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# Nitrogen Recovery Using a New Generation MaX+ Anammox Reactor for Urine Treatment

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**Sustainable space habitation systems must maintain operations without depending on regular resupply of materials. Wastewater recycling can facilitate sustainable habitation by reducing resupply costs. Biological treatment has previously been shown to be a promising technology to reduce resupply costs compared to only physical/chemical treatment systems. To date most research has evaluated biological reactors that oxidize organic carbon and nitrogen with or without denitrification. These systems can remove >90% of the organic carbon, oxidize ~50-60% of the organic nitrogen but can only remove ~ 50% of the oxidized N due to carbon limitations. A new process, anaerobic ammonium oxidation (Anammox), which can convert >90% of the total N to $N_2$ gas has been demonstrated for treatment of urine. This work evaluates a new generation of these reactors designed to allow for greater volumetric reaction rates and more robust performance. The MaX+ represents a simpler design for Anammox application compared to the previous PaX and MaX reactor. The MaX+ reactor was able to treat a biologically pretreated 1 crew-d urine load (~2L/day). Conversion rates approached 83 g-N/m$^3$-d. Over 91 % of the total N was converted to $N_2$ gas, the equivalent of 7.7 g-N/crew-day or ~7 L-$N_2$/crew-day. Results suggest that the MaX+ Anammox system may allow for nearly complete N recovery to a useful end product ($N_2$), reducing resupply costs and providing an effluent that can be more easily desalinated without chemical pretreatment.**

## Nomenclature

| | | |
|---|---|---|
| *Anammox* | = | Anaerobic Ammonium Oxidation |
| *MABR* | = | Membrane Aerated Bioreactors |
| *PAX* | = | Pancopia Anaerobic Ammonium Oxidation |
| *MaX+* | = | Mixed Anammox |
| *LEO* | = | Low Earth Orbit |
| *TN* | = | Total Nitrogen |
| *DOC* | = | Dissolved Organic Carbon |

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| TOC | = | Total Organic Carbon |
| IC | = | Ion Chromatography |
| ISS | = | International Space Station |
| WPA | = | Water Processing Assembly |

## I. Introduction

As humanity travels further into space, sustainable life support systems are necessary to maintain crews for long duration space missions. Biological wastewater treatment is a standard on Earth. Traditionally this includes carbon oxidation followed by oxidation of ammonium and organic nitrogen. In the past few decades this has expanded to include complete nitrogen removal using denitrification reactors. However, denitrification is often limited by a lack of organic carbon. A relatively new process to remove both ammonium ($NH_4^+$) and ammonia ($NH_3$) or Total Ammonia Nitrogen (TAN) is the Anammox process (Anaerobic Ammonium Oxidation) [3]. In this process specialized bacteria use TAN as a substrate and $NO_2^-$ as an electron acceptor to produce $N_2$ and a small amount of $NO_3^-$. As such, it allows for nearly complete N removal and production of $N_2$ gas without any organic carbon requirement. This is a significant advantage for wastewater with high nitrogen to carbon (N/C) ratios. For long term space habitation, wastewater recycling is mandatory. One of the most challenging wastewaters for any space habitat is urine. Urine contains total nitrogen (TN) concentrations that commonly exceed 3,000 mg/L and Total Organic Carbon (TOC) concentrations that exceed 2,000 mg/L. Removal of the TOC and TN has been a significant research focus. Excessive amounts of TOC in wastewater effluent may contribute to bacteria growth downstream which has led to documented issues on the International Space Station (ISS), specifically in the Water Processing Assembly (WPA). [5] Reducing the TN in the wastewater allows for the reduction of $NH_3$ to allow for water purification and reuse, and even the potential to capture $N_2$ gases on station for recycling. The Anammox process to remove the TN has also been investigated for space habitation systems as it not only can reduce the TN by over 90% but also produces $N_2$ gas, a valuable commodity for space habitation systems.

A previous study on Anammox bacteria demonstrated how a two-stage nitritation-anammox biological reactor was able to remove N and organic C from a combination of urine and flush water. [3] This study proposed that $N_2$ gas from urine treatment could be used as a makeup gas on station. [3] This two-stage system consisted of a Membrane Aerated Bioreactor (MABR) and Pancopia Anaerobic Ammonium Oxidation (PAX) system which were operated for 21 and 15 months respectively. [3] Effluent from the MABR system flowed to the anammox PAX reactor. [3] The PAX reactor included four 10-liter treatment modules making a total liquid volume of 50 liters for each reactor. Each of the four treatment modules was filled with a low-density foam and used heating jackets to maintain temperatures between 27°C and 32°C. A schematic of the MABR-PAX system can be seen in Figure 1.The systems PAX reactor were fed with pretreated urine + flush from a Membrane Aerated Bioreactor (MABR) that oxidized 90% of the organic matter

