Methanogenesis-induced pH-Eh shifts drives aqueous metal(loid) mobility in Sulfide Mineral systems under CO₂ enriched conditions

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ABSTRACT

To better understand the role of CO₂-utilizing processes in determining geochemical outcomes in CO₂-impacted, reduced subsurface environments; we studied hydrogenotrophic methanogenesis (a key process across a range of subsurface systems) and associated changes in CO₂-CH₄, pH-Eh and metal(loid) dynamics in CO₂-enriched pyrite-, arsenopyrite- and galena-containing batch reactors. Hydrogenotrophic methanogenesis proceeded via two first-order, microbially-mediated steps. The first involved microbially-enhanced gas-to-solution transfer of CO₂ with a rate constant of $0.95 \pm 0.20 \text{ d}^{-1}$ (compared to $0.17 \pm 0.34 \text{ d}^{-1}$ in non-methanogenic reactors). The second step involved the pseudo-first order reduction of HCO₃⁻-to-CH₄ with a rate constant of $0.46 \pm 0.10 \text{ d}^{-1}$. Bicarbonate reduction accounted for 76% of the CO₂-C removed from the reactors' headspace and triggered a decrease in Eh (-0.215 to -0.332V) and an increase in pH (6.93 to 8.03). No significant effect on dissolved Pb (or its release from galena) was observed but dissolved Fe decreased and As increased during methanogenesis. Changes in dissolved Fe and As were proportional to methanogenesis-induced pH-Eh shifts and attributable to an initial CO₂-induced release of As and Fe from arsenopyrite (and Fe from pyrite) with subsequent methanogenesis-induced increase in pH and electron activity triggering the precipitation of Fe, as amorphous FeCO₃·6H₂O. In addition to the role of methanogenesis in enhancing, 1) aqueous dissolution of CO₂, 2) CO₂ to carbonate mineralization and 3) immobilization of some metals, our findings suggested that due to its occurrence after CO₂ dissolution and before carbonate precipitation, great extents of HCO₃⁻-to-CH₄ reduction would lower solubility- and mineral-trapping of CO₂. Such considerations are central to assessments of how CO₂-utilizing processes may alter long-term outcomes at geologic storage sites.

1. INTRODUCTION

With current climatic trends favoring elevated levels of CO₂ in the subsurface, a comprehensive understanding of the fate and behavior of CO₂ in these environments is central in predicting any potential risk to environmental quality. An increasing number of studies have been focused on developing such an understanding by elucidating links between elevated CO₂ concentrations and the geochemistry of crucial subsurface resources such as potable aquifers and soils. Lu et al. (2010) suggested that increasing CO₂ concentration in potable aquifers may enhance the mobility of some metals via acidity-induced mineral dissolution, but reduce the net mobility of others via promotion of their adsorption to oxides and organic matter. Altevogt and Jaffe (2005) modeled the potential effect of elevated CO₂ levels on selected soil biogeochemical processes and found that in addition to decreasing solution pH, shifts in redox conditions and subsequently, nitrogen speciation and organic matter degradation could result. They predicted that CO₂ displacement of O₂ in soil air would result in reducing conditions that favors lower rates of organic matter degradation and the immobilization of NO₃⁻ [via its chemical reduction to NH₄⁺, dissimilatory nitrate reduction to NH₃, or denitrification to N₂ (Rivett et al., 2008)].

Significant progress has been made in understanding potential effects of CO₂ on subsurface geochemistry via this direct CO₂-systems response approach. Such progress has been reviewed in detail (Harvey et al., 2012; Lions et al., 2014). As highlighted in these reviews, key knowledge gaps still exist including the role of CO₂-transforming processes (e.g. autotrophic methanogenesis and acetogenesis) in determining geochemical outcomes in CO₂-impacted subsurface environments (Harvey et al., 2012). In other words, how different would the system's geochemical trajectory be in the presence, versus absence, of CO₂ transforming processes? To begin to answer this question, two key drivers of geochemical change must be considered. These

