Biofilms have played a significant role on the effectiveness of life support hardware on the Space Shuttle and International Space Station (ISS). This presentation will discuss how biofilms impact flight hardware, how on orbit biofilms are analyzed from an engineering and research perspective, and future needs to analyze and utilize biofilms for long duration, deep space missions.
Biofilms On Orbit and On Earth:

Current Methods, Future Needs

Leticia Vega
Overview

- Impact of biofilms on space exploration
- Biofilm research
  - On orbit
    - Space Shuttle
    - ISS
  - On earth
- Impact of biofilms flight hardware
- Lessons learned
- Next steps/ future needs
Challenges Associated With Analysis of Biofilms in Space

- Closed system
  - Working volume of ISS: 29,600 ft³
    - Size of a house
- Small fluid volumes
  - ~2m³ water volume on ISS
- Time constraints
  - Daily activities
  - Station maintenance
  - Research
Biofilms on Orbit and on Earth: Current Methods, Future Needs

Biofilm Research
Characteristics of a Bacterial Culture in Microgravity

- Typical physical stresses are absent
  - Low shear forces
  - No convection forces
- Shorter lag and longer log phase growth
- Increased resistance to antibiotics
Examples of Devices Used in Spaceflight
Spaceflight Biofilm Experiments

- Pyle (STS-81, 1997)
  - Compared on-orbit biofilm formation and growth *Burkholderia cepacia* to a parallel experiment performed at KSC
- McLean (STS-95, 1998)
  - Evaluated the effect of microgravity on *Pseudomonas aeruginosa* biofilms
- Wilson et al. (STS-115, 2006)
  - Compared colony morphology and expression of virulence genes in *Salmonella typhimurium* to a parallel experiment
- Kim et al. (STS-132 and-135, 2010 and 2011)
  - Evaluated the growth *Pseudomonas aeruginosa* biofilms on orbit and determined that there is an altered biofilm morphology as compared to those growth in identical conditions on orbit.
Mimicking Microgravity: Rotating Wall Vessels

- Developed at JSC to perform mammalian cell culture experiments
  - Axis of rotation is perpendicular to the gravity force, mimicking microgravity
- Modified for biofilm research
  - Lynch, et al. 2006 (E.coli)
  - Crabbe, et al 2010 (PAO-1)

Source: http://www.synthecon.com/autoclavable-vessel-batch-systems.html
Impacts of Biofilms to Flight Hardware
Biofilms impact flight hardware by
- Affecting fluid quality
- Fouling fluid lines
- Decreasing lifetime of hardware and hardware components

From “Microbial Characterization of the International Space Station Water Processor Assembly External Filter Assembly”
AIAA 2012-3595, 2012
Biofilms on Orbit and on Earth: Current Methods, Future Needs

Example #1: A Potable Water Biofilm

- **Potable Water Dispenser (PWD)**
  - Provides drinking water to the crew for consumption and food rehydration
  - Source water is recycled water from humidity condensate and urine
    - Bacterial limit: 50 CFU/mL

From "International Space Station United States On-orbit Segment Potable Water Dispenser On-orbit Functionality vs. Design" AIAA 2010-6250, 2010
Biofilms on Orbit and on Earth: Current Methods, Future Needs

- **Issue**
  - Counts exceed NASA potable water limits

- **Cause**
  - System was wetted with non sterile water prior to launch and left stagnant for 6 months
  - Exacerbated by corrugated tubing in PWD

- **Flight Solution**
  - Flush with iodinated water

From “International Space Station United States On-orbit Segment Potable Water Dispenser On-orbit Functionality vs. Design” AIAA 2010-6250, 2010
The Making of a Space Biofilm

- Scientists at JSC inoculated several stainless steel hoses with a consortium of bacteria
  - Mimic the type of biofilm likely present in the PWD
- Challenged biofilm with several disinfectants to identify potential alternative biocides
  - 6% hydrogen peroxide, colloidal silver were effective
Example #2: Serendipitous Finding of a Biofouling Resistant Heat Exchanger

- Service and Performance Check Out Unit Heat Exchanger (SPCU HX)
  - Provides thermal control to the spacesuits before and after a spacewalk
  - Issues
    - Changes in coolant chemistry
    - Increased bacterial concentration

Analysis of Heat Exchanger

- Heat exchanger was replaced and analyzed for biofilm & MIC
  - Evaluated using
    - Scanning electron microscopy (SEM)
    - Epifluorescence microscopy
    - Auger microscopy

- Results
  - Planktonic growth without biofilm
Verification of Biofilm Inhibition

- **Objective:** to confirm biofilm inhibition on heat exchanger materials

- **Method**
  - Coupons were inoculated and incubated for 10 weeks
  - Samples were collected for
    - Heterotrophic plate count
    - Microscopy analysis

- **Results**
  - No microcolony formation

Microscopy of control (top) and SPCU HX (bottom) coupons test day 65.
Biofilms on Orbit and on Earth: Current Methods, Future Needs

Lessons Learned

- **Research**
  - Biofilm growth is prolific in spaceflight and can subsequently impact virulence
  - Microgravity can affect biofilm morphology

- **Practical**
  - “Start clean, stay clean”
Future Needs for Long Duration Spaceflight

- Water quality verification
  - Prevent problems before they start
- Ability to recover from an event
  - Non-toxic
  - Fast acting
  - Regenerative
Acknowledgements

- Hank Rotter (NASA Technical Fellow, JSC)
- Dr. Barry Pyle and the members of the CBE
- Dr. Mark Ott and Dr. Duane Pierson (Biomedical Research and Environmental Sciences (BR&ES) Division)
- Dr. Daniel Barta and Kate Toon (Crew and Thermal Systems Division)
Biofilms on Orbit and on Earth: Current Methods, Future Needs

References