Life Support Systems Microbial Challenges

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Agenda

- Environmental Control and Life Support Systems (ECLSS) What is it?
- A Look Inside the International Space Station (ISS)
- The Complexity of a Water Recycling System
- ISS Microbiology Acceptability Limits
- Overview of Current Microbial Challenges
- In a Perfect World What we Would Like to Have
- The Future

Environmental Control and Life Support Systems

Human Needs and Effluents Mass Balance (per person per day)

Needs

- Oxygen = 0.84 kg (1.84 lb)
- Food Solids = 0.62 kg (1.36 lb)
- Water in Food = 1.15 kg (2.54 lb)
- Food Prep Water = 0.76 kg (1.67 lb)
- Drink = 1.62 kg (3.56 lb)
- Metabolized Water = 0.35 kg (0.76 lb)
- Hand/Face Wash Water = 4.09 kg (9.00 lb)
- Shower Water = 2.73 kg (6.00 lb)
- Urinal Flush = 0.49 kg (1.09 lb)
- Clothes Wash Water = 12.50 kg (27.50 lb)
- Dish Wash Water = 5.45 kg (12.00 lb)
- Total = 30.60 kg (67.32 lb)

Effluents

- Carbon Dioxide = 1.00 kg (2.20 lb)
- Respiration & Perspiration Water = 2.28 kg (5.02 lb)
- Food Preparation, Latent Water = 0.036 kg (0.08 lb)
- Urine = 1.50 kg (3.31 lb)
- Urine Flush Water = 0.50 kg (1.09 lb)
- Feces Water = 0.091 kg (0.20 lb)
- Sweat Solids = 0.018 kg (0.04 lb)
- Urine Solids = 0.059 kg (0.13 lb)
- Feces Solids = 0.032 kg (0.07 lb)
- Hygiene Water = 12.58 kg (27.68 lb)
- Clothes Wash Water
  Liquid = 11.90 kg (26.17 lb)
  Latent = 0.60 kg (1.33 lb)
  Total = 30.60 kg (67.32 lb)

Note: These values are based on an average metabolic rate of 136.7 W/person (11,200 BTU/person/day) and a respiration quotient of 0.87.
The values will be higher when activity levels are greater and for larger than average people. The respiration quotient is the molar ratio of CO_2 generated to O_2 consumed.

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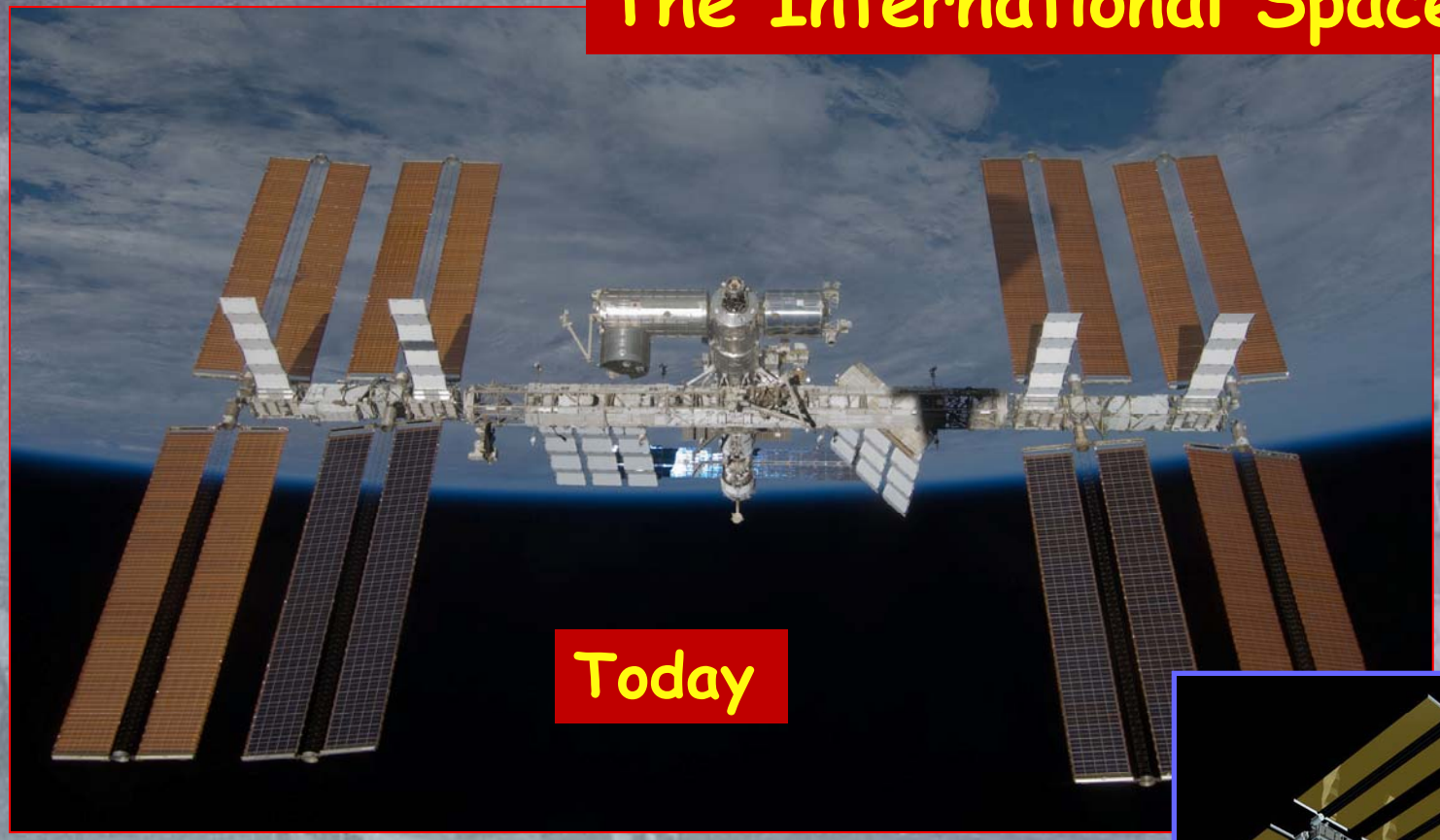
ECLSS- What is it?

Control Atmosphere Pressure	Condition Atmosphere	Respond to Emergency Conditions	Control Internal CO_2 & Contaminants	Provide Water	Prepare for EVA Operations
• O_2/N_2 Pressure Control Assemblies (USO/RS)	• Cabin Air Temperature & Humidity Control Assemblies (All)	• Smoke Detectors (All)	• CO_2 Removal Assembly (USOS/RS)	• Potable Water Processor (USOS/RS)	• O_2/N_2 Pressure Control Assemblies (USOS)
• Positive & Negative Pressure Relief (USOS-Transport)	• Ventilation Fans (USOS, RS, MPLM)	• Portable Fire Extinguishers (All)	• CO_2 Vent (USOS/RS)	• Urine Processor (USOS/RS)	• O_2/N_2 Distribution (USOS)
• O_2/N_2 Storage (USOS, RS, Pro-gress)	• Air Particulate Filters (All)	• Fire Indicators and Fire Suppression Ports (All)	• Trace Contaminant Control Assembly (USOS/RS)	• Process Control Water Quality Monitor (USOS)	• O_2/N_2 Storage (USOS)
• O_2 Generation Assembly, O_2 Solid Chemicals (RS)	• Intermodule Ventilation Fans & Valves (All)	• Portable Breathing Apparatus and Masks (All)	• Major Constituent Analyzer (USOS)	• Condensate Storage (USOS/RS)	• Major Constituent Analyzer (USOS) (Shared)
• Major Constituent Analyzer (USOS) (Share)	• Ducting (All)	• O_2/N_2 Pressure Control Assemblies (USOS) (Shared)	• CO_2 Reduction Assembly (RS)	• Fuel Cell Water Storage (USOS)	
• Gas Analyzer (RS) (Shared)			• CO_2 LIOH Removal (RS)	• Waste Water Distribution (USOS)	
			• Manual Sampling Equipment (USOS)	• Hygiene Water Processor (RS)	
			• Gas Analyzer (RS)		
Atmosphere Control & Supply (ACS) & AR	**Temperature Humidity Control**	**Fire Detection & Suppression & ACS**	**Atmosphere Revitalization (AR)**	**Water Recovery & Mgmt/ Waste Mgmt**	**ACS & AR**

The International Space Station



Today

Artist Concept

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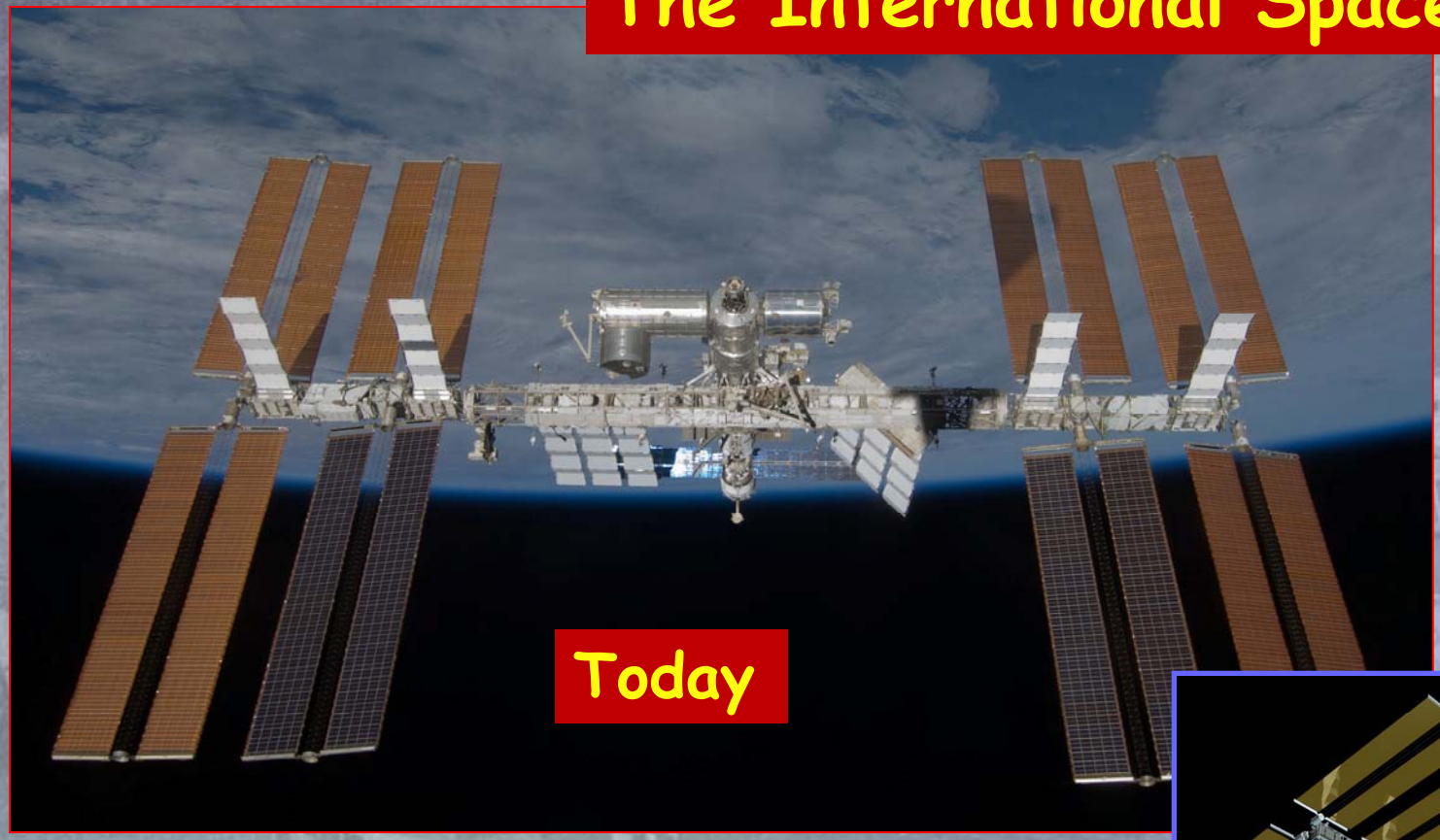
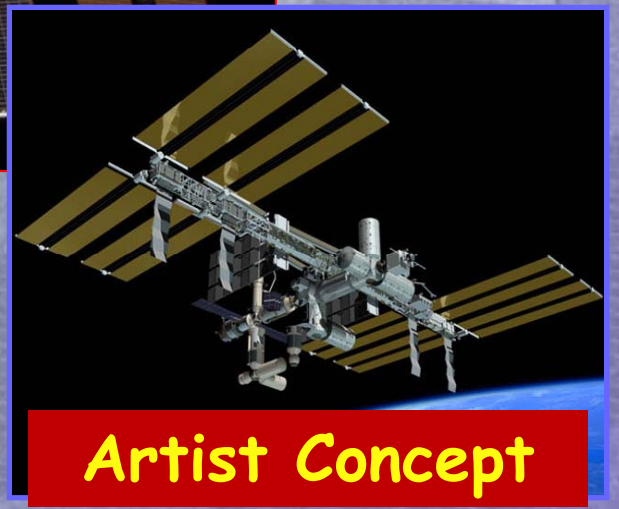
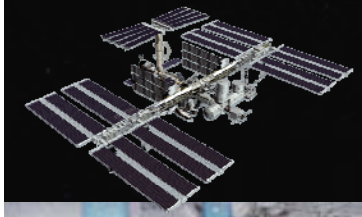
The International Space Station