are, 1) the CO₂-induced alteration of aqueous pH towards more acidic conditions, and 2) the CO₂-induced alteration in redox (Eh) potentials towards more reducing conditions. The extent of pH and Eh alteration and consequently, geochemical change will be heavily reliant on the systems capacity to buffer such change. Experimental and modeling studies suggest that elevated CO₂ levels in the vadose zone or potable aquifers may decrease aqueous pH by 1– 3 units; with values at the lower end expected in well buffered environments where organic matter and/or alkalinity-producing minerals (e.g. carbonate-, feldspar- and clay-rich systems) can buffer against shifts in pH (Kharaka et al., 2010; Wilkin and DiGiulio, 2010). Conversely, CO₂-induced pH changes at the higher end of the range are expected in poorly buffered, quartz-dominated environments.

The consumption of CO₂, the production of reduced carbon species (e.g. CH₄ or CH₃COOH) and the shorter timescale needed to initiate autotrophic processes (weeks to months), compared to more permanent CO₂ sequestration mechanisms (e.g. mineral trapping), dictate that such processes are likely to alter the long term trajectory of pH and redox response in CO₂-impacted system. For example, the removal of CO₂ from solution (during its autotrophic consumption) would minimize against the CO₂-induced increase in H⁺ concentration typical of CO₂ impacted systems. Consequently, the propensity for H⁺-driven processes (such as carbonate and silicate weathering) to dominate would be less in CO₂-impacted subsurface systems where autotrophy is active. The reduction of CO₂ and the associated production of more reduced species would also be expected to lower the redox potential of the system and the propensity for oxidative processes.

Despite, theoretical arguments pointing to the potential for autotrophic processes to modify the impact of elevated CO₂ on subsurface geochemistry, what these changes look like

1 from a quantitative perspective and, to what magnitude they may alter geochemical outcome is
2 still unexplored. Given the diversity of autotrophs in the subsurface (Angel et al., 2012; Bharti et
3 al., 2014; Kotelnikova and Pedersen, 1997) and their capacity to reduce CO₂ via different
4 biological pathways (Costa and Leigh, 2014; Kaster et al., 2011; Rotaru et al., 2014) the
5 expectation is that quantifying the overall effects of autotrophy on geochemical outcome from
6 elevated CO₂ is a multivariate problem and requires a systematic approach.

7 As a first step in systematically quantifying relationships between autotrophic CO₂
8 utilization and geochemical outcome in CO₂-impacted environments, we studied the mechanism,
9 kinetics and effects on solution chemistry of microbially-mediated transformation of CO₂ to CH₄
10 via hydrogenotrophic methanogenesis. Our overarching goal was to assess the extent to which
11 hydrogenotrophic methanogenesis could alter the aqueous pH-redox signatures and solution
12 chemistry. Hydrogentrophic methanogenesis was chosen from among the possible autotrophic
13 pathways because it is the most well understood and, hydrogenotrophs are among the most well
14 known class of subsurface lithautotrophs (Chapelle et al., 2002; Kotelnikova and Pedersen, 1997;
15 Stevens and McKinley, 1995). In addition to considerations of biological pathway we wanted to
16 constrain the experiments to a (near) worst-case scenario. Therefore, experiments were designed
17 to compare extremes of no versus optimal hydrogenotrophic methanogenesis. To accomplish our
18 goal, we conducted a series of batch-reactor experiments in three sulfide mineral systems under
19 optimized methanogenic conditions, with and without the model hydrogentrophic methanogen -
20 *Methanococcus maripaludis*. Specific objectives were to, 1) assess the mechanism and kinetics
21 of methane production, 2) evaluate the effect of methanogenesis on aqueous pH and redox
22 signatures and, 3) compare changes in aqueous metal(loid) concentrations with and without
23 methanogenesis.