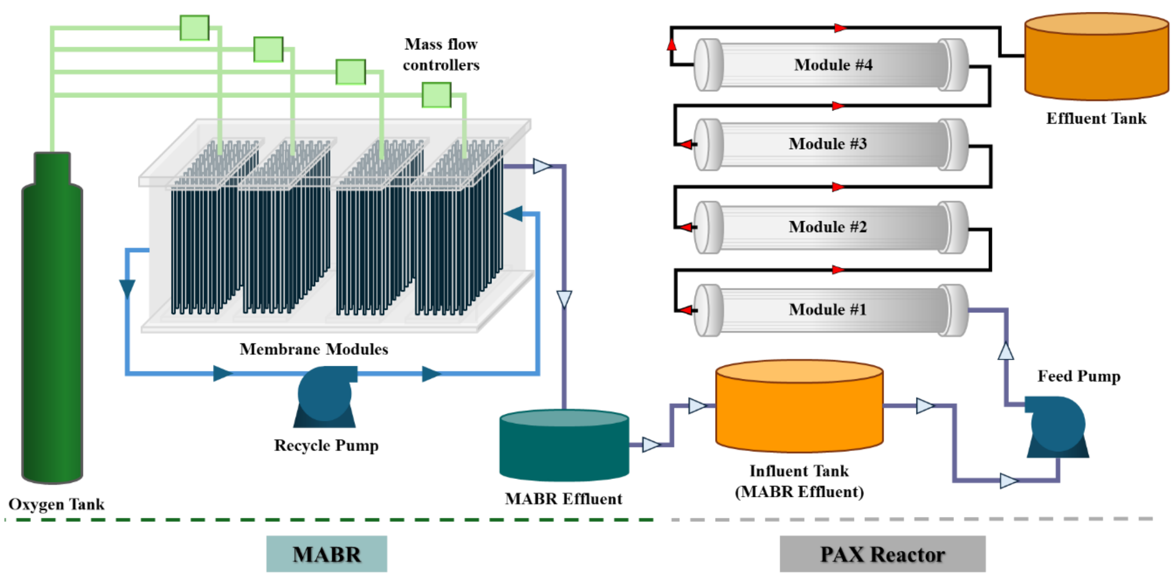



**Figure 1. MABR-PAX system for treatment of urine and flush water (Jalalieh et al., 2022).**

and ~50% of the TN to $NO_2^-$. The PAX reactor converted over 89% of the remaining TAN and over 99% of $NO_2$- to $N_2$ gas using anammox bacteria. [3] This previous study suggested that anammox could be a sustainable alternative bacterium for biological wastewater treatment in LEO. Despite the success of this previous study, the design of the PAX system was not optimal for treatment. Produced $N_2$ gas from PAX treatment was often trapped inside the reactor reducing wetted volumes and occasionally required the system to be vented.. This prompted a simpler design to study the Anammox culture that may allow for better treatment of high nitrogen-based wastewaters.

## II. Objectives

There were two main objectives for this study: 1) to design a new simple more robust anammox system (MAX+) to treat high strength nitrogen-based wastewater and 2) to measure the performance, reliability, and impact of off gas production ($N_2$, $CO_2$, $N_2O$, NO, $NO_2$, and $NH_3$).

