International Journal of Greenhouse Gas Control 47 (2016) 386–395

Contents lists available at ScienceDirect

International Journal of Greenhouse Gas Control

journal homepage: www.elsevier.com/locate/ijggc

Interplay between microorganisms and geochemistry in geological carbon storage

Matthew F. Kirk[a], Susan J. Altman[b,*], Eugenio-Felipe U. Santillan[c,1], Philip C. Bennett[c]

[a] Department of Geology, Kansas State University, Manhattan, KS 66506, United States
[b] Geochemistry Department, Sandia National Laboratories, Albuquerque, NM 87185, United States
[c] Department of Geological Sciences, Jackson School of Geosciences, The University of Texas at Austin, Austin, TX, United States

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 26 January 2016
Accepted 29 January 2016
Available online 28 February 2016

Keywords:
Carbon trapping
Porous media
Subsurface microbiology
Bioenergetics
Capnophile
Bioreactor experiments

ABSTRACT

Researchers at the Center for Frontiers of Subsurface Energy Security (CFSES) have conducted laboratory and modeling studies to better understand the interplay between microorganisms and geochemistry for geological carbon storage (GCS). We provide evidence of microorganisms adapting to high pressure CO_2 conditions and identify factors that may influence survival of cells to CO_2 stress. Factors that influenced the ability of cells to survive exposure to high-pressure CO_2 in our experiments include mineralogy, the permeability of cell walls and/or membranes, intracellular buffering capacity, and whether cells live planktonically or within biofilm. Column experiments show that, following exposure to acidic water, biomass can remain intact in porous media and continue to alter hydraulic conductivity. Our research also shows that geochemical changes triggered by CO_2 injection can alter energy available to populations of subsurface anaerobes and that microbial feedbacks on this effect can influence carbon storage. Our research documents the impact of CO_2 on microorganisms and in turn, how subsurface microorganisms can influence GCS. We conclude that microbial presence and activities can have important implications for carbon storage and that microorganisms should not be overlooked in further GCS research.

1. Introduction

Geologic carbon storage (GCS) involves the capture, compression, injection, and storage of anthropogenic carbon dioxide (CO_2) in order to mitigate carbon emissions to the atmosphere. Deep (>1 km below the ground surface) sedimentary formations are one of the largest sets of likely injection targets. Pore waters in potential storage reservoirs are typically saline with ionic strengths ranging from that of seawater to levels near those of fluids saturated with halite. Injected CO_2 will exist as a supercritical phase, given the ranges of pressures and temperatures at these depths (10–30 MPa and 310–380 K). High concentrations of dissolved CO_2 will alter groundwater pH and dissolved inorganic carbon (DIC) concentration, increase levels of dissolved ions, and cause both mineral dissolution and precipitation (Kaszuba and Janecky, 2009; Lu et al., 2010).

Benson et al. (2005) describes the four trapping mechanisms for GCS: structural, residual, solubility, and mineral. It is well recognized that these mechanisms are driven by geochemical and hydrological processes. Microbial processes may also be important, however, because microorganisms can influence hydrological and geochemical processes in subsurface environments (Baker et al., 2010; Banks et al., 2010; Davidson et al., 2011; Fredrickson et al., 1998; Gorbushina, 2007; Onstott et al., 1998; Pedersen et al., 1996; Sahl et al., 2008). For example, microbial biomass can enhance precipitation of carbonate minerals (Cunningham et al., 2009; Kandianis et al., 2008; Mitchell et al., 2010), clog porous media (Baveye et al., 1998), and alter water chemistry on a regional scale (Flynn et al., 2013; Kirk et al., 2015).

Microbial life extends deep into the subsurface, including depths of interest to GCS. The depth limit of microbial life in the subsurface is somewhat uncertain. However, active microorganisms have been confirmed at depths greater than 3 km (Kieft et al., 2005). Their ability to adapt to a wide range of environmental conditions (Pikuta et al., 2007) together with the vast size of the habitable subsurface allow subsurface microbes to play a major role in mediating

* Corresponding author at: P.O. Box 5800 MS0754, Sandia National Laboratories, Albuquerque, NM 87185, United States.
E-mail address: sjaltma@sandia.gov (S.J. Altman).
[1] Currently at: Smithsonian Environmental Research Center, Edgewater, MD 21037, United States.

http://dx.doi.org/10.1016/j.ijggc.2016.01.041
1750-5836/

global-scale biogeochemical processes (Colwell and D'Hondt, 2013; Orcutt et al., 2013; Parkes et al., 2014).

Changes in conditions following CO_2 injection will impose stress on indigenous microorganisms, potentially triggering changes in community composition (Mu et al., 2014; Peet et al., 2015; Wilkins et al., 2014). Where CO_2 exists as a supercritical phase, it may dissolve cell membranes and cause cell death (Dillow et al., 1999; White et al., 2006). High levels of CO_2 in an aqueous solution can also be toxic to microbes because CO_2 can pass through cell membranes, acidify cytoplasm, and disrupt cellular functions (Ballestra et al., 1996).

In addition to changes in community composition driven by CO_2 stress, CO_2 injection may also shift community composition by altering redox disequilibrium. When CO_2 dissolves into water, carbonic acid is produced, which can then dissociate into protons and dissolved inorganic carbon species:

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

Because many of the redox reactions used as a source of energy by microbes include dissolved inorganic carbon species as well as hydrogen ions, changes in CO_2 abundance affects the extent to which those reactions are out of equilibrium (Harvey et al., 2013; Kirk, 2011; Mayumi et al., 2013; Ohtomo et al., 2013). Such changes can significantly affect microbial activity because the amount of energy that is available in the environment for microbial reactions affects the ability of microorganisms to compete with one another. Microorganisms that conserve energy from more energetically favorable reactions can grow faster, and thus catalyze their reaction more rapidly, than those using less favorable reactions (Jin, 2012; LaRowe and Amend, 2015; Lovley and Goodwin, 1988; Roden and Jin, 2011).