2. EXPERIMENTAL SECTION

2.1. Materials, Characterization and Experimental Set-up

The sulfide minerals used were natural samples of pyrite (FeS_2), arsenopyrite (AsFeS) and galena (PbS). Sulfides, as a class of minerals, were selected for their reduced nature and occurrence in methanogenic environments such as wetlands, coal-beds, anaerobic groundwaters. Specific sulfides were selected solely to provide chemical and mineralogical variability, with no assumptions of minerals being representative of a given system. Mineralogy and elemental composition of the samples was confirmed by XRD analysis (Figure S1) and microwave acid-digestion with ICP-OES analysis of the digest (Table S1). Prior to their use, the minerals were crushed, sieved (<150 , $150\text{-}250$ and $250\text{-}500\text{ }\mu\text{m}$), rinsed (1% HCl) and dried (room temperature) in an anaerobic glovebox (Coy Laboratory, Grass Lake, MI). Portions of the $<150\text{ }\mu\text{m}$ fractions were milled to very fine powders for XRD and microwave digestion and the $150\text{-}250\text{ }\mu\text{m}$ fraction was used for batch-reactor experiments. Crushing and sieving of samples was done to maintain experimental uniformity and minimize particle size effects on outcomes.

Batch reactors were autoclaved 10 ml crimp vials. A mineral: solution ratio of 1: 20 and an incubation temperature of 37°C were used in all experiments. A temperature of 37°C was chosen based on recommendations for the optimal growth of the methanogen (*M. maripaludis* strain *JJ*; Jones et al., 1983). The methanogen was purchased as a single strain (ATCC, Manassas, VA) and pre-cultured according to supplier's instructions. All preparations and experimental setup were done under anaerobic conditions. First, $0.25\pm0.01\text{ g}$ of the mineral was placed in each reactor with 5 ml of microbial growth media (non-methanogenic treatment) or 4.5 ml of growth media + 0.5 ml live methanogen inoculum (methanogenic treatment). Live

inoculum was added to the reactors at an approximate optical density (OD₆₆₀) of 0.15. The reactors were then stoppered (20 mm butyl rubber stoppers), crimped sealed and the headspace flushed and filled with a certified 80/20 H₂/CO₂ gas mix. The microbial growth media and the methanogen inoculum were prepared according to procedures outlined by Jones et al. (1983). After filling with the H₂/CO₂ gas mix, reactors were incubated in the dark at 37°C for 1, 3, 5, 7 or 14 days. Each reactor was prepared in duplicate (non-methanogenic) or triplicate (methanogenic). A third treatment (non-methanogenic gas blanks) comprising of 4.5 ml of growth media, 0.5 ml methanogen inoculum and a nitrogen filled headspace was also carried through the experiments. Gas blanks were single replicates and were incubated for 1, 7 and 14 days.

Following incubation, a nitrogen-flushed syringe (+ needle) was used to sample the headspace of each reactor and the sample analyzed immediately for CO₂ and CH₄ using gas chromatography (SRI-GC Model 6810C, Menlo Park, CA). Signals for CO₂ and CH₄ were obtained from an electron capture detector (ECD) and a flame ionization detector (FID), respectively. Reactors were then returned to the glovebox, decrimped, destoppered, the pH of the solution measured, the solution filtered (0.22 µm) and the solids washed (de-aerated water) and dried under anaerobic conditions. Solids from selected treatments were analyzed using scanning electron microscopy/energy dispersive spectrometry (SEM/EDS). Filtered solutions were analyzed using ICP-OES, for aqueous Fe and a suite of other elements (including Ca, Mg, Mn, P, S), and ICP-MS for aqueous As and Pb.

