A Modeling Study on Microbial Growth for the ISS Wastewater Process System: An Update

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Wastewater processing systems on spacecraft are environments where microbial growth can cause problems. The International Space Station (ISS) wastewater processing assembly (WPA) has adopted various operational methods to minimize biofilm accumulation in the water recovery system (WRS). Long periods of system dormancy are likely necessary for future manned exploration missions, which introduces new challenges to the microbial control in the WRS. Previously, a biomass mathematical model was established based on the nutrient balance and energy consumption conditions relevant to the WPA wastewater system. The previous model was based on bacterial cell composition and nutrient consumption input. This paper provides a detailed background on the ISS WPA biofilm challenge, an overview of these models, an illustration of their application in the ISS WPA system, and also report the latest effort to update the models.

Nomenclature

AES	=	Advanced Exploration Systems	*AOC*	=	Assimilable Organic Carbon
ATP	=	Adenosine triphosphate	*C*	=	Carbon
CFU	=	Colony-forming unit	*CHX*	=	Condensing Heat Exchanger
DA	=	Distillation Assembly	*EFA*	=	External Filter Assembly
E_G	=	growth energy	E_M	=	maintenance energy
G	=	generation time	*hr*	=	Hour
ISS	=	International Space Station	*L*	=	liter
LSS	=	Life Support System	*MCO*	=	Mars Campaign Office
MF	=	Multifiltration	*MLS*	=	Mostly Liquid Separator
NASA	=	National Aeronautics and Space Administration	O_2	=	Oxygen
P	=	Phosphorus	*ppm*	=	Parts Per Million
PWD	=	potable water dispenser	*t*	=	time
TOC	=	Total Organic Carbon	*UPA*	=	Urine Processing Assembly
WPA	=	Water Processing Assembly	*WRM*	=	Water Recovery and Management
WRS	=	Water Recovery System	*WW*	=	wastewater
η	=	metabolic efficiency			

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I. Introduction

WASTEWATER recovery systems on spacecraft are environments where microbial growth can cause problems.[1,2,3] In the past, the International Space Station (ISS) Wastewater Processing Assembly (WPA) experienced biofilm accumulation that obstructed flow paths, leading to increased pressure drop and hardware replacement.[3] Since then, various measurements have been taken to help maintain the ISS WPA components, such as tanks, solenoid valves, and tubing, from accumulating biofilm growth. Examples of some effective measurements include downstream filter addition, tank cycling, and regular biocide water flushing. For exploration missions, ground water resupply is not an option; thus the reliability of the water processing system becomes even more critical. Understanding biofilm growth in relevant environments is essential for successful biofilm mitigation. Quantitative or semi-quantitative relationships between environmental factors and bacterial growth can provide valuable insights to guide the selection and optimization of biofilm mitigation strategies.

Preliminary mathematical models were established to understand biomass accumulation in the WPA system during continuous operation and dormancy. These models were built to predict biomass production by applying fundamental laws of mass and energy conservation and considering the nutrient and energy needs for bacterial growth and maintenance.[4] They were further developed to reflect the nutrient limitation (including the oxygen availability), and to adopt the tank cycling operation condition. These changes further improve these models' accuracy and increase the flexibility for future adaptations.[5]

The previous model was based on bacterial composition and metabolic information; since then, some literature reviews have been conducted to extend this model to fungal growth. Some relevant experiments were also initiated. This paper provides a detailed background on the ISS WPA biofilm challenge, an overview of these models and illustration of their application in the ISS WPA system, and also reports the latest effort to update the models.

II. ISS WPA Biofilm Challenge and Mitigation

The International Space Station (ISS) Water Recovery and Management (WRM) System ensures the availability of potable water for crew drinking and hygiene, oxygen generation, urinal flush water, and payloads as required.

To support this function, crew urine is collected, treated with an oxidizer and an inorganic acid, and then delivered to the Urine Processing Assembly (UPA) to produce urine distillate and brine through a distillation process at low pressure. The oxidizer and the acid help maintain microbial control during urine collection, but no biocide is present in the urine distillate. Biomass is consistently observed in the Distillation Assembly (DA) after it is returned to the ground, but has not caused operational issues yet in the UPA on ISS.[6]

Condensate is collected from the cabin air by the Condensing Heat Exchanger (CHX), fed through an air/water separator to remove excess gas, and then sent to the WPA Wastewater Tank. The wet surface provides the opportunity for microbial growth. Silver was added to the coating for biofilm mitigation, and a monthly “dry-out” of the coating is performed to reduce fungal growth.[6]

Urine distillate, humidity condensate, and Sabatier product water (when available) are delivered to the WPA wastewater tank for processing. The typical input of the WPA includes humidity condensate and urine distillate. It has an average organic carbon level of around 100 ppm. Condensate contains oxygen from the CHX operation, though urine distillate is degassed under vacuum during the UPA operation. According to the initial WPA configuration (shown in Figure 1), the wastewater passes through the Particulate Filter and the Multifiltration (MF) Beds to remove particulates, inorganic and organic impurities. The Catalytic Reactor then treats it to remove residual volatile organics. Next, it passes through the Ion Exchange Bed to remove the dissolved reaction byproducts and add iodine biocide before being delivered to the Water Storage tank as product water.

The 0.5-micron Particulate filter protects the MF beds, while the Catalytic Reactor provides a thermal “sterile barrier” between the upstream wastewater and the product water. Potable water is maintained by reactor sterilization, low organic carbon level, and addition of iodine. However, the WPA wastewater tank and the plumbing before the Particulate Filter remained vulnerable to microbial growth due to the presence of microbial nutrients at higher levels.

