

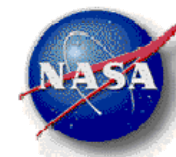


Microbiological Tests Performed During the Design of the International Space Station ECLSS: Part 1, Bulk Phase Water and Wastewater

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Introduction

- Many microbiological studies were performed during the development of the Space Station Water Recovery and Management System from 1990-2009. Studies include assessments of:
 - bulk phase (planktonic) microbial population
 - biofilms,
 - microbially influenced corrosion
 - biofouling treatments



Introduction

- This presentation will summarize the studies performed to assess the bulk phase microbial community during the Space Station Water Recovery Tests (WRT) from 1990 to 1998.
- A series of related studies, involving biofilms, microbially influenced corrosion and biofouling control strategies, were also conducted. These studies will be summarized in a future report.

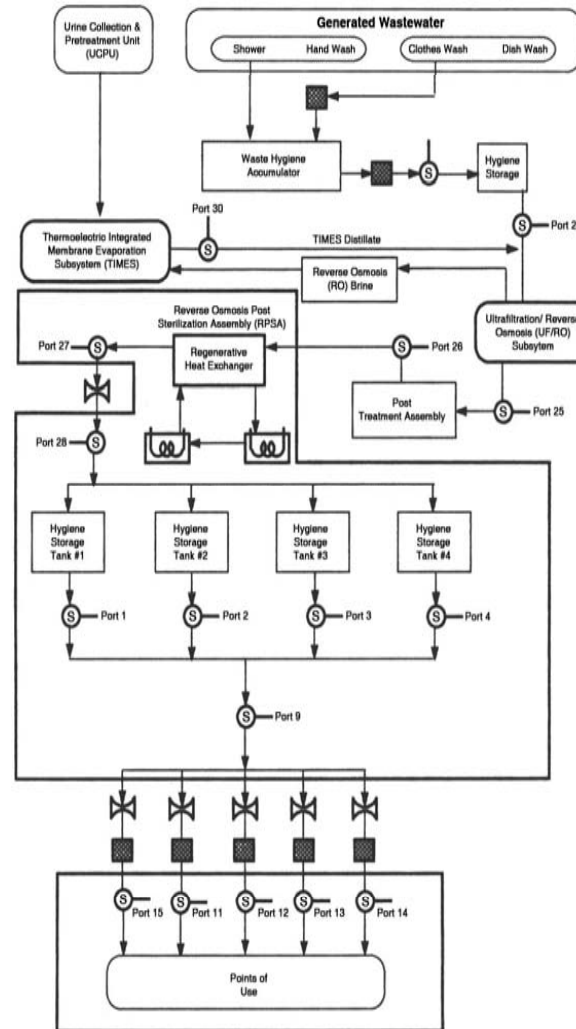


Water Recovery Test Stages 1A, 2A and 3A

- SSF/ 2-loop system/ 1990
 - Hygiene Loop (urine, shower, hand-wash, dishwasher, laundry)
 - Urine Processor: Thermoelectrically Integrated Membrane Evaporation Subsystem (TIMES)
 - Ultrafiltration (UF)/Reverse Osmosis (RO) subsystem
 - 4 hygiene processed water storage tanks
 - Potable Loop (humidity condensate)
 - Multifiltration (MF) Subsystem (series of ion exchange resins and organic adsorbents)
 - MF “post-Sterilization” Assembly
 - 4 potable processed water storage tanks



WRT Stages 1A, 2A, 3A Processing Schematic (Hygiene Loop)