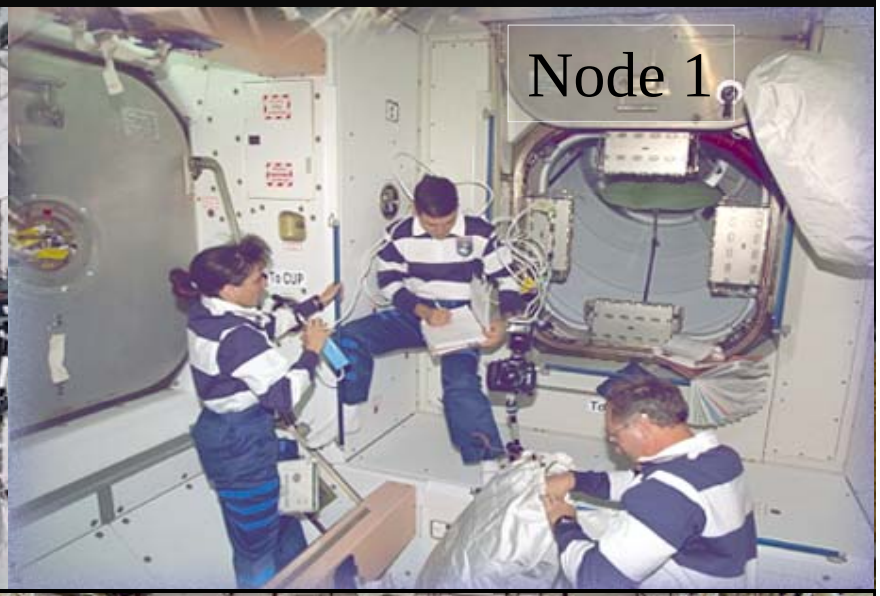
Today



Artist Concept



A Look Inside ISS



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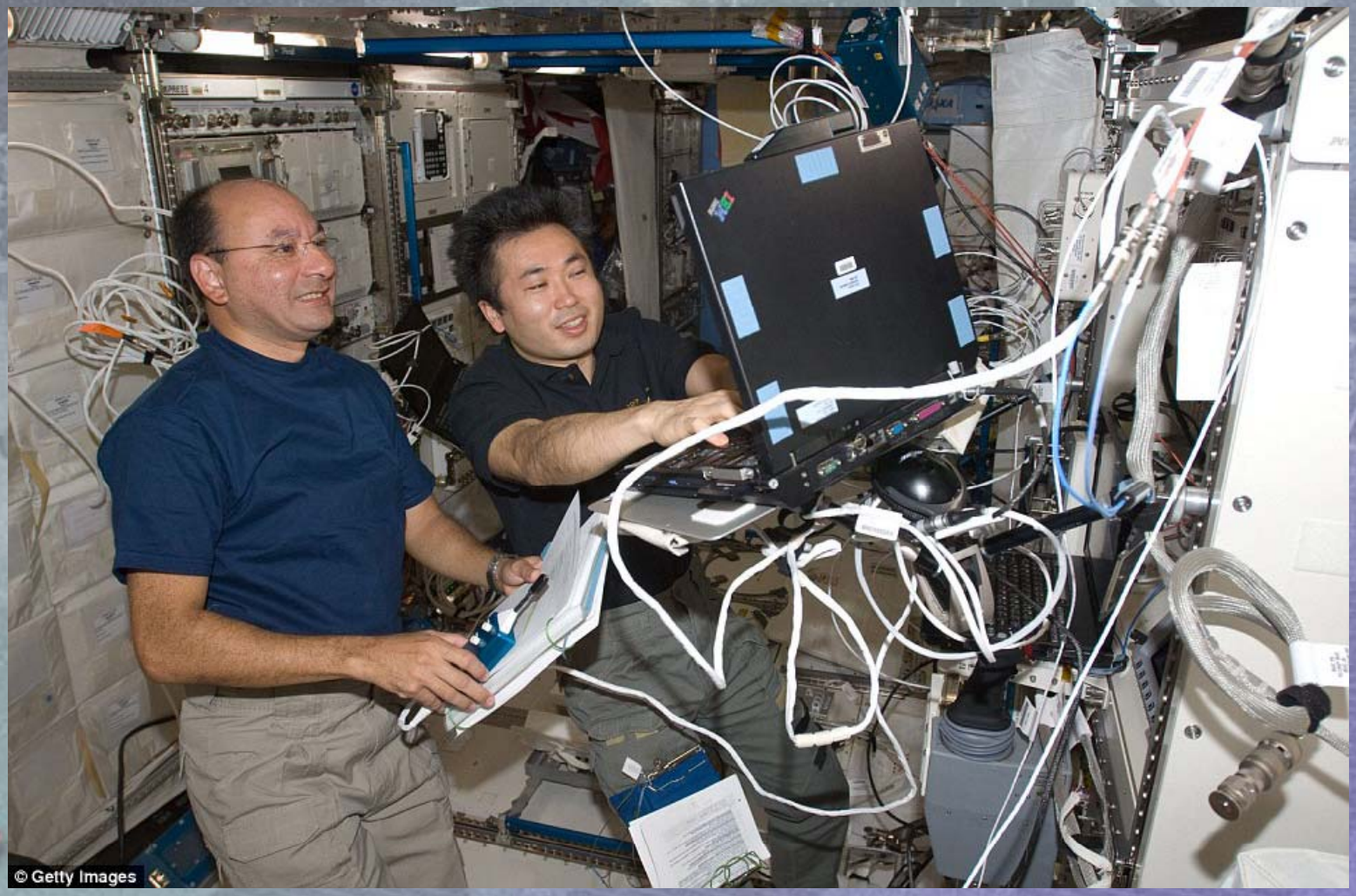
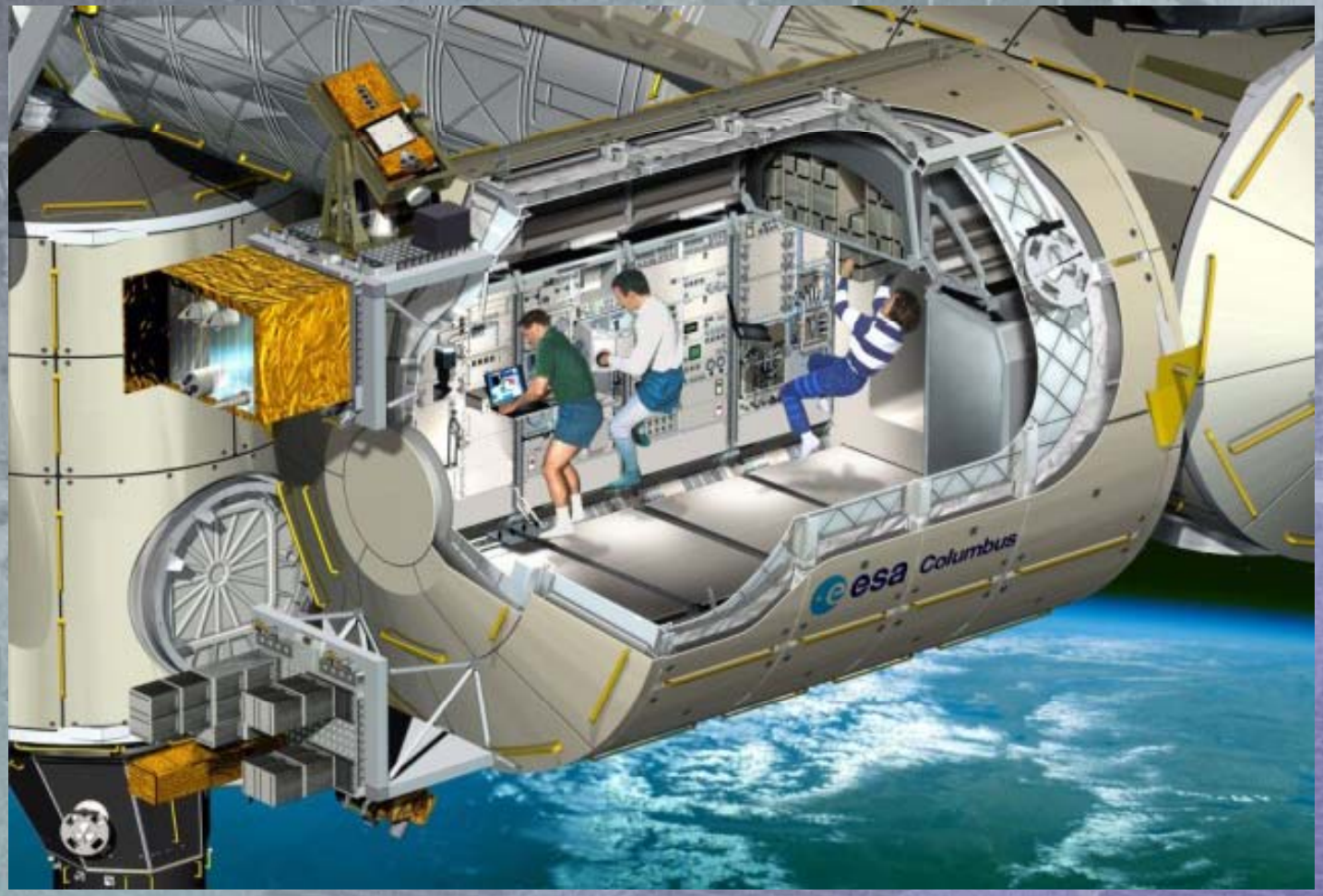
A Look Inside ISS

A Look Inside ISS

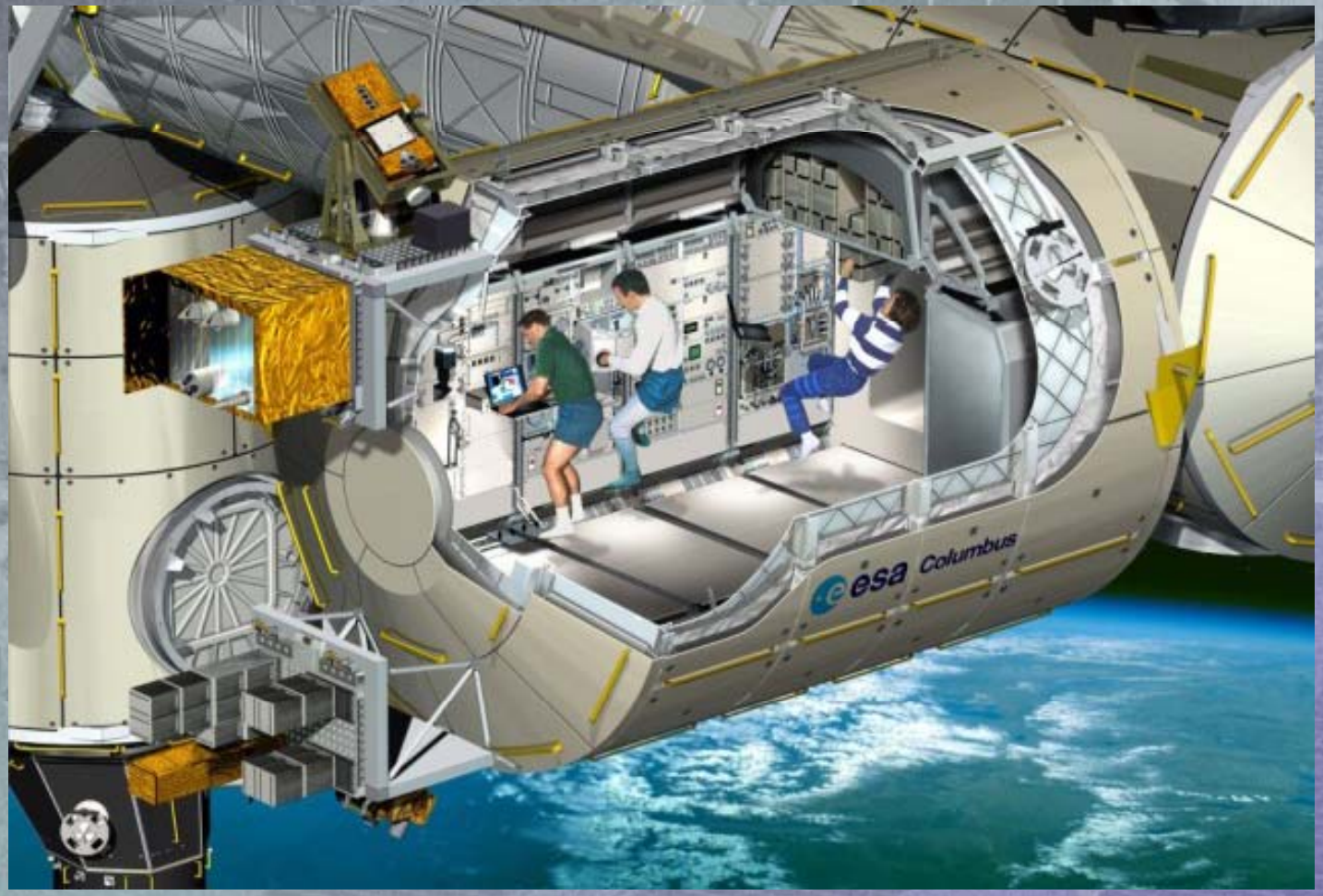


Hope

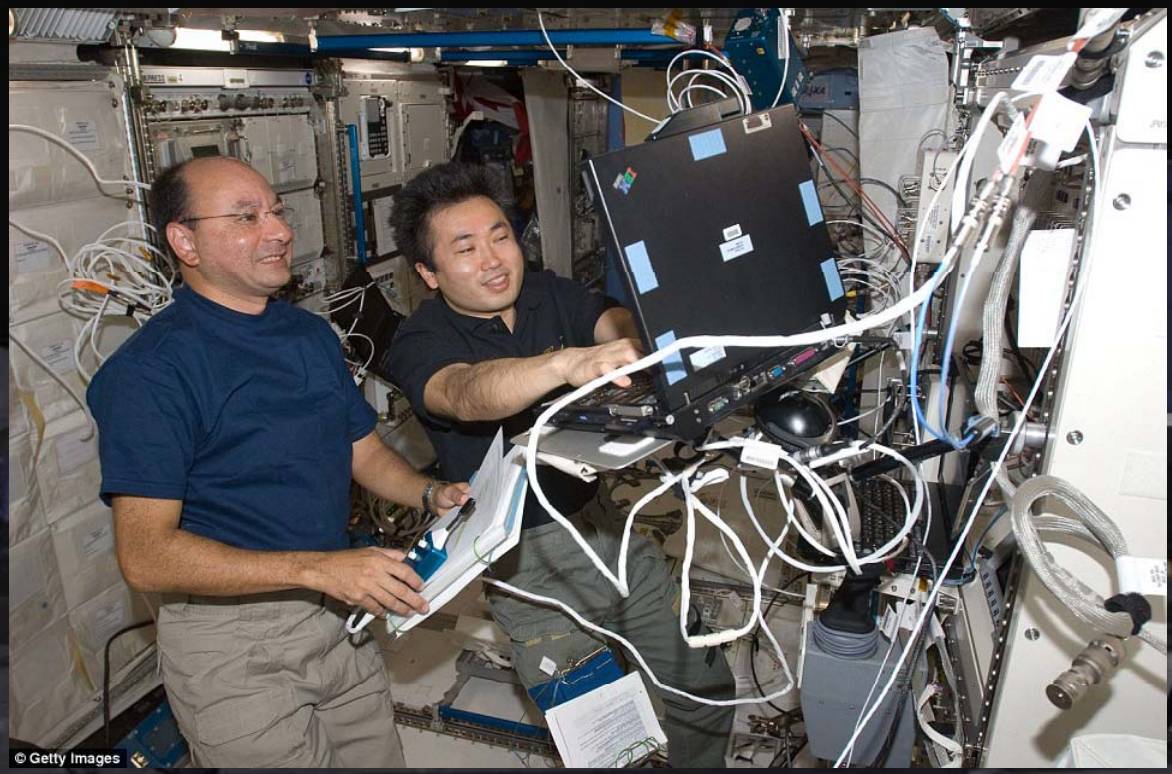
A Look Inside ISS



A Look Inside ISS



ECLSS- What is it?



