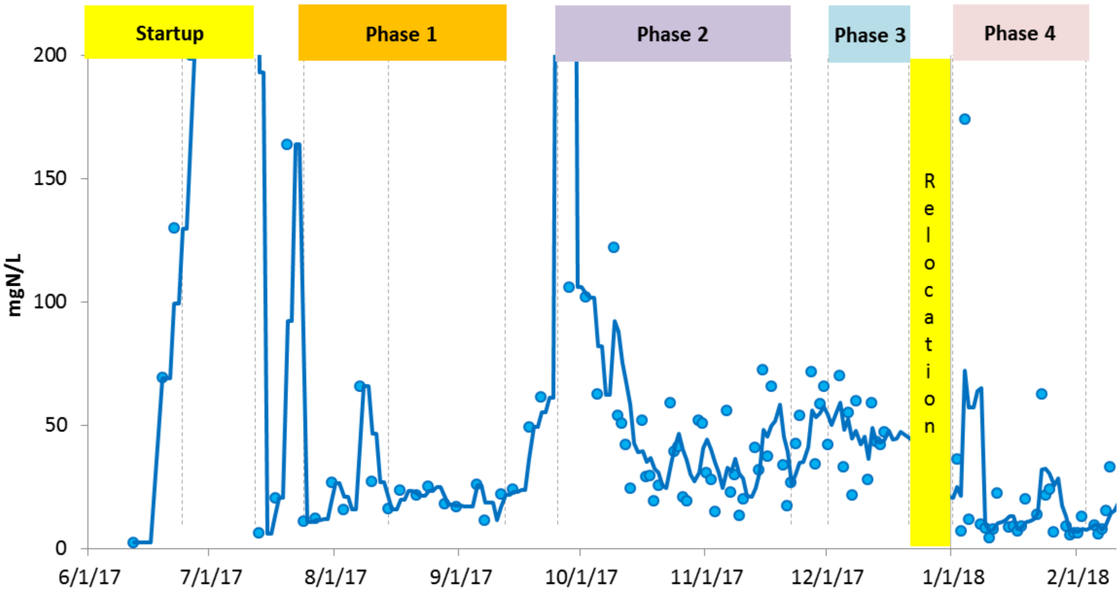

Figure 14 Effluent TSS

(4) Compatibility with Enhanced Biological Phosphorus Removal (EBPR)

Soluble ortho-phosphorus (SOP) was monitored in the pilot system's influent and effluent during the study (Figure 15). After installation of a selector and switching to fresh PE as the supplemental carbon (Phase 4 and later), the system exhibited consistent phosphorus removal, with median effluent SOP of 0.14 mgP/L. SOP profiling indicated phosphorus release within the selector, consistent with EBPR. This result shows that MD can co-occur with EBPR.

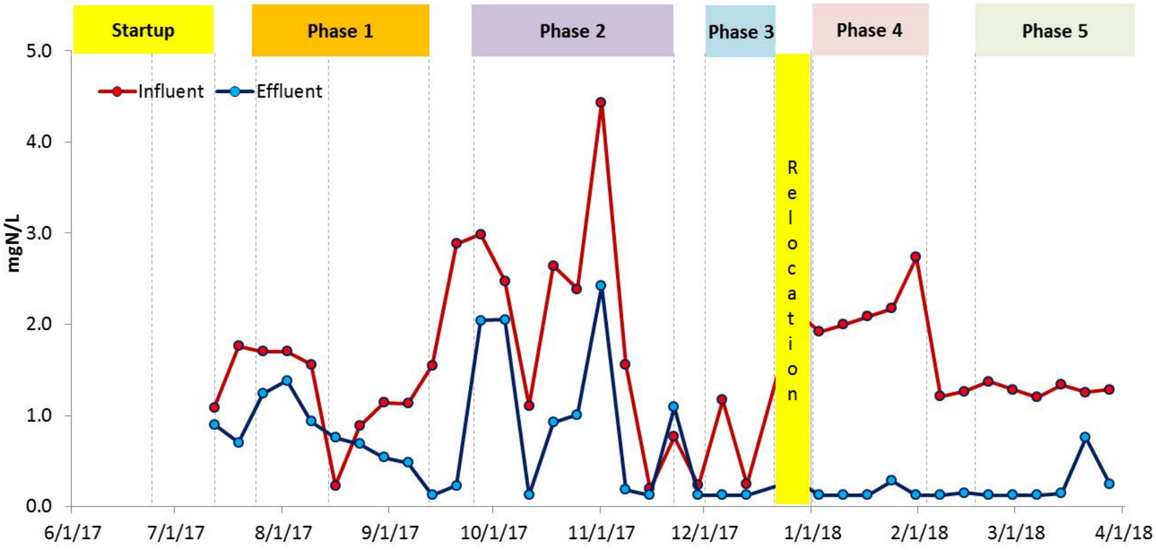

Figure 15 Influent and Effluent SOP

(5) Stability of Media Biomass Density and Anammox Activity

A key challenge to implementing MD stems from AnAOB's slow growth rate, which is further exacerbated under mainstream conditions where lower temperature and ammonia concentration prevail. To help address this challenge, other MD processes (i.e., mainstream DEMON) employ continuous seeding/augmentation from a sidestream system. In mainstream ANITA Mox there is no such augmentation, so the system's stability with respect to biomass population and Anammox activity is of great interest.

During the study, media biomass density was monitored weekly for four months, while Anammox activity was monitored initially and more intensively during the latter seven months. Over the course of the monitoring periods, both media biomass density and Anammox activity exhibited stable/increasing trends (Figures 16 and 17). These observations suggest that a population of active AnAOB can be stably maintained in mainstream conditions, without the need for augmentation from a sidestream system.

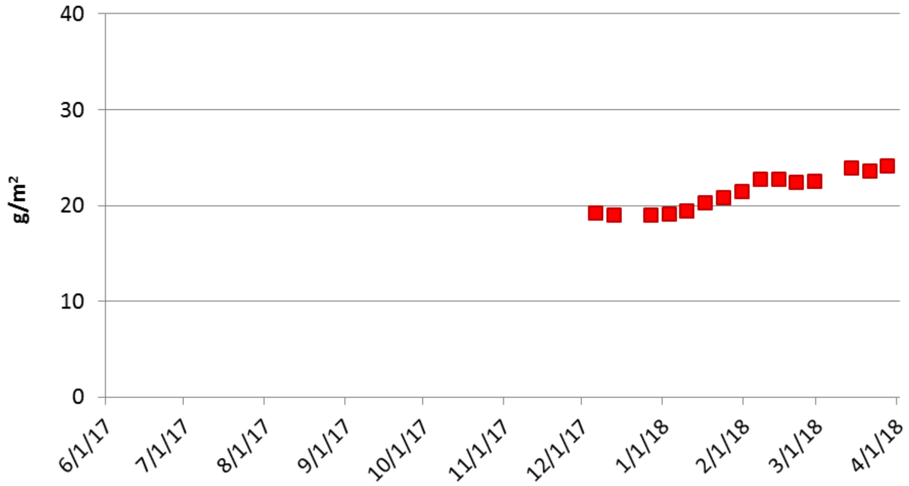

Figure 16 Media Biomass Density

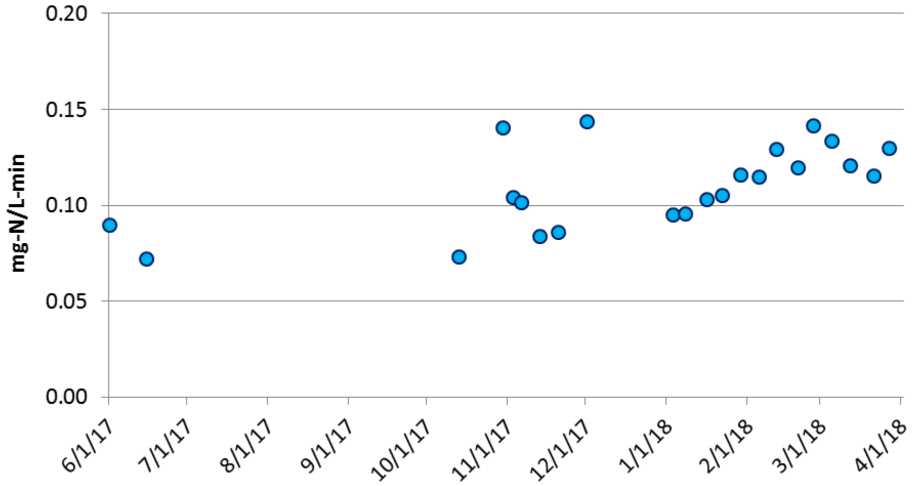

Figure 17 Media AnAOB Activity (NH_4 Consumption Rate, Corrected to 20 ℃)

CONCLUSIONS

In summary, the work being reported extends previous work in the following ways:

1. Verified the ability of ANITA Mox to remove nitrogen from mainstream wastewater at a North American facility;
2. Demonstrated that stable AnAOB activity can be maintained under mainstream conditions without augmentation over a ten-month period;
3. Evaluated the impact of feed C/N on various process performance parameters;
4. Identified sludge settling as a potential operating issue with mainstream ANITA Mox; and
5. Demonstrated an ANITA Mox configuration where EBPR can co-occur with deammonification.

