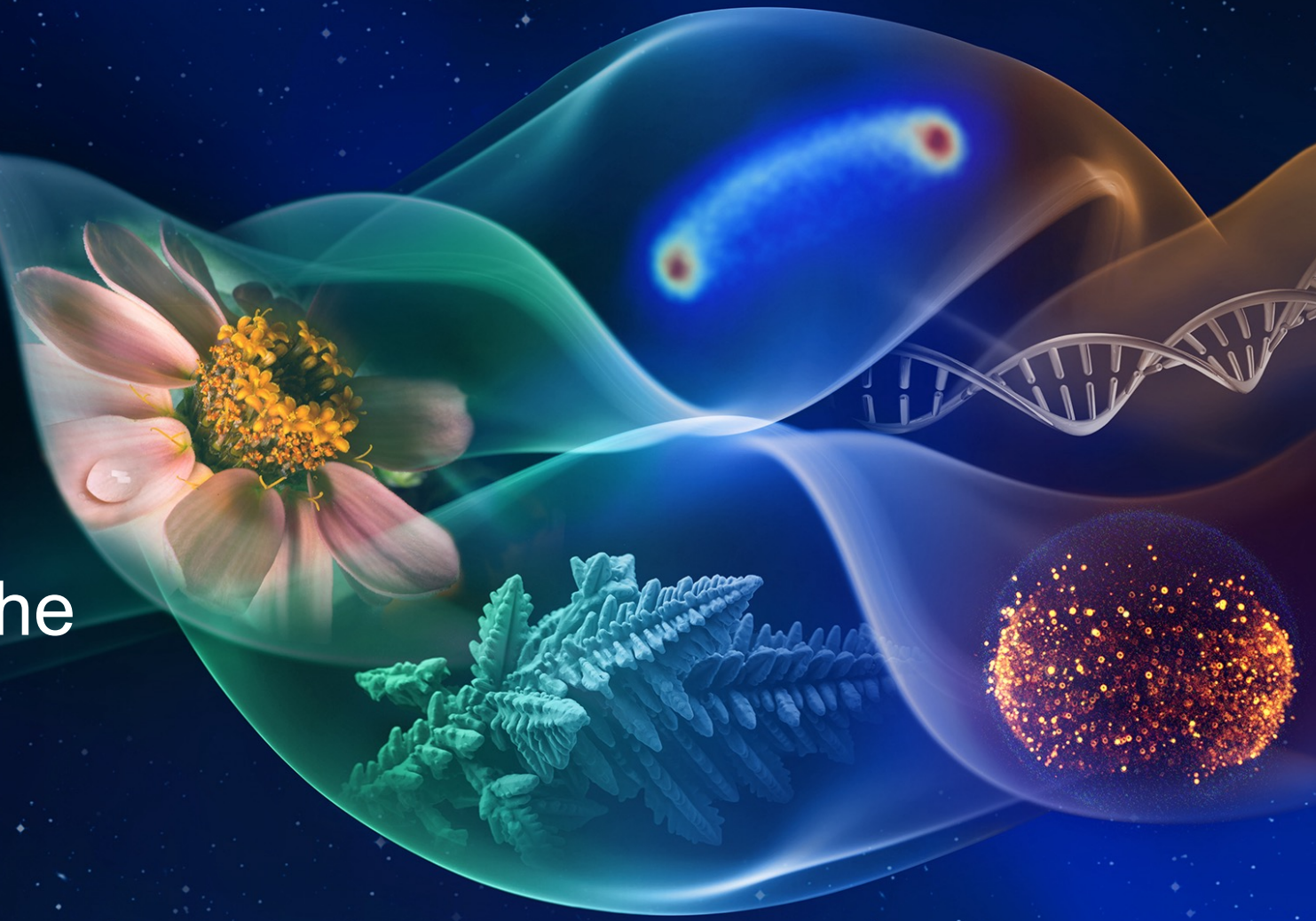
BPS
BIOLOGICAL AND
PHYSICAL SCIENCES

Characterization of Microbe Resistant Coatings for Use in the ISS Water System



Ashley Keeley
Summer 2022 Ames Intern

Ashley Keeley

- Year: Rising junior
- School: University of Idaho
- Major: Chemical Engineering
- Mentor: Jessica Lee
- Internship: virtual, but I'm working in Dr. Bernards lab at my school
- Lab experience
  - Bioremediation
  - SPOCS box 1 (ISS student project)
- Fun fact: I've been to a launch in Florida :)

