

Ground Test Validations for Uncrewed Dormancy Preparation and Recovery

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In future long duration space flight and habitation missions, periods of uncrewed dormancy where life support systems are shutdown or in low power-modes will occur. To examine the impacts of these dormant periods, along with potential pre- and post-dormancy procedures, Marshall Space Flight Center has been building an uncrewed dormancy test stand. The uncrewed dormancy test stand will include a urine separator from the Universal Waste Management System, a condensate separator, urine filter, brine filter and distillate filter. In conjunction to the test stand, the Center for Biofilm Engineering (CBE) has ongoing long-term dormancy experiments to assess microbial survival in a variety of simulated shutdown procedures including shut-in, flushing with and without biocide, and dry-out conditions. This publication will serve as a discussion of dormancy considerations, criteria for success, and experimental designs for testing dormancy conditions and shutdown procedures. It will include an update of ongoing test stand construction, including a description of the current lower-fidelity versions of the urine and condensate separators being produced. It will also examine dormancy work being conducted by the CBE and discuss how this may factor into procedures tested on the uncrewed dormancy test stand.

Nomenclature

ASTM = American Society for Testing and Materials
CBE = Center for Biofilm Engineering
CDC = Center for Disease Control
HIP = Hot Isostatic Pressing
ISS = International Space Station
MSFC = Marshall Space Flight Center
NI = National Instruments
TOC = Total Organic Carbon
UWMS = Universal Waste Management System
WRS = Water Recovery System
WPA = Water Processing Assembly

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I. Introduction

The Marshall Space Flight Center (MSFC) understands the need to determine the effect of long-term dormancy in water systems, as well as the requirement to establish a pre-dormancy shutdown procedure to give the wastewater system adequate preparation for extended dormant conditions. The primary concern of extended dormancy periods within the wastewater system is bacterial and fungal growth within components. Potential startup post-dormancy could run into bacterial and fungal overgrowth and biofouling conditions, overloading the system and clogging components when operation resumes. In this paper two forms of dormancy will be discussed. First is the engineering terminology of system dormancy when a water system is shutdown during uncrewed operations. While we will generally use dormancy to refer to this definition, from a microbiology perspective, microbial dormancy (1, 2), or the ability for organisms to cease dividing but actively transition into a dormant cellular state while maintaining viability is an important phenomenon to understand. A lack of nutrients does not cause bacterial death, and resuscitation of dormant cells with the influx of nutrients upon restart of the system plays a major role in the success of mitigation practices during system dormancy.

As there has yet to be a comprehensive look at how dormancy would affect each individual piece of wastewater equipment, the uncrewed dormancy effort at MSFC seeks to complete the build of wastewater hardware by constructing a urine separator, condensate separator, and several of the filter assemblies, as alternative means of acquiring this equipment were not feasible. These pieces of equipment are not intended to mimic externally the flight units, as externals have little effect on internal biofilm formation. Instead, this paper seeks to provide an update to the work completed thus far in the creation of the separators, along with an update on addressing the work being completed for dormancy effects on various bacterial mitigation factors. The paper will primarily focus on the efforts to build the separators and less on the physical makeup of the separators.

Two primary approaches to the dormancy effort will similarly be discussed. The first, involving flushing tests will seek to determine the flushing volumes of each piece of equipment, and the effectiveness of flushing on long-term dormancy situations when completed. The second, involving a comprehensive look at biocidal mitigation efforts will also lend insight into strategies that could be tested once a full-scale dormancy test stand is operational. We will first assess shutdown considerations and potential shutdown procedures for successful mitigation. We will then discuss experimental designs of two ongoing studies to compare shutdown procedures building in complexity to the future testing of the uncrewed dormancy test stand.

II. Shutdown Considerations

A primary concern when water systems are under uncrewed, dormant conditions is biofouling during shutdown or upon resumed operation of the system. Microorganisms are well adapted to periods of nutrient limitation and the ability for bacteria to go dormant or persist in a non-dividing but viable state for very long periods of time is well documented (1-5). When sensing nutrient limitation, cells may become smaller (6), reduce membrane potential (7), and hibernate ribosomes (8-10) as they actively transition (11) into a non-dividing or dormant state. Ribosomes are the bacterial machinery to build new proteins for bacterial processes and protection versus consumption of these large molecules allows for rapid deployment of bacterial processes when sensing environmental cues such as available nutrients without the need to remake these large ribosomal subunits *de novo*. The ability for organisms to maintain dormancy for years and resume activity in minutes means biofouling can present itself quickly, particularly when systems resume operation and nutrients again flood the system. Additionally, microorganisms in these systems grow as surface-associated biofilms and while systems may be drained or flushed, these organisms can remain in the system. Biofilms are 100-1,000 times more tolerant to biocides than planktonic cells (12-14) and removal can be challenging. Biofilm growth may particularly challenge smaller apertures of these water systems as minimal growth may quickly occlude smaller regions and be problematic.