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Attachment 4 – Concurrent Mainstream Deammonification and Phosphorus Removal Using the ANITA Mox Process: Pilot-Scale Evaluation at the Joint Water Pollution Control Plant (JWPCP)

Concurrent Mainstream Deammonification and Phosphorus Removal Using the ANITA Mox Process: Pilot-Scale Evaluation at the Joint Water Pollution Control Plant (JWPCP)

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ABSTRACT

The IFAS (Integrated Fixed-film Activated Sludge) variant of Kruger's ANITA Mox process was tested for thirteen-months at the Joint Water Pollution Control Plant (JWPCP) for mainstream nitrogen removal via deammonification. The system treated mixtures of primary effluent (PE) and non-nitrified secondary effluent (SE) from the facility's High Purity Oxygen Activated Sludge (HPOAS) process. Depending on test conditions, removal efficiency and rate were 60% -85% and 0.20-0.25 kg/m3-d, respectively. Results indicated that higher feed C/N correlated with lower total nitrogen (TN) removal rates and poorer settling sludge. Ammonia and nitrogen removal performance exhibited temperature dependence with an apparent Arrhenius constant (1.1) similar to that reported for ammonium oxidizing bacteria (AOB), which may be the rate limiting step. Under temperate conditions, mainstream ANITA Mox exhibited modest sensitivity to short-term disturbances in dissolved oxygen (DO) and ammonia concentration; recoveries from such disturbances were fast (within 24 hours). The system exhibited strong sensitivity and slow recovery to solids loss, similar to a conventional nitrification/denitrification (NDN) system. Long-term stability (8~10 months) with respect to media biomass density and anaerobic ammonium oxidizing bacteria (AnAOB) activity was demonstrated at temperate temperatures; however AnAOB activity declined substantially following a temperature shock event (delta of 7°C). The installation of an anaerobic selector in the process enabled co-occurrence of enhanced biological phosphorus removal (EBPR) and deammonification. EBPR was demonstrated to be mediated by both polyphosphate accumulating organisms (PAOs) and denitrifying PAOs (DPAOs), with the former being the dominant pathway.

KEYWORDS

Mainstream Deammonification, Anammox, Nitrogen Removal

INTRODUCTION

The Sanitation Districts of Los Angeles County operate the Joint Water Pollution Control Plant (JWPCP), an ocean-discharge wastewater treatment facility with a capacity of 400 MGD located in Carson, CA. The facility employs the High Purity Oxygen Activated Sludge (HPOAS) process with short solids retention time (SRT) for secondary treatment, and consequently does not nitrify nor remove nitrogen from the wastewater. While nitrogen removal is not currently required for the JWPCP, future regulations and reuse requirements may make nitrogen removal necessary. When such a need arises, implementation of nitrogen removal is expected to be costly. In exploring opportunities to reduce cost, the utilization of different biochemical pathways to remove nitrogen was assessed.

Typically, the removal of nitrogen from wastewater is achieved via the nitrification / denitrification (NDN) pathway, which involves nitrification by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), followed by denitrification by denitrifiers (Figure 1, red and blue arrows). This pathway requires the input of Biological Oxygen Demand (BOD) and oxygen (4~6 gBOD/gN and 4.57 gO_2/gN), which can escalate power and chemical costs. An alternative is employing the deammonification pathway (Figure 1 green arrows).

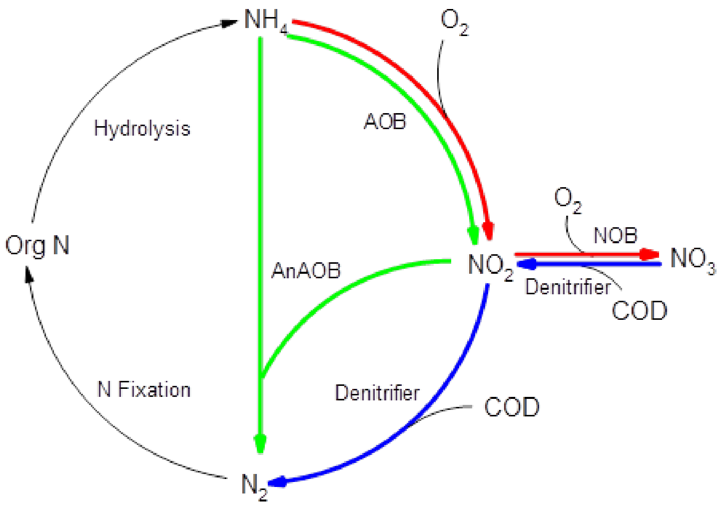

Figure 1. The Nitrogen Cycle. Red: Nitrification Pathway; Blue: Denitrification Pathway; Green: Deammonification Pathway.

Deammonification offers reduced carbon and oxygen requirements compared to conventional NDN. These attributes can translate into substantial O&M cost savings and help facilities achieve energy-neutrality. While the application for deammonification to treat sidestreams (e.g., centrate) is considered mature and has experienced rapid growth, its application to treat the mainstream has lagged. Some success has been reported (Thomson et al., 2016; Lotti et al., 2015); however, difficulties in growing and retaining the key anaerobic ammonia oxidizing bacteria (AnAOB) and repressing the competing NOB, especially at lower process temperatures, remain major barriers.

Kruger developed a mainstream deammonification process (“mainstream ANITA Mox”) based on the Integrated Fixed-film Activated Sludge (IFAS) variant of its ANITA Mox process. The success of this process was previously reported by Thomson et al. (2016) and involved pilot-scale testing at two facilities in France. The following work represents a pilot-scale demonstration at a North American facility and includes the following assessments: the effect of feed C/N (defined as the soluble biodegradable COD to ammonia-nitrogen ratio) on process performance, the impact of temperature on process performance, process robustness to short-term operational disturbances, long-term stability of the key AnAOB organism, and the observation and characterization of Enhanced Biological Phosphorus Removal (EBPR) co-occurring with deammonification.

METHODOLOGY

Pilot System Description

The system contained the following major components: (1) Feed Tanks and Pumps; (2) Reactor; (3) Clarifier with sludge return; (4) Selector; and (5) Instrumentation and Control. Figure 2 shows the pilot system's process flow diagram. Each component is described in more detail in the following subsections.

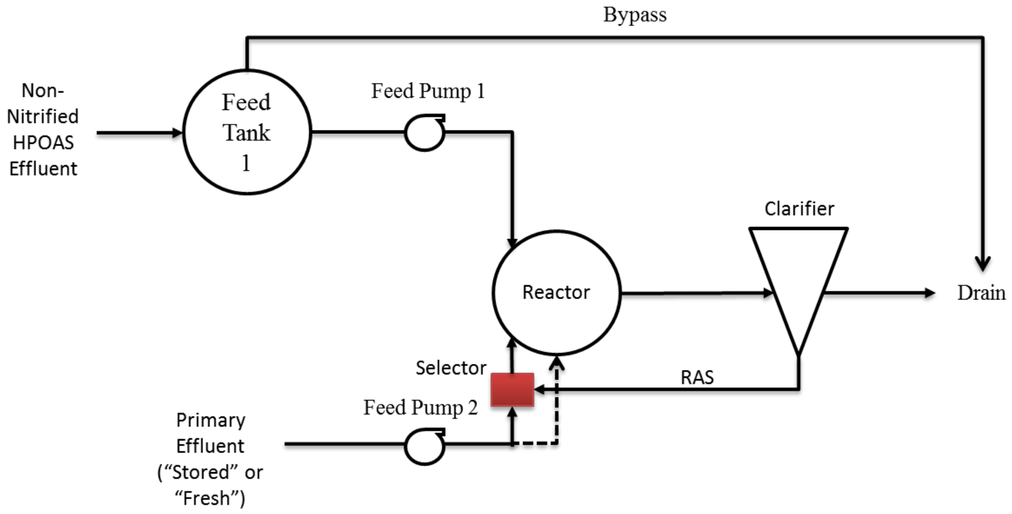

Figure 2. Process Flow Diagram

<u>Feed Tanks and Pumps</u>

The system included feed tanks and pumps to allow feeding of two streams independently. Feed Tank 1 served as a break tank for non-nitrified HPOAS secondary effluent (SE) and operated at a typical working volume of 194 gallons. Two interconnected cylindrical tanks with a combined working volume of 20,000 gallons and a pump-driven recirculation system served as storage for primary effluent (PE), which was transported as needed using tanker trucks. Mid way through the study it was discovered that such storage of PE significantly altered its characteristics, so the pilot system was moved to a location with direct access to fresh PE and SE. Two progressive cavity pumps were used to deliver the feeds to the reactor.

<u>Reactor and Seeded Media</u>

The reactor was a 1,030 gallon stainless steel cylindrical vessel with a typical working volume of 701 gallons. During startup, approximately 295 gallons of seeded AnoxKaldnes K5 media (Figure 3 protected surface area = 800 m^2/m^3), equivalent to a media fill of 37% by volume, was transferred into the reactor. The seeded media originated from a full-scale sidestream reactor at the South Durham Wastewater Treatment Plant (Durham, NC). The suspended growth sludge was not seeded, but instead grown on-site during the start-up phase. Media retention was achieved by screens installed on the tank outlets. Process air was provided by a mobile air compressor with moisture knockout by two air chillers. Process air flow was regulated by two inline air mass flow controllers and distributed within the reactor via a medium bubble aeration grid located at the bottom of the reactor. The reactor was also equipped with a mechanical mixer that was used to facilitate mixing during non-aerated periods.