2.2. Geochemical Modeling

Data for headspace gas and aqueous elemental concentrations were combined with thermodynamic calculations to estimate redox potential (Eh), alkalinity and mineral saturation indices (SI) of carbonates and oxides for solutions from each replicate. Calculations were performed using the expanded LLNL thermodynamic database (thermo.com.V8.R6+.dat) in the React module of Geochemist's Workbench® 9 (Aqueous™ Solutions LLC, Champaign, IL). The thermodynamic database was used without alterations. For each set of calculations, fugacity of the gases ($f = \text{mol\%/100}$) were incorporated through basis swaps between $\text{CH}_{4(\text{aq})}$ and $\text{CH}_{4(\text{g})}$, HCO_3^- and $\text{CO}_{2(\text{g})}$ and, $\text{N}_{2(\text{aq})}$ and $\text{N}_{2(\text{g})}$ (Bethke, 2008). Fugacity of N_2 in gas blank treatments was calculated as $(1 - f_{\text{CO}_2})$. SI was defined as $\log(IAP/K_{sp})$, where IAP is the ion activity product of the species comprising the mineral and K_{sp} is the mineral solubility product at 37 °C.

2.3. Statistical Analysis

A two-stage statistical approach was used to evaluate data sets of measured and modeled parameters. The first stage (performed on data sets that span more than one mineral) was carried out to assess sulfide mineral and temporal effects on outcome within a fixed treatment (i.e. methanogenic and non-methanogenic). The second level of analysis (performed on all data, except CH_4) evaluated treatment effects (methanogenic versus non-methanogenic) on outcome. Both stages were executed using two-way, repeated-measures ANOVA ($\alpha = 0.05$). In the first stage, repeated measures were constrained to replicates for a given sulfide mineral and treatment. In the second stage repeated measures were dependent on if mineral effects were significant in stage 1. If mineral effects were significant, then repeated measures were constrained by minerals with comparisons across treatments. If minerals effects were not significant, then repeated measures were replicates for all minerals. In addition to facilitating the differentiation of kinetics and mineral driven processes, this staged approach to statistical

analysis allowed for a higher number of replicates (in the case of no mineral effects) and consequently improved rigor in assessing methanogenic versus non-methanogenic effects. All statistical analyses were done using GraphPad Prism[®] 6.0.

3. RESULTS

3.1. Headspace gas dynamics

Headspace CO₂ and CH₄ concentrations measured over the course of the study are shown in Figure 1. Values were aggregated across sulfide minerals because there were no statistical evidence of mineral effects on CO₂ (*P*-value = 0.633 and 0.059 for non-methanogenic and methanogenic treatment, respectively) or CH₄ (*P*-value = 0.052; Table S2). The borderline significance of mineral effects in methanogenic treatments was due to lower amounts of methanogenesis on day 3 in PbS-containing samples, compared to FeS₂ and AsFeS. Any PbS-induced inhibition was quickly overcome as there was no statistical evidence for mineral effects on CO₂ consumption and CH₄ production beyond day 3 (Table S3). On day 1, there were equivalent declines in headspace CO₂ concentration in both non-methanogenic and methanogenic treatments to approximately 17 mol%. After day 1, headspace CO₂ in non-methanogenic treatments averaged 16.1±2.4 mol% with no further significant change with time (*P*-value = 0.623). In contrast, significant temporal effects (*P*-value < 0.0001) were observed in methanogenic samples. Headspace CO₂ decreased from day 1 levels (17.6 mol%) to gas blank levels (1.9 mol%) by day 7, with no further decline thereafter (Figure 1a). Temporal effects accounted for 89% of within-treatment (and 49% of between-treatment) variance in headspace CO₂ in methanogenic treatments- significantly higher than the 10.6% attributable to temporal effects on headspace CO₂ in non-methanogenic samples and orders of magnitude higher than the

1 1.9 (*P*-value = 0.633) and 1.93% (*P*-value = 0.059) attributable to sulfide mineral effects in non-
2 methanogenic or methanogenic treatments, respectively.

3 No CH₄ was detected in non-methanogenic reactors. Hence, assessments of temporal or
4 mineral effects on CH₄ production were not possible in this treatment. In contrast, average
5 headspace CH₄ in methanogenic reactors increased significantly over time from 1.4 mol% on day
6 1 to 13.3 mol% on day 14 (Figure 1b). The increase in headspace CH₄ occurred in conjunction
7 with the decrease in headspace CO₂ in methanogenic reactors. Approximately 76% of the total
8 headspace CO₂-C lost over the course of the study was directly attributable to CH₄-C production.
9 As with headspace CO₂ in methanogenic reactors, time was the main source of variance for
10 headspace CH₄ - accounting for 89% (*P*-value < 0.0001) of total variability. Mineral effects
11 accounted for less than 1% of total CH₄ variability.