The most simple case of a shutdown scenario is a shut-in condition where flow is stopped, inlets and outlets are closed, and residual biomass and wastewater are left in the system. Indeed, the International Space Station's (ISS) Water

Processing Assembly (WPA) has likely seen this scenario during intermittent periods of repair. The WPA tank already has a relatively slow inflow in relationship to WPA microbial growth rates and we predict nutrients are rapidly consumed while the tank is filled prior to processing through the WPA in batch; ISS microbial isolates can reach stationary phase within 24hrs in synthetic wastewater while the WPA tank fills and is processed in batch every 1-3 days. In the case of a shut-in scenario, we can assume that microorganisms will continue to utilize nutrients until a limiting nutrient is depleted. The amount of growth will be contingent on the excess nutrient load, the organisms present, and biofilm formation during transition from a high nutrient to a nutrient-limiting condition. While this situation may be considered a worst-case scenario, it depends on the operating conditions of the water system prior to dormant conditions. In addition to shutdown procedures, we have assessed nutrient limitation and biocide dosing to mitigate biofilm formation in the system during crewed operations. Preliminary results in an ongoing study suggest that if the state of the microbial load in the water system is sufficiently managed (in the study condition, through phosphate limitation with daily silver dosing), a shut-in scenario with wastewater can perform quite well, up to the month of dormant conditions tested thus far, with minimal growth when the system resumes operation.

The most frequently discussed shutdown procedure includes flushing the wastewater recycling system with potable water prior to dormancy, reducing residual nutrients in the system and processing remaining wastewater prior to shutdown. This type of flushing could be monitored with total organic carbon (TOC), conductivity, or other means suitable to monitor product water to assess reductions and the dilution of wastewater during this process. It should be noted that while TOC may drop several fold, we have found that significant biofilm can remain in the system and while the system is nutrient-limited, microorganisms remain present and biofouling issues may reappear when nutrient inflow resumes. If feasible, increasing the flowrate during flushes may present shear stresses to detach biofilm from adhered surfaces during this flushing process. Some organisms we have studied from the WPA, particularly fungal isolates, create significant amounts of visual biomass and biofilm sloughing of large aggregates during system flushing may clog small apertures or mesh downstream of the WPA tank however; removal of these large aggregates may aid in reducing the microbial load in the system during shutdown. Flushing would reduce planktonic loads and potentially remove significant amounts of biofilm, however flushing procedures should assess successful removal, potentially aided by mechanical removal (increased flowrates) or chemical agents that successfully disaggregate attached biofilm from internal surfaces of system components.

One strategy to additionally reduce the microbial load is use of biocides in the flushes or in the final storage volume to reduce the microbial load prior to dormancy and prevent growth during system shutdown. The features of this type of biocide treatment may be different from preventative or maintenance dosing during operation. These biocides must have sufficient killing efficacy to reduce the microbial load or have minimum reactivity to maintain a long-term biocidal residual to reduce growth. This may be achieved with passively released biocides however duration of efficacy would need to be assessed. For long-term dormancy, periodic biocide dosing could be considered or systems could be engineered to have intermittent maintenance to flush and cycle biocides. Alternatively, engineered maintenance systems could allow for cycling or flushing maintenance only during entry or prior to resumed operations of the dormant system. These systems would also be useful for remediation if biofouling reduces flow during standard operation.

