Abstract

Water is a limiting factor in space exploration. Biological systems such as Aerobic Rotating Membrane Systems (ARMS) are an effective means of water resource regeneration. Bacteria involved in this reactor are identified using fluorescent in situ hybridization (FISH). Distribution of organisms is examined in relation to dissolved oxygen gradients within the reactor. Bacteria within the ARMS are capable of up to 70% conversion of ammonia to nitrate.

New research is now being conducted in benchtop tidal wetland reactors to determine the benefits of this low-yield low-energy reactor capable of complete nitrogen removal from wastewater. Sequential filling and draining of tidal wetland reactors provides both anoxic and aerobic phases. Simultaneous nitrification, denitrification, and COD removal are observed in these reactors. Previous research has determined the predominance of heterotrophic nitrifiers and ammonia oxidizers under certain conditions. The presence of anaerobic ammonia oxidizing (anammox) bacteria is consistent with results under other conditions.

FISH will be used to identify nitrogen cycling bacteria within benchtop columns. Benchtop column research will determine the presence of novel nitrogen cycle bacteria such as ANAMMOX. Research within these reactor systems will not only quantify nitrogen-processing bacteria and examine their spatial distribution but also determine optimum operating conditions for these organisms.

Introduction

Extended space flight missions require water recycling systems in order to sustain crew members. The International Space Station (ISS) has been an invaluable tool for testing engineered systems for sustaining life in outer space and it has demonstrated the need for water regeneration 3. Biological purification of water is a valid component of water purification systems 17. Engineered biological systems exist on Earth that are capable of almost complete removal of nitrogen, phosphorous and organic wastes from water 5, 18. Recent improvements in our understanding of biological nitrogen cycling have lead scientists and engineers to develop systems, such as Completely Autotrophic Nitrogen removal Over Nitrate (CANON), that take advantage of anammox (eq. 1), the anaerobic oxidation of \( \text{NH}_4^+ \) to \( \text{NO}_2^- \) or \( \text{NO}_3^- \), to produce low biomass biofilms capable of near complete nitrogen removal from waste waters 15, 19. CANON combines anammox with anaerobic respiration of nitrite to reduce volatile suspended solids (VSS).

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \quad [\text{AG}^{\circ} = -357 \text{kJ/mol}] \quad \text{eq. 1}
\]

\[
\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^- \quad [\text{AG}^{\circ} = -74 \text{kJ/mol}] \quad \text{eq. 2}
\]

The waste water stream in outer space is 13 times more concentrated with respect to urine than the waste waters on Earth 17. According to Wallace and Austin 19 and Schmidt et al 15 CANON is best suited to process waters with a high nitrogen load and low organic matter content: Treating the high nitrogen content waste water stream occurring on extended space flight missions with a CANON based reactor system is a logical progression. Tidal wetland simulators create a habitat capable of supporting CANON 7, 19 and are the focus of current studies.

Removing nitrogen species from waste water in space is challenging due to many constraints (e.g. confined space and microgravity) 17. The ARMS took advantage of silastic, a gas permeable rubber, to provide oxygen to bacteria inside the unit without the formation of bubbles. Bubbleless aeration is necessary in microgravity environments because bubbles will not disperse in the absence of gravity 1, 4. Nitrification only converts ammonia to nitrate. For complete nitrogen removal from water processes such as canon, via anammox, reduce reactive nitrogen species all the way to \( \text{N}_2 \) (Figure 1) 15. In addition to this canon biofilms are low yield and low energy. Anaerobic ammonia oxidizers form lean biofilms and require less organic matter for complete nitrogen reduction.

\[
\text{NH}_4^+ \quad (100) \quad \text{Canon} \quad \text{N}_2/\text{NO}_3^- \quad (90/10)
\]

**Figure 1**: The Canon process uses anammox to produce \( \text{N}_2 \) and \( \text{NO}_3^- \). The resultant \( \text{NO}_3^- \) can be cycled back into the canon processor for reduction to \( \text{N}_2 \) gas.

This is important for biological reactor management because lean biofilms will biofoul the reactor less frequently therefore reducing maintence 9. Also a typical waste stream during space exploration...
tends to be high in reactive nitrogen species but low in organic matter \(^{11, 17}\).

Understanding the ecology of nitrogen processing organisms here on Earth will better enable scientists and engineers to utilize them in engineered biological reactors used in extended space flight missions. Ground based reactor systems will also be necessary for water resource regeneration on potential lunar and Martian colonies.

In this study we are using wetland simulators packed with pea size gravel made of Stealite and lightweight shale aggregate (LESA) to examine the distribution and activity of organisms within wetland simulators. Stealite and LESA are both lightweight, high volume media that could be easily adapted for a water regeneration system on extended space flight missions. Chemical data from existing columns (Figure 2) suggests that CANON processes are occurring in the columns, however we have yet to quantify the organisms responsible or examine the lost component of total nitrogen inputs for greenhouse gasses such as N\(_2\)O and CH\(_4\). While greenhouse gasses may not be of interest on space missions, these reactors have application here on Earth as well. Therefore greenhouse emissions should be further explored.

