Rhamnolipids Compositions for Hydrocarbon-Contaminated Soil Remediation: *Part II*

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Rhamnolipid Chemistry and Rhamnolipid-Assisted Bioremediation Mechanism

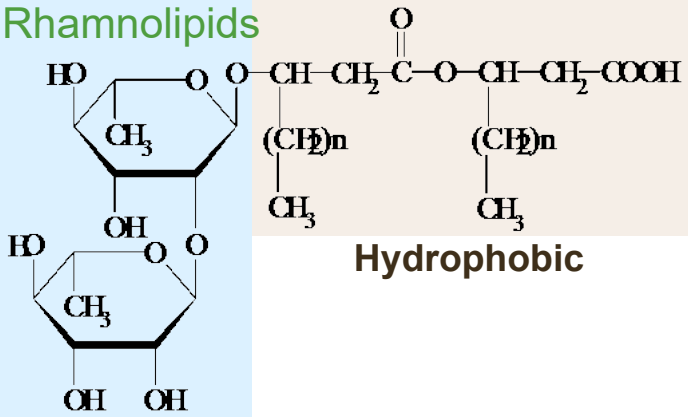
Oils

Sugars

Fermentation

Pseudomonas aeruginosa
-Kateryna Kon, 2022

Rhamnolipids

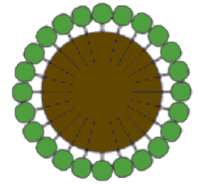


Hydrophobic

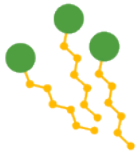
Hydrophilic

1. Emulsify HC making it more accessible to bacteria
2. Attach to bacteria and improve their contact with HC

Hydrocarbon polluted soil

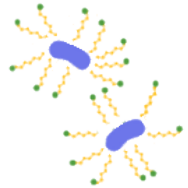


Emulsified hydrocarbons



Rhamnolipids (RL)

Bacteria in soil



Bio-activated Bacteria

Hydrocarbon and rhamnolipids interaction with soil during the bioremediation process adapted from Fenibo (2019)

Conducting Lab and Field Studies in Parallel

Objective: To collect data from both the lab and field to validate the concept of using rhamnolipid to improve indigenous bacteria and degrade hydrocarbon in soil.

Hydrocarbon-contaminated soil from a field site in Ontario, Canada

Comparative Study

Laboratory study using simulated biopiles in a continuous pulse-flow respirometer

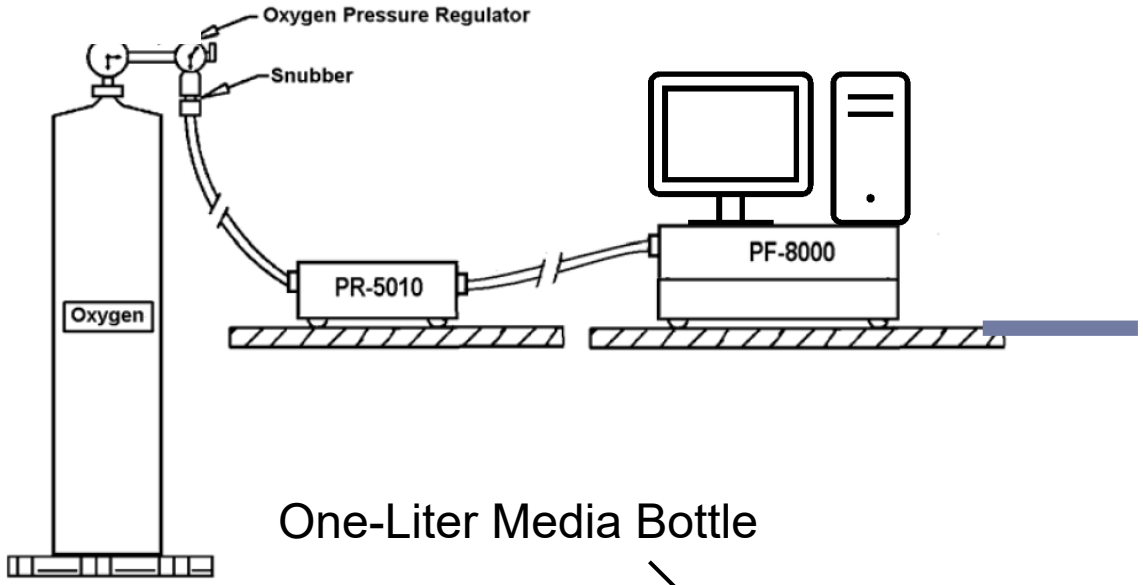
On-site field study using ex-situ biopiles

