DAGSI RX21-29, Degradation analysis of *Naganishia* yeast isolates associated with materials degradation on aircraft

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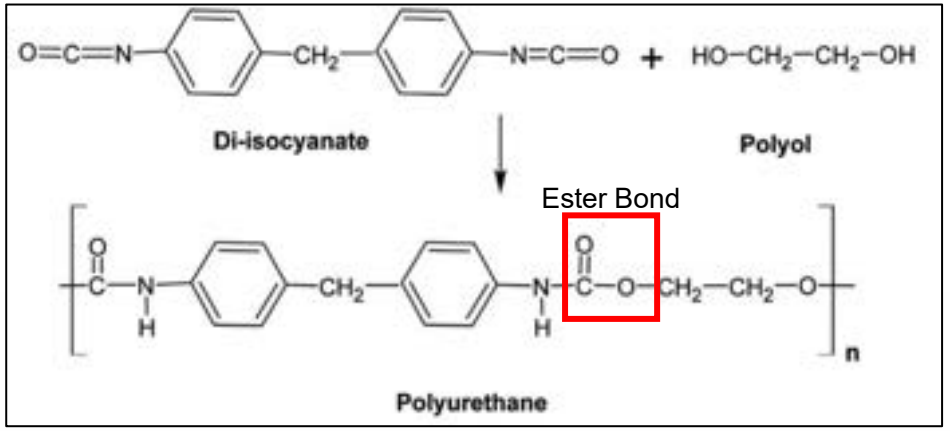
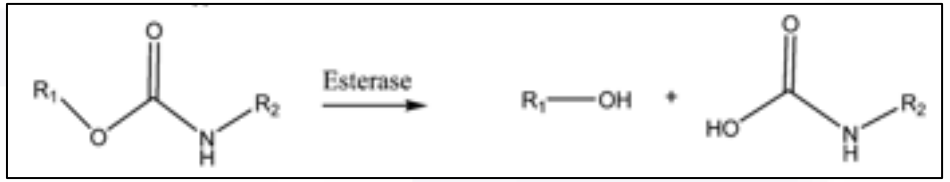
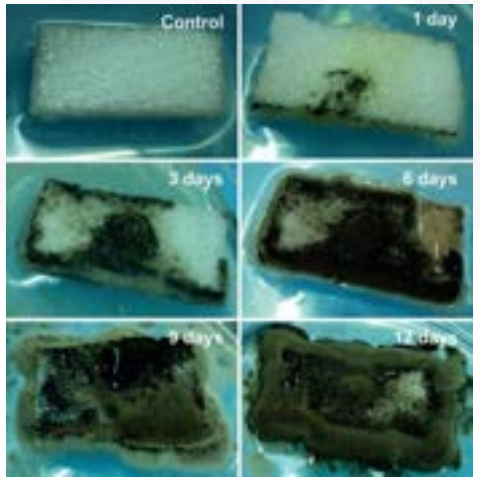
Background and Motivation

- Polyester polyurethane foams are multi-purpose materials that are formed by combining a Di-isocyanate and a polyol
- Polyester polyurethane foams are susceptible to microbial degradation
  - Degradation = Any physical or chemical change in a polymer as a result of environmental factors (Shah AA et al., 2008)
  - It was found the United States Air Force spent ~$1.2 billion annually replaced degraded materials (Jordan, 2016)
- *Naganishia albida* strains excrete enzymes called esterases that target and degrade ester bonds in the polyurethane
  - Ester bonds are a key component in the polyurethane backbone
  - These enzymes require water in order to catalyze their reactions
- The goal is to determine how environmental water impacts the degradative function of *Naganishia*
  - Investigating the impact of relative humidity and condensed water





