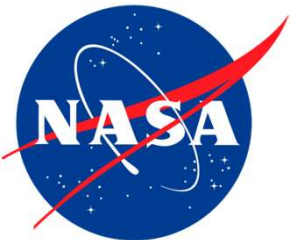


Biofilm Mitigation in Spaceflight Utilizing Phytoremediation

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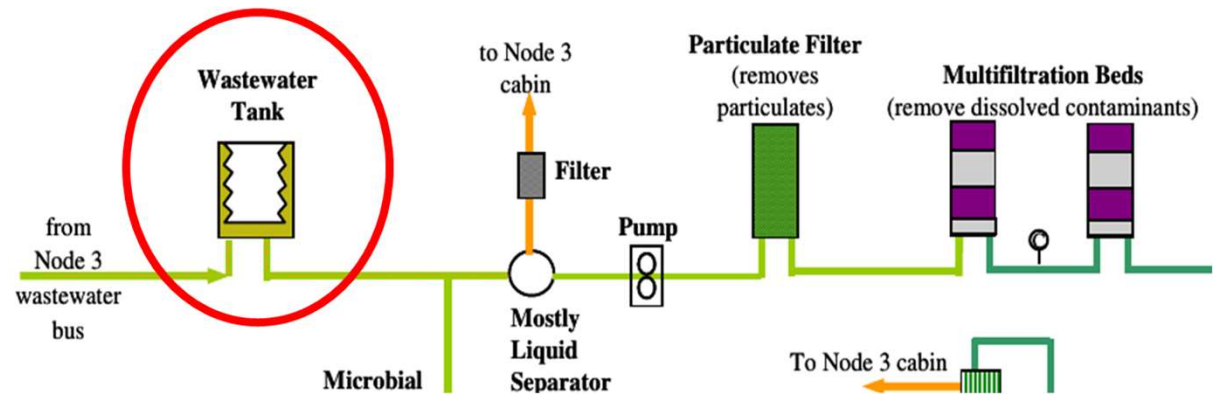
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Introduction

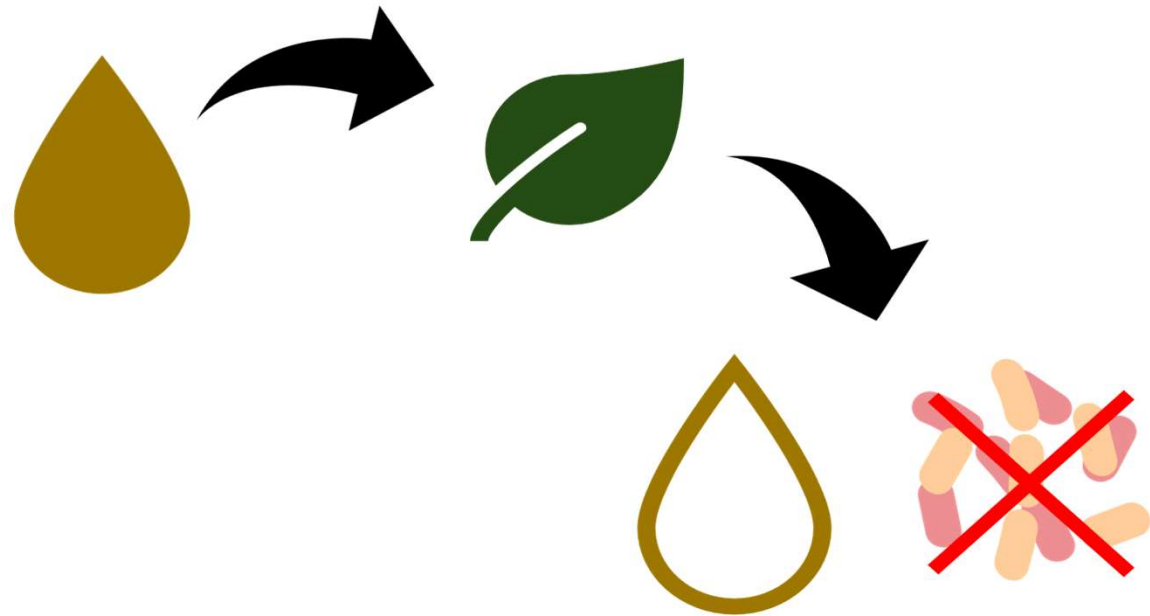


Biofilm formation in the water processing assembly on the International Space Station is a current and ongoing issue. Biofilm causes clogging of equipment, downstream issues and degradation of hardware. Multiple biofilm mitigation strategies are currently being explored to limit microbial growth in the wastewater tank.



