Development of Resting Cell Assay Protocol to Characterize Sulfolane Degrading Bacteria

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URSA Undergraduate Research & Scholarly Activity

Abstract

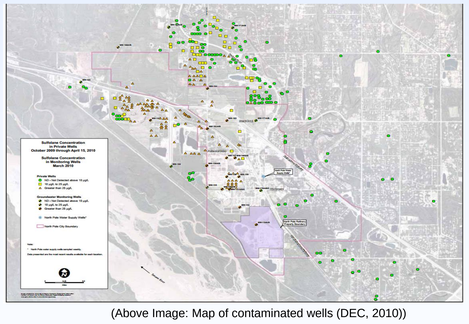
Sulfolane is a chemical contaminant present in hundreds of commercial and residential drinking wells in the North Pole area. Due to the possible health impacts of consumption, degradation/removal of the sulfolane from contaminated wells is necessary. Microbial isolates taken from sulfolane contaminated sites have shown the potential to degrade sulfolane. It is the purpose of my research to design a protocol by which sulfolane tolerant bacteria could be screened for their potential to utilize sulfolane as a sole carbon source (SOCS), and characterize them as sulfolane degraders.

To develop my protocol, I chose 3 strains of potential sulfolane degrading bacteria from a group of 23 isolates and incubated them in a sulfolane-containing liquid medium. After the cells had achieved a resting phase of growth, they were removed from culture and set in a resting cell assay using sulfolane as the only carbon source.

The final concentration of sulfolane present in the assays was measured via GC/MS. Although no conclusive data has been drawn as to degradation potential, several isolates still need to be screened and the methods refined.

Introduction

In 2009, sulfolane, an industrial chemical solvent used in the “sweetening“ of natural gas, was discovered to have contaminated nearly 300 drinking wells in the North Pole Area (ADEC, 2013). Because sulfolane has proven toxic in acute toxicity studies and the health impacts of chronic consumption are unknown, remediation of sulfolane from the contaminated aquifer is necessary (CCME, 2006)(Parnell, 2010).



(Above Image: Map of contaminated wells (DEC, 2010))

It is possible that the sulfolane spill is being attenuated by microbes in the environment. In fact, researchers have already identified several possible sulfolane degrading bacteria (Greene, Beatty, Fedorak, 2000). For this purpose, investigations into bacterial potential to degrade sulfolane are needed. A protocol by which bacteria can be screened for sulfolane degradation potential is a pivotal step in laying way for future research and assessing the degradation capabilities of known isolates.

Methods

Isolates

Bacterial isolates were obtained from slants present in the Leigh microbiology lab. The bacteria contained within the slants were initially isolated from sulfolane contaminated soil and granularly activated carbon (GAC) filters. Isolates were streaked for pure cultures on a sulfolane containing, selective medium agar plates.

Incubations

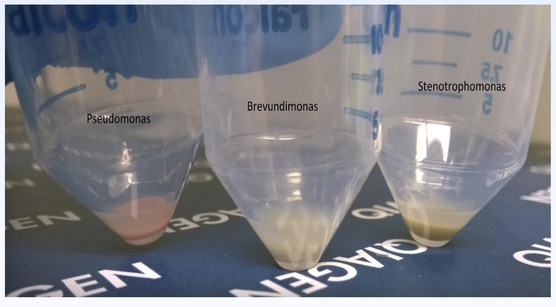
Bacterial isolates were incubated at 30^0 C in a selective liquid broth medium containing a concentration of 100 parts per million (ppm) sulfolane. All cultures were shaken at 150rpm. Separate incubations were established for assessment of bacterial growth and preparation for resting cell assay.

Growth Curve

Assessment of bacterial growth in liquid cultures was conducted by measuring their absorbance at a wavelength of 600nm with a corrective pathlength. Optical density measurements were taken 8 times over the course of 13 days to generate growth curves.