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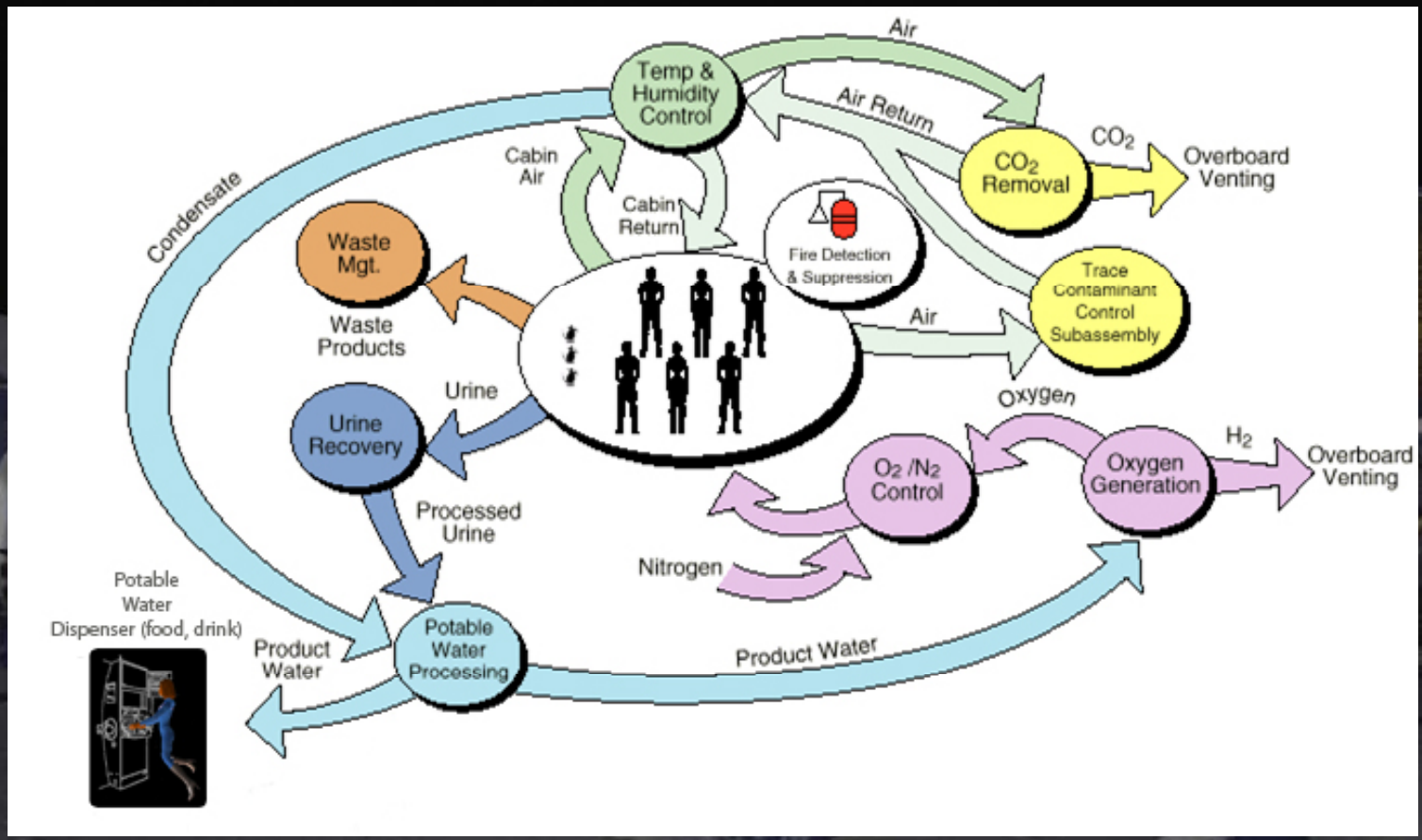

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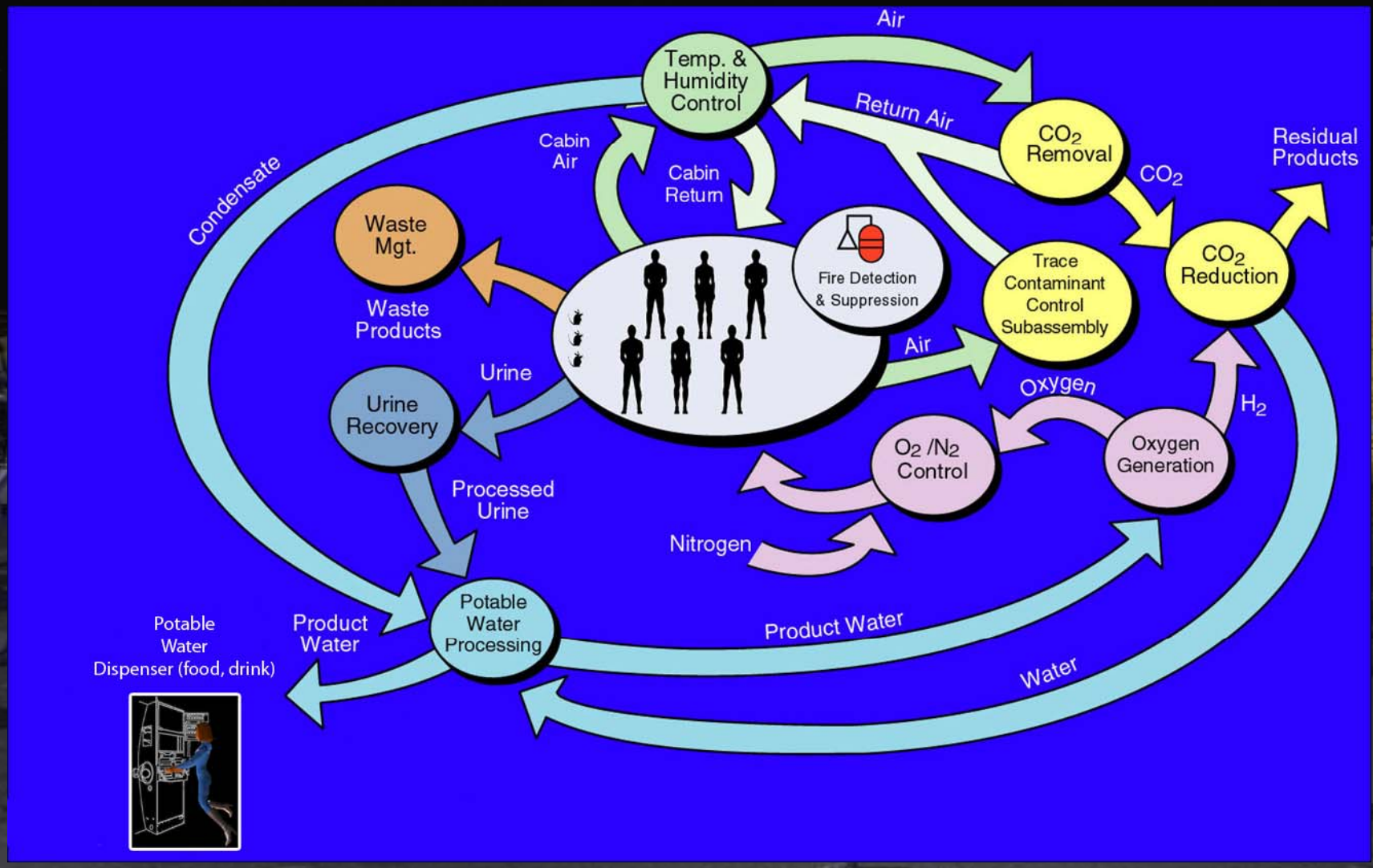

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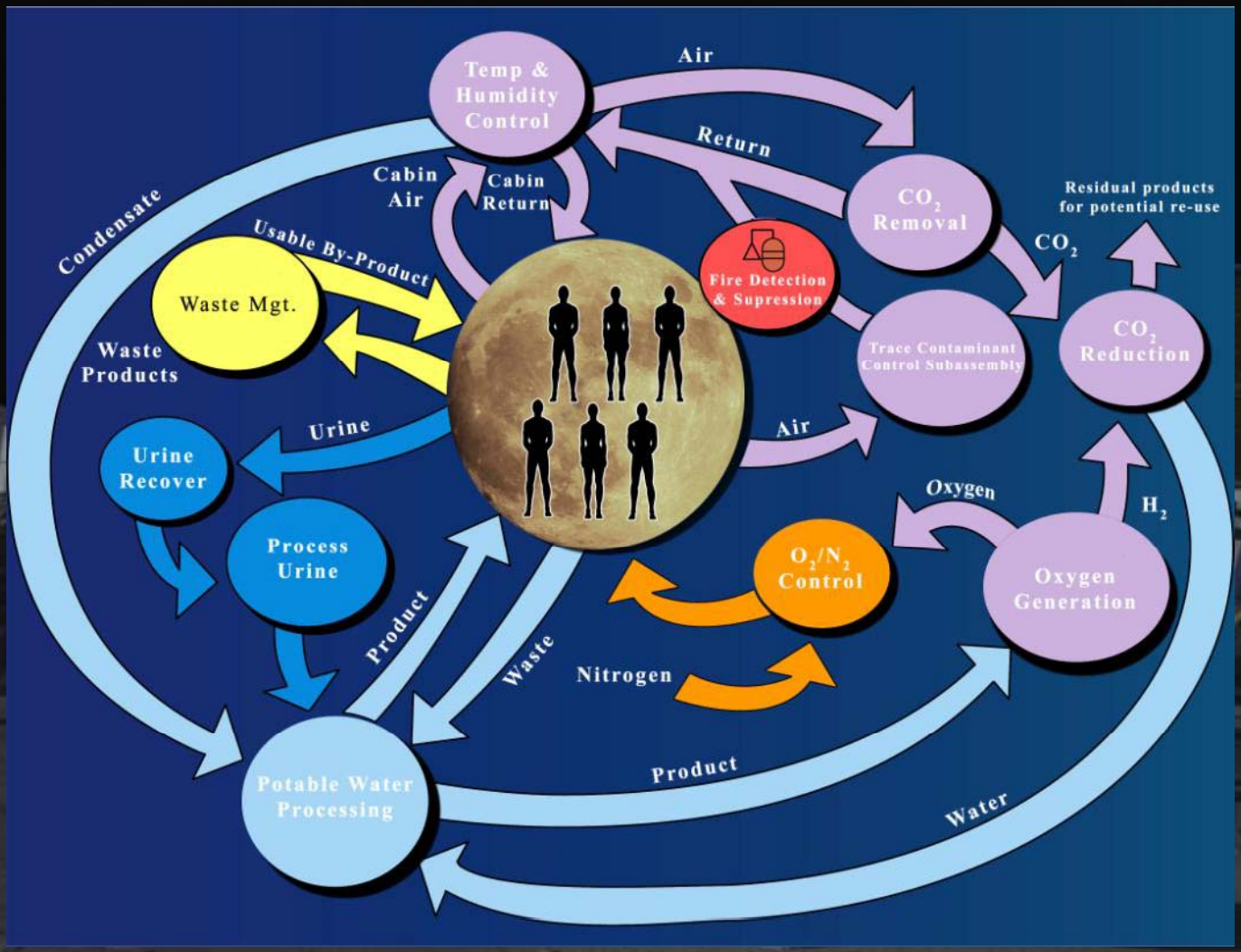

ECLSS Microbial Challenges

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ECLSS Microbial Challenges

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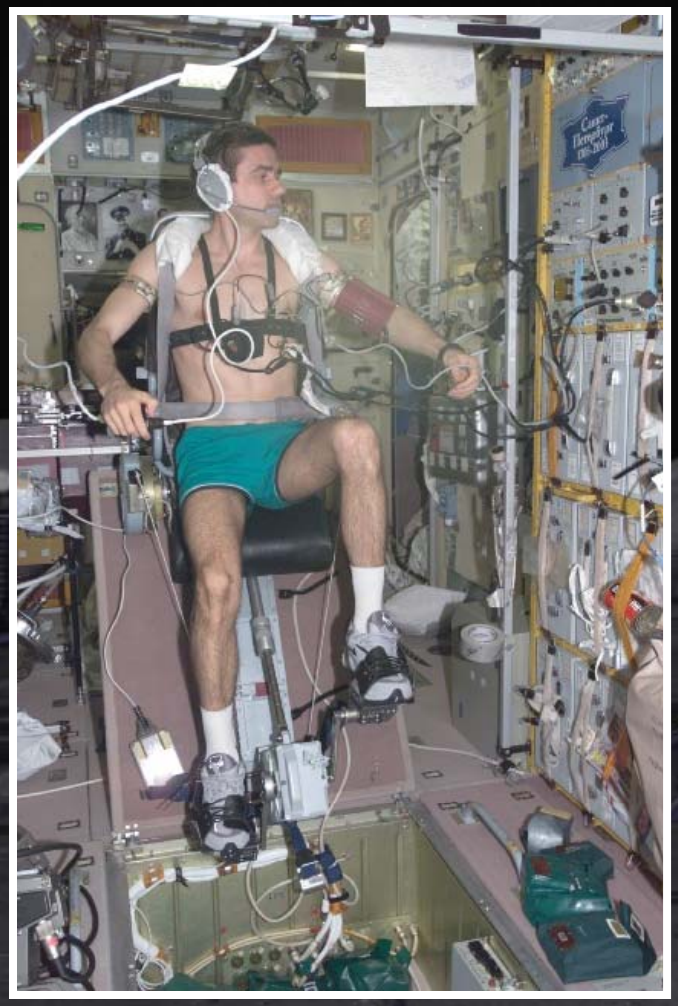