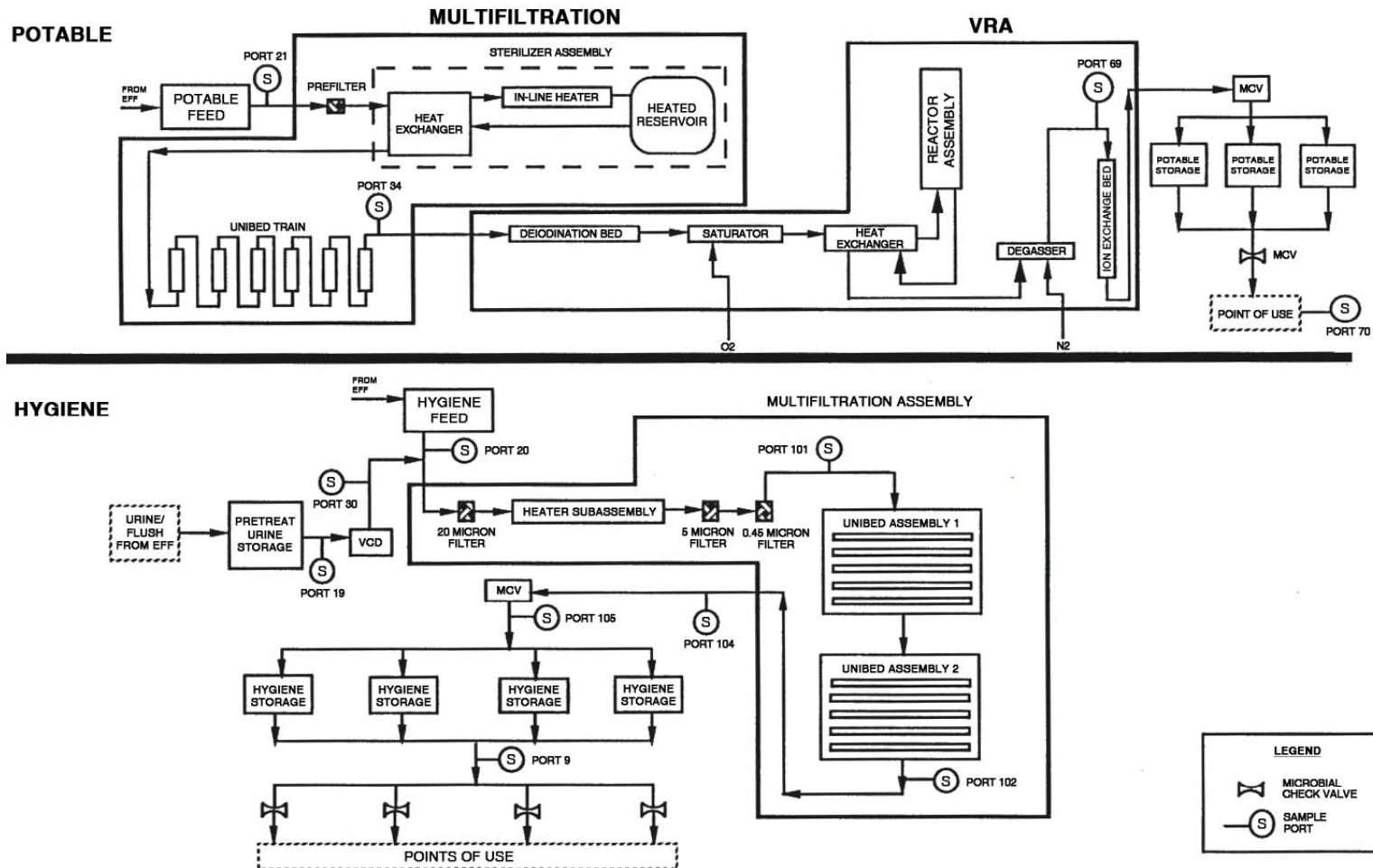


Water Recovery Test Stages 4/5

- SSF/ 2-loops system/ 1991
 - Hygiene Loop (urine, shower, hand-wash, ~~dishwasher~~, laundry)
 - Urine processor: Vapor Compression Distillation (VCD) subsystem
 - MF Subsystem
 - 4 hygiene processed water storage tanks
 - Potable Loop (humidity condensate)
 - MF pre-"Sterilization" Assembly (250°F for 20 minutes/ 2 log reduction)
 - MF Subsystem (~~MF post-"Sterilization" Assembly~~)
 - Volatile Removal Assembly (VRA)- catalytic oxidation reactor/260°F
 - 4 potable processed water storage tanks



WRT Stages 4/5 Processing Schematic (Potable and Hygiene Loop)