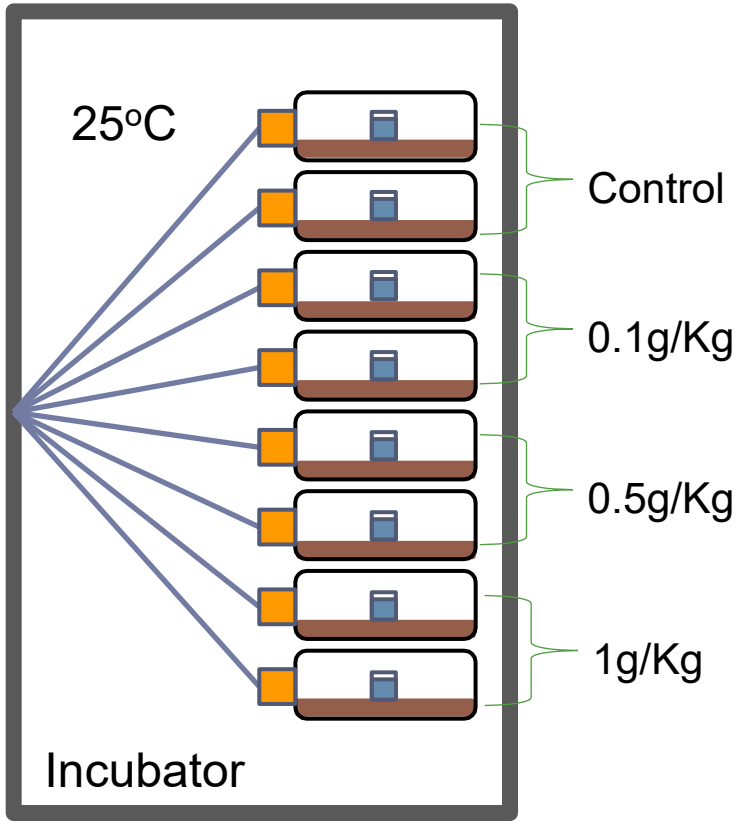
Experimental Variables

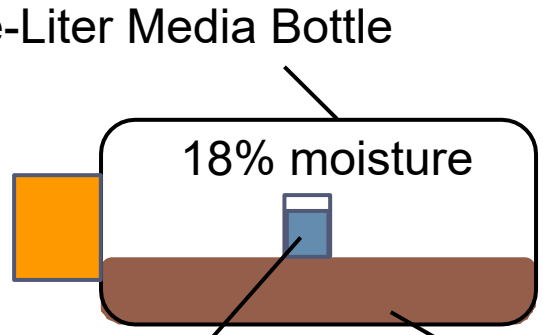
- Rhamnolipid concentration (0.1, 0.5, and 1g RL/kg soil)
- Nutrient addition (NH_4Cl)
- Commercial benchmark – Enzymes (field trial only)

Laboratory Setup – Continuous Oxygen Uptake Monitoring

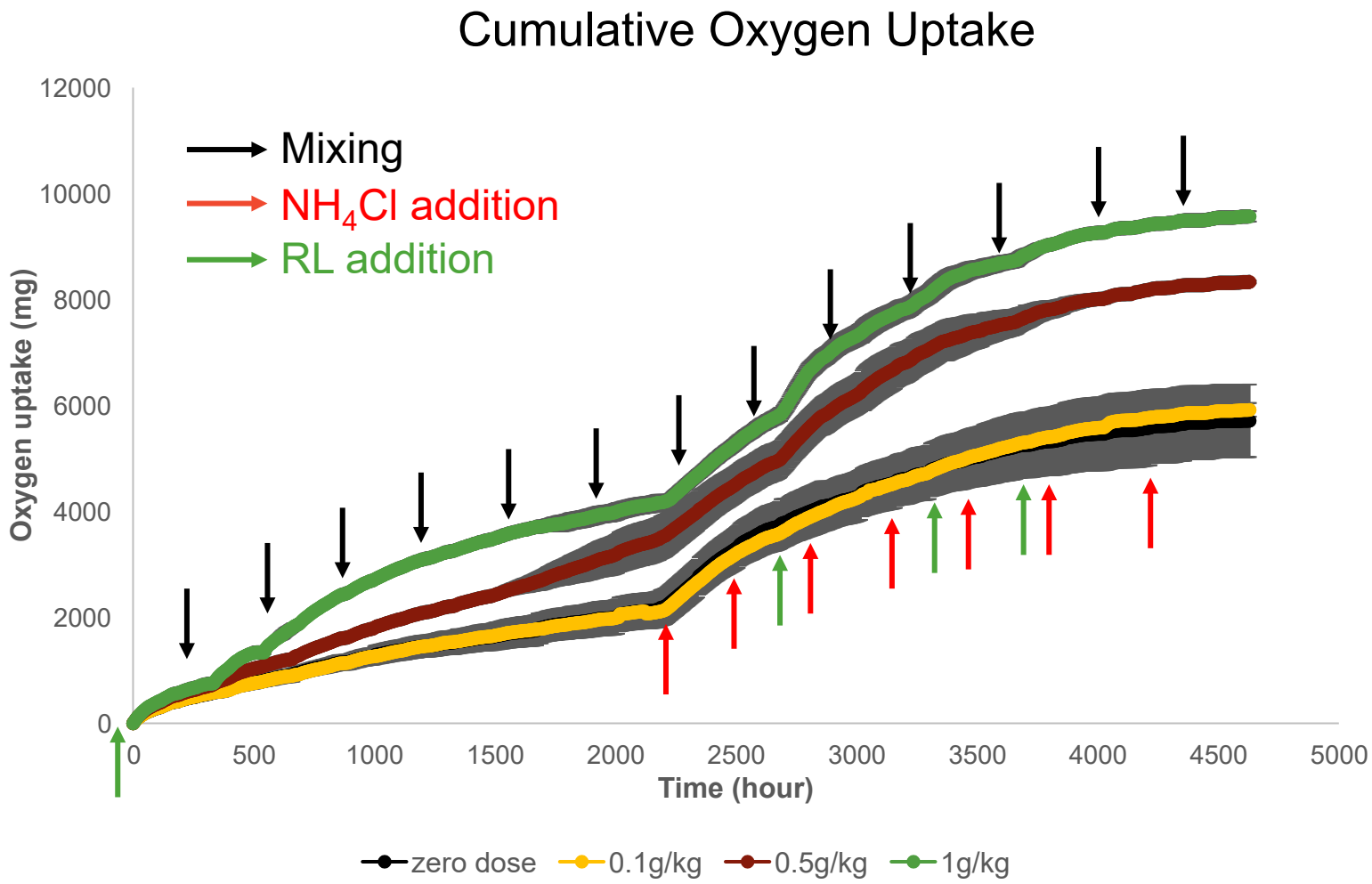
Respirometer can monitor eight soil samples simultaneously