**Methods**

**Pilot Scale Reactors:**

Pilot scale reactors, currently in operation, are packed with stealite, and LESA and then fed a whey and urea broth. Columns undergo a biological commissioning phase to allow bacteria to colonize the columns. After this phase columns are cycled 24 times per day between submerged and emptied stages. Cycle time decreases and the number of cycles per day is increased, thereby extending the time spent under reducing conditions inside the columns. Fluid sampling occurs routinely for nitrogen speciation and concentration.

**Bench Scale Reactors:**

Bench scale reactors will mimic test scale reactor loading and cycle rates. The smaller scale reactors will enable easy sampling for enumerating bacterial claves as well as making \(^{15}\)N tracer studies possible to determine anammox rates within the column. Reactors were constructed following the blue prints in Figure 1. At the end of each cycling period media from within the column is removed and stored in a -80°C freezer, preserved in glycerol to prevent cell lyses.

**Fluorescent in situ Hybridization (FISH):**

Anammox bacteria will be identified using FISH. The probe S*-AMX-0368-a-A-18, sequence 5’CCCTTGGGATATTGCGAA 3’ will be used to identify anammox organisms \(^{12}\). The 5’ end of the probe is modified to contain the fluorescent molecule 6-Carboxyfluorescein (6-FAM). The hybridization process will be carried out according to Manz and Amann \(^{10}\), and Schmid et al. \(^{12, 13}\). Total bacterial counts will be performed using probe EUB338, sequence 5’GCC GCC TCC CGT AGG AGT 3’, labeled with a Cy3 fluorescent molecule on the 5’ end. Reaction protocols will be carried out according to Manz\(^{10}\).

**Nutrient Assays:**

Colorimetric techniques are used to monitor products and reactants within test columns, such as NH\(_4\)^+, NO\(_2\)-, and NO\(_3\)- or by Ion Chromatograph\(^5\).

**Results**

Based on nitrogen speciation we can infer the dominant microbial reaction occurring within the wetland simulators, and factors that are influencing process selection (Figure 3). Anammox appears to be encouraged by high loading rates, a decrease in the cycling rate and low CEC. To confirm this FISH and a \(^{15}\)N tracer study will be required. Monitoring the production of \(^{29}\)N\(_2\) in a \(^{15}\)N labeled NH\(_4\)^+ tracer study is the definitive means to determine anammox is occurring \(^{6, 7}\). In the presence of \(^{15}\)NH\(_4^+\) only anammox produces \(^{29}\)N\(_2\), whereas without the tracer study the end
products of anammox and aerobic nitrification are indiscriminant. While FISH may prove the ability to anaerobically oxidize ammonia to N\(_2\) gas, it does not demonstrate that it is occurring. We expect to see the abundance of anammox capable organisms increase over time. When abundance levels of anammox organisms are high the \(^{15}\text{N}\) tracer study should show a high ratio of \(^{28}\text{N}_2\) gas production.

**Figure 2:** Nitrogen speciation in effluent from reactor columns run under two different dosing protocols is displayed in the bar graphs. Columns 2 and 4 under protocol 2, and column 5 under protocol 1 show evidence of annamox and a Cannon like process. Change in N represent N lost from the system, but is not well quantified (e.g. N\(_2\) or N\(_2\)O gas production, or N uptake as biomass.

**Figure 3:** The percentage of nitrogen processed by different guilds of bacteria is altered by the cation exchange capacity (CEC) of the media packed into the wetland simulators as well as loading rates and cycling times.
Data from these experiments will provide insight to the distribution and function of tidal wetland simulators. It is expected that anammox will play a large role in the removal of nitrogen from waste waters. Although it is difficult to correctly quantify the activity of anammox organisms, nitrifiers and denitrifiers with the help of \(^{15}\)N tracer studies this is possible. Combined with FISH, a isotope tracer study will make it possible to both quantify organisms and their functionality within the reactor system. Research by Kindaichi has shown that the abundance of anammox capable organisms changes not only within biofilms structures, but also along a unidirectional flow path of a fixed bed reactor system. After passing through 75% of the reactor system (240 mm) anammox activity was no longer detected and the abundance of anammox organisms declined from 90% to 60% of the total biomass. It will be interesting to see how abundance data from tidal wetland simulators compares with a unidirectional flow reactor system. The slow growth rate of anammox organisms is important for the sustainability of reactor systems. Waste water treatment systems based on CANON require flushing to remove biosolids less frequently. Low maintenance systems are important for space missions.

**Conclusions**

Although we have established a wetland simulator further research is required to better understand environmental factors controlling the microbial reactions ongoing within the columns.

**Works Cited**