The final consideration would be a draining or dry-out strategy used in many terrestrial systems operated seasonally or during periods of repair or modification. In the context of space-based systems, a drain or dry-out condition may not be feasible as the product water (a critical resource) would need to be stored elsewhere. However, for considerations, this could be done with or without the flushing scenarios listed above. Dried or dry biofilms in industrial applications is an ongoing area of research and draining is not the same as a fully dry system. Residual moisture in the system may favor survival of microorganisms particularly those that thrive in humid conditions like fungus. If an additional dry-out could be considered, it is anticipated that most organisms would experience a significant reduction in total viable cells. From preliminary results, we have found that three WPA isolates (*Bukholderia*, *Cupriavidus*, and *Ralstonia*), have little to no desiccation tolerance and are not detected in dryout conditions however *Methylobacterium* and the fungus *Lecythophora* have initial decreases in cell counts but then maintain viability for months and quickly recolonize when fresh wastewater is introduced to the system. While the

overall reduction in organisms during dryout conditions seems promising, recolonization of the surviving organisms in the absence of competition with faster growing organisms, warrants further assessment.

A. Criteria for Success

A successful dormancy strategy will define a procedure for entry into uncrewed, dormant operation, will ideally involve minimal to no maintenance during system dormancy, and result in an unfouled water system allowing for a successful restart when crewed operations resume. Several criteria could be considered to define success.

First would be microbial survival or presence. These water systems have constant influx of organisms during operation and microbial monitoring of the ISS WPA suggests many long-term resident populations of microorganisms while the system is in operation. Wastewater systems are not sterile nor would we expect future wastewater systems to be. The WPA has operated successfully with resident organisms which aid in removal of nutrients as long as growth does not present biofouling issues. Thus, microbial survival or presence may or may not be a primary criteria although it will inherently be assessed. More importantly is the biofouling potential. We feel this targets a slightly different metric of microbial presence by the amount of biomass produced by these organisms and clogging potential presented by associated biomass. Certain organisms such as filamentous fungi produce significant biomass in relationship to viable cell counts compared to many of the ISS bacterial isolates. Certain organisms may be specific targets for dormancy management.

Another main criteria is material compatibility. Some microorganisms in biofilms have means to utilize material components in microbial influenced corrosion processes and storage as a wetted system should assess this potential. Scenarios involving potable water flushing are likely benign to the system as this is a purer version of the process water however biocides have the potential to corrode metal components or plate out on surfaces.

Finally, if biocides are considered, a final consideration is maintenance of a residual biocide concentration either through biocide concentrations much higher than those currently considered in the potable water, through the use of passively released biocides or through maintenance dosing during dormancy. This is a challenging task and in our several month dormancy durations to date we have seen indications that biocide residuals after dosing 3-fold higher than potable water concentrations are difficult to maintain and microorganisms counts rebound even after simulated potable water flushes and storage in nutrient depleted, sterile water with biocide.

B. Experimental Systems to Assess Dormancy Strategies

Marshall Space Flight Center (MSFC) and the Center for Biofilm Engineering (CBE) at Montana State University have active projects investigating biofouling during shutdown or dormancy procedures to address the considerations and concerns discussed above. Unfortunately, in contrast to many operations that can utilize accelerating testing, the real test of these procedures is time and these projects are long in duration. All of these approaches offer advantages and limitations making a comprehensive approach useful in gaining an understanding of feasible strategies. A comparison of these approaches can be seen in Figure 1.