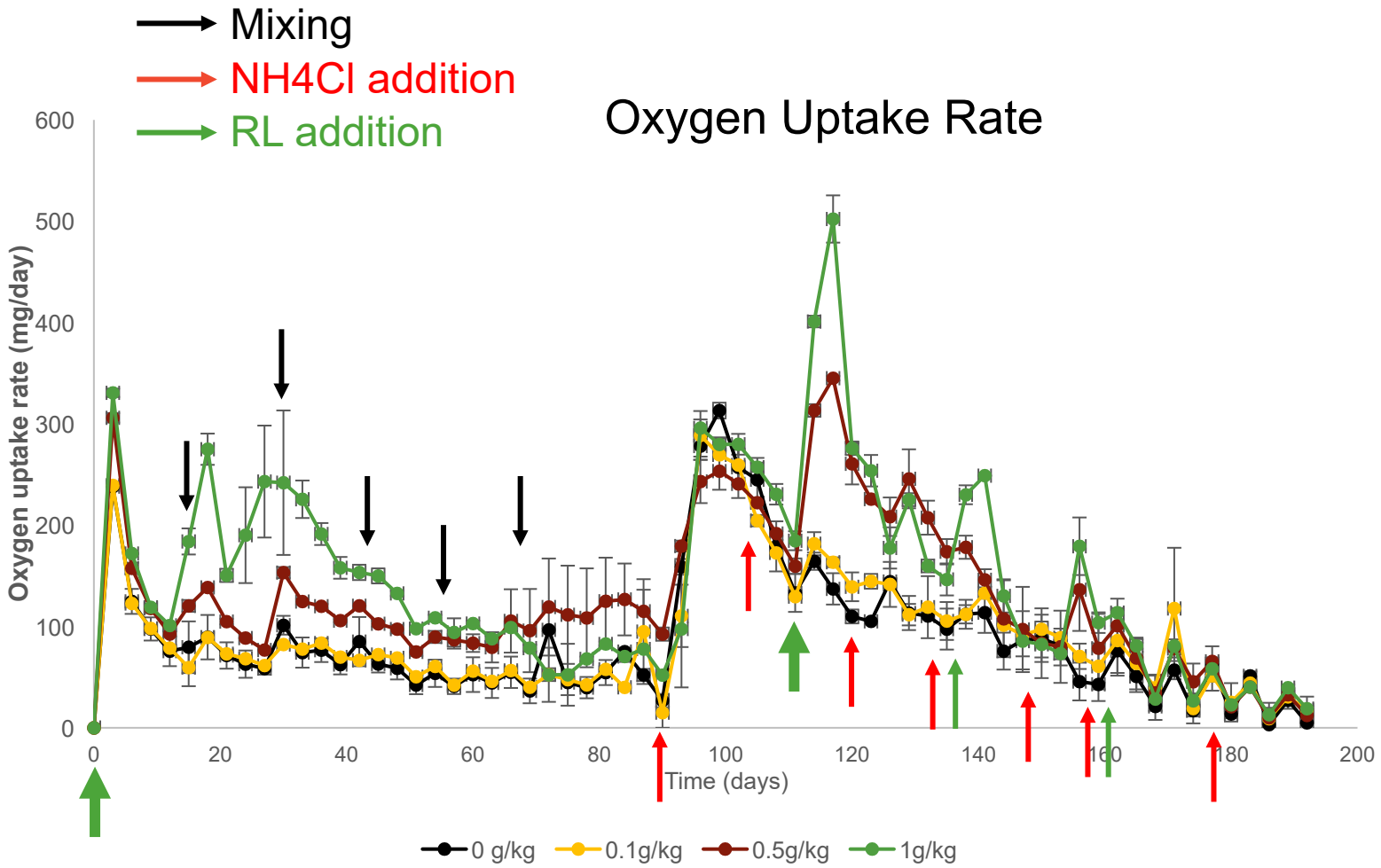


Lab Respirometer Results Over 193 Days (4650 hours) of Operation



- Cumulative oxygen uptake
  - Indication of bacteria activity in the soil
- Scheduled mixing and treatment
  - Aeration and water
  - Nitrogen source
  - Rhamnolipid

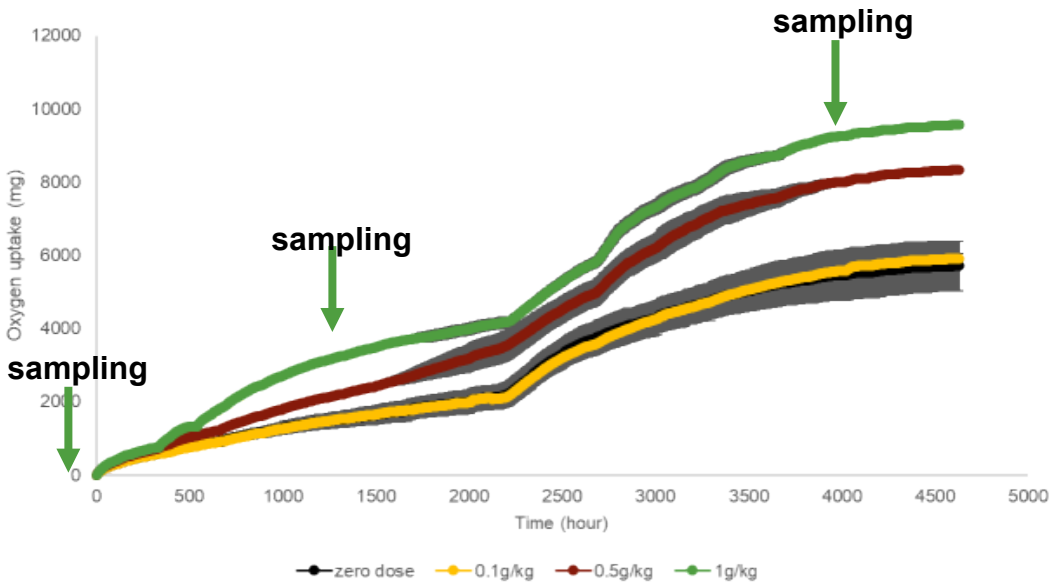
Impact of Mixing, Amendments Applied to the Soil Bottles