## III. Methods

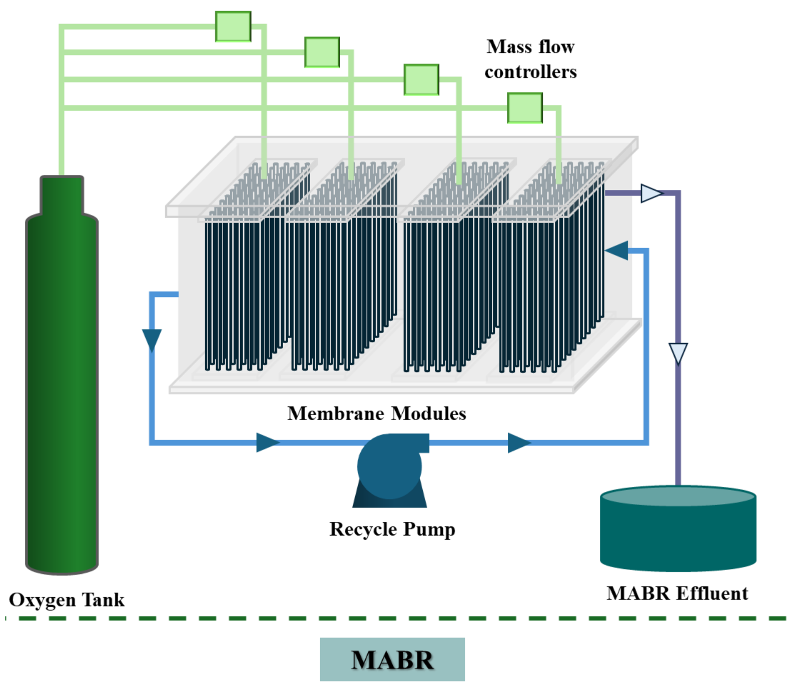



**Figure 2: Schematic of MABR**

There are two stages for the MABR-MaX+ system. The first stage, as with the PAX based system, uses membrane aerated bioreactors (MABRs)to oxidize organic carbon and perform partial nitritation. The MABRs used for this study were used in the previous research conducted on the PAX reactors. Two MABRs were utilized for the purpose of the study on the MaX+ system. A schematic of the MABR can be seen in Figure 2. The second stage of the MABR-MAX+ System is then used to perform the Anammox reaction (conversion of ammonium to $N_2$ gas and some $NO_3^-$) using the stage one effluent from the MABRs. The MABRs were designed with four membrane modules, a recycle pump (Blichmann Engineering), and a feeding pump (Neptune Systems), not shown. The feeding pumps stopped operating properly during the study period leading to batch feeding of the MABRs. The MABRs each had a total volume of 107 liters with a liquid volume of 48 liters. Baffle walls separated designated influent and effluent sections of the reactors from the membrane modules. The membrane modules contained 282 semi-permeable hollow siloxane membranes that were 30 centimeters in length and had an inner diameter of 0.556 centimeters. For one MABR, there was a total of 1,128 membranes for the entire system with a total specific surface area of 119 $m^2/m^3$. [3] Both of the MABRs were initially inoculated with 1 liter of activated sludge from a local municipal wastewater treatment plant and 65 grams of a lyophilized bacterial culture that contained both nitrifying and denitrifying bacteria (Pancopia, Inc.). Compressed oxygen was supplied to the system via mass flow controllers (Cole-Parmer).

Each system was fed with donated urine and flush water generated by Reverse Osmosis (RO). A consistent feed (1 crew-day) volume of 2.1 L of pure urine and 300 mL of flush water was delivered to each of the MABRs. Effluent from each MABR was collected and fed to the MaX+ reactor. To maintain a 1:1 ratio of $NH_4^+$ and $NO_2^-$ when feeding MABR effluent into the MaX+ reactor , additional ammonia or nitrite salts were added, as needed. Utilizing two MABRs allowed for over 4 liters of effluent to be collected per day and feed the MaX+ reactor ensuring there was consistent flow throughout the study period.

Similar to the PAX reactors, the MaX+ reactor converted $NO_2$- to $N_2$ gas using anammox bacteria. The MaX+ reactor operated as an anaerobic system that was sealed off to atmosphere preventing oxygen from entering the reactor. For this study, the MaX+ reactor was operated continuously from October of 2023 to May of 2024. A schematic of the MaX+ reactor can be seen in Figure 3. MaX+ contained three sections: the first and third sections contained two low-density foam cores each of which were inoculated with anammox bacteria (Core 1,2,3,4). The system itself was 100 liters and operated with a total liquid volume of 90-95 liters. In the second section, a heater was placed to keep the temperature between 26°C and 28°C. Anammox bacteria provided by Pancopia Inc. were fed directly into the reactor for inoculation. The reactor solution was recycled from the two cores in section one and injected into two cores in section three. Section two was separated by baffle walls as well as one inch foam mounted onto the sides of the baffles. Internal recycling was maintained by two pumps from Stenner Pumps. Effluent was removed through tubing and wasted after analysis. Effluent was removed from the MABR and fed into the MaX+ reactor at rates varying from 1.5 to 2.4 L/day.

The reactions occurring within the MABR (1 and 2) and MaX+ (3) reactors are shown below:

$$(1)\ Organics + O_2 \rightarrow CO_2 + H_2O$$

$$(2)\ AN + 1.5O_2 \rightarrow NO_2 - + H_2O + 2H^+$$

$$(3)\ AN + 1.32NO_2 + 0.066HCO_3 - + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3 - + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

