Interplay between Microorganisms and Geochemistry in Geological Carbon Storage

Matthew F. Kirk[‖], Susan J. Altman[†*], Eugenio-Felipe U. Santillan[§¥], Philip C. Bennett[§]

[‖]Department of Geology, Kansas State University, Manhattan, Kansas 66506
[†]Geochemistry Department, Sandia National Laboratories, Albuquerque, New Mexico 87185
[§]Department of Geological Sciences, Jackson School of Geosciences
The University of Texas at Austin, Austin, Texas
[¥]Currently at: Smithsonian Environmental Research Center, Edgewater, Maryland 21037

*Corresponding author: P.O. Box 5800 MS0754, Sandia National Laboratories, Albuquerque, New Mexico 87185. Telephone: 505 844 2397 email: sjaltma@sandia.gov

International Journal of Greenhouse Gas Control

Submitted: July 24, 2015
Revised version submitted: November 4, 2015
Second revision submitted: January, 2016 --- ACCEPTED

Key words: Carbon trapping, porous media, subsurface microbiology, bioenergetics, capnophile, bioreactor experiments

Classification: Geological storage – By mechanisms, processes and discipline
Geological storage - other
Geochemical – Coverage/Type

ABSTRACT **(200 words maximum)**

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 on carbon storage and that their presence 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 to 30 MPa and 310 to 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

82 microorganisms have been confirmed at depths greater than 3 km (Kieft et al., 2005). Their
83 ability to adapt to a wide range of environmental conditions (Pikuta et al., 2007) together with
84 the vast size of the habitable subsurface allow subsurface microbes to play a major role in
85 mediating global-scale biogeochemical processes (Colwell and D'Hondt, 2013; Orcutt et al.,
86 2013; Parkes et al., 2014).

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

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

97 $$CO_2(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

98 Because many of the redox reactions used as a source of energy by microbes include dissolved
99 inorganic carbon species as well as hydrogen ions, changes in CO_2 abundance affects the extent
100 to which those reactions are out of equilibrium (Harvey et al., 2013; Kirk, 2011; Mayumi et al.,
101 2013; Ohtomo et al., 2013). Such changes can significantly affect microbial activity because the
102 amount of energy that is available in the environment for microbial reactions affects the ability
103 of microorganisms to compete with one another. Microorganisms that conserve energy from

104 more energetically favorable reactions can grow faster, and thus catalyze their reaction more
105 rapidly, than those using less favorable reactions (Jin, 2012; LaRowe and Amend, 2015; Lovley
106 and Goodwin, 1988; Roden and Jin, 2011).

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

115 CFSES is an Energy Frontier Research Center established by the Office of Science, Basic
116 Energy Sciences program in the U.S. Department of Energy in 2009 and chosen for renewal until
117 2018. Researchers at CFSES have taken many different approaches to better understand the
118 interplay between GCS and subsurface microbiology. Our research has identified and
119 characterized an isolate from a CO_2-rich spring (Santillan et al., 2015). We used pure-culture
120 batch reactor experiments to test the influence of mineralogy on the ability of cells to survive
121 exposure to high-pressure CO_2 (Santillan et al., 2013). We considered how decreasing pH, a
122 geochemical change caused by CO_2 injection, will affect the stability of bioclogging in porous
123 media (Kirk et al., 2012). And, we used bioenergetics and mixed-community bioreactor
124 experiments to assess potential changes in the relative significance of different microbial
125 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

147 containing Tryptic soy broth with 15 g L^{-1} NaCl. After three transfers, the cultures were diluted
148 to extinction to obtain an isolate.
149 The isolate discussed in this paper, designated CG-1, was assessed for growth under
150 various conditions that focused on CO_2, temperature, salinity, pH, carbon substrates, electron
151 acceptors, and fermentation capability. Cloning was performed on GC-1 to determine its 16S
152 gene identity through the Basic Local Alignment Search Tool search (BLASTn) search
153 (http://blast.ncbi.nlm.nih.gov/). A phylogenetic tree relating the isolate to related sequences
154 was made using CLUSTALX (Chenna et al., 2003). Cell morphology was characterized using
155 transmission electron microscopy (TEM). Lipid samples were processed according to Rodriguez-
156 Ruiz et al. (1998) and analyzed using gas chromatography mass spectrometry (GCMS).
157
158 *2.2. Pure-culture experiments*
159 Pure-culture experiments were performed to examine factors influencing the ability of
160 cells to survive exposure to high-pressure CO_2 (Santillan et al., 2013). Experiments were
161 conducted with three model organisms: *Shewanella oneidensis* strain MR-1 (ATCC BA-1096),
162 *Geobacillus stearothermophilus* (ATCC 7953), and *Methanothermobacter thermoautitrophicus*
163 (ATCC 29096). These organisms allowed the experiments to include variation in metabolic
164 reactions as well as cell wall structure and composition. *S. oneidensis* is a Gram negative
165 bacterium that was grown under iron-reducing conditions, *G. stearothermophilus* is a Gram
166 positive aerobic bacterium that is capable of sporulation, and *M. thermoautitrophicus* is a
167 methanogenic archaeaon. Species closely related to *G. stearothermophilus* and *M.*
168 *thermoautitrophicus* have been detected in the deep subsurface (Kawaguchi et al., 2010;

169 Nazina et al., 2001). *S. oneidensis* is widespread in soils and shallow sediment and has been
170 studied within the context of CO_2 leakage to shallow groundwater from deep storage (Wu et al.,
171 2010).

172 Organisms were grown to stationary phase in batch cultures and then placed in pressure
173 vessels (Parr instruments) and exposed to elevated CO_2 pressure at 30°C for time periods
174 ranging from 1 to 24 hr. CO_2 pressures tested ranged from 0.3 to 6.5 MPa. At the end of the
175 exposure period, pressure was slowly released over a period of about 2 min to limit potential
176 impacts of pressure change on cell survival. The cultures were then removed from the pressure
177 vessels and sonicated to disperse biofilm and attached cells. Cell survival was quantified using
178 cultivation. Cultivable *S. oneidensis* and *G. stearothermophilus* cells were enumerated using the
179 pour plate method. *M. thermoautitrophicus* cells were was cultivated in liquid anaerobic
180 cultures with low CO_2 content. Growth was periodically assessed in the cultures by measuring
181 optical density at 680 nm. Iron reducing activity of *S. oneidensis* was evaluated by measuring
182 ferrous iron concentration using the ferrozine method (Stookey, 1970). Methanogenesis by *M.*
183 *thermoautitrophicus* was evaluated by measuring CH_4 partial pressure using gas
184 chromatography.

185 *S. oneidensis,* the model organism most susceptible to CO_2 exposure of those tested,
186 was selected for a second set of experiments that examined the effects of mineral solid phases
187 on CO_2 toxicity (Santillan et al., 2013). Minerals and rock samples (Ward's Natural Science,
188 Rochester, NY) were crushed to the size of coarse sand, cleaned of any magnetite they may
189 have contained using a hand magnet, and sterilized at 121 °C for 30 min. Test tubes with 10 mL
190 of growth medium and 1 g of autoclaved mineral were inoculated with *S. oneidensis* and

anaerobically incubated at 30 °C for 3 days. Test cultures were then exposed to 2.5 MPa CO_2 for up to 8 h. The impact of CO_2 exposure on cell survival was assessed by comparing the culturable cell content of test cultures to identical cultures that were not exposed to high-pressure CO_2. In both cases, the cultures were sonicated prior to culturing to disperse cells and cell survival was evaluated using pour plating. Samples of minerals and cells were imaged using scanning electron microscopy (SEM) following termination of the experiments.

For our pure-culture tests, control experiments were performed to assess the impact of sonication and pressure changes on cell survival. Results indicate that neither factor significantly impacted the culturable cell concentrations we measured. A set of control experiments was also included to examine the extent to which biofilm protected cells during exposure to high-pressure CO_2. For those controls, the cultures were sonicated prior to CO_2 exposure to disperse biofilm cells.

2.3. Bioclogging experiments

Column experiments were performed to examine how sudden acidification of water would impact the stability of biofilm in porous media (Kirk et al., 2012). The experiments were run in 10 cm long square capillary tubes with a 1 mm^2 cross-sectional area packed with 105–150 µm diameter glass beads. Each experiment had three phases: pre-growth, growth at pH 7.2, and acidic pH, which started four days after inoculation. The acidic phases of six biologically-active experiments received medium with an average pH of 4.0 and six additional experiments received medium with an average pH of 5.7. Abiological-control experiments were

212 also performed at pH 4 (two) and pH 5.7 (one). Experiments were terminated after hydraulic
213 conductivity was stable for at least 24 h.

