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 CO₂ generated to O₂ consumed.

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# ECLSS - What is it?

<table>
<thead>
<tr>
<th>Control Atmosphere Pressure</th>
<th>Condition Atmosphere</th>
<th>Respond to Emergency Conditions</th>
<th>Control Internal CO₂ &amp; Contaminants</th>
<th>Provide Water</th>
<th>Prepare for EVA Operations</th>
</tr>
</thead>
<tbody>
<tr>
<td>• O₂/N₂ Pressure Control Assemblies (USO/RS)</td>
<td>• Cabin Air Temperature &amp; Humidity Control Assemblies (All)</td>
<td>• Smoke Detectors (All)</td>
<td>• CO₂ Removal Assembly (USOS/RS)</td>
<td>• Potable Water Processor (USOS/RS)</td>
<td>• O₂/N₂ Pressure Control Assemblies (USOS)</td>
</tr>
<tr>
<td>• Positive &amp; Negative Pressure Relief (USOS-Transport)</td>
<td>• Ventilation Fans (USOS, RS, MPLM)</td>
<td>• Portable Fire Extinguishers (All)</td>
<td>• CO₂ Vent (USOS/RS)</td>
<td>• Urine Processor (USOS/RS)</td>
<td>• O₂/N₂ Distribution (USOS)</td>
</tr>
<tr>
<td>• O₂/N₂ Storage (USOS, RS, Progress)</td>
<td>• Air Particulate Filters (All)</td>
<td>• Fire Indicators and Fire Suppression Ports (All)</td>
<td>• Trace Contaminant Control Assembly (USOS/RS)</td>
<td>• Process Control Water Quality Monitor (USOS)</td>
<td>• O₂/N₂ Storage (USOS)</td>
</tr>
<tr>
<td>• O₂ Generation Assembly, O₂ Solid Chemicals (RS)</td>
<td>• Intermodule Ventilation Fans &amp; Valves (All)</td>
<td>• Portable Breathing Apparatus and Masks (All)</td>
<td>• Major Constituent Analyzer (USOS)</td>
<td>• Condensate Storage (USOS/RS)</td>
<td>• Major Constituent Analyzer (USOS) (Shared)</td>
</tr>
<tr>
<td>• Major Constituent Analyzer (USOS) (Share)</td>
<td>• O₂/N₂ Pressure Control Assemblies (USOS) (Shared)</td>
<td>• CO₂ Reduction Assembly (RS)</td>
<td>• CO₂ LIOH Removal (RS)</td>
<td>• Fuel Cell Water Storage (USOS)</td>
<td></td>
</tr>
<tr>
<td>• Gas Analyzer (RS) (Shared)</td>
<td></td>
<td>• Manual Sampling Equipment (USOS)</td>
<td>• Gas Analyzer (RS)</td>
<td>• Waste Water Distribution (USOS)</td>
<td></td>
</tr>
</tbody>
</table>

**Atmosphere Control & Supply (ACS) & AR**

**Temperature Humidity Control**

**Fire Detection & Suppression & ACS**

**Atmosphere Revitalization (AR)**

**Water Recovery & Mgmt/ Waste Mgmt**

**ACS & AR**

*NASA/ M. Roman*
The International Space Station

Today

Artist Concept

NASA/ M. Roman
A Look Inside ISS

Lab

Node 1

FGB

SM

NASA/M. Roman
A Look Inside ISS

Columbus
A Look Inside ISS

Hope
A Look Inside ISS

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A Look Inside ISS
ECLSS - What is it?

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ECLSS—What is it?
ECLSS - What is it?
ECLSS- What is it?
ECLSS Microbial Challenges
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ECLSS Microbial Challenges

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ECLSS Microbial Challenges
ECLSS Microbial Challenges
ECLSS Microbial Challenges
ECLSS Microbial Challenges