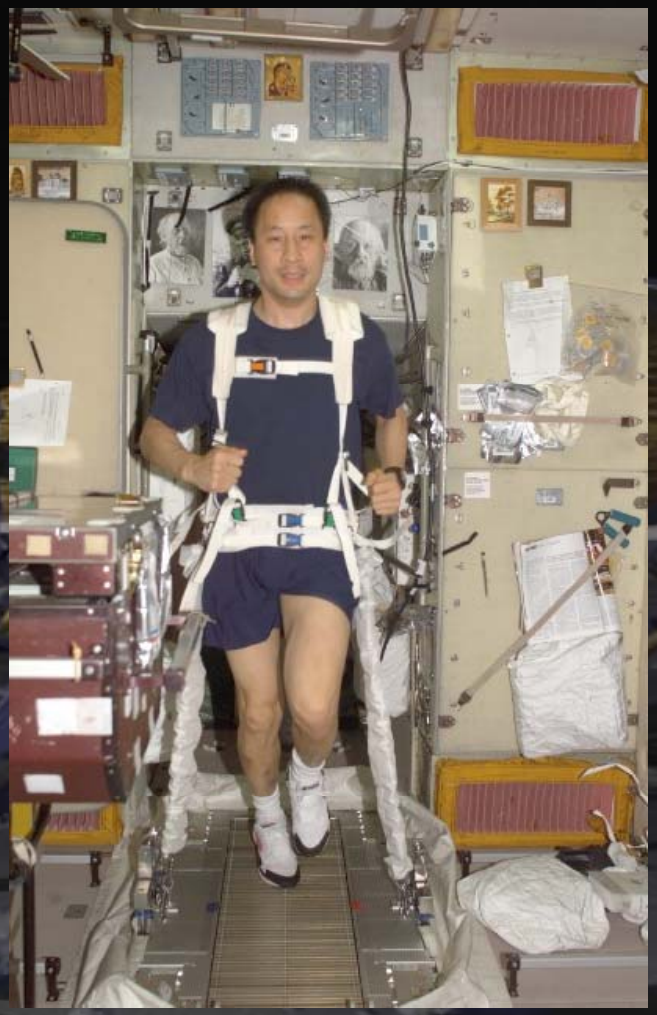

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ECLSS Microbial Challenges

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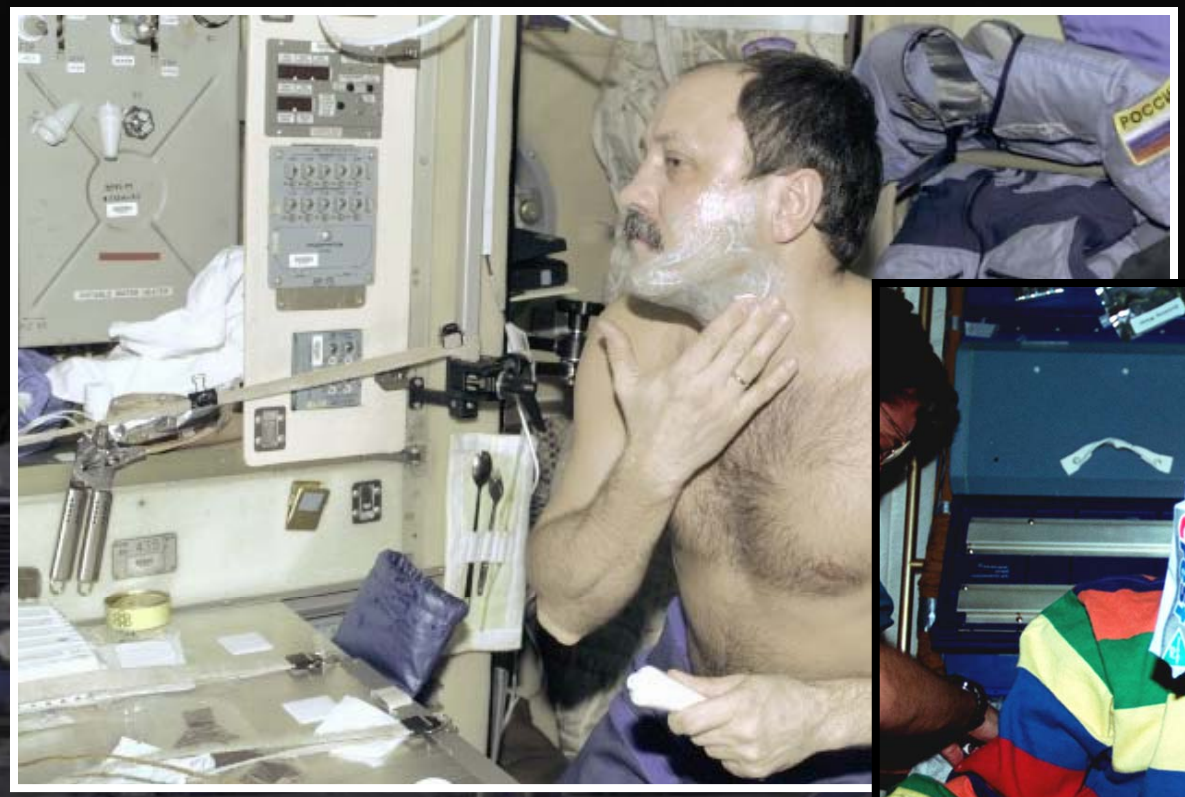
ECLSS Microbial Challenges



ECLSS Microbial Challenges

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ECLSS Microbial Challenges

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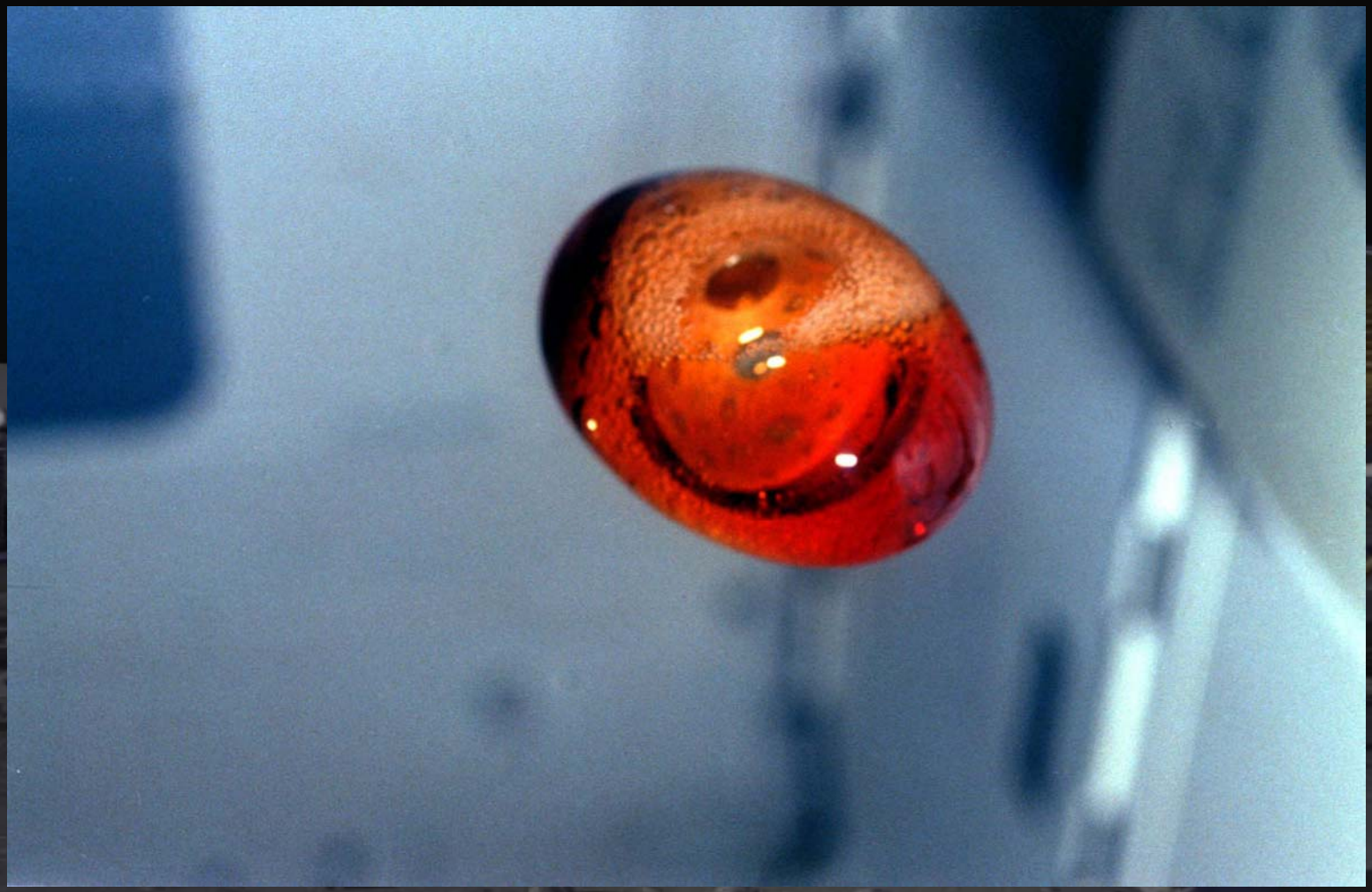
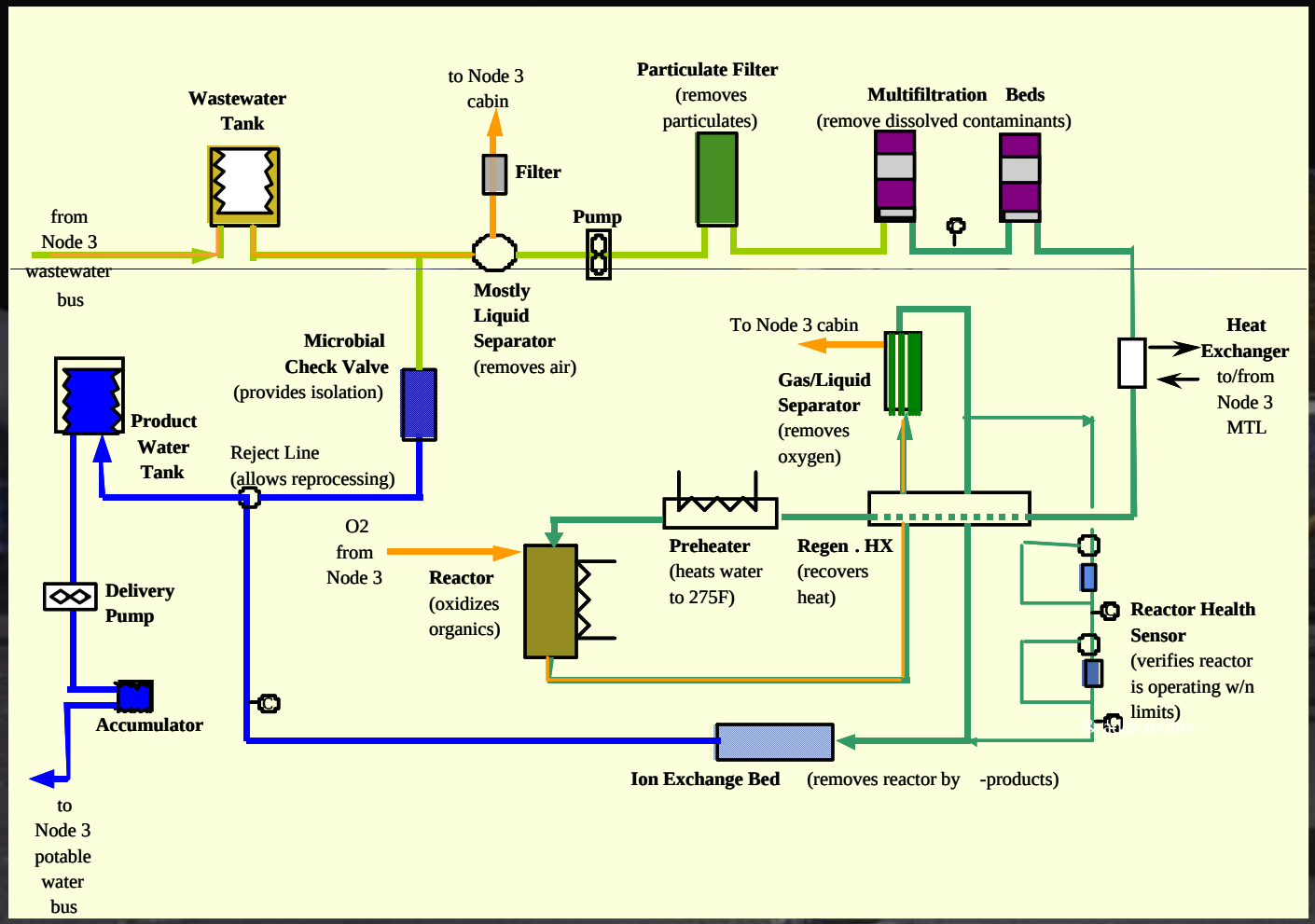


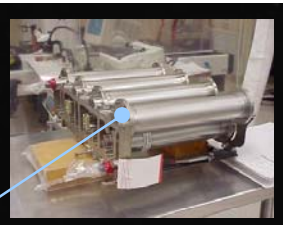
ECLSS Microbial Challenges

Filling up a bag of water in the Zvezda, SM

ISS Water Processor Diagram



Water Processor Assembly



Wastewater Tank

to Node 3 cabin

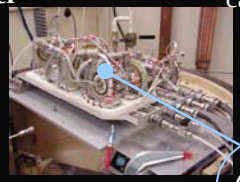
Filter

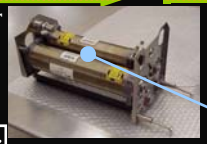
Pump

Particulate Filter (removes particulates)

Multifiltration Beds (remove dissolved contaminants)

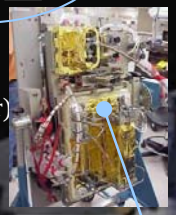
from Node 3 wastewater bus





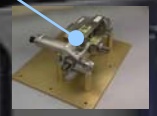
Microbial Check Valve (provides isolation)

Mostly Liquid Separator (removes air)



To Node 3 cabin

Gas/Liquid Separator (removes oxygen)



Heat Exchanger to/from Node 3 MTL

Product Water Tank

Reject Line (allows reprocessing)

O2 from Node 3

Reactor (oxidizes organics)

Preheater (heats water to 275F)

Regen. HX (recovers heat)

Delivery Pump

Accumulator

Reactor Health Sensor (verifies reactor is operating w/n limits)

to Node 3 potable water bus

Ion Exchange Bed (removes reactor by-products)

ECLSS Microbial Challenges

- Wetted Materials in space life support systems include:
  - Titanium
  - 316L Stainless Steel
  - Teflon
  - Viton O-rings
  - Nickel-Brazed Stainless Steel

ADVERSE EFFECTS OF MICROBIAL CONTAMINATION

Short-term Effects (days to weeks)

Air/Surfaces:

- Release of volatiles (e.g., odors)
- Allergies (e.g., skin, respiratory)
- Infectious diseases (e.g., Legionnaire's)

Water:

- Objectionable taste/odor
- Gastrointestinal distress

Long-term Effects (weeks to years)

Air/Surfaces (same as short-term plus):

- Release of toxins (e.g., mycotoxins)
- Sick building syndrome
- Environmental contamination
- Biodegradation of materials
- Systems performance

Water (same as short-term plus):

- System failure
  - Clogging, corrosion, pitting, antimicrobial resistance/regrowth potential (biofilm)

From Victoria Castro, ICES 2006, JSC

ECLSS- What is it?