214 Artificial Na-Cl type groundwater with glucose and bicarbonate was used as the aqueous
215 medium. Rhodamine, a fluorescent dye, was included for pore-space imaging. pH was adjusted
216 using HCl. Medium was pumped through the columns at 0.015 mL min^{-1} (specific discharge of
217 22 m day^{-1}) using syringe pump. After the hydraulic properties were allowed to stabilize for at
218 least three days, the system was inoculated with an average of 8.4 log colony forming units
219 (CFU; stdev 0.3) of *Pseudomonas fluorescens* tagged with a green fluorescent protein (GFP).
220 Biofilm production by *P. fluorescens* is well characterized, including growth in flowing systems
221 (e.g., Pereira et al., 2002; Simoes et al., 2007; Simoes et al., 2005). A strain tagged with GFP was
222 chosen to allow biomass growth to be monitored nondestructively. Following inoculation, flow
223 was stopped for 2 h to allow initial cell attachment and growth to occur. Cells injected into the
224 control experiments were heat-sterilized before injection.

225 The average saturated hydraulic conductivity over the entire length of each column was
226 evaluated for each of the three phases of the experiments based on pressure measurements.
227 Pores and biomass were imaged with a scanning laser confocal microscope during the
228 experiments. Culturable cell concentrations in column effluent were measured periodically
229 throughout the experiment by plating effluent samples. For two pH 4 and three pH 5.7
230 experiments, effluent cell abundance was also quantified using live-dead staining. This
231 approach provides a measure of cell viability that, unlike plating, is not influence by any
232 cultivation bias. After the experiments were terminated, the culturable cell content of 1 cm
233 column segments was measured in one pH 4 and one pH 5.7 experiment.

234

235 *2.4. Mixed-community experiments*

236 Experiments were carried out with bioreactors containing a mixed-microbial community
237 to examine how changes in CO_2 abundance could alter interactions between groups of
238 microbes that naturally co-exist (Kirk et al., 2013). Unlike the pure-culture experiments, which
239 isolate factors that influence cell survival, these experiments consider how an increase in CO_2
240 could affect interactions between different functional groups of microorganisms.

241 The experiments were carried out in duplicate using anoxic semi-continuous
242 bioreactors. Microbes and groundwater for the experiments were obtained from a freshwater
243 aquifer. Two sets of experiments were performed: one with low CO_2 partial pressure (~0.002
244 MPa) in the headspace of the reactors and one with high CO_2 partial pressure (~0.1 MPa).
245 Hereafter, we refer to these experiments as the low-CO_2 bioreactors and high-CO_2 bioreactors,
246 respectively. A fluid residence time of 35 days was maintained in the reactors by replacing one-
247 fifth of the aqueous volume with fresh medium every seven days. The aqueous medium was
248 composed of groundwater amended with small amounts of acetate (250 μM), phosphate (1
249 μM), and ammonium (50 μM) to stimulate microbial activity. Synthetic goethite (1 mmol) and
250 sulfate (500 μM influent concentration) were also available in each reactor to serve as electron
251 acceptors.

252 Reactors were incubated for 15 weeks. During that time, influent medium and reactor
253 effluent were regularly sampled and analyzed using a variety of techniques. The ferrozine
254 method was used to analyze ferrous iron concentration (Stookey, 1970). Ion chromatography
255 was used to analyze anion concentrations. Gran alkalinity titrations were used to evaluate

alkalinity. Atomic adsorption and inductively coupled plasma optical emissions spectroscopy were used to measure cation concentrations. Rates of acetate oxidation, iron reduction, and sulfate reduction were directly evaluated using mass-balance calculations based on measured reactor chemistry.

Well-mixed samples of reactor solids and fluid were collected at the end of the incubations for analysis of reactor solid phases and microbial community composition. Total community DNA was extracted from microbial samples using an Ultraclean® Microbial DNA Isolation Kit (MO BIO) and then sequenced using 454 pyrosequencing. Sequences were then processed using QIIME (Caporaso et al., 2010). During processing, the software used AmpliconNoise to remove sequencing errors (Quince et al., 2011).

2.5. Numerical analysis

Bioenergetics calculations were used to consider how increasing CO_2 abundance affects redox disequilibrium and, in turn, microbial activity. Calculations were performed using data collected during two field CO_2-injection experiments (Kirk, 2011) and with data collected from the mixed-community experiments (Kirk et al., 2013). In both cases, the calculations assessed changes in energy available (ΔG_A) for microbial metabolism. As defined previously (Bethke et al., 2011), ΔG_A is the negative of the free energy change of microbial metabolic reaction (ΔG_r) and can be calculated in units of kJ·mol^{-1} as follows:

$$\Delta G_A = -\Delta G_r = -[\Delta G_T^\circ + RTln \prod_i (\gamma_i \times m_i)^{\nu_i}]$$

where ΔG_T° is the standard Gibbs free-energy change for reaction *r* at temperature *T* (°K), *R* represents the gas constant (kJ·mol^{-1}·K^{-1}), γ_i and m_i are the activity coefficient (molal^{-1}) and

molality of the ith chemical species in the reaction, and v_i is the stoichiometric coefficient of that species, which is positive for products and negative for reactants.

Standard Gibbs free energy values at *in situ* temperature were calculated using the Geochemists Workbench® software package (Bethke, 2009) and the Lawrence Livermore National Laboratory thermodynamic database (Delany and Lundeen, 1990). Activities were calculated from chemical data with Geochemists Workbench® software using an extended form of the Debye-Hückel equation, the *B*-dot equation (Helgeson, 1969).

Calculations for the mixed-community experiments considered iron reduction and sulfate reduction, the two groups of microorganisms that account for all of the microbial activity during the experiments. Calculations for the field studies considered iron reduction, sulfate reduction, and methanogenesis. Those groups were selected because they are the three most common groups of respiring microorganisms in the subsurface (Bethke et al., 2011; Lovley and Chapelle, 1995; McMahon and Chapelle, 2008). As such, they are likely present in many potential storage reservoirs that contain active microbial populations.

Field experiment data used in our calculations was collected during the Frio Formation experiment and the Zero Emissions Research and Technology (ZERT) experiment (Kharaka et al., 2006; Kharaka et al., 2010). To account for errors associated with activity modeling and uncertainty regarding electron donor concentrations, results from the bioenergetics analysis of the field data are normalized relative to conditions present prior to CO_2 injection, as follows:

$$\Delta G_A^{CO_2} - \Delta G_A^{initial} = \Delta G_A^n$$

where the superscript "CO_2" designates each value calculated during or after CO_2 injection began, "initial" designates the value calculated prior to injection, and "n" represents the

normalized value. As such, our analysis of the field data considered how energy available changed as a result of CO_2 injection, not absolute values of energy available.

3. Results and discussion

The integration of our studies yields insight into the interplay between subsurface microbes and GCS beyond that possible within each individual study. In the subsections that follow, we examine the results of our studies within the context of these two-way interactions.

3.1 Impacts of GCS on microbiology

3.1.1. *Factors influencing cell survival*

Results of our isolation and pure-culture experiments indicate that cells that have properties that limit CO_2 accumulation in their cytoplasm are better able to survive exposure to high pressure CO_2. These properties include the make-up of their cell wall and membranes, the nature of their metabolic reactions, and whether they exist within biofilm.

We found that isolate CG-1 exhibits a fermentative metabolism and was most related (98.5%) to *Lactobacillus casei* (Santillan et al., 2015). It grows at CO_2 partial pressures between 0 and 1.0 MPa and is able to survive for at least 5 days at 2.5 MPa CO_2 and for at least 1 day at 5 MPa CO_2. CG-1 morphology and fatty acid composition both vary with CO_2 partial pressure. Images collected from cultures with 0.1 MPa CO_2 show rod-shaped cells. In images collected from cultures with 1 MPa CO_2, however, cells are generally smaller and encased in capsular material (Figure 1). With increasing CO_2 partial pressure, monounsaturated fatty acids decreased in relative abundance while saturated fatty acids increased. Production of capsular

material and the changes in lipid composition at high CO_2 levels are consistent with a decrease in the flexibility and perhaps permeability of the cells.

Strains tested in our pure-culture experiments varied in their ability to survive exposure to high-pressure CO_2 (Santillan et al., 2013). For all organisms, survival was best at low CO_2 pressures but decreased as pressures increased. *S. oneidensis* cells were the most sensitive to increased CO_2 while *G. stearothermophilus* cells were the most resilient.