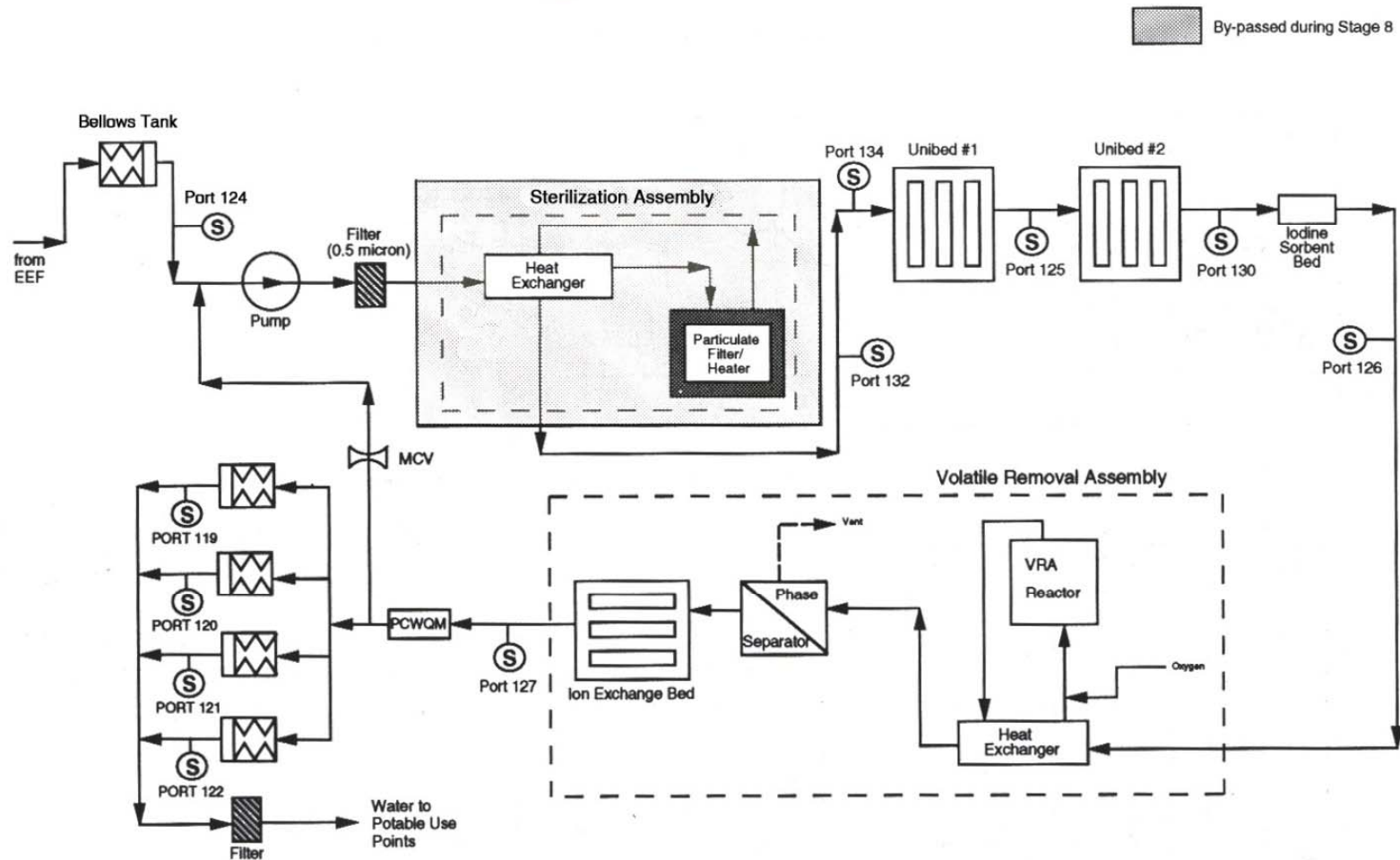


Water Recovery Test Stages 7/8

- SSF/ 1-loop system/ 1992
 - Potable/Hygiene Loop (urine, shower, hand-wash, laundry, humidity condensate)
 - Urine processor: Vapor Compression Distillation (VCD) subsystem
 - MF Subsystem (~~MF pre-”Sterilization” Assembly~~)
 - VRA
 - 4 processed water storage tanks



WRT Stages 7/8 Processing Schematic (Hygiene / Potable Loop)