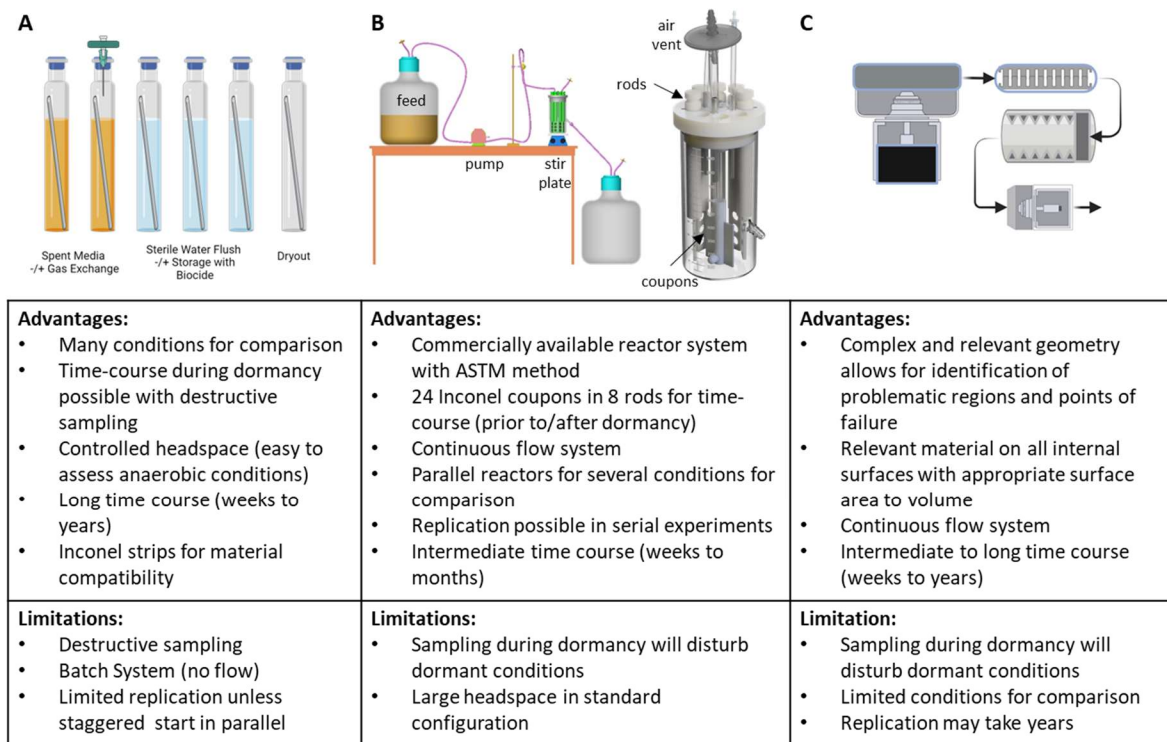


Figure 1 Advantages and limitations of experimental designs for testing dormancy. A) Batch tube dormancy study with Inconel strips. B) CDC Biofilm Reactor System for continuous flow studies. C) Complex geometries and buildout of the dormancy test stand.

The most simplistic experimental design is the tube dormancy initiated in November 2023 at the CBE (Figure 1A). Each tube in this study contains a 0.25 x 4.5 inch strip of Inconel and biofilm grows both on this surface and the walls of the glass tube. A consortium of ISS WPA isolates four bacteria (*B. contaminans*, *C. metallidurans*, *M. organophilum*, and *R. insidiosa*), and one fungus (*L. mutabilis*) were grown in synthetic wastewater for two weeks in Balch tubes in shaking, aerated conditions. At this point, tubes were subject to six simulated shutdown procedures. The first two were shut-in conditions in which tubes were sealed with a butyl rubber stopper with a headspace of ambient air with the second condition having a 0.22µm pore air filter with a needle through the stopper. The next three conditions simulated potable water flushes without and with a biocide during storage. For these three conditions, the residual growth liquid is dumped and refilled with sterile water twice with the later two conditions containing silver fluoride or iodine. The final condition was a dry-out condition in which residual growth liquid is dumped and tubes were dried overnight in a biosafety cabinet prior to sealing. At weekly then monthly intervals tubes are assayed for endpoint bulk fluid and biofilm, Inconel strips are stained for confocal microscopy, and other tubes are subjected to regrowth assays in which residual bulk fluid is removed and fresh synthetic wastewater is added to simulate regrowth on resumed operations. This study has sufficient tubes to reach a two-year duration and will be presented in a future publication.

The next experimental design utilizes the CDC Biofilm Reactor System, a robust modified chemostat or continuous stir tank reactor that grows biofilm on disks or coupons suspended in the bulk fluid (Figure 1B). This commercially available reactor system has been designed and tested for repeatability and has an associated international standard method through the American Society for Testing and Materials International (15). The CBE has modified this procedure to model the ISS WPA with the same multi-domain consortium of the tube study grown in synthetic wastewater. In this study reactors are operated with continuous flow for two weeks achieving steady state viable counts between day 3 and 4. Three of the reactors used standard operating conditions and were subjected to three simulated shutdown procedures 1) shut-in (turned off with spent media), 2) potable water flush (drain and refill twice with sterile water), 3) potable water flush ending with biocide during dormancy (drain and refill twice with sterile water with

second refill containing silver fluoride). The fourth reactor simulated the effects of dormancy in a system where biofilm growth is managed during ongoing operations. This reactor was fed synthetic wastewater without phosphate and with daily silver fluoride doses. This condition went in a shut-in condition with no flushes. All reactor influents, effluents, and vent ports were clamped and reactors were stored in the dark at room temperature during four week dormant conditions. Reactors are sampled at the end of dormancy and then monitored for two weeks of resumed continuous operation. This study is ongoing and will be presented in a later publication.

