Biofilm Management in a Microgravity Water Recovery System

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Biofilm growth continues to be a significant concern for NASA's current and future water systems. The International Space Station (ISS) Water Processor Assembly (WPA) produces potable water from a combination of humidity condensate and urine distillate. While infrequent, fouling events originating in or downstream of the WPA waste tank have threatened system performance thus management to prevent biofilm growth from impacting downstream components is being considered. This issue is magnified for future NASA manned missions due to the need to place the vehicle's life support system in a dormant state during uncrewed operations (e.g., when the vehicle is in Mars orbit during the surface mission). NASA personnel and the Center for Biofilm Engineering at Montana State University are evaluating various methods for mitigating biofilm growth, including reduced nutrient levels, thermal treatment, sonic waves treatment, and identifying effective biocides in this application. This paper provides an overview of efforts to develop a synthetic wastewater medium to assess nutrient limitation and biocide control and the status of shockwave mitigation efforts.

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Nomenclature

CFU	=	colony forming units
ISS	=	International Space Station
TOC	=	total organic content/carbon
WPA	=	Water Processor Assembly
R2A	=	Reasoner's 2A agar
CBE	=	Center for Biofilm Engineering
PWB	=	Potable Water Bus
WRS	=	Water Recovery System
OD	=	Optical Density
MSFC	=	Marshall Space Flight Center

I. Introduction

Water recovery is fundamental for manned space missions. The following decade is set to be marked by the Artemis program missions, which aim to return humans to the Moon and make way for further exploration of other celestial bodies and resources[1]. One such celestial body is Mars, for which life support systems will require fewer spare parts and maintenance, and a much higher safety factor[1]. These requirements for Mars missions can be applied and tested in orbit, following the longest operating life support system example in space: the one in the ISS[2]. One of the lessons gathered from ISS has been the biofilm-related issue observed in grounded Water Recovery System (WRS) parts[3]. Such parts have been clogged[4], representing a hazard to the systems, and an increase in risk for system failures. Such concerns must be addressed prior to long-term space missions. Tending to them requires performing an analysis of the current system while testing probable solutions. The Potable Water Bus (PWB) already employs a biocide with a filter system that has proven useful and robust with time[5] however additional biofilm control in the upstream processes in the Wastewater Processor Assembly (WPA) is being considered. The target of biofilm control strategies discussed in this paper is the bellowed WPA tank.

Promising control strategies include selective nutrient removal to reduce biofilm formation, the possibility of adding a new chemical constituent that aids in microbial control, reduction of biofilm attachment with shockwaves, and thermal treatment. In the effort to employ lower mass and more robust compatible methods, a variety of potentially complementary approaches can be tested for the specific purpose of biofilm mitigation. Multiple biocide options were mentioned in a previous publication focusing on the same goals[6]. Another previously explored topic[6] has been the use of shockwaves for biofilm prevention and mitigation. This highly mechanical technique has been used for some time in the treatment of high bioburden ulcers in diabetic patients[7]. Further insight into this technique will be provided in this report, while a multiple bioreactor approach and thermal testing will be discussed in a separate publication. All of these control strategies require an understanding of the nutrient interaction with the existing microbial community to represent ISS fouling concerns in ground testing. Synthetic wastewater formulations or ersatz have been developed for a multitude of testing applications including within the WRS[8]. One potential impediment to performing a nutrient analysis in the current WPA ersatz media is the unparalleled 32 components, developed with the purpose of mimicking the current water in ISS as a chemical challenge to system components and in some cases for being an ionic challenge for the ion exchange resin employed in station[9]. Another challenge is the changes in chemical and microbial characteristics of ISS water samples that occur during spaceflight and landing[10]. Such changes have allowed for enough variation in the chemical constituent data that exists and thus result in less accurate modeling of the *in-vivo* interactions occurring in the WPA. In this article, growth issues in existing ersatz will be discussed and components of a new ersatz modeled on available chemical analysis from the WPA will be listed. Approximating the chemical composition with ersatz allows for a more relevant system. An ersatz that is closer to the WPA tank composition is

explored as an option that allows microbial growth to be representative of existing life support system conditions and present a challenge to assess biofilm mitigation strategies.

II. Synthetic Wastewater Media to Study Biofouling in the WPA

The ISS WPA processes wastewater to product water through a series of water treatment processes. While successfully recycling water to potable water, intermittent fouling events have occurred in and around the WPA wastewater tank[4]. These events can lead to clogging in the system and threaten the longevity and function of WPA components, sometimes requiring hardware replacements to restore operation. For this reason, additional biofouling control is being considered for the wastewater tank where no biocide or biofouling control is currently used. Influent to the tank is a 50:50 mix of urine distillate and humidity condensate with effluent running to downstream multi-filtration beds. Ongoing work at the Center for Biofilm Engineering (CBE) is evaluating nutrient limitation and biocide addition as control strategies to reduce biofilm formation in the wastewater tank. Both evaluations implement screens of potential growth-driving nutrients or biocides and will be followed by testing top candidates in biofilm reactors in a synthetic wastewater medium, termed Ersatz. In this section we will discuss growth issues identified in an existing Wastewater Ersatz and development of a Microbial Ersatz formulation for the study of microbial growth in the WPA tank.