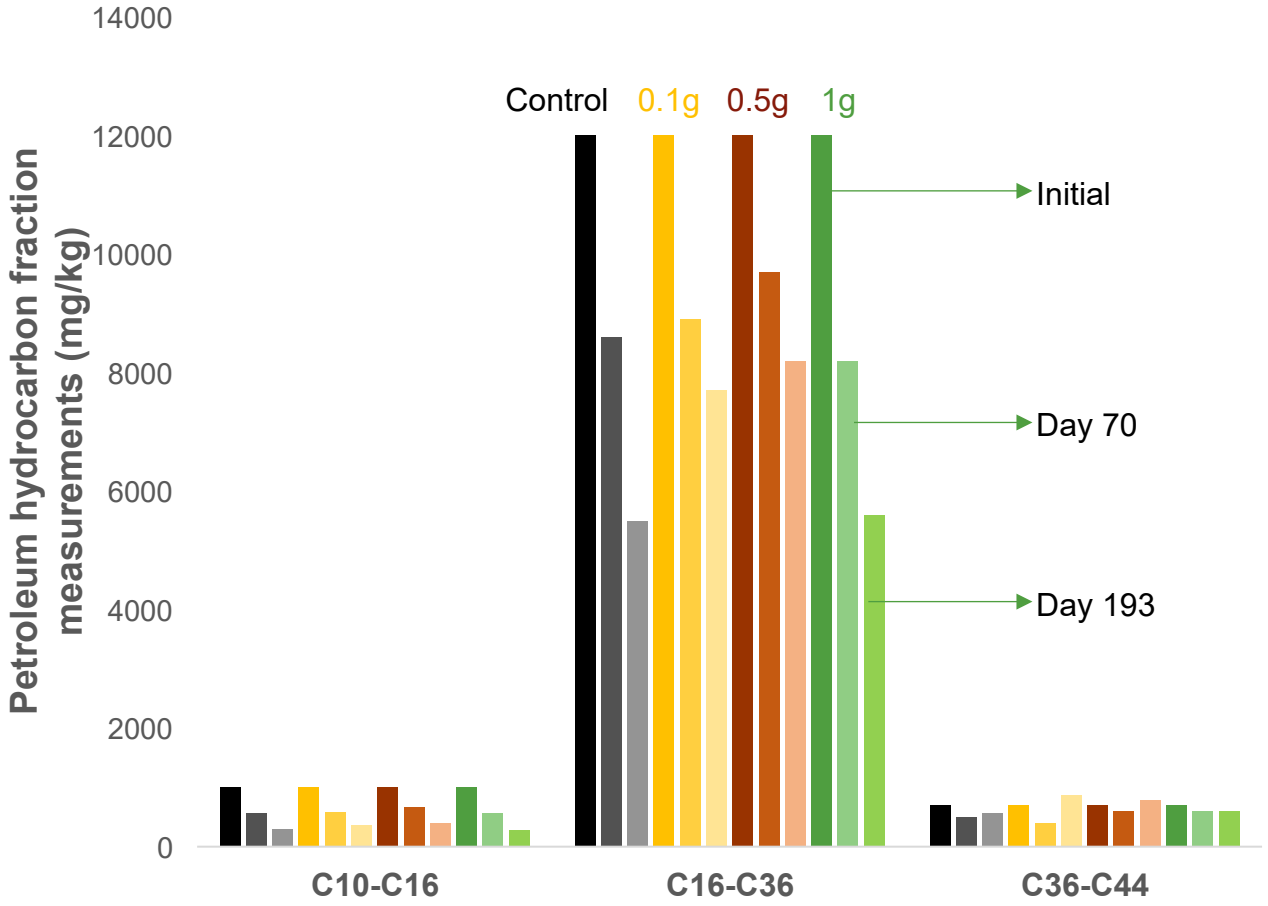


- Oxygen uptake rate (calculated)
  - Easier way to look at the impact of mixing and amendment addition
- Scheduled mixing and treatment
  - Aeration had a bigger impact at the beginning of the experiment
  - The first two RL addition showed a significant boost in bacteria activity