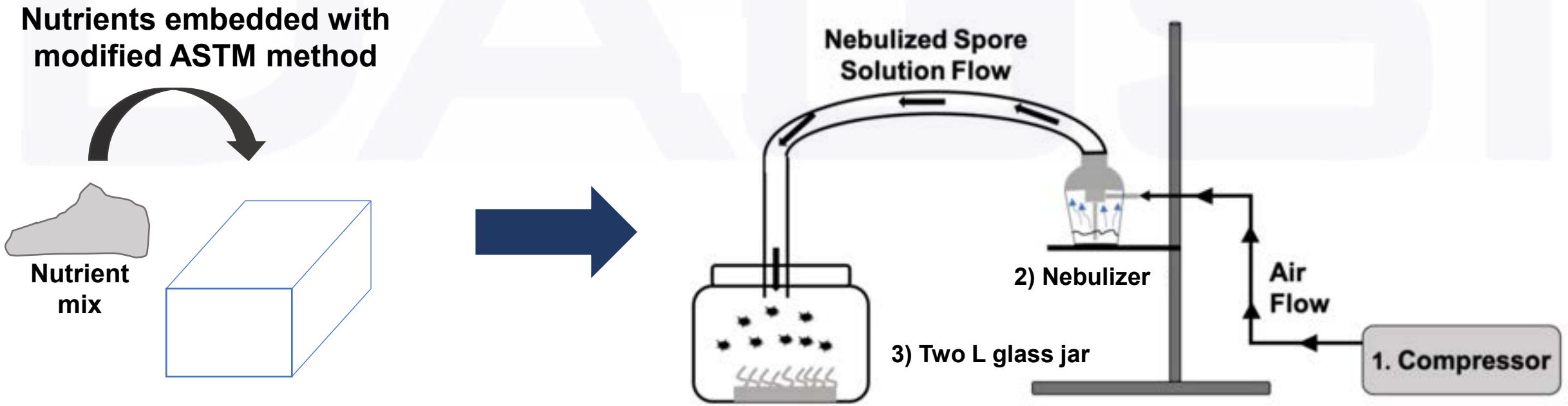


Cladosporium growth on polyurethane foam. Álvarez-Barragán *et al,* 2016

Aureobasidium growth on polyurethane foam

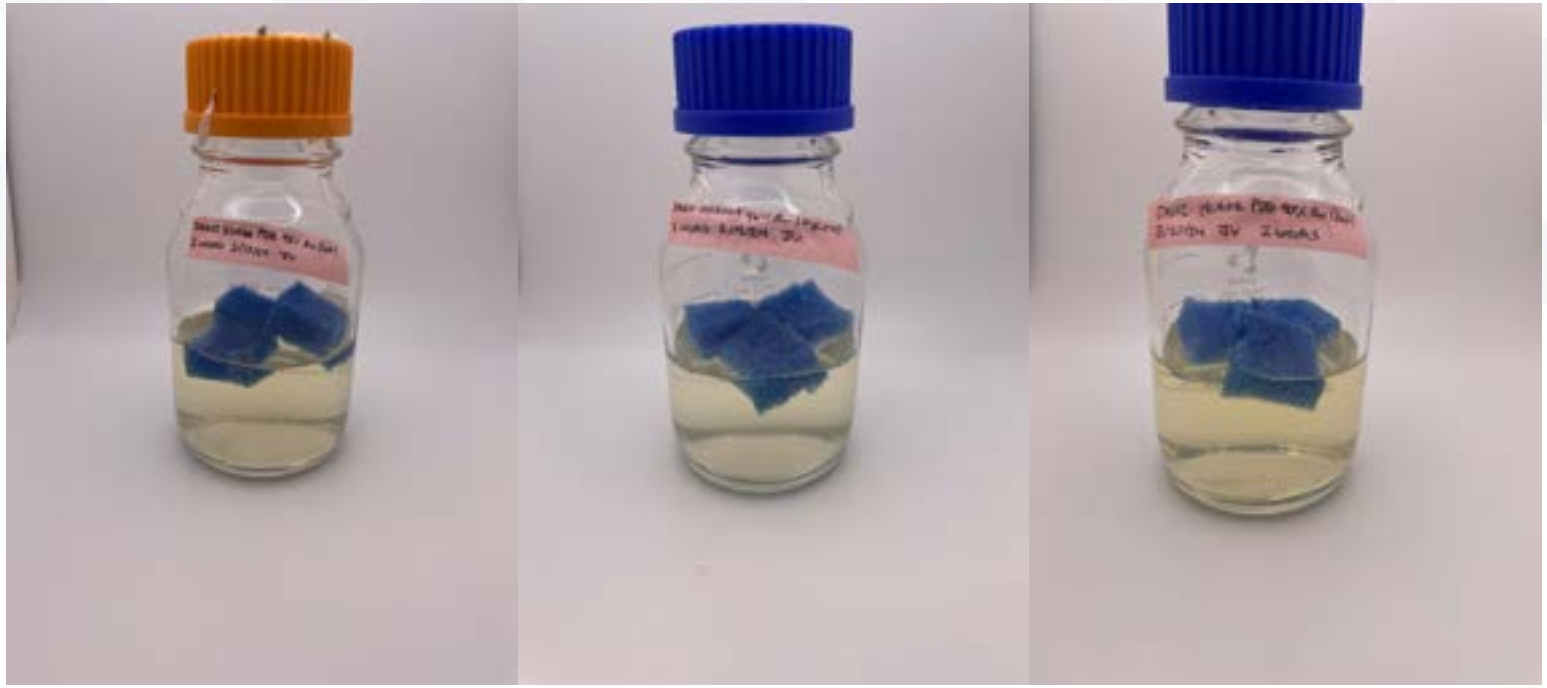
Relative Humidity Testing Methods

- Foam is loaded with nutrient powders to mimic the presence of dust in the foam
  - Dust was found to contain more than enough nutrients for fungal growth (Dannemiller, K.C., Weschler, C.J. and Peccia, J. 2017)
  - Potato dextrose broth powder, a rich, complex media, and a minimal media with glucose as a carbon source were used
- Yeasts were nebulized on to the foam to mimic the deposition of organisms in nature
  - The cell solution used for nebulization had a concentration of 10^7 cells/mL
  - Three strains were used: 19CA02, 24CA02A, and 5301AA
- Experiments were incubated at 25°C at varying relative humidities
  - 50% RH was achieved with a solution of MgCl, 85% with a NaCl solution, and 100% with DI water