ECLSS Microbial Challenges

ISS Microbial Acceptability Limits (U.S.)

	Bacteria	Fungi
Surfaces	10,000 CFU/100 cm^2	100 CFU/100 cm^2
Water	50 CFU/ 100 (no detectable coliforms per 100 ml; treatment technique* to prevent parasitic protozoa)	N/A
Air	1,000 CFU/m^3	100 CFU/m^3

CFU/cm^2= colony forming units per square centimeter; CFU/ m^3= colony forming units per cubic meter; CFU/ ml= colony forming units per milliliter

* Current potable water treatment is filtration

ECLSS Microbial Challenges

Exploration Microbial Acceptability Limits

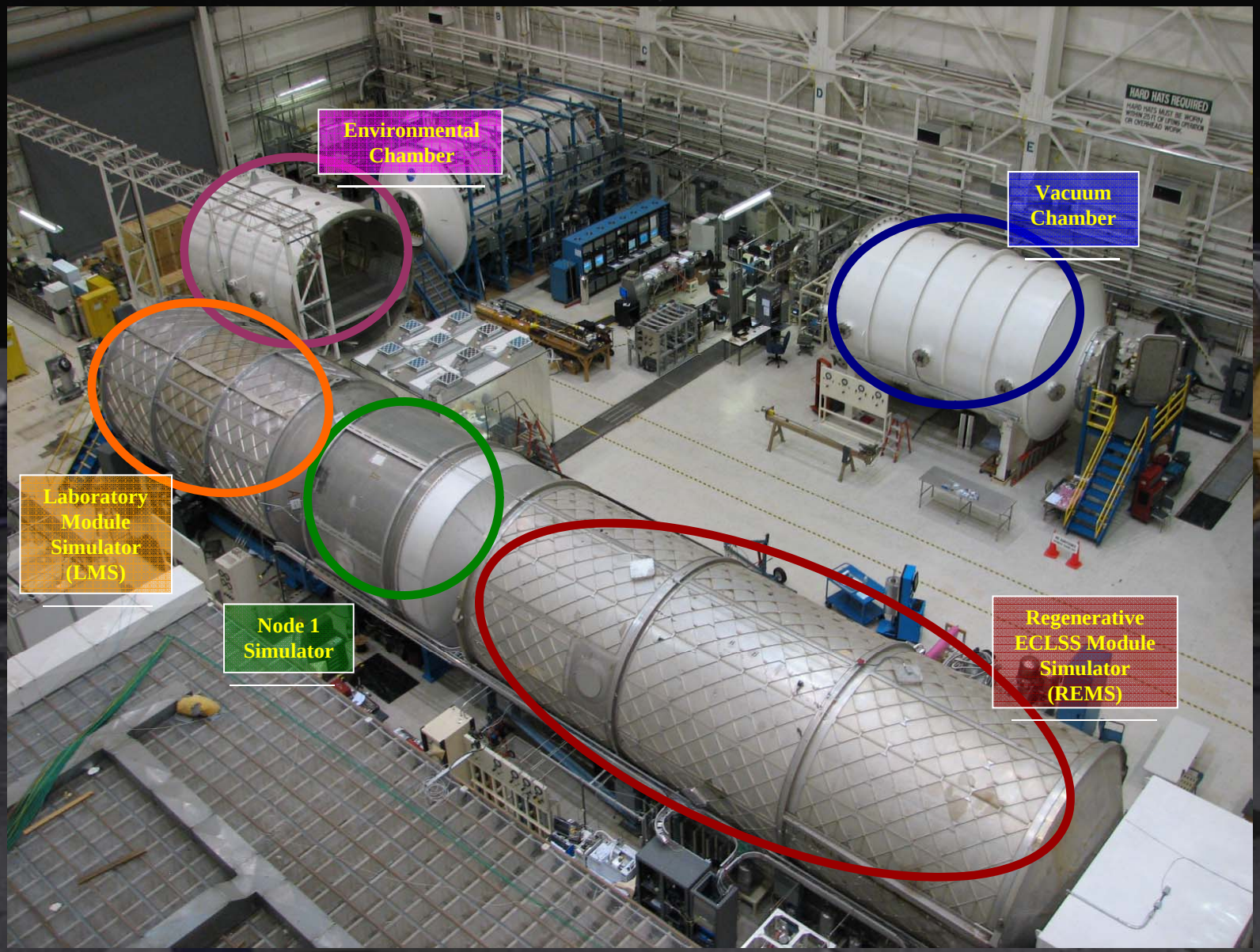
	Bacteria	Fungi
Surfaces	500 CFU/100 cm^2	10 CFU/100 cm^2
Water	50 CFU/ 100 (no detectable coliforms per 100 ml; no detectable fungi per 100 ml; 0 parasitic protozoa)	N/A
Air	1,000 CFU/m^3	100 CFU/m^3

CFU/cm^2= colony forming units per square centimeter; CFU/ m^3= colony forming units per cubic meter; CFU/ ml= colony forming units per milliliter

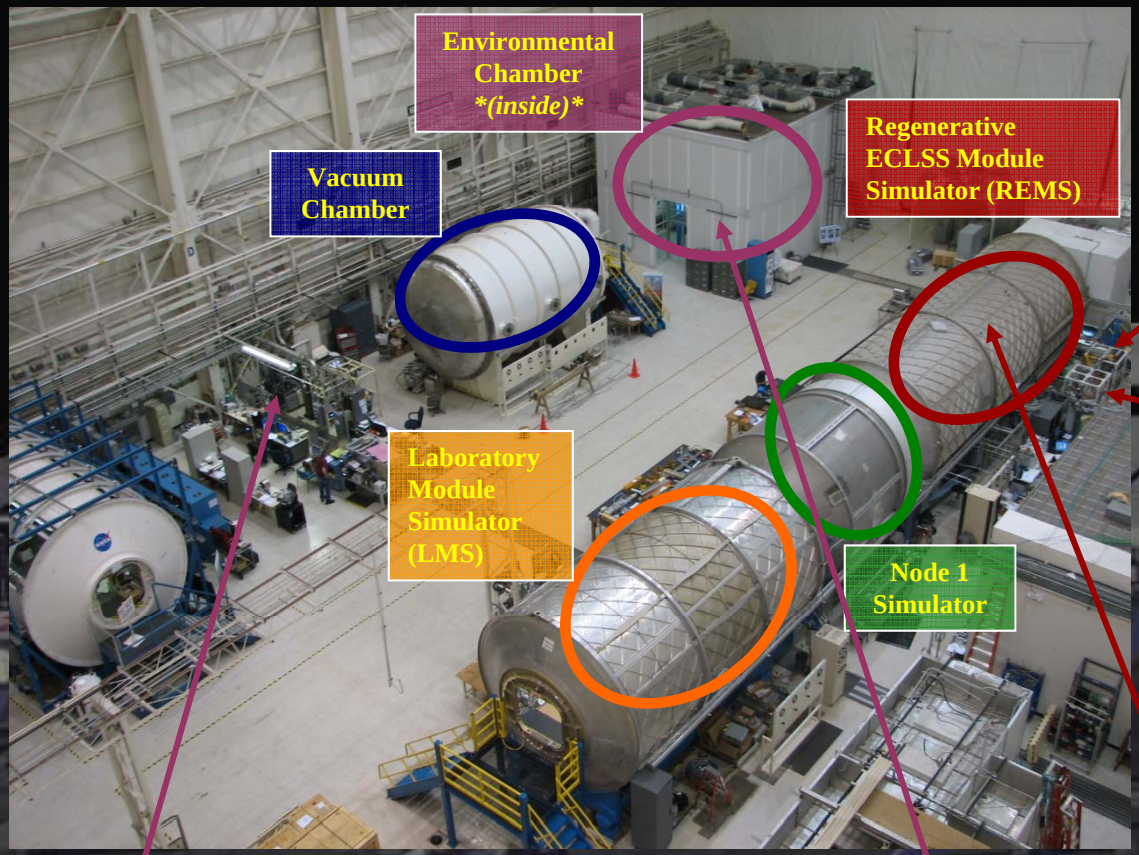
ECLS Microbial Challenges

- **Urine/Pretreated Urine**
  - Hardware Performance Issues
    - Control of biofilm on wetted surfaces
    - Control of fungal growth in pretreated urine
- **Water (potable/wastewater)**
  - Health and Hardware Performance/Life Issues
    - Control of biofilm on wetted surfaces
      - Conditions of flight equipment unknown
    - Control of microorganisms in potable water
      - Re-growth potential/resistance to antimicrobials/MIC
    - Control microorganisms in humidity condensate

ELS/ECLS Module Switch



MSFC Exploration Life Support (ELS) Test Facility (Present/ Final Configuration)



Exploration Water Recovery System (EWRS)

Vapor Phase Catalytic Ammonia Removal (VPCAR)

Sorbent Base Atmosphere Revitalization System (SBAR)

Sabatier

Humans in the Loop Generating Waste Water in the REMS

ECLS Microbial Challenges

- **Coolant**
  - Health and Hardware Performance/Life Issues
    - Control of microorganisms in the fluid
    - Control of biofilm on wetted surfaces
    - Microbiologically Influenced Corrosion (MIC)
- **Surfaces**
  - Health and Hardware Performance/Life Issues
    - Fungi, bacteria
- **Air**
  - Health and Hardware Performance/Life Issues
    - Fungi, bacteria

ECLSS Microbial Challenges (Design and Test)

- Flow rates: low, intermittent or no flow
- Dead-legs
- Potential long term storage of water in Teflon bags
- Limitations with the use of antimicrobials
- Gravity/microgravity effects
- Wastewater in narrow tubes

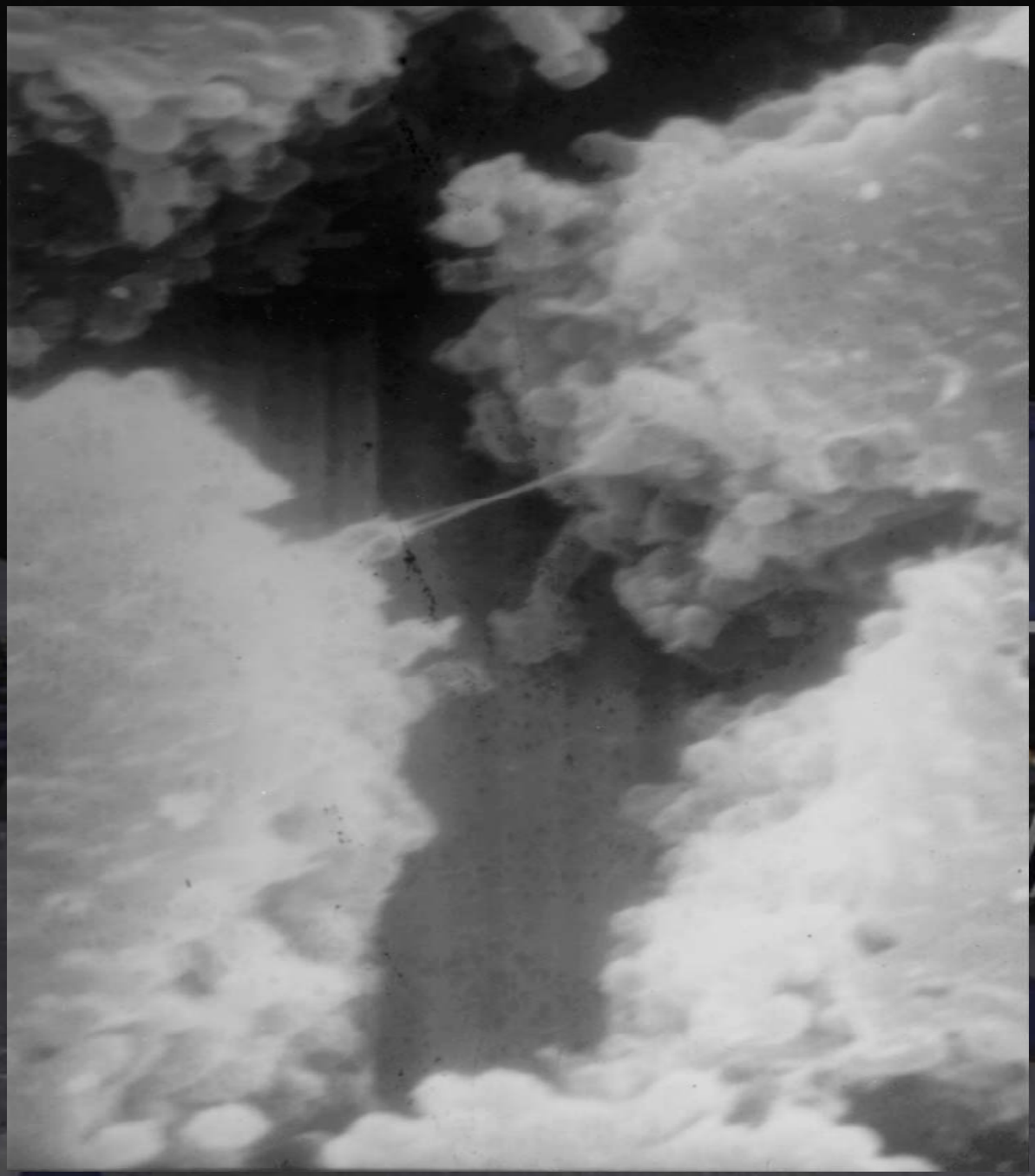
ECLSS Microbial Challenges (Design and Test)

- Holding time (between sample and analysis)
- Limited monitoring technology available
- Data interpretation
- Acceptable levels of microorganisms/biofilm
- Need for long term ground testing
- Replicate applicable flight conditions to ground tests