A. Microbial consortia and synthetic wastewater selection.

CBE's initial goals were to establish sufficient microbial growth under untreated control conditions to mimic microbial concentrations and biofilm in the WPA wastewater during a fouling event and thereby present a realistic challenge to test biofilm treatment strategies. A consortium of four bacterial isolates from ISS and one fungal strain frequently found in the ISS wastewater system were selected for use in this study. The ISS isolates were kindly provided by Boeing: *Burkholderia cepacia* complex (Boeing isolate 15-1563-1), *Cupriavidus metallidurans* (Boeing isolate 15-1564-4), a *Methylobacteria* strain identified to be either *M. organophilum* or *M. fujisawense* (Boeing isolate 15-1561-1), and *Ralstonia insidiosa* (Boeing isolate 19-597-yn-2). The fungal strain is an ATCC strain of *Lecythophora mutabilis* (ATCC 44304) now called *Coniochaeta mutabilis*. For media, NASA has used a simulated wastewater called ISS Wastewater Ersatz[6] formulated on the influent stream to the multi-filtration bed to test long-term material durability of components of the WPA. While a representative, worst-case ionic and chemical challenge, in discussions with NASA colleagues, this Ersatz is known to poorly supported microbial growth[11]. CBE's work began with an untested variation to the Ersatz formulation which had a 10-fold decrease in KCl that was conjectured to improve growth. This medium was called Modified Ersatz.

B. Microbial survival in Modified Ersatz.

To define the growth potential of organisms in the synthetic wastewater, monoculture growth curves were performed in 25mL of Modified Ersatz in baffled flasks at room temperature with shaking (225rpm). Cultures were grown in rich media, washed twice in Modified Ersatz and inoculated to an optical density (OD_{600nm}) of 0.005 or 0.05. Daily monitoring of optical density failed to show growth of any organism over the course of two weeks and a batch tube assay with viable cell counts was adopted as follows: Overnight cultures of each microorganism were grown in 4mL of tryptic soy broth (bacteria) or Sabouraud dextrose broth (fungus) in culture tubes in an environmental shaker at room temperature, 225 rpm. Due to potential growth concerns at the onset of this test, *Pseudomonas aeruginosa* PAO1, a known laboratory strain capable of growing in very low nutrient systems, was used to test the growth potential of the medium. As these methods were developed to eventually test nutrient limitation, all cultures were washed prior to inoculation of synthetic wastewater to remove carryover of undefined rich medium components from the overnight cultures. Overnight cultures of *L. mutabilis* were filtered through sterile glass wool in 1mL pipette tips to improve pelleting. Cultures were washed twice by centrifuging for 3 minutes at 8,000 rpm in microcentrifuge tubes, removing the supernatant and resuspending in sterile saline (0.85% NaCl). Each inoculum was adjusted to an OD_{600nm} to establish 10^4 CFU/mL when 80µL of washed inoculum was added to 4mL of Modified Ersatz or saline as a nutrient-free control. Cultures were returned to the environmental shaker and daily samples were diluted and drop plated on R2A agar for enumeration of colony forming units (CFU) following up to five days of incubation at 30°C. Spread plating was additionally used in some experiments when toxicity was anticipated. The limit of detection was one colony in the plated volume and was 1.3 $\log_{10}$(CFU/mL) for drop plating or 1.0 $\log_{10}$(CFU/mL) for spread plating. Samples appearing below the limit of detection had no CFUs in the undiluted sample and represent an arbitrary 0.5 CFU in the plated volume.