  - Activities of bacteria increase as increase the dosage of rhamnolipid

TPH from the Lab Data by GC

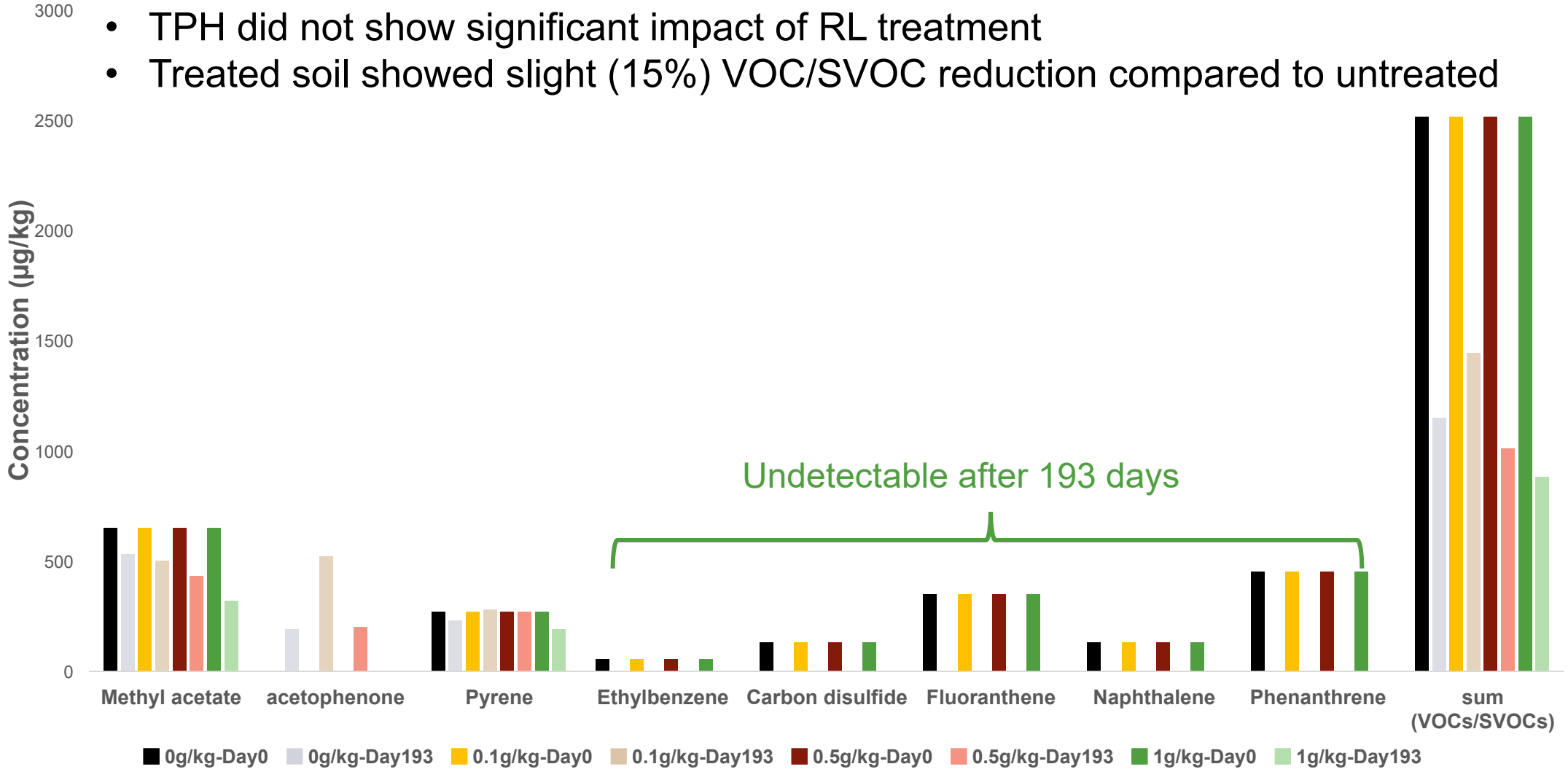
- Method:
  - GC/FID following EPA 8015C
- Results:
  - No significant impact of Rhamnolipid addition on the TPH