Introduction (con.)

- One biofilm mitigation strategy is nutrient depletion through phytoremediation
- Nutrients are depleted upstream of the wastewater tank by duckweed and the downstream microbial growth starves



Why Duckweed?



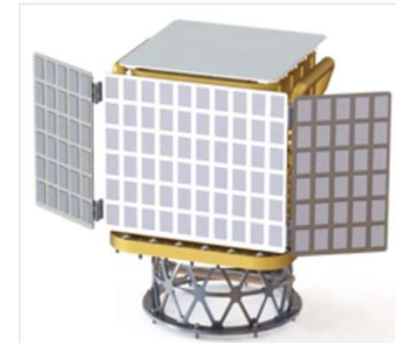
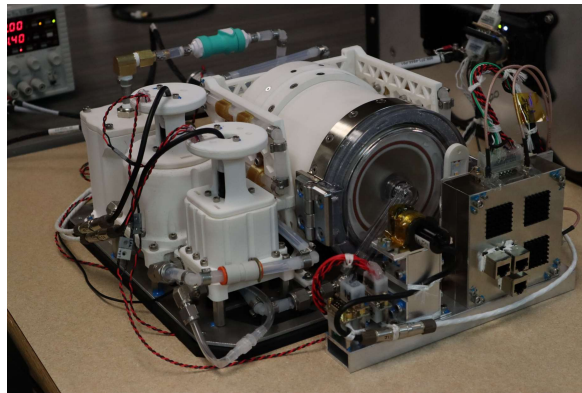
Table 2
Phytoremediation potentials of aquatic plants for industrial wastewater.

Plants	Type of industrial wastewater	Nutrient uptake of interest/ Removal Efficiency	Duration	Authors'
<i>Eichhornia crassipes</i> (Water hyacinth)	Industrial wastewater	BOD (16.4–8.1 mg/L), COD (122–33 mg/L), DO (7.69–5.59 mg/L), pH (7.78–7.35), Fe (139.4%), Nitrite (183.4%), oil & grease (0.8–1.43 mg/L);	2 months	[83]
<i>Pistia stratiotes</i> (Water lettuce)		BOD (17–8.3 mg/L), COD (126–52 mg/L), DO (7.54–5.33 mg/L), pH (7.81–7.37), Fe (137%), Nitrite (0.7 to –0.2 mg/L), oil & grease (0.91–1.54 mg/L).		
<i>Ceratophyllum demersum</i> L. and <i>Potamogeton perfoliatus</i> .	Danube river (Romania)	<i>C. demersum</i> : Cu (22.71 µg/g), Cd (3.52 µg/g), Pb (20.06 µg/g), and Zn (104.23 µg/g); <i>P. perfoliatus</i> : Cu (13.14 µg/g), Cd (1.88 µg/g), Pb (13.32 µg/g), and Zn (57.96 µg/g). BCFs Cd > Zn > Pb > Cu	19 months	[59]
<i>Egeria densa</i> (Brazilian waterweed); <i>Myriophyllum aquaticum</i>	Heavy metal effluent	Accumulation of Ni (10 mg/L), Pb (15 mg/L), and Zn (20 mg/L); Accumulation of Ni (10 mg/L), Pb (15 mg/L), Zn (20 mg/L).	7 days	[38]
<i>Alisma lanceolatum</i> , <i>Carex cuprina</i> ; <i>Epilobium hirsutum</i> ; <i>Juncus inflexus</i> (Hard rush, European meadow rush)	Industrial effluents (heavy metals)	Al, As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Sn, Zn and organic pollutants	1 year/ Constructed wetlands	[33]
<i>Ipomea aquatica</i> (Water spinach)	Synthetic hydroponic solution	Pb uptake		[18]
<i>Ceratophyllum demersum</i> L. (coontail, hornweed);	Synthetic aqueous wastewater	Cd (82.01%) and Ni (50%), Bioconcentration factors (BCFs): Cd (75–707.92), Ni (104.16–200); Cd (91%) and Ni (50%)	3 months/(8th day batch experiment)	[72]
<i>Lemna gibba</i> (Duckweed)	Industrial wastewater (Ammonium rich wastewater)	BCF: Cd (942.79–5093.27), Ni (27.19–638.95)	30 days	[64]
<i>Nasturium officinale</i> (Watercress)		Total hardness (25%), Ca (23%), NH ₃ (66%), BOD (22%), and COD (38%).		
<i>Lemna minor</i> (Lesser duckweed)	Combination of textile, distillery and institutional wastewater;	COD (92%), BOD (92%), electrical conductivity (68%), pH (8–9), TDS (68%);	28 days	[11]
	Treated industrial wastewater	BOD (85.58%), COD (90.57%), TP (104.99%), TN (102.55%), pH (97.65%), Cu (103.70%), Pb (107.49%), Zn (100.62%)	7 days (summer and winter)	[17]
<i>Typha angustifolia</i> L.	1,2-Dichloroethane wastewater (petrochemical)	1,2-DCA (100%)	42 days/ subsurface batch system	[8]