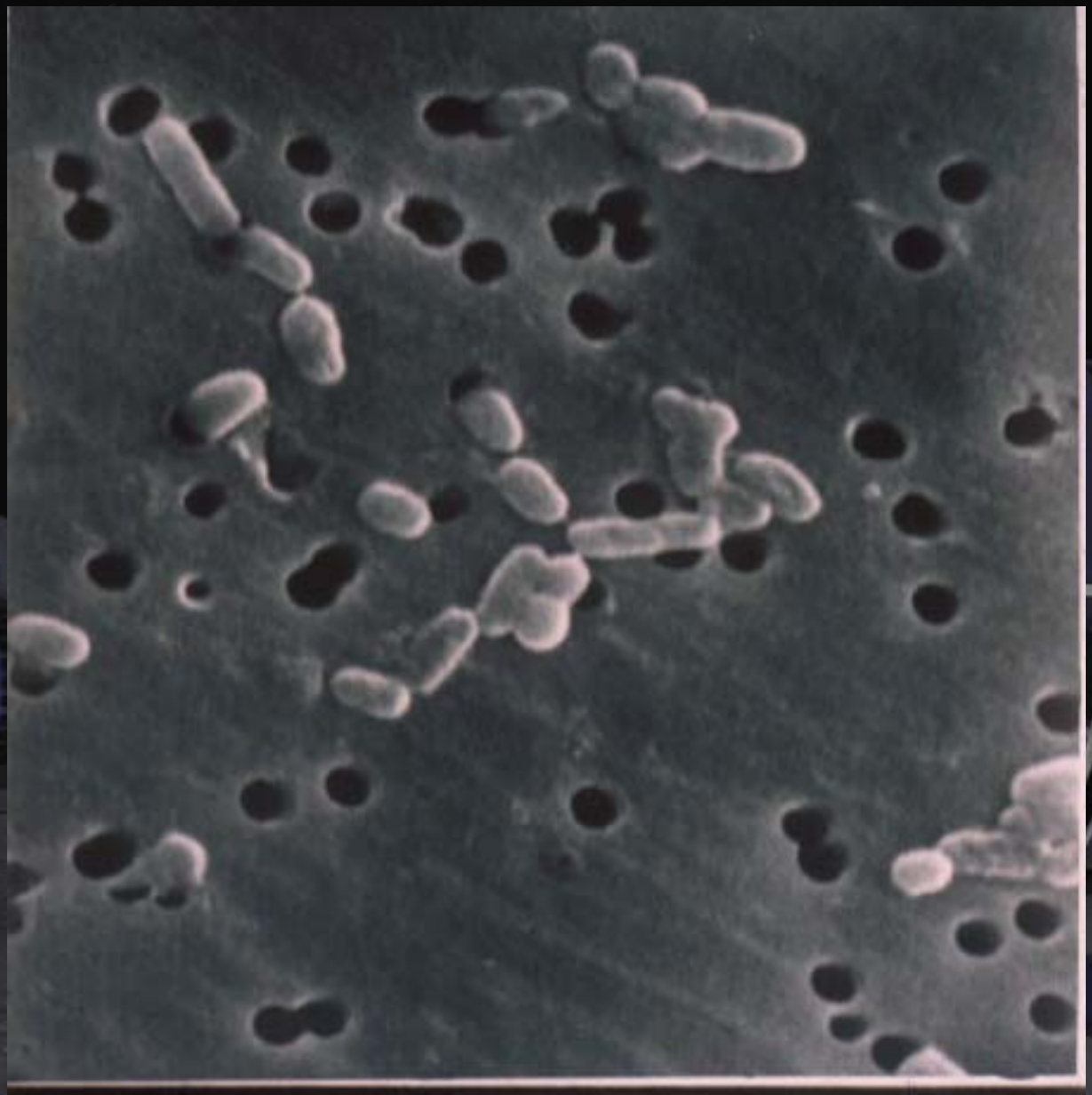
ECLSS- What is it?

	Fleet Leader (Ground Test)	ISS LTL (Flight Sample)	ISS MTL (Flight Sample)
Acidovorax avenae		X	
Acidovorax delafieldii	X	X	X
Acidovorax facilis	X		X
Acidovorax konjaci	X		X
Acidovorax temperans		X	
Acinetobacter lwoffii/genospecies 9			X
Brevibacterium casei			X
Brevundimonas vesicularis			X
Burkholderia glumae	X		
Comamonas acidovorans	X	X	
Flavobacterium resinovorum			X
Janthinobacterium lividum	X		
Oligella species			X
Ralstonia eutropha (very similar genetically to R. paucula)			X
Ralstonia paucula		X	X
Ralstonia pickettii	X		X
Sphingobacterium spiritovorum		X	
Sphingomonas paucimobilis			X
Stenotrophomonas maltophilia	X		
Unidentified non-fermenting Gram Negative Rod (GNR)	X	X	X
Variovorax paradoxus	X		X

Biofilm Bacteria



Planktonic Bacteria



Biodeterioration in Water Distribution Systems

Mechanical Fouling

microbially influenced corrosion (MIC)

Copper Lead

Corrosion

High Bioburden

ECLSS Microbial Challenges

Challenges with monitoring ECLS systems in-flight include:

- Microbial count (quantification)
  - Viable vs non-viable
  - How will it compare with culture methods?
- Real-time identification
  - Bacteria, Fungi, Viruses
- Flexible
  - Integrated to systems (in-line)
  - Hand-held (for clinical applications)
- Robustness
  - Will the hardware survive qual/acceptance testing?

ECLSS Microbial Challenges

- If gene-base technology will be used what challenges, like damage to genetic material due to radiation, will need to be addressed?
- Expendables (how much waste will be generated)
- Consumables (reusable is preferred)
- Low power consumption
- Equipment size
- Non-hazardous reagents
- Non-generation of hazardous waste

ECLSS Microbial Challenges

- Calibration (positive/negative controls?)
- Cleaning/disinfection of the sample collection areas
  - How to avoid cross contamination?
- What chemicals/conditions(temp, humidity, etc) could cause a problem (void the reaction)?
- Maintenance/repair (ORU's?)
- Construction materials
  - Are the materials acceptable in a close environment?

ECLSS Microbial Challenges

- Sample size
- Detection limit (currently <300 CFU/100 mL)
- Microgravity sensitivity
- Sensitivity to particles/precipitates in the fluid
- A system that can be upgraded as needed is preferable (as “target” organisms are identified)
- Will the crew be able to “read” the results on-orbit; can the results be sent to the ground?
- Sample archival for later analyses

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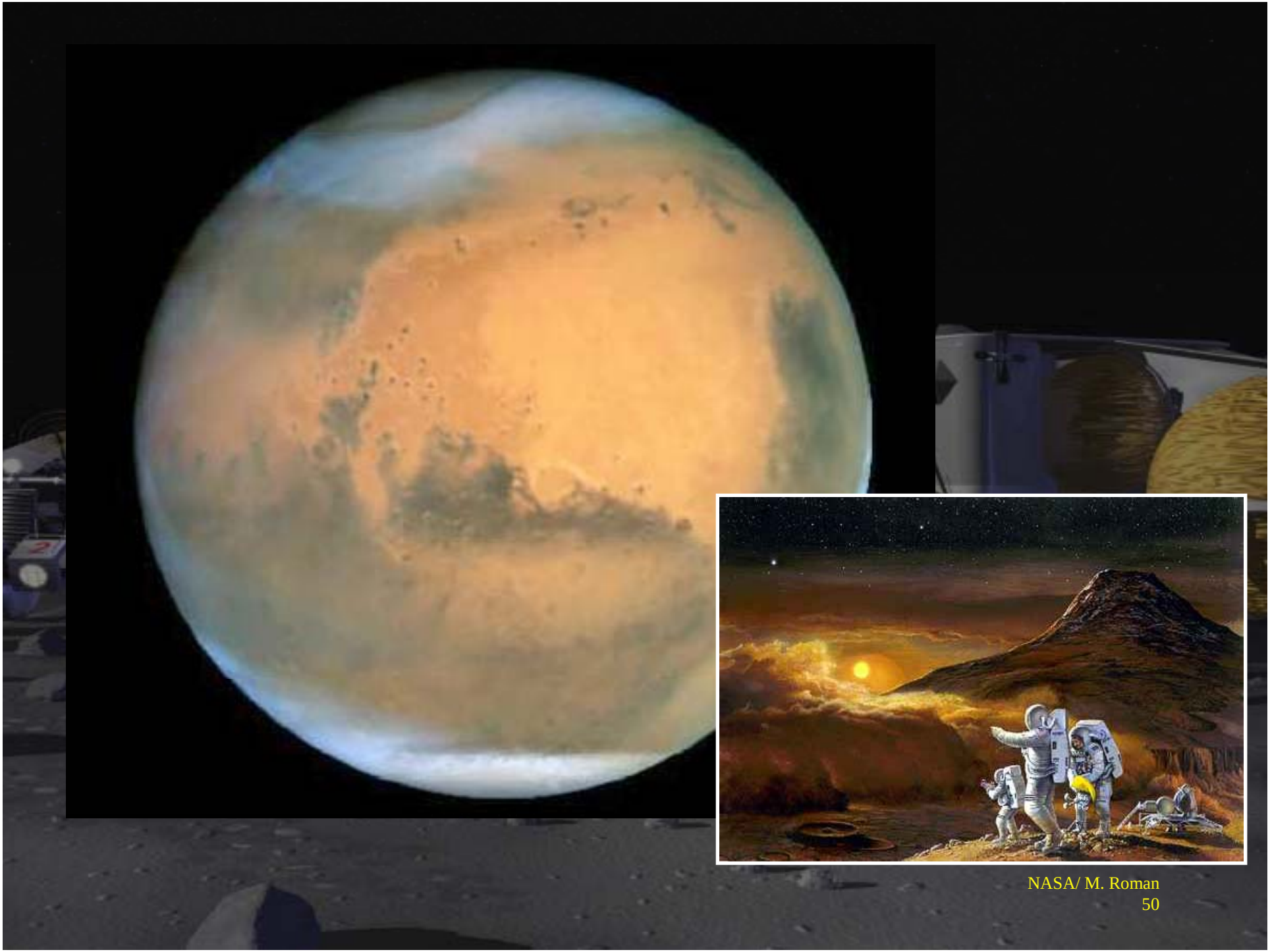

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BACK UP

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

NASA MSFC / Monsi C. Roman

Exponent and Harvard University / Marc W. Mittelman

40th ICES, 11–15 July 2010, Barcelona, Spain

Introduction

- Many microbiological studies were performed during the development of the Space Station Water Recovery and Management System from1990-2009. Studies include assessments of:
  - bulk phase (planktonic) microbial population
  - biofilms,
  - microbially influenced corrosion
  - biofouling treatments

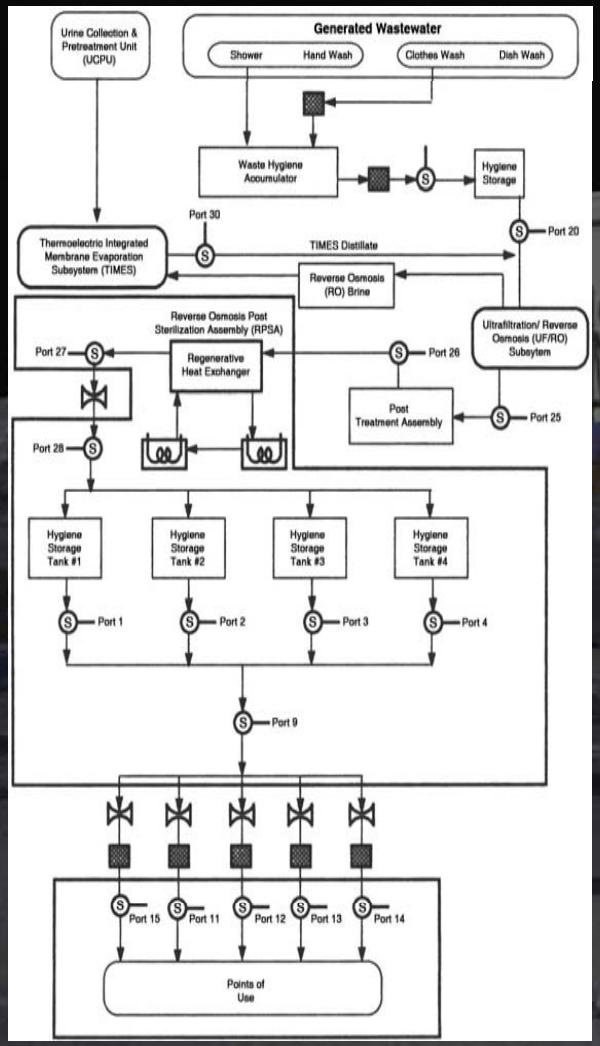
Introduction

- 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.

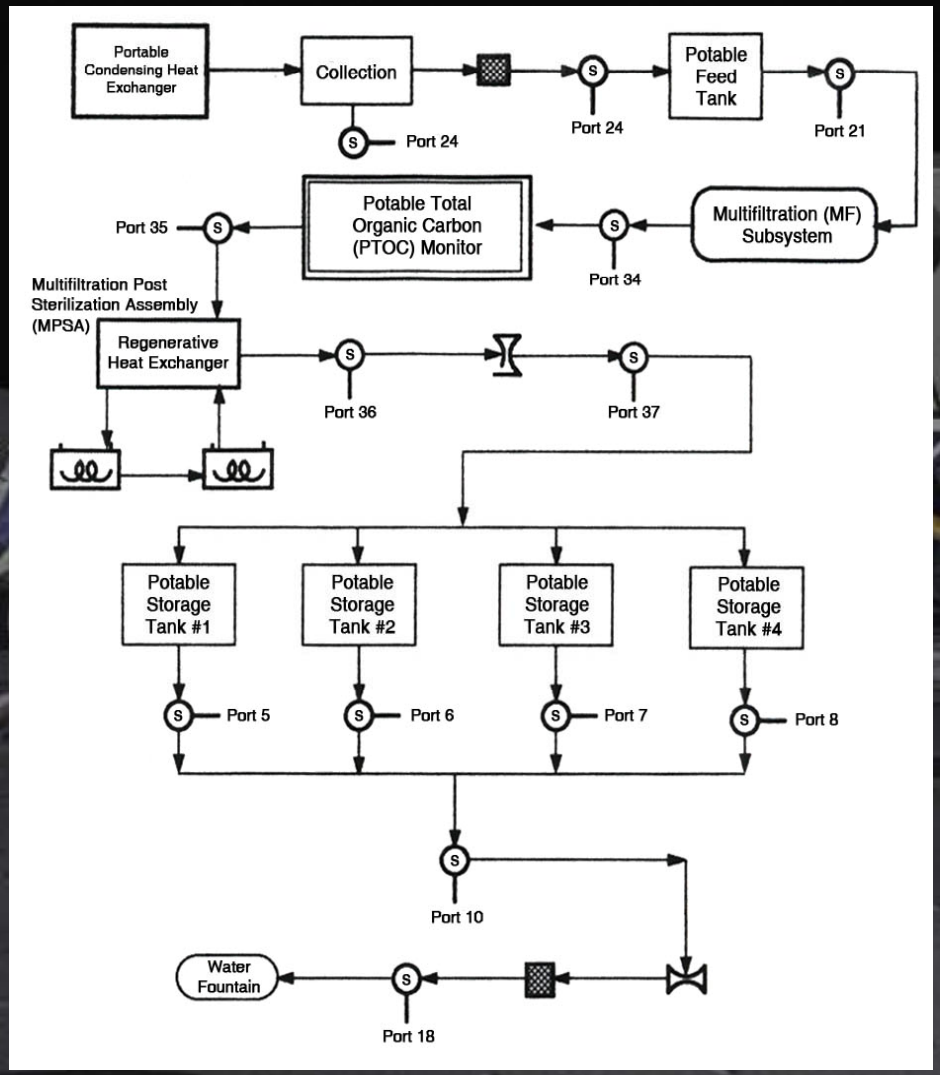
Water Recovery Test Stages 1A, 2A and 3A