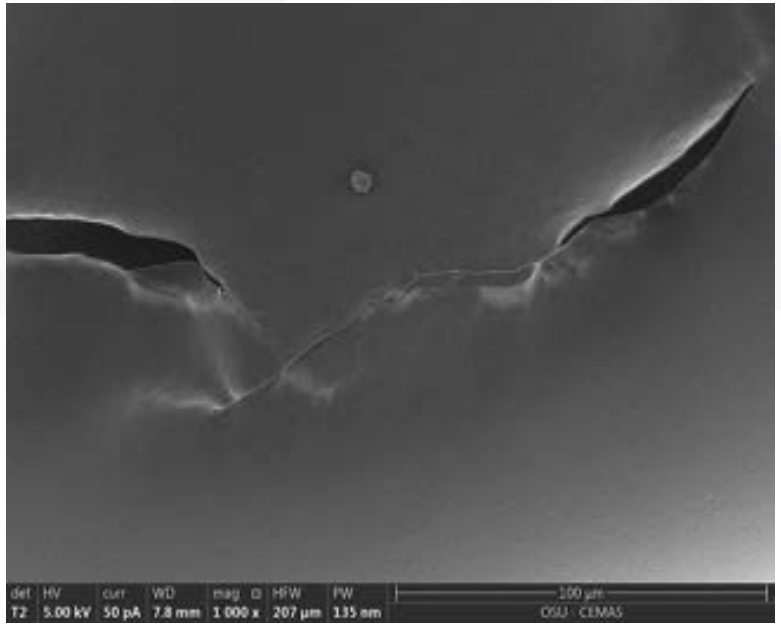
Condensed Water Testing Methods

- Foam pieces were added directly to the liquid solution the strains were grown in
  - The media used for the growth solution was potato dextrose broth
    - This is the same solution used in the nebulization process
  - Strains were incubated at 25°C for 2 weeks on a shaker table moving at 160 rpm to keep cells suspended
- Additives were used to control the amount of available water in the growth solution
  - NaCl and glycerol were added to separate jars to test if limited water affected degradation
  - Different additives were also used to see if their presence had an impact on the strains
  - Water availabilities measured were 85%, 90%, and 95% with NaCl and 92-93% and 95-96% with glycerol
    - We could not reach ~85% water availability with glycerol due to the volume of the additive required

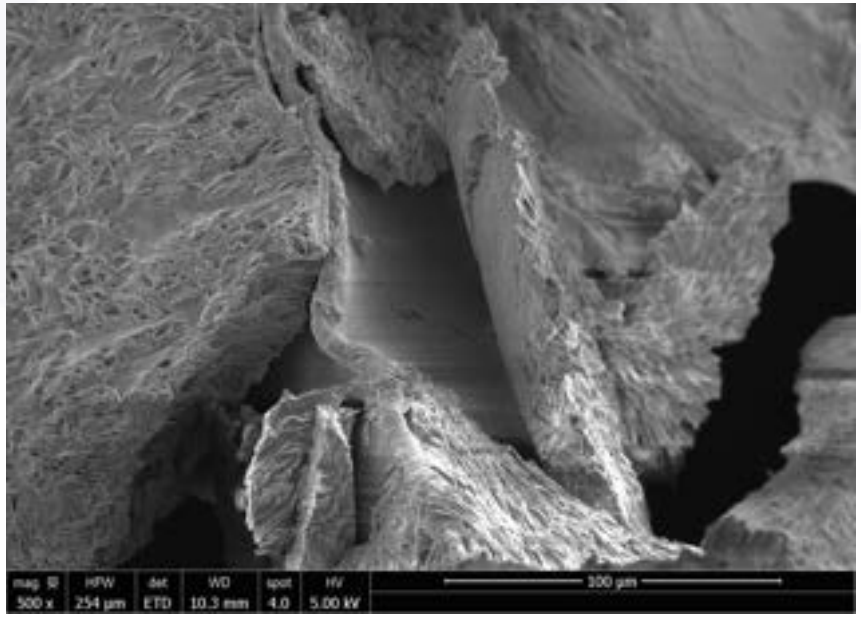


Post-Incubation Methods

- Following both types of incubation, foam pieces were cleaned to remove any fungal growth or residual nutrients
  - Foam pieces were cut in half for cleaning
  - Each half was vortexed twice in 10 mL of 70% ethanol
  - Washed foam pieces were placed in a 100°C oven for two days to dry off any ethanol or water
- Three methods are used to analyze any degradation by the *Naganishia* strains
  - Scanning Electron Microscopy (SEM) Imaging
  - Weight Comparisons
  - Differential gene expression analysis



