360. A Novel Approach for Characterizing the Ultra-Micro Size-Fraction Community

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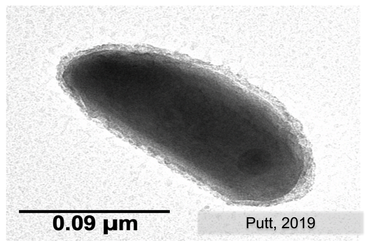
Introduction

- Ultra-micro size-fraction (UMSF; pictured right): bacteria that can pass through the 0.2 µm pore membranes employed in environmental surveys.
- Despite being ubiquitous and having high metabolic activity, UMSF remain elusive and largely uncultured.
- Investigations of UMSF are skewed by difficulties in culturing and a lack of techniques for measuring UMSF biogeochemical signatures.
- According to a 2019 study , one out of seven global rivers surveyed had antibiotic levels above safe water standards.[1]



Rationale:

To study the impact of man-made carbon sources, introduced through human movement and migration, on the metabolic response of a local stream water UMSF community.

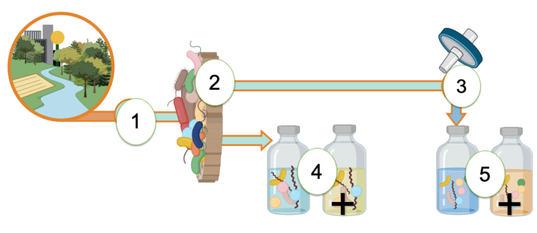
Driving Question:

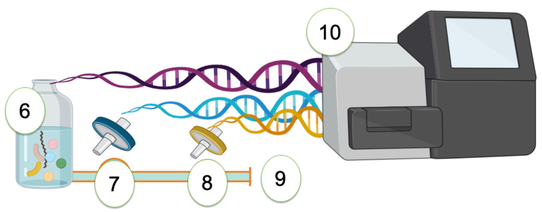
What effect does the introduction of man-made carbon sources have on the UMSF community?

Hypotheses:

H1: In the presence of cyclodextrin, the UMSF community will undergo significant metabolic response activity.
H2: In the presence of cyclodextrin, the UMSF community will experience significant growth in terms of cell counts and DNA concentration.
H3: During this experiment, we will identify effective methods & procedures for filtration and culturing of the UMSF community.

Methods





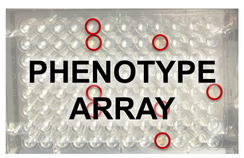
Microcosm Preparations

1. Water sample collection at Beaver Creek
2. Initial (1F) 0.2 µm filtration
3. Re-filtering with second 0.2 µm filter (2F)
4. 1F inoculation (+ Cyclodextrin in ½)
5. 2F inoculation (+ Cyclodextrin in ½)

Timepoint Measures

6. Biofilm DNA extracted from bottle walls
7. DNA extraction & analysis – 0.2 µm filters
8. DNA extraction & analysis – 0.1 µm filters
9. Geochem: anions, organic acids, pH
10. 16S rRNA amplicon sequencing

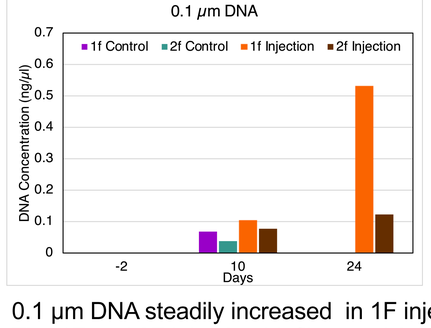
(+) Community Metabolic Analysis

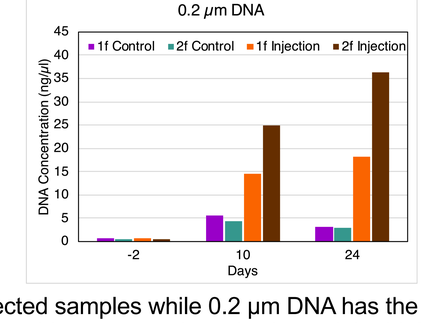


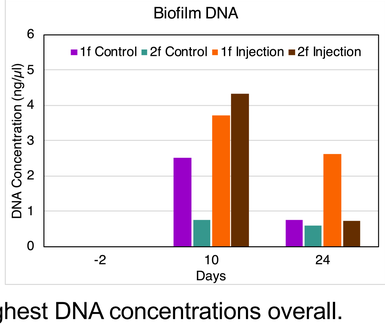
Well	Carbon Source
…	…
B5	D-Arabinose
F5	Oxalomalic Acid
A5	Cyclodextrin
E12	5-Keto-D- Gluconic Acid
…	…