The WPA was initially activated in November 2008. In June 2009, the WPA began experiencing (transient) increased pressure drop between the wastewater tank and the Mostly Liquid Separator (MLS). In September 2009, this condition worsened, and the flow from the wastewater tank became too low to maintain the regular operation of the MLS pump. This led to replacing the Pump/Sep ORU in January 2010 and adding the External Filter Assembly (EFA), a 300 µm mesh screen filter, to protect the clearances in the MLS inlet solenoid valve. Post-analysis showed no significant biofouling in the pump/Sep ORU, except for the MLS inlet solenoid valve. It was occluded with a mixture of fungal and bacterial biomass.[7] Figure 2(A) provides a picture of the internal section of the valve, illustrating

the biofilm accumulation in all but one channel.[8] The identified fungal isolates were *Acremonium* and *Penicillium* species, and the bacterial isolates are typically *Ralstonia*, *Cupriavidus*, and *Burkholderia* species.[6]

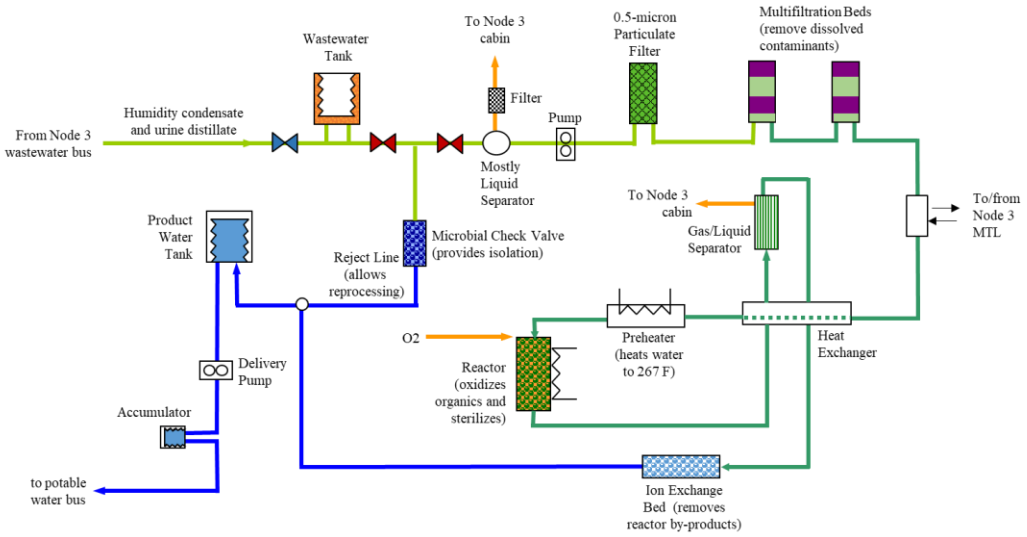

Figure 1. Simplified schematic of the initial WPA configuration.

In January 2011, the pressure drop between the wastewater tank and the MLS increased again. The EFA filter was replaced, and the wastewater tank outlet solenoid valve was replaced with a flow-through plug. Post-analysis showed the EFA filter surface area was covered with biomass, which had extruded and/or grown through the filter.[7,9] Figure 2 (B) shows the mesh filter in the EFA returned in 2011, with a close-up of its inlet section shown in Figure 2 (C).

Figure 2. (A) Biofilm Present in the MLS inlet Solenoid Valve of the Pump/Sep ORU returned in 2010.[8] (B) The mesh filter in the EFA returned in 2011, and (C) a close-up of its inlet section.[9]

WPA tank cycling has also been implemented since 2011 to ensure the bellow is cycled "fully" over its normal range at least once a month, and when possible, during each cycle. Figure 3 shows the simplified wastewater tank geometry. Regular tank cycling, from nearly full to nearly empty, would limit the duration and the amount of undisturbed biofilm accumulation on the tank bellows; this reduces the biofilm sent downstream during each cycle, to avoid premature filter clogging and replacement.

In April 2013, an operational modification was also implemented to flush the EFA filter and MLS inlet with iodinated product water at the end of each process cycle. This provides additional biofilm mitigation by removing nutrients and introducing a biocide.[10]

After all the modifications, including full tank cycling, regular biocide water flushing, and the addition of the EFA mesh filter, the WPA has been operated normally in the presence of microbial growth. The EFA was designed to be replaced annually, but often lasts up to 18 months.[10] Figure 4 shows the current WPA configuration, reflecting some changes described and other changes, such as from two multifiltration beds to one.

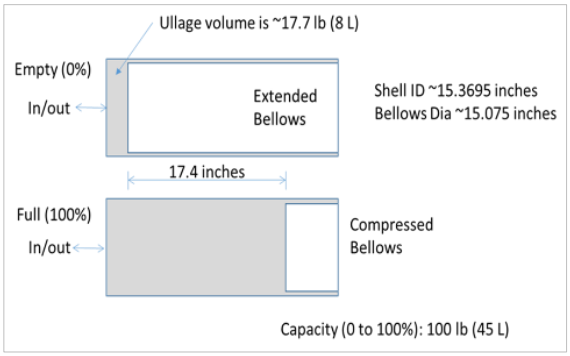

Figure 3. Simplified WPA wastewater tank geometry

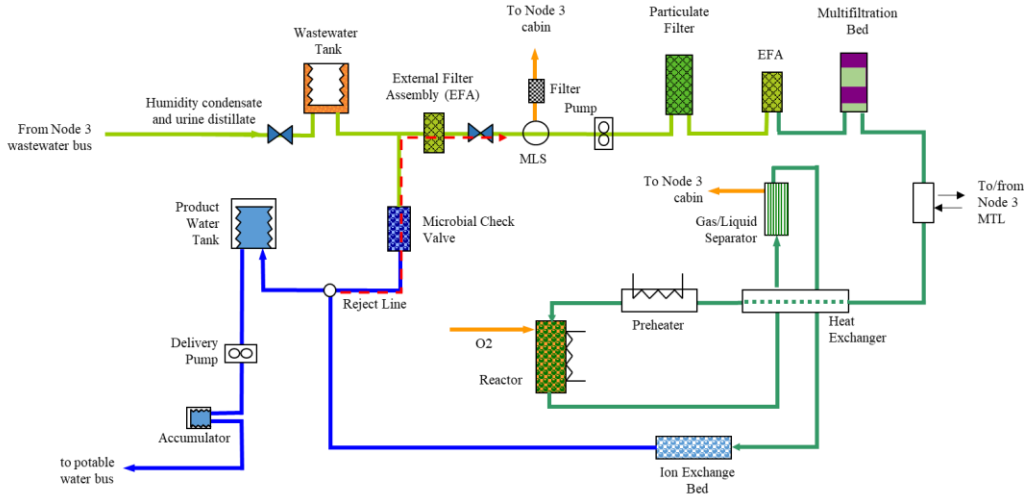

Figure 4. Simplified schematic of the current WPA configuration, with biocide water flushing path shown in red dotting line.

