Oregon State University

Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.

Culturing and Identifying Microorganisms in Basalts Targeted for Geologic Carbon Sequestration

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Abstract

Today's fossil fuel resources exceed 5000 gigatons of carbon(GtC) with the world consuming 6 GtC a year, which suggests that current CO_2 emissions will become unsustainable unless greenhouse gas emissions are reduced(Lackner, 2003). One proposed solution is to inject CO_2 into geologic formations capable of undergoing mineralization reactions to permanently store carbon; however, the interaction between the diverse communities of microorganisms and CO_2 is unknown. The purpose of this study is to examine the changes in a microbial community during a supercritical CO_2 ($scCO_2$) injection simulation experiment and to culture model microorganisms for future reference. Basalt cores and natural formation water samples were placed in small autoclaves at 35°C and 1200 psi. Each set of autoclaves were opened after 55, 106, and 146 days of incubation. Water samples containing basalt subsamples were then sparged and vortexed for 15 minutes and 1 mL was injected into Hungate tubes filled with media designed to enrich for sulfate reducers, iron reducers, and hydrogen utilizing bacteria, respectively. Results have yet to be obtained, but similar studies support the predicted result of shifting microbial community composition towards hydrogen utilizing microbes with sulfate reducing and iron reducing bacteria suffering a sudden drop in biomass.

Introduction

Carbon dioxide emissions are major contributors to global climate change. Geologic carbon sequestration is one of many technologies being developed to help alleviate the effect of carbon emissions on the environment.

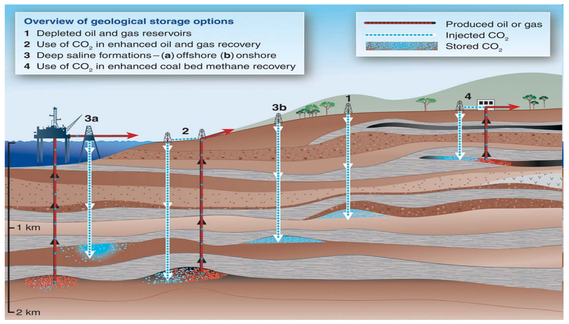

Fig.1: Different types of geologic carbon sequestration, with 3b being the best depiction of the Wallula pilot well.(Orr, 2009)

Geologic carbon sequestration in basalts is considered a long-term storage method due to mineralization reactions that convert CO_2 to carbonate. A pilot well located along the Columbia River at Wallula Gap has been drilled in order to investigate the feasibility of geologic carbon sequestration in basalts.

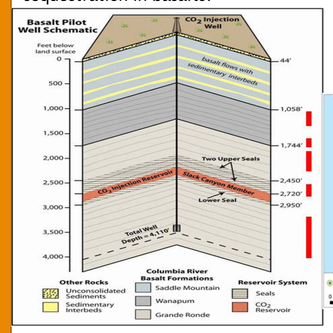

Figure 2: A schematic of the Wallula pilot well

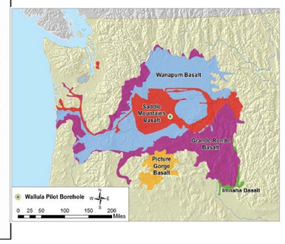

Figure 3: A map depicting the location of the Wallula well

This study is conducting an experiment on the role of microorganisms in carbon sequestration by determining the most prevalent organisms in the post-injection environment. In addition, this study is providing potential model organisms for future injection simulation experiments.

Questions

What are the most abundant culturable microorganisms present in groundwater collected from the Wallula pilot well prior to supercritical carbon dioxide injection?

What are the most abundant culturable microorganisms present after the injection of carbon dioxide into the basalts following high temperature/high pressure incubations?

Objectives

Enrich iron reducing, sulfate reducing, and hydrogen utilizing microbes that are potentially present in water samples from the Wallula well in the Columbia River Basalts group.

Develop model organisms that can be used in future geologic carbon sequestration and aquifer studies.

Materials & Methods

Three positive control microorganisms were chosen:

- *Desulfovibrio vulgaris* to represent sulfate-reducing bacteria(SRB)
- *Shewanella oneidensis* to represent the iron-reducing bacteria(IRB)
- Methanogen to represent the hydrogen utilizing bacteria(HUM).

Specific media were made for SRB, IRB, and HUM to isolate the different microbes.