By-passed during Stage 8

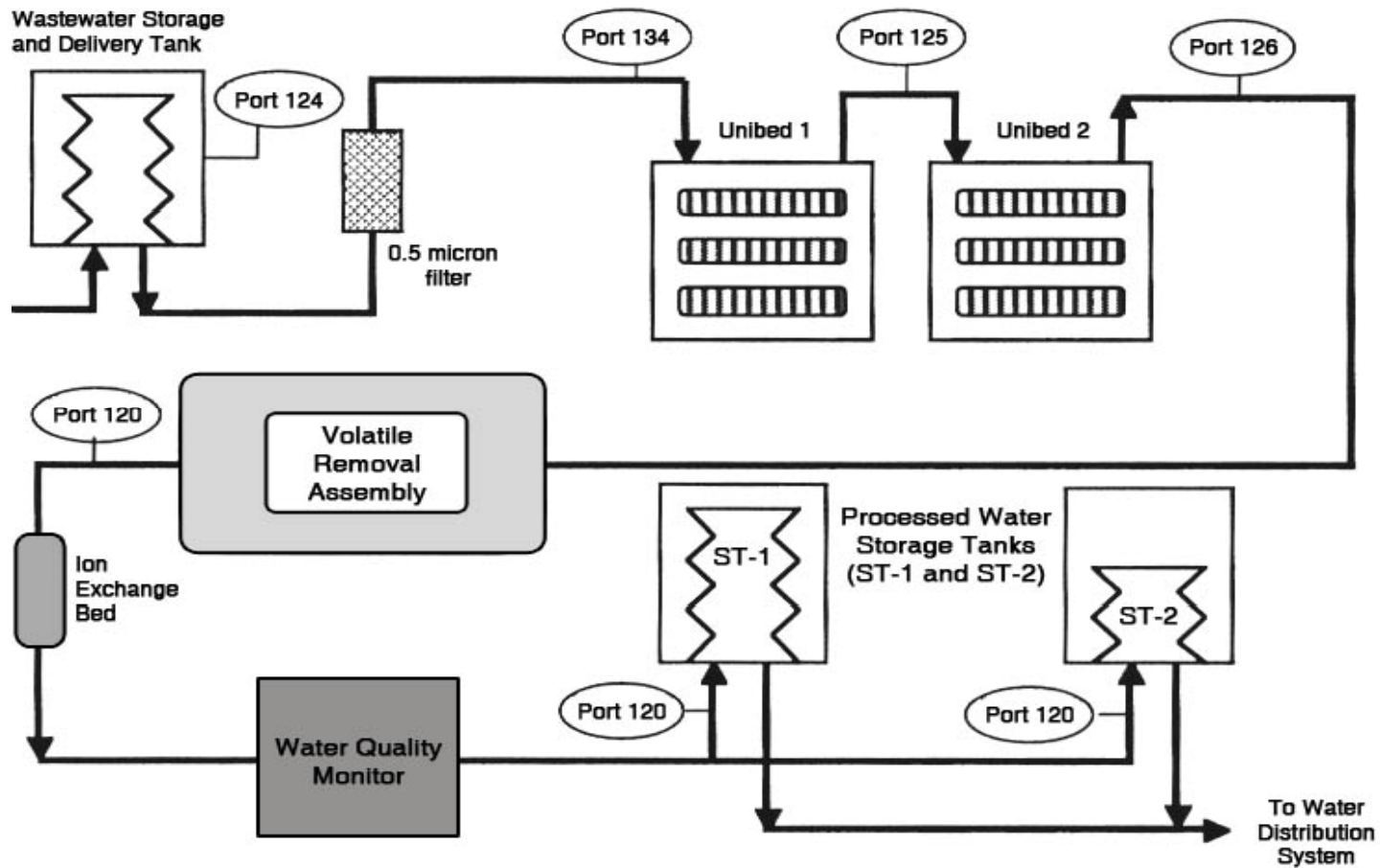


Water Recovery Test Stages 10/11

- ISS/ 1-loop system/ 1996-97
 - Potable/Hygiene Loop (urine, shower, hand-wash, laundry, humidity condensate)
 - Urine processor: Vapor Compression Distillation (VCD) subsystem
 - MF Subsystem
 - VRA
 - 2 processed water storage tanks



WRT Stages 10/11 Processing Schematic (Hygiene / Potable Loop)





Potable Water Requirements

Target Microorganism	U.S. EPA Requirement	NASA/WRT Requirement
total coliforms	<1/100 mL	Not detectable
heterotrophic bacteria	<500/mL	1 CFU/100mL
Total	99.9% reduction (¹ MCLG= 0)	GI
<i>Giardia lablia</i>	99.9% reduction (MCLG= 0)	GI
enteric viruses (adenovirus as most resistant)	99.99% reduction	GI; systemic
<i>Legionella spp.</i>	(MCLG= 0)	respiratory

¹MCLG, maximum contaminant level goal



Microbiological Tests Performed During the WRT

- Microbial Tests
 - Microbial Characterization of Processed Water
 - Viral Survival Study
 - Water Storage Test
 - Endotoxin Test
 - Analysis of Multifiltration Beds
 - Assessment of shower (point of use) water
 - Assessment of Assimilable Organic Carbon