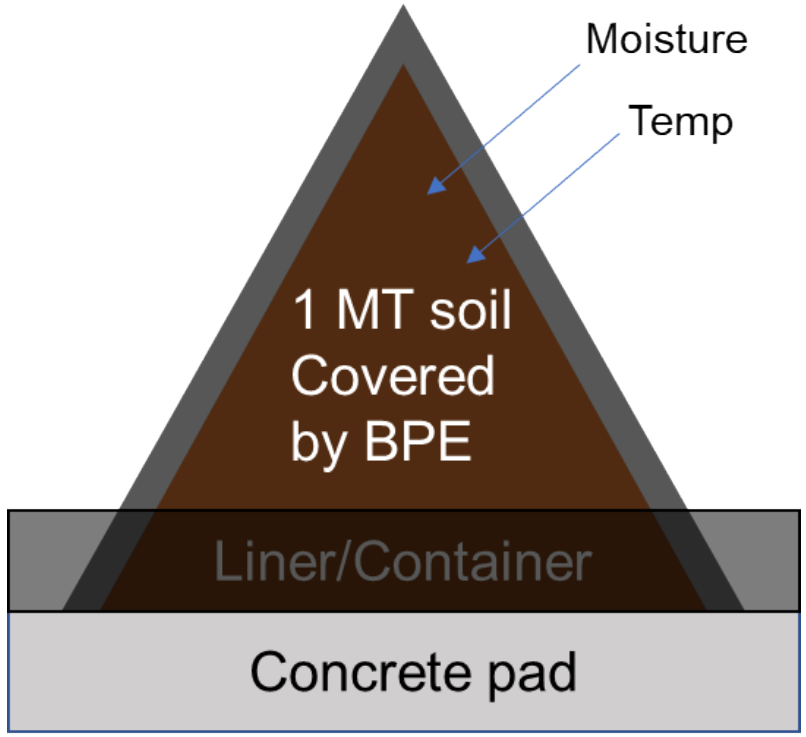


Lab VOC and SVOC Analysis Before and After 193 Days

- TPH did not show significant impact of RL treatment
- Treated soil showed slight (15%) VOC/SVOC reduction compared to untreated