Filling up a bag of water in the Zvezda, SM

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ISS Water Processor Diagram

- **Wastewater Tank**: from Node 3 wastewater bus to Node 3 cabin
- **Particulate Filter**: (removes particulates)
- **Multifiltration Beds**: (remove dissolved contaminants)
- **Filter**: (removes particulates)
- **Multifiltration Beds**: (remove dissolved contaminants)
- **Gas/Liquid Separator**: (removes oxygen)
- **Mostly Liquid Separator**: (removes air)
- **Product Water Tank**: (provides isolation)
- **Microbial Check Valve**: (provides isolation)
- **Reject Line**: (allows reprocessing)
- **O2 from Node 3**: Reactor
- **Reactor**: (oxidizes organics)
- **Preheater**: (heats water to 275F)
- **Regen. HX**: (recovers heat)
- **Heat Exchanger**: to/from Node 3 MTL
- **Reactor Health Sensor**: (verifies reactor is operating w/n limits)
- **Ion Exchange Bed**: (removes reactor by-products)
- **Accumulator**: (verifies reactor is operating w/n limits)
- **Delivery Pump**: (allows reprocessing)
- **Wastewater Bus**: to Node 3 potable water bus

**Subsystems**:
- **Reactor Health Sensor**: Reactor health monitoring
- **Delivery Pump**: Potable water delivery
- **Accumulator**: Reactor by-product accumulation
- **Product Water Tank**: Final product storage
- **Particulate Filter**: Particulate matter removal
- **Multifiltration Beds**: Dissolved contaminant removal
- **Gas/Liquid Separator**: Oxygen removal
- **Mostly Liquid Separator**: Air removal
- **Microbial Check Valve**: Isolation
- **Reject Line**: Water reprocessing
- **O2 from Node 3**: Oxygen source
- **Reactor**: Organic oxidation
- **Preheater**: Water heating
- **Regen. HX**: Heat recovery
- **Heat Exchanger**: Water temperature control

**System Details**:
- **Wastewater Tank**: Collects wastewater from Node 3
- **Particulate Filter**: Removes particulate matter
- **Multifiltration Beds**: Removes dissolved contaminants
- **Gas/Liquid Separator**: Removes oxygen
- **Mostly Liquid Separator**: Removes air
- **Microbial Check Valve**: Provides isolation
- **Reject Line**: Allows reprocessing
- **O2 from Node 3**: Oxygen supply
- **Reactor**: Oxidizes organics
- **Preheater**: Heats water to 275°F
- **Regen. HX**: Recovering heat
- **Heat Exchanger**: Temperature control
- **Reactor Health Sensor**: Monitors reactor performance
- **Ion Exchange Bed**: Removes reactor by-products
- **Accumulator**: Stores reactor by-products
- **Delivery Pump**: Delivers potable water

**Source**:
NASA/M. Roman

**Page**: 26
Water Processor Assembly

- **Wastewater Tank**: from Node 3 wastewater bus
- **Microbial Check Valve**: (provides isolation)
- **Filter**: Particulate Filter (removes particulates), Multifiltration Beds (remove dissolved contaminants)
- **Pump**: to Node 3 cabin
- **Mostly Liquid Separator**: (removes air)
- **O2 from Node 3**: delivery pump
- **Product Water Tank**: to Node 3 potable water bus
- **Reject Line**: (allows reprocessing)
- **Gas/Liquid Separator**: (removes oxygen)
- **Preheater**: Reactor (heats water to 275°F), Regen. HX (recovers heat)
- **Ion Exchange Bed**: (removes reactor by-products)
- **Heat Exchanger**: to/from Node 3 MTL
- **Microbial Reactor Health Sensor**: (verifies reactor is operating w/n limits)
- **Ion Exchange Bed**: to/from N Node 3 C
- **Reactors**: to/from MTL
- **Potable Water Bus**: NASA/ M. Roman
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.)

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfaces</td>
<td>10,000 CFU/100 cm²</td>
<td>100 CFU/100 cm²</td>
</tr>
<tr>
<td>Water</td>
<td>50 CFU/100 (no detectable coliforms per 100 ml; treatment technique* to prevent parasitic protozoa)</td>
<td>N/A</td>
</tr>
<tr>
<td>Air</td>
<td>1,000 CFU/m³</td>
<td>100 CFU/m³</td>
</tr>
</tbody>
</table>

CFU/cm² = colony forming units per square centimeter; CFU/ m³ = colony forming units per cubic meter; CFU/ ml = colony forming units per milliliter

* Current potable water treatment is filtration

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# ECLSS Microbial Challenges