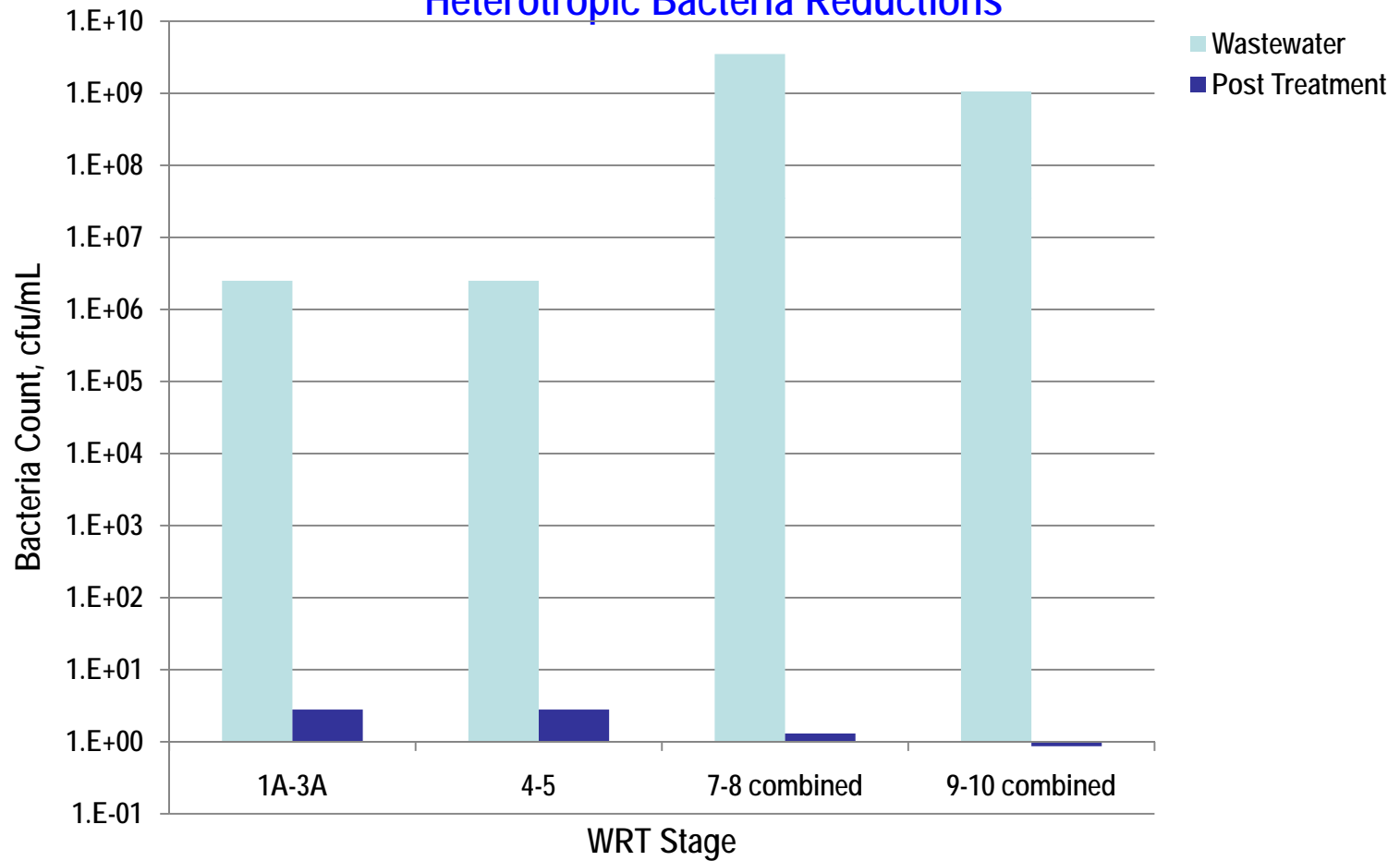
WRT Microbiological Methodology

Method	Microorganisms Recovered	Comments
epifluorescence microscopy	direct counts of total microbial bioburden	detection limit of 10^4 cells/mL
R2A culture	heterotrophic bacteria (nutrient limited)	7 d incubations
enriched chocolate agar with incubation in 5% CO ₂	aerotolerant bacteria	recovery of fastidious human isolates; 2 d incubations
Emmon's medium	yeast; filamentous fungi	5 d incubations
membrane fecal coliform (MFC)	fecal coliforms	24 h
viral plaque assay	challenge bacteriophage viruses	performed at U.S. EPA labs
microbial identification	bacteria, fungi	MIDI, Vitek, Biolog test systems employed