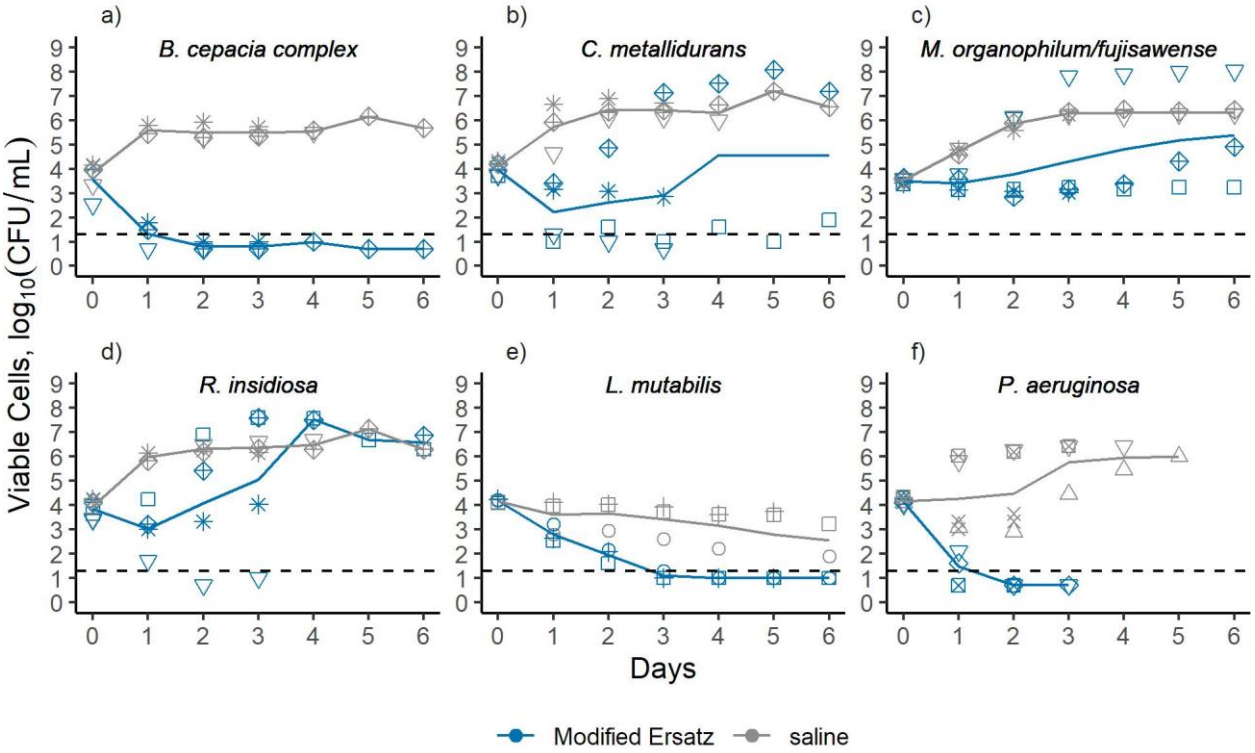

Figure 1. Monoculture viable cell counts of organisms in 4mL of Modified Ersatz versus saline. Symbols represent samples within the same experiment. Solid lines are the mean within the respective medium. The dashed line is the limit of detection=1.3 $\log_{10}$(CFU/mL) for drop plating.

Viable cell counts of organisms in Modified Ersatz are shown in Figure 1. All organisms had poor or variable survival in Modified Ersatz and only two organisms in one experiment demonstrated viable counts that might indicate growth in the medium above the nutrient-free saline control (Figure 1b,1c). *B. cepacia* complex, *L. mutabilis*, and *P. aeruginosa* all lost viability below the limit of detection in Modified Ersatz within 1 to 3 days of inoculation (Figure 1a, 1e, 1f). *C. metallidurans* and *R. insidiosa* had variable survival, losing viability similar to the most susceptible organisms in several experiments but maintaining viability or reaching viable cell counts similar to saline in others (Figure 1b, 1d). In two of three experiments *M. organophilum/fujisawense* was bacteriostatic, maintaining viability over the course of 6 days but demonstrated growth above the saline control in one experiment (Figure 1c). The evidence of growth for any organism in this medium but was not repeatable. Most important, is the comparison of survival in Modified Ersatz to survival in the nutrient-free control (saline) in which all organisms maintained viable cell counts or increased slightly as cells transitioned into starvation. If Modified Ersatz only lacked nutrients for microbial growth, viable cell counts should be maintained similar to the saline control, however, cultures in Modified Ersatz lost viability. These results suggest that Modified Ersatz not only fails to support growth but is toxic towards the organisms tested. We next sought to identify the source of Modified Ersatz toxicity.

Modified Ersatz is a complex defined medium with 32 components made from four concentrated stock solutions and a separate phosphate addition[8]. To identify toxic components, we reduced or removed components by reducing or removing select stock solutions in the medium. The organic and inorganic concentrates both contained essential nutrients, and nothing thought to be toxic. However, the high zinc and nickel concentrations introduced in the acetate concentrate as zinc acetate and nickel acetate were a potential toxicity concern. While we could not identify toxic components in the direct addition containing mostly organosilicon compounds, we also selectively reduced or removed this concentrate, supplementing $CaSO_4$ in this concentrate back at the original concentration as a primary nutrient. *P. aeruginosa* PAO1 was used as the test organism for these variation of Modified Ersatz and was assayed using the tube assay adding a third inoculum wash for these and subsequent assays (Figure 2). The results demonstrate that nickel and zinc toxicity is the cause of *P. aeruginosa* viable cell loss in Modified Ersatz. All variations, including

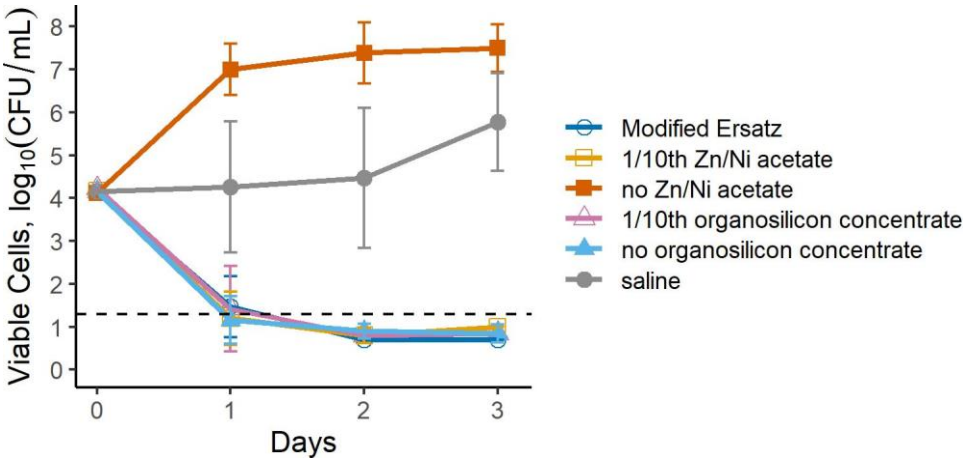

Figure 2. Identifying toxic components in Modified Ersatz. P. aeruginosa PAO1 viable cell counts in Modified Ersatz with reduced or absent zinc and nickel acetate concentrate or reduced or absent direct addition (supplementing $CaSO_4$ in direct addition back at 1X). Symbols and lines represent mean +/- standard deviation of N>=3 experiments. The dashed line is the limit of detection=1.3 $\log_{10}$(CFU/mL) for drop plating.

decreasing the zinc and nickel acetate to 10% of the Modified Ersatz concentration, exhibited toxicity similar to Modified Ersatz. *P. aeruginosa* growth in Zn/Ni-free Modified Ersatz was 2-3 $\log_{10}$(CFU/mL) higher than the nutrient-free control on all days suggesting not only survival but growth in Modified Ersatz when zinc and nickel were absent.