G. stearothermophilus cells may have been better able to survive than the other strains because they possess Gram positive cell walls as well as the capacity to form endospores. Cell wall and membrane composition influence the extent to which CO_2 can penetrate cells (Bertoloni et al., 2006; Zhang et al., 2006). Gram positive cell walls are more rigid and less permeable than Gram negative cell walls. Sporulation can provide a mechanism by which cells can reduce themselves into a more durable form until CO_2 stress is removed (Furukawa et al., 2004; Watanabe et al., 2003).

Differences in survival between *M. thermoautitrophicus* and *S. oneidensis* cells may also reflect differences in the ability of CO_2 to penetrate the cells. Archaea, such as *M. thermoautitrophicus*, possess cell membranes that differ considerably from those of Bacteria. Because of those differences, they are thought to generally be better able to withstand extreme conditions (Arakawa et al., 1999; Gambacorta et al., 1994). In addition, differences in metabolism between the strains may have also contributed to variation in cell survival. Unlike *S. oneidensis*, *M. thermoautitrophicus* cells consume CO_2 in their catabolic reaction, potentially helping them limit accumulation of CO_2 within their cytoplasm. The isolation process of CG-1 suggests it may similarly benefit from intracellular CO_2 consumption. Many fermenters utilize

344 CO_2 in metabolic processes, such as amino acid synthesis or through C_1 metabolism (Arioli et al.,
345 2009; Bringel et al., 2008; Song et al., 2007).
346 Results from our experiments that included minerals, indicate that the mere presence of
347 a mineral can enhance the ability of *S. oneidensis* cells to survive exposure to high pressure CO_2
348 (Santillan et al., 2013). With the exception of kaolinite, cell survival was higher in cultures
349 containing minerals than those without (Figure 2). We hypothesize that these results reflect the
350 shelter provided by biofilm. Unlike planktonic cells, biofilm cells are surrounded by extracellular
351 polymeric substances (EPS), which limits their exposure to environmental stresses such as high-
352 pressure CO_2 (Mitchell et al., 2008; Mitchell et al., 2009). Surface area available for biofilm
353 formation was greater in cultures that contained minerals than those that did not. SEM images
354 (not shown) confirm that biofilm formation did occur on mineral surfaces during the
355 experiment.
356
357 *3.1.2. Persistence of attached biomass*
358 Results from our column experiments show that biofilm can remain largely intact
359 following sudden acidification of water, even if considerable cell death occurs (Kirk et al., 2012).
360 After 4 days of growth at pH 7.2, a 0.67 log reduction in the overall hydraulic conductivity of the
361 columns occurred, on average (Figure 3). Acidification caused hydraulic conductivity to increase
362 significantly in all but one pH 5.7 experiment as well as extensive cell death and stress,
363 particularly in pH 4 experiments. However, the columns remained significantly clogged relative
364 to pre-growth conditions. Following acidification, log reductions in hydraulic conductivity
365 averaged 0.43 and 0.65 in pH 4 and pH 5.7 experiments, respectively.

366

367 *3.1.3. Shifts in microbial reactions*

368 Our mixed-community experiments and numerical analyses show that increasing CO_2
369 concentration favors microbial reactions that consume acid. As a result, microbial communities
370 that emerge following injection of CO_2 may differ from indigenous communities not only
371 because they are better at tolerating CO_2 stress but also because the balance between different
372 microbial reactions has shifted.

373 Microbial activity differed considerably between the high- and low-CO_2 bioreactors in
374 our mixed-community experiments (Kirk et al., 2013). Mass-balance calculations demonstrate
375 that sulfate reduction was dominant in reactors with low CO_2 content. The reaction consumed
376 85% of the acetate after acetate consumption reached steady state while iron reduction
377 accounted for only 15% on average (Figure 4). In contrast, iron reduction was dominant during
378 that same interval in reactors with high CO_2 content, accounting for at least 90% of the acetate
379 consumption while sulfate reduction consumed a negligible amount (<1%).

380 Results of our microbial community analyses agree with our mass-balance calculations
381 (Kirk et al., 2013). Sequences classified in groups that contain species related to iron reduction
382 were abundant in samples from all biologically-active reactors but more than twice as abundant
383 in the high-CO_2 reactor samples compared to the low-CO_2 reactor samples. Moreover,
384 sequences classified in groups relating to sulfate reducers were abundant in the low-CO_2
385 reactor samples but nearly absent from the high-CO_2 reactor samples.

386 Bioenergetics calculations show that the rate of microbial iron reduction may have
387 varied in response to differences in thermodynamic controls (Kirk et al., 2013). Iron reduction

was much more energetically favorable in reactors that hosted more rapid iron reduction, the high-CO_2 reactors, than those with slower iron reduction rates, the low-CO_2 reactors. After acetate consumption stabilized, energy available for microbial iron reduction was 114 kJ mol^{-1} and 60 kJ mol^{-1}, on average in the high- and low-CO2 bioreactors, respectively.

In contrast, thermodynamic controls on microbial sulfate reduction could not be responsible for variation in the rate of that reaction. Energy available for sulfate reduction varied little, averaging a maximum of 65 kJ mol^{-1} and 62 kJ mol^{-1} in the high- and low-CO_2 reactors, respectively. Instead, we hypothesize that the rate of sulfate reduction varied in response to competition for electron donor from iron reduction (Kirk et al., 2013). Where energy available for microbial iron reduction was high, the reaction occurred rapidly and little electron donor remained for sulfate reduction. However, where energy available for iron reduction was low, the reaction slowed, allowing sulfate reduction to consume excess electron donor.

Bioenergetic calculations performed using data from the field CO_2-injection experiments provide results that parallel those from the mixed-community experiments. CO_2 injection benefitted iron reduction much more than sulfate reduction or methanogenesis at both field sites (Kirk, 2011). For both acetotrophic and hydrogentrophic reactions, the energy available for iron reduction increased considerably for all three iron minerals considered as electron acceptors in iron-reduction reactions (Figure 5). In contrast, energy available for sulfate reduction and methanogenesis varied relatively little.

In both sets of calculations, the energy advantage gained by iron reduction with increased CO_2 levels primarily reflects changes in pH. Reduction of ferric iron in oxides and

oxyhydroxides consumes a large number of protons. As such, the energy yield of iron reduction increases sharply as pH decreases. Sulfate reduction and methanogenesis, however, consume relatively few protons. As such, the energy yield of those reactions does not vary strongly with pH.

Our numerical and mixed-culture studies indicate that CO_2 injection has the potential to stimulate microbial iron reduction where ferric iron is available. At first glance, these results seem to be in conflict with our isolate experiments. In those experiments, *S. oneidensis*, an organism capable of dissimilatory iron reduction, showed greater sensitivity to elevated CO_2 than *M. thermoautitrophicus*, a methanogen. However, individual isolates are not representative of an entire metabolic group of microorganisms. Cells capable of dissimilatory iron reduction, for example, have broad phylogenetic diversity and have been identified across a wide range of chemical and physical conditions, including at extreme acidic pH and salinity (Emmerich et al., 2012; Itoh et al., 2011; Lu et al., 2010; Weber et al., 2006). The mixed-community of iron-reducing microorganisms that may exist in a GCS reservoir, therefore, may be better able to adapt to an increase in the abundance of CO_2 than the individual isolate we tested.

3.2. Impacts of microbiology on GCS

3.2.1. Impacts of microbiology on flow

Similar to our findings, previous studies have shown that biofilm can remain largely intact in porous media during exposure to supercritical CO_2 (Mitchell et al., 2008; Mitchell et al., 2009). Combined with our efforts, the results of these studies provide compelling evidence that

hydraulic conductivity will change little in response to biofilm redistribution following injection of CO_2 into GCS reservoirs where biofilms are present. If microbial biomass influences hydraulic conductivity before CO_2 is injection into a GCS, our results and those of previous studies suggest it will influence hydraulic conductivity afterward as well.

These findings imply that, in biologically activity GCS reservoirs, microbial biofilms can influence the flow of CO_2 and water away from injection wells. Consistent with this implication, previous studies found that microbial activity significantly decreased the injectivity of a CO_2-injection well at the Ketzin site (Morozova et al., 2010; Zettlitzer et al., 2010). In addition, biofilm on a mineral surface may alter the wettability of those minerals, which is a major control on residual trapping of CO_2 (Chaudhary et al., 2013).

3.2.2. Impacts of microbiology on solution and mineral trapping

Results of the mixed-community experiments show that, where CO_2 injection stimulates microbial iron reduction, solubility trapping may be enhanced. Because microbial reduction of ferric iron in iron oxides and oxyhydroxides consumes a large number of protons, the reaction works to convert CO_2 into carbonate alkalinity, thereby enhancing storage of inorganic carbon in solution (Kirk et al., 2013). Reflecting this relationship, the increase in carbonate alkalinity caused by microbial activity in high-CO_2 bioreactors was six-fold greater than that in the low-CO_2 bioreactors (Figure 6). Mitchell et al. (2010) describe a similar effect during bacterial hydrolysis of urea batch reactor experiments containing elevated CO_2 content. The results of these studies suggest that we may need to consider the response of the microbial community to CO_2 injection in order to accurately predict rates of solution trapping in GCS reservoirs.

