

CDC Bioreactor Configuration Method for Volume Level Control with Controlled Inlet and Outlet Flow

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Environmental Control Life Support Systems and other microbiological biofilm studies often utilize small scale bioreactors. Among these options are the popular BioSurface CDC bioreactors, currently being utilized by Marshall Space Flight Center groups researching the impact of biofilms on life support systems. After a recent experimental regime, it was determined additional equipment could be added to augment the capabilities of the bioreactors. Previous research configurations such as ASTM E3161-21 relied on an outlet stream gravity draining from a side drain port located above sampling coupons. This limited applications to experimental conditions with a controlled inlet and uncontrolled outlet flow. With the introduction of a small single pole single throw (SPST) reed float switch and a peristaltic pump connected to the outlet drain and a chassis controller, the bioreactor is able to maintain a set level. The modification allows additional variables to be tested, including highly adjustable fill and flush cycles, bioreactor volume, draining and filling control. Once the configuration modifications were implemented through the installation of the new equipment, data was collected to ensure the stability of the level measurements. As the level control switch is a float switch, consideration was taken into account for effects of internal stirring speed, along with effects of inlet and outlet flow rate. Data presented in this study will illustrate the stability and effectiveness of the configuration changes in equipment made to the bioreactor. These configuration changes have demonstrated a control method to conduct biofilm mitigation techniques for ECLSS hardware research. The controlled level capabilities allow for a constant drip feed flow rate into the bioreactors, a key aspect of the biofilm mitigation testing.

Nomenclature

ASTM	=	American Society for Testing and Materials
CDC	=	Center for Disease Control and Prevention
SPST	=	Single Pole Single Throw
ECLSS	=	Environmental Control and Life Support Systems
MSFC	=	Marshall Space Flight Center
DAQ	=	Data Acquisition Systems
NI	=	National Instruments

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I. Introduction

Biofilm growth occurs when microorganisms, such as various bacteria or fungi, adhere to a surface and clump up around each other on said surface. The microorganisms then produce an extracellular polymeric substance to protect the community of microorganisms. This biofilm can eventually detach from the surface, which can lead to clogging of filters or small orifices when the detached biofilm comes into contact with these components. This clogging has been a topic of concern for space travel, as the clogs caused by the biofilms can lead to impaired functionality of life support systems and the need to carry spare components, which limits the supplies that can be brought. In order to better understand how these biofilms are formed and determine what methods best mitigate or breaking down biofilms, they can be grown and monitored by using CDC bioreactors. The CDC bioreactors have utilized several different ASTM standards for growing bacterial and fungal biofilms, such as *Pseudomonas aeruginosa*[1]. The bioreactors have additional ASTM standards developed for them that instruct on how to determine the effectiveness of surface treatments and mitigation techniques[1]. However, all of these standards operated with a controlled inlet flow into the top of the reactor and the fluid inside would simply gravity drain once the volume reached the outlet spout. Due to the drain spout of the CDC bioreactors being situated around the 350 mL mark, a method to control the outlet flow was required. After working through several design schemes to control the volume, a simple yet effective method of using a single pole single throw (SPST) float switch to command a peristaltic pump was decided upon for the final design. Similar to the inlet of the original CDC bioreactor design, a peristaltic pump is situated downstream of the bioreactor. This allows for fine control over the drain rate and bioreactor volume level, along with different testing conditions to be obtainable, such as a higher volume or a flushing simulation.