Field Study Design

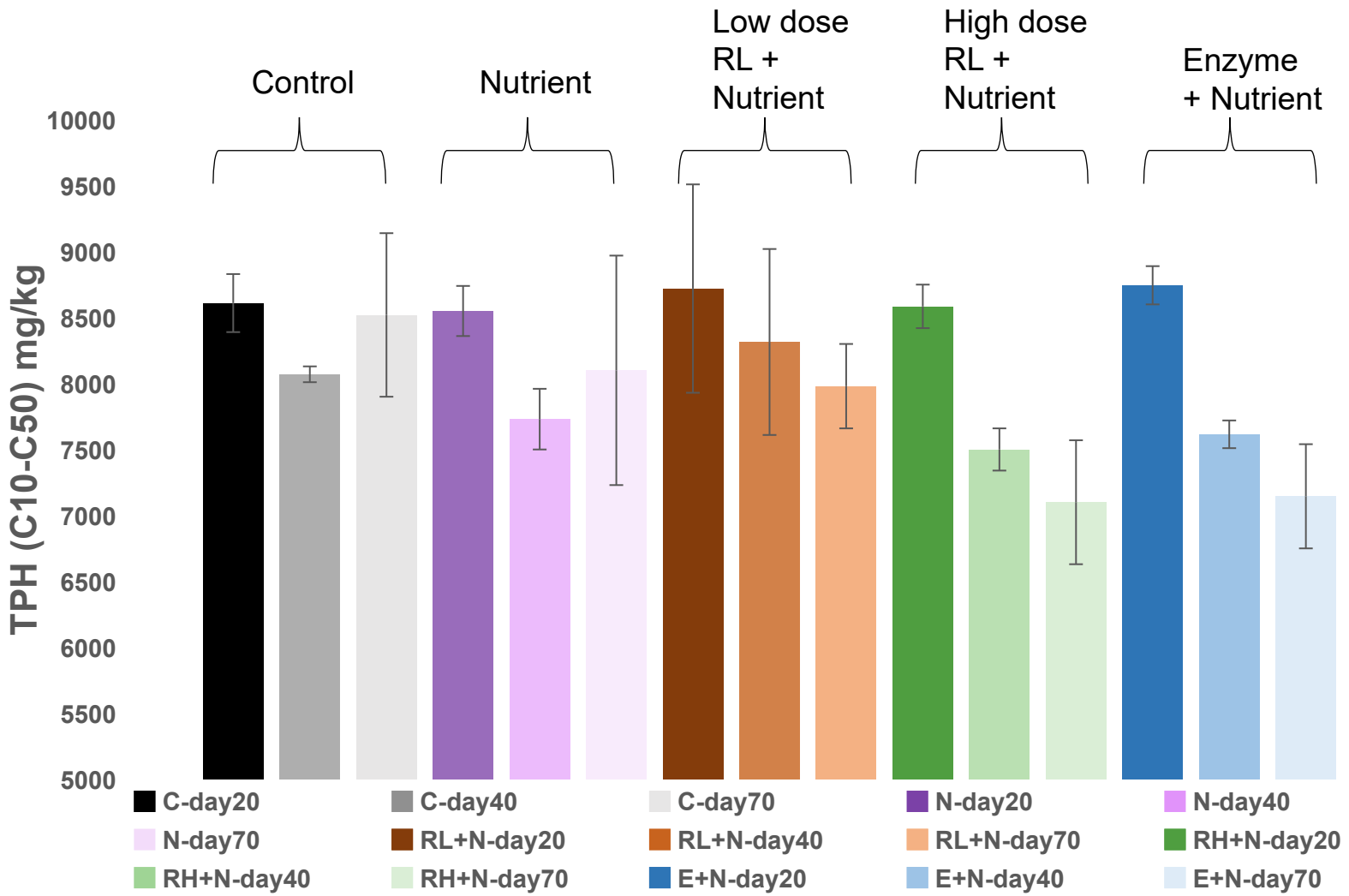


- Pile size: 1000 kg soil/pile, 1.5m x 1.5m concrete pad
- 5 Pairs of Treatments
  - 2x control (no amendments)
  - 2x nutrient control
  - 2x RL @ 0.5 g/kg
  - 2x RL @ 1.0 g/kg
  - 2x Enzyme @ 10 g/kg
- Monitoring/Sampling plan:
  - Moisture meter (weekly measurement)
  - Temperature (daily measurement)
  - Nutrient (External Lab, every 3 wks)
  - Total organics (External Lab, every 3 wks)
  - Initial/final bacteria (External Lab)
  - Hydrocarbon analysis (External Lab, every 3 wks)
- Timeline: Aug 2022 – Oct 2023

Field Soil Pile Setup

TPH Analysis/Results (70 days) of Field Trial in Canada Showed Positive Impact of RL and Enzyme Treatment

- Field vs. Lab
  - Less mixing
  - Air vs. oxygen
  - Inconsistent moisture level due to rain and condensation
  - Only initial treatment and 2-month experiment/data collecting window
- Results
  - Adding nutrients only had almost no impact
  - Higher (1g/Kg) RL dosage showed 18% more HC reduction than the untreated
  - Better results than the lab
  - Comparable results with the commercial enzyme solution



Additional Lab Soil Tests

- **Texas diesel-contaminated soil**
  - Contamination level >15%, the addition of RL has no impact due to the extremely low population of indigenous bacteria

- **New Jersey aromatic solvent contaminated soil**
  - Contamination level <1% (0.5%), the addition of RL seems to have no detectable impact of increasing the biodegradation of HC but other organics in the soil

Conclusions and Next Steps

- **Conclusions:**
  - ✓ Rhamnolipid addition can promote bacterial activity in hydrocarbon contaminated soil
  - ✓ Rhamnolipid was most effective in helping indigenous bacteria remediate hydrocarbon when the contamination level was in an intermediate range (1-15%)
  - ✓ Field results showed better remediation than the lab study (ideal conditions in the lab enabled bacteria activity in the absence of RL)
- **Next steps:**
  - ➢ Complete the second part of the field trial (2023)
  - ➢ Seek partnerships more field pilot trials

