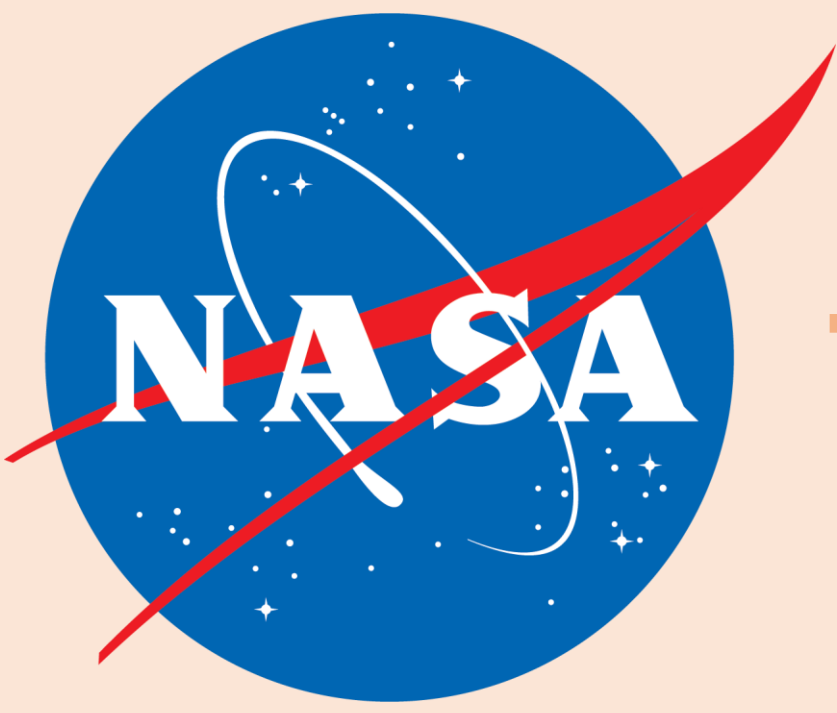
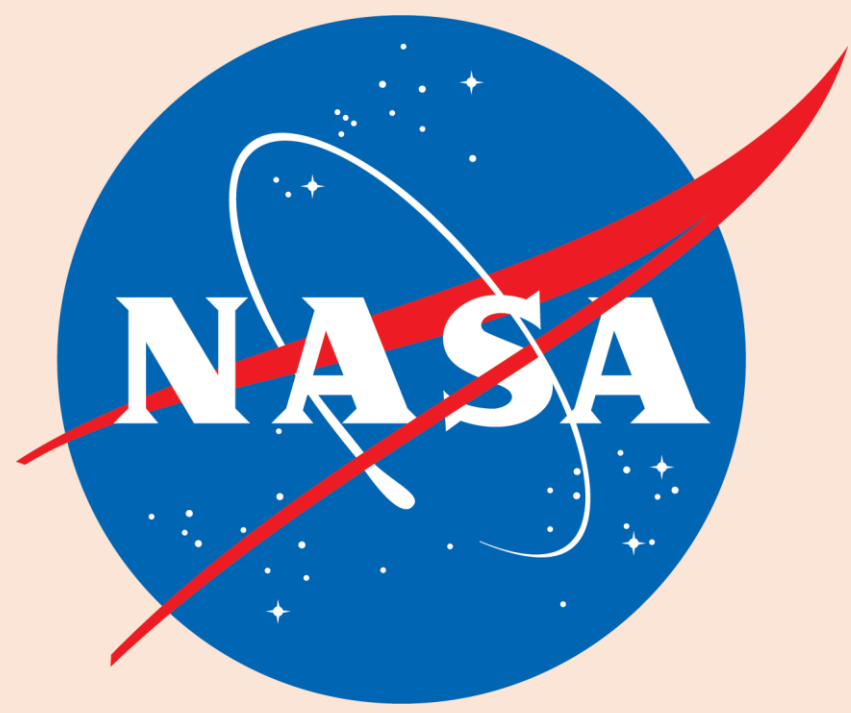