In addition to solution trapping, microbial activity also has the potential to impact mineral trapping. Alkalinity generation by acid-consuming microorganisms works to increase the saturation state of carbonate minerals such as calcite ($CaCO_3$) and siderite ($FeCO_3$) (Kirk et al., 2013; Mitchell et al., 2010). Moreover, cells and biofilms can also facilitate carbonate mineralization by providing nucleation cites (Benzerara et al., 2011; Mitchell and Ferris, 2006). Hence, rates of mineral trapping may also be influenced by the response of the microbial community to CO_2 injection.

3.5. Future research

Our efforts and those of many other researchers have to date been weighted toward understanding one side of the interplay between microbiology and GCS: the impact of GCS on microbial activity. This area of research is important. We can understand how microbes will affect GCS without knowing what physical and functional characteristics GCS reservoirs will select for. However, we suggest that more attention needs to be paid to the impact of microbiology on GCS.

Many questions about this component of GCS geomicrobiology remain unresolved. Little is known about the nature of microbial impacts on GCS and their relative significance. For example, how will alkalinity production by acid-consuming microorganisms compare to that generated by abiological reactions between CO_2 and minerals? We also do not have a clear basis for identifying which GCS reservoirs are more likely to host significant microbial impacts. Should our attention focus on organic-rich reservoirs (e.g., depleted oil reservoirs and coalbeds) or will microbial reaction rates be significant relative to the time scale of GCS in all reservoirs?

476 Answering these questions will constrain the extent to which numerical models need to include
477 microbial activity to accurately simulate the long-term fate of CO_2 in the subsurface.
478 Future laboratory research needs to simulate conditions consistent with GCS reservoirs.
479 GCS reservoirs will commonly be anoxic, with heterogeneous mineralogy and microbiology and
480 elevated pressure, temperature, and salinity. Many recent laboratory studies were performed
481 under relevant conditions (e.g., Dupraz et al., 2013; Mayumi et al., 2013; Ohtomo et al., 2013;
482 Peet et al., 2015; Wilkins et al., 2014). However, most of what we know about the impact of
483 high pressure CO_2 on microbiology stems from food industry research into CO_2 as a sterilizing
484 agent (e.g., Amanatidou et al., 1999; Spilimbergo et al., 2002; Watanabe et al., 2003; Zhang et
485 al., 2006). Follow-up experiments are warranted to test some of the research questions in those
486 studies under conditions consistent with GCS reservoirs.
487 Lastly, we suggest that addition research should examine microbiological mechanisms
488 that could create an energy return on subsurface CO_2 injection. For example, recent research
489 has found evidence that CO_2 injection can stimulate biological conversion of crude oil into
490 natural gas (Mayumi et al., 2013). CO_2 injection into depleted or heavy oil reservoirs, therefore,
491 may provide a strategy to enhance energy recovery from those systems and alleviate some of
492 the economic burden of GCS.
493
494 **4. Conclusions**
495 Geomicrobiology studies performed by CFSES examine impacts of GCS on subsurface
496 microbiology. Pure-culture and isolation studies identify factors that may influence survival,
497 including environmental, biochemical, and structural characteristics. Our column experiments

show that biofilm can remain largely intact following sudden acidification of water, even if significant cell death and stress occurs. Mixed-community experiments and thermodynamic calculations show that the balance between microbial reactions can shift in response to changes in fluid chemistry caused by increasing CO_2 levels. Collectively, these efforts add to the growing body of evidence that microbial life will persist in GCS reservoirs, likely defined by communities that differ from those present prior to injection. Our work suggests that communities will change in response to differences in the ability of cells to tolerate elevated CO_2 levels as well as shifts in the balance of microbial reactions.

These studies also shed light on potential impacts of subsurface microbial communities on GCS. Subsurface biomass may influence the hydrodynamics of porous media in GCS reservoirs, affecting flow away from injection wells and capillary trapping of CO_2. Coupled with this effect, by catalyzing oxidation-reduction reactions, microorganisms can affect the rate and form of solubility and mineral trapping. The potential importance of microbial activity in GCS reservoirs, therefore, should not be overlooked.

Acknowledgements

This material is based upon work supported as part of the Center for Frontiers of Subsurface Energy Security, an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Award Number DE-SC0001114. Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-

94AL85000. Matthew Kirk conducted the laboratory and modeling work presented in this paper while at Sandia National Laboratories.

References

Amanatidou, A., Smid, E. J., and Gorris, L. G. M. (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. *Journal of Applied Microbiology* 86, 429-438. 10.1046/j.1365-2672.1999.00682.x

Arakawa, K., Kano, H., Eguchi, T., Nishiyama, Y., and Kakinuma, K. (1999). Significance of the 72-membered macrocyclic structure found in archaeal membrane lipids: Model studies of the macrocyclic tetraether diphospholipids by calorimetric, P-31 NMR, and electron microscopic analyses. *Bulletin of the Chemical Society of Japan* 72, 1575-1581. 10.1246/bcsj.72.1575

Arioli, S., Roncada, P., Salzano, A. M., Deriu, F., Corona, S., Guglielmetti, S., Bonizzi, L., Scaloni, A., and Mora, D. (2009). The relevance of carbon dioxide metabolism in *Streptococcus thermophilus*. *Microbiology-SGM* 155, 1953-1965. 10.1099/mic.0.024737-0

Baker, B. J., Comolli, L. R., Dick, G. J., Hauser, L. J., Hyatt, D., Dill, B. D., Land, M. L., VerBerkmoes, N. C., Hettich, R. L., and Banfield, J. F. (2010). Enigmatic, ultrasmall, uncultivated Archaea. *Proceedings of the National Academy of Sciences* 107, 8806-8811. 10.1073/pnas.0914470107

Ballestra, P., Dasilva, A. A., and Cuq, J. L. (1996). Inactivation of *Escherichia coli* by carbon dioxide under pressure. *Journal of Food Science* 61, 829-&. 10.1111/j.1365-2621.1996.tb12212.x

Banks, E. D., Taylor, N. M., Gulley, J., Lubbers, B. R., Giarrizo, J. G., Bullen, H. A., Hoehler, T. M., and Barton, H. A. (2010). Bacterial calcium carbonate precipitation in cave environments: A function of calcium homeostasis. *Geomicrobiology Journal* 27, 444-454. 10.1080/01490450903485136

Baveye, P., Vandevivere, P., Hoyle, B. L., DeLeo, P. C., and de Lozada, D. S. (1998). Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials. *Critical Reviews in Environmental Science and Technology* 28, 123-191. 10.1080/10643389891254197

Benson, S. M., Cook, P., Anderson, J., Bachu, S., Hassan, B. N., Basu, B., Bradshaw, J., Deguchi, G., Gale, J., von Goerne, G., Heidug, W., Holloway, S., Kamal, R., Keith, D., Lloyd, P., Rocha, P., Senior, B., Thomson, J., Torp, T., Wildenborg, T., Wilson, M., Zarlenga, F., Zhou, D., Celia, M., Gunter, B., King, J. E., Lindeberg, E., Lombardi, S., Oldenburg, C., Pruess, K., Rigg, A., Stevens, S., Wilson, E., and Whittaker, S. (2005). Underground geological storage, *in* Metz, B., Davidson, O., de Coninck, H., Loos, M., and Meyer, L., eds., Carbon Dioxide Capture and Storage: Cambridge, England, Cambridge University Press, p. 431.

Benzerara, K., Miot, J., Morin, G., Ona-Nguema, G., Skouri-Panet, F., and Ferard, C. (2011). Significance, mechanisms and environmental implications of microbial biomineralization. *Comptes Rendus Geoscience* 343, 160-167. 10.1016/j.crte.2010.09.002

Bertoloni, G., Bertucco, A., De Cian, V., and Parton, T. (2006). A study on the inactivation of micro-organisms and enzymes by high pressure CO_2. *Biotechnology and Bioengineering* 95, 155-160. 10.1002/bit.21006

Bethke, C. M. (2009). The Geochemist's Workbench: Champaign, IL, Aqueous Solutions, LLC.