Zinc and nickel toxicity was then tested against the consortium organisms (Figure 3). Similar to *P. aeruginosa*, all organisms were able to grow in the Zn/Ni-free Modified Ersatz with growth 1-2 $\log_{10}$(CFU/mL) above

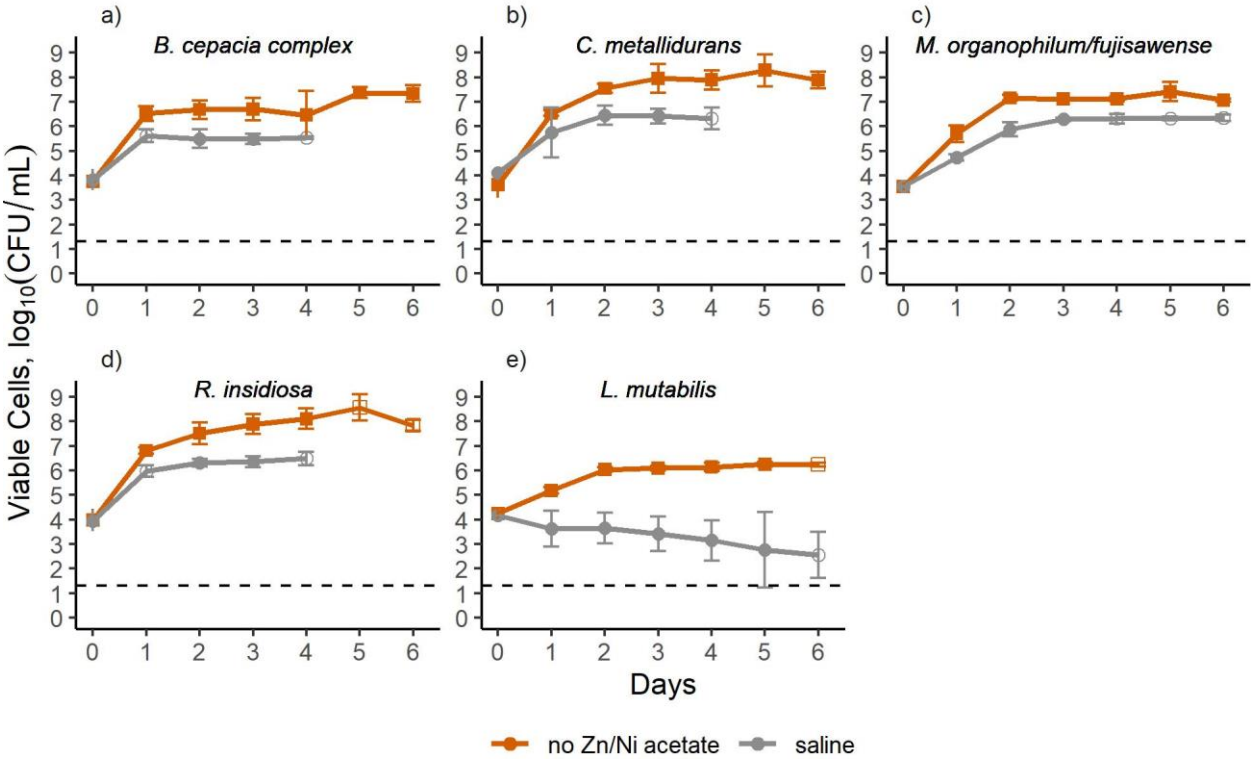

Figure 3. Viable cell counts of test organisms in Modified Ersatz with no zinc and nickel acetate. Solid symbols and lines represent mean +/- standard deviation of N>=3 experiments, open symbols represent N=2 experiments. The dashed line is the limit of detection=1.3 $\log_{10}$(CFU/mL) for drop plating.

the nutrient-free control. While an improvement from a toxic medium, the growth was just reaching a range high enough for a representable challenge to test biofouling control. We compared zinc and nickel concentrations to ISS wastewater chemical analysis data from US Expeditions 21 to 59[12] (Table 1) and found that for the ionic challenge for which the Ersatz was originally developed, total zinc and nickel was slightly overrepresented in the wastewater recipe but was still present in the system. While microbial growth was possible when metals were absent, completely eliminating the zinc and nickel in a synthetic wastewater may not accurately depict the situation on-station. An additional strategy to increase growth came from examining sampling locations throughout the ISS wastewater data (Table 1). The wastewater Ersatz recipe was developed from the effluent of the wastewater tank and comparison to the condensate stream (half of the influent to the wastewater tank) showed presence of many potential nutrients that were absent in the effluent, suggesting microbial consumption between the tank influent and effluent. With these considerations, we sought to develop a new formulation based on available data for the tank influent streams specifically focused on nutrient sources for microbial growth.

Table 1. Chemical analysis data of Ersatz components used to develop Microbial Ersatz. Condensate (half of the tank influent) and wastewater (effluent) streams from the WPA tank were means of ISS US Condensate and Wastewater data form Expeditions 21 to 59. Distillate data is from MSFC ground testing, values not listed were either not assayed or not detected. The calculated influent is the average of condensate and distillate streams or half of the known data if data was only present for one stream.