Biofilm Mitigation Testing Phase III



Koellner, Liezel A., Velez-Justiniano, Yo-Ann, Beitle, Eric R., Murphy, Connor P., & McCool, Chelsea

INTRODUCTION

Biofilm is produced from bacterial or fungal organisms that can survive in extreme conditions. The microorganisms attach themselves to surfaces and produce an extracellular polymeric matrix that develops into a community structure and ecosystem.¹ Biofilm has been known to cause blockage and clogging inside the Water Processor Assembly (WPA) and Water Recovery System (WRS) piping. The WPA and WRS on the International Space Station (ISS) is responsible for recycling humidity condensate and urine distillate to produce potable water for the astronauts to survive. The Environmental Control and Life Support System (ECLSS) department is developing a way to mitigate biofilm accumulation inside the wastewater tank.

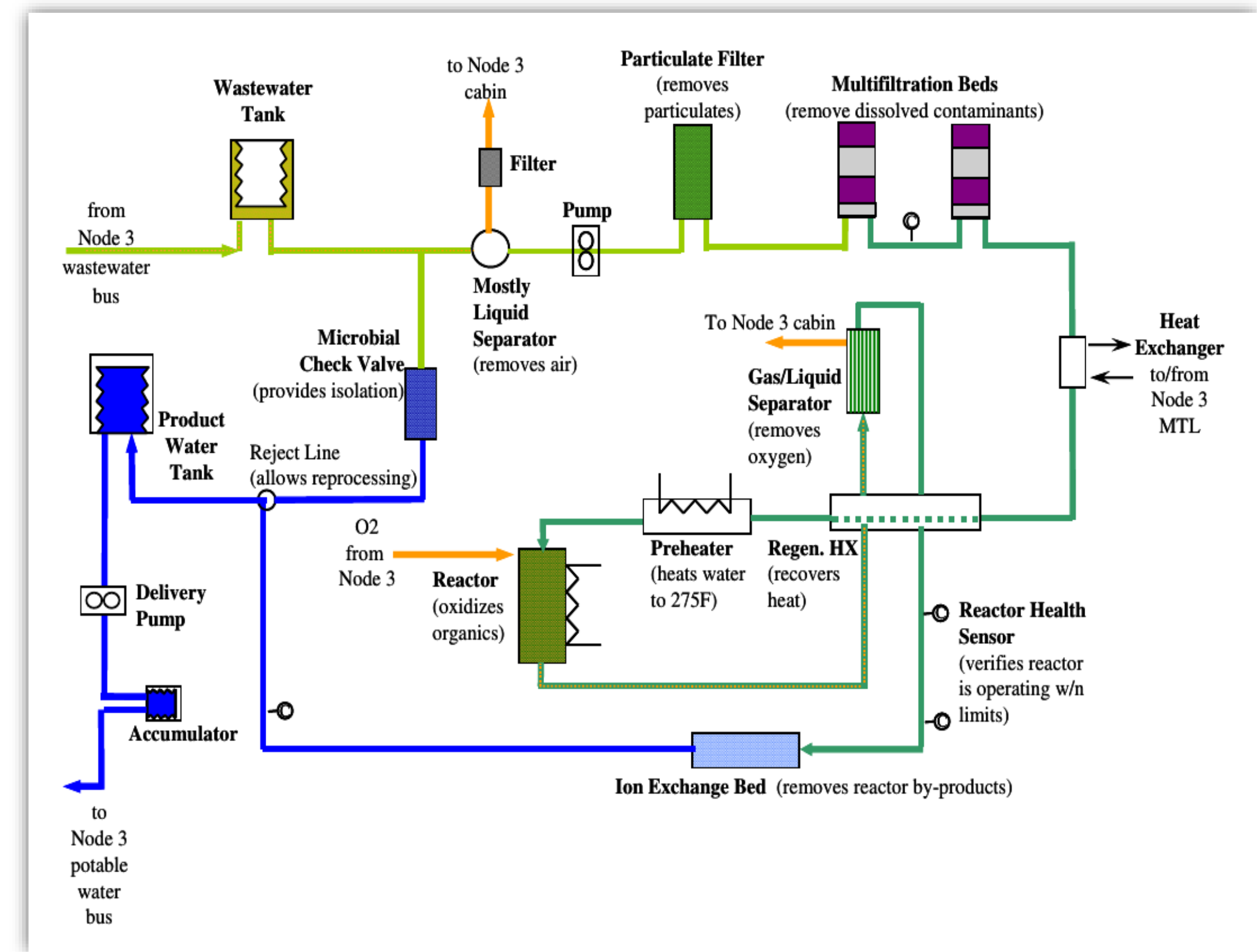


Figure 1: Waste Processor Assembly Schematic



Figure 2: Biofilm accumulation in piping²

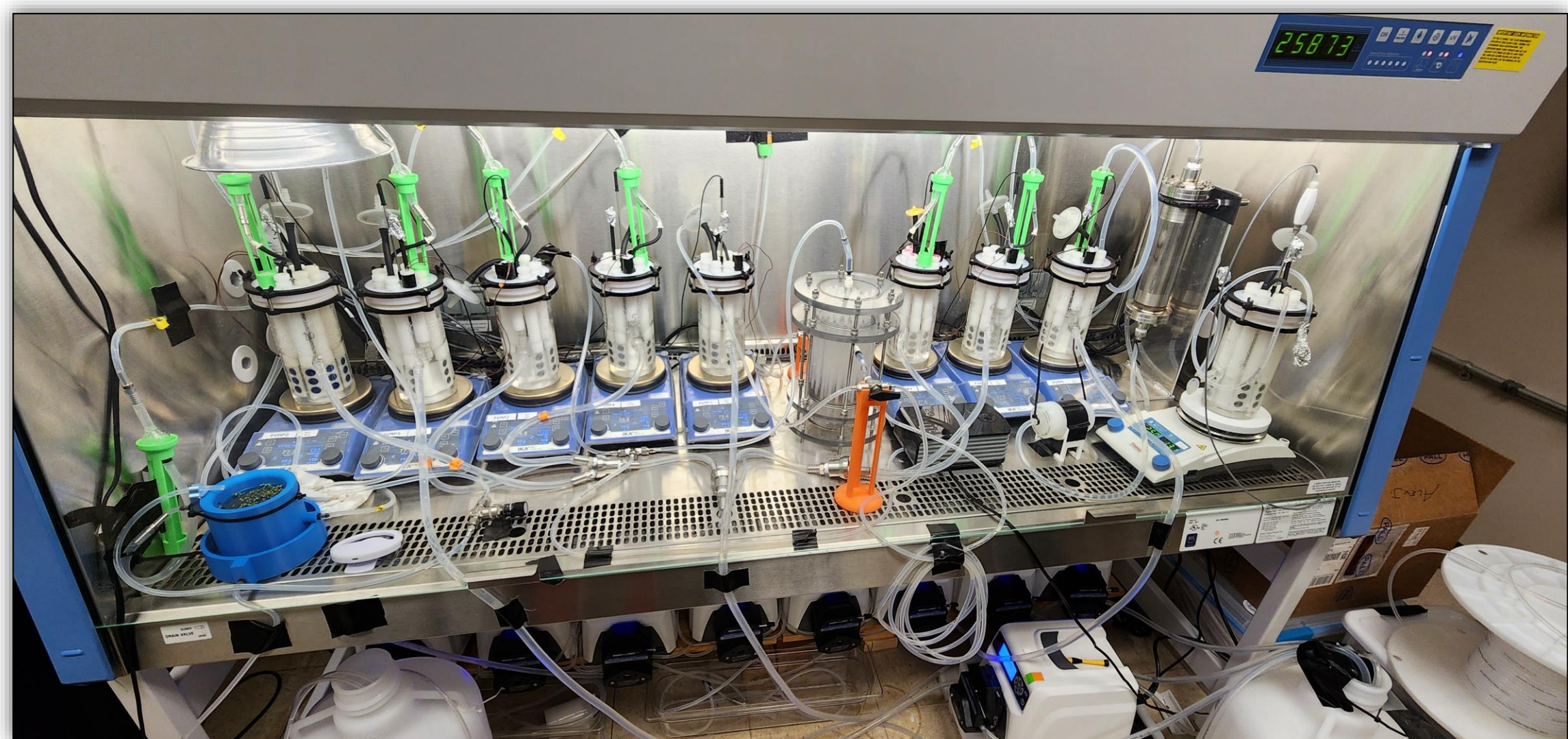


Figure 3: Phase III Bioreactors

Bioreactor (BR)	Mitigation Description
1	Cu infused poly lactic acid (PLA) coupons
2	Phytoremediation – Nutrient limitation by upstream duckweed
3	Aerated control (compare to BR5)
4	High [Microbial Organisms] = 10 ⁶ every 7 days
5	Membrane Aerated Bioreactor (MABR)
7	Inoculated Control – No mitigation
8	Nutrient filter – Nutrient limitation by upstream resin bed
9	Control with pure ersatz – No inoculation

Table 1: Mitigation strategies of each bioreactor

OBJECTIVES

The bioreactor test stand was intended to mimic the conditions within the wastewater tank on the International Space Station to evaluate different mitigation strategies to determine which was the most effective against biofilm formation. One mitigation strategy was nutrient limitation. In the case of BR 2, 5, and 8, the nutrients were removed upstream of the bioreactors that resulted in the biofilm forming organisms being starved of essential nutrients, resulting in death.

EXPERIMENTAL METHODS

- Cultured microbial organisms from frozen
- 4',6-diamidino-2-phenylindole (DAPI) stain microscopy on coupons from bioreactors
- Biofilm analysis to determine area percent using IMAGE J Fiji
- Bulk water & coupon assay on Reasoner's 2A (R2A) and Sabouraud Dextrose Agar (SDA) agar plates to determine CFU/mL & CFU/cm²
- Water analysis to determine total organic carbon, conductivity, and pH

DATA

DAPI Stained Coupons:

Below is the progression of coupons sampled from the bioreactors for week 3, 6, and 9. The coupons were imaged using DAPI microscopy.