Acknowledgements

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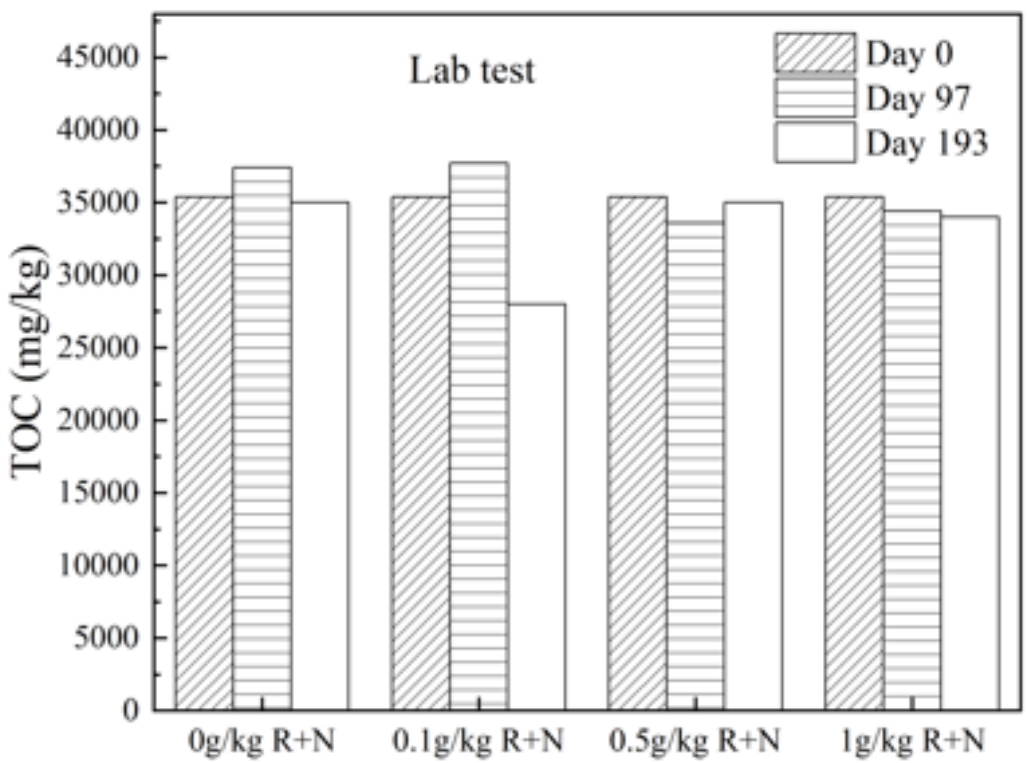
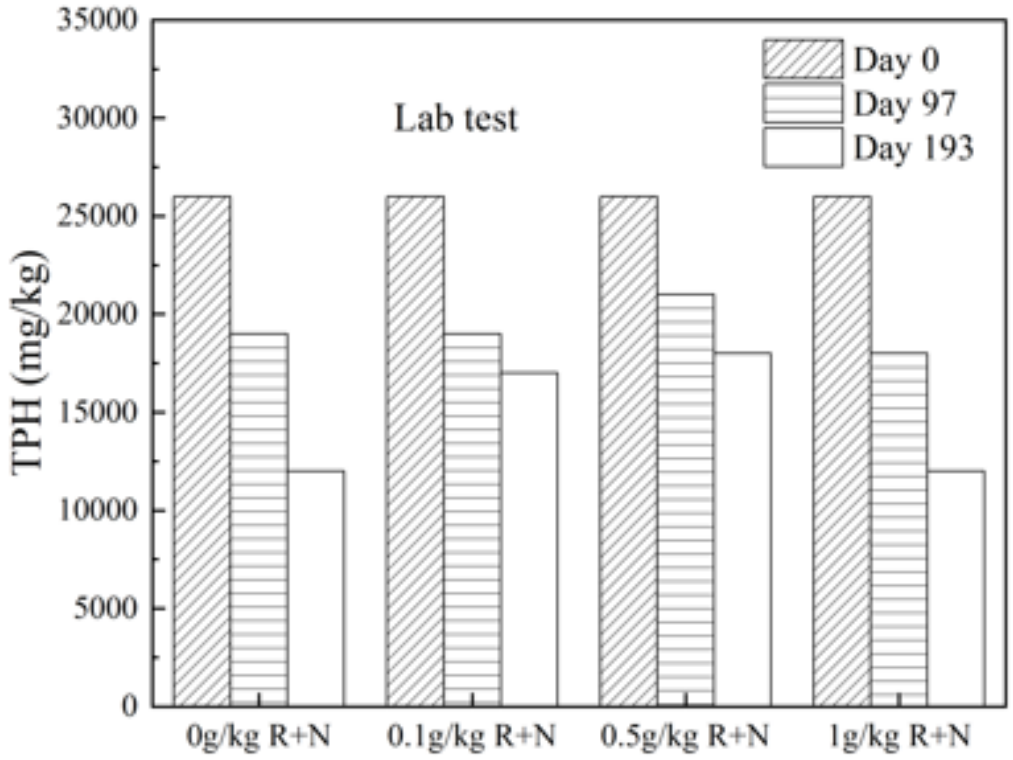
Appendix 1. Lab TOC vs. TPH





Appendix 2. Field TOC vs. TPH