Source: Mustafa, H. M., & Hayder, G. (2021). Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: A review article. *Ain Shams Engineering Journal*, 12(1), 355-365.

Why Duckweed (con.)?

- Duckweed has been flown before on Space Shuttle in 1996
- Duckweed is currently being examined as a space crop potential
- Microgravity growth beds are already being designed and tested



Source: (Left) Space Lab® μ G LilyPond Suborbital Payload, Space Lab® μ G LilyPond EDU (Middle) LEAF β Payload Concept from Space Lab®

There were 3 species of vascular plants in the ABS: the rootless, submerged aquatic macrophyte, *Ceratophyllum demersum*, and the tiny floating aquatics, *Lemna minor* and *Wolffia* sp. The aquatic invertebrate animals were introduced as populations, and included: two species of gastropods, including *Physa* sp.; ostracods; *Daphnia* sp.; the amphipod *Hyalella azteca*, cyclopoid copepods and *Planaria* sp. Three individuals of both snail species,

Source: MacCallum, T. K., Anderson, G. A., Poynter, J. E., Ishikawa, Y., Kobayashi, K., Mizutani, H., ... & Leigh, L. S. (2000). The ABS (Autonomous Biological System): Spaceflight results from a bioregenerative closed life support system (No. 2000-01-2340). SAE Technical Paper.

Biofilm Test Stand



Test Stand Overview

- Biosurface CDC bioreactors are continuously fed ersatz and hold 21 coupons
- Ersatz is fed at a rate of 0.1 mL/min to match the ratio of the flow rate into the wastewater tank
- Stir plates are used to provide constant temperature of 25°C and stir 125 rotations per minute (RPM)
- Each bioreactor is inoculated with a combination of *Burkholderia cephia complex*, *Cupriavidus metallidurans*, *Methylobacterium fujisawaense*, *Pseudomonas aeruginosa*, *Ralstonia insidiosa*, *Aureobasidium pullulans*, *Exophiala spinifera*, and *Lecythophora mutabilis* at a concentration of 10^6 CFU/mL
- Each duckweed round of testing was run for a 3-month duration with re-inoculation at a lower dose of 10^3 CFU/mL and this re-inoculation occurred every week



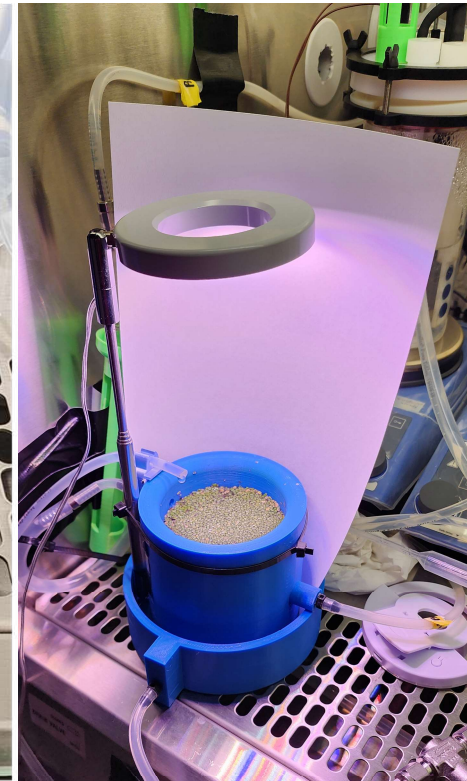
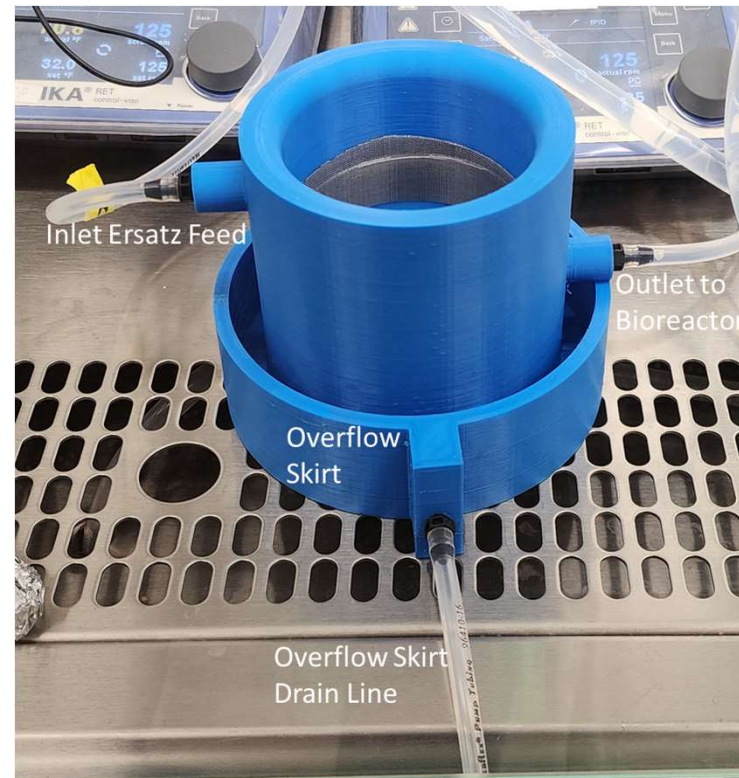
Test Stand Sampling