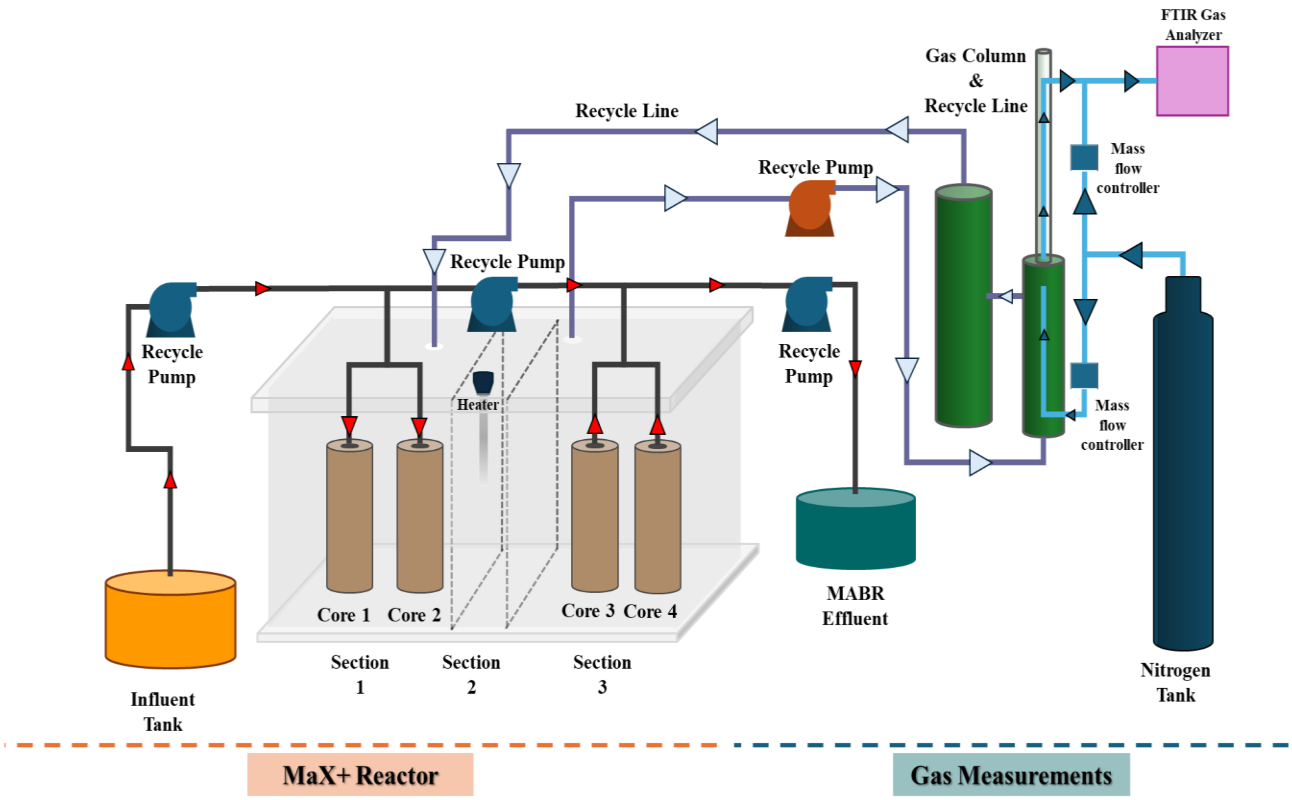



**Figure 3: Schematic of MaX+ Reactor**

### A. Water Quality Analysis

Daily water quality analysis was documented to understand the treatment occurring in each reactor and to maintain both partial nitritation and the Anammox reaction. The effluent of each of the MABRs and the influent and effluent of the MaX+ reactor were evaluated. Daily pH and Dissolved Oxygen (DO) measurements were taken for both the MABRs and MaX+. Effluent samples were collected from the MABR on Monday, Wednesday, and Friday each week.

Both influent and effluent samples were collected daily from MaX+ reactor. Influent samples were collected from the influent tanks of MaX+ while the effluent samples were collected by syringe directly from section 2 of the reactor itself. All samples were filtered with Whatman, 45 µm pore size, before analysis. After filtering, all samples were stored at 4°C. For all samples, both the Total Nitrogen (TN) and Dissolved Organic Carbon (DOC) concentrations were measured on Shimadzu TOC/TN analyzer. All samples were further analyzed on a Dionex Ion Chromatograph (IC) to measure both nitrite ($NO_2^-$) and nitrate ($NO_3^-$) concentrations. Total Ammonia Nitrogen (TAN) was calculated based on the subtraction of $Nox^-$ ($NO_2^-$ + $NO_3^-$) from TN measured in each sample.

### B. Gas Measurements

To measure the gas produced from the MaX+ reactor, a gas stripping column was set up for direct measurements from recycled MaX+ reactor fluid (Figure 3). $N_2$ gas was purged through the reactor solution in the gas stripper which then flowed to a FTIR Gas Analyzer (GasMet). The flow of nitrogen gas was maintained by a mass flow controller (Cole-Parmer). Nitrogen gas bubbled through effluent from the reactor for at least 24 hours prior to any gas measurements. Continuous gas measurements were taken over 4–6-hour periods during testing.