Results- Microbial Characterization

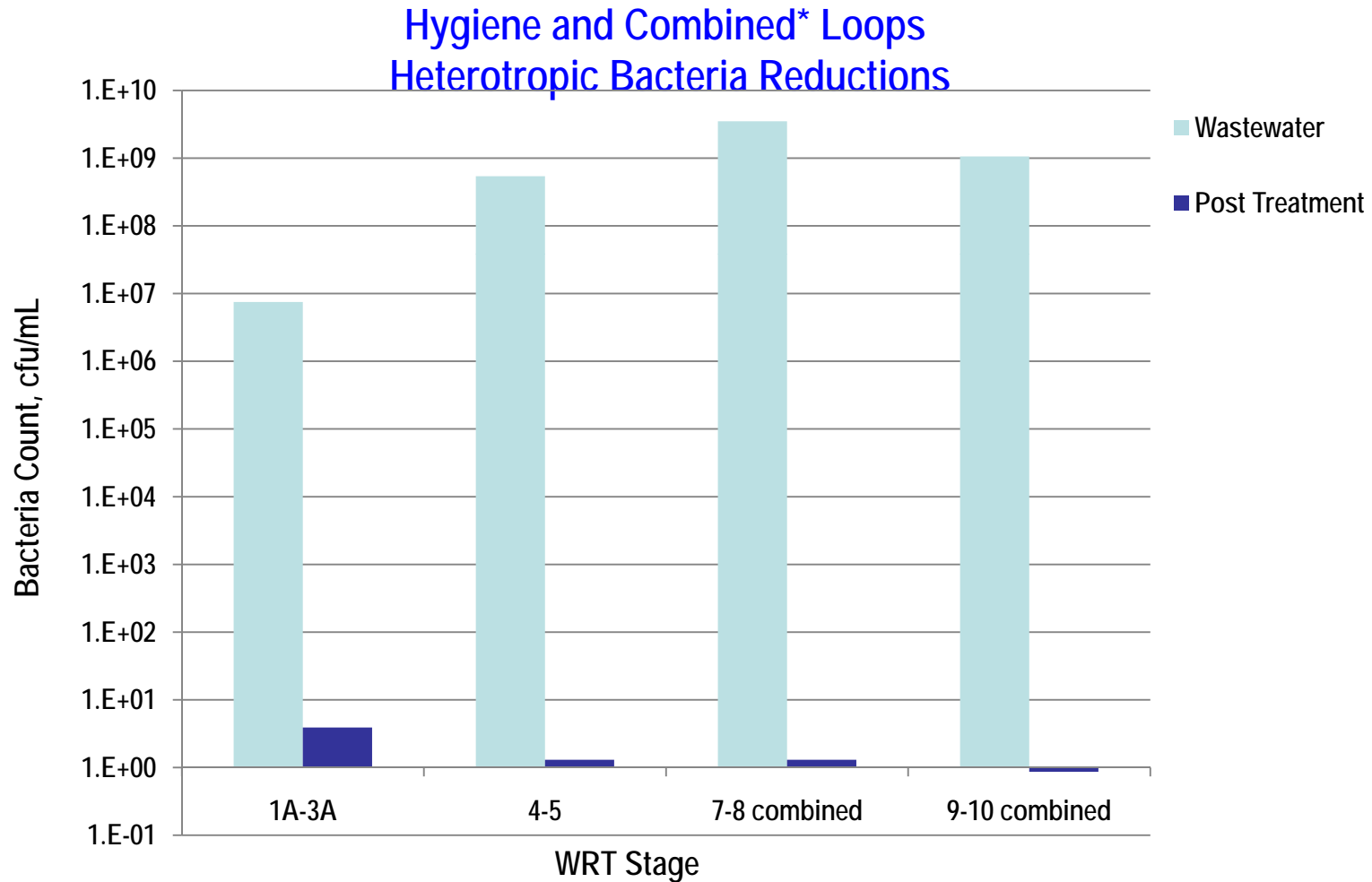
Potable and Combined* Loops Heterotrophic Bacteria Reductions