Compound	Units	Condensate Stream	Distillate Stream	Calculated Influent	Effluent Stream
1,2-Propanediol (Propylene glycol)	µg/L	18,588	---	9,294	5,656
1-Methyl-2-pyrrolidinone	µg/L	568	---	284	192
1-Propanol	µg/L	1,409	---	704	<RL
2-(2-Butoxyethoxy)ethanol	µg/L	1,855	---	927	396
2-Ethoxyethanol	µg/L	725	---	362	216
2-Ethyl-1-hexanol	µg/L	323	---	161	83
2-Propanol (Isopropanol)	µg/L	1,201	205	703	<RL
4-Ethylmorpholine	µg/L	173	---	86	41
Acetate	µg/L	38,974	29,300	34,137	2,896
Acetone, Alcohols (DAI/GC/MS)	µg/L	2,534	39,425	20,979	3,093
Acetone, Volatile Organics-Targets	µg/L	144	39,425	19,785	<RL
Ammonium (as N)	mg/L	36.0	---	18.0	18.0
Benzoic acid	µg/L	2,039	---	1,020	542
Benzothiazole	µg/L	105	---	52	79
Benzyl alcohol	µg/L	12,677	20	6,348	3,502
Calcium	mg/L	0.19	0.15	0.17	0.44
Caprolactam	µg/L	3,026	---	1,513	408
Chloride	mg/L	RL	---	---	<RL
Decamethylcyclopentasiloxane (DMCPS)	µg/L	249	---	124	119
Diethylphthalate	µg/L	999	---	499	427
Dimethylsilanediol (DMSD)	µg/L	43,243	---	21,622	17,345
Dodecamethylcyclohexasiloxane	µg/L	157	---	79	55
Ethanol	µg/L	63,072	8,500	35,786	12,905
Fluoride	mg/L	0.5	---	0.26	0.4
Formate	µg/L	11,516	54,100	32,808	115
Lactate	µg/L	18,263	<1,000	9,131	<RL
Methanol	µg/L	5,828	4,580	5,204	4,386
Methyl sulfone	µg/L	244	44	144	144
N,N-Dimethylformamide	µg/L	748	---	374	310

Nickel	µg/L	2,272	172	1,222	846
Octamethylcyclotetrasiloxane (OMCTS)	µg/L	48	---	24	<RL
Phosphate (as P), Anions IC/ISE	mg/L	0.16	0.27	0.21	0.1
Phosphate (as P), Minerals ICPMS	mg/L	0.07	0.27	0.17	0.11
Potassium	mg/L	0.31	0.03	0.17	0.22
Silicon (ICP/MS)	µg/L	14,839	---	7,419	7,407
Sodium	mg/L	0.61	0.33	0.47	0.24
Sulfate	mg/L	1.45	1.20	1.33	<RL
Total I	mg/L	0.031	---	0.016	0.200
Trimethylsilanol	µg/L	336	22.75	179	205
Urea	µg/L	2,768	1,030	1,899	142
Zinc	µg/L	8,476	12.25	4,244	5,032
Total Inorganic Carbon (TIC)	mg/L	29.4	4.88	17.15	18.9
Total Organic Carbon (TOC)	mg/L	110	58.19	84.15	43.9
pH		7.39	2.96		7.21

C. Formulation of a microbial medium specific to the ISS wastewater system to study biofouling.

To address microbial growth constraints, we utilized chemical analysis data from ISS monitoring and ground testing, and compared microbial nutrients sources (C, N, S, P, and trace nutrients) in the current medium recipes to an approximate tank influent based on known 50:50 inputs of the condensate and distillate streams (Table 1). The ISS condensate stream is routinely monitored with chemical analysis however no ISS distillate samples were available. Chemical analysis data for the distillate stream are from MSFC ground testing[13]. We identified numerous potential carbon sources that could be utilized by microorganisms that were underrepresented or absent in the Modified Ersatz makeup. Additionally, as a worst-case scenario, the total zinc and nickel concentrations were over-represented in Modified Ersatz, and presence as zinc acetate and nickel acetate may additionally present more bioavailable zinc and nickel than the corrosion products suspected to be the source of those metals in the system. In total, twelve components across common nutrients in the previous Ersatz formulation were increased or decreased to the calculated influent concentrations, acetic acid was added to increase the acetate concentration, and formic acid and lactic acid were introduced to represent top C-sources based on the calculated 50:50 influent stream.

Noting the potential metal toxicity and/or overrepresentation of bioavailable zinc and nickel by representing total zinc and nickel as zinc or nickel acetate, we tested this new formulation at 100% and 10% of the total zinc and nickel influent in the tube assay (see levels in Table 2, Figure 4). All organisms in the target consortium grew in the new formulation at 10% total zinc and nickel however representation of the total zinc and nickel concentrations as zinc acetate and nickel acetate remained toxic to *B. cepacia* complex (Figure 4a) and *L. mutabilis* (Figure 4e) and was largely bacteriostatic against *M. organophilum/fujisawense* (Figure 4c). With improved nutrient availability, metal tolerance also improved although it should be noted that this 10% total zinc and nickel is slightly lower than that in Modified Ersatz as it is based on the calculated influent data (See Table 2). Interestingly, the new formulation at either metal concentration remained toxic against the laboratory test strain *P. aeruginosa* PAO1 suggesting further metal

Table 2. Monoculture growth in Modified and Microbial Ersatz. Mean viable cell counts +/- standard deviation are shown below after three days of growth for the data plotted above. P. aeruginosa was below the limit of detection for all samples in Modified Ersatz and 100% Zn/Ni Microbial Ersatz.