III. Biofilm Challenge for the WRS for Exploration Mission with Dormancy

For exploration missions, such as a crewed Mars mission where ground water resupply is not an option, the reliability of the water processing system becomes even more critical. In addition, long periods of system dormancy (up to a year) are likely necessary for those missions, which could make microbial control in the WRS more challenging.[6]

Stagnant water can be susceptible to biofilm growth. Adequate preparation is needed to ensure the smooth operation of the water recovery system after dormancy. One of the challenges of biofilm control during dormancy is the limited options for biofilm control compared with normal operation. Current ISS WPA biofilm mitigation approaches include active managements, such as tank cycling and regular flushing, which are not good options during system dormancy.

The current option proposed for biofilm mitigation is to flush the system with biocidal water before dormancy.[11] This might also require more active biofilm control during operation to minimize the biofilm presence in the system, and to ensure the system flush's effectiveness before dormancy. Other options, such as drain/dry and continuous recirculation, are not considered viable.

Understanding biofilm growth in relevant environments is essential for successful biofilm mitigation. Preliminary mathematical models were established to estimate biomass accumulation in the WPA system during continuous operation and dormancy. They can provide valuable insights to guide the selection and optimization of biofilm mitigation strategies.

IV. Microbial Growth Model for WPA Wastewater Tank: Key Concepts

Current microbial growth models were developed based on mass balance and energy conservation to predict biomass production based on nutrient input. The nutrient requirements and the nutrient limitation are the key concepts for these models. Nutrients are required for biomass production as building blocks for cellular synthesis and energy supplies.

A. Building Block Nutrients and Stoichiometric Limitation

Building block nutrients contribute to the cellular elemental composition (such as C, N, O, H, P, S, K, Mg, Ca, and Fe),[12,13,14] as they participate in the synthesis of the cellular components such as proteins, nucleic acids, and other essential materials.[15]

Suppose the culture media is unbalanced to supply different nutrients in a closed system. In that case, the one in the shortest supply becomes the stoichiometric limiting nutrient and will control the maximum biomass production in a closed system. For an open system, a limiting nutrient will eventually limit the growth rate before the steady state biomass is reached.[4,5]

Oxygen and hydrogen are major components of bacterial cellular composition. They are generally not limiting nutrients as building blocks, due to their abundance in the culture media, such as in water. However, molecular oxygen and organic carbon compounds play essential roles in energy production, and oxygen can become the limiting factor as an energy source (as shown later).

Table 1 shows the typical averages and ranges of bacterial cell composition and the element growth yield factors.[9] A representative WPA input composition is used to determine the limiting nutrient by calculating the biomass yield from each element. Phosphorus (P) yields the lowest biomass and is therefore the building block limiting nutrient.

Table 1. Determine the building block limiting nutrient in the WPA input.

Bacterial Cell Composition and Range					WPA Wastewater Tank Input		
Element	**Average (dry wt%)**	**Range**	**Average $Y_{X/E}$**	**Max $Y_{X/E}$**	**WPA Input (ppm)**	**Biomass Yield (ppm)**	**Biomass Max Yield (ppm)**
C	50	45 – 58	1	1	104	104	-
O	21	18 – 31	1	1		-	-
N	12	5 – 17	8	20	36	288	-
P	3	1.2 – 10	33	83	0.16	5.3	13.3
S	1	0.3 – 1.3	100	333	1.45	145	-
K	1	0.2 – 5	100	500	0.31	31	-
Ca	1	0.02-2.0	100	500	0.17	17	-
Mg	0.5	0.1 – 1.1	200	1000	0.09	18	
Fe	0.5	0.01–0.5	200	10000	0.044	8.8	

B. Energy Supplies and Oxygen Limitation

Energy sources are needed during the bacterial life cycle, including growth energy (E_G) for the biosynthesis of the new cellular material and maintenance energy (E_M) for structural and functional upkeep of an active cell. [16]

Energy can be acquired through photosynthesis, chemosynthesis, and the consumption of organic matter by heterotrophs.[17] Based on the operation conditions and the microbial species identified in WPA,[18] heterotrophic microbial metabolism is expected. Thus, organic compounds are both carbon building blocks and energy sources (electron donors). Depending on the bacterial metabolism and the availability of electron acceptors, organic compounds can be metabolized through different pathways with different energy generation efficiencies. For instance, one molecule of glucose can yield 36, 2, and 20-28 adenosine triphosphate (ATP) molecules, through aerobic respiration, fermentation, and anaerobic respiration (with other electron acceptors such as nitrates or sulfates), respectively.[19]

The presence of the electron acceptors, including oxygen, in the WPA tank input was analyzed. It was found that free oxygen is too limited to support complete aerobic respiration, and the other electron acceptors' concentrations are also too low to support anaerobic respiration.[5] This is expected, as the WPA system is closed to the ISS atmosphere.

As oxygen is the limiting nutrient for energy supply, both aerobic respiration and fermentation are considered in the models with a metabolic efficiency (η) to differentiate them. When oxygen is spent, microbes switch from aerobic

respiration (η = 100%) to fermentation (η = 2/36 =5.6%). During fermentation, more organic carbon will be required to meet the energy source requirements for growth and maintenance.