*Combined Loop= Potable and Hygiene Loops



Results- Microbial Characterization



*Combined Loop= Potable and Hygiene Loops

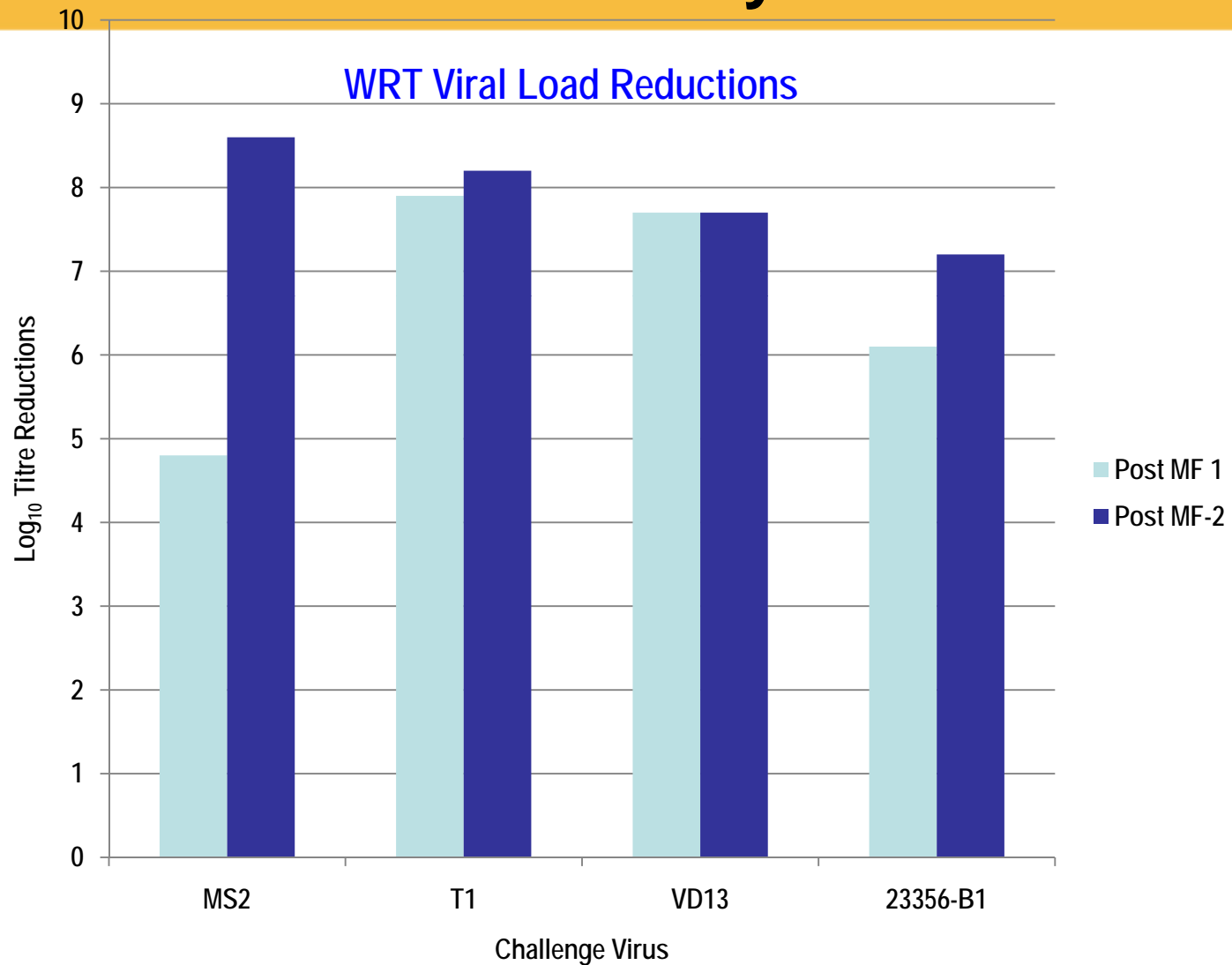


Results- Viral Survival Study

- Bacteriophages MS2, T-1, VD13 and 23356-B1 were chosen for this study because of their similarity to viruses that could be found in the Space Station wastewater.
- A minimum of 10⁷ PFU/mL were mixed with human generated wastewater.
- The viral population was removed after the 2nd multifiltration bed; VRA was not challenged with viruses in WRT Stage 9.
- After the completion of WRT Stage 10, the same concentration of viruses was injected in the system, prior of the VRA.
- Test showed that the VRA has a viral removal capability of 6 log₁₀ units.
- Test demonstrated that the WP has an excellent capacity to remove viruses in wastewater.



