Research Project Summary

Project Details

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Title: Anaerobic Bioremediation – Chlorine to Hydrogen (Reductive Dehalogenation & Microbial Chain Elongation)
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Background and Rationale

Contaminants in the environment pose health risks to humans and other organisms.
Using organisms to convert harmful contaminants to less harmful compounds can help redeem the environment (e.g., soil and groundwater) such as, cleaning of superfund sites - locations the EPA has deemed hazardous and in need of cleanup.
An example of one such hazardous chemical are chlorinated solvents; e.g., perchloroethylene (PCE) and trichloroethylene (TCE), common in groundwater. Industrial chlorinated solvents are found in the majority of many Superfund sites.
Bioremediation can be used to clean up contamination at a superfund site. A common form of bioremediation to cleanup chlorinated solvents is reductive dechlorination (or reductive dehalogenation), an anaerobic metabolism done by *Dehalococcoides mccartyi*. These bacterial microorganisms convert PCE, TCE, and other chlorinated solvents to non-toxic ethene or ethane.
Reductive dechlorination needs H_2 typically provided by fermentation. Fermentation is the breakdown of long-chain, complex organic molecules to produce hydrogen.
In a process called microbial chain elongation (MCE), anaerobic microorganisms produce H_2 through elongation of short-chain simple organic molecules to long-chain, complex organic molecules (the opposite of fermentation). MCE can support reductive dechlorination, like fermentation, through hydrogen production. Whether fermentation or MCE occurs in the subsurface will overwhelmingly depend on the substrate introduced: either long-chain, complex organics or short-chain, simple organics.
Current methods of bioremediation can lead to subsurface bioclogging, incomplete transformation of chlorinated solvents, methane production (a greenhouse gas), along with other issues.
Using MCE to support reductive dechlorination could be the only solution at certain sites where chemical/physical remediation has been shown to be ineffective. This is an import process to aid a return of sites to a more environmentally safe setting and can do so without high costs.

Research Objectives

- Set up calibration points for MCE products (volatile fatty acids C1-C8, lactate, ethanol, butanol, and hexanol) on High Performance Liquid-Chromatography (HPLC) to evaluate MCE products at a superfund site.
- Through DNA extraction assess microbial community abundance and quantify relevant organisms (methanogens and *D. mccartyi*) using qPCR.
- Data comparison (MCE products, microbial community abundance, quantity of relevant microorganisms) to evaluate groundwater at superfund site with new MCE substrate of ethanol and acetate (9:1, respectively), versus, more complex organic fermentable substrates of potassium lactate, sodium lactate, molasses, and emulsified vegetable oil, previously used.

Methods and Materials

Conduct laboratory testing (DNA extraction, HPLC) using Superfund site groundwater received.

- Superfund site injectate mixture (9:1 ethanol-to-acetate molar ratio)
- Superfund site baseline (groundwater *before* MCE-driven operation began starting June)
- Superfund site groundwater (groundwater *after* MCE-driven operation began and throughout June)
    - We have received this weekly.
- High-performance liquid chromatography instrument (company is Shimadzu)
    - The HPLC is used to measure MCE products (i.e., carboxylates and alcohols)
- Anaerobic glovebox
    - We have been using this to take our samples in case we want to use the groundwater in a project moving forward and do not kill the anaerobic bacteria of interest (i.e., *C. Kluyveri*, *D. Mccartyi)*
- 0.2 μm filter
    - We use this filter to clarify our samples for HPLC analysis. Particle sizes over 0.2 μm in a sample can damage the HPLC instrument and give inaccurate data.
- 3 mL sterile syringe
    - We use these to draw up liquid sample in the anaerobic glovebox.
- Sterile microcentrifuge tubes
    - We use these to collect sample in the anaerobic glovebox for DNA extraction and qPCR.
- HPLC vials
    - These are the vials that run on the HPLC
- DNA Blood & Tissue Kit (company is Qiagen)
    - This is the kit we use for DNA extraction
    - https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjTrPydy9D_AhU9LkQIHdF_CJUQFnoECBIQAQ&url=http%3A%2F%2Fwww.qiagen.com%2Fus%2Fresources%2Fdownload.aspx%3Fid%3D68f29296-5a9f-40fa-8b3d-1c148d0b3030%26lang%3Den&usg=AOvVaw3YbZ5mcdZw40JfiPLFcKvf&opi=89978449
    - We follow the protocol for “gram-positive bacteria” on page 50.
- Supplies to create calibration curves:
    - We made a 5 point calibration curve on the HPLC for:
        - Lactic, formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, heptanoic, and octanoic acid (these are all carboxylates listed from short-carbon chain to long-carbon chain)
        - Ethanol, butanol, hexanol (these are all alcohols)

Experimental Results

Data collected on days 0-13 showed propionate was produced. Acetate was consumed until day 3, and after day 3, acetate was produced. Ethanol was rapidly consumed after day 6.

Research Conclusions

Propionate production the major MCE product. Although acetate was consumed until day 3, after day 3 acetate production was likely a product of MCE.
Further research is needed to assess the microbial community and determine if MCE microorganisms fed with the new substrates are driving reductive dechlorination.

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References

[1] Microbial Chain Elongation and Subsequent Fermentation of Elongated Carboxylates as H2-Producing Processes for Sustained Reductive Dechlorination of Chlorinated Ethenes
Aide Robles, Theodora L. Yellowman, Sayalee Joshi, Srivatsan Mohana Rangan, and Anca G. Delgado
Environmental Science & Technology 2021 55 (15), 10398-10410

DOI: 10.1021/acs.est.1c01319 [2]

[2] Robles, A. (2019). *Reductive Dechlorination Sustained by Microbial Chain Elongation* (Order No. 13864349). Available from Dissertations & Theses @ Arizona State University; ProQuest Dissertations & Theses Global. (2228126831). http://login.ezproxy1.lib.asu.edu/login?url=https://www.proquest.com/dissertations-theses/reductive-dechlorination-sustained-microbial/docview/2228126831/se-2

[3] Chain Elongation with Reactor Microbiomes: Open-Culture Biotechnology To Produce Biochemicals
Largus T. Angenent, Hanno Richter, Wolfgang Buckel, Catherine M. Spirito, Kirsten J. J. Steinbusch, Caroline M. Plugge, David P. B. T. B. Strik, Tim I. M. Grootscholten, Cees J. N. Buisman, and Hubertus V. M. Hamelers
Environmental Science & Technology 2016 50 (6), 2796-2810
DOI: 10.1021/acs.est.5b04847