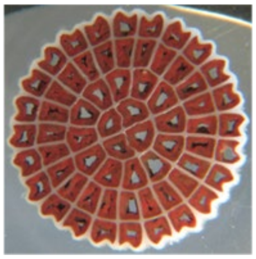

Figure 3. AnoxKaldnes K5 media

Clarifier

A lamella clarifier, with a working volume of 480 gallons, was installed downstream of the reactor. Sludge was recycled by pumping from the sludge collection box of the clarifier back to the reactor. While the system was equipped with sludge wasting capability from the reactor (mixed liquor) and the clarifier (returned activated sludge), the former was used for better control of the sludge wasting rate.

Selector

A selector was installed mid-way through the study to help address sludge settling issues. The selector tank consisted of a cylindrical tank with a typical working volume of 150 gallons. Returned activated sludge (RAS) and PE were routed to the selector, slowly mixed, and gravity fed back to the reactor tank with a typical hydraulic retention time (HRT) of 30-60 minutes.

Instrumentation and Control

A Programmable Logic Controller (PLC) with a customized Human Machine Interface (HMI) maintained the system within the prescribed parameters and set points. The following mixed liquor parameters were monitored online: pH (Hach; pHD), DO (Hach; LDO2), NH_4 and NO_x (Hach; ANISE).

Feed Sources

Three sources of feed were available to the system: (1) SE; (2) Stored PE; and (3) Fresh PE. Characteristics of the feeds are summarized in Table 1.

Table 1. Feed Characteristics*

Parameter	Unit	HPOAS Effluent	Primary Effluent (“Stored”)	Primary Effluent (“Fresh”)
COD	mg/L	56.0	221	620
sCOD	mg/L	43.5	88.1	232
TKN	mgN/L	45.2	57.7	68.4
NH_4	mgN/L	42.9	48.1	51.3
NO_x	mgN/L	0.82	<0.10	<0.10
SOP	mgP/L	0.23	3.40	5.10
TSS	mg/L	13.6	112	276

* Median values observed during the study

Operational Strategies for NOB Control

NOB was controlled by a two-prong approach: intermittent aeration and SRT control. Intermittent aeration was enabled by the software/PLC which allowed the operator to adjust the aeration timing. SRT control was achieved by controlling the wasting rate. The aerobic SRT was typically targeted at three days, though this criterion was relaxed when the system was judged to have insufficient biomass inventory.

Test Phases

The study consisted of eight test phases. Phases 1 through 5 evaluated the effects of feed C/N on process performance. Phases 6 and 7 examined the impact of process temperatures on process performance. Phase 8 explored the system's robustness to short-term operational disturbances. Table 2 summarizes the key operating parameters for the test phases conducted. The methods for feed C/N, low temperature, and robustness testing are described in the subsequent subsections.

Table 2. Test Phases

Phase	Date Range	Carbon Supplement	Q_{PE}:Q_{SE}	Target Feed C/N	Target Temp (°C)
1	7/24/17 – 9/11/17	Primary effluent (stored)	1:3	0.9	Ambient
2	9/25/17 – 11/21/17	Primary effluent (stored)	2:1	2.2	
3	12/1/17 – 12/15/17	Primary effluent (stored)	1:1	1.7	
4	1/1/18 – 2/2/18	Primary effluent (fresh)	1:1	1.7	
5	2/17/18 – 3/30/18	Primary effluent (fresh)	1:3	0.9	
6	5/15/18 – 7/6/2018	Primary effluent (fresh)	1:3	0.9	15
7	7/14/18 – 7/23/18	Primary effluent (fresh)	1:3	0.9	19
8	7/31/18 – 8/26/18	Primary effluent (fresh)	1:3	0.9	19

Feed C/N Testing

During Phases 1-5, PE and SE feed flows were adjusted to yield various target C/N ratios. During Phases 1 through 3, the observed feed C/N was typically lower than the target, and the discrepancy increased as the study progressed (Table 3). It was subsequently discovered that storage of PE resulted in COD loss, presumably due to biodegradation within the storage tanks. To address this issue, the pilot system was relocated between phases 3 and 4 to a new location with access to fresh PE. After the relocation, the observed feed C/N tracked the target more closely. Because stored and fresh PE were essentially different feeds, they were also assessed for their impact on process performance.

Table 3 Target vs. Observed Feed C/N by Test Phase

Phase	Carbon supplement	Q_{PE}:Q_{SE}	Feed C/N	
			Target	Observed
1	Primary effluent (stored)	1:3	0.9	0.6
2	Primary effluent (stored)	2:1	2.2	1.4
3	Primary effluent (stored)	1:1	1.7	0.5
Relocation				
4	Primary effluent (fresh)	1:1	1.7	2.0
5	Primary effluent (fresh)	1:3	0.9	1.1

Low Temperature Testing

Phases 6 and 7 assessed the impact of temperature on process performance, particularly below the range encountered during the initial testing. To minimize other confounding factors, the target feed C/N utilized in Phase 5 was held constant for Phases 6 and 7, while the process temperature was changed by chilling the SE prior to it being fed to the reactor. The modified process flow diagram is shown in Figure 4; the target reactor temperature for each phase in summarized in Table 2

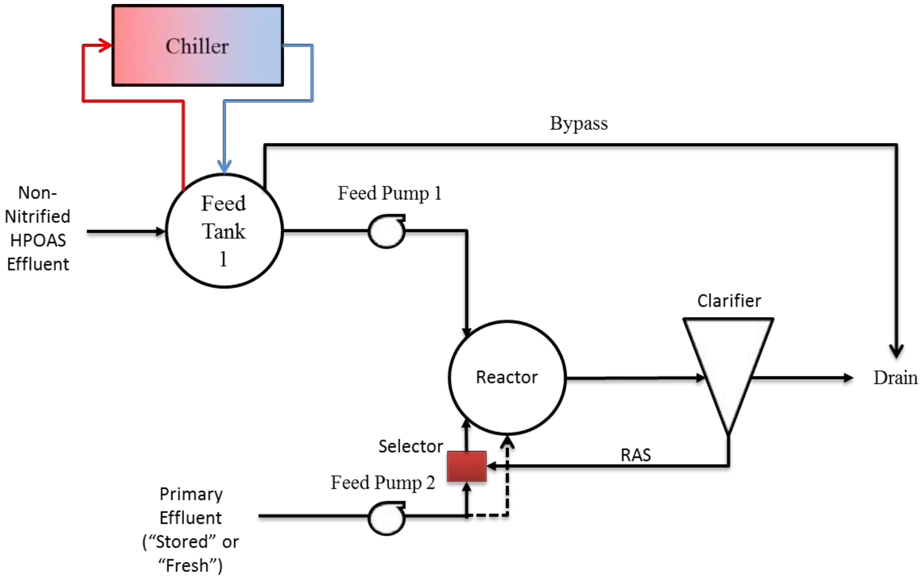

Figure 4. Process Flow Diagram (With Chiller)

Robustness Testing

Phase 8 assessed the system's ability to regain its treatment performance after being subjected to short-term disturbances commonly encountered at wastewater treatment plants. Five scenarios were examined, including under-aeration, over-aeration, under-feed, over-feed, and solids loss. Table 4 provides additional details on the specific testing parameters for each scenario examined.

Table 4. Robustness Testing – Scenarios Examined

Phase	Parameter	Test Conditions	Scenario
8-A	DO	DO=0.5mg/L for 24 hours	Under-aeration
8-B	DO	DO=2.0mg/L for 24 hours	Over-aeration
8-C	Feed	Effluent $NH_4 < 1$mg-N/L for 24 hours	Underfeed
8-D	Feed	Effluent $NH_4 > 10$mg-N/L for 24 hours	Overfeed
8-E	MLSS	MLSS drop to 1000 mg/L	Solids loss

The testing conditions selected were well outside of typical operating ranges but were considered reasonable "edge-cases". The duration of each disturbance was selected to be 24-hours as equipment failures at this facility are typically resolved within that timeframe. Scenarios involving aeration were implemented by altering the system's DO set point. Scenarios involving feed were implemented by altering the system's feed rate. The scenario involving solids loss was implemented by wasting sufficient mixed liquor until the target MLSS concentration was achieved. Subsequent to each disturbance, process performance was monitored at 8-hour intervals until the recovery criterion was met or exceeded.

Two metrics were used to assess the system's performance during and after each disturbance event: NH_4 and TIN volumetric removal rates (VRR). Effect of each disturbance event was determined by the performance reduction (Δp) and the recovery time ($t_{recovery}$) relative to the baseline. Baseline performance was defined as the 14-day median NH_4 and TIN VRRs before robustness testing began. Performance reduction (Δp) was defined as the maximum performance loss from baseline. Recovery time ($t_{recovery}$) was defined as the time required for the performance to reach a recovery threshold, defined as 95% of baseline. A robust process exhibits both small Δp and $t_{recovery}$.

Sampling and Analysis

Samples were collected and analyzed routinely to assess system performance and to aid operational decisions. Samples collected included: (1) SE; (2) PE; (3) Clarifier Effluent; (4) Mixed Liquor; (5) RAS; and (6) Media. Grab samples were collected by the operator; composite samples were collected with an autosampler on an hourly basis and stored at 4°C until they were analyzed. Table 5 summarizes the sampling and analysis program conducted. Testing involving proprietary methods is described in more detail below.