Bethke, C. M., Sanford, R. A., Kirk, M. F., Jin, Q., and Flynn, T. M. (2011). The thermodynamic ladder in geomicrobiology. *American Journal of Science* 311, 183-210. 10.2475/03.2011.01

Bringel, F., Hammann, P., Kugler, V., and Arsene-Ploetze, F. (2008). *Lactobacillus plantarum* response to inorganic carbon concentrations: PyrR(2)-dependent and -independent transcription regulation

of genes involved in arginine and nucleotide metabolism. *Microbiology-SGM* 154, 2629-2640. 10.1099/mic.0.2008/018184-0

Burnside, N. M., Shipton, Z. K., Dockrill, B., and Ellam, R. M. (2013). Man-made versus natural CO_2 leakage: A 400 k.y. history of an analogue for engineered geological storage of CO_2. *Geology* 41, 471-474. 10.1130/g33738.1

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Tumbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335-336. 10.1038/nmeth.f.303

Chaudhary, K., Cardenas, M. B., Wolfe, W. W., Maisano, J. A., Ketcham, R. A., and Bennett, P. C. (2013). Pore-scale trapping of supercritical CO_2 and the role of grain wettability and shape. *Geophysical Research Letters* 40, 3878-3882. 10.1002/grl.50658

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., and Thompson, J. D. (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* 31, 3497-3500. 10.1093/nar/gkg500

Colwell, F. S., and D'Hondt, S. (2013). Nature and Extent of the Deep Biosphere, *in* Hazen, R. M., Jones, A. P., and Baross, J. A., eds., Carbon in Earth, Volume 75, p. 547-574. 10.2138/rmg.2013.75.17

Cunningham, A. B., Gerlach, R., Spangler, L., and Mitchell, A. C. (2009). Microbially Enhanced Geologic Containment of Sequestered Supercritical CO_2, *in* Gale, J., Herzog, H., and Braitsch, J., eds., Greenhouse Gas Control Technologies 9, Volume 1, p. 3245-3252. 10.1016/j.egypro.2009.02.109

Davidson, M. M., Silver, B. J., Onstott, T. C., Moser, D. P., Gihring, T. M., Pratt, L. M., Boice, E. A., Lollar, B. S., Lippmann-Pipke, J., Pfiffner, S. M., Kieft, T. L., Seymore, W., and Ralston, C. (2011). Capture of Planktonic Microbial Diversity in Fractures by Long-Term Monitoring of Flowing Boreholes, Evander Basin, South Africa. *Geomicrobiology Journal* 28, 275-300. 10.1080/01490451.2010.499928

Delany, J. M., and Lundeen, S. R. (1990). The LLNL thermochemical database: Lawrence Livermore National Laboratory, LLNL report UCRL-21658.

Dillow, A. K., Dehghani, F., Hrkach, J. S., Foster, N. R., and Langer, R. (1999). Bacterial inactivation by using near- and supercritical carbon dioxide. *Proceedings of the National Academy of Sciences of the United States of America* 96, 10344-10348. 10.1073/pnas.96.18.10344

Dupraz, S., Fabbri, A., Joulian, C., Dictor, M. C., Battaglia-Brunet, F., Menez, B., Crouzet, C., Henry, B., and Garrido, F. (2013). Impact of CO_2 concentration on autotrophic metabolisms and carbon fate in saline aquifers - A case study. *Geochimica et Cosmochimica Acta* 119, 61-76. 10.1016/j.gca.2013.05.027

Emerson, J. B., Thomas, B. C., Alvarez, W., and Banfield, J. F. (2015). Metagenomic analysis of a high carbon dioxide subsurface microbial community populated by chemolithoautotrophs and bacteria and archaea from candidate phyla. *Environmental Microbiology*. 10.1111/1462-2920.12817

Emmerich, M., Bhansali, A., Loesekann-Behrens, T., Schroeder, C., Kappler, A., and Behrens, S. (2012). Abundance, Distribution, and Activity of Fe(II)-Oxidizing and Fe(III)-Reducing Microorganisms in Hypersaline Sediments of Lake Kasin, Southern Russia. *Applied and Environmental Microbiology* 78, 4386-4399. 10.1128/aem.07637-11

Flynn, T. M., Sanford, R. A., Ryu, H., Bethke, C. M., Levine, A. D., Ashbolt, N. J., and Domingo, J. W. S. (2013). Functional microbial diversity explains groundwater chemistry in a pristine aquifer. *Bmc Microbiology* 13. 10.1186/1471-2180-13-146

610 Fredrickson, J. K., Zachara, J. M., Kennedy, D. W., Dong, H., Onstott, T. C., Hinman, N. W., and Li, S.-m.
611 (1998). Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric
612 oxide by a groundwater bacterium. *Geochimica et Cosmochimica Acta* 62, 3239-3257.
613 10.1016/S0016-7037(98)00243-9
614 Furukawa, S., Watanabe, T., Tai, T., Hirata, J., Narisawa, N., Kawarai, T., Ogihara, H., and Yamasaki, M.
615 (2004). Effect of high pressure gaseous carbon dioxide on the germination of bacterial spores.
616 *International Journal of Food Microbiology* 91, 209-213. 10.1016/0168-1605(03)00372-6
617 Gambacorta, A., Trincone, A., Nicolaus, B., Lama, L., and Derosa, M. (1994). Unique features of lipids of
618 archaea. *Systematic and Applied Microbiology* 16, 518-527.
619 Gorbushina, A. A. (2007). Life on the rocks. *Environmental Microbiology* 9, 1613-1631. 10.1111/j.1462-
620 2920.2007.01301.x
621 Harvey, O. R., Qafoku, N. P., Cantrell, K. J., Lee, G., Amonette, J. E., and Brown, C. F. (2013). Geochemical
622 implications of gas leakage associated with geological CO_2 storage - a qualitative review.
623 *Environmental Science & Technology* 47, 23-36.
624 Helgeson, H. C. (1969). Thermodynamics of hydrothermal systems at elevated temperatures and
625 pressures. *American Journal of Science* 267, 729-804.
626 Itoh, T., Yamanoi, K., Kudo, T., Ohkuma, M., and Takashina, T. (2011). *Aciditerrimonas ferrireducens* gen.
627 nov., sp nov., an iron-reducing *thermoacidophilic actinobacterium* isolated from a solfataric
628 field. *International Journal of Systematic and Evolutionary Microbiology* 61, 1281-1285.
629 10.1099/ijs.0.023044-0
630 Jin, Q. (2012). Energy conservation of anaerobic respiration. *American Journal of Science* 312, 573-628.
631 10.2475/06.2012.01
632 Kandianis, M. T., Fouke, B. W., Johnson, R. W., Veysey, J., II, and Inskeep, W. P. (2008). Microbial
633 biomass: A catalyst for $CaCO_3$ precipitation in advection-dominated transport regimes.
634 *Geological Society of America Bulletin* 120, 442-450. 10.1130/b26188.1
635 Kaszuba, J. P., and Janecky, D. R. (2009). Geochemical Impacts of Sequestering Carbon Dioxide in Brine
636 Formations, in Carbon Sequestration and Its Role in the Global Carbon Cycle, *in* Mcpherson, B. J.,
637 and Sundquist, E. T., eds., Carbon Sequestration and Its Role in the Global Carbon Cycle:
638 Washington, D. C., American Geophysical Union. 10.1029/2006GM000353
639 Kawaguchi, H., Sakuma, T., Nakata, Y., Kobayashi, H., Endo, K., and Sato, K. (2010). Methane production
640 by *Methanothermobacter thermautotrophicus* to recover energy from carbon dioxide
641 sequestered in geological reservoirs. *Journal of Bioscience and Bioengineering* 110, 106-108.
642 10.1016/j.jbiosc.2010.01.008
643 Kharaka, Y. K., Cole, D. R., Hovorka, S. D., Gunter, W. D., Knauss, K. G., and Freifeld, B. M. (2006). Gas-
644 water-rock interactions in Frio Formation following CO_2 injection: Implications for the storage of
645 greenhouse gases in sedimentary basins. *Geology* 34, 577-580. 10.1130/g22357.1
646 Kharaka, Y. K., Thordsen, J. J., Kakouros, E., Ambats, G., Herkelrath, W. N., Beers, S. R., Birkholzer, J. T.,
647 Apps, J. A., Spycher, N. F., Zheng, L. E., Trautz, R. C., Rauch, H. W., and Gullickson, K. S. (2010).
648 Changes in the chemistry of shallow groundwater related to the 2008 injection of CO_2 at the
649 ZERT field site, Bozeman, Montana. *Environmental Earth Sciences* 60, 273-284. 10.1007/s12665-
650 009-0401-1
651 Kieft, T. L., McCuddy, S. M., Onstott, T. C., Davidson, M., Lin, L. H., Mislowack, B., Pratt, L., Boice, E.,
652 Lollar, B. S., Lippmann-Pipke, J., Pfiffner, S. M., Phelps, T. J., Gihring, T., Moser, D., and van
653 Heerden, A. (2005). Geochemically generated, energy-rich substrates and indigenous
654 microorganisms in deep, ancient groundwater. *Geomicrobiology Journal* 22, 325-335.
655 10.1080/01490450500184876