- 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)



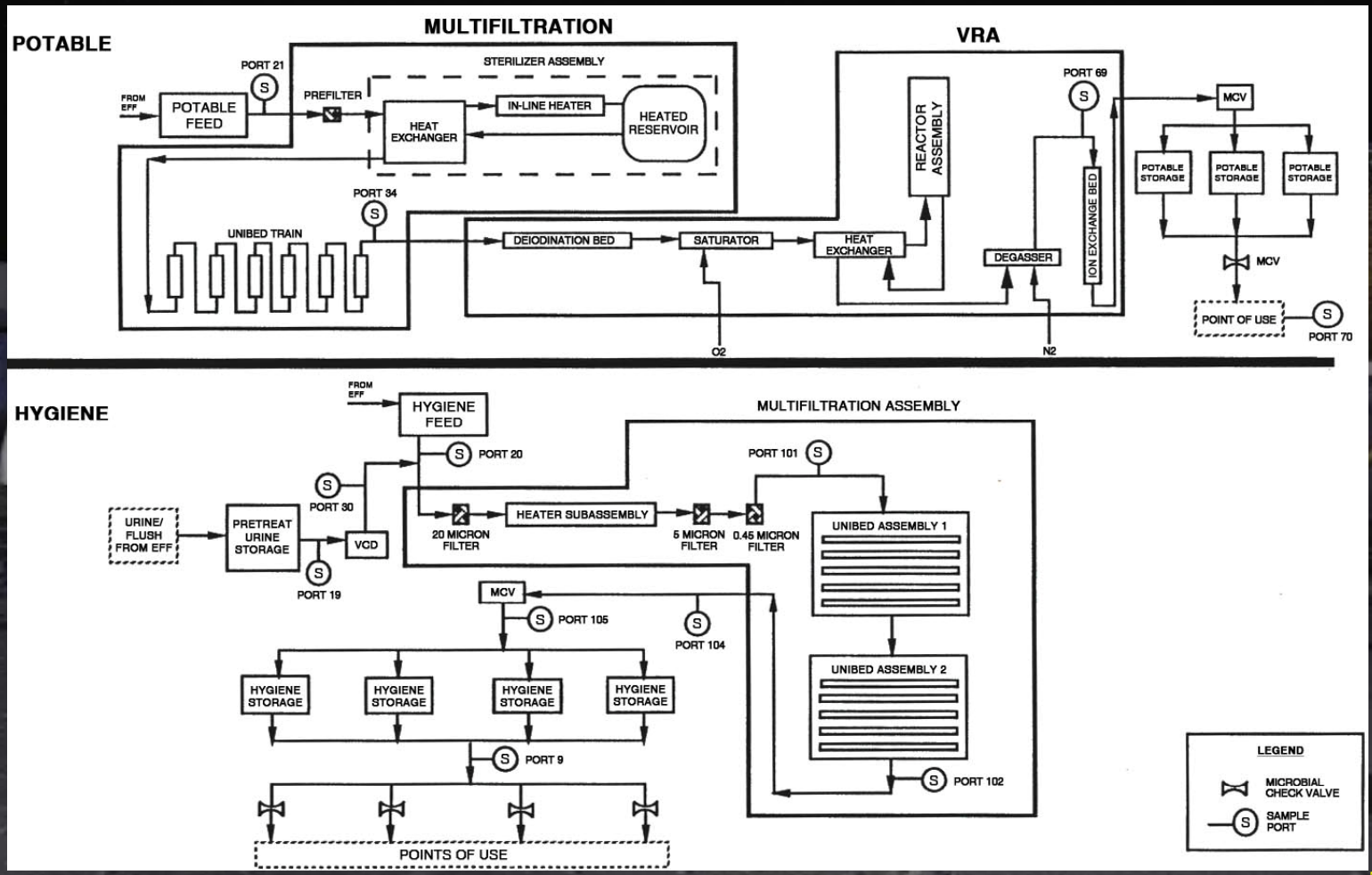
WRT Stages 1A, 2A, 3A Processing Schematic (Potable Loop)



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 (250^0F for 20 minutes/ 2 log reduction)
    - MF Subsystem (~~MF post-"Sterilization" Assembly~~)
    - Volatile Removal Assembly (VRA)- catalytic oxidation reactor/260^0F
    - 4 potable processed water storage tanks

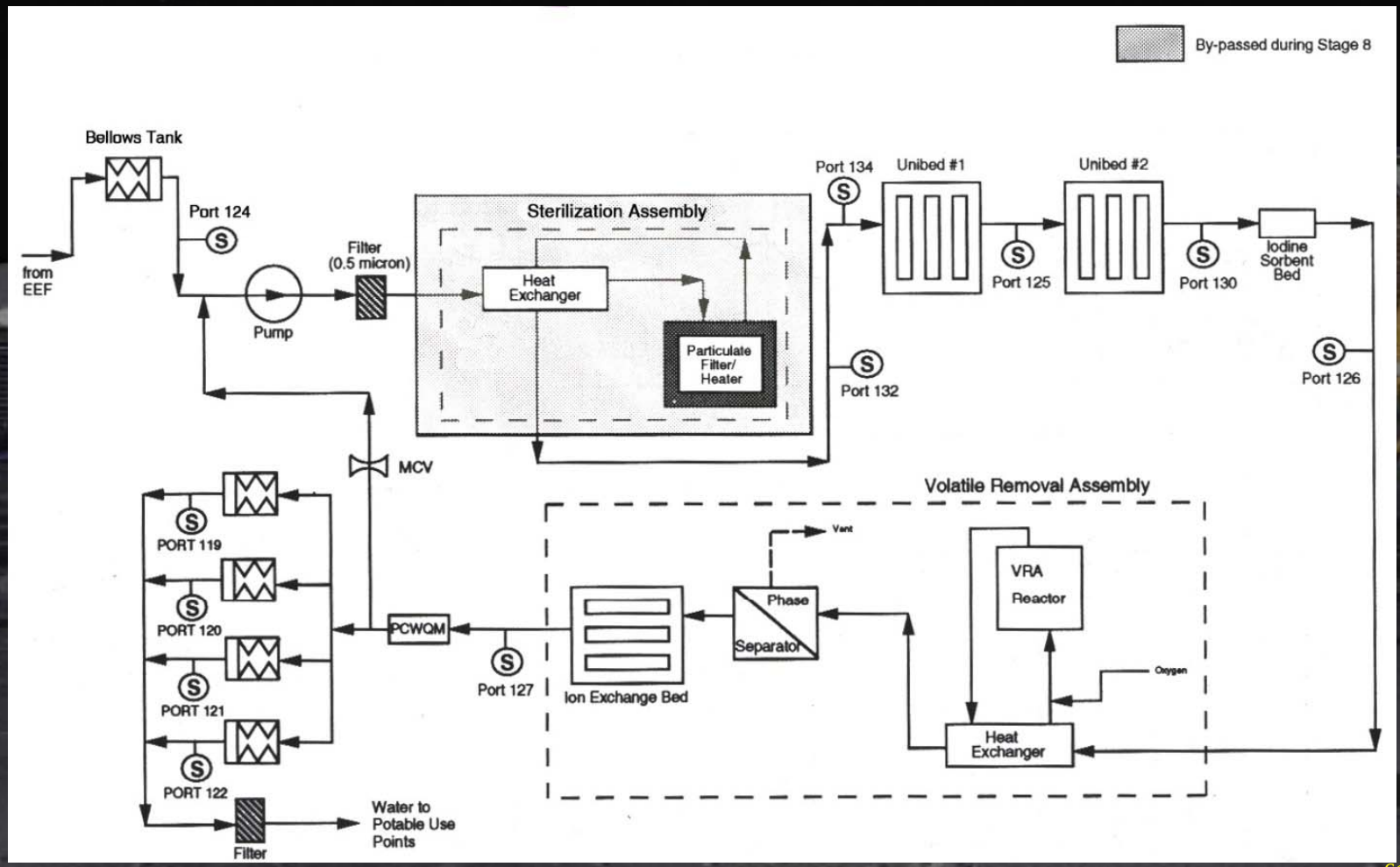
WRT Stages 4/5 Processing Schematic (Potable and Hygiene Loop)



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

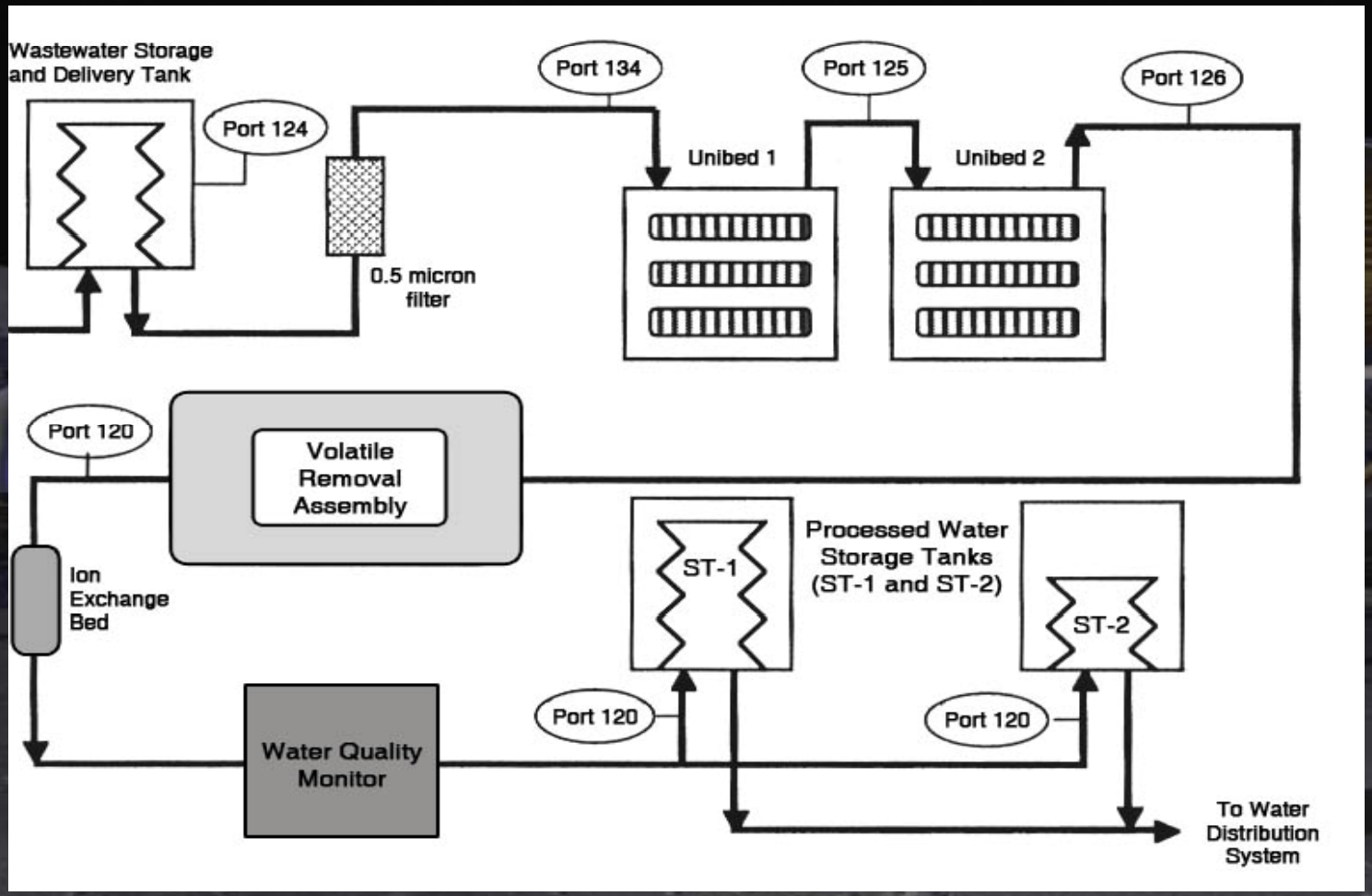
WRT Stages 7/8 Processing Schematic
(Hygiene / Potable Loop)



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
Giardia lablia	99.9% reduction (MCLG= 0)	GI
enteric viruses (adenovirus as most resistant)	99.99% reduction	GI; systemic
Legionella spp.	(MCLG= 0)	respiratory