Table 5. Summary of the Sampling and Analysis Program

Category	Analysis	Sampling Location						Method
		SE	PE	Effluent	Mixed Liquor	RAS	Media	
Oxygen Demands	COD	C-W2	C-W2	C-W2				SM 5220D
	sCOD	C-W2	C-W2	C-W2				SM 5220D
Solids	TSS	C-W2	C-W2	C-D	G-D	G-D		SM 2540D
	VSS				G-W2	G-W2		SM 2540E
	Biomass Density						G-W	Proprietary
Nitrogen	TKN	C-W	C-W	C-W				EPA 351.2
	NH_4	C-D G-D	C-D G-D	C-D G-D				SM 4500NH3G Hach TNT832
	NO_x	C-W	C-W	C-D				SM 4500NO3F
	NO_2	G-D	G-D	G-D				Hach TNT840
	NO_3	G-D	G-D	G-D				Hach TNT835
Phosphorus	Ortho-P	G-W	G-W	G-W				EPA 365.1 Hach TNT843
Activity	Anammox Activity Assay						G-W	Proprietary

C: 24h composites; G: Grabs; D: Daily; W2: Twice per week; W: Once per week

Biomass Density Tests

To assess the system's stability with respect to the retention and maintenance of the AnAOB biofilm, media biomass density tests were conducted weekly for eight months. Media biomass density testing was conducted by randomly sampling 50 pieces of media from the reactor, followed by gravimetrical determination of the biomass dry mass. The results are normalized by the media's protected surface area.

Batch Activity Assays

AnOB activity assays were performed during the last 10 months of the study to measure AnAOB activity and stability over time. EBPR activity assays were also performed to characterize the observed phosphorus removal, specifically the activity of polyphosphate accumulating organisms (PAOs) and denitrifying PAOs (DPAOs). The methods used to perform batch activity assays are described in further detail below.

(1) AnAOB Activity Assays

AnAOB activity assay solution was prepared by equilibrating 2L of SE to reactor temperature and spiking it with $NaNO_2$ to a concentration of 30 mg-N/L. Two hundred pieces of media were transferred from the reactor to the assay solution and mixed at 60 rpm. Liquid samples were collected from the mixture every 30 minutes and immediately filtered and analyzed for ammonia (Hach; TNT832), nitrite (Hach; TNT840) and nitrate (Hach; TNT835). Temperature of the assay solution was monitored and recorded throughout the assay. The data collected were used to estimate the Anammox rates on the basis of NH_4 or NO_2 consumption. Average temperature during the assay was used to standardize the observed rates to 20°C to allow comparison across different assays.

(2) PAO Activity Assays

PAO activity assay solution was prepared by collecting 2L of mixed liquor from the selector and spiking it with acetate to a concentration of 100 mg/L. The solution was stirred at 30 rpm and samples were collected at t=0, 20, 40, 60, and 80 minutes. After 100 minutes, the solution was aerated via an aquarium stone. The subsequent aerobic samples were taken at t=100, 120, 140, 160, 180 minutes. Immediately after collection, each sample was filtered and analyzed for orthophosphate (Hach; TNT845). No temperature control/correction was applied throughout each test.

(3) DPAO Activity Assays

The method for determining the DPAO activity was similar to the procedure outlined for PAOs. As before, 2L of mixed liquor was spiked with acetate, the solution was mixed at 30 rpm, and samples were collected at t=0, 20, 40, 60, and 80 minutes. Instead of supplying oxygen after 100 minutes, the solution was spiked with sodium nitrate to achieve a concentration of 168 mg NO_3/L. The subsequent anoxic samples were then taken at t=100, 120, 140, 160, 180 and all samples were filtered immediately and analyzed for orthophosphate (Hach; TNT845) and nitrate (Hach; TNT835). No temperature control/correction was applied throughout each test. Typically the PAO and DPAO activity assays were performed side-by-side.

RESULTS

Effects of Feed C/N on Process Performance

The effect of feed C/N on process performance was of interest because previous reports indicated that the introduction of organics into a deammonification system may promote denitrification in conjunction with deammonification, potentially resulting in higher nitrogen removal efficiency (Du et al., 2014). In addition, it was thought that higher feed C/N would promote additional heterotrophic bacteria growth, which may then promote the formation of better settling sludge and consequently increase the system's capacity. As such, the impact of feed C/N on treatment performance was assessed during Phases 1 through 5.

Figure 5 shows the average TN removal efficiency observed during each phase plotted against the corresponding feed C/N. Note that the dataset from Phase 3 was limited (n=1) and may not represent steady state conditions. The figure shows that higher feed C/N correlated with higher TN removal efficiency. This observation appears to be consistent with hypothesis described in Du et al. (2014), where higher feed C/N would promote additional denitrification, and consequently contribute to the system's overall nitrogen removal efficiency.

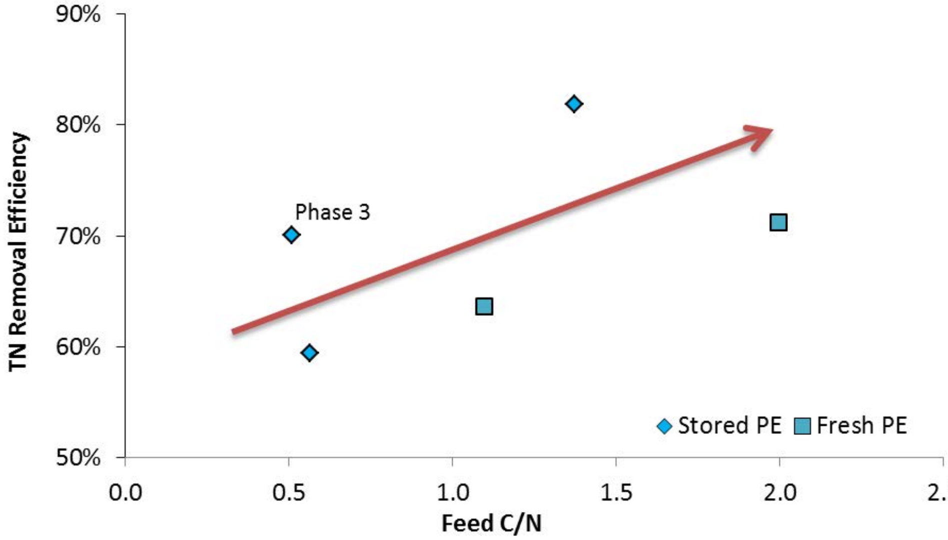

Figure 5. TN Removal Efficiency vs. Feed C/N

Figure 6 shows the average TN removal rate observed during each phase plotted against the corresponding feed C/N. Interestingly, the figure shows an opposite trend than in Figure 5: that higher feed C/N correlated with lower TN removal rate. This discrepancy can be explained by the TN loading rate not being equal across all phases. From a design perspective, the removal rate results would be more relevant. Based on these results, it appears that higher feed C/N reduced rather than enhanced the system's nitrogen removal performance. The observation may be explained by the additional heterotrophs competing with AOBs (for oxygen) and with AnAOB (for nitrite) rather than working cooperatively.

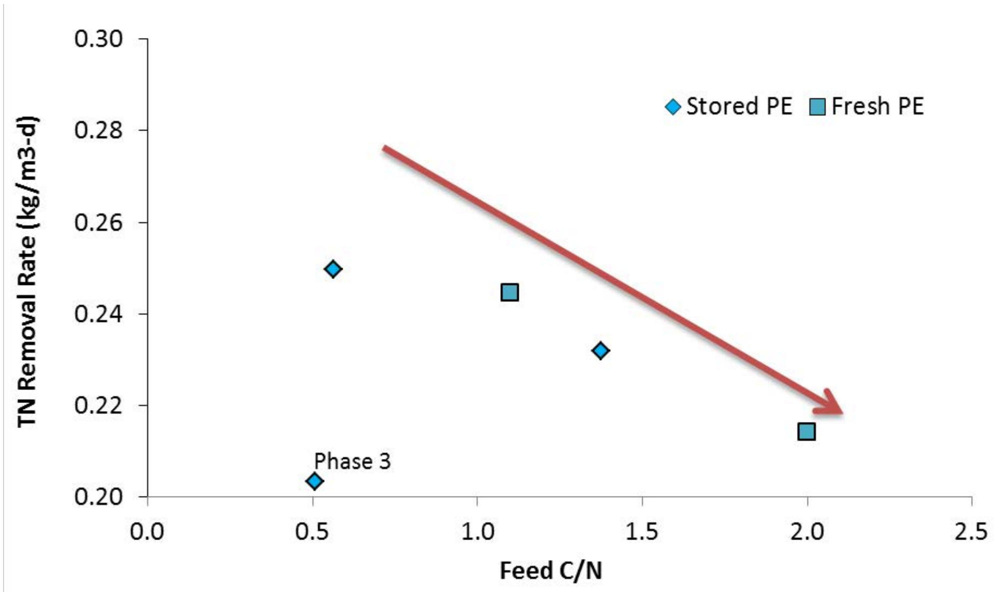

Figure 6. TN Removal Rate vs. Feed C/N

Figure 7 shows the average mixed liquor SVI observed during each phase plotted against the corresponding feed C/N. The figure shows that higher feed C/N correlated with higher mixed liquor SVI and consequently poorer sludge settling characteristics. Such correlation may have significant design and operational implications, as higher C/N operation may require substantially higher clarifier volume and may be subject to greater operational risk with respect to sludge settling.