Intro

- **Question: How do antimicrobial polymer coatings affect microbial colonization?**

- **Goals:**
  - **Develop procedures for performing bacteria adhesion assays, which is the main focus for my efforts this summer and final presentation**
  - **Write a review paper about previous ISS biofilm research**

Background

- **Dr. Bernards and polyampholyte polymers**
- **SPOCS box 1**

Multifunctional Polyampholyte Hydrogels with Fouling Resistance and Protein Conjugation Capacity

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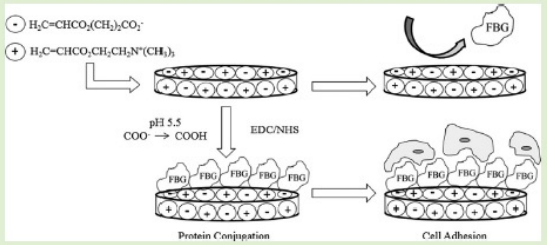
S *Supporting Information*

ABSTRACT: Materials that are resistant to nonspecific protein adsorption are critical in the biomedical community. Specifically, nonfouling implantable biomaterials are necessary to reduce the undesirable, but natural foreign body response. The focus of this investigation is to demonstrate that polyampholyte hydrogels prepared with equimolar quantities of positively charged [2-(acryloyloxy)ethyl] trimethylammonium chloride (TMA) and negatively charged 2-carboxyethyl acrylate (CAA) monomers are a viable solution to this problem. TMA/CAA hydrogels were prepared and their physical and chemical properties were characterized. The fouling resistance of the TMA/CAA hydrogels were assessed at varying cross-linker densities using enzyme-linked immunosorbant assays (ELISAs). The results clearly demonstrate that TMA/CAA hydrogels are resistant to nonspecific protein adsorption. A unique advantage of the fouling resistant TMA/CAA system is that bioactive proteins can be covalently attached to these materials using standard conjugation chemistry. This was demonstrated in this study through a combination of ELISA investigations and short-term cell adhesion assays. The multifunctional properties of the TMA/CAA polyampholyte hydrogels shown in this work clearly demonstrate the potential for these materials for use as tissue regeneration scaffolds for many biomedical applications.