II. Description of MSFC Biofilm Test Stand

The 8 CDC bioreactors that were used in the MSFC biofilm test stand are one-liter glass vessels with an outlet spout at approximately the 350 mL marker. Each bioreactor has eight polypropylene removable coupon rods, with three Inconel coupons per rod and an example bioreactor is shown in Figure 1. Those CDC bioreactors were connected to an upstream reservoir and were fed by using peristaltic pumps. The upstream portion follows a setup found in both ASTM E3161−21 and BioSurface Technology's operation manual, shown in Figure 2, with a slight variation of each peristaltic pump driving two bioreactor inlets, such that only 4 pumps were needed instead of 8[1,2]. The system downstream of the reactors' outlets deviated from the ASTM E3161−21 as well, as there were peristaltic pumps between the outlet drain and the end tank[1,2]. Traditionally the CDC bioreactors would gravity drain once the fluid reached the outlet spout into a collection tank, shown in Figure 2[1,2]. The peristaltic pumps downstream of the bioreactors blocked the fluid flow out of the bioreactors until they were activated, which occurred when the targeted volume was reached. The peristaltic pumps downstream of the bioreactors also allowed for the fine tuning of the outlet flow rate, allowing for a flow rate of 0.1 mL/min for this test stand. An example schematic for the pump configuration for the MSFC Biofilm test stand is shown below in Figure 3. All pumps utilized in this setup were Masterflex® L/S® 100RPM 115/230 pump drives with a dual Pump Head L/S® Easy-Load® II CRS. Both the inlet and outlet flow rates were verified by volumetric flow checks to be truly operating at 0.1 mL/min. Each of the bioreactors sat atop an individual IKA® RET control-visc stir plate that provided the heating and stirring to the bioreactors. Heating was controlled by a thermocouple measuring the fluid temperature through a thermowell to preserve sterility and the thermocouple output was fed into the integrated PID controller within the stir plate which heated the bioreactor accordingly to reach the set temperature. The magnetic stir bar within the CDC bioreactors ran at a constant 125 RPM. The IKA® RET control-visc stir plates also could output the weight atop the plate, which will be discussed further below.

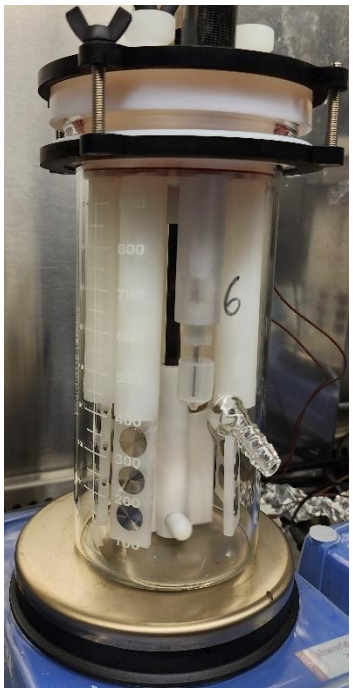

Figure 1. BioSurface CDC Bioreactor

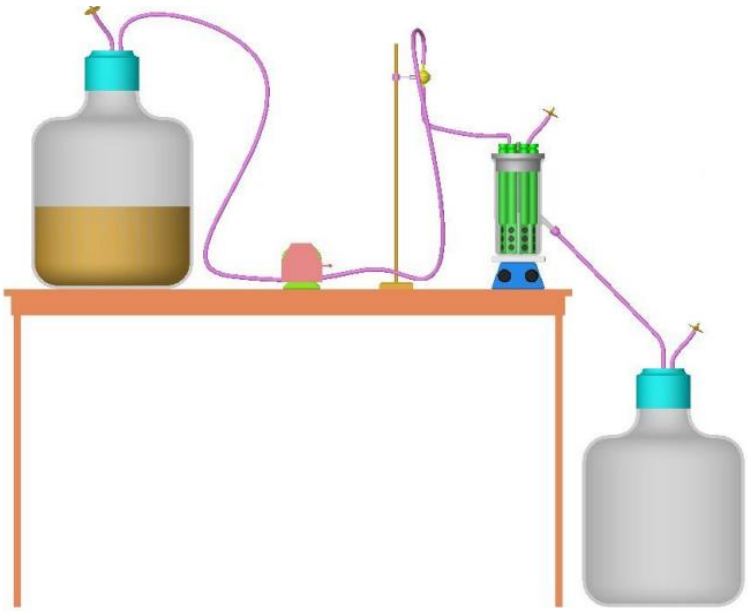

Figure 2. Assembled Reactor System Schematic[1,2]
Image credits: P. Norris and BioSurface