	Modified Ersatz		**Microbial Ersatz**	
	standard	no Zn/Ni acetate	100% Zn/Ni	standard
mg/L Zn	7.54	0.00	4.24	0.42
mg/L Ni	1.42	0.00	1.22	0.12
B. cepacia complex	0.80 +/- 0.17	6.70 +/- 0.46	0.90 +/- 0.17	7.67 +/- 0.39
C. metallidurans	2.92 +/- 2.97	7.95 +/- 0.58	7.96 +/- 0.10	8.26 +/- 0.48
L. mutabilis	1.10 +/- 0.17	6.10 +/- 0.10	1.78 +/- 0.60	4.90 +/- 0.78
M. organophilum/fujisawense	4.31 +/- 2.34	7.12 +/- 0.01	4.38 +/- 2.21	7.83 +/- 0.23
R. insidiosa	5.06 +/- 3.19	7.89 +/- 0.40	7.59 +/- 0.27	8.19 +/- 0.46
P. aeruginosa	≤0.7 +/- 0.00	7.50 +/- 0.55	≤1.00 +/- 0.00	1.45 +/- 0.77

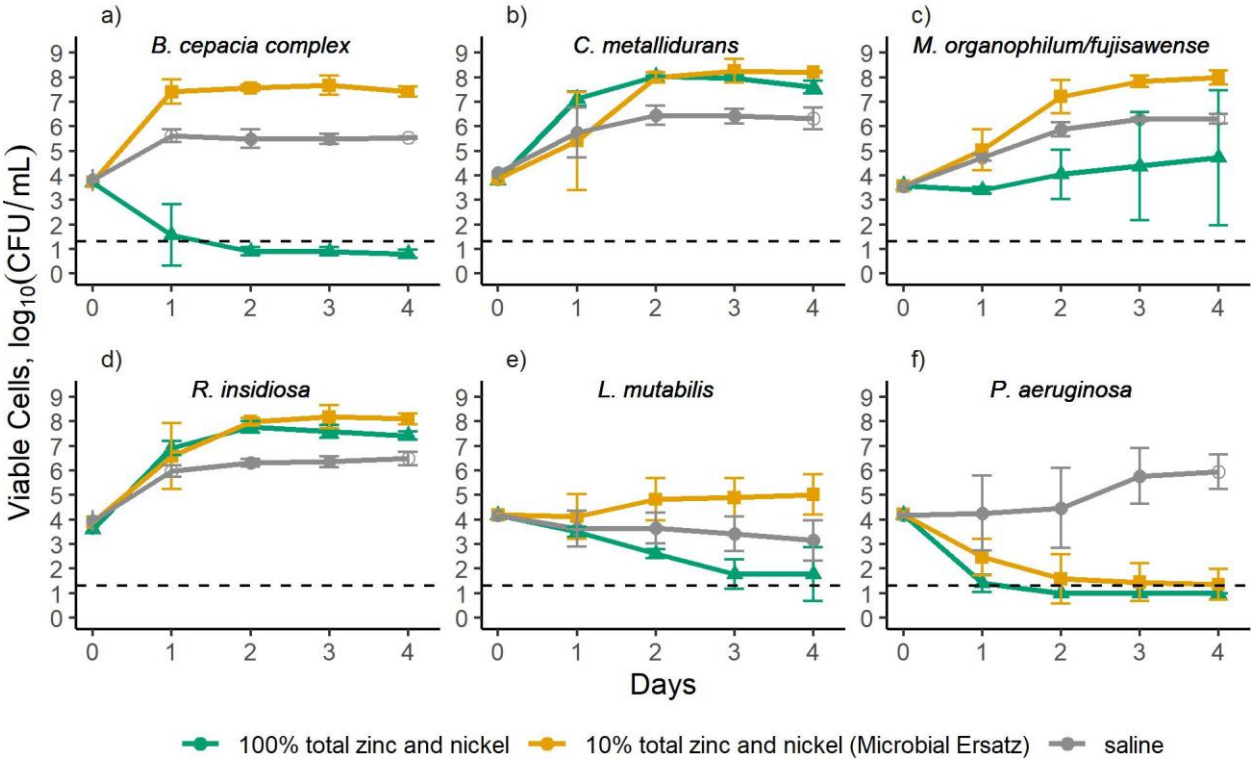

Figure 4. Monoculture growth in Microbial Ersatz. The impact of zinc and nickel toxicity was tested with 100% of the calculated influent as zinc and nickel acetate versus Microbial Ersatz which has 1/10th of the calculated influent. Solid symbols and lines represent mean +/- standard deviation of N>=3 experiments, open symbols represent N=2 experiments. The dashed line is the limit of detection=1.3 log10(CFU/mL) for drop plating

elimination if this strain was a topic of study (Figure 4f). Within the consortium species, all four bacterial strains had the highest growth in standard Microbial Ersatz of the Modified and Microbial variations tested, while *L. mutabilis* grows in Microbial Ersatz but had the highest growth in Zn/Ni-free Modified Ersatz suggesting some metal sensitivity may be limiting the growth potential of this organism (Table 2). Based on these data, the Microbial Ersatz standard solution contains 10% of the total zinc and nickel as zinc acetate and nickel acetate. A list of stock component and final makeup for Modified Ersatz and the final Microbial Ersatz is found in Table 3. Microbial Ersatz is currently being used to identify nutrient drivers of microbial growth and as the growth medium for a biocide screen.

Table 3. Chemical makeup of Modified Ersatz and Microbial Ersatz. Both media are made from four concentrated stock solutions and a phosphate addition. Solutions and final media are filter sterilized.

