Duckweed: A Helpful Plant for Biofilm Reduction

National Aeronautics and Space Administration

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INTRODUCTION

The International Space Station (ISS) wastewater tank currently experiences bacterial growth producing biofilm. Biofilm formation in the wastewater tank forms due to microorganisms adhering to surfaces and generating an extracellular polymeric matrix.[1] The formed biofilm contributes to issues downstream from the tank, causing clogging of equipment and effecting equipment reliability life. The MSFC biofilm test stand is actively exploring multiple mitigation efforts. Early-stage testing indicates that duckweed may be a successful mitigation strategy. Aquatic plants upstream of the wastewater tank consume nutrients in the water, such as total organic carbon, causing downstream bacteria to starve. The next step in this research is developing a duckweed unit that can function in microgravity and be integrated into the current ECLSS architecture.

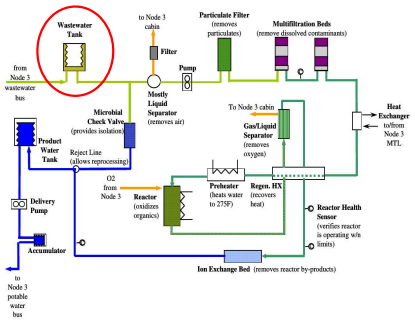

Figure 1: The Wastewater Tank Location in the Wastewater Processing Assembly

Figure 2: Biofilm clog in ISS quick disconnect[2]

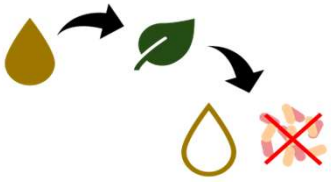

Figure 3: Basic diagram of nutrient depletion phytoremediation[6]

DUCKWEED TESTING

Through Jacobs innovation grant funding, a proof-of-concept test run occurred on the biofilm test stand in August of 2024 and ran for the standard 9-week sampling period. Two types of duckweed were evaluated, *Lemna Minor* and *Wolffia Arrhiza*. The duckweed ran on the biofilm test stand in the Phytoremediation Operated Nutrient Depletion (POND) units.

Figure 4:*Wolffia Arrhiza* (left) and *Lemna Minor* (right) running in the PONDs on the biofilm test stand.[5]

The duckweed in the POND units operated on a nutrient starvation mitigation setup. The ersatz, or simulated inlet wastewater liquid, was first pumped through the PONDs. The duckweed living in the PONDs pulled nutrients out for food, starving the downstream bacterial and fungal microbes living in the simulated small scale wastewater tanks bioreactors.

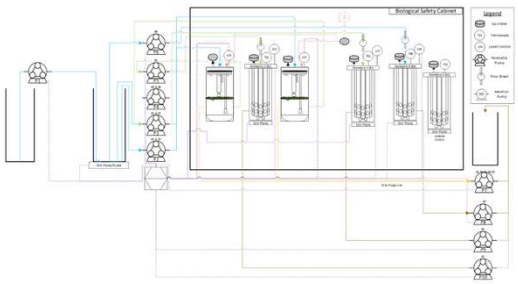

Figure 5: P&ID of the upstream PONDs and the downstream biofilm bioreactors during the duckweed testing run.

During the 9-week sampling run coupon samples were pulled every three weeks. The coupon material being tested in the bioreactors (simulated small scale wastewater tanks) was Inconel-718, the internal wetted material of the ISS wastewater tank. The samples were swabbed and plated to quantify the CFU/cm^2 per the standard Center for Disease Control (CDC) BioSurface bioreactor method ASTM- E3161-18[3]. The mean average mean log reduction was then calculated and compared to the un-mitigated control to determine biofilm prevention.

$$LOG_{10}\left(Control\ Bioreactor\ \frac{CFU}{cm^2}\right) - LOG_{10}\left(Lemna\ Minor\ Bioreactor\ \frac{CFU}{cm^2}\right) = Average\ Coupon\ Mean\ Log\ Reduction\ of\ Biofilm$$

RESULTS

Lemna Minor succeeded in preventing approximately **97%** of bacterial and **99%** fungal formation of biofilm based on the average coupon mean log reduction. *Wolffia arrhizal* also demonstrated promise in the first 3-week sampling period but died week four of fungal contamination.[5]

Average Coupon Mean Log Reduction of Biofilm (Log10 CFU/cm^2)		
	Bacterial	
	Wolffia arrhiza	*Lemna minor*
Week 3	1.5	**1.46**
Week 6	Test Stopped (Died)	**2.00**
Week 9		**1.4**
	Fungal	
Week 3	1.49	**1.62**
Week 6	Test Stopped (Died)	**2.5**
Week 9		**2.47**

Lemna Minor also succeeded in reducing the overall total organic carbon (TOC) by an average of 73.5 from 86.6 ppm.[5]

ONGOING WORK

We have been awarded a Marshall Space Flight Center Technology Innovation Development grant to begin planning an integrated system. Below is the concept of the full-scale system.

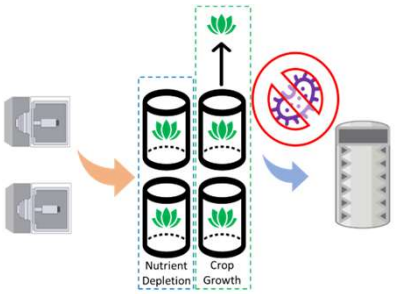

Figure 6: Full scale system concept[5]

The full-scale system would utilize a crop growth bed design by Space Lab Tech for a zero-gravity growth unit to house the duckweed. The MSFC TID is funding receipt of their µG-LilyPond™ to begin testing design and behavior of an integrated system. The integrated system would utilize half of the bed infrastructure to pull nutrients out upstream of the wastewater tank, and the other half for crop growth usage. The beds would switch in between functions and could provide additional duckweed either to replenish die out on the nutrient depletion side or for crop usage.

Figure 7:Space Lab Tech µG-LilyPond™[4]

SOURCES

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5. Beitle, Eric, et al. "Biofilm Mitigation Through Nutrient Starvation Utilizing Duckweed Phytoremediation" *54th International Conference on Environmental Systems (ICES)*. 2025 (Approved).
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