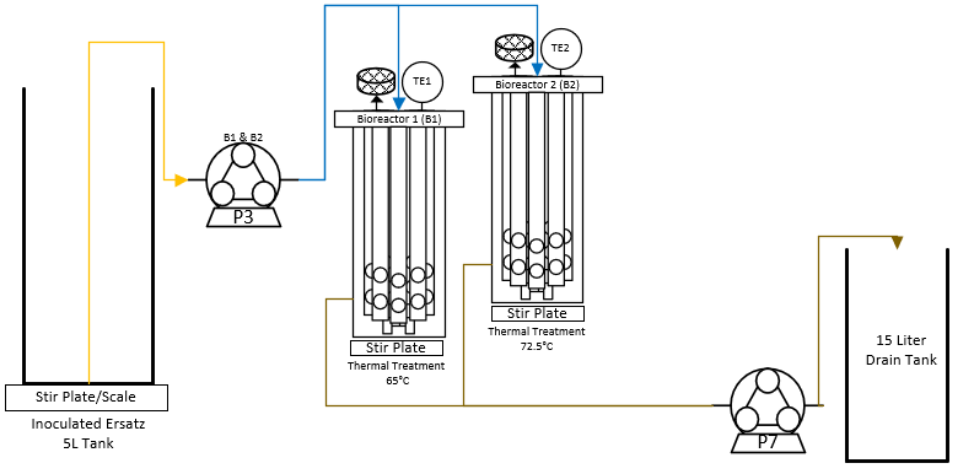

Figure 3. Schematic of the MSFC Biofilm Test Stand Bioreactor Assembly

III. Description of First Iteration of the MSFC Biofilm Test Stand

The first iteration of the MSFC Biofilm Test Stand operated similarly to the aforementioned description with the only difference being that the first iteration had 10 bioreactors integrated instead of 8. Additionally, the set point for the level control was 330 mL for the first iteration as opposed to the 550 mL used in the current iteration. This change in setpoint was solely to better replicate the testing conditions for the biofilm within the bioreactors and is unrelated to the volume control. As mentioned previously, the IKA® RET control-visc stir plates, can enter "PC mode" by communicating with a computer. In the PC mode, the stir plates heated and stirred the bioreactors and send the weight to the program controlling the MSFC Biofilm Test Stand. Before this mode was activated, the stir plates were tared with the empty CDC bioreactors with the coupons and coupon rods inserted. Once the stir plates were tared, they could then enter the "PC Mode" and continuously report the measured weight to the software as the bioreactors were filled using the upstream peristaltic pumps. When the program received notification that the weight of a bioreactor had crossed this threshold, the peristaltic pump downstream of the bioreactor activated and drained the bioreactor at a rate of 0.1 mL/min. For the first iteration, this setpoint occurred when the stir plates returned a weight above 350g as the fluid was mostly water with some nutrients and minerals contained within the mixture, so the approximation of 1 mL = 1 g was used.

The first iteration test stand configuration ran continuously for 120 hours before stopping issues with the contamination of the feed fluid. During this 120-hour run, an issue with the draining function was noticed. After approximately 24 hours into this run, the weight values that the IKA® RET control-visc stir plates were outputting to the program appeared to be slightly out of line with the visible volume within the bioreactors. It was only a slight discrepancy at first, with a maximum of around 20 mL difference between the reported and actual volume. Approximately 72 hours into the run, it became apparent that the weight measurements reported from the stir plates were significantly incorrect. At the end of the test run, one of the stir plates was reading 250 mL under the actual volume and the bioreactor filled to a volume of 800 mL. This inaccuracy was seen across all ten bioreactors, with varying, seemingly random amounts of drift.

Due to these observations, using the reported weight of each bioreactor was not a reliable method of determining their volume. Two possible causes of error were considered. The first was that the very low flow rate of 0.1 mL/min could have not been a significant enough increase and the stir plate could have seen it as a drift and auto-tared accounting for it. The second was that the stirring function of the stir plate could have somehow interfered with the load cell, causing inaccuracies that continued to compound over the 120-hour run time. The two potential sources of error were not investigated further due to both the low flow rate and stirring being requirements of the test. In figure 4, a sample of the weight values received from the stir plates is shown 50 hours into the test. The lower and upper bounds are designated by dashed red lines at 280 and 380 mL respectively. Figure 4 shows the failure of the method of utilizing the stir plates weight measurements to remain within the acceptable bounds. Delineation was noted between the recorded bioreactor weight, and external graduated markings present on the bioreactors.