## IV. Results & Discussion

The MABR performance was typical of past studies. In general, it produced a well stabilized effluent with >90% organic carbon (OC) removal and > 50% oxidation of TAN resulting in nearly equal concentrations of TAN and $NO_2^-$. For this paper, the discussion will focus on the performance of the MaX+ reactor.

### A. Max+ pH and DO

The MaX+ reactor maintained a steady pH between 6 and 8 throughout the operational period. Hydrochloric acid (HCL) or sulfuric acid ($H_2SO_4$) were occasionally added if a high pH occurred. Results show that the pH fluctuated from October to December of 2023 above 6, but below 7.

The DO content of MaX+ remained below 1 mg/L for the general operation of the reactor. When the DO reached above 1 mg/L, 50 mL of pure urine was added directly to the influent tank of MaX+ and nitrogen gas was bubbled into the reactor. The reactor was sealed with silicone to reduce the amount of available oxygen entering the system. This seal was applied twice to the reactor. The original seal application was on 11/3/2023 to reduce the number of available openings that may allow oxygen to0 enter the reactor. The second seal application was on 8/12/2024, continuous monitoring probes were removed from the reactor requiring the seal to be broken and replaced.

### B. DOC Concentrations (mg/L)

Results showed that there was over a 90% reduction in DOC concentrations over the eight-month operational period for MaX+ (Figure 4).

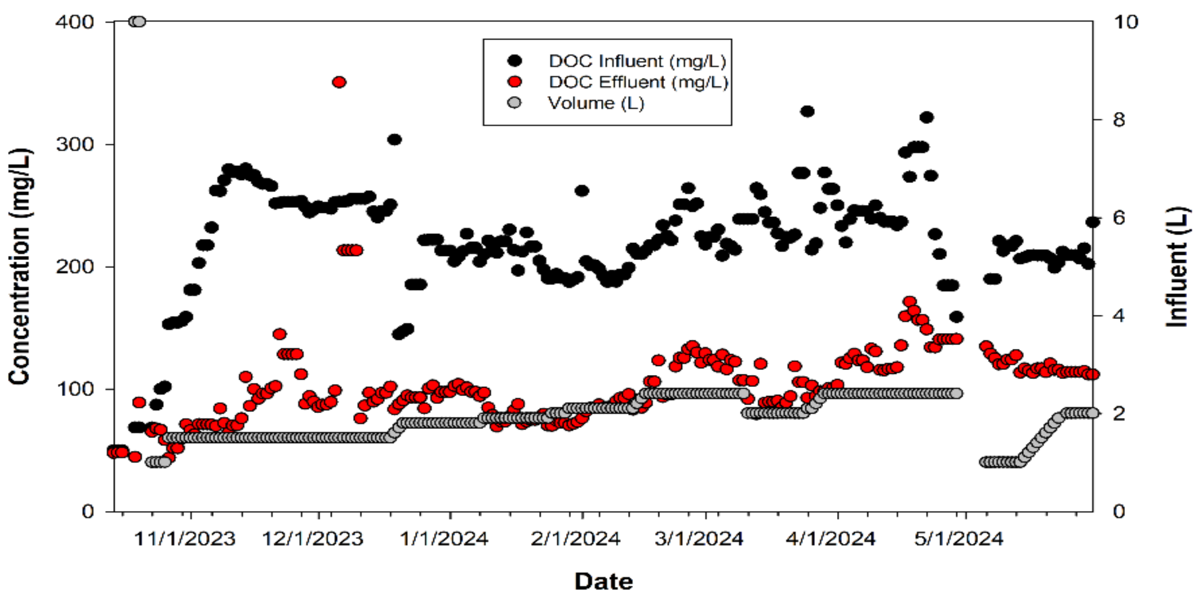



**Figure 4: DOC Concentrations (mg/L) for MaX+ and Influent Loading Rate (L)**

### C. TN Concentrations (mg/L)

MaX+ was able to reduce the TN by 93% over the entire eight-month operational period (Figure 5). Influent concentrations (~4,000 mg/L) were reduced to ~ 200 mg/L for loading rates ranging from 1.5-2.4 L/day. TN peaked near 5000 mg/L for effluent concentrations when feed rates were raised to 2.4 L/day. These feed rates exceeded the reactor capacity. The maximum loading rate of MaX+ was 142 g-N/m$^3$-day. Over the entire study period, only ~144 grams of TN were not transformed out of a total of 1,823 grams which were fed to the reactor (Figures 6 and 7). In Figure 8, the daily patterns of TN, $NH_3$, $NO_2^-$, $NO_3^-$, are displayed against the influent loading rate. These changes describe internally what was happening to nitrogen within the MaX+ reactor throughout the study period. An average reaction rate was calculated to be about 83.51 g/m$^3$-day.