Stock Solution	Chemical	Concentration of Stock Solution			Final Media Concentration (µM)	
		Modified Ersatz	Microbial Ersatz	SS units	Modified Ersatz	Microbial Ersatz
Organics Concentrate (100X)	Propylene Glycol	1.6995	0.8971	mL/L	222.60	117.50
Organics Concentrate (100X)	Ethanol	1.6857	4.4946	mL/L	291.33	776.77
Organics Concentrate (100X)	Acetone	0.3417	2.5799	mL/L	46.47	350.92
Organics Concentrate (100X)	2-(2-butoxyethoxy) Ethanol	0.2356	0.2356	mL/L	13.94	13.94
Organics Concentrate (100X)	N, N-Dimethylformamide	0.1486	0.1486	mL/L	19.21	19.21
Organics Concentrate (100X)	2-Ethoxyethanol	0.1303	0.1303	mL/L	13.45	13.45

Organics Concentrate (100X)	1-Methyl-2-Pyrrolidinone	0.0782	0.0782	mL/L	8.13	8.13
Organics Concentrate (100X)	2-Propanol	0.0255	0.0896	mL/L	3.33	11.70
Organics Concentrate (100X)	1-Propanol	0.0249	0.0876	mL/L	3.33	11.72
Organics Concentrate (100X)	4-Ethylmorpholine	0.2265	0.2265	mL/L	17.90	17.90
Organics Concentrate (100X)	Formic Acid	---	2.7489	mL/L	---	728.58
Organics Concentrate (100X)	DL-Lactic Acid	---	0.8550	mL/L	---	102.51
Organics Concentrate (100X)	Benzoic Acid	0.3015	0.3015	g/L	24.69	24.69
Organics Concentrate (100X)	Caprolactam	0.1111	0.1111	g/L	9.82	9.82
Organics Concentrate (100X)	Urea	0.0603	0.1899	g/L	10.03	31.62
Inorganic Concentrate (100X)	Potassium Chloride	0.5065	0.5065	g/L	67.94	67.94
Inorganic Concentrate (100X)	Ammonium Bicarbonate	3.0002	10.1457	g/L	379.50	1283.37
Inorganic Concentrate (100X)	Sodium Fluoride	0.0880	0.0880	g/L	20.96	20.96
Inorganic Concentrate (100X)	Potassium Iodide	0.0260	0.0260	g/L	1.57	1.57
Acetate Concentrate (100X)	Zinc (II) Acetate dihydrate	2.5300	0.1425	g/L	115.26	6.49
Acetate Concentrate (100X)	Nickel (II) Acetate tetrahydrate	0.6020	0.0518	g/L	24.19	2.08
Acetate Concentrate (100X)	Acetic Acid	---	3.2088	mL/L	---	561.06
Organosilicon Concentrate (15X)	Benzyl Alcohol	0.2121	0.0911	mL/L	136.65	58.70
Organosilicon Concentrate (15X)	Diethylphthalate	0.0302	0.0302	mL/L	10.11	10.11
Organosilicon Concentrate (15X)	Trimethyl Silanol	0.0095	0.0095	mL/L	5.71	5.71
Organosilicon Concentrate (15X)	Benzothiazole	0.0031	0.0031	mL/L	1.93	1.93
Organosilicon Concentrate (15X)	2-Ethyl-1-Hexanol	0.0072	0.0072	mL/L	3.07	3.07
Organosilicon Concentrate (15X)	Decamethylcyclopentasiloxane	0.0155	0.0155	mL/L	2.67	2.67
Organosilicon Concentrate (15X)	Dodecamethylcyclohexasiloxane	0.0150	0.0150	mL/L	2.18	2.18
Organosilicon Concentrate (15X)	Octamethylcyclotetrasiloxane	0.0153	0.0153	mL/L	3.30	3.30
Organosilicon Concentrate (15X)	Dimethoxydimethylsilane	0.5265	0.5265	mL/L	252.53	252.53
Organosilicon Concentrate (15X)	Calcium Sulfate	0.0673	0.0283	g/L	32.98	13.85
Organosilicon Concentrate (15X)	Dimethyl Sulfone	0.0031	0.0031	g/L	2.17	2.17
Organosilicon Concentrate (15X)	Hexamethylcyclotrisiloxane	0.0153	0.0153	g/L	4.59	4.59

	Chemical	**Final Media Concentration (mg/L)**		**Final Media Concentration (µM)**	
		Modified Ersatz	Microbial Ersatz	Modified Ersatz	Microbial Ersatz
Direct Addition	Monobasic Potassium phosphate	0.1576	0.8349	1.16	6.13

III. Shockwaves

As part of the efforts to mitigate biofilms in the WPA, an innovative solution such as the use of shockwaves is being explored. Previous tests performed by the Center for Biofilm Engineering (CBE) at Montana State University, showed that SANUWAVE shockwave technology is effective in eliminating medical[14], marine[15], and monument[16] biofilms containing both Gram-positive or Gram-negative bacterium species. Furthermore, experiments performed at University of Georgia, showed that shockwaves have bactericidal effects against planktonic bacteria[17], by destroying bacterial wall integrity via either tensile forces produced by shockwaves[18] or by creation of opened sensitive channels in bacterial shell via mechanotransduction that bloats/burst the bacteria[19]. Based on these shockwave mechanisms of action, we believe that short periodic exposure to only shockwaves or in combination with any other anti-bacterial and/or anti-biofilm technologies, will prevent the biofilm formation, remove patchy biofilms, and reduce the overall

free bacteria implementing population in the Water Reclamation System (WRS) aboard the International Space Station (ISS).