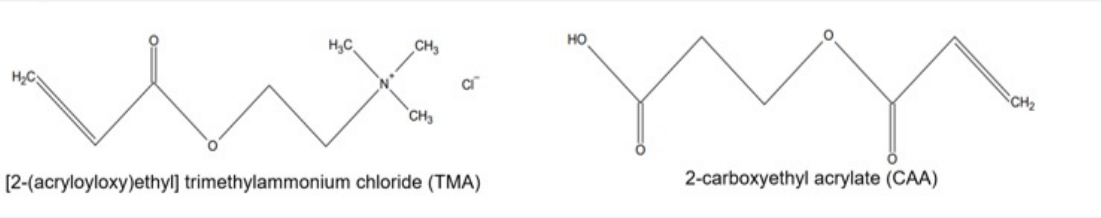


Scroeder et al, 2013, Biomacromolecules

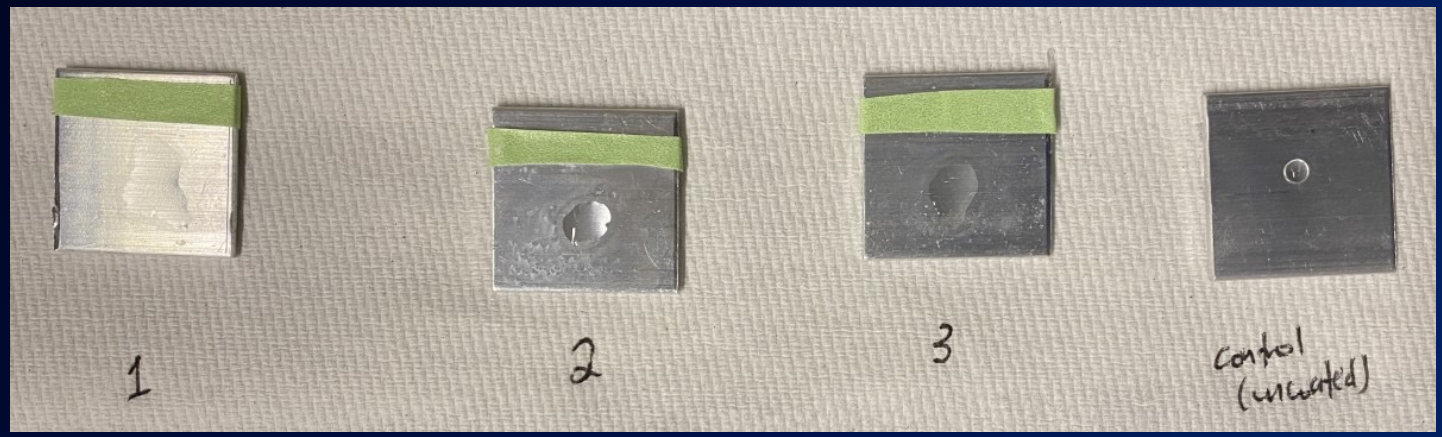
Background (coatings)

- Prevent biofouling
- 2 types of coatings
- Water layer
- Changes in the hydrophilicity of the aluminum surface also indicates polymer adhesion
- Potential applications
  - High touch surfaces
  - ISS water system
  - Medical tools

TMA/CAA:

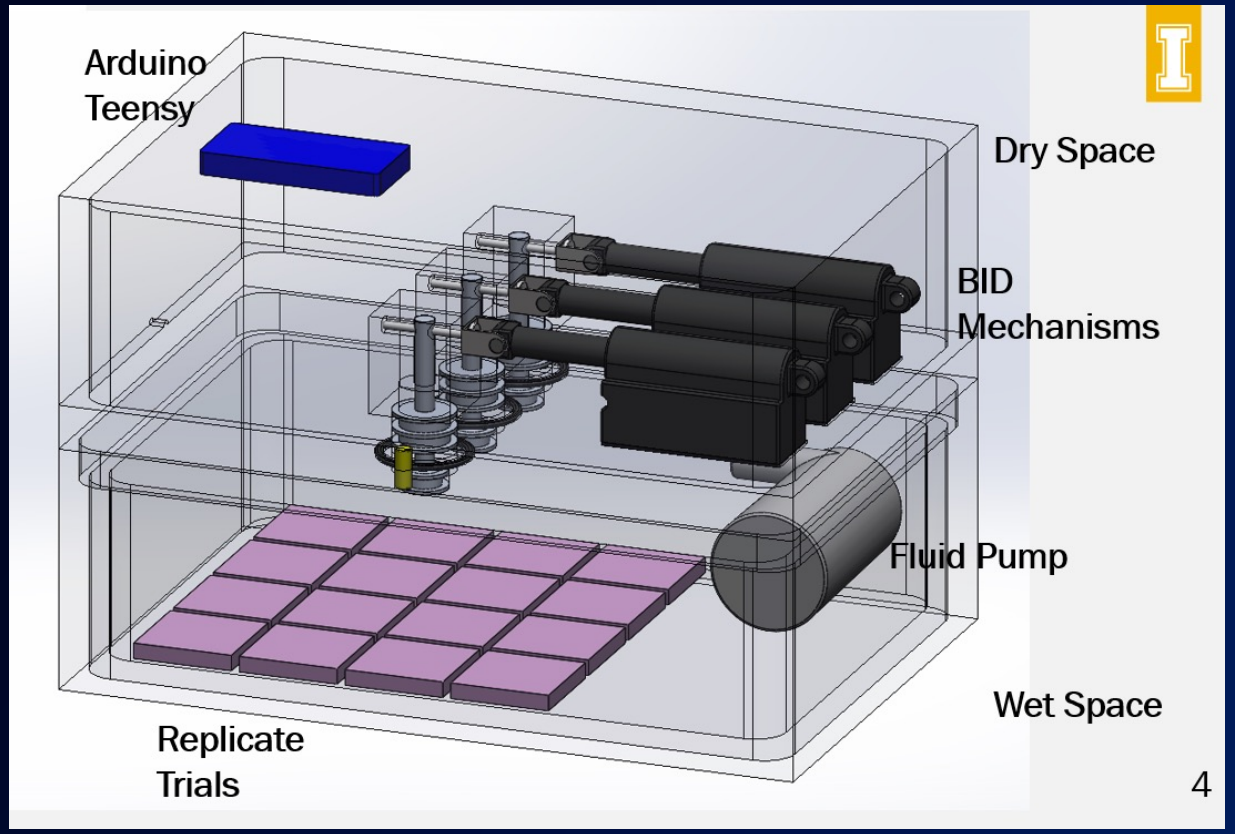


TMA/SA:



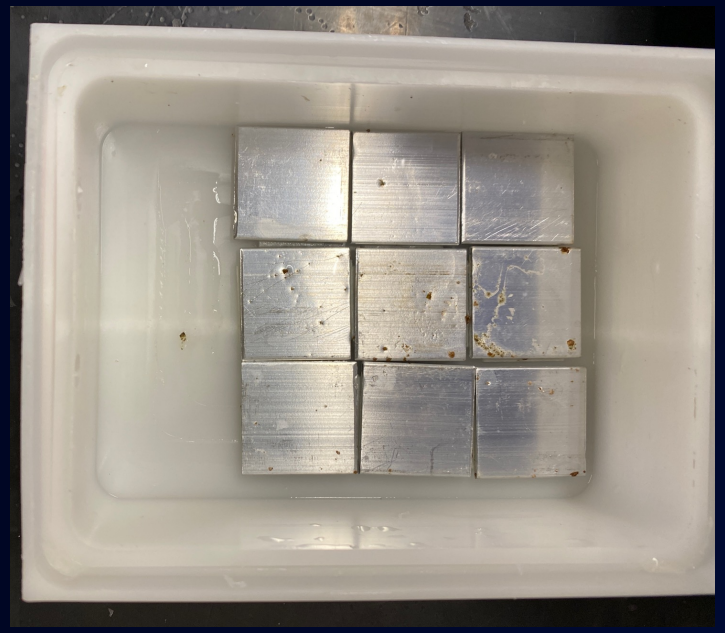
Background

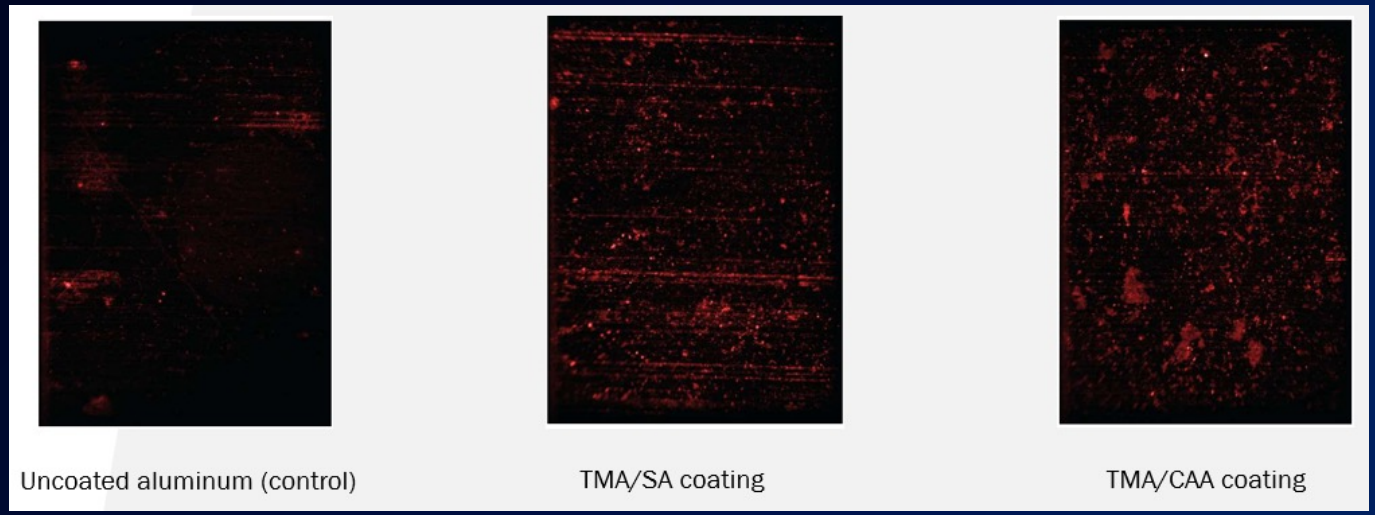
- Spocs 1 experiment layout
- 9 aluminum coupons, 6 coated, 3 uncoated
- Nutrient broth, pump mixed it
- S. epidermidis and BIDS