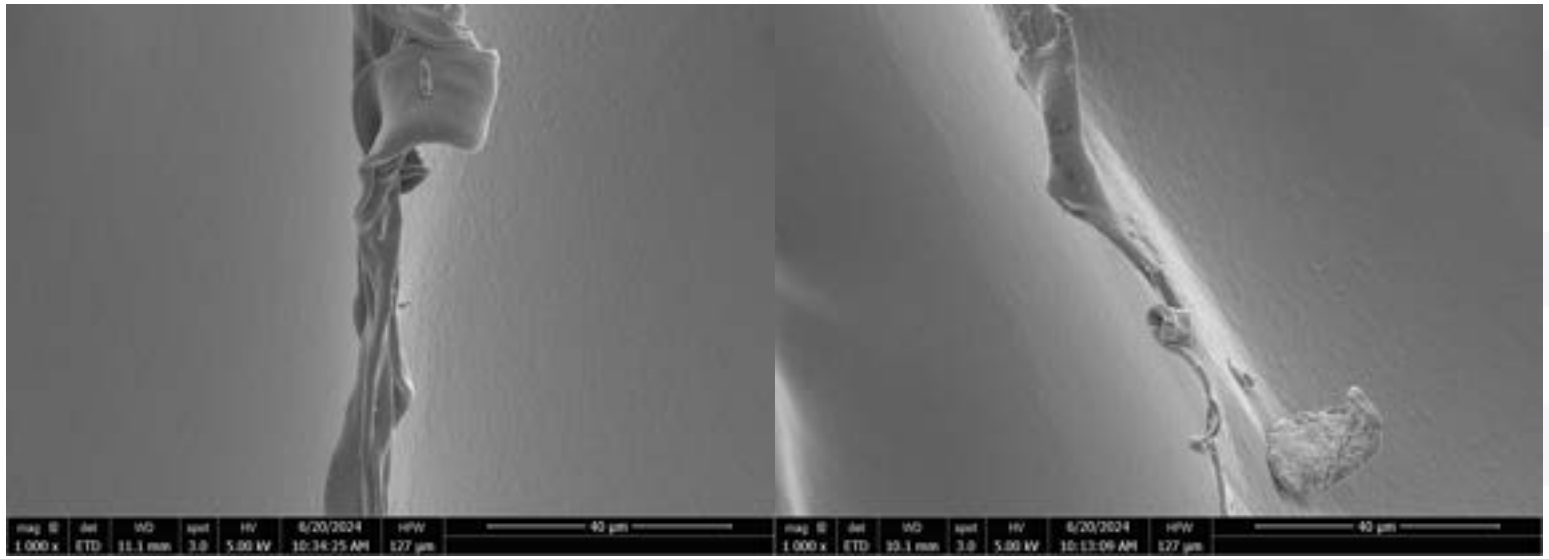
SEM of plain foam



SEM of foam with *Naganishia*

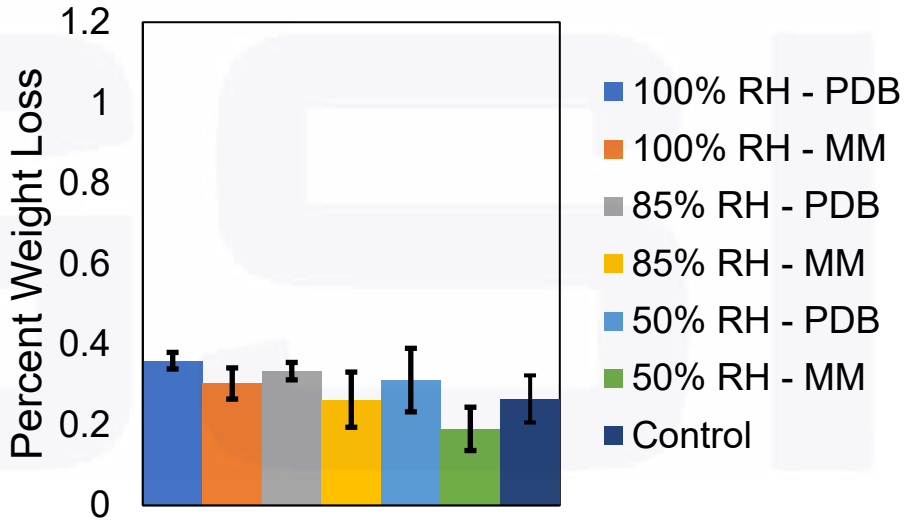
Relative Humidity Results

- Relative Humidity did not have an observable impact on foam degradation by our *Naganishia* strains
  - No signs of degradation were observed under SEM (no pitting, cracking, etc.)
  - No significant difference between media types or the control when doing weight comparisons
  - Even after incubating for 5 weeks, strains showed less than 1% foam weight loss
    - Strains were incubated for 2,3, and 5 weeks. Inconsistent weight loss was observed between the different time periods