Shockwaves are asymmetric significant bursts of pressure that last 5 to 8 microseconds. This burst is denoted by a single and fast acoustic pressure pulse that rises in tens or hundreds of nanoseconds to a maximum positive compressive pressure of 50 to 110 MPa (500 to 1100 bar), and it is followed by exponentially decrease to negative pressures of -5 to -15 MPa (-50 to -150 bar)[20]. The positive compressive pressures generate "acoustic streaming" that is the move of fluid in the direction of shockwave propagation, which produces high tensile and shear forces[10] against bacterial biofilms. The negative pressures create cavitation bubbles that can grow for 300 to 800 microseconds and then collapse/implode through the bubble medial portion, generating secondary shockwaves and microjets, with speeds in excess of 100 meter/second (328 foot/second)[20]. These microjets create "acoustic microstreaming" in fluids producing additionally localized tensile and shear forces, which contribute to the cracking, dislodging, and fragmentation of bacterial biofilms. The cavitation bubbles' collapse also induces localized transient high temperatures and sonoluminescence[20, 21, 22], which both contribute to sterilization of the surrounding fluid medium.

The SANUWAVE proposed set of activities are focused on its proprietary technology on a modification to an existing accumulator bellows tank, representative of those found in the WRS, to fit a total of six (6) shockwave reflectors, to efficiently clean the biofilm formed inside the tank from different directions, as seen in Figure 5. The shockwave reflectors are oriented at different angles relatively to the bellows tank longitudinal axis, with the top three reflectors positioned at 120 degrees apart and off-set with 60 degrees relatively to the ones from the bottom, to reach all sections of the tank and clean thoroughly the bellows. Although the actuation of the shockwave reflectors can be done simultaneously, it is preferred to be done subsequently. Based on the reflector geometry, the shockwaves can be focused (semi-ellipsoidal reflector) or unfocused (parabolic reflector), both of which will be tested. The focused shockwaves are powerful and more localized and the unfocused ones are less powerful but with larger spatial distribution. At first, the shockwaves will be generated via electrohydraulic principle, by discharging high voltage in between two electrodes of a spark gap, to determine the most effective shockwave dosage (input energy setting, frequency, and total number of shockwaves) needed to disrupt the biofilm and kill bacteria, as a standalone technology or in combination with biocides or ultraviolet light. Later, the shockwaves might be generated via piezoelectric principle, to increase system longevity.

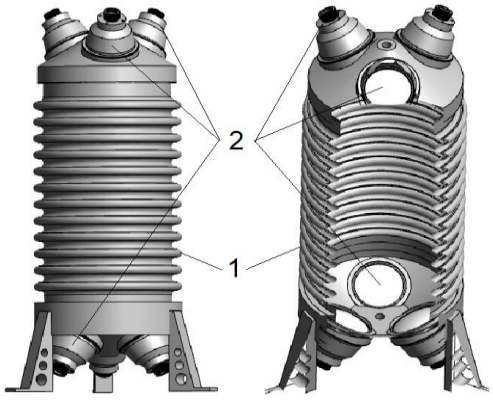

Figure 5. Accumulator bellow tank (1) with six shockwave reflectors (2)

The existing design will be tested as a ground component for its feasibility. This feasibility testing will assess complete biofilm removal using simulated biofilm material made of 5% (W/V) gelatin with Fluorescein Isothiocyanate and then actual biofilms, using assessment methods developed by CBE. Generally, the tank must be filled with water and reasonable extended, to expose the biofilm from bellows' pleats to acoustic streaming, microjets, or microstreaming generated by shockwaves. The shockwaves system is suitable for both dormant (no crew on board) and non-dormant periods, being operated for short time periods (minutes) at set time intervals, as determined from the feasibility testing. The shockwave system design was done to easily integrate with existing ISS WRS hardware, to avoid air entrapment, to have minimum weight of additional components, to easily access the shockwave reflectors for a rapid exchange in case of failure, and to reduce crew time involvement in system maintenance or recovery from dormant periods. Shockwave energy action can be adjusted to maintain bellows tank integrity and predicted life. The shockwave systems have completely non-movable parts, which increase their longevity. The average power needed during functioning of the shockwave system from Figure 5 is approximately 100 Watts, which is comparable with the energy consumption of an average laptop.

IV. Conclusion

Biofilm management within water recovery systems is critical to maintain system function and prolong system longevity as manned spaceflight moves to longer and more distant missions and the feasibility of component

replacement decreases. *Ralstonia spp.* organisms have been an almost permanent resident in the ISS WRS[23]. *Lecythophora mutabilis* has also been used in tests related to LSS for its presence in the grounded biofilm samples[24]. Growth of these microorganisms is driven by nutrients flowing through the system and it could follow that higher, preferred nutrients be consumed in the upstream portion of the WPA and decrease during microbial growth through the system. The previous WPA Ersatz was developed for a different type of testing and a downstream challenge (the multifiltration beds) and as demonstrated here, was toxic against the organisms we tested. In targeting the WPA tank, examination of the estimated influent to the WPA tank identified many potential nutrients, particularly C-sources, that were decreased or absent in the effluent. These changes indicate microbial consumption. Inclusion of those nutrients supported growth of the microbes of the test consortia moving toward representing the type of microbial growth that could lead to system clogging and a suitable control against which to test our current biofilm control strategies. Future studies of the metadata from biocide, shockwave and other internal MSFC testing will help in determining an optimal technology for biofilm mitigation.

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