Results- Viral Survival Study





Results- Water Storage Test

- After the completion of WRT Stage 8 iodinated processed water was stored in 2 316L stainless steel bellows tank for up to 183 days.
- Samples were taken once a week and the heterotrophic microbial population was assessed.
- The microbial population in the tank was maintained at an average of 1 CFU/100mL.
- This test confirmed that the microbial population can be controlled for at least 183 days, if the water quality is controlled and the storage vessel is properly disinfected before use.



Results- Endotoxin Test

- During WRT Stage 8 processed water, deionized water and Birmingham city water were analyzed for endotoxins using the Limulus amoebocyte lysate (LAL) test.
- Birmingham (drinking) water contained endotoxin levels between 0.125 and 0.250 EU/mL.
- Deionized water contained endotoxin levels between 0.060 and 0.125 EU/mL.
- WRT water endotoxin level was reduced from >103 EU/mL in the wastewater tank to <0.060 EU/mL in the processed water.



Results- Analysis of Multifiltration Beds

- The resins inside the WP multifiltration beds were analyzed after they became saturated with contaminants during the WRT Stage 8 test.
- The inside of the multifiltration beds was exposed by aseptically cutting the stainless steel casing with a saw at predefined locations.
 - between 2 to 7 grams of each material was placed in a sterile test tube containing a phosphate buffer solution.
 - Material included iodinated resins (inlet and outlet/ imparts 1 to 4 ppm of iodine), ion exchange resins and carbon mix.
- The microbial loads in most of the multifiltration bed media reflected a reduction from the feed wastewater.
- The microorganisms identified in the media were similar to those isolated in the wastewater



Results- Assessment of Shower Water

- To compare the quality of reclaimed water used by test subjects while showering in the EEF, with municipally-treated water used in showers at home, samples from selected homes in north Alabama were collected and analyzed on June 28, 1991.
- Three samples were collected from home showers in 3 different cities in Al.
- Viable counts were higher on R2A than on CAE and ranged from 2.9×10^2 to 1.2×10^4 CFU/100 mL.
- The bacterial counts from the home showers were similar or higher than the counts recorded during the sampling of the WRT shower.
- Predominant genera isolated included Pseudomonas, Methylobacterium, and Bacillus.



Results- Assessment of AOC

- During WRT Stage 4/5, a bioassay to measure the assimilable organic carbon (AOC) concentration, was performed to assess bacterial regrowth potential.
- Nine clean water samples were analyzed, 5 from the potable water storage tank and 4 from the hygiene water storage tank.
- The AOC levels in the potable water samples had an average of recorded as: 102.8 $\mu\text{g/L}$. The average of culturable bacteria was maintained at <1.0 CFU/100mL.
- In the hygiene water samples, the AOC levels steadily increased during the 2 week study from 103 to 150 $\mu\text{g/L}$. This increase in AOC levels could have been reflected in the microbial count increase from <1 CFU/100mL to 6 CFU/100mL on CAE reported by the laboratory.



Summary

- Information gained during the design and testing of a partially closed water recovery system for Space Station provided a basis for understanding the activity of microbial communities in relevant test environments.
- With a better understanding of the microbial ecology in closed-loop life support systems, technologies/system designs can be improved to minimize negative effects and unnecessary requirements.
- Even with the incorporation of the best life support design improvements, real-time microbial monitoring will be needed to assess the changes that will occur overtime in the microbial population.



Summary

- This report provides an overview of some of the microbiological analyses performed during the Space Station WRT program. These tests not only integrated several technologies with the goal of producing water that met NASA's potable water specifications, but also integrated humans, and therefore human flora into the protocols. At the time these tests were performed, not much was known (or published) about the microbial composition of these types of wastewater. It is important to note that design changes to the WRS have been implemented over the years and results discussed in this report might be directly related to test configurations that were not chosen for the final flight configuration.



Conclusion

Results from the microbiological analyses performed during the WRT showed that it was possible to recycle water from different sources, including urine, and produce water that can exceed the quality of municipally produced water.



A Final Note

A significant amount of valuable information was gathered during WRT ground testing, with humans in the loop. The uniqueness of a microgravity environment and the possibility of extending the stay of humans in closed environments, away from Earth, will pose a constant challenge and many learning opportunities. Microbes will always be a significant inhabitant of the life support systems in space.



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