[1]MCLG, maximum contaminant level goal

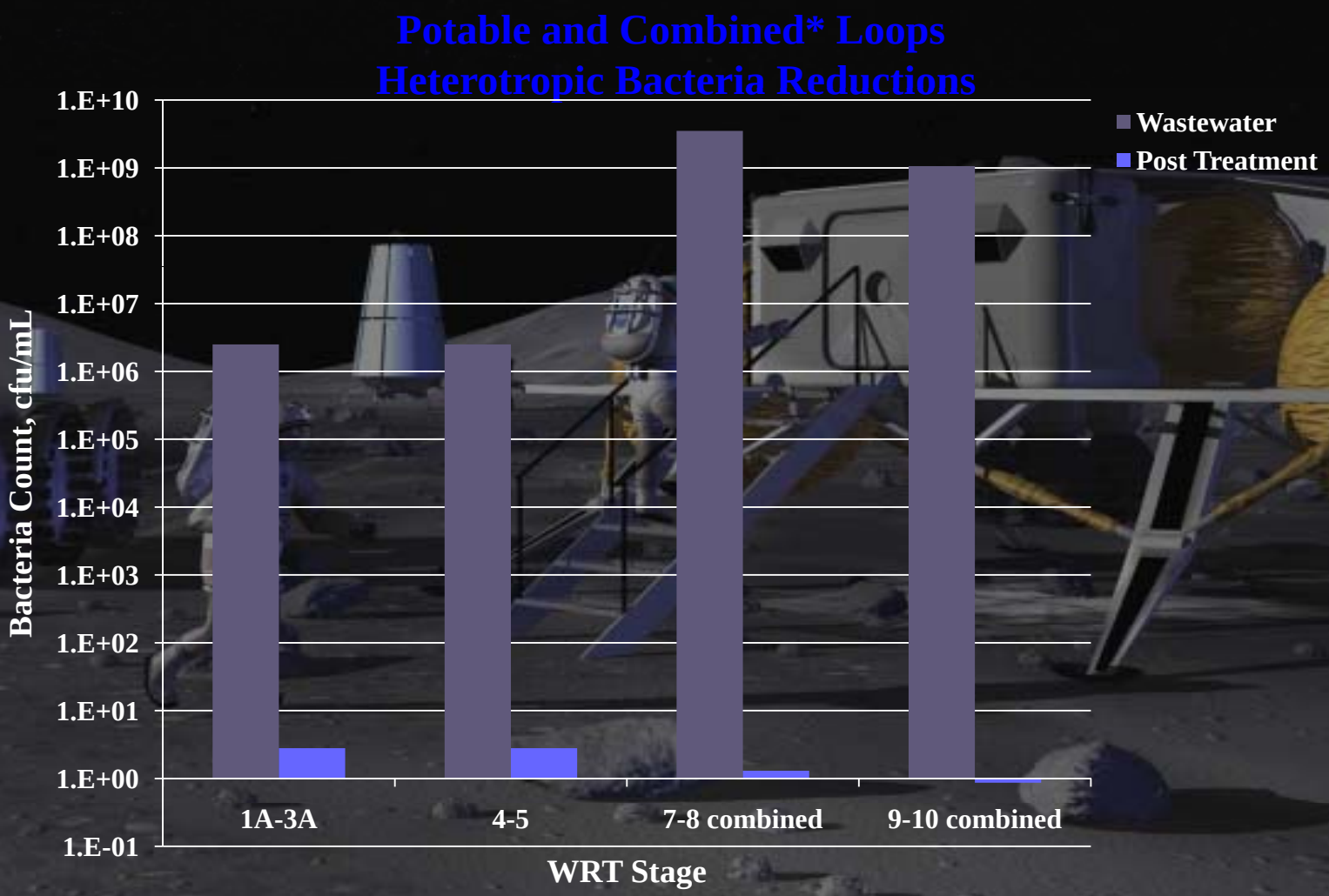
Microbiological Tests Performed During the WRT

- 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

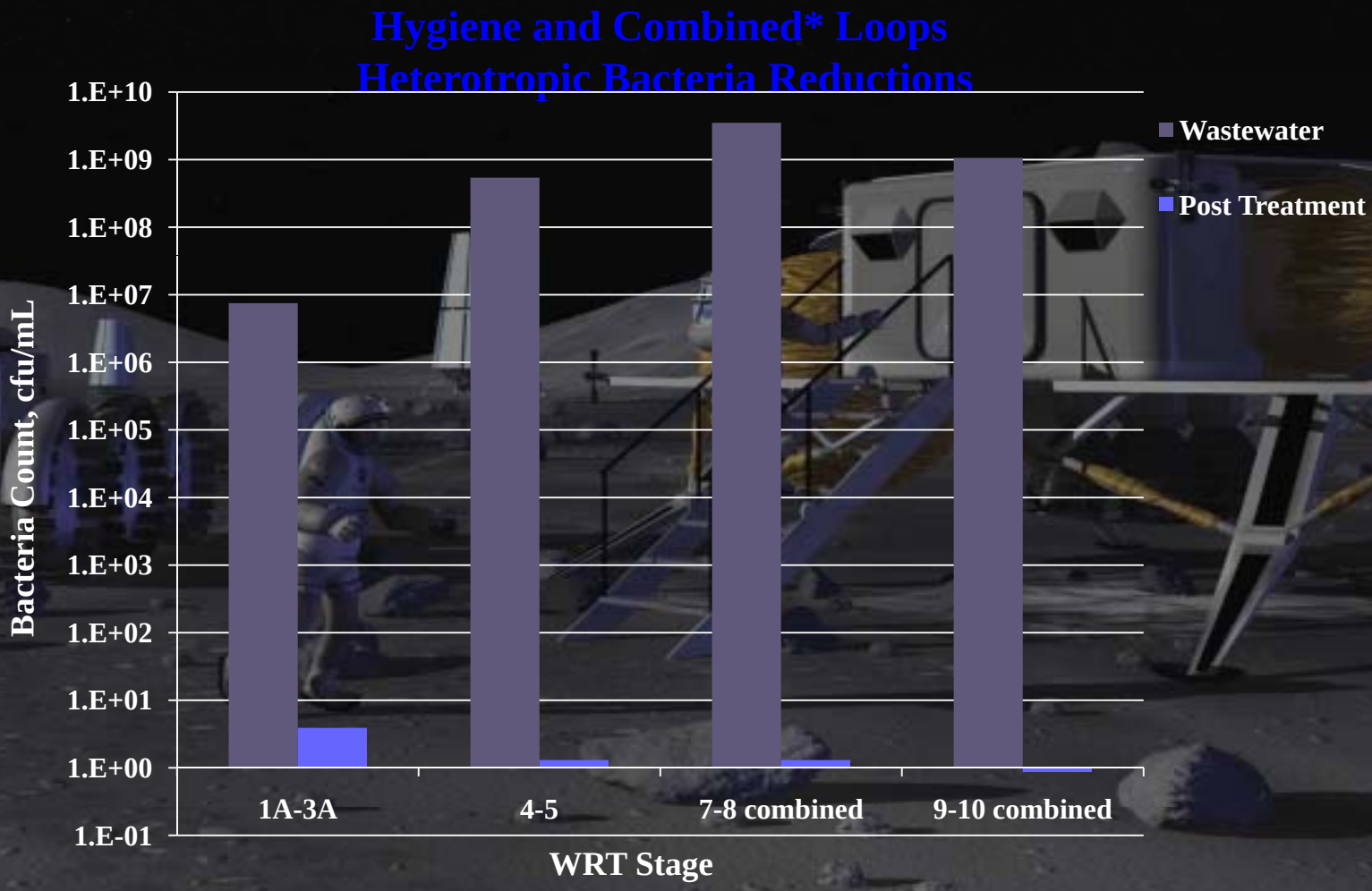
Method	Microorganisms Recovered	Comments
epifluorescence microscopy	direct counts of total microbial bioburden	detection limit of 10^4 cells/mL
R2A culture	heterotrophic bacteria (nutrient limited)	7 d incubations
enriched chocolate agar with incubation in 5% CO_2	aerotolerent bacteria	recovery of fastidious 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



*Combined Loop= Potable and Hygiene Loops

Results- Microbial Characterization

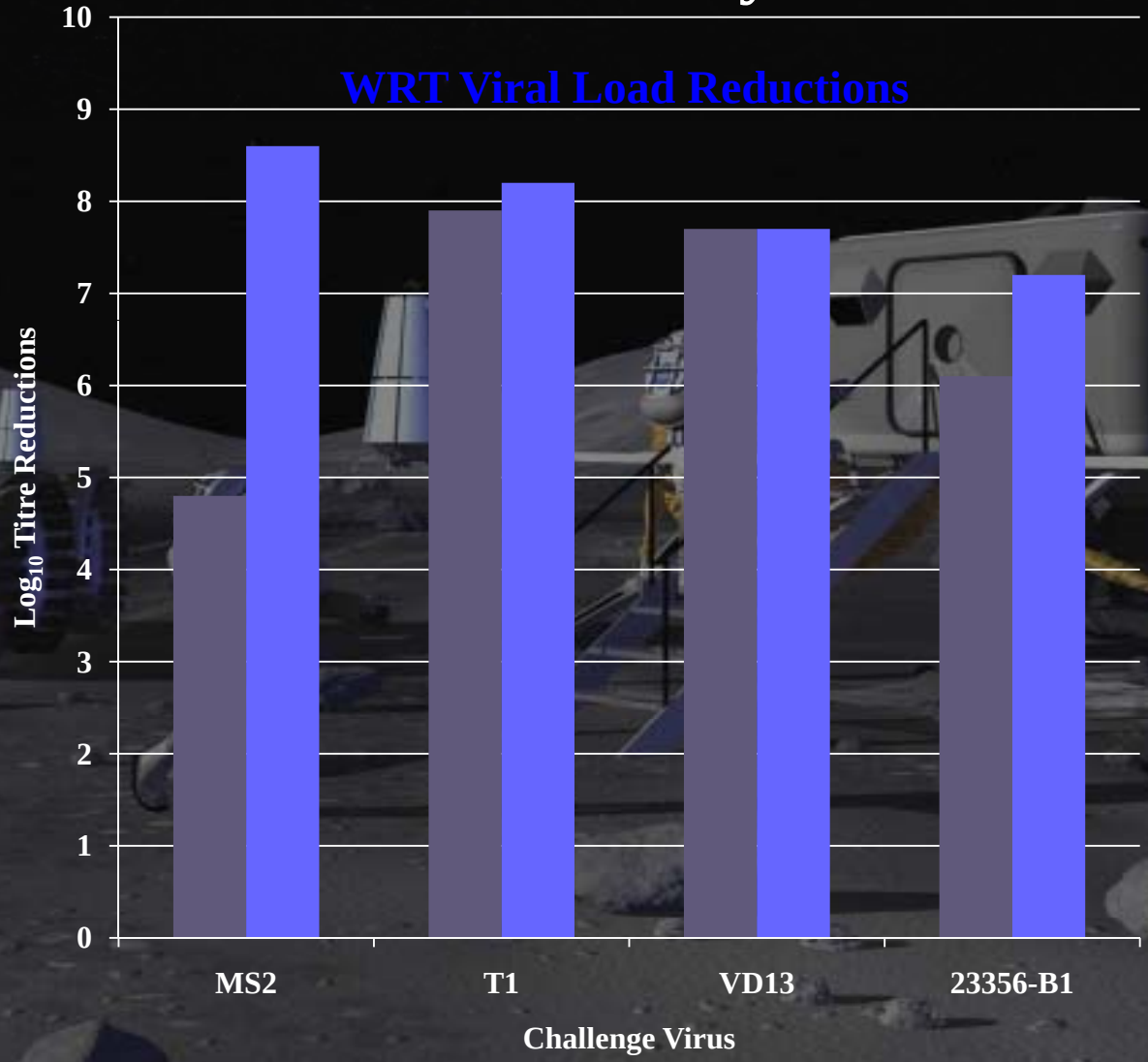


*Combined Loop= Potable and Hygiene Loops

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 us 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 amebocyte 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 X 102 to 1.2 X 104 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

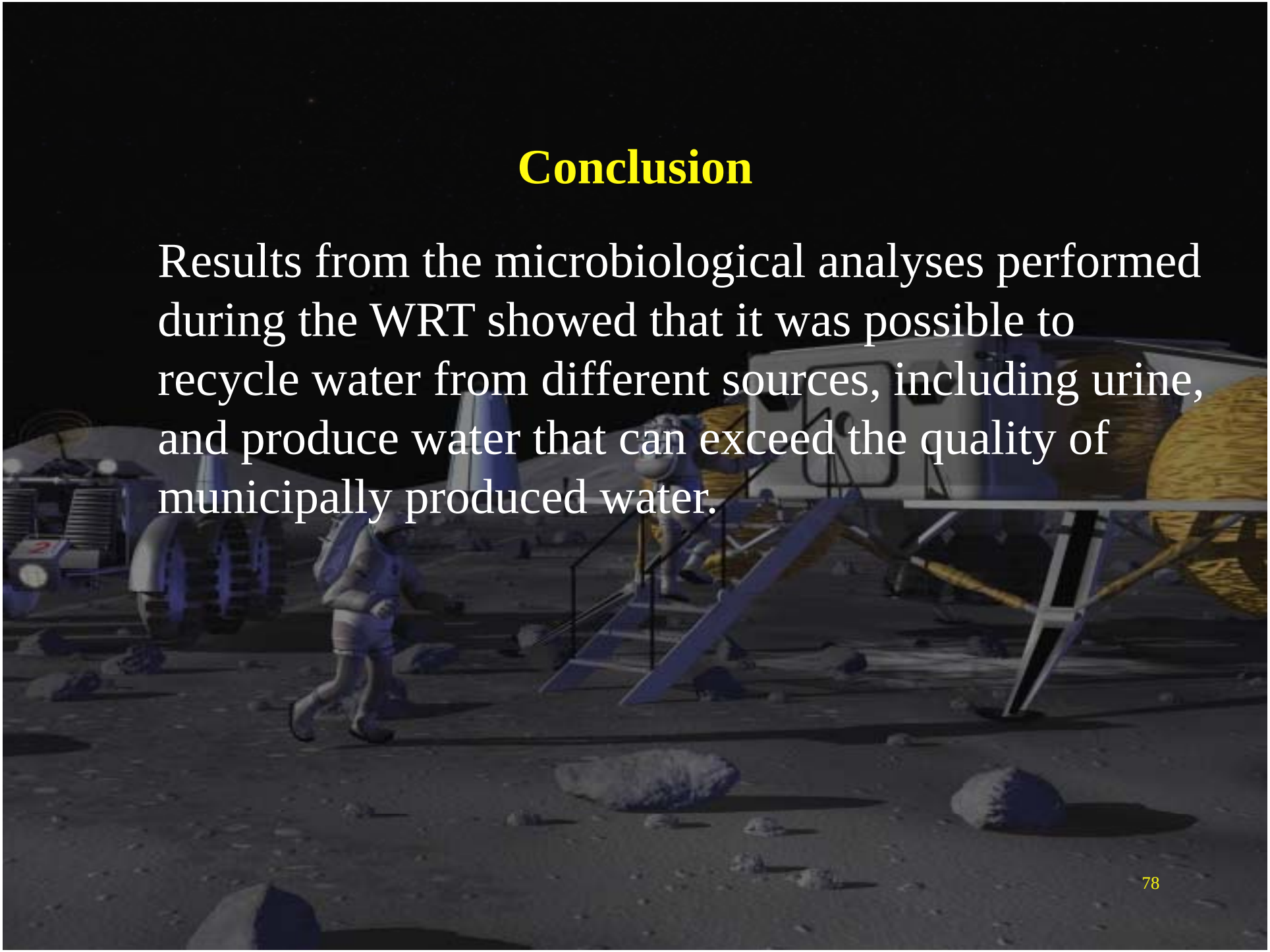
- During WRT Stage 4/5, a bioassay to measure the assimilable organic carbon (AOC) concentration, was performed to assess bacterial regrowth potential.
- Nine clean water samples were analyzed, 5 from the potable water storage tank and 4 from the hygiene water storage tank.
- The AOC levels in the potable water samples had an average of recorded as: 102.8 µg/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 µg/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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Visiting Scientist, Harvard University
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