12 The opposite response in CO₂ versus CH₄ in methanogenic reactors was consistent with
13 expected trends for methanogen-mediated reduction of CO₂ to CH₄. The fact that 89% of the
14 total within-treatment variability in CO₂ and CH₄ in these samples was attributable to temporal
15 effects, indicated a strong reliance on reaction kinetics. Nevertheless, kinetic analysis of
16 headspace CO₂ consumption and CH₄ production suggested that hydrogenotrophic
17 methanogenesis was not straightforward and included at least two distinct, sequential steps. For
18 example, reactant-based first-order rate modeling of CO₂ consumption from day 1 to day 14 in
19 methanogenic and non-methanogenic reactors yielded rate constants (*k*) of 0.95±0.20 and
20 0.17±0.34 d⁻¹, respectively (Figure S2a). The rate constant for CO₂ consumption in the
21 methanogenic reactors was much higher than the 0.45±0.06 d⁻¹ obtained via first-order product-
22 based kinetics for CH₄ production, indicating that CO₂ was removed from the headspace about 2
23 times faster than it was being reduced to CH₄ (Figure S2b). The faster rate of CO₂ removal in

methanogenic reactors was well supported by the fact that despite headspace CO₂ reaching a minimum by days 3 or 5, peak headspace CH₄ was not observed until after 7 days; consistent with a 2 to 4 day lag time between CO₂ removal from the headspace and its later reduction to CH₄. This suggested that the removal of CO₂ and the addition of CH₄ to the headspace were decoupled processes. The absence of a significant decline in headspace CO₂ beyond day 1 in the non-methanogenic reactors was indicative of both processes being mediated by the methanogen. The most plausible explanation is that of an initial gas-to-solution step (where CO_{2(g)} dissolution into the aqueous phase is microbially enhanced), followed by a solution-to-gas step (where the dissolved CO₂ species is microbially reduced to CH_{4(g)} and exsolved into the headspace). A generalized equation for gas-to-solution step (Step 1) can be written as:

Based on measured pH values (6.9 to 8.4; no statistical difference across minerals) HCO₃⁻ would be the dominant aqueous CO₂ species in our experiments and hence the most likely reaction for CH₄ generation (the solution-to-gas step) would be:

Trends in proton concentration (calculated as 10^{-pH}; Figure S3) were supportive of a H⁺ consuming reaction but also indicated that HCO₃⁻ reduction to CH₄ was zeroth order with respect to H⁺ consumption. This leaves HCO₃⁻ and H_{2(aq)} as the potential limiting factor(s) in methanogenesis. Under hydrogen-limited conditions, H_{2(aq)} would be the primary limiting factor and hence the pseudo-first order rate expression for methanogenesis would be, rate = -*k'* [H_{2(aq)}] with *k'* representing the pseudo first-order rate constant. However, since our experiments were conducted under non-H_{2(g)} limited conditions, the reactant-based kinetics for hydrogenotrophic

methanogenesis could be approximated using the pseudo-first order expression of rate = $-k'$ $[\text{HCO}_3^-]$. Using this latter rate expression, and calculated HCO_3^- concentrations (determined using Equation S1), an overall k' value of $0.46 \pm 0.10 \text{ d}^{-1}$ was obtained (Figure 2). It is worthwhile mentioning that in addition to k' ($0.46 \pm 0.10 \text{ d}^{-1}$) being statistical equivalent to k for CH_4 production ($0.45 \pm 0.06 \text{ d}^{-1}$), calculated HCO_3^- concentrations were also comparable to $\text{CO}_2(\text{aq})$ from our geochemical modeling exercise (data not shown).