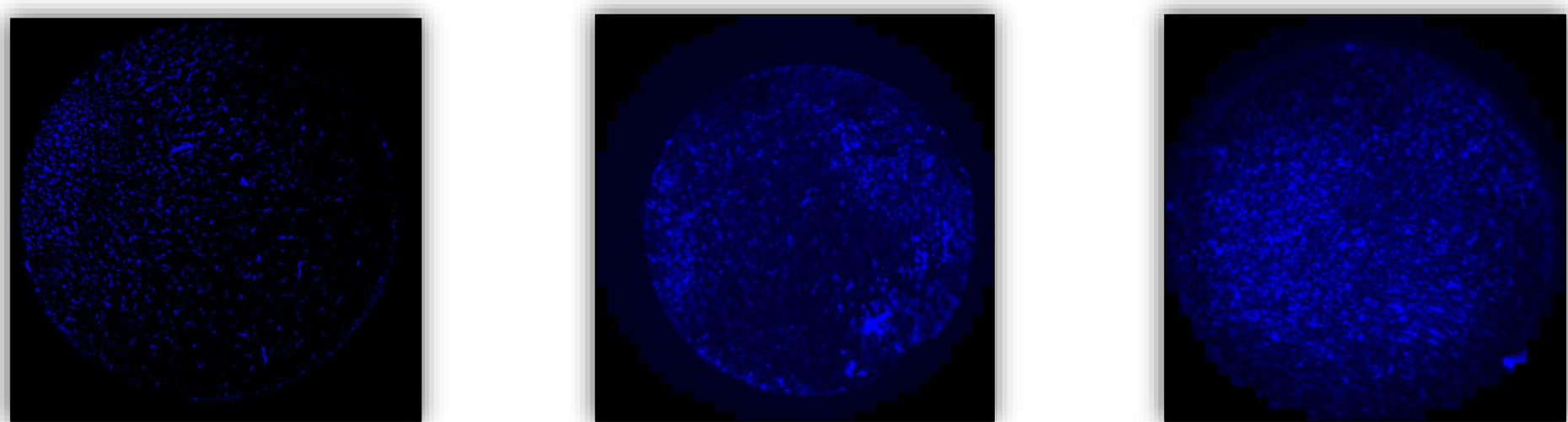


Figure 4: BR 7 Inoculated Control

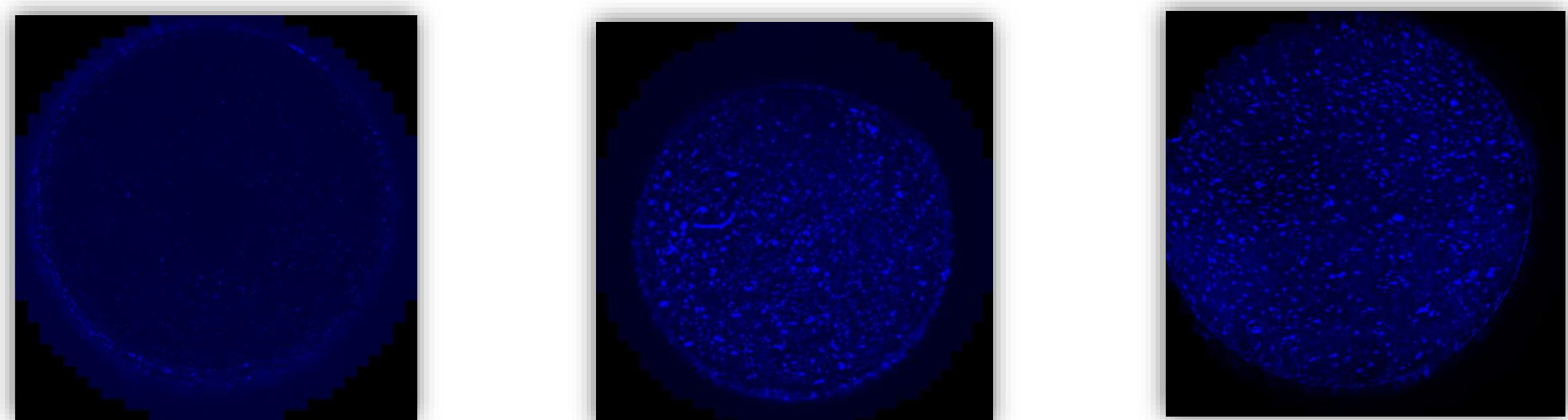