656 Kirk, M. F. (2011). Variation in energy available to populations of subsurface anaerobes in response to
657 geological carbon storage. *Environmental Science & Technology* 45, 6676-6682.
658 10.1021/es201279e
659 Kirk, M. F., Jin, Q., and Haller, B. R. (2015). Broad-scale evidence that pH influences the balance between
660 microbial iron and sulfate reduction. *Groundwater*. 10.1111/gwat.12364
661 Kirk, M. F., Santillan, E. F. U., McGrath, L. K., and Altman, S. J. (2012). Variation in hydraulic conductivity
662 with decreasing pH in a biologically-clogged porous medium. *International Journal of*
663 *Greenhouse Gas Control* 11, 133-140. 10.1016/j.ijggc.2012.08.003
664 Kirk, M. F., Santillan, E. F. U., Sanford, R. A., and Altman, S. J. (2013). CO_2-induced shift in microbial
665 activity affects carbon trapping and water quality in anoxic bioreactors. *Geochimica et*
666 *Cosmochimica Acta* 122, 198-208. 10.1016/j.gca.2013.08.018
667 LaRowe, D. E., and Amend, J. P. (2015). Catabolic rates, population sizes and doubling/replacement
668 times of microorganisms in natural settings. *American Journal of Science* 315, 167-203.
669 10.2475/03.2015.01
670 Lovley, D. R., and Chapelle, F. H. (1995). Deep subsurface microbial processes. *Reviews of Geophysics* 33,
671 365-381.
672 Lovley, D. R., and Goodwin, S. (1988). Hydrogen concentrations as an indicator of the predominant
673 terminal electron-accepting reactions in aquatic sediments. *Geochimica et Cosmochimica Acta*
674 52, 2993-3003. 10.1016/0016-7037(88)90163-9
675 Lu, S., Gischkat, S., Reiche, M., Akob, D. M., Hallberg, K. B., and Kuesel, K. (2010). Ecophysiology of Fe-
676 Cycling Bacteria in Acidic Sediments. *Applied and Environmental Microbiology* 76, 8174-8183.
677 10.1128/aem.01931-10
678 Mayumi, D., Dolfing, J., Sakata, S., Maeda, H., Miyagawa, Y., Ikarashi, M., Tamaki, H., Takeuchi, M.,
679 Nakatsu, C. H., and Kamagata, Y. (2013). Carbon dioxide concentration dictates alternative
680 methanogenic pathways in oil reservoirs. *Nature Communications* 4. 10.1038/ncomms2998
681 McMahon, P. B., and Chapelle, F. H. (2008). Redox processes and water quality of selected principal
682 aquifer systems. *Ground Water* 46, 259-271. 10.1111/j.1745-6584.2007.00385.x
683 Mitchell, A. C., Dideriksen, K., Spangler, L. H., Cunningham, A. B., and Gerlach, R. (2010). Microbially
684 enhanced carbon capture and storage by mineral-trapping and solubility-trapping.
685 *Environmental Science & Technology* 44, 5270-5276. 10.1021/es903270w
686 Mitchell, A. C., and Ferris, F. G. (2006). The Influence of *Bacillus pasteurii* on the nucleation and growth
687 of calcium carbonate. *Geomicrobiology Journal* 23, 213-226. 10.1080/01490450600724233
688 Mitchell, A. C., Phillips, A. J., Hamilton, M. A., Gerlach, R., Hollis, W. K., Kaszuba, J. P., and Cunningham,
689 A. B. (2008). Resilience of planktonic and biofilm cultures to supercritical CO_2. *Journal of*
690 *Supercritical Fluids* 47, 318-325. 10.1016/j.supflu.2008.07.005
691 Mitchell, A. C., Phillips, A. J., Hiebert, R., Gerlach, R., Spangler, L. H., and Cunningham, A. B. (2009).
692 Biofilm enhanced geologic sequestration of supercritical CO_2. *International Journal of*
693 *Greenhouse Gas Control* 3, 90-99. 10.1016/j.ijggc.2008.05.002
694 Morozova, D., Wandrey, M., Alawi, M., Zimmer, M., Vieth, A., Zettlitzer, M., Würdemann, H., and
695 CO2SINK-Group. (2010). Monitoring of the microbial community composition in saline aquifers
696 during CO_2 storage by fluorescence in situ hybridisation. *International Journal of Greenhouse*
697 *Gas Control* 4, 981-989. 10.1016/j.ijggc.2009.11.014
698 Mu, A., Boreham, C., Leong, H. X., Haese, R., and Moreau, J. W. (2014). Changes in the deep subsurface
699 microbial biosphere resulting from a field-scale CO_2 geosequestration experiment. *Frontiers in*
700 *Microbiology* 5. 10.3389/fmicb.2014.00209
701 Nazina, T. N., Tourova, T. P., Poltaraus, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A.
702 M., Petrunyaka, V. V., Osipov, G. A., Belyaev, S. S., and Ivanov, M. V. (2001). Taxonomic study of
703 aerobic thermophilic bacilli: descriptions of Geobacillus subterraneus gen. nov., sp nov and

Geobacillus uzenensis sp nov from petroleum reservoirs and transfer of Bacillus stearothermophilus Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermoglucosidasius and Bacillus thermodenitrificans to Geobacillus as the new combinations G-stearothermophilus, G-thermocatenulatus, G-thermoleovorans, G-kaustophilus, G-thermoglucosidasius and G-thermodenitrificans. *International Journal of Systematic and Evolutionary Microbiology* 51, 433-446.

Ohtomo, Y., Ijiri, A., Ikegawa, Y., Tsutsumi, M., Imachi, H., Uramoto, G. I., Hoshino, T., Morono, Y., Sakai, S., Saito, Y., Tanikawa, W., Hirose, T., and Inagaki, F. (2013). Biological CO_2 conversion to acetate in subsurface coal-sand formation using a high-pressure reactor system. *Frontiers in Microbiology* 4. 10.3389/fmicb.2013.00361

Onstott, T. C., Phelps, T. J., Colwell, F. S., Ringelberg, D., White, D. C., Boone, D. R., McKinley, J. P., Stevens, T. O., Long, P. E., Balkwill, D. L., Griffin, W. T., and Kieft, T. (1998). Observations pertaining to the origin and ecology of microorganisms recovered from the deep subsurface of Taylorsville Basin, Virginia. *Geomicrobiology Journal* 15, 353-385.

Orcutt, B. N., LaRowe, D. E., Biddle, J. F., Colwell, F. S., Glazer, B. T., Reese, B. K., Kirkpatrick, J. B., Lapham, L. L., Mills, H. J., Sylvan, J. B., Wankel, S. D., and Wheat, C. G. (2013). Microbial activity in the marine deep biosphere: progress and prospects. *Frontiers in Microbiology* 4. 10.3389/fmicb.2013.00189

Parkes, R. J., Cragg, B., Roussel, E., Webster, G., Weightman, A., and Sass, H. (2014). A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere:geosphere interactions. *Marine Geology* 352, 409-425. 10.1016/j.margeo.2014.02.009

Pedersen, K., Arlinger, J., Hallbeck, L., and Pettersson, C. (1996). Diversity and distribution of subterranean bacteria in groundwater at Oklo in Gabon, Africa, as determined by 16S rRNA gene sequencing. *Molecular Ecology* 5, 427-436. 10.1111/j.1365-294X.1996.tb00332.x

Peet, K. C., Freedman, A. J. E., Hernandez, H. H., Britto, V., Boreham, C., Ajo-Franklin, J. B., and Thompson, J. R. (2015). Microbial Growth under Supercritical CO_2. *Applied and Environmental Microbiology* 81, 2881-2892. 10.1128/aem.03162-14

Pereira, M. O., Kuehn, M., Wuertz, S., Neu, T., and Melo, L. F. (2002). Effect of flow regime on the architecture of a *Pseudomonas fluorescens* biofilm. *Biotechnology and Bioengineering* 78, 164-171. 10.1002/bit.10189

Pikuta, E. V., Hoover, R. B., and Tang, J. (2007). Microbial extremophiles at the limits of life. *Critical Reviews in Microbiology* 33, 183-209. 10.1080/10408410701451948

Quince, C., Lanzen, A., Davenport, R. J., and Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 12. 10.1186/1471-2105-12-38

Roden, E. E., and Jin, Q. (2011). Thermodynamics of microbial growth coupled to metabolism of glucose, ethanol, short-chain organic acids, and hydrogen. *Applied and Environmental Microbiology* 77, 1907-1909. 10.1128/aem.02425-10