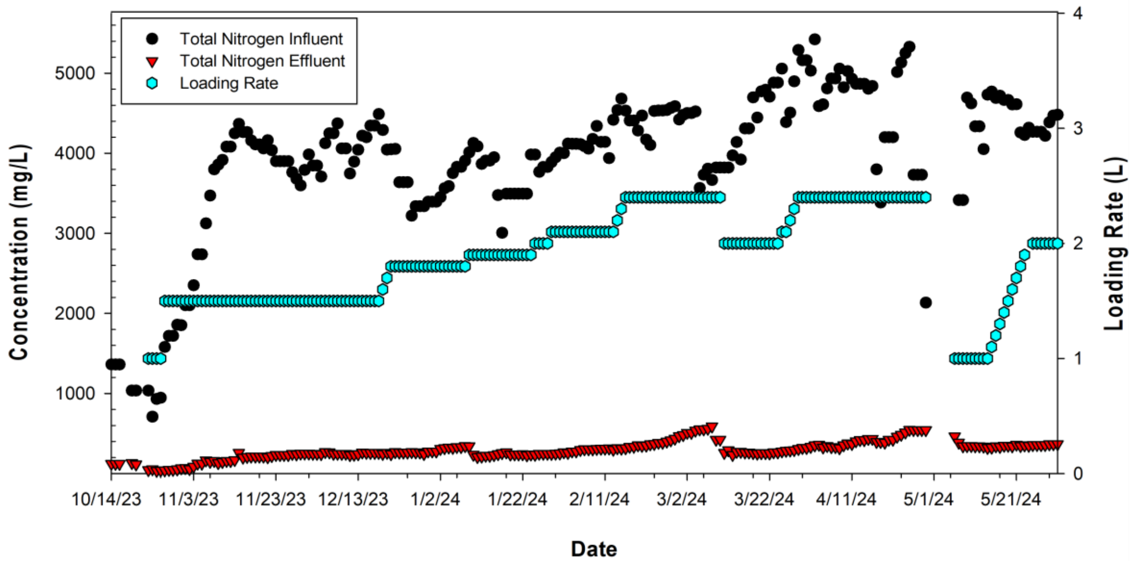
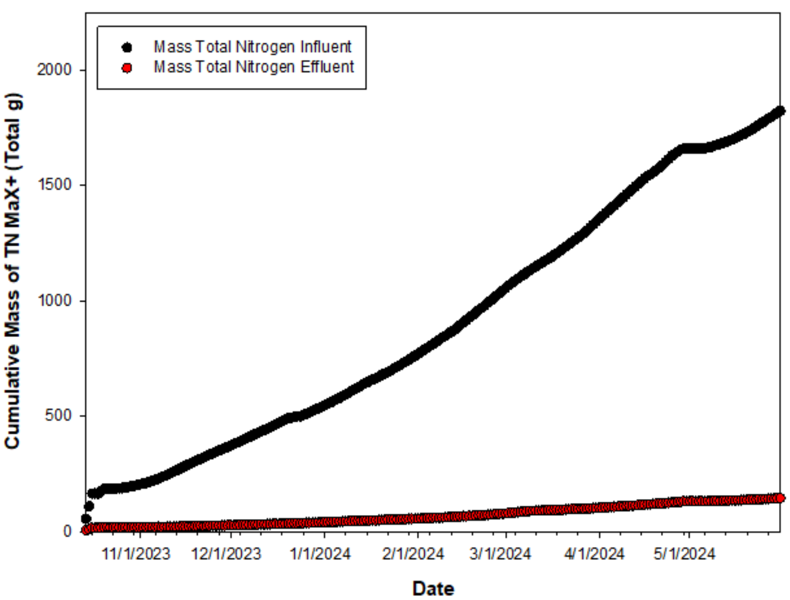
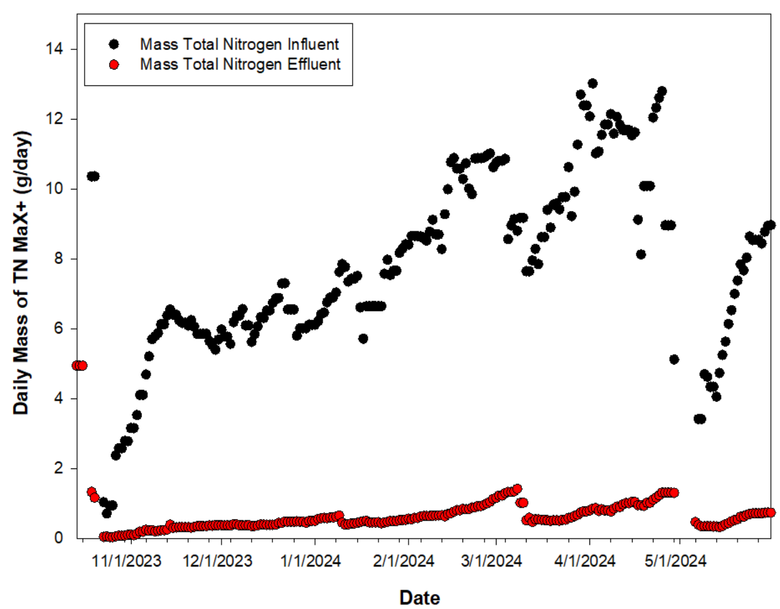