Figure 5: BR 2 Duckweed Upstream

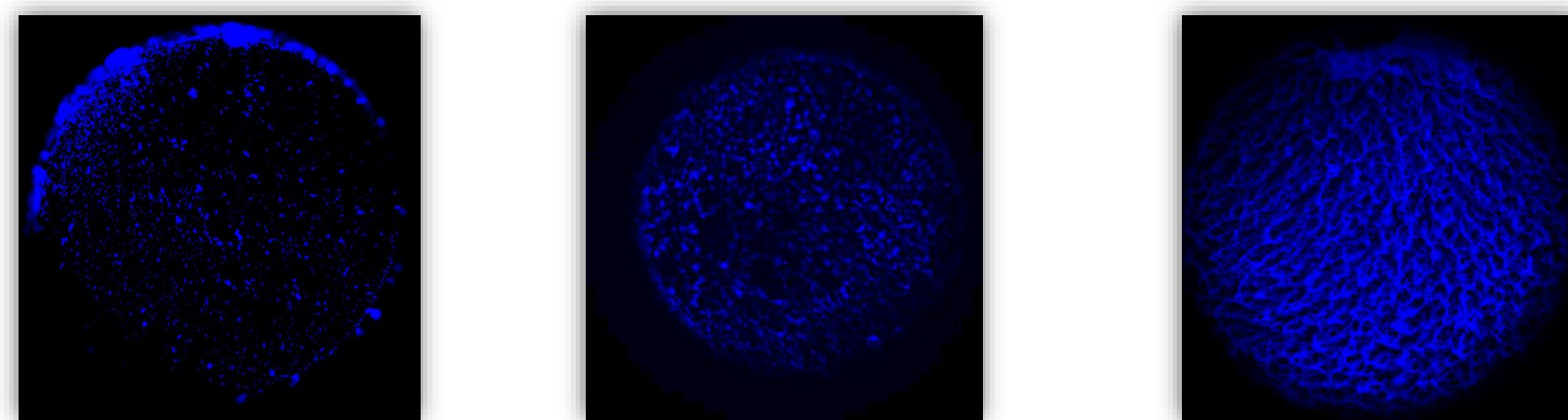