V. Closed System: A Simplified Model for the WPA Tank during Dormancy[4,5]

During system dormancy, the WPA is closed off with water, as drain/dry is likely not a viable option. Microbes will be present, as whole system sterilization is unlikely. System components downstream of the WPA wastewater tank are flushed with biocide water at the end of each operation, so the most vulnerable location is the WPA wastewater tank. A simplified model was developed for the WPA tank during dormancy: a batch culture in a closed system with the initial nutrient input and a bacterial inoculation, with no further input or output.

A. Closed System: Growth Phases and Generalized Equations

A typical growth curve of a batch culture in a closed system has three phases (shown in Figure 5):
(1) Exponential: bacteria grow until the building block limiting nutrient in the tank is spent ($t_0 < t < t_{m1}$).
(2) Stationary: bacteria live until the energy source is spent ($t_{m1} < t < t_{m2}$).
(3) Decline: bacteria decline until biomass is spent ($t > t_{m2}$).

The input conditions at t_0: $C\ substrate\ (X) = X_0, P\ substrate\ (P) = P_0,\ biomass\ Y = Y_0$

The equations at each phase:

(1) Exponential ($t_0 < t < t_{m1}$):

Biomass (Y): $Y = Y_0 \cdot 2^{t/G}$

C substrate (X): $\frac{dX}{dt} = -(1 + E_G)\frac{dY}{dt} - E_M Y$

Where: E_G = growth energy, and E_M = maintenance energy
G = generation time

(2) Stationary ($t_{m1} < t < t_{m2}$):

Biomass (Y): $dY = 0,\ Y = Y_{max}$

C substrate (X): $\frac{dX}{dt} = -(1 + E_G)\frac{dY}{dt} - E_M Y = -E_M Y_{max}$

(3) Decline ($t > t_{m2}$):

Biomass (Y): $\frac{dY}{dt} = -E_M Y$

C substrate (X): $X = 0$

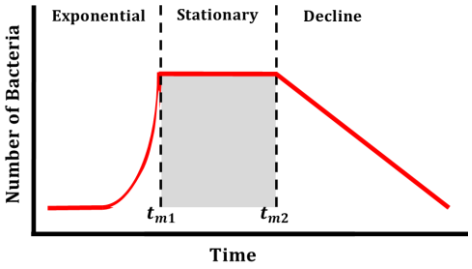

Figure 5. Growth phases of a batch culture in a closed system.

B. Closed System Model for WPA Wastewater Tank

To adopt the closed system model for the WPA wastewater tank, model inputs were derived from the available WPA information, when possible, otherwise estimated, or using relevant data from the literature:

(1) Influent concentration (X_0 and P_0): WPA tank input as shown in Table 1. X_0 = 104 ppm C, P_0 = 0.16 ppm, then Y_{max} = 13.3 ppm or 6.7 ppm C.
(2) Initial microbe inoculation (Y_0): 0.01 ppm C, roughly 4×10^4 CFU/mL.
(3) Generation time (G): 0.5 hour, an estimation that can be updated later.
(4) Growth energy (E_G): 0.17 $C_{substrate}/C_{biomass}$ during aerobic respiration, and 3 $C_{substrate}/C_{biomass}$ during fermentation. This is estimated from the biosynthesis energy of an *E. coli* cell, which is about 10^{10} ATP, or 1 ATP per carbon atom in biomass, as each cell also contains about 10^{10} carbon atoms.[12] Consider that a glucose molecule with 6 carbon atoms can yield 36 ATP during aerobic respiration, and 2 ATP during fermentation, which is 0.17 C (respiration) or 3 C (fermentation) of substrate for 1 ATP.
(5) Maintenance energy (E_M): 0.013 $C_{substrate}/C_{biomass}$/hour for respiration, and 0.235/hour for fermentation.
(6) Two levels of oxygen are considered: without air bubble, or with air bubble. Typically, the only oxygen is from the condensate stream, which is about 10 ppm, after mixing with urine distillate (free of oxygen) at 1:1 ratio, the input oxygen is 5 ppm (without air bubble). Occasionally, air can be introduced when reprocessing the product water stored in gas-permeable bags, which could introduce 8 L air in the ullage of the 45 L wastewater tank, which would result in about 53.8 ppm (with air bubble).

Based on the WPA influent composition and the oxygen availability, the biomass production is limited by P (building block) and O (electron acceptor). Figure 6 shows the growth curves for the closed system under P-limited and O-limited conditions, without the air bubble.

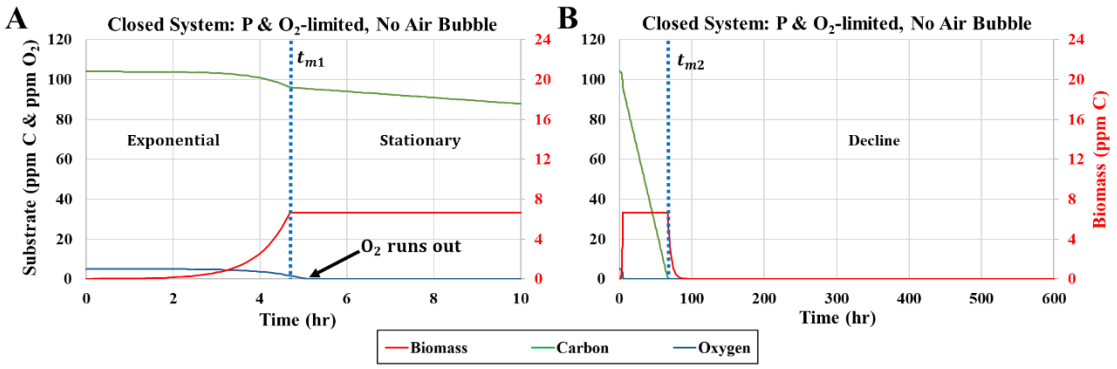

Figure 6. Bacterial growth curve for the closed system model under the nutrient condition: P & O_2-limited, no air bubble, during 0 to 10 (A) and 0 to 600 hours (B).