100% RH Control

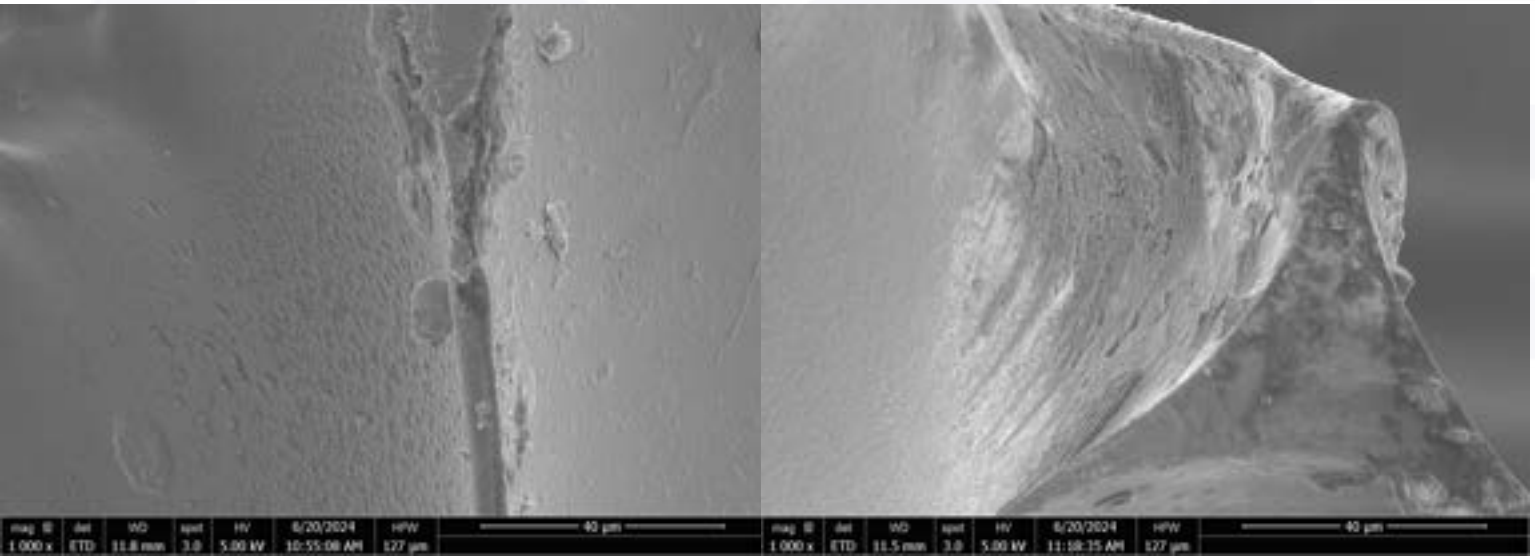
100% RH Strain 19CA02



Strain 19CA02 after 5 weeks

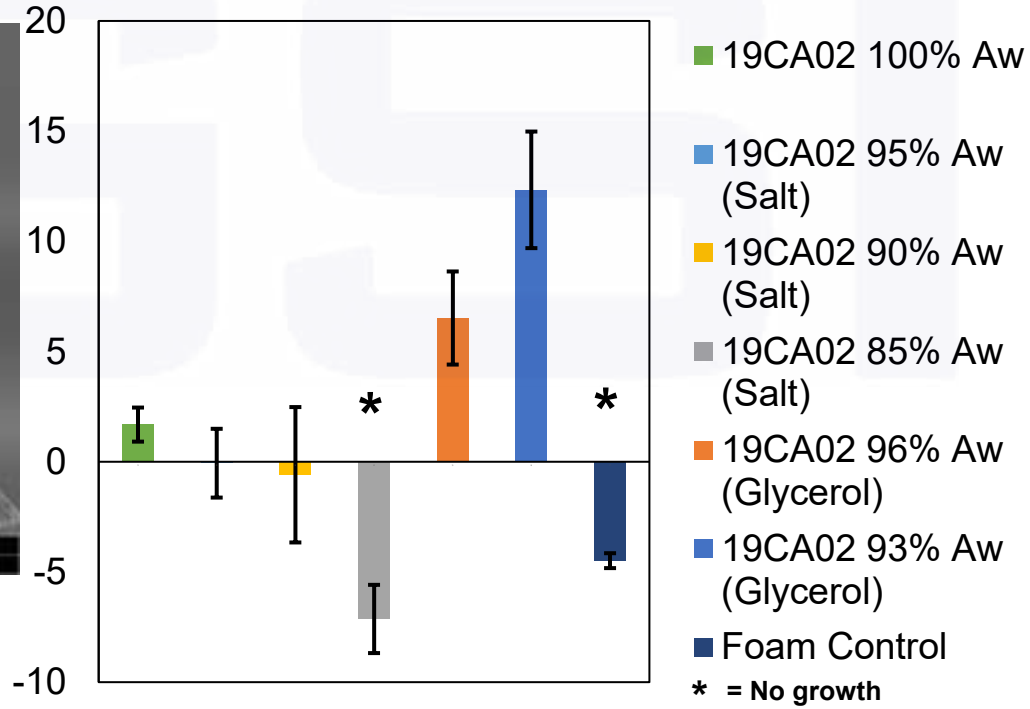
Condensed Water Results

- The presence of condensed water appears to have some impact on the degradation by our strains
  - SEM imaging revealed several signs of degradation including discoloration of the material, pitting/cracking, and the remnants of biofilms
  - Weight comparisons showed that pieces shed between 2-14% of their weight following the incubation
    - Following the incubations in condensed water, small chunks of foam were observed floating in the liquid solution. These small chunks were not collected for the reweighing. Only the main foam piece was weighed
    - It is unclear if this weight change is due to the *Naganishia* degrading the foam directly, altering the pH through their metabolism of the growth solution, or if the mechanical function of shaking the solution caused chunks to break off
    - Experiments that did not feature fungal growth showed an *increase* in foam weight, possibly due to salts getting trapped in the foam pieces
- Strains showed altered weight loss patterns based on additive used



Foam Control 100% Aw

19CA02 95% Aw



Strain 19CA02

Summary

- Relative humidity was not observed to have a role in foam degradation by *Naganishia albida* strains
  - SEM did not show any signs of degradation
  - Foam weight loss tests showed less than 1% weight loss and were inconsistent
- *Naganishia* strains may require condensed water to degrade foam
  - SEM images showed pitting, cracking, remnants of biofilms, and discoloration which are all signs of degradation
  - Weight loss comparisons ranged from 2-14% weight loss, depending on strain and additives