3.2. pH, Eh and aqueous metal(loid) chemistry

Aqueous pH and Eh for gas blanks, non-methanogenic and methanogenic treatments are shown in Figures 3 and S4. As with gas concentrations, there was no evidence of a significant mineral effect on pH (Table S2). Average pH in gas blank and non-methanogenic treatments were 8.04 and 6.93 respectively, and (with the exception of day 5) did not change significantly with time. The lower aqueous pH observed in non-methanogenic (versus gas blank) samples were reflective of CO_2 –induced acidification; attributed to proton release into solution as a result of carbonic acid dissociation ($\text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+$), particularly above its first pK_a ($\text{pH}=6.33$). For gas blanks, the dissolution of N_2 into the aqueous phase would have no effect on H^+ concentration and therefore were largely reflective of baseline pH conditions without CO_2 impact.

In contrast to the gas blank and non-methanogenic treatments, aqueous pH in methanogenic reactors changed significantly with time ($P\text{-value} < 0.0001$). Average aqueous pH in methanogenic reactors was 6.93 on day 1 (the same as in non-methanogenic reactors) but increased with methanogenesis to 7.98, 8.37, 8.00 and 7.95 on day 3, 5, 7 and 14, respectively. These higher pH values were, 1) comparable to pH in gas blanks, 2) indicative of H^+

consumption with methanogenesis (Equation 2), and 3) reflective of the capacity of hydrogenotrophic methanogenesis to “buffer” against CO₂-induced acidification. Such changes pointed to a very different chemical environment and potential for contaminant mobility than if methanogenesis was not considered.

Redox potentials (Eh) also reflected very different chemical environments in non-methanogenic versus methanogenic reactors (Figure 3b). Average estimated Eh in gas blanks was -0.218V and was constant throughout the study. Average estimated Eh in non-methanogenic reactors was -0.238, -0.242, -0.272, -0.248 and -0.248V on day 1, 3, 5, 7 and 14 respectively; indicating CO₂-induced decrease in redox potential. Except on day 1, when Eh was -0.248 V redox potentials in methanogenic reactors were significantly lower (compared to non-methanogenic reactors; *P-value* < 0.0001), with an average Eh across day 3, 5, 7 and 14 (*n* = 36) of -0.332±0.011 V; consistent with typical methanogenic systems (Hungate, 1967). A favorable environment for hydrogenotrophic methanogenesis was created in methanogenic reactors via microbially-enhanced CO₂ dissolution and subsequent microbially-mediated reduction of HCO₃⁻ to CH₄.

The combined effect of methanogenesis-induced pH-Eh changes on metal(loid) mobility was highlighted by differences in both mineral saturation states and dissolved Fe, As and Pb concentrations (Table S3). Methanogenesis-induced increase in pH (and decrease in Eh) favored an increase in saturation state with respect to both carbonate and Fe-oxide mineral phases (Table 1). Among the carbonates, methanogenesis-induced changes in mineral saturation state (*ΔSI*) were up to 5 orders of magnitude. Trends in *ΔSI* (hydromagnesite > huntite > dolomite > siderite = magnesite > rhodocrosite = calcite) pointed to methanogenesis promoting the precipitation of hydrated and mixed-metal carbonate phases, than single-metal, non-hydrated carbonates. A lack

1 of a significant treatment effect on aqueous Mg concentration excluded the possibility of
2 precipitating Mg-bearing minerals (i.e. hydromagnesite, huntite, dolomite, magnesite). Such
3 observations were consistent with low temperature precipitation studies and indicated that while
4 methanogenesis increased thermodynamic favorability, the kinetic barrier to their precipitation
5 still existed in our study (Lin and Singer, 2005; Saldi et al., 2009).