Fig.4: The media and components of media shown here are Magot's N,M, and Acidphilium media for iron reducers. (Basso, 2009) Postgate's B and C media for sulfate reducers. (Geomicrohandbook citation) No hydrogen utilizing media were selected.

Tubes were prepared by autoclaving for 15 minutes and filled with nitrogen gas for 10 seconds.The samples were placed on vortexers for 15 minutes to ensure that bacteria mixed well with the water. Media and sample water was injected into the Hungate tubes with a syringe. Incubation occurred in Hungate tubes filled with IRB,SRB, and HUB media with 1 mL of sample water and 5 mL of media. A set of tubes for one medium consisted of one tube for sterile control, one tube for positive control by inoculation of a control species, and duplicates of each sample. The tubes were left to incubate for 2 months at 30°C.

Fig.5 : Culturing is done under anaerobic conditions by sparging the media and samples under nitrogen gas for 15 minutes in the gassing station on the left. Inoculation occurs in the glovebox on the right.

The samples were prepared by incubating basalt cores and well water for 55, 106, and 146 days respectively.

Fig.6: Autoclaves in which the samples were incubated in at 35°C and 1200 psi.

Results

No results to date have been obtained from this study because the microorganisms require 2 months to reach significant growth; however, results from similar studies will be cited to show predicted results. The tubes have been incubated at 30°C and will be opened on October 24th for cell counts, isolation, and cloning.

The expected *in situ* result is a community, at or close to the carrying capacity, that will experience a sudden loss in biomass due to the sudden change in conditions and regrowth to a lower biomass with a shift in community composition.

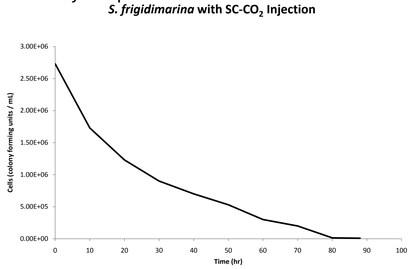

Fig.7: Data from Mitchell for S. frigidimarina shows a drop in biomass after supercritical CO_2 injection. The trend is slow since the microbes exist in biofilm form.

Mitchell's study on supercritical CO_2 and biolfilms supports the sharp decrease in biomass by providing some experimental results of the population biomass before and after the supercritical carbon dioxide injection(Mitchell, 2008). The difference between this study and the Mitchell study is the absence of a starvation period. Microbes in biofilms are able to resist changes in pH and chemical composition better than planktonic microbes(Mitchell, 2008).

Discussion

The expected shift in microbial community composition should show an initial community dominated by sulfate and iron reducers. Once the supercritical carbon dioxide is injected the community should show an increase in hydrogen utilizing microbes due to the release of hydrogen from the basalt due to mineralization and the amount of carbon dioxide available in the water providing a rich nutrient source for those microbes. The community should return to a composition similar to the initial composition at some point, but will have a much lower biomass than the initial community. The diversity of the microbial community will also be reduced due to the Bottleneck Effect.

Some issues with this study are problems with water loss in the autoclaves, the effect of pH on the microbes, and guessing ingredients from international papers such as using tripticase for biotrypcase in the Magot N medium.

Future Work

The most probable number method with five tubes will be used to determine the quantity of IRBs, SRBs, and HUBs . Isolates will be made by injecting the media that has been incubated on to solid media and streaking individual colonies. The isolates are cloned and undergo DNA extraction and amplification. The clones are sent for sequencing to determine the phylotypes of the isolated organism. The isolates can then be used as model organisms in future studies of the interactions between basalts and native microorganisms.

The rate of mineralization is also an issue due to the sudden change in pH and the permeability of the biofilms. Current studies show debate on whether hydrogen gas could be produced in quantities capable of support hydrogen utilizing bacteria, which would affect the biofilm composition and the ability to store CO_2 in carbonate form. There is the possibility of seismic events causing cracks that could lead to CO_2 leaking back into the atmosphere.

Methanogens provide an extremely interesting platform for future work. They require hydrogen gas and carbon dioxide as energy and carbon sources, which could allow more injections into the well. Methanogens also produce methane gas, which could transform wells into potential renewable natural gas reservoirs. This would suggest that geologic sequestration would not be a repository, but could become a potential resource. This notion would require substantial testing to determine the viability of methanogens.

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