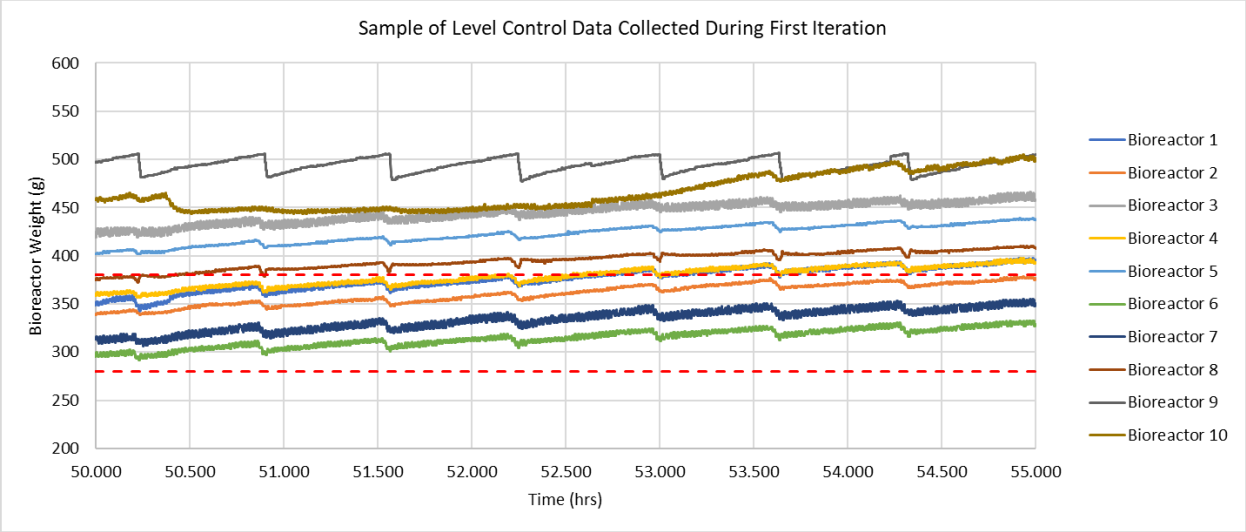

Figure 4. Sample of Recorded Weights of the Bioreactors During First Iteration of Testing

IV. Determining an Alternative Method to Measure Volume

Due to the large inaccuracies in the weighing function of the stir plates, a new method to maintain the volume in the CDC bioreactors was needed. A simple solution was desired along with either non-intrusive hardware or for sterilizable wetted hardware, preferably autoclavable, made of a compatible material. After researching different methods of level control and looking for simple to implement options, float switches were found to be the most ideal to add to the system. The challenges in using a float sensor was to select one that was made of a material that is already contained within the CDC bioreactor to avoid introducing any additional variables for biofilm growth. Additionally, the limited space and geometry of the bioreactor made selection of a wetted sensor difficult. However, a float switch meeting both of these conditions was found after searching for available commercial off-the-shelf options. The float switches selected were ProSense® FLS-VS-200s. The wetted materials were all polypropylene, the same material that the coupon holder rods are constructed of, and the float switches have a diameter of 0.58 inches. This small diameter allowed for them to be inserted through one of the holes in the top of the bioreactor, in place of one of the coupon holder rods. In Figure 5, a ProSense® float switch is shown next to the standard coupon holder rods used with the bioreactors for a size comparison. The float switches were sterilized at their maximum operating temperature of 105° C for 90 minutes to ensure sterility.

To set the float switch to a consistent height, fixtures were manufactured by taking rod stock of polypropylene and machining it down to the same diameter as the standard CDC bioreactor control rod. A through-hole was drilled to allow passthrough of the wires and a threaded base to mount the float switch. An example of this fixture is shown in Figure 6. The modified level control sensor does take the place of one of the coupon sample rods, but the ability to accurately control the level in the bioreactor for the remaining seven sample rods negates the loss of three coupons. For the MSFC Biofilm Test stand, this was acceptable since only 21 samples needed to be pulled during the operational test, which the other seven coupon rods provided.