- Coupon rods are pulled every 3 weeks. Three coupons (one coupon rod) are sampled per ASTM ES2562-22. Coupons are scrapped and plated to determine colony forming units (CFU/mL) on R2A and SDA plates
- An additional coupon rod is pulled every 3 weeks for DAPI staining for imaging
- Coupons are Inconel 718 to match the internal material of the wastewater bellows tank



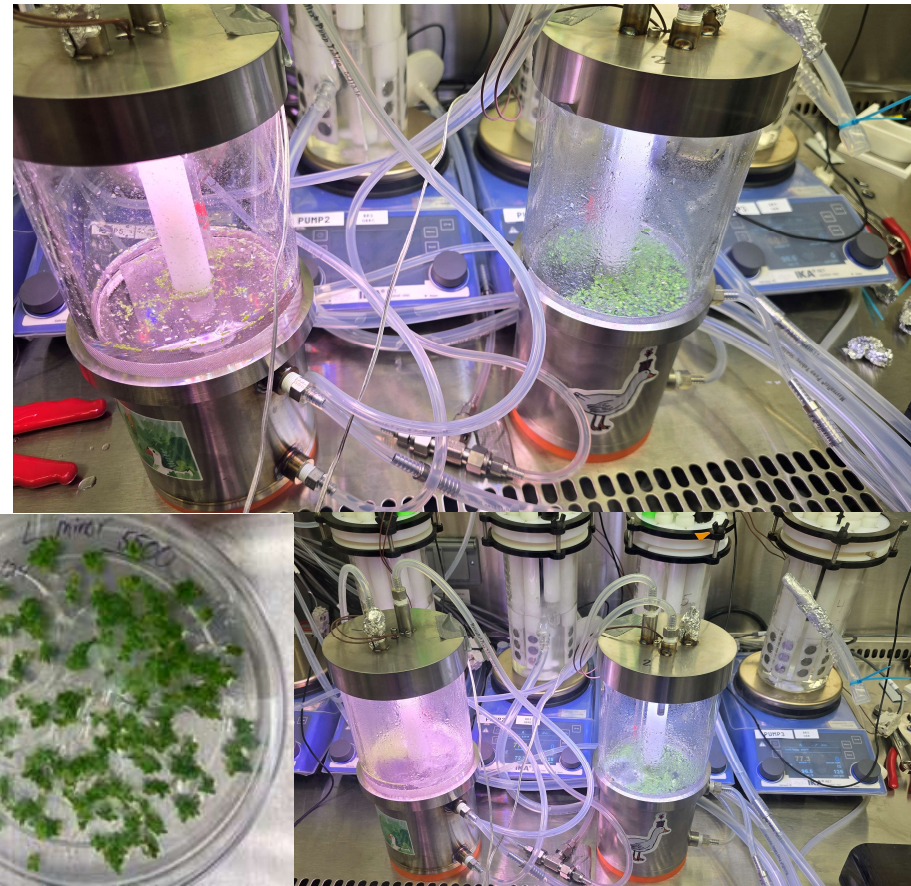
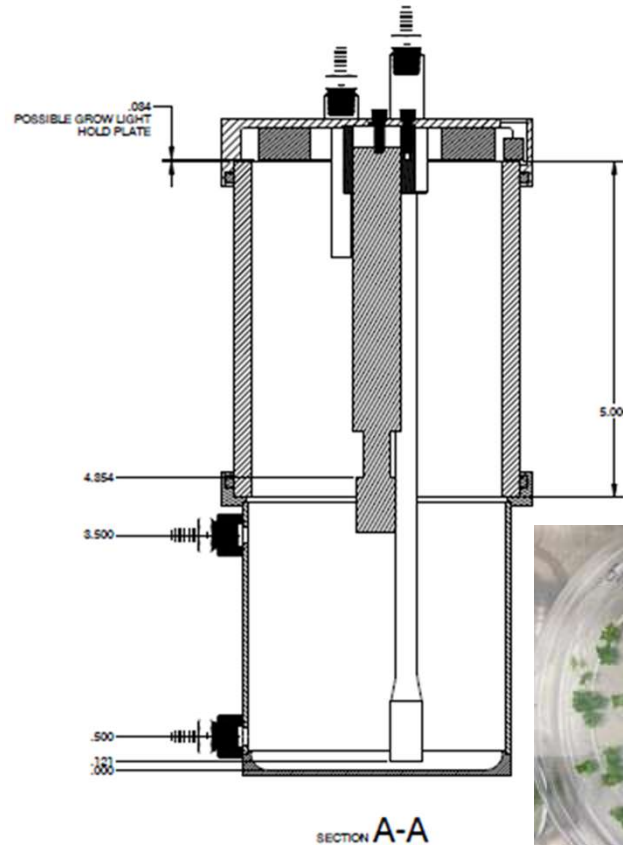
Integration of Duckweed