Background

- What's next for Vandal Voyagers 1?
  - Second payload has been approved, senior design project
  - Water system focus
    - Stainless steel
    - Co-culture (R. picketti and B. cepacia)
    - Additional levels of containment
    - Improved coating techniques
    - More than microscopy

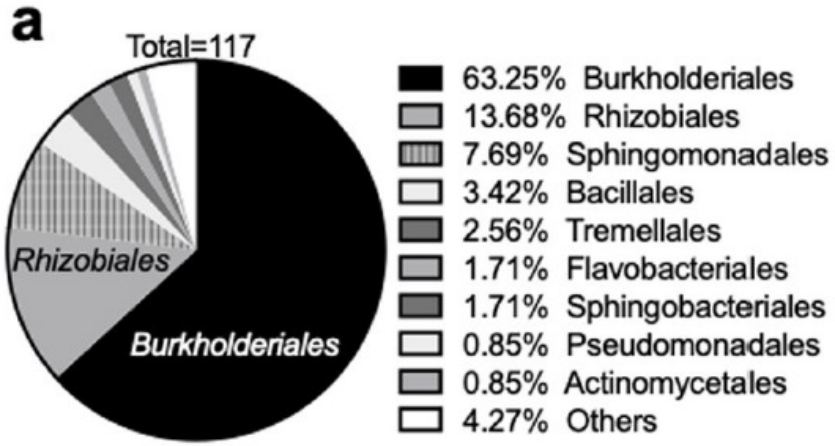




Relevance

- **International Space Station water treatment system**
- **Where the new bacteria came from**

of biofilms[1–3]. Changes that occur to the microbial ecosystem and the water-system integrity or function can impact the balance and population dynamics of resident microbiota, resulting in the release of pathogens or toxic materials to users and the environment. In addition to the health risks associated with microbial contaminants and their by-products, biofouling can cause materials degradation and systems' failure[6].



Yang et al, 2021, Nature

Methods

- Ralstonia picketti
- Difco nutrient broth
- Incubator at 30C
- Growth curve using optical density

- Goals:
  - –Make a growth curve
  - –Correlate CFU to OD
  - –Do an adhesion experiment

Methods

Making broth [500 mL]

1. Measure 4g Difco nutrient broth and 500mL DI water into a 1L glass bottle (because it's supposed to be 8g/L water).
2. Add a stir bar and stir the bottle on a stir plate until it looks completely mixed.
3. Remove the stir bar, put a piece of autoclave tape on the bottle, and make sure the lid is loose.
4. Put the bottle in the autoclave at setting 2-1 (liquid sterilization, 121°C, 27 min) with a few inches of water in the bottom of the autoclave.
5. Store the bottle at room temp.
6. Screw the bottle lid all the way closed after cooling.

Making nutrient agar [250 mL, approximately 8-12 plates]

1. Measure 2g Difco nutrient broth, 3.75g agar (15g agar/L broth), and 250mL DI water into a 500 mL glass bottle.
2. Add a stir bar and stir the bottle on a stir plate until it looks completely mixed.
3. Remove the stir bar, put a piece of autoclave tape on the bottle, and make sure the lid is loose.
4. Put the bottle in the autoclave at setting 2-1 (liquid sterilization, 121°C, 27 min) with a few inches of water in the bottom of the autoclave.
5. Store the bottle at room temp.
6. Screw the bottle lid all the way closed after cooling.