6 Figure 4 show measured dissolved Fe, As and Pb (the major metal(oids) in the sulfide
7 minerals) concentrations. Concentrations ranged from 5 to 90, 4 to 8 and 0.03 to 0.09 μM for Fe,
8 As and Pb respectively. Galena and arsenopyrite were the only mineral source of Pb and As,
9 respectively. Consequently, no assessment of mineral effects on the aqueous Pb or As was
10 possible. Assessment of mineral effects was however possible for Fe with both pyrite and
11 arsenopyrite as sources. No definitive temporal trend ($P\text{-value} = 0.6904$) or significant treatment
12 effects ($P\text{-value} = 0.2985$) was observed for aqueous Pb (Figure 4a). This was attributable to the
13 low solubility and slow dissolution kinetics of PbS in aqueous solution around near neutral pH
14 (Barrett and Anderson, 1988; Uhler and Helz, 1984). In contrast to Pb, dissolved As
15 concentration increased significantly with time ($P\text{-value} < 0.0001$) and in methanogenic relative
16 to non-methanogenic treatments ($P\text{-value} = 0.0035$). Treatment effects (13.4% of variability)
17 were however low compared to temporal effects (69.5%); suggesting a strong kinetic effect on
18 As dynamics with shifts in pH and Eh.

19 Mineral effects accounted for 60.3 and 84.5% of the variability in dissolved Fe
20 concentrations within methanogenic and non-methanogenic treatments, respectively; suggesting
21 strong mineral-specific controls. Dissolved Fe in both pyrite- ($P\text{-value} < 0.0001$) and
22 arsenopyrite-containing ($P\text{-value} = 0.0004$) reactors decreased in methanogenic relative to non-
23 methanogenic treatments (Figure 4b). In pyrite-containing reactors, treatment effects accounted

for 51.5% of the variability in dissolved Fe, whereas temporal effects accounted for 11.2%. Similar trends were apparent in arsenopyrite-containing reactors (52.9 and 33.2%, respectively) indicating stronger treatment than kinetic effects. The stronger kinetic effect on dissolved Fe concentrations in arsenopyrite-containing reactors was consistent with that observed for dissolved As concentrations but, the fact that As increased while Fe decreased in their respective methanogenic treatments suggested very different effects of methanogenesis-induced pH and Eh shifts on the mobility of the two metal(oids).

4. DISCUSSION

4.1. Quantitative effects of hydrogenotrophic methanogenesis on shifts in pH and redox conditions

As depicted in equation 2, HCO_3^- to CH_4 reduction involved H^+ consumption, the acceptance of 8 electrons per mole of HCO_3^- , and consequently the capacity to provide both pH and redox buffering. Buffering via methanogenesis is however very different from that via mineral weathering. For example, pH buffering via mineral weathering typically involves the $\text{H}_2\text{CO}_{3(\text{aq})}/\text{HCO}_3^-$ acid-base pair. Hence, minerals such as carbonates, feldspars and clays that produce HCO_3^- upon weathering are good pH buffers due to their H^+ consumption capacity. With methanogenesis, pH buffering is via the $\text{HCO}_3^-/\text{CH}_4$ redox couple. Therefore rather than the kinetics of mineral dissolution, it is the kinetics of methanogenesis that regulates buffering response. This was apparent from data that showed no reversal of CO_2 -induced acidity without methanogenesis. The highest rate of decline in H^+ activity also coincided with the highest rates of methanogenesis (Figure 3a).

Differences in H^+ activity between respective non-methanogenic and methanogenic treatments (0.91 ± 0.05 pH unit) put the pH buffering effect of methanogenesis at approximately an order of magnitude. This was in line with that expected in carbonate systems impacted by elevated CO_2 (Shao et al., 2015; Wilkin and DiGiulio, 2010). However, given that our experiments were conducted under optimal conditions, is likely to be representative of the maximum pH buffering effect that could be expected for methanogenesis. Lower H_2 concentrations, competition from more thermodynamically favorable H_2 -utilizing processes (e.g. sulfate-, nitrate-, Fe(III)-reduction) and the involvement of other methanogenic pathways would constrain the reaction kinetics and extent of methanogenesis in natural environments (Achnich et al., 1995; Kotelnikova, 2002; Reiche et al., 2008). A generalized pH buffering effect of hydrogenotrophic methanogenesis (BH_{HM}^+) in natural environments can be estimated using the equation