- Prototype unit piped in upstream of the test bioreactor
- The prototype did experience a few issues
 - Prototype did not have level control
 - Prototype wasn't sterile or fed with sterile air
- The prototype did provide data indicating with better application, the duckweed concept could be a viable mitigation strategy

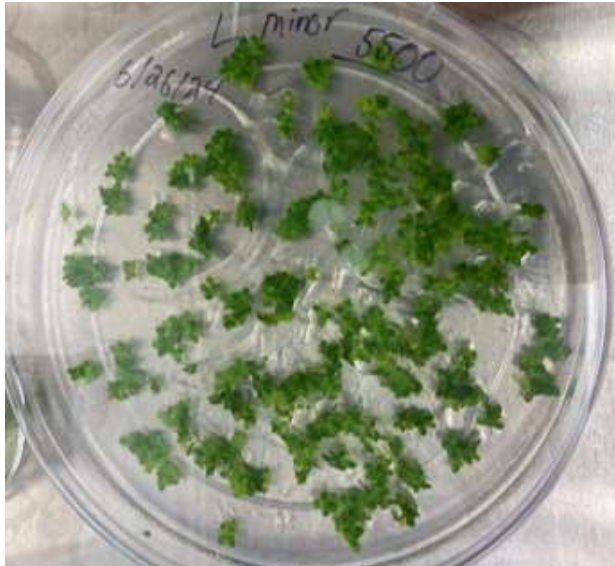


Better Integration of Duckweed

- Jacobs Innovation Grant provided funding for the design and build up of the new units, Phytoremediation Operated Nutrient Depletion (POND) Units
- POND units had level control, could be sterilized due to material selection and had a sterile bubbler system for feed air
- Each POND unit was loaded with a unique sterile duckweed

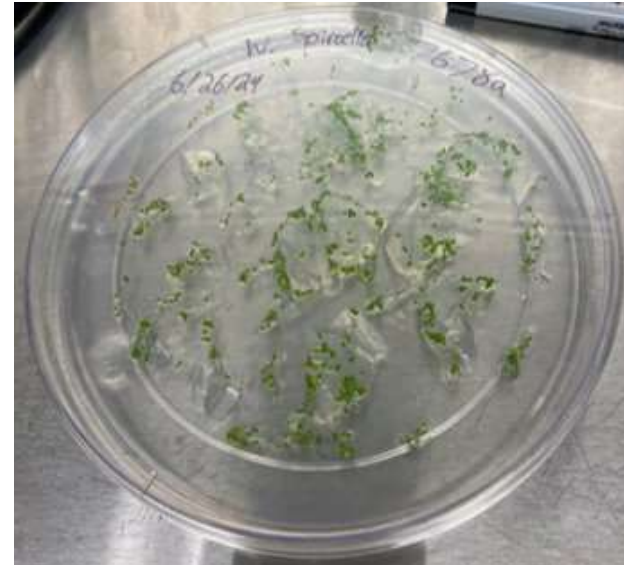


Duckweed Samples



Lemna minor – Blarney 5500

- Used in phase 3 biofilm test stand
- Has been flown on shuttle
- Well studied clone with fully sequenced genome