In this paper, we examine geomicrobiological studies conducted at the Center for Frontiers of Subsurface Energy Security (CFSES) within the context of the interplay between microbiology and GCS. In other words, we consider what our findings tell us about how GCS could affect subsurface microbes and in turn, how subsurface microbes could affect GCS. Given the potential for microorganisms to influence the geochemistry and hydrodynamics of the subsurface, understanding this interplay may be a key to ensuring secure carbon storage. Moreover, this knowledge can provide a basis for developing biological strategies to enhance GCS reservoir performance (Mitchell et al., 2010).

CFSES is an Energy Frontier Research Center established by the Office of Science, Basic Energy Sciences program in the U.S. Department of Energy in 2009 and chosen for renewal until 2018. Researchers at CFSES have taken many different approaches to better understand the interplay between GCS and subsurface microbiology. Our research has identified and characterized an isolate from a CO_2-rich spring (Santillan et al., 2015). We used pure-culture batch reactor experiments to test the influence of mineralogy on the ability of cells to survive exposure to high-pressure CO_2 (Santillan et al., 2013). We considered how decreasing pH, a geochemical change caused by CO_2 injection, will affect the stability of bioclogging in porous media (Kirk et al., 2012). And, we used bioenergetics and mixed-community bioreactor experiments to assess potential changes in the relative significance of different microbial processes in response to increasing CO_2 abundance (Kirk, 2011; Kirk et al., 2013). These efforts provide insight into both sides of the two-way interactions between GCS and subsurface microorganisms.

2. Methods

The content below provides a brief summary of methods used in our investigations. For more details about these methods as well as our results, please refer back to the publications associated with each study.

2.1. Isolation

A capnophile, an microbe capable of growth in the presence of high concentrations of CO_2, was isolated and characterized as part of our effort to learn about properties of microbes in aqueous environments with high CO_2 levels (Santillan et al., 2015). The isolate was collected from Crystal Geyser spring, Utah, USA. The site is considered an analog site for GCS research and provides the opportunity to study a subsurface microbial community that has been exposed to elevated CO_2 over a long period of time (Emerson et al., 2015). CO_2 has been leaking from the subsurface near the geyser for over 400,000 years (Burnside et al., 2013).

Samples of water and microbial biomass were collected at 9.7 m depth in the spring outlet using aseptic techniques. Cultures were prepared immediately by placing filtered biomass in serum bottles that contained Luria Bertain broth amended with 15 g L^{-1} NaCl. The bottles were then placed within a pressure vessel and pressurized to 1 MPa with ultrapure CO_2. Cultures were incubated for about 1 month and then re-cultured multiple times to cultures containing Tryptic soy broth with 15 g L^{-1} NaCl. After three transfers, the cultures were diluted to extinction to obtain an isolate.

The isolate discussed in this paper, designated CG-1, was assessed for growth under various conditions that focused on CO_2, temperature, salinity, pH, carbon substrates, electron acceptors, and fermentation capability. Cloning was performed on GC-1 to determine its 16S gene identity through the Basic Local Alignment Search Tool search (BLASTn) search (http://blast.ncbi.nlm.nih.gov/). A phylogenetic tree relating the isolate to related sequences was made using CLUSTALX (Chenna et al., 2003). Cell morphology was characterized using transmission electron microscopy (TEM). Lipid samples were processed according to Rodriguez-Ruiz et al. (1998) and analyzed using gas chromatography mass spectrometry (GCMS).

2.2. Pure-culture experiments

Pure-culture experiments were performed to examine factors influencing the ability of cells to survive exposure to high-pressure CO_2 (Santillan et al., 2013). Experiments were conducted with three model organisms: *Shewanella oneidensis* strain MR-1 (ATCC BA-1096), *Geobacillus stearothermophilus* (ATCC 7953), and *Methanothermobacter thermoautitrophicus* (ATCC 29096). These organisms allowed the experiments to include variation in metabolic reactions as well as cell wall structure and composition. *S. oneidensis* is a Gram-negative bacterium that was grown under iron-reducing conditions, *G. stearothermophilus* is a Gram-positive aerobic bacterium that is capable of sporulation, and *M. thermoautitrophicus* is a methanogenic archaeaon. Species closely related to *G. stearothermophilus* and *M. thermoautitrophicus* have been detected in the deep subsurface (Kawaguchi et al., 2010; Nazina et al., 2001). *S. oneidensis* is widespread in soils and shallow sediment and has been studied within the context of CO_2 leakage to shallow groundwater from deep storage (Wu et al., 2010).

Organisms were grown to stationary phase in batch cultures and then placed in pressure vessels (Parr instruments) and exposed to elevated CO_2 pressure at 30 °C for time periods ranging from 1 to 24 h. CO_2 pressures tested ranged from 0.3 to 6.5 MPa. At the end of the exposure period, pressure was slowly released over a period of about 2 min to limit potential impacts of pressure change on cell survival. The cultures were then removed from the pressure vessels and sonicated to disperse biofilm and attached cells. Cell survival was quantified using cultivation. Cultivable *S. oneidensis* and *G. stearothermophilus* cells were enumerated using the pour