- Strains showed altered weight loss patterns based on additive used
  - 24CA02A showed similar weight loss between 100% Aw (no additives) and 95% Aw (NaCl), but did not show any weight loss with Glycerol or higher concentrations of NaCl
  - 5301AA showed similar weight loss between 100% Aw (no additives) and 93% Aw (Glycerol), but did not show any weight loss with any concentration of NaCl
  - 19CA02 showed increased weight loss with both concentrations of glycerol when compared to 100% Aw, but did not show any loss with any concentration of NaCl

Future Directions

- Re-run condensed water experiments and collect sub-samples for RNA sequencing
  - We are interested in identifying any gene products relating to esterases that the *Naganishia* may be excreting into the liquid solution
- Develop a method for collecting and cleaning the small chunks floating in the liquid solution for more accurate weight loss measurements
- Examine the pH of the liquid solutions before and after incubations
  - If the *Naganishia* are altering the pH of the solution, this could be the cause for the small chunks falling off during incubation
- Investigate the metabolism of *Naganishia albida* in relation to the additives
  - The additives are being used to control the amount of available water inside the liquid solution, but we are unaware of the direct impact their presence has on the strains
    - We do know that at 85% water availability, which takes about 150 grams of NaCl, there is no growth observed
- Investigate if the amount of dissolved oxygen has a role in foam degradation
  - *Naganishia* are usually found in aerobic environments such as the exteriors of plants or buildings

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