Wolffia arrhiza – Campania 7678a

- Scheduled to fly on Artemis III as part of a Space Lab Technologies payload named μ G-LilyPond™
- Hearty, temperate-climate clone



Results of the Phase 3.5 Test

Average Coupon Mean Log Reduction of Biofilm (Log10 CFU/cm²)

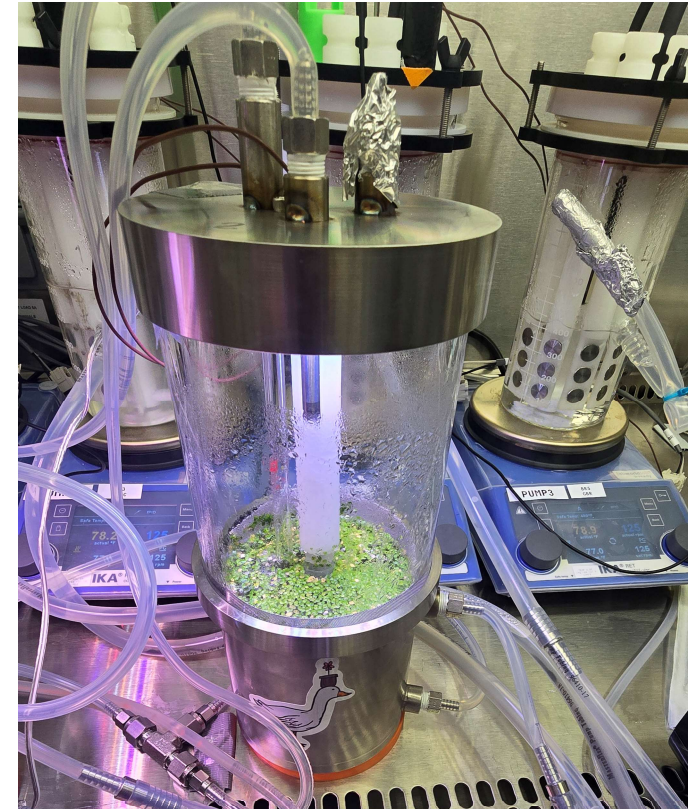
Mean log reduction: ▪ 1-log = 90% ▪ 2-log = 99% ▪ 3-log = 99.9% ▪ 4-log = 99.99%	Bacterial		
	<i>Wolffia arrhiza</i>	<i>Lemna minor</i>	Nutrient Filter
Week 3	1.5	1.46	0.61
Week 6	Test Stopped (Died)	2.00	-1.02
Week 9		1.4	-0.72
	Fungal		
Week 3	1.49	1.62	-0.15
Week 6	Test Stopped (Died)	2.5	0
Week 9		2.47	0

We reduced approximately **97%** of the biofilm growth from the bacterial strains over the 9 weeks, and approximately **99.4%** reduction of biofilm growth from the fungal strains.

Discussion



- In addition to the biofilm reduction, the *Lemna Minor* growth unit produced approximately 24 grams of plant mass starting from 0.5 grams (dry weight)
- *Wolffia arrhiza* suffered from an apparent fungal infection, and had the majority of plant mass die off after week three. This exhibits the risk of starting with sterile strains
- Future crop growth systems could receive the combination urine distillate and humidity condensate for nutrient limitation to reduce biofilm build up in the wastewater processing tank
- A two-growth unit system with one unit operating on nutrient limitation while the other “recovers” and is fed regular growth water for crop production could be implemented



Future Work

- Work continuing in the next phase through the MSFC TID program
 - Tier Three Grant
 - Space Lab® μ G-LilyPond Engineering Demonstration Unit (EDU) being purchased to start evaluating growth of duckweed and biofilm remediation on a full scale size
- Discussion with the external partners to expand duckweed research to contribute to biofilm prevention through phytoremediation
- Examination of pathogenicity and cleanliness of an in-use system
- Experimentation with specifically modified duckweed and a targeted pre-established microbiome



Images provided by Space Lab®

Questions?

Thank you for your time!