## Exploration Microbial Acceptability Limits

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surfaces</strong></td>
<td>500 CFU/100 cm²</td>
<td>10 CFU/100 cm²</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td>50 CFU/100 (no detectable coliforms per 100 ml; no detectable fungi per 100 ml; 0 parasitic protozoa)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Air</strong></td>
<td>1,000 CFU/m³</td>
<td>100 CFU/m³</td>
</tr>
</tbody>
</table>

CFU/cm² = colony forming units per square centimeter; CFU/m³ = 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

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ELS/ECLS Module Switch

Environmental Chamber

Vacuum Chamber

Laboratory Module Simulator (EMIS)

Node 1 Simulator

Regenerative ECLSS Module Simulator (REMS)
MSFC Exploration Life Support (ELS) Test Facility (Present/ Final Configuration)

Environmental Chamber *(inside)*

Vacuum Chamber

Regenerative ECLSS Module Simulator (REMS)

Laboratory Module Simulator (LMS)

Node 1 Simulator

Sabattier

Exploration Water Recovery System (EWRS)

Vapor Phase Catalytic Ammonia Removal (VPCAR)

Sabattier

Sorbent Base Atmosphere Revitalization System (SBARS)

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?

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fleet Leader (Ground Test)</th>
<th>ISS LTL (Flight Sample)</th>
<th>ISS MTL (Flight Sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidovorax avenae</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Acidovorax delafieldii</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Acidovorax facilis</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidovorax konjacii</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidovorax temperans</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter lwofii/genospecies 9</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Brevibacterium casei</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Brevundimonas vesicularis</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Burkholderia glumae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comamonas acidovorans</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Flavobacterium resinovorum</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Janthinobacterium lividum</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligella species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ralstonia eutropha (very similar genetically to R. paucula)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ralstonia paucula</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ralstonia pickettii</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sphingobacterium spiritovorum</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified non-fermenting Gram Negative Rod (GNR)</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Variovorax paradoxus</td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
Planktonic Bacteria
Biodeterioration in Water Distribution Systems

- Mechanical Fouling
- Copper Lead
- Corrosion
- High Bioburden
- Microbially influenced corrosion (MIC)
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
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 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)
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°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)
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

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

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

<table>
<thead>
<tr>
<th>Method</th>
<th>Microorganisms</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>epifluorescence microscopy</td>
<td>direct counts of total microbial bioburden</td>
<td>detection limit of $10^4$ cells/mL</td>
</tr>
<tr>
<td>R2A culture</td>
<td>heterotrophic bacteria (nutrient limited)</td>
<td>7 d incubations</td>
</tr>
<tr>
<td>enriched chocolate agar with incubation in 5% CO₂</td>
<td>aerotolerent bacteria</td>
<td>recovery of fastidious human isolates; 2 d incubations</td>
</tr>
<tr>
<td>Emmon’s medium</td>
<td>yeast; filamentous fungi</td>
<td>5 d incubations</td>
</tr>
<tr>
<td>membrane fecal coliform (MFC)</td>
<td>fecal coliforms</td>
<td>24 h</td>
</tr>
<tr>
<td>viral plaque assay</td>
<td>challenge bacteriophage viruses</td>
<td>performed at U.S. EPA labs</td>
</tr>
<tr>
<td>microbial identification</td>
<td>bacteria, fungi</td>
<td>MIDI, Vitek, Biolog test systems employed</td>
</tr>
</tbody>
</table>
Results - Microbial Characterization

Potable and Combined* Loops
Heterotrophic Bacteria Reductions

*Combined Loop = Potable and Hygiene Loops
Results - Microbial Characterization

Hygiene and Combined* Loops
Heterotrophic Bacteria Reductions

Bacteria Count, cfu/mL

WRT Stage

*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

WRT Viral Load Reductions

<table>
<thead>
<tr>
<th>Challenge Virus</th>
<th>Post MF 1</th>
<th>Post MF-2</th>
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<tbody>
<tr>
<td>MS2</td>
<td></td>
<td>8</td>
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<td>T1</td>
<td>8</td>
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<tr>
<td>VD13</td>
<td>8</td>
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<tr>
<td>23356-B1</td>
<td>8</td>
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</tbody>
</table>

Log_{10} Time Reductions
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 $2.9 \times 10^2$ to $1.2 \times 10^4$ 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 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
School of Engineering and Applied Science
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