**21-4:** **Biofilm Study Under Simulated Microgravity**

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**Primary Taxonomy:** TX06.1.2 Water Recovery and Management

**Start TRL:** 1 **End TRL:** 3

**Executive Summary:** The goal of this study was to understand biofilm formation under microgravity (µg), in support of biofilm mitigation efforts in exploration water recovery systems. The technical approach was to conduct a mass transfer and bacterial culture study under both simulated µg and ambient gravity. The aim was to correlate nutrient consumption to gene expression to better understand biofilm formation.

A representative species of bacteria that is commonly cultured from the International Space Station (ISS) Water Processor Assembly (WPA) was cultured in a WPA influent water ersatz formulation that is tailored for microbiology studies. A mass transfer rate study was carried out using the ersatz WPA influent water by introducing a water-soluble dye to represent dissolved nutrients and nutrient particles. Imaging of dye diffusion over time allowed for the comparison of mass transport rates under a series of rotation per minute (RPM) speeds for the High Aspect Ratio Vessels (HARVs) on a Rotating Wall Vessel (RWV). This was done to determine the speed that will most accurately simulate the low convective rates experienced under actual µg conditions. Three biological replicates of the *Burkholderia contaminans* (*B. contaminans*) microbe were cultured under simulated µg with a rotating (R) control in the horizontal plane at the determined optimal RPM of 15, along with a stationary (S) reference culture. At T=0, and then at T=1,2,3 (in exponential phase) and T=4 (in S phase), the bacterial culture and ersatz were harvested for transcriptomic and nutrient content analysis, respectively. The experimental results illustrated that phosphate is a limiting nutrient in the WPA ersatz formula. Nutrient analysis illustrated that the µg treatment culture took up essential nutrients more rapidly than the R and S control cultures, yet non-essential nutrients remained higher in the µg treatment than in the controls at later timepoints. The rapid uptake and subsequent starvation of phosphate in the culture under µg conditions is further illustrated in the transcriptomic response when compared to that of the R control condition. The subsequent starvation response may serve as one element to explain a moderate enhancement of biofilm formation in the µg treatment. One implication of this work is that biofilm mitigation in the ISS environment could be supported by ensuring a steady flow of water as a vehicle for phosphate within the WPA to avoid complete phosphate consumption, which occurs in times of no flow and leads to undesired biofilm formation.