"Novel" Rapid Biofilm Extraction

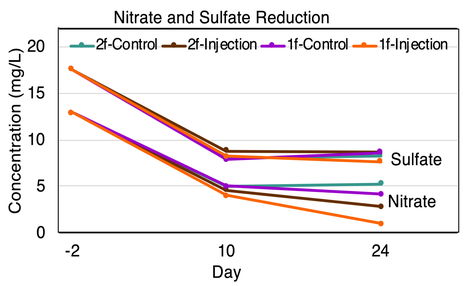
Results

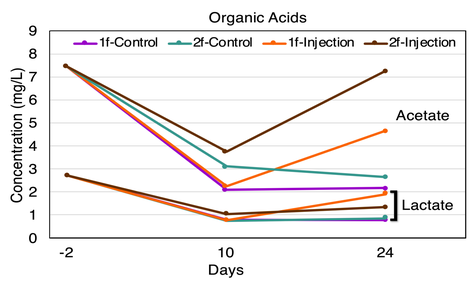






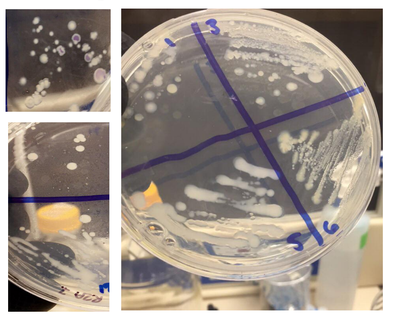
0.1 µm DNA steadily increased in 1F injected samples while 0.2 µm DNA has the highest DNA concentrations overall. The 1F and 2F injection biofilms and 0.2 µm biomass consistently showed the highest DNA concentrations.

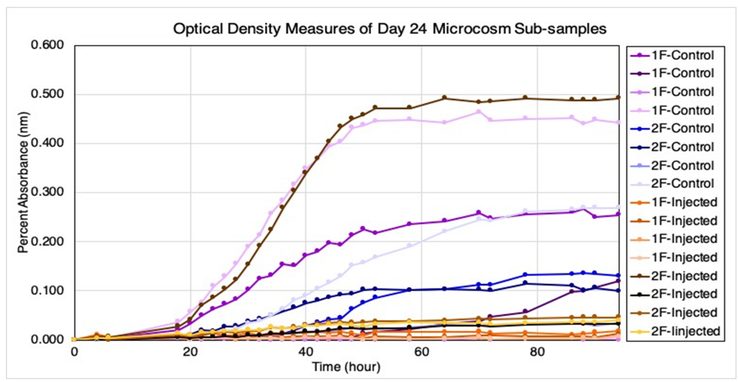


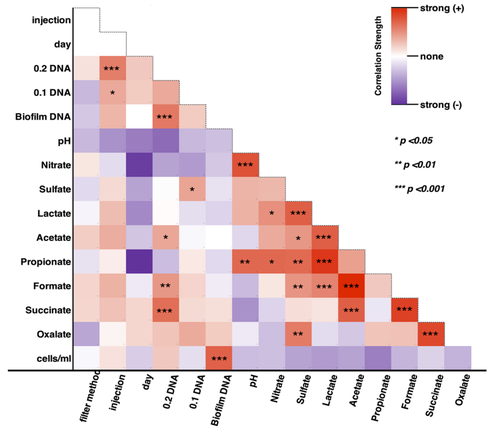


Nitrate and sulfate decreased 3x and 2x respectively while lactate and acetate synthesis occurred around day 24.

Day 24 Isolates







Conclusions

H1: In the presence of cyclodextrin, the UMSF community underwent significant metabolic response activity as indicated by increased sulfate and nitrate reduction post-injection as well as the increase in organic acid production from day 10 to day 24.
H2: In the presence of cyclodextrin, the UMSF community experienced significant growth as indicated by higher DNA concentration counts observed post-injection.
H3: We developed methods & procedures for effective filtration and culturing of the UMSF community.

Future work

- Isolate characterization and 16S rRNA amplicon community analysis.
- The lab techniques employed here will guide future research into the UMSF and understanding their relationship with man-made carbon sources.
- Future research directions include looking at the potential utilization of UMSF to biodegrade antibiotics and pharmaceutical by-products in rural community water systems.

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References: [1]A. Boxall and J. Wilkinson. Identifying hotspots of resistance selection from antibiotic exposure in urban environments around the world. SETAC Europe 29th Annual Meeting, Helsinki, Finland. May 27, 2019.