The final experimental design addresses the complex geometry of the ISS WPA components and is led by the team at MSFC. The buildup and design of these dormancy test stands will be discussed in the next section as well as future plans for dormancy testing based on the findings of the ongoing studies at CBE. This approach assesses the specific surface area to volume, small apertures, and complex architecture of the internal components of the WPA and will aid in identifying problematic regions or failure points during dormant conditions. Plans for this system is to assess optimal flushing and control strategies under one year operation. Biofilm will be assessed on defined surface areas on varying regions of the internal structure.

III. Flushing Volume and Dormancy Quantification

For uncrewed dormancy conditions, and in instances where a flushing regime would be implemented, flushing volumes for the individual separators and downstream equipment is needed. At MSFC, there is currently an effort to build such equipment with high fidelity internal geometry while excluding the high fidelity on external geometry. Figure 2 illustrates the planned separator and equipment that will be examined for flushing volumes. Once effective flushing volumes are determined, and each piece of equipment adequately flushed, a dormancy period will occur. After said dormancy period, normal inlet flow will be introduced to evaluate biofilm build up along with any potential issues post dormancy.

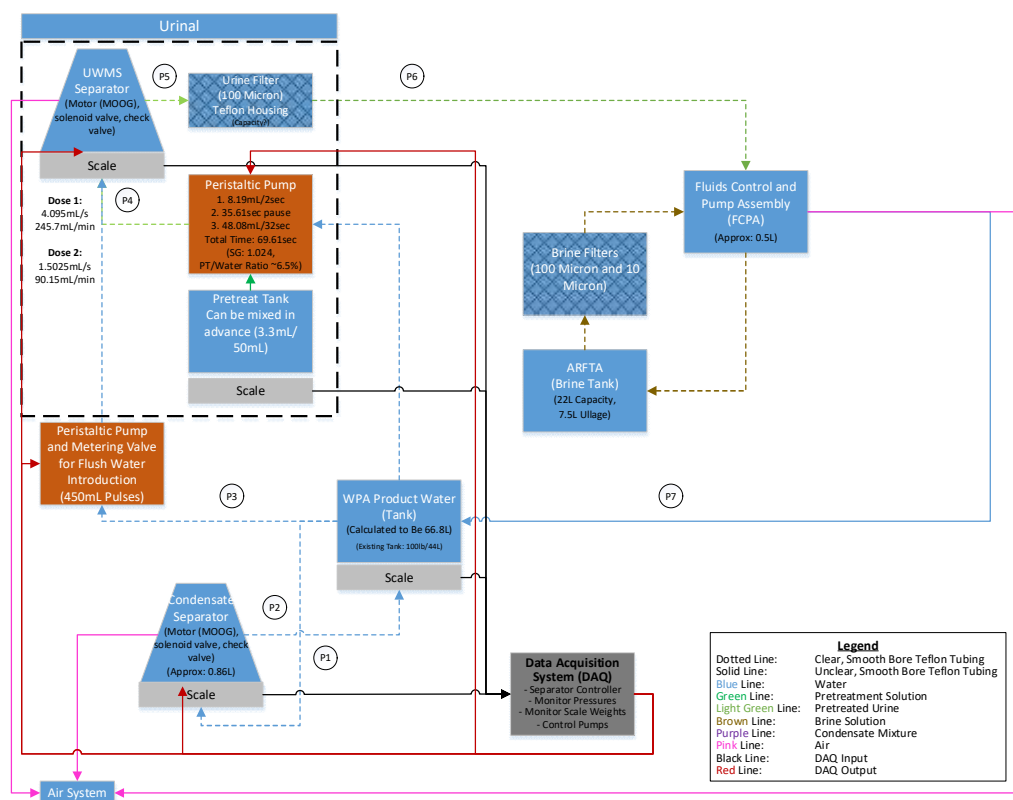


Figure 2: Current Planned Dormancy Hardware for Flushing Evaluation

Due to the unique nature of the separator design, and feasibility of obtaining a separator, MSFC is currently working on fabrication of each individual piece in Figure 3 with high fidelity internal geometry. Once this work is completed, quantification of the flushing volumes can be conducted, and post dormancy biofilm evaluated.

A. Urine Separator

The urine separator contained within the Universal Waste Management System is a primary concern for uncrewed dormancy, as it sees pretreated urine within the separator. While active, the separator has not experienced a biofilm issue, however during long durations of uncrewed dormancy, this could occur. An adequate flushing regime has also not been determined.