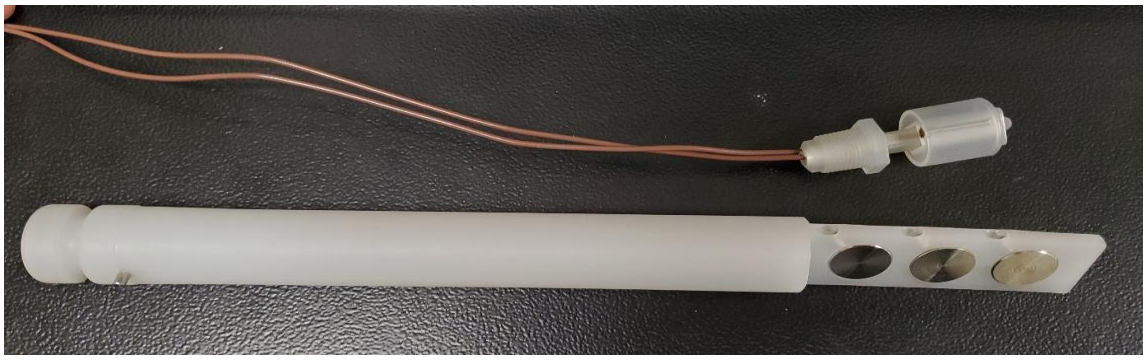

Figure 6. Prosense® Float Switch Next to a Coupon Sample Rod

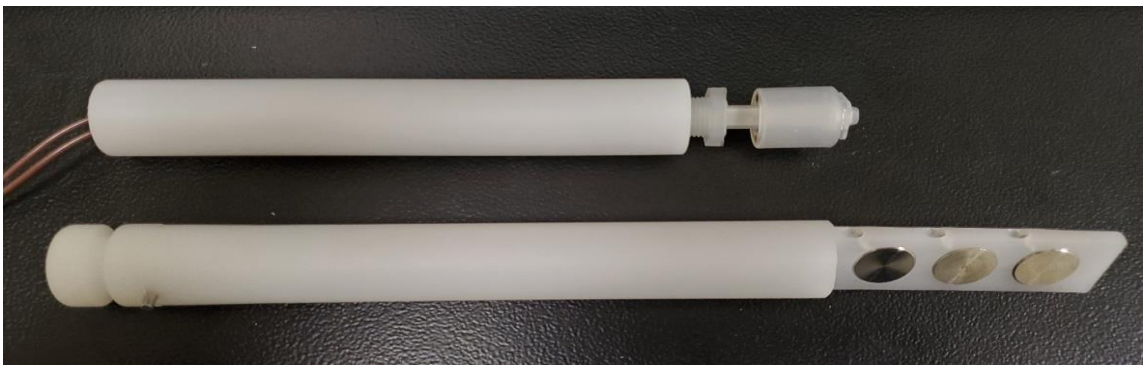

Figure 5. Fabricated Housing for the Float Switch Next to a Coupon Sample Rod

The initial design had a slight flaw in that the float switches would occasionally sink due to the fixture sliding down deeper into the rod holes atop the bioreactors. The slippage of the float switches occurred due to the gasket within the lid of the bioreactor experiencing expansion from the heat of the stir plate. This however was quickly remedied by adding a metal notch press fit into the float switch fixture to further mimic the coupon holder rods. With the notches added to the float switch fixtures, all of the actuation points of the switches were aligned with the visual 550 mL mark on each bioreactor. Once this modification was made, several tests were run at flow rates varying from 0.1 to 25 mL/min, with an operating temperature within the bioreactor around 90° C and there was no noticeable or measurable change in the float switch height within the bioreactor. An assembled bioreactor with the coupon rods and float switch housing installed is shown in Figure 7.

Once the method of maintaining the volume within the bioreactors and the hardware to do so was selected, it was implemented into the MSFC Biofilm Test Stand. The float switch was connected between a 24V power supply and a NI 9375 card. In the float switch's default state, the NI card read the 24V signal and when the float switch was actuated, the NI card would receive no signal. A default read state was chosen so that if any damage was suffered to the float switch during autoclaving the failure would be immediately identifiable. In the floated state, the inlet pump is off and the outlet pump is on, and in the non-floated state, the inlet pump is on and the outlet is off.

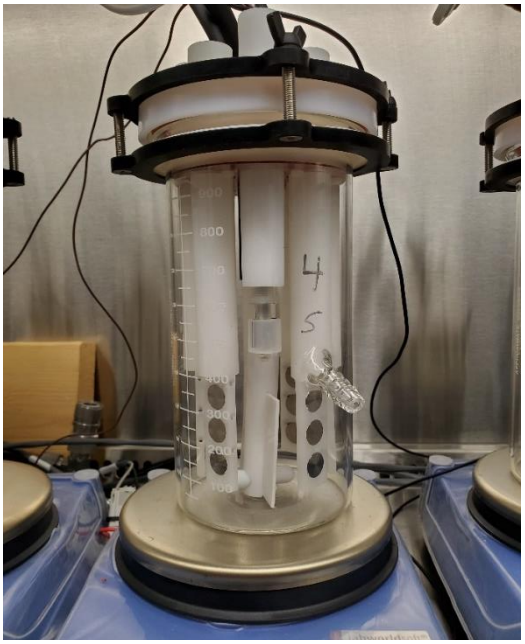

Figure 7. Assembled CDC Bioreactor with Float Switch Housing

In order to validate the accuracy and stability of the actuation of the float switches, three sets of data were recorded from 6 bioreactors every 30 minutes for 5 hours. The data recorded was the visual volume of each of the 6 bioreactors. The plot showing the averaged volumes of the bioreactors is shown below in figure 8. The graph demonstrates that there is some variation in the volume within each bioreactor, but that this control method keeps the volume within the desired minimum volume of 500 mL and the maximum volume of 600 mL.