Resting Cell Assay

The resting cell assay was conducted on isolates that exhibited growth in high concentrations of sulfolane. Pure cultures at resting phase were transferred to the resting cell assay through pelleting, washing, and suspension in phosphate-buffered saline with a 5ppm concentration of sulfolane as the only carbon source. Pelleting was conducted by centrifugation of liquid culture. For each live sample in the assay, a control containing the respective bacteria was established with autoclaved cells. Samples were frozen upon reaching assay time point.

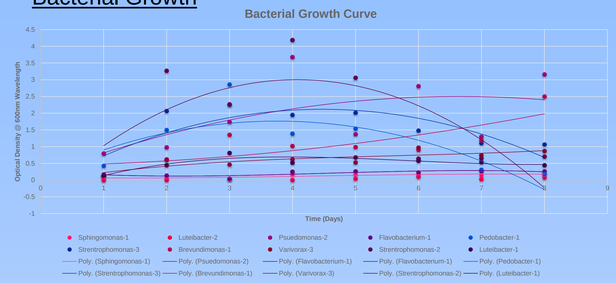


Gas Chromatography / Mass Spectroscopy Analysis of Sulfolane Concentration and Organic Extractions

Sulfolane concentration was derived from data generated through GC/MS analysis. Sulfolane was extracted from samples by washing 4.5mL of sample with 7.5mL dichloromethane ($CHCl_2$). 0.5mL of D8 sulfolane was added to evaluate organic extraction efficiency. Internal controls were established with nitrobenzene to ensure reading consistency.

Results

Bacterial Growth



(Fig. 1: A graph of the bacterial growth measured in optical density. Several strains reached resting phase despite the 100ppm concentration of sulfolane)

Several of the cultures grew quick in the presence of sulfolane. The fastest growing cultures were of the genera *Stenotrophomonas*, *Pseudomonas* and *Pedobacter*.

Resting Cell Assay

(Fig. 2: A graph of final sulfolane concentrations present in the dead controls and live samples of Stenotrophomonas. Lines integrated into the bars represent standard deviation.)

Analysis of sulfolane concentrations showed no significant difference in the concentration of sulfolane remaining between 0 hr. (T=0) and 48 hr. (T=2) in the samples collected from *Stenotrophomonas*.

Ongoing Work

Analysis of final time points (T=3, 72 hr.) and samples (Brevundimonas-1 and Pseudomonas-2), are still ongoing. After extractions have been conducted and sulfolane concentrations calculated, updates to experimental procedure will be provided. In addition to my ongoing assays, I am currently attempting to grow several of the isolates in a SOCS medium to ensure expression of sulfolane degrading genes.

Conclusion/Discussion

Though no significant difference can be discerned between the two time points I had defined, or between the controls, it is still possible that the *Stenotrophomonas* isolate is able to degrade sulfolane. Lowering the initial concentration of sulfolane to 500ppb, increasing my sample size and the addition of a sterile control (no cells) are all considerations when refining the methods I employed. Data collection is ongoing for the other samples, and it is possible that either one of them may exhibit sulfolane degradation in the resting cell assay.

Furthermore, there is the chance of genetic regulation inhibiting the expression of sulfolane degrading genes when other carbon sources are present, and sulfolane degrading genes could be evacuated on plasmids when they are not needed. Growing the bacteria in a SOCS medium before the addition to a resting cell assay may work to prevent the inhibition or loss of genes correlated or responsible for sulfolane degradation pathways.

In growing the bacterial cultures, sufficient replication should be utilized to ensure statistical veracity of the growth curves. Additionally, growing the cultures alongside sterile controls would establish a baseline for experimental error.

Several other notes were taken during the process of my research in regards to the tools used, growth media recipes, and techniques employed for utilizing equipment and technology. Future research with refined methods and sufficient replication is needed to fully characterize the remaining isolates. Upon validation of the isolates ability to degrade sulfolane, identification of the isolate to the species level should be conducted through 16s rRNA sequencing.

References

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