Due to the hexavalent chromium pretreat, the flight urine separator is comprised of titanium. Current plans are to operate the unit with an equivalent pre-treated feed prior to flushing and dormancy testing, thus the uncrewed dormancy unit will also be titanium. First, each individual piece was examined comprising the separator to determine what could be machined and what was necessary to be either cast or three dimensionally (3D) printed. Once these non-machinable pieces were identified, a collaboration with the Army allowed for 3D printed titanium pieces to be fabricated. After fabrication, these pieces underwent a destress and Hot Isostatic Pressing (HIP) treatment. 3D support material attached to the parts were removed.

Remaining work for the urine separator includes welding the 3D printed components together, along with the addition of non-3D printed machine parts and final full assembly of the separator. Additionally, a gear box assembly was needed to drive the internals of the separator. As obtaining an exact version of the flight unit was unfeasible, a gearbox needed to be designed.

B. Urine Separator Gear Box Design

As with the urine separator, obtaining an original gearbox was unfeasible, necessitating a custom gearbox and motor to drive the system. The requirements of the gearbox were known, but the internal structure of the gear box was an unknown. The gear diameters and profiles consequently were created based on calculated inputs.

To increase time efficiency, material was sourced through gear blanks, making the custom gearing easier by simply modifying commercial of the shelf (COTS) gear components where applicable. Extensive use of Grasshopper visual programming language helped to create custom algorithms to aid and iterate different gearing options to meet gearbox specifications. An example of these complex custom algorithms can be seen in Figure 3.

