Investigating the Effect of Microencapsulation of Bacteria and Fungi on PAH Biodegradation Success

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Background

EPA Superfund sites are areas contaminated with hazardous levels of constituents that pose potential harm to the environment and human health. Superfund sites often contain polyaromatic hydrocarbons (PAHs) due to their mutagenic and carcinogenic properties[1,2].

There are numerous remediation methods to reduce PAH concentrations, most being ecologically intrusive. Bioremediation is an alternative approach that is minimally invasive and cost effective[3]. However, problems arise due to limited establishment success of planktonic microbes, which results in limited PAH degradation success.

This project involves microbes isolated directly from sediments within a PAH contaminated Superfund site that are known to degrade at least one PAH[4]. Through this project, we aim to develop methods to encapsule isolated bacteria to promote the survival and delivery of these PAH degrading microbes back into the environment[5].

Methodology

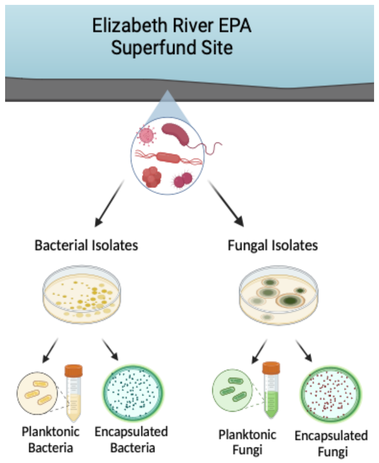

Figure 1: Schematic depicting the monoculture process

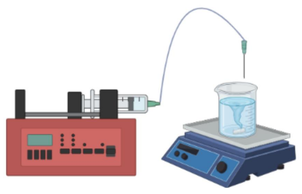

Figure 2: The syringe pump setup used for making microcapsules via extrusion

Monocultures
Fungal and bacterial isolates were isolated from Sediments of the Elizabeth River. Monocultures were then grow in LB media at 30 C as both planktonic and encapsulated

Biopolymers
Three different capsule biopolymers were investigated:

- Sodium Alginate
- Chitosan Coated Alginate
- Polyvinyl Alcohol

Microencapsulation via extrusion
Capsules were generated via extrusion using a 2% alginate gel a 10% liquid culture through a 30G needle into a 0.1 calcium chloride bath[6]

Measuring Growth
In a 48-well plate, OD600 is used to measure growth of the following[7]

- Intact microcapsules in LB
- Microcapsules dissolved with sodium citrate
- Planktonic culture in LB

Results by Amelia Foley

1 What biopolymer supported bacterial growth best?

Found that microcapsules made of 2% Sodium Alginate, with or without a Chitosan coat showed the best results. Polyvinyl alcohol was not a supportive capsule material.

2 Is absorbance readings is an effective method to measure growth?

OD600 was taken over a 24 hour period for three sodium alginate beads. Figure 3 indicated that OD is adequate in measuring growth for bacterial species

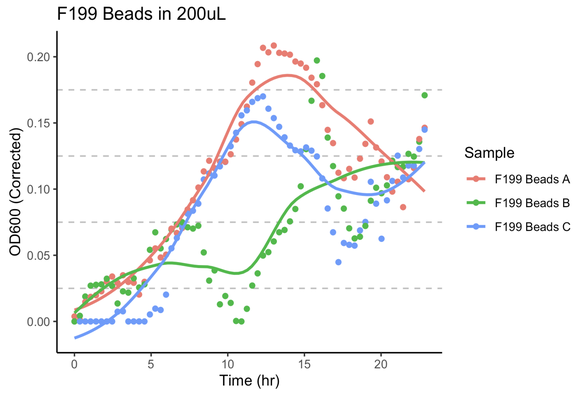

Figure 3. OD600 measurements of microcapsules in LB after 24 hour growth period

3 How does OD readings in encapsulated bacteria compare to planktonic cultures?

There was leakage of bacteria from the capsule to the environment. By looking at the OD600 of encapsulated bacteria, we observed that Alginate beads support growth, but there is lots of leakage whereas chitosan beads are stronger, and don't leak as much.

4 What are ways that we could measure the bacteria growth in the capsule without considering leakage?

Microcapsules were rinsed with PBS and dissolved in sodium citrate and prepared to get a OD600 reading. This prepared sample only will have bacteria growth within the capsule. Different bacteria have different growth curves in the beads

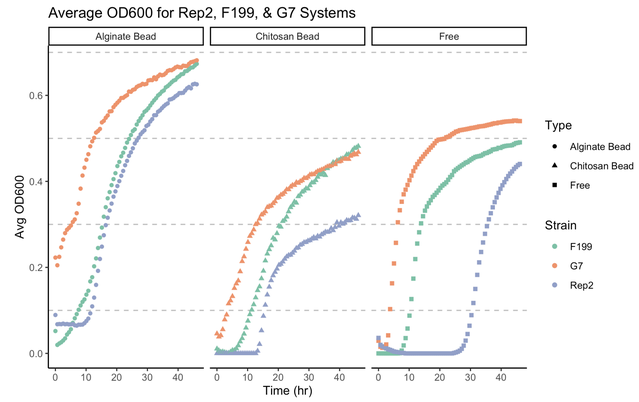

Figure 4. Average OD600 of encapsulated and planktonic cultures after 48 hours of growth at 30C

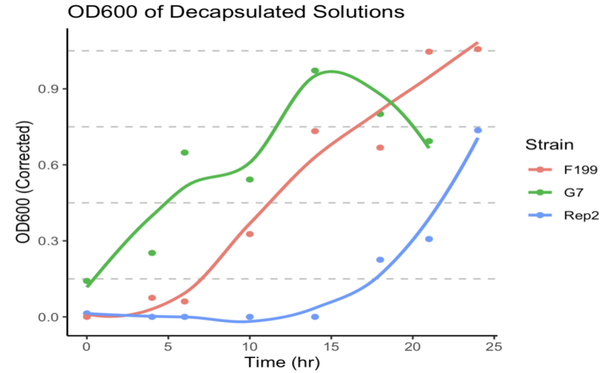

Figure 5. OD600 of decapsulated cultures at multiple time points within 24 hour period

Future Directions

- Expand upon this research in respect to fungi to develop proper methods of fungal encapsulation and investigate which biopolymers best support fungal growth
- Develop alternative methods for quantifying microbial growth. Currently looking at fluorescence tagged bacteria to understand growth/ leakage dynamics within the capsule
- Evaluate if microcapsules keep bacteria and fungi cultures viable over extended periods of time compared to planktonic cultures
- Test the capsuled microbes in different environmental properties (i.e., pH, ionic strength) to determine if capsules can persist in *in-situ* like ecological conditions

References

[1] Di Giulio, R. T., & Clark, B. W. (2015). *J Tox and Env Health* [2] Baek, et al. (1991) *Water Air Soil Pollut*. [3] Seo, J.-S., Keum, Y.-S., & Li, Q. X. (2009). Bacterial Degradation of Aromatic Compounds. [4] Valdivia-Rivera, et al. (2021). *Reviews in Environmental Science and Bio/Technology*. [5] Volkoff, et al. (2019) *Duke University*. [6] Yueng, et al. (2016) *Front. Microbial*. [7] Zhou et al (1998) J Applied Micro.

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