The max biomass (6.7 ppm C) is determined only by the building block limiting nutrient (P_0 at 0.16 ppm) and is thus not affected by oxygen level. However, the availability of oxygen directly impacts energy efficiency. After the oxygen input (5 ppm) is spent, the organic carbon (as energy supply) is quickly utilized, which leads to a short stationary phase in Figure 6.

Figure 7 shows the growth curves for the closed system under P-limited and O-limited condition, with air bubble. The max biomass (determined by the building block limiting nutrient P_0) is at the same level as without the air bubble. However, the availability of oxygen directly impacts the duration of the stationary phase. The higher oxygen input (53.8 ppm) leads to the longer stationary phase in Figure 7.

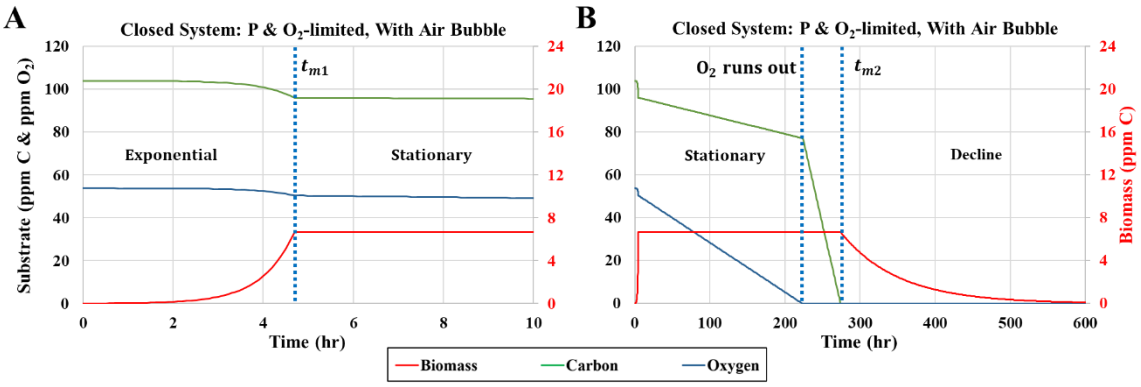

Figure 7. Bacterial growth curve for the closed system model under the nutrient condition: P & O_2 -limited, with air bubble, during 0 to 10 (A) and 0 to 600 hours (B).

C. Closed System Model: Application to Biofilm Mitigation with System Dormancy

Based on the closed system model, during dormancy, the biomass growth in the wastewater tank can reach 6.7 ppm C, or 13.3 ppm biomass, consider the tank volume (45L), the max biomass would be around 0.6 gram.

As biofilm can contain up to 90% of extracellular polymeric substances (EPS), which are highly hydrated, with 98% of water,[20,21] the volume of the biofilm is roughly ten times of the dry biomass. Then, the 0.6 gram of biomass can contribute to about 6 mL of biofilm. This does not seem to be overwhelming, considering the size of the EPA filter (5-inch long with 2-inch diameter).

During long term dormancy, the total biomass is expected to decrease due to nutrient starvation. It is worth mentioning that the maintenance energy used in this model is close to the energy used to maintain healthy, non-growing bacteria, not at the starvation-survival level.[16] To understand the long-term microbial starvation survival behavior, a literature review was carried out.[22] It was found that during long term starvation, most bacterial species will likely survive, in a dormant or resting metabolic state, starvation survival is the rule, with a few exceptions. However, this is the survival of the species, not the whole population. For future exploration missions that include periods of dormancy, bacterial growth and biofilm formation will be directly affected by the residual nutrients in the water recovery system. Therefore, nutrient deprivation likely will not eliminate the presence of microbes, but it will be effective for biomass control.

For instance, if the whole system including the wastewater tank is flushed and refilled with product water, the biomass growth would be further reduced. Because of the incomplete nutrient data of the ISS potable water (due to the low nutrient levels), its biomass yield cannot be predicted. However, as shown in Figure 8, when comparing the ISS historical microbial data sets, it is clear that the max biomass in potable water is about 100 times lower than that of the wastewater tank effluent.[23]

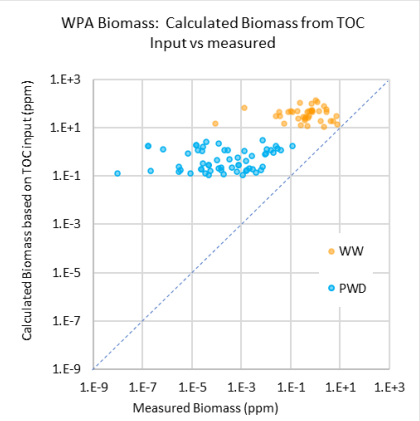

Figure 8. Comparing the calculated biomass (from TOC) to the measured biomass in WPA wastewater (WW) and the potable water (PWD).

To minimize residual nutrient during the dormancy, ensuring leachate-free materials of the system components is at least equally critical. The biofilm grown in dental unit waterlines is notorious problem, one of main reasons is that these water lines are often built of porous rubber or plastic which contains organic carbon. They are easy to be moved around but also provide a perfect surface for biofilm accumulation, and must be cleaned often, especially after weekends.[24] Any system components with polymer contact surface must be carefully evaluated and replaced with metallic ones when possible.

Last, but not the least, is the existing biomass in the system before dormancy. This can be the most challenging to control, short of mechanical cleaning currently being investigated.[25] Frequent tank cycling can limit the biofilm accumulation between cycles, but there is still some space (about 2.6 L) between the tank wall and the billow where biofilm can accumulate over time. This volume is big enough that, if filled with biofilm, the subsequent sloughing would be a serious concern.