Rodriguez-Ruiz, J., Belarbi, E. H., Sanchez, J. L. G., and Alonso, D. L. (1998). Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. *Biotechnology Techniques* 12, 689-691. 10.1023/a:1008812904017

Sahl, J. W., Schmidt, R. H., Swanner, E. D., Mandernack, K. W., Templeton, A. S., Kieft, T. L., Smith, R. L., Sanford, W. E., Callaghan, R. L., Mitton, J. B., and Spear, J. R. (2008). Subsurface microbial diversity in deep-granitic-fracture water in Colorado. *Applied and Environmental Microbiology* 74, 143-152. 10.1128/aem.01133-07

Santillan, E. F. U., Shanahan, T. M., Omelon, C. R., Major, J. R., and Bennett, P. C. (2015). Isolation and characterization of a CO_2-tolerant Lactobacillus strain from Crystal Geyser, Utah, U.S.A. *Frontiers in Earth Science* 3. 10.3389/feart.2015.00041

Santillan, E. U., Kirk, M. F., Altman, S. J., and Bennett, P. C. (2013). Mineral influence on microbial survival during carbon sequestration. *Geomicrobiology Journal* 30, 578-592. 10.1080/01490451.2013.767396

Simoes, M., Pereira, M. O., Sillankorva, S., Azeredo, J., and Vieira, M. J. (2007). The effect of hydrodynamic conditions on the phenotype of *Pseudomonas fluorescens* biofilms. *Biofouling* 23, 249-258. 10.1080/08927010701368476

Simoes, M., Pereira, M. O., and Vieira, M. J. (2005). Effect of mechanical stress on biofilms challenged by different chemicals. *Water Research* 39, 5142-5152. 10.1016/j.watres.2005.09.028

Song, H., Lee, J. W., Choi, S., You, J. K., Hong, W. H., and Lee, S. Y. (2007). Effects of dissolved CO_2 levels on the growth of *Mannheimia succiniciproducens* and succinic acid production. *Biotechnology and Bioengineering* 98, 1296-1304. 10.1002/bit.21530

Spilimbergo, S., Elvassore, N., and Bertucco, A. (2002). Microbial inactivation by high-pressure. *Journal of Supercritical Fluids* 22, 55-63. 10.1016/S0896-8446(01)00106-1

Stookey, L. L. (1970). Ferrozine - A new spectrophotometric reagent for iron. *Analytical Chemistry* 42, 779-781. 10.1021/ac60289a016

Watanabe, T., Furukawa, S., Hirata, J., Koyama, T., Ogihara, H., and Yamasaki, M. (2003). Inactivation of Geobacillus stearothermophilus spores by high-pressure carbon dioxide treatment. *Applied and Environmental Microbiology* 69, 7124-7129. 10.1128/aem.69.12.7124-7129.2003

Weber, K. A., Achenbach, L. A., and Coates, J. D. (2006). Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology* 4, 752-764. 10.1038/nrmicro1490

White, A., Burns, D., and Christensen, T. W. (2006). Effective terminal sterilization using supercritical carbon dioxide. *Journal of Biotechnology* 123, 504-515. 10.1016/j.jbiotec.2005.12.033

Wilkins, M. J., Hoyt, D. W., Marshall, M. J., Alderson, P. A., Plymale, A. E., Markillie, L. M., Tucker, A. E., Walter, E. D., Linggi, B. E., Dohnalkova, A. C., and Taylor, R. C. (2014). CO_2 exposure at pressure impacts metabolism and stress responses in the model sulfate-reducing bacterium *Desulfovibrio vulgaris* strain Hildenborough. *Frontiers in Microbiology* 5. 10.3389/fmicb.2014.00507

Wu, B., Shao, H. B., Wang, Z. P., Hu, Y. D., Tang, Y. J. J., and Jun, Y. S. (2010). Viability and metal reduction of *Shewanella oneidensis* MR-1 under CO_2 stress: Implications for ecological effects of CO_2 leakage from geologic CO_2 sequestration. *Environmental Science & Technology* 44, 9213-9218. 10.1021/es102299j

Zettlitzer, M., Moeller, F., Morozova, D., Lokay, P., Wuerdemann, H., and Grp, C. S. (2010). Re-establishment of the proper injectivity of the CO2-injection well Ktzi 201 in Ketzin, Germany. *International Journal of Greenhouse Gas Control* 4, 952-959. 10.1016/j.ijggc.2010.05.006

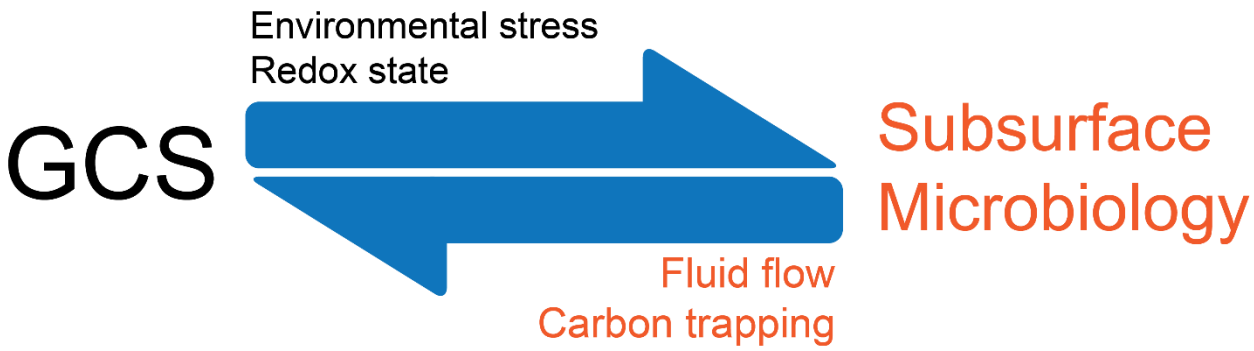
Zhang, J., Davis, T. A., Matthews, M. A., Drews, M. J., LaBerge, M., and An, Y. H. (2006). Sterilization using high-pressure carbon dioxide. *Journal of Supercritical Fluids* 38, 354-372. 10.1016/j.supflu.2005.05.005

792 TOC figure. This paper integrates geomicrobiology research performed by the Center for

793 Frontiers in Subsurface Energy Security to better understand the interplay between geological

794 carbon storage (GCS) and subsurface microorganisms.

795



796

797

798

Figure 1. Bright-field TEM images of CG-1 at 0.1 MPa CO_2 (A,C) and 1.0 MPa CO_2 (B,D). Arrows in: (B) show invaginations in CO_2 exposed cells that may suggest cell division; (C) show the intact cell wall for organisms at low CO_2 exposure; (D) show the capsular material present for CO_2 exposed cells. Modified after Santillan et al. (2015).

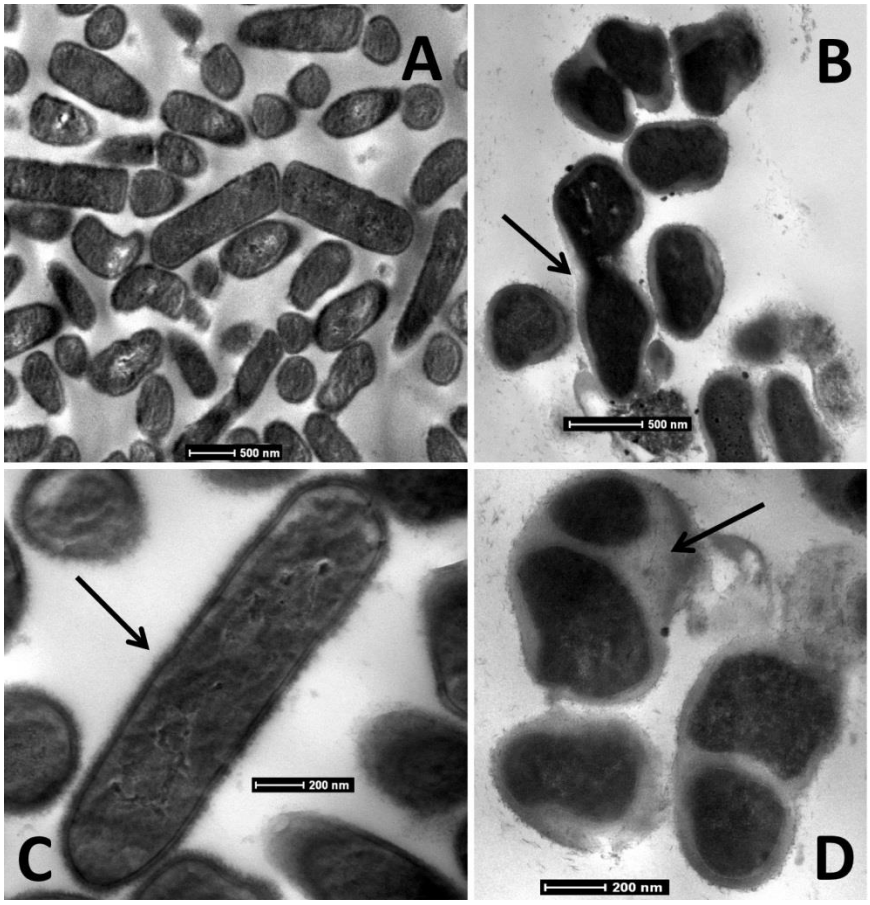


Figure 2. Variation with culture mineralogy in the abundance of culturable *Shewanella oneidensis* MR1 cells following incubation in the presence and absence (control) of high-pressure CO_2. Chart modified after Santillan et al., 2013.