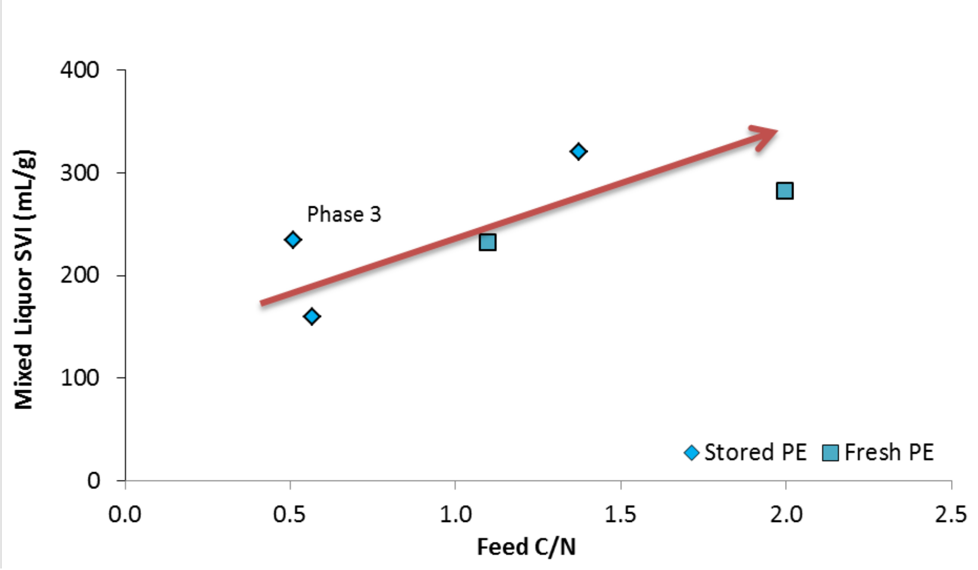

Figure 7. Mixed Liquor SVI vs. Feed C/N

Note that in Figures 5 through 7, the PE source (stored or fresh) associated with each data point is also specified. In general, the relationships described previously between the feed C/N and various process performance metrics (i.e., TN removal efficiency and rate and mixed liquor SVI) applied regardless of the PE source. This observation suggests that the predominant factor influencing these performance metrics was likely the feed C/N.

Effects of Reactor Temperature on Process Performance
To assess the applicability of mainstream ANITA Mox for facilities that may encounter colder process conditions, the pilot system's feed water was chilled to below ambient temperature during Phases 6 and 7. Ammonia and TN removal rates during Phases 6 and 7 were compared to those from Phase 5. The median reactor temperature during Phase 5 was 22.3°C ± 1.5°C while median reactor temperatures for Phases 6 and 7 were 15.8°C ± 1.6°C and 19.0°C ± 0.4°C respectively.

Figures 8 and 9 show the observed ammonia and TN removal rates for Phases 5 through 7, plotted against the reactor temperatures.

Ammonia and TN removal rates exhibited strong temperature dependence, corresponding to an apparent Arrhenius coefficient of 1.1 (Figure 8 and Figure 9; red lines). This value is similar to those reported for AOB (Hwang et al., 2007; Ficara et al., 2000). This observation suggests that the rate-limiting step for mainstream ANITA Mox is likely biological rather than physico-chemical (i.e., mass transfer of substrate).

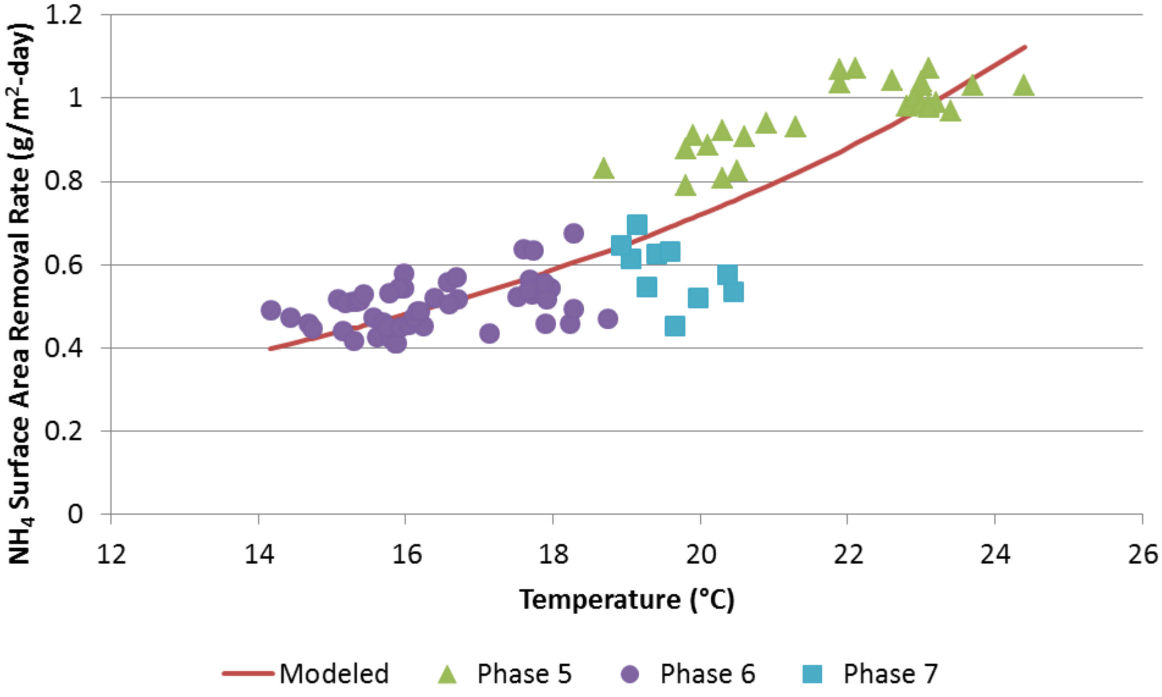

Figure 8. Surface Area Removal Rate vs. Reactor Temperature

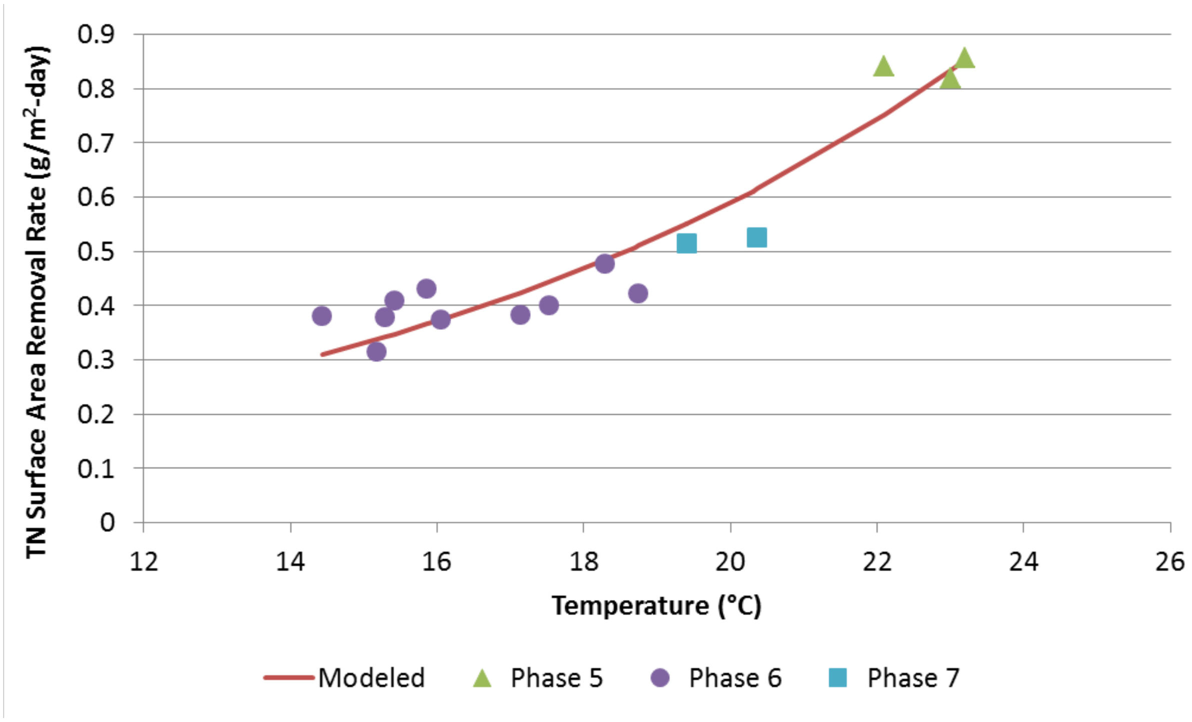

Figure 9. Nitrogen Surface Area Removal Rate vs. Reactor Temperature

Short-Term Process Stability - Robustness Testing

The process' robustness was evaluated by subjecting the system to various disturbances as described previously. Test conditions included: under-aeration, over-aeration, underfeeding, overfeeding, and solids loss. Table 6 summarizes the disturbances that were tested along with the resulting performance reductions (ΔP) and recovery times ($T_{recovery}$).

Table 6. Summary of Robustness Testing Results

				ΔP		$T_{recovery}$ (hr)	
Phase	Parameter	Disturbance	Scenario	NH_4 (%)	TIN (%)	NH_4	TIN
8-A	DO	DO=0.5mg/L for 24 hours	Under-aeration	-14	-20	8	8
8-B	DO	DO=2.0mg/L for 24 hours	Over-aeration	9*	-11	0	8
8-C	Feed	Effluent NH4 <1mg/L for 24 hours	Underfeed	-16	-18	8	8
8-D	Feed	Effluent NH4 >10mg/L for 24 hours	Overfeed	-3	-18	0	8
8-E	MLSS	MLSS drop to 1000mg/L	Solids loss	-25	-23	>144	>144

*positive values represent a performance gain

For the under-aeration scenario (Phase 8A), both ammonia and TIN VRRs declined, which can be explained by reduced AOB activity due to DO limitation.