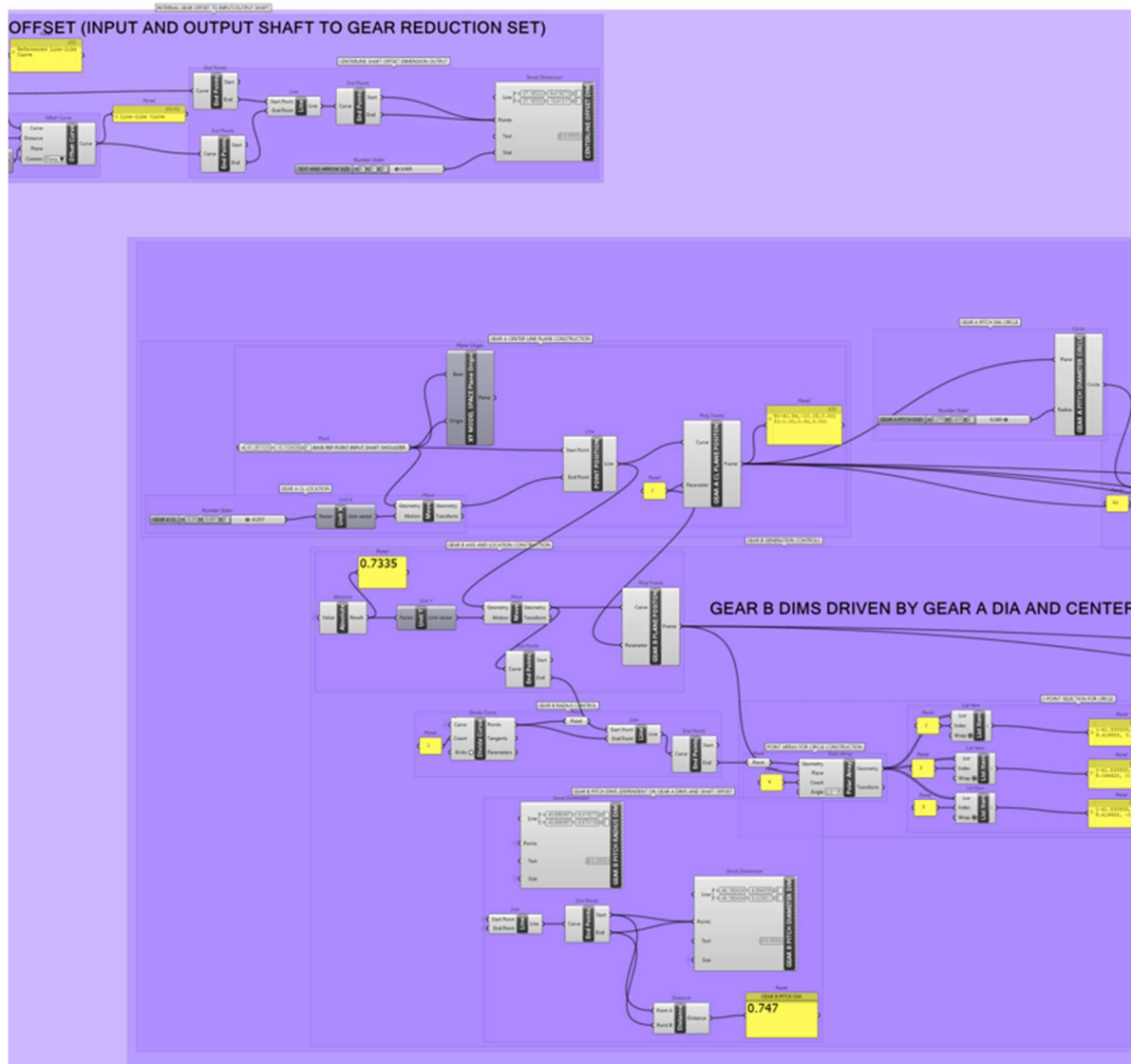


Figure 3: Example of the complex algorithms required for the gearbox design.

Additional design details included custom seal designs and modification, shim selection, incorporation of miscellaneous spacers and addition of snap rings as need, bearing selection, seal selection and lubricant selection. Remaining work includes machining the gears and shaft, assembly of the gearbox, and attachment to the urine separator.

C. Condensate Separator

Another piece of equipment under primary concern is the water condensate separator. Potential biofilm formation during an uncrewed dormancy period could occur while the separator is not active. This phenomenon has not been observed before, and as such there is no procedure for flushing to prevent biofilm formation or cleaning. Uncrewed dormancy efforts at MSFC seek to determine an adequate flushing procedure for the water condensate separator and quantify how much biofilm may be seen in dormant condition.

The original water condensate separator is cast aluminum with machined pieces. For design of the condensate separator dormancy test unit, as with the urine separator, the parts were carefully examined and divided into machinable parts, and unmachinable parts. Casting was unable to be pursued for the MSFC uncrewed dormancy test

stand build. As the urine separator dormancy test unit, previously mentioned, was being 3D printed out of titanium in collaboration with the Army, the decision was made to similarly print the originally cast pieces of the water condensate separator in titanium. Aluminum material is additionally generally difficult to 3D print, further attributing to the decision to utilize the titanium 3D printing. The water condensate separator cast pieces were returned from printing and underwent destress with planned HIP treatment. The machinable parts are currently being machined and assembled.

Remaining work for the dormancy test units for the water condensate separator is similar to that of the urine separator. The 3D printed pieces must be welded together to complete the separator assembly, along with assembly and addition of the machined parts consisting of the separator. Additionally, since the HIP treatment has not been completed yet, HIP is needed prior to machining and welding of the 3D printed pieces. The 3D supports also need to be removed from the pieces post HIP treatment.

D. Water Recovery System Filters

Other areas of concern for potential biofilm build up in dormancy situations are the various filters distributed across the wastewater system. To account for this, a urine brine processing filter, purge gas filter, and a urine filter are being obtained. During dormancy testing, filters need to be accessible for sampling. To allow this, the urine filter unit needed to be modified to allow for access. As previous biofilm clogging in the ISS WPA has been observed in this filter, this redesign was necessitated.

The urine filter casing was redesigned with a flange in the middle allowing for the assembly to easily open and provide access to the filter for microbial sampling and imaging. The material of the outer filter housing consists of polyvinylidene difluoride (PVDF) due to potential contact with the hexavalent chromium pretreat. The ISS filter cartridge is a Pall 100 micron filter. Filter caps on each end, as well as a diverter cone in the housing, direct flow through the filter cartridge. Each end connects into a transfer hose. Fittings for the Russian side have also been machined as the traditional US design. The internal tertiary seals within each transfer hose have remained, as internal geometry is a primary factor of biofilm formation to clog the internal dimensions.

IV. Conclusion

A complex and thorough look at uncrewed dormancy is being conducted at both MSFC and CBE. While CBE focuses primarily on quantification of biofilm growth, and the effect of biocides on dormancy conditions, MSFC is focusing heavily on hardware with identical internal geometry to flight units. Through work in both the CBE and MSFC work, a strategy to approach uncrewed dormancy will be developed for future long duration space flight.

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