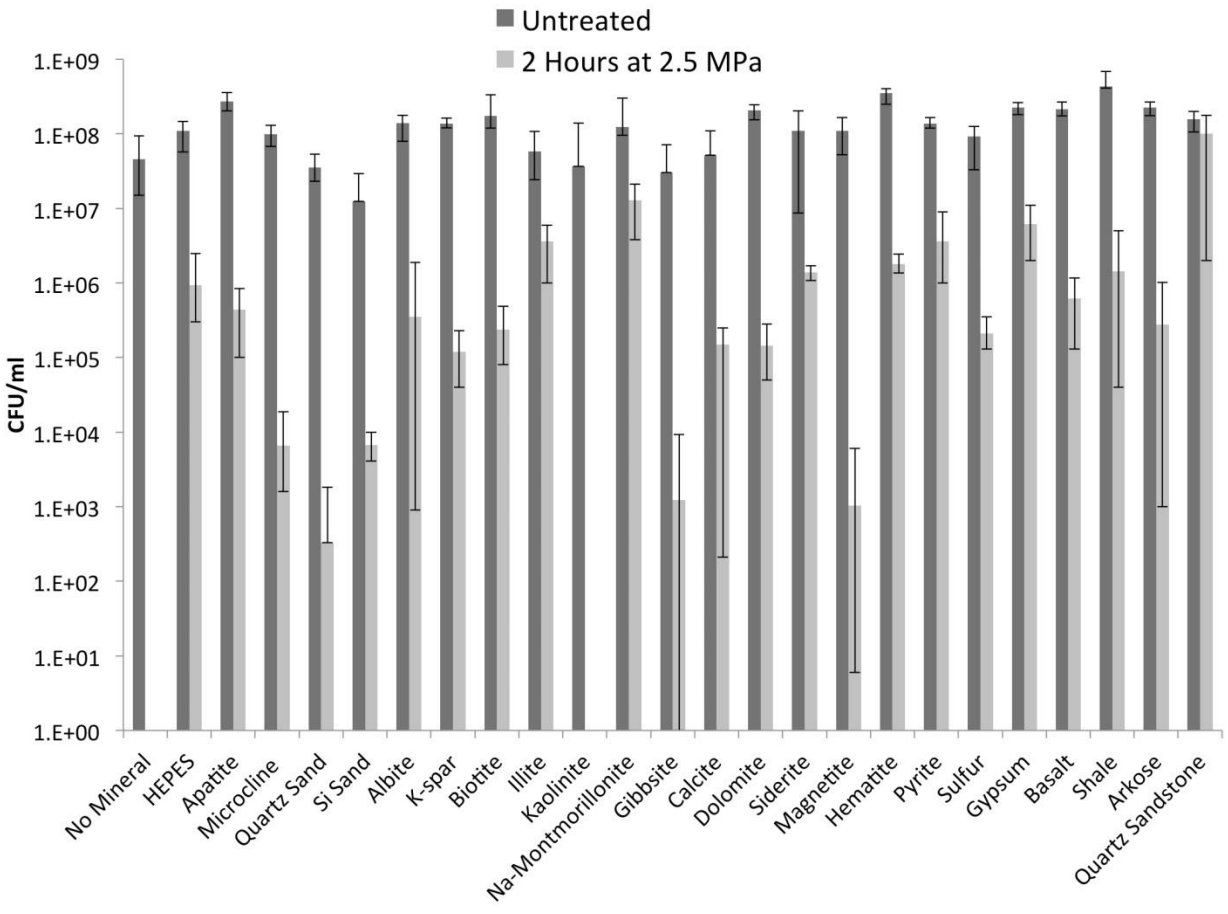


Figure 3. Typical variation in hydraulic conductivity of column reactors during bioclogging experiments.

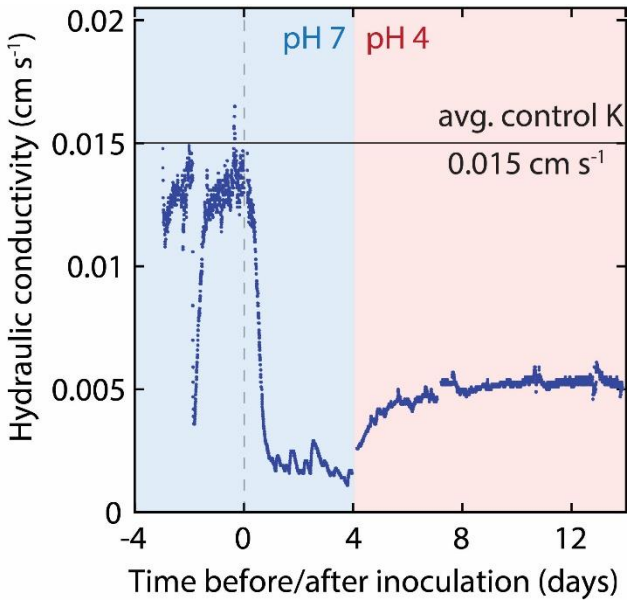


Figure 4. Average overall rate of acetate oxidation and the rate of acetate oxidation by iron reducers and sulfate reducers in the mixed-culture bioreactor experiments during the final 8 weeks of the incubations. Error bars show standard deviation.

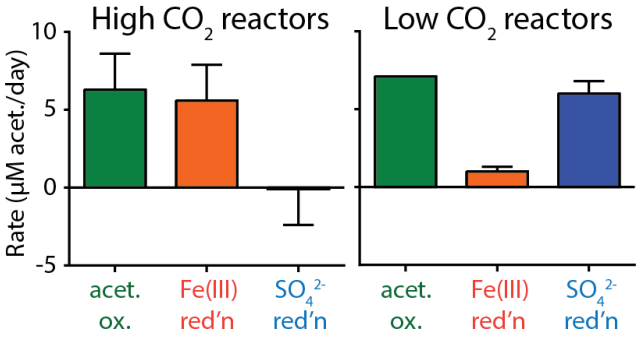


Figure 5. Change in energy available for iron reduction, sulfate reduction, and methanogenesis as a result of CO_2 injection during field CO_2-injection experiments. Values show the average difference between energy available prior to CO_2 injection and during. Three values were averaged for the Frio Formation experiment and eight for the ZERT experiment. Error bars show standard deviation. Calculations for iron reduction considered three sources of ferric iron (Fe(III)): goethite (FeOOH), hematite (Fe_2O_3), and magnetite (Fe_2O_3). All reactions were written on the basis of eight electron transfers with acetate or hydrogen serving as electron donors.

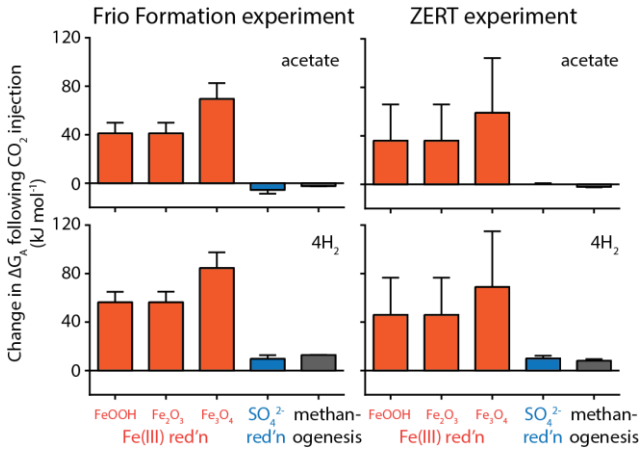


Figure 6. Average alkalinity content of effluent from the mixed-culture bioreactor experiments during the final 8 weeks of the incubations. Results are shown for biologically-active (i.e., live) bioreactors as well as corresponding sterile control reactors. Error bars show standard deviation.