For the over-aeration scenario (Phase 8B), ammonia VRR increased likely due to the higher DO promoting AOB activity; however TIN VRR decreased likely due to the higher DO inhibiting AnAOB activity.

For the underfeeding scenario (Phase 8C), both ammonia and TIN VRRs declined, which can be explained by reduced AOB activity due to NH_4 limitation.

For the overfeeding scenario (Phase 8D), NH_4 VRR remained relatively unaffected. This observation can be explained by the baseline NH_4 levels being sufficiently higher than the half saturation constant for ammonia ($K_{s,NH4}$) for AOB, therefore further increase in the NH_4 levels did not translate into substantially higher AOB activity. Surprisingly, TIN VRR declined modestly during overfeeding. Closer inspection of the data in terms of the activities of the major groups in the system (assuming negligible denitrifier activity) suggested that the decline in TIN VRR was due to proliferation in NOB activity at the expense of AnAOB activity (Figure 10).

For the last scenario tested (Phase 8E), a substantial amount of the mix liquor was deliberately wasted. Not surprisingly this scenario resulted in substantial loss in both ammonia and TIN VRRs, which can be explained by substantial loss in AOB activity.

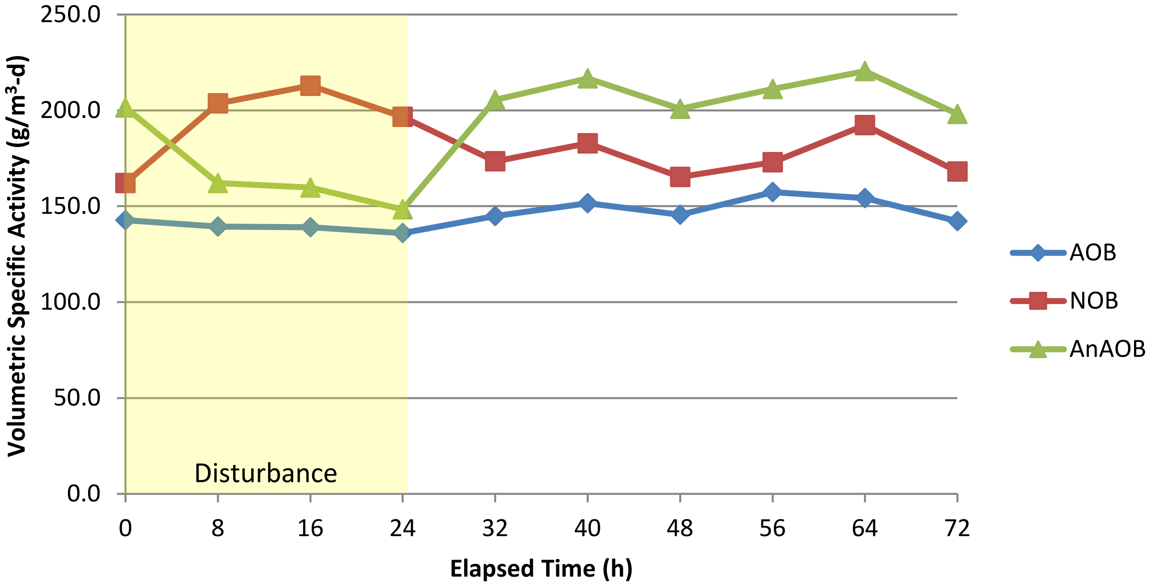

Figure 10. Nitrogen Utilization Rate of the Major Groups during Phase 8-D. Overfeeding

From the recovery time perspective, for the first four scenarios tested, process performance returned to normal within eight hours. For the solids loss scenario, the system had not fully recovered by the time the test was terminated (6 days). However, such lengthy recovery is to be expected even for conventional NDN systems.

Long-Term Process Stability - Media Biomass Density

A key challenge to implementing mainstream deammonification stems from AnAOB's slow growth rate, which is further exacerbated under mainstream conditions where lower temperature and ammonia concentration prevail. To help address this challenge, other mainstream deammonification processes (i.e., mainstream DEMON) employ continuous seeding/augmentation from a sidestream system which helps maintain the AnAOB biomass population and activity within the process. In mainstream ANITA Mox there is no such augmentation, so understanding the system's stability with respect to AnAOB biomass population and activity was of great importance.

During an eight-month period of the study, media biomass density was routinely monitored. The media biomass density exhibited stable/increasing trends at ambient reactor temperatures (Figure 11, Phases 3-5; average temperature = 23°C). A small, but observable decrease in biomass density occurred at the beginning of Phase 6 when the reactor temperature was lowered to 15°C; and an apparent increase in biomass density coincides with the start of Phase 7 when the reactor temperature was increased to 19°C.

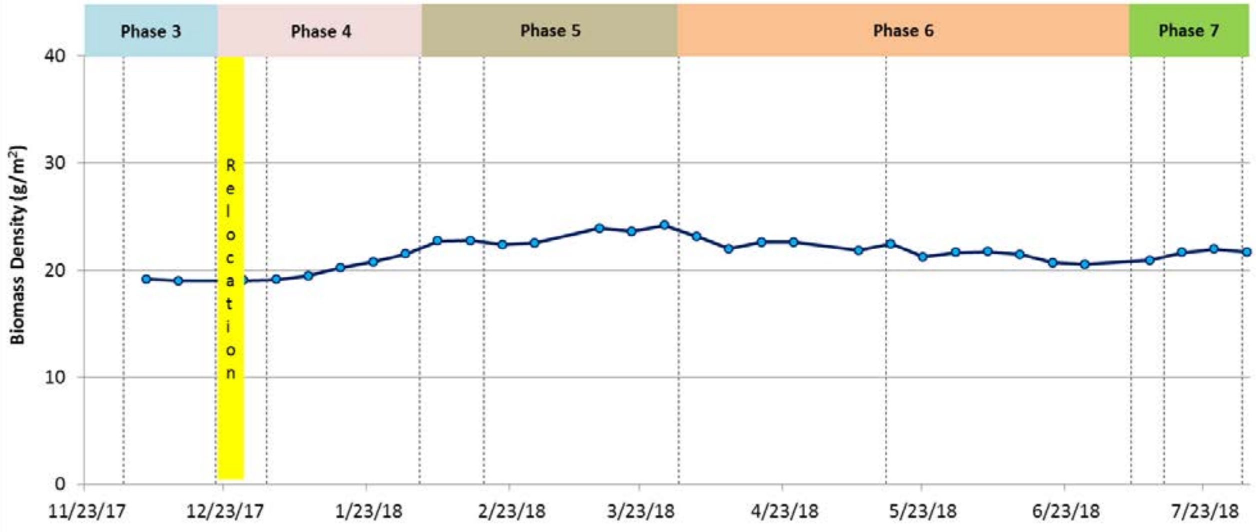

Figure 11. Media Biomass Density

Long-Term Process Stability - Anammox Activity Testing

While media biomass density remained stable over the eight-month monitoring period, the result did not necessarily indicate maintenance of AnAOB activity. To fully assess the long term-stability of the ANITA Mox process, media were periodically removed from the reactor and assayed for their AnAOB activity during a similar period lasting about 10 months. The results were expressed in terms of the media's NH_4 and NO_2 utilization rates, corrected to 20°C assuming an Arrhenius constant of 1.1. The temperature correction enabled an assessment of the AnAOB activity without temperature as a confounding variable.

Media collected from the pilot system exhibited stable Anammox activity at warm/temperate process temperatures (Figure 12 and Figure 13, startup through Phase 5; average temperature =25°C). The activity declined sharply after the process temperature was decreased to 16°C

(Phase 6). Note that as the activity measurements were already corrected for temperature effect, this decline in activity was attributed to AnAOB inactivation. Subsequent elevation of the process temperature (Phase 7; average temperature = 19°C) did not restore the activity, further supporting that the prior activity decline was irreversible. It was speculated that the temperature shock between Phase 5 and 6 may have irreversibly inactivated a substantial fraction of the AnAOB population. This aspect should be examined in more detail to improve understanding of the issue's pervasiveness, which may be particularly relevant for facilities that undergo seasonal temperature transitions. Nonetheless, it should be noted that under temperate mainstream conditions, ANITA Mox appears capable of sustaining AnAOB activity for long periods without continuous reseeding from a sidestream process as is required in other mainstream deammonification processes.