Pouring plates

1. Take the agar out of the autoclave and pour it right away, or, if it solidified since then, microwave it back to liquid using half power and swirling every 1-2 minutes. If molten agar needs to be stored before pouring, keep it in a 50C water bath.
2. In the biosafety cabinet, pour agar into approximately 8 petri dishes and make sure it covers the bottom.
3. If there's bubbles, swirl the plate until the bubbles go to the edge.
4. Wait for it to solidify.
5. Put the lids on and store the plates in ziploc bags or the original petri dish bag.
6. Label the bag, store it in the fridge.
7. Rinse out the agar bottle right away so it doesn't clog the sink.

Resuscitating bacteria [See https://www.atcc.org/products/14990 , "Handling Information"]

1. Clean the inside of the biosafety cabinet and everything I'm going to use with 70% ethanol.
2. Transfer 6mL of broth to a 50mL centrifuge tube.
3. Open the bacteria vial.
4. Use a micropipette to pipette 500 µL broth into the bacteria vial.
5. Recap the vial and mix it with the vortex mixer until there is no visible bacteria stuck to the edges.
6. Use a micropipette to transfer the contents of the vial into the centrifuge tube.
7. Remove the centrifuge tube from the biosafety cabinet and put it in the incubator set to 37C.
8. Shake it by hand every few hours.
9. Wait for it to get cloudy, ~12-36 hours.

Making glycerol [50mL]

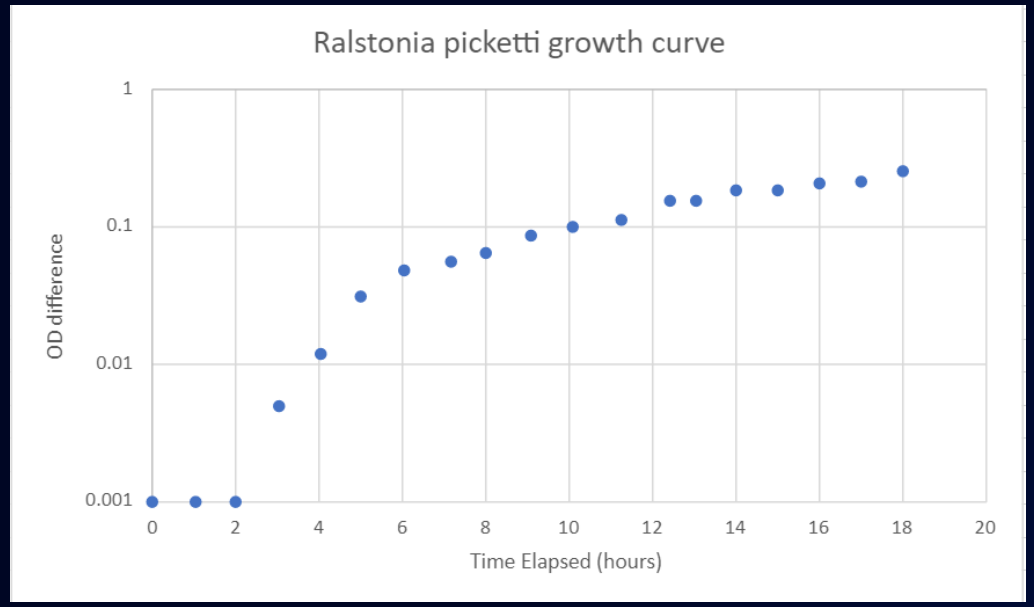
1. Mix 50% water and 50% glycerol, so 25mL of each, in a 100 mL glass bottle.
2. Add a stir bar and stir the bottle on a stir plate until it looks completely mixed.
3. Remove the stir bar, put a piece of autoclave tape on the bottle, and make sure the lid is loose.
4. Put the bottle in the autoclave at setting 2-1 (liquid sterilization, 121°C, 27 min) with a few inches of water in the bottom of the autoclave.
5. Store the bottle at room temp.
6. Screw the bottle lid all the way closed after cooling.

Making freezer stock

1. Autoclave cryovials on equipment setting in a glass beaker with a loose foil lid.
2. Label the cryovials with bacteria strain, date, and initials.
3. Clean the inside of the biosafety cabinet and everything I'm going to use with 70% ethanol.
4. Pipette 500 µL of glycerol solution into each vial.
5. Pipette 500 µL of bacteria culture into each vial.
6. Cap the vials and invert each several times to mix the liquids.
7. Store the cryovials in the cryofreezer or in a -80 freezer.