Figure 6: BR 3 Aerated Control

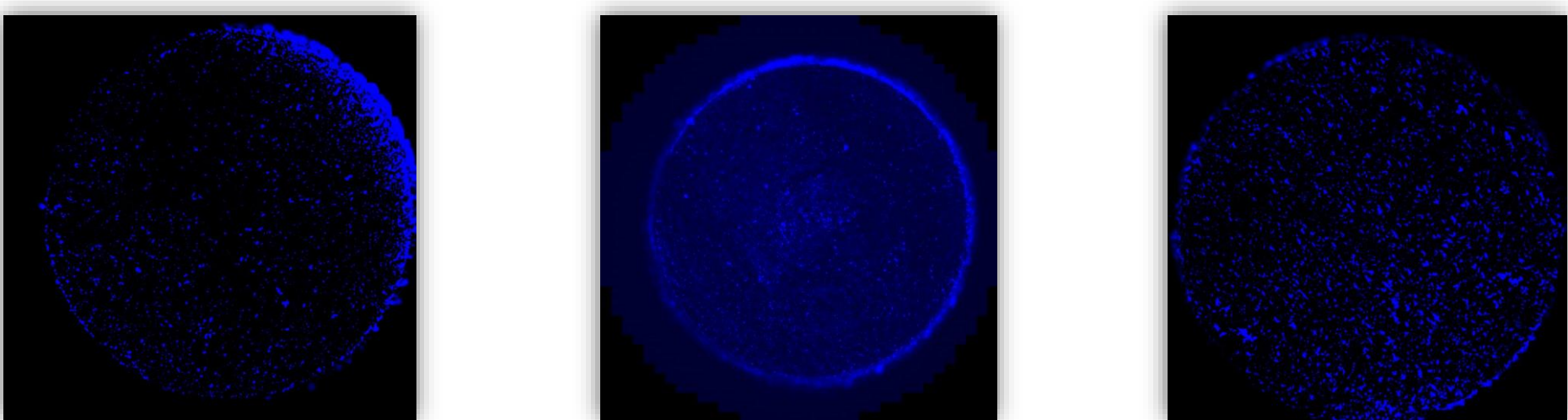
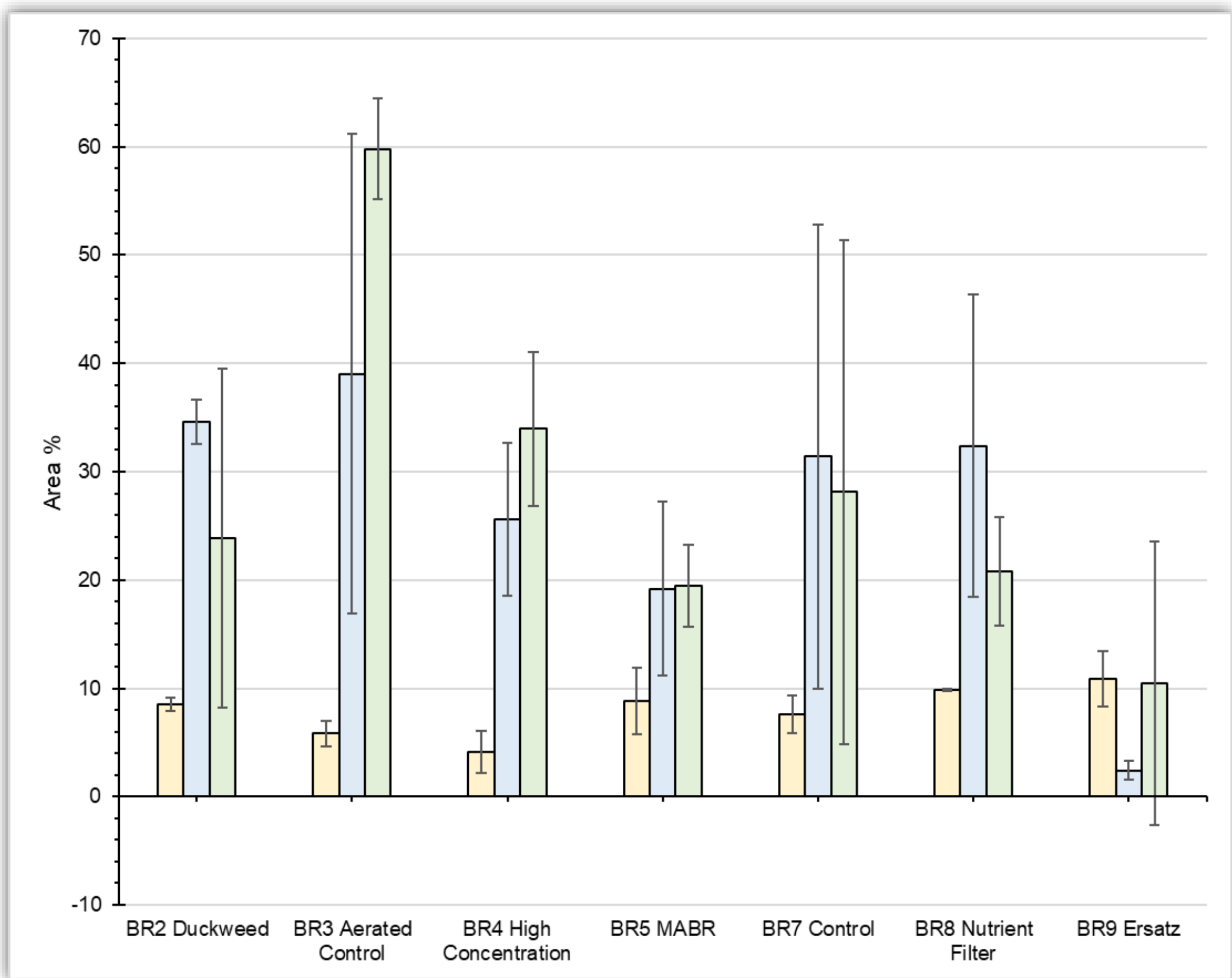