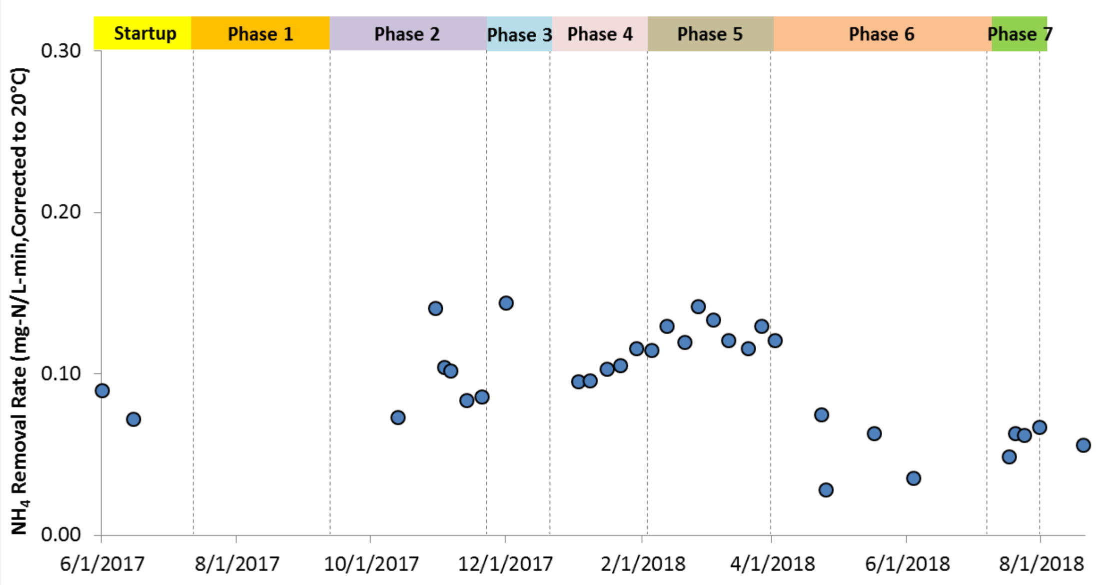

Figure 12. Media AnAOB Activity (NH_4 consumption rate, corrected to 20°C)

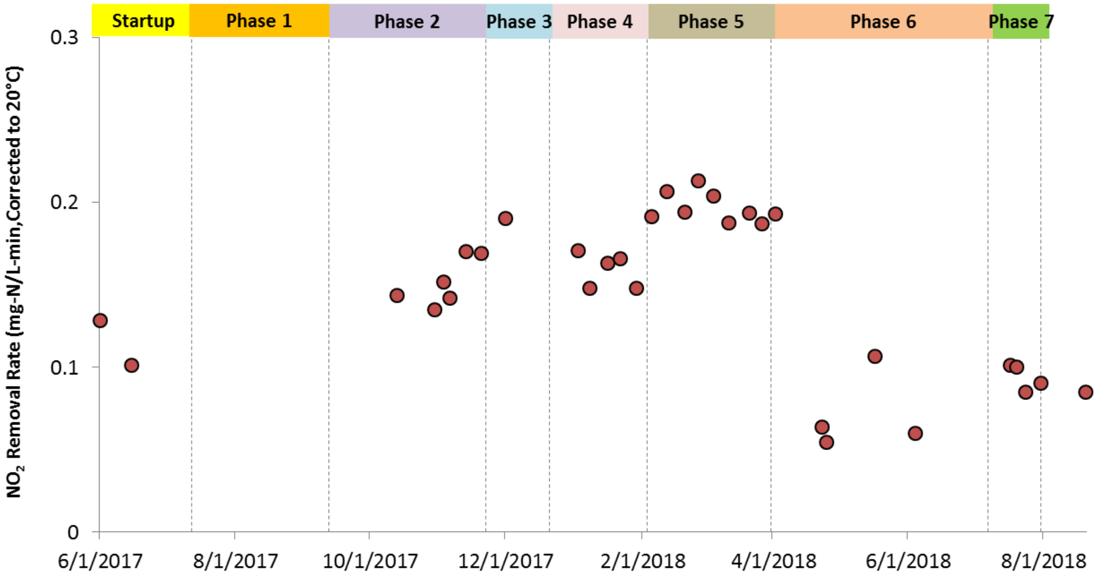

Figure 13. Media AnAOB Activity (NO_2 consumption rate, corrected to 20°C)

Concurrent Enhanced Biological Phosphorus Removal

While relocating the pilot in January 2018 for access to fresh PE, an anaerobic selector was installed to improve sludge settling. Prior to its relocation, the pilot system achieved consistent nitrogen removal but inconsistent phosphorus removal. After the relocation, the pilot exhibited consistent phosphorus removal (Figure 14). This observation could be potentially explained by EBPR. Although co-occurrence of deammonification and EBPR has been previously reported in an incidental deammonification system (Cao et al., 2017), to the authors' knowledge it has not been reported in a system designed for deammonification. As EBPR typically requires an abundance of volatile fatty acids (VFAs), its occurrence in a low C/N feed environment (1.1-2.0) was unexpected. Additional data analysis and assays were conducted to verify the observation and the mechanism of phosphorus removal, which are described below.

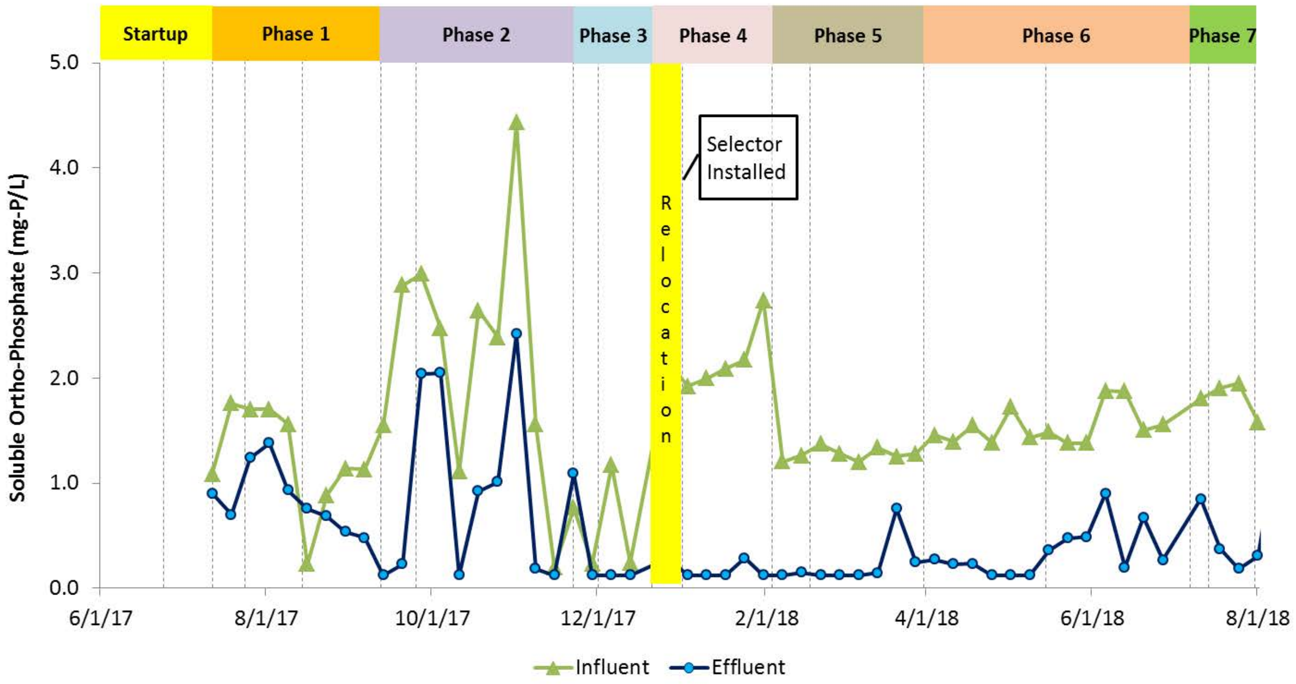

Figure 14. Influent and Effluent SOP

<u>Confirmation of EBPR via Batch Activity Assays</u>

The left panel of Figure 15 illustrates the typical soluble orthophosphate (SOP) profile in a system without EBPR; the right panel of the same figure shows such profile in a system with EBPR. In the latter, SOP markedly increases during the anaerobic phase and declines during the aerobic phase.

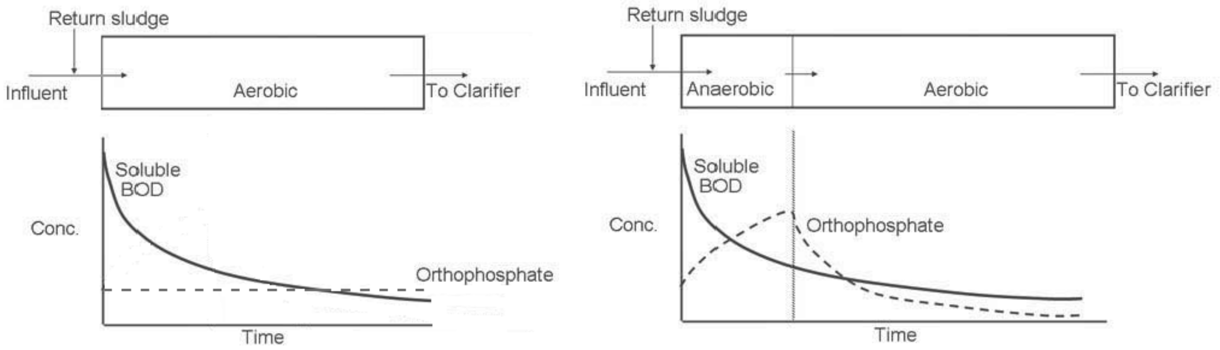

Figure 15 Left: SOP profile in a system without PAO activity; Right: SOP profile in a system with PAO activity (adapted from WDNR 2009)

PAO activity assays were routinely conducted using samples collected from the pilot system's selector. A typical SOP profile during such an assay is shown in Figure 16. These results match the pattern expected for sludge from an EBPR system and therefore confirmed EBPR as the mechanism behind the observed SOP removal within the pilot system.