In summary, based on the closed system model, both the max biomass growth and the length of the stationary phase are determined by the input nutrient level. After the energy supply is spent, the biomass starts to decline due to nutrient starvation, and some microbes likely enter the starvation survival phase in a dormant or resting metabolic state. The biomass growth and starvation survival are directly affected by the residual nutrient, thus nutrient deprivation is an effective approach for biomass control (but not disinfection). However, there are other important factors for biofilm control with system dormancy. First is that the materials of the system component must be leachate-free, otherwise they become the nutrient source during dormancy, biofilm accumulation will occur unchecked. Second is the existing biomass level of the system before dormancy must be minimized to an acceptable level, which can be challenging short of mechanical cleaning with the current tank design.

VI. Open System: A Simplified Model for the WPA Operation

The open system provides a simplified model for the WPA daily operation conditions. There are two WPA tank input streams: UPA distillate and humidity condensate. The generation and collection of the condensate is relatively steady while the generation and collection of the UPA distillate occurs less frequently (a few hours every other day). After the water in the WPA tank reaches a certain level (80 to 90% full), it is sent downstream to be further processed till the tank reached a low level (<4% full). The average flowrate is about 0.83L per hour (based on 40L every 48 hours).

The open system (fed-batch) provides a simplified representation of the nutrient conditions in the WPA tank:

(1) Starting with a full tank of wastewater and an initial inoculation.
(2) Tank influent and effluent at a constant flow rate.
(3) All biomass is captured in biofilm, with no loss to effluent.

A. Open System: Growth Phases and Generalized Equations.

A typical open system model has four stages, as shown in Figure 9.

(1) Exponential: bacteria grow until the building block limiting nutrient in the tank is spent ($t_0 < t < t_{m1}$).
(2) Substrate Limited: bacteria grow at a rate limited by the limiting substrate influx rate ($t_{m1} < t < t_{m2}$).
(3) Energy Limited: bacterial growth rate decreases as the biomass approaches the maximum biomass that the energy source can support ($t_{m2} < t < t_{max}$).
(4) Steady State: bacterial growth stops; biomass remains at the max value ($t > t_{max}$).

The input conditions:

(1) Flow rate = R_{flow}, Tank volume =V_{tank}
(2) P Cell Content =%P, C Cell Content =%C
(3) Influent and Initial Input: Carbon Substrate = X_0, Biomass = Y_0 Phosphate = P_0

The equations at each phase:

(1) Exponential ($t_0 < t < t_{m1}$):

Biomass (Y): $Y = Y_0 \cdot 2^{t/G}$

C substrate (X): $\frac{dX}{dt} = -(1 + E_G)\frac{dY}{dt} - E_M Y$

Where: E_G = growth energy, E_M = maintenance energy, and G = generation time

(2) Substrate Limited ($t_{m1} < t < t_{m2}$):

Biomass (Y): $\frac{dY}{dt} = P_0 \frac{R_{flow}}{V_{tank}} \times \frac{\%C}{\%P} = constant$

C substrate (X): $\frac{dX}{dt} = -(1 + E_G)\frac{dY}{dt} - E_M Y$

(3) Energy Limited ($t_{m2} < t < t_{max}$):

Biomass (Y): $X_0 * \frac{R_{flow}}{V_{tank}} dt = (1 + E_G)dY + E_M Y dt$

C substrate (X): $X = 0$

(4) Steady State ($t > t_{max}$):

Biomass (Y): $\frac{dY}{dt} = 0,\ Y = Y_{max}$

C substrate (X): $X = 0$

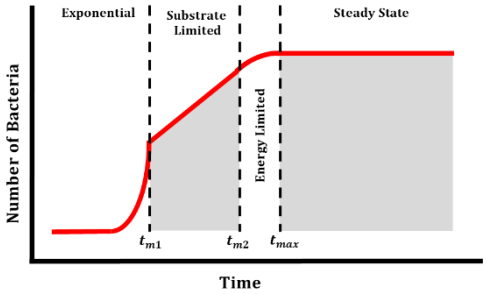

Figure 9. Growth phases of a fed-batch culture in the open system.

B. Open System Model for WPA Wastewater Tank

The model inputs for the WPA wastewater tank during daily operation:

(1) Initial Input:
   a. X_0 = 104 ppm, P_0 = 0.16 ppm.
   b. two levels of initial oxygen: without air bubble (5 ppm), or with air bubble (53.8 ppm).
   c. inoculation (Y_0): 0.01 ppm C.
(2) Microbe characteristic:
   a. Generation time (G): 0.5 hour.
   b. Growth energy (E_G): 0.17 $C_{substrate}$/ $C_{biomass}$ during aerobic respiration, and 3 $C_{substrate}$/ $C_{biomass}$ during fermentation.
   c. Maintenance energy (E_M): 0.013 $C_{substrate}/C_{biomass}$/hour for respiration, and 0.235/hour during fermentation.
(3) Reactor:
   a. Flow rate = 0.83 L/hr
   b. Tank size = 45 L

Figure 10 shows the growth curve of the open system model under P and O-limited conditions without an air bubble, i.e., the only oxygen source is the dissolved oxygen in the condensate.

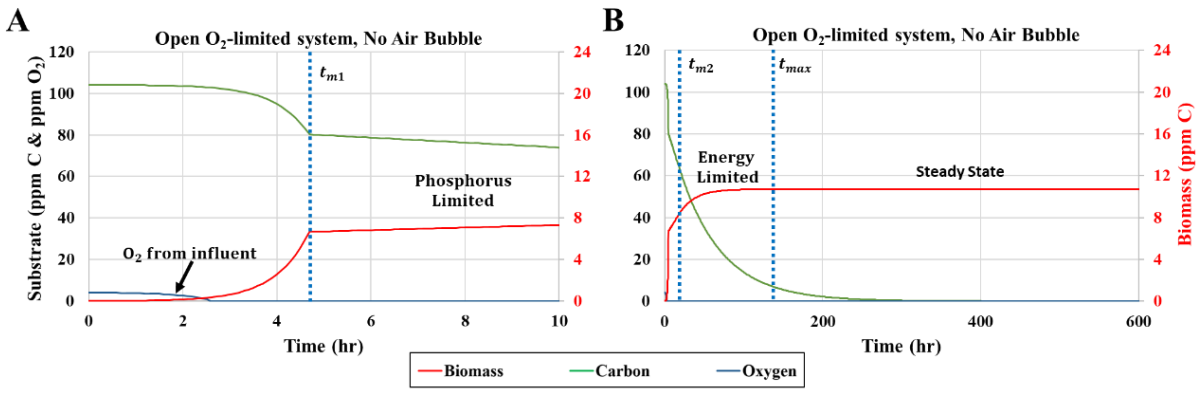

Figure 10. Bacterial growth curve for the open system model under the P-limited and O-limited (no bubble) condition, during 0 to 10 (A) and 0 to 600 hours (B).