Figure 7: BR 5 MABR

DATA

Biofilm Area Percent:

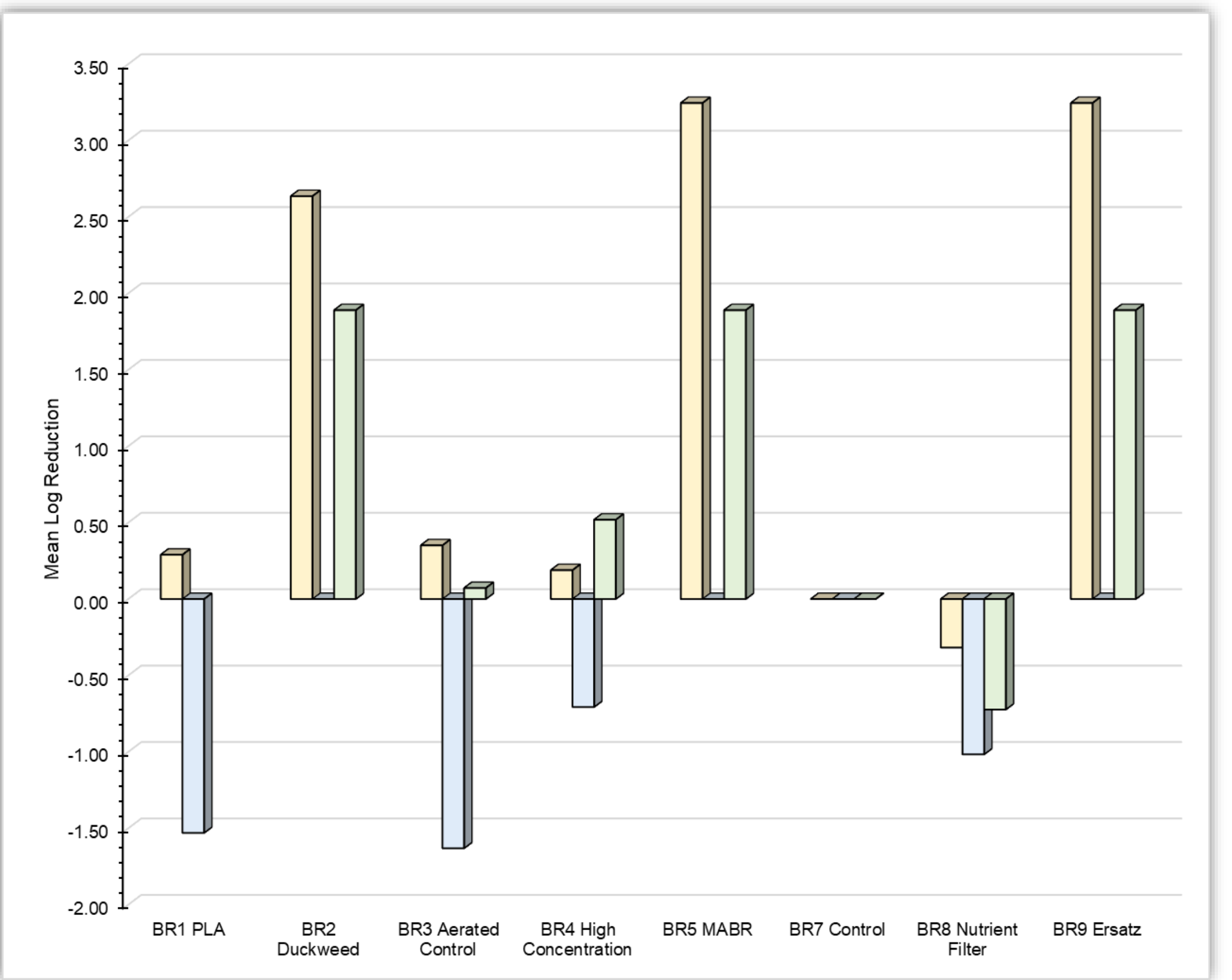
Graph 1 represents the biofilm area percent to the coupons using ImageJ Fiji. Each week included triplicate coupons for sampling.



Graph 1: Biofilm area percent on the coupons

Mean Log Reduction:

Graph 2 represents the mean log reduction that was obtained from the coupons inside the bioreactors for each week.



Graph 2: Mean log reduction for Log₁₀ CFU/cm²

CONCLUSION

The mitigation strategies from BR 2 and BR 5 had the least amount of biofilm accumulation on the coupons and the largest mean log reduction. A mean log reduction is the measure of how thoroughly a decontamination process reduced the concentration of the contaminant (i.e., biofilm) with 1-log = 90%, 2-log = 99%, 3-log = 99.9%, and 4-log = 99.99%.

REFERENCES

- Bamford, Natalie C., Cait E. MacPhee, and Nicola R. Stanley-Wall. "Microbial Primer: An introduction to biofilms–what they are, why they form and their impact on built and natural environments." *Microbiology* 169.8 (2023): 001338.
- Zea, Luis, et al. "Design of a spaceflight biofilm experiment." *Acta astronautica* 148 (2018): 294-300.

ACKNOWLEDGEMENTS

Jill P. Williamson the ISS Water Subsystems Manager
Paul Tatum the Manager of Advanced Life Support Systems & The Mars Campaign Office.