V. Conclusions and Future Applications

There are some downsides to utilizing this design, with the largest being that the float switch assembly replaces one of the coupon sample rods, leaving 21of the original 24 samples within the bioreactor. For the MSFC Biofilm Test Stand, this was acceptable, as maintaining level control was significantly more important than receiving three additional samples. An additional drawback is that it complicates the operation of the bioreactor, as an external logic controller is needed. The necessary drain pumps downstream of the bioreactors also add complexity and cost to the test design. In the MSFC Biofilm test stand this also was not a significant drawback, as a computer DAQ was already utilized to control the operation of the pumps and stir plates and spare pumps were available for use.

The design could also be better improved by having separate pumps for each bioreactor. The MSFC Biofilm Test Stand had a single peristaltic pump control the inlet to two bioreactors due to a limited number of pumps available for use. This resulted in having less control over the volume of each bioreactor, but as shown in figure 8, the volumes of the bioreactors remained within our tolerances, so it was deemed acceptable. For future tests that need more control or have a tighter tolerance, it would be recommended to have a separate inlet and outlet pump for each bioreactor.

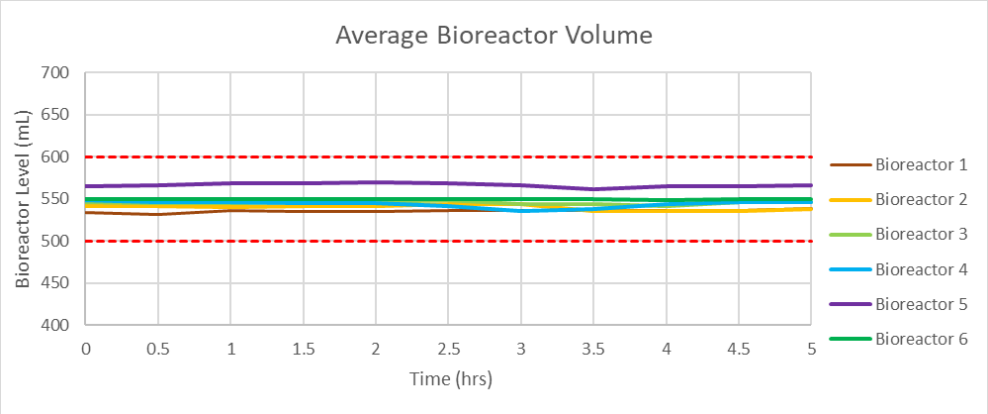

Figure 8: Average Results of Level Stability Tests

This method of holding the CDC bioreactors to a higher volume than that of the drain spout can be used for several applications in future tests. Any test that requires an operating volume greater than 350 mL, which is dictated by the height of the outlet port, such as the MSFC Biofilm Test stand, can benefit from the float switch volume control method. Any volume between 350 mL to 950 mL can be targeted by adjusting the height of the float switch within the bioreactor. This could be accomplished by simply changing the location of the metal notch that was press fit into the float switch housing.

An additional application would be utilizing the float switch to allow for recreating a flushing scenario. An example would be to have a bioreactor with 350 mL of liquid, which is the minimum dictated by the height of the drain port, and fill that bioreactor up to a volume of 700 mL at a flow rate of 50 mL/min. Once the 700 mL is reached, several different actions depending on the test could be done, such as heating the fluid to a certain temperature, simply holding that volume for a period of time, or immediately running the drain pump and pulling the volume back down to 350 mL. This could allow for a simulation of filling and emptying a wastewater tank in batches akin to the flushing of a toilet, which could allow for a better understanding of biofilm growth and impact on systems downstream for future habitation considerations.

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Stambaugh. We would like to acknowledge the NASA MSFC Space Systems Department for their management and support.

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