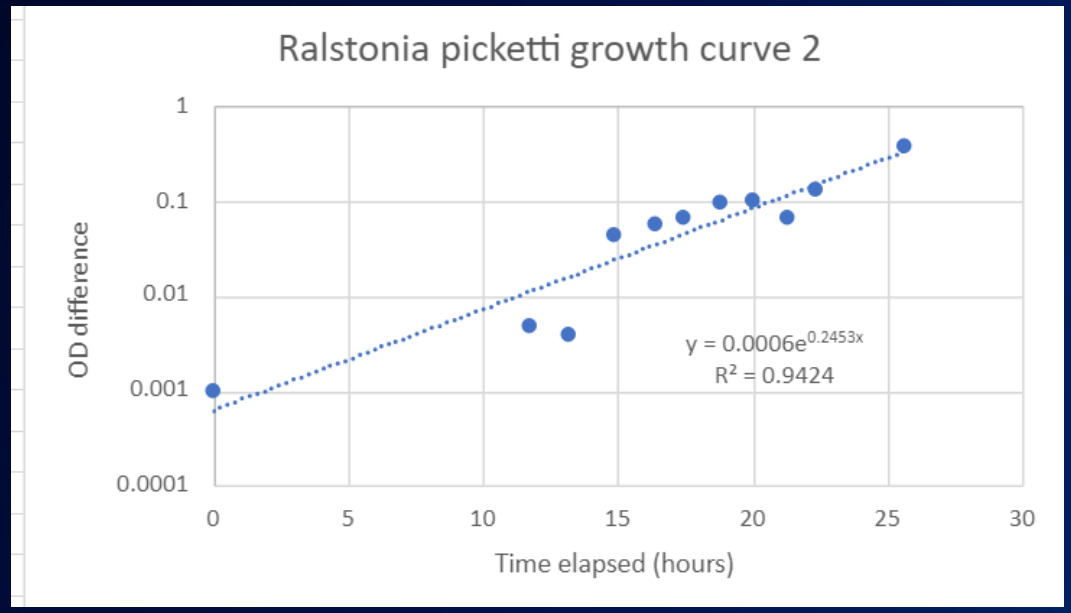
Results

- New organism, no one knew what to expect



doubling time
Td = (t2 – t1) * [ln(2) / ln (q2 / q1)]
4.881318

- Unusual conditions
  - Not shaken
  - In sealed centrifuge tubes

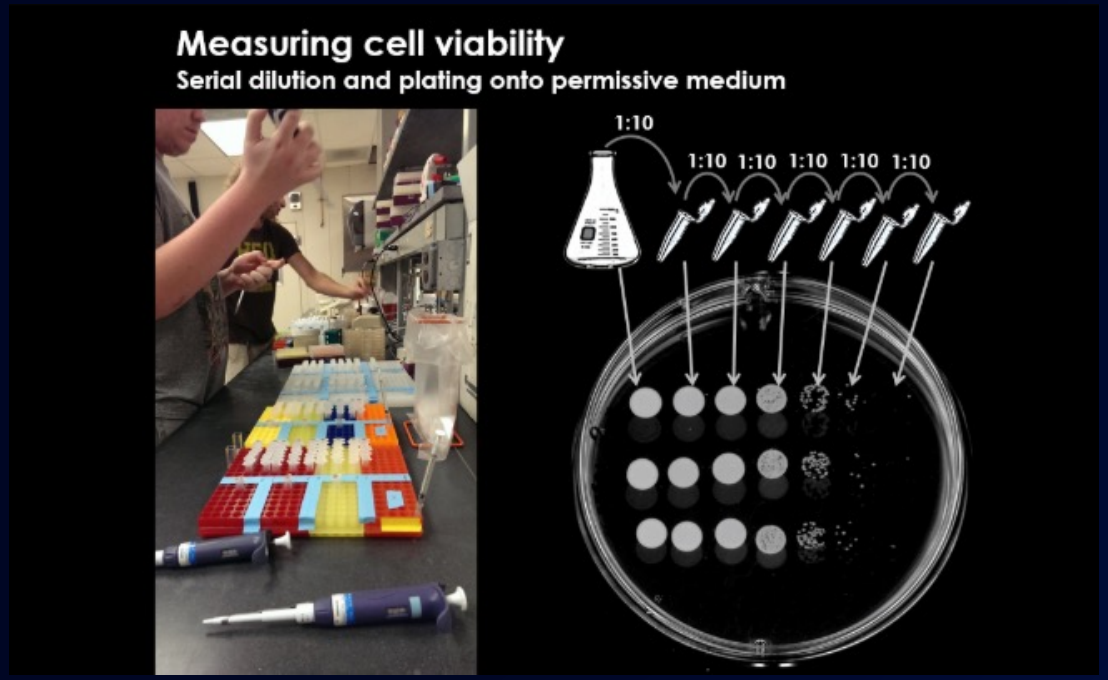


doubling time
Td = (t2 – t1) * [ln(2) / ln (q2 / q1)]
2.825712

Plans

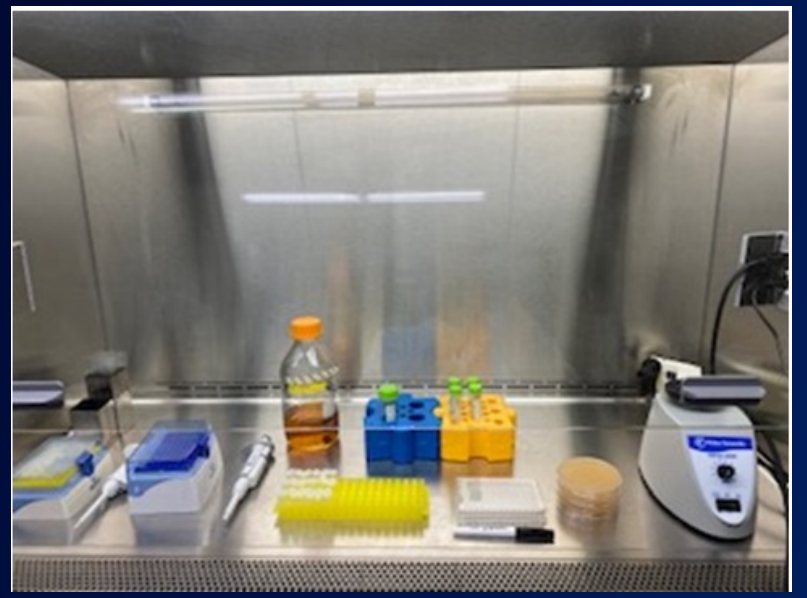
1) Correlate CFU to OD

- Dilutions and plating



2) Coupons and sonicate experiment

- Incubate coupons with Ralstonia
- Assay bacterial adhesion



Impact

- Standardized lab methods around microbiology
  - Consolidated documentation
  - Freezer stock for future experiments in this lab
  - Organized the lab and improved lab processes
  - Ralstonia does form colonies, it also grows fast and spreads making colony counting difficult

- Assisted with surface chemistry work
  - Finalizing SPOCS 1 analysis (contact angle, ellipsometry)
  - Ruled out unpolished substrates

What I Learned

- How to use(and troubleshoot) the autoclave, spin coater, incubator, plate reader, cryofreezer, microscope, sonicator
- How to dispose of solid and liquid biohazard waste
- How to make freezer stock
- How to make growth curves
- How to resuscitate freeze-dried bacteria, culture bacteria in general

- How to make and streak onto agar plates
- Practiced sterile technique
- Professional emails
- Project management
- Work computer/VPN setup
- Learned about NASA careers
- Creative problem solving
- Working independently

Acknowledgements

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Adrianne Shea

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- **Juliana Gesztesi**
- **Maha Ulhaq**

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- **Niko Hansen**
- **Kael Stelck**
- **Roslyn McCormack**
- **Hannah Johnson**
- **Travis Lindsay**
- **Adriana Bryant**
- **Kaitlyn Harvey**
- **Chelsea Barerra**

Review Paper

- Biofilms
- Comparison of several coating types
  - N-halamine
  - Zwitterions
  - Silver
  - Cations