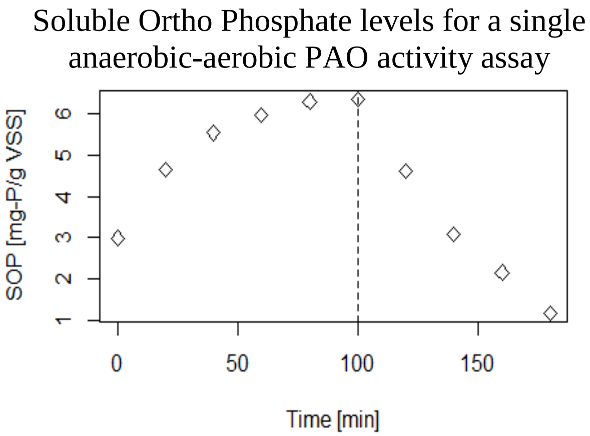

Figure 16. SOP Profile for a Typical PAO Activity Assay

Characterization of PAO Community

To further understand the community responsible for the observed EBPR activity, seven side-by-side PAO/DPAO activity assays were conducted. Figure 17 shows data from such assays -the "aerobic" representing the PAO activity assay while the "anoxic" representing the DPAO activity assay.

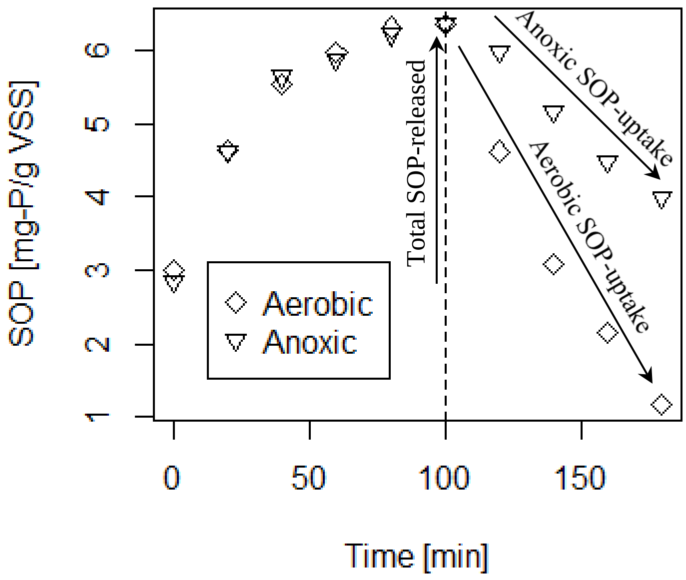

Figure 17. SOP Profiles for a Side-by-Side PAO/DPAO Activity Assay

Figure 18 shows the results for all 14 assays fitted with appropriate regression functions; logarithmic for SOP release and linear for SOP uptake. In general, the data were uniform with the exception of two days when lower than typical SOP release and uptake rates were observed (Figure 18, dotted lines).

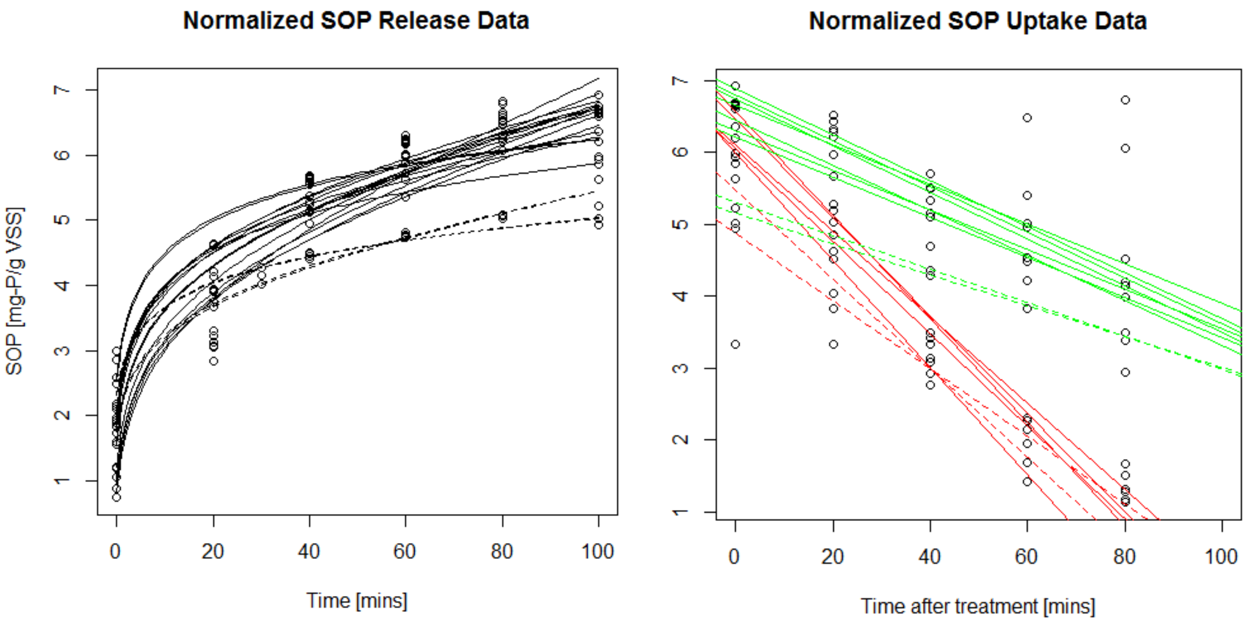

Figure 18. Left: Anaerobic SOP release data fitted with logarithmic trends; Right: Aerobic SOP uptake data (red) and anoxic SOP uptake data (green) fitted with linear trends

Table 7 summarizes the total SOP uptake and SOP uptake rate, and the total nitrate uptake and nitrate uptake rate, as calculated from the slopes of the linear regression lines. The PAO activity assays showed a higher mean uptake and a faster mean uptake rate compared to the DPAO activity assays. In addition under aerobic conditions, net nitrate production was observed likely due to nitrifier activity; whereas under anoxic conditions, net nitrate consumption was observed likely due to denitrification activity.

Table 7. Change in SOP and NO_3 uptake and uptake rates for aerobic and anoxic assays

Treatment	ΔSOP [mg P/g VSS] Mean ± St. Dev.	SOP Uptake Rate [mg P/g VSS h] Mean ± St. Dev.	ΔNO_3 [mg N/g VSS] Mean ± St. Dev.	NO_3 Uptake Rate [mg N/g VSS h] Mean ± St. Dev.
Aerobic/O_2 (PAO activity)	4.67 ± 0.716	3.83 ± 0.548	-2.52 ± 1.45	-1.89 ± 1.09
Anoxic/NO_3 (DPAO activity)	1.94 ± 0.483	1.70 ± 0.235	3.36 ± 2.40	2.31 ± 1.41

Relative PAO and DPAO activities were estimated using three different methods proposed by Wachtmeister et al. (1997) and Meinhold et al. (1999). Table 8 summarizes the results of the three methods using data from the seven side-by-side activity assays. All three methods yielded similar estimates: PAO accounted for 65~70% of the total EBPR activity; DPAO accounted for 30 to 35%. Note that this result does not necessarily indicate that PAO was numerically more dominant than DPAO in the pilot system; only that PAO contributed more than DPAO to the overall EBPR activity.

Table 8. Mean relative PAO and DPAO activities calculated using three different methods

DPAO/PAO Fraction Estimation Method	X_{DPAO}	X_{PAO}	**St. Dev.**
SOP-uptake rate (Wachtmeister et al. 1997)	30.4 %	69.6 %	2.29 %
Corrected SOP-uptake rate (Meinhold et al. 1999)	35.3 %	64.7 %	2.48 %
Total P-uptake (Meinhold et al. 1999)	31.8 %	68.2 %	2.70 %

The aforementioned results together demonstrated one configuration in which mainstream deammonification can co-occur with EBPR. In this particular system, the dominant group responsible for EBPR appeared to be PAOs, though DPAOs contributed to a substantial portion of the EBPR activity.

CONCLUSIONS

The key findings for the work being reported here are:

(1) Operation of the ANITA Mox pilot at higher feed C/N ratios correlated with lower TN removal rates and poorer settling sludge.
(2) Impact of PE type (stored or fresh) on system performance (i.e., TN removal rate, TN removal efficiency, and mixed liquor SVI) could be explained by the changes in feed C/N alone.

(3) Ammonia and nitrogen removal performance of mainstream ANITA Mox exhibited temperature dependence with an apparent Arrhenius constant (1.1), similar to that reported for AOB, which may be the rate limiting step.
(4) Under temperate conditions, mainstream ANITA Mox exhibited modest sensitivity to short-term disturbance in DO and ammonia concentration. However recovery from such disturbance was fast (within 24 hours). The system exhibited strong sensitivity and slow recovery to solids loss, similar to a conventional NDN system.
(5) Under temperate conditions, mainstream ANITA Mox exhibited long-term stability (8~10 months) with respect to media biomass density and AnAOB activity. However AnAOB activity declined substantially following a temperature shock event (delta of 7°C).
(6) Installation of an anaerobic selector in the process enabled co-occurrence of EBPR and deammonification. EBPR was demonstrated to be mediated by both PAOs and DPAOs, with the former being the dominant pathway.

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