The initial oxygen in the tank is quickly spent, afterwards, energy generation switches from aerobic respiration to mainly fermentation. After the initial P is spent, exponential growth stops, and growth rate is limited by the P influx. Soon after that, the growth becomes energy limited (rate decreases), as it approaches the max biomass at steady state. Note that unlike in the closed system, the max biomass is determined by the influx energy supply, not the building block limiting nutrient (P), which only control the growth rate. At the steady state, the influx energy equals the maintenance energy demand of the (metabolically active) biomass, leaving no excess energy for growth.

Oxygen is the limiting energy supplier, thus determines the biomass at the steady state. Note the substrate here is the Assimilable Organic Carbon (AOC) level under O-limited condition (mostly through fermentation), which is at a much lower level than TOC.

Figure 11 shows the growth curve of an open system model under P-limited and O-limited conditions with an air bubble. In the presence of additional initial oxygen, a higher biomass was produced before the initial oxygen was spent. Note that the final biomass is the same with or without an air bubble, as determined by the energy supply influx. Thus, the effort of the air bubble seems to be short-lived. However, part of the initial higher biomass would likely become a part of the biofilm that is metabolically dormant, which can still cause issues for operation.

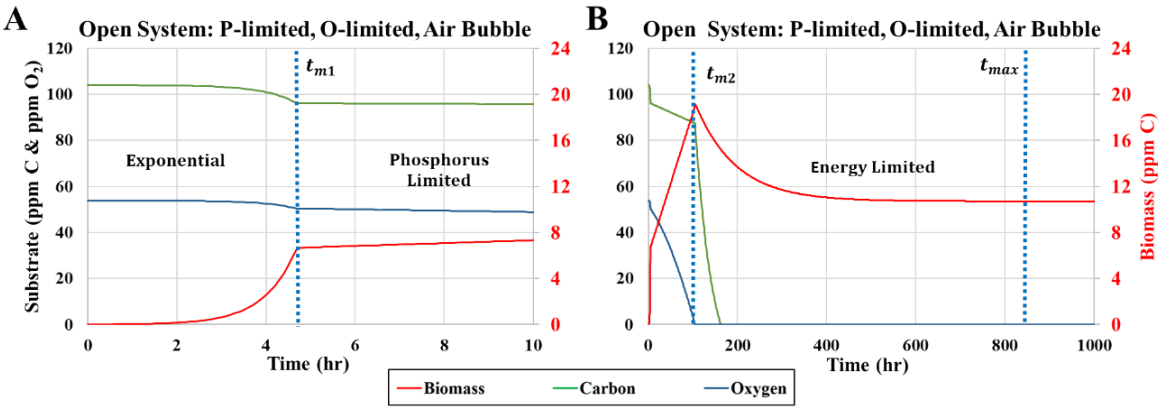

Figure 11. Bacterial growth curve for the open system model under the P-limited and O-limited (no bubble) condition, during 0 to 10 (A) and 0 to 600 hours (B).

C. The Effect of Tank Cycling

The current WPA operation includes a 48-hour tank cycling condition, during which the bellows move within proximity of the tank walls, from its compressed state to its extended state. The frequent full tank cycling helps to limit the biofilm accumulation and sequential loss to downstream by scraping or sloughing.

To observe the tank cycling effect, an open system model of P-limited and O-limited conditions and no air bubble is used to establish the steady state before the nutrient input pattern is changed to mimic the tank cycling:

(1) The influent flow pattern:
   a. Empty tank over 8 hours, to 3L, flow rate = -3.75 L/hr
   b. Fill tank over 40 hours, to 33L, flow rate = 0.75 L/hr.

(2) Assume 10% of the (metabolically active) biomass is scraped off during each tank cycle, mainly from the biofilm attached to the bellows.

Figure 12 shows the open system with tank cycling. At first glance, the effect of tank cycling is not apparent. However, a closer examination reveals that the tank cycling limits the biofilm inside the tank to the sum of (1) the biofilm left after each scraping and (2) the biomass growth during each cycle.

When the nutrient level fluctuates, such as oxygen introduction by makeup water, the biomass can reach a higher level. Frequent tank cycling is effective to limit the biomass accumulation and subsequent sloughing to a manageable amount (by limiting the time for growth). This becomes even more necessary when the building block limiting nutrient (such as P) also increases, which leads to a higher growth rate.

D. Open System Model: Application to Biofilm Mitigation during WPA Operation

In an open system, the (metabolic active) biomass at the steady state is determined by the energy sources availability, while the building block limiting nutrient influences the growth rate to reach the steady state.