**Figure 5: Total Nitrogen Concentration (mg/L) of MaX+ Reactor and Loading Rate (L)**




**Figures 6 and 7: Comparison of Cumulative and Daily Mass of TN for MaX+**

TAN concentrations varied throughout the operational period but generally stayed below 200 mg/L in the effluent of MaX+. A peak occurred in March of 2024 which was observed after an increase in loading rates in February of 2024. $NO_3^-$ effluent concentrations increased throughout the operational period, reaching as high as 370 mg/L near the end of the study. $NO_3^-$ in the influent was below 50 mg/L throughout the study.

$NO_2^-$ influent concentrations were over 1,000 mg/L for most days. $NO_2^-$ effluent concentrations were typically below the detection limit of 5 mg/L. An increase in $NO_2^-$ effluent concentrations was observed after increasing loading rates to 2.4 L/day. The cumulative influent mass of $NO_2^-$ was 835 grams and was reduced to 11.20 grams over the eight-month operational period. This suggests that most of the nitrogen in the system was converted through the anammox bacteria.

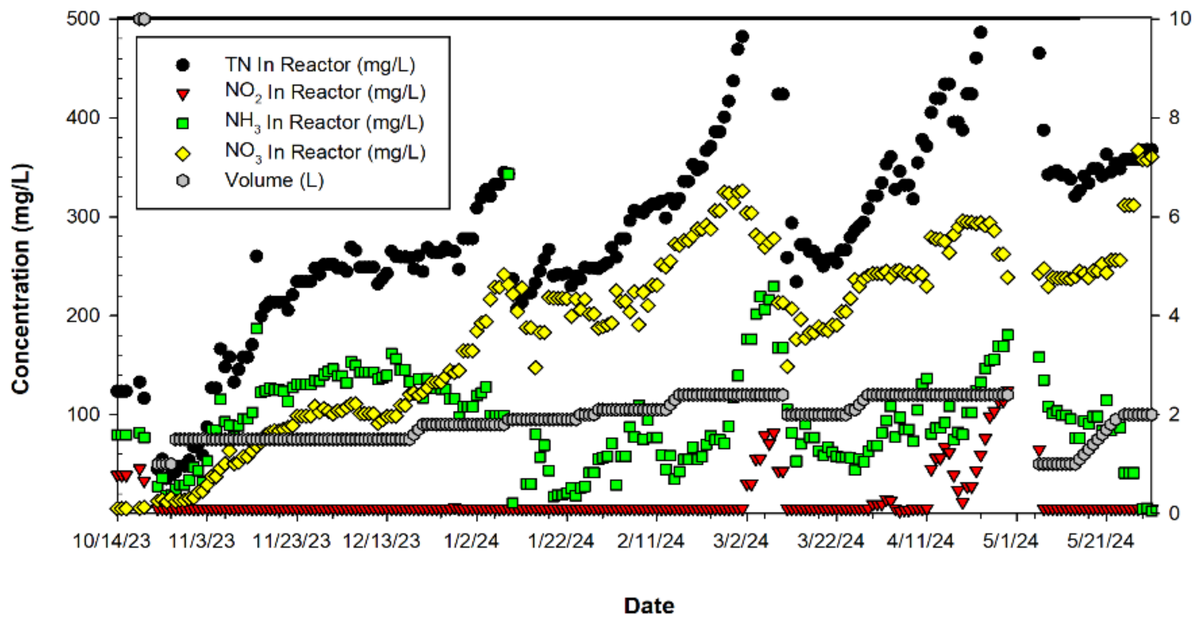



**Figure 8: TN, $NH_3$, $NO_2^-$, $NO_3^-$ Concentrations (mg/L) vs Influent Volume (L) for MaX+ Reactor**

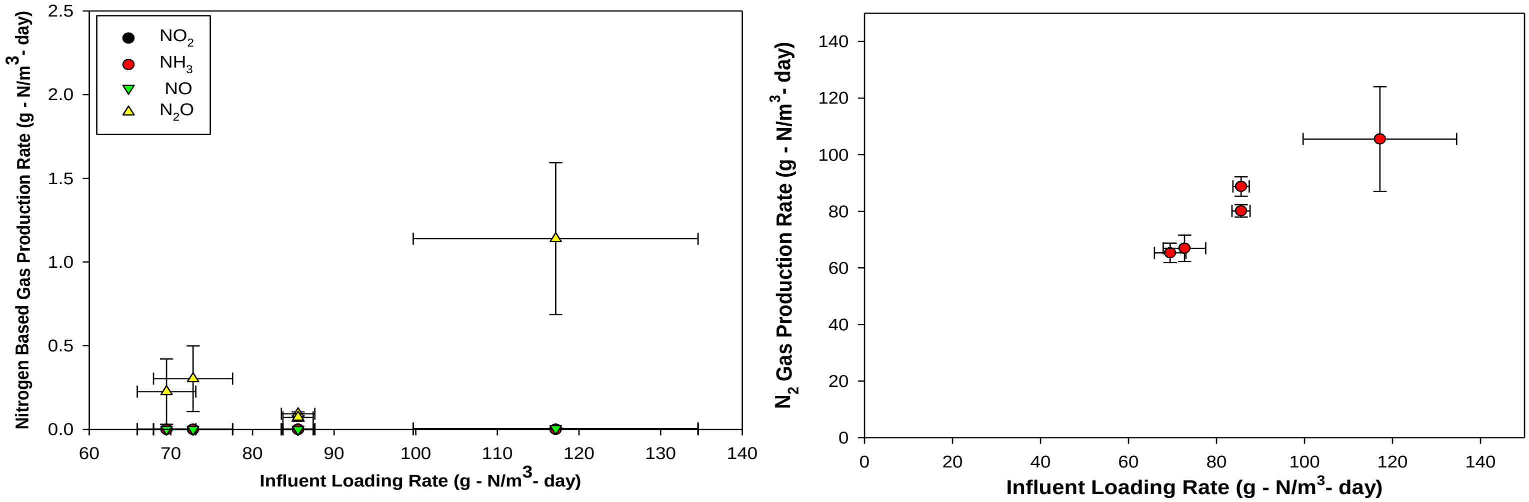
### D. Gas Measurements

Gas measurements started in mid-December of 2023 and ran until May of 2024. $N_2$ gas was not measured directly but rather calculated based on the difference of TN from the influent and effluent concentration of the reactor, feed rate, and volume of the reactor. Gases measured included $CO_2$, $N_2O$, NO, $NO_2$, and $NH_3$ all in ppm. $N_2$ gas production rates ranged from 60 to 120 g-N/m$^3$-day and linearly corelated with influent N loading rates. Production rates of other N gases were much lower. $N_2O$ was produced in the next highest amount but the highest rate was < 2 g-N/m$^3$-day which only occurred at the highest N loading rate. Mass production of other species was insignificant. Results for daily $N_2$ gas production and $NO_x$ species gas production for the MaX+ reactor are seen in Figures 9 and 10.