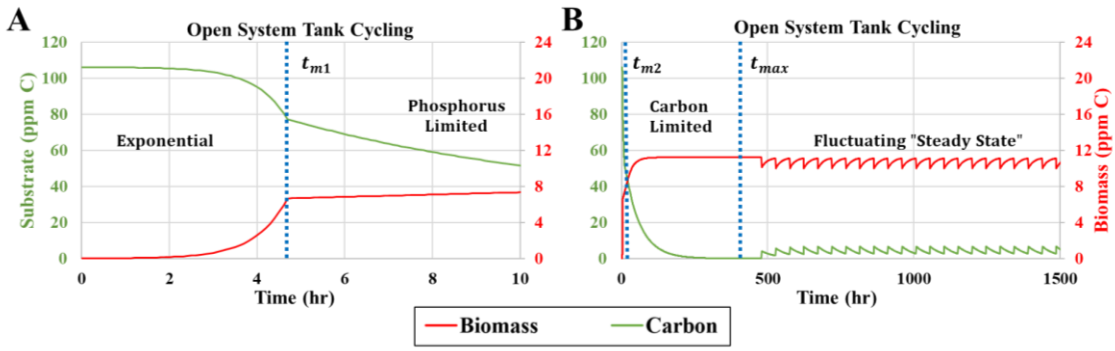

Figure 12. An open system model under conditions of P-limited, O-limited, no bubble; flow pattern changes to tank cycling after steady state is established: (A) 0 to10 hours, and (B) 0 to 1500 hours.

During WPA operation, biomass growth occurs due to the nutrient input of the wastewater processed (about 4000L per year). Based on a closed system model, 4000 L wastewater can yield about 50 grams of biomass (at 6.67 ppm C, or 13.3 ppm). However, in an open system, the steady state (metabolically active) biomass is limited by the energy influx (11 ppm C, or 22 ppm in 45L, about 1 gram). Using the tank cycling model, the total biomass scrapped off is somewhere in between (8.8 gram, at 10% of 22 ppm and 4000 L).

The EFA filter, likely the main biofilm output of the WPA, has a size of 5 in (length) × 2 in (diameter), can hold a calculated 80 mL of biofilm at 30% loading, or about 8 gram of dry biomass (assuming 90% water in biofilm). This biomass calculation is based on internal EFA volume. The EFA is designed to be replaced every year, but in operation has seen replacement up to 18 months. This seems to be consistent with the result of the tank cycling model, but the assumed biofilm loading (30%) at EFA replacement (dictated by pressure drop) must be verified by testing.

The open system model illustrates the effect of tank cycling and nutrient fluctuation on biomass accumulation, and their potential impact on biofilm growth and detachment. Though the model shed light on the WPA operation biofilm mitigation approaches, it should be noted that the steady state biomass calculated is metabolically active, not the dormant or resting cells, that can also be found in biofilm. To determine the actual amount of biofilm in the WPA system can be challenging, as the percentage of the metabolically dormant cells in the biofilm can be influenced by nutrient condition, biofilm structure, and flow patterns.

During WPA daily operation, the biofilm amount in the tank likely becomes relatively stable, limited by the tank geometry and the full tank cycling. Afterwards, the biofilm scrapped off during each cycle should be at same level of the biomass growth, which can be estimated by the open system model. However, the amount of the existing biofilm (including dormant cells) can be critical for the preparation of the system dormancy, due to the possible event that the biofilm becomes unstable during or after the dormancy.

Considering the possible biomass output of the WPA system, the upstream filters, the EFA (250 to 300 micron) and the Particulate Filter (0.5 micron) are likely responsible for catching most of the biomass. The EFA is designed to be replaced every year, but it can last up to 18 months. The Particulate Filter consists of a bank of seven filter elements in parallel, filtering out anything bigger than 0.5 micron; while it is not a sterile barrier, it does filter out most of the microbes that pass the EFA. The Particulate Filter is designed to last for 2.5 years, and it was replaced (for the first time) in July 2013, after more than 4 years of service. If the biomass loading of the two filters can be tested on ground, it would provide the most relevant data needed to verify the models, and to prepare for system dormancy.

In summary, the open system model is useful to understand biomass growth and biofilm control for the WPA daily operation, such as the effect of the nutrient input and the tank cycling effect, but it is more desirable if the model can include the biomass that is in a dormant metabolic state, and consider the factors influencing biofilm sloughing. The experimental data for the EFA and the Particulate Filter loading would be very valuable to verify the model and prepare for the system dormancy.

VII. Summary, Future Plan and Ongoing Work

Biomass growth and biofilm accumulation can be challenging for the water recovery system (WRS) on spacecraft. For crewed exploration mission, the reliability of the WRS is critical, while the system dormancy presents new challenges to biofilm management.

Preliminary mathematical models were established to understand biomass accumulation in the WPA system during continuous operation and dormancy. They were built to correlate the biomass production with the nutrient input, to illustrate that nutrient deprivation is an effective biofilm mitigation approach.

By applying the models to the biofilm mitigation of the WPA system, it becomes clear that nutrient removal (both building block and energy suppliers) can be effective for biofilm mitigation during normal operation and system dormancy. It also becomes clear that there are other important factors for biofilm control with system dormancy.

The materials of the system component must be leachate-free, otherwise they become the nutrient source during dormancy, and biofilm accumulation will occur unchecked.

The existing biomass level of the system before dormancy must be minimized to an acceptable level, which can be challenging short of mechanical cleaning with the current tank design.

While the current models are useful to understand biomass growth and biofilm control for the WPA under daily operation, such as the effect of the nutrient input and the tank cycling effect, it is more desirable if the model could also include the biomass that is in a dormant metabolic state, and consider the factors influencing biofilm sloughing. This will be included in future model development. The experimental data for the EFA and the Particulate Filter loading would be very valuable to verify the model and prepare for the system dormancy.

There is also an ongoing effort to extend the current models to include fungal growth. The previous model was based on bacterial cell composition and nutrient consumption input. As the ISS WPA water microbial data indicates, the most frequently identified microbes were gram-negative bacteria; this is likely a reasonable assumption in general. However, some fungi were also identified in those water samples, though less frequent and generally in much lower counts; there are a few cases where filamentous fungi counts were too high to ignore.

In order to establish a fungal grow model, a literature review was carried out to understand the growth stage of fungal growth and the role of nutrient input. The initial experimental studies are being carried out to identify fungal cell composition and substrate yield efficiency. Those results will be reported in future publications.

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