While off-gas concentrations were measured from the MaX+ system, measured gases were too low of concentration to have an impact on the cabin atmosphere. If the flow rate increased

### E. Reactor Improvements

The simpler design of the MaX+ reactor performed more efficiently compared to the PAX reactor. Instead of sustaining flow through separate contained modules, flow was visible as it went in and out of the MaX+ reactor. The walls of the MaX+ system were designed to be clear so that the system was able to be monitored directly. This allowed consistent maintenance for the MaX+ reactor. Produced gas was able to exit the MaX+ system through effluent lines, or measured directly through the GasMet system, without getting trapped compared to the PAX system.




**Figures 9 and 10: $N_2$ Gas Production and NOx Species Gas Production for MaX+ Reactor**

## V. Conclusion

The performance for the MaX+ reactor suggests a proportional relationship between $N_2$ gas production and the influent loading rate of MABR effluent. These results suggest that over 90% of the nitrogen entering the MaX+ reactor system is converted into $N_2$ gas which may be viable as a makeup gas for long-term space missions. This recycling of gas may help to reduce consumables and cargo for long-term space missions and allow for improved sustainability onboard spacecraft.

While the success of the MaX+ reactor shows that the Anammox bacteria can convert high strength nitrogen-based wastewaters into $N_2$ gas, the reactor itself is not optimized for performance. To increase the amount of feed delivered and treated by the system, more surface area could be provided at the same volume as much of the reactors internal volume was not utilized. Future studies are necessary to understand the off-nominal operation of the MaX+ reactor. Stress testing would help to understand how the reactor can adapt to increased rates of $NO_2$- concentrations entering the system.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