$$BH_{HM}^+ = -\Delta H_{HM}^+ \int_{i=0}^{i=t} R_{TM,i} \phi_{HM,i} dt \quad (3)$$

where, ΔH_{HM}^+ is the change in H^+ activity due to hydrogenotrophic methanogenesis ($-5.26(\pm 0.78) \times 10^{-3}$ mmol L^{-1} mmol $^{-1}$ CH_4 ; from this study), R_{TM} is the rate of total methane production within the system of interest, ϕ_{HM} is the fraction of total methane due to hydrogenotrophic methanogenesis and t is the duration of active hydrogenotrophic methanogenesis. Reported values for R_{TM} in natural environments range from 1.7×10^{-5} to 1.30 mmol d^{-1} with ϕ_{HM} in the 0.15-0.46 range (Conrad, 1999; Kotelnikova and Pedersen, 1997; Mayumi et al., 2013). This suggests daily BH_{HM}^+ of between 1.34×10^{-8} and 3.15×10^{-3} mmol L^{-1} in natural environments. The range for BH_{HM}^+ in our experiments ($R_{TM} = 0.22$ to 2.53 mmol d^{-1} and $\phi_{HM} = 1$) was 1.16×10^{-3} to

1 $1.33 \times 10^{-2} \text{ mmol L}^{-1}$; about 0.4 to 4 times the maximum expected under normal *in situ*
2 methanogenic conditions.

3 In contrast to H^+ activity, which decreased, methanogenesis-induced changes in redox
4 potential (Eh) indicated an increase in electron activity (e^-) and consequently a “buffering”
5 against oxidative processes. Conversion of Eh to (e^-) indicated a 19.7 ± 2.85 times higher electron
6 activity in methanogenic versus non-methanogenic reactors (Equation S2). Despite free
7 electrons not existing in solution a similar expression to equation 3 can be used to estimate the
8 “virtual” electron activity contribution of hydrogenotrophic methanogenesis (Be_{HM}^-), with
9 Be_{HM}^- replacing BH_{HM}^+ and the change in electron activity (Δe_{HM}^-) replacing Δe_{HM} . Δe_{HM}^- has a
10 value of $1.18(\pm 0.65) \times 10^{10}$ with units of mol^{-1} while Be_{HM}^- is unitless with daily values ranging
11 from 2.59×10^9 to 2.97×10^{10} in our experiments and, 3.00×10^4 to 7.03×10^9 in natural
12 environments

13 The significance of Be_{HM}^- is more intuitive when considering its impact on Gibb’s free
14 energy. Without consideration of Be_{HM}^- free energy can be defined as $\Delta G = nF(Eh) = -0.062nF(-$
15 $\log e^-_{initial})$, where $n = 8$ (the number of electrons involved in HCO_3^- to CH_4 reduction), F is
16 faraday’s constant ($96.485 \text{ kJ V}^{-1} \text{ mol}^{-1}$) and $e^-_{initial}$ is the initial electron activity. With Be_{HM}^- the
17 free energy expression becomes $\Delta G_{e^-} = 47.86 \log (e^-_{initial} + Be_{HM}^-)$ indicating a reduction in free
18 energy of the system, with methanogenesis. Under natural methanogenic conditions (Eh = -0.35
19 to -0.26V), this effect ($\Delta G_{e^-} - \Delta G$) ranges from 1 to 270 kJ mol^{-1} of CH_4 produced; with the
20 impact being greatest in less reducing environments (Figure 5).

21 **4.2. Links between methanogenesis, metal(loid) geochemistry and carbon cycling in** 22 **reduced environments**

Our results indicate that hydrogenotrophic methanogenesis will trigger very different geochemical signatures, in the both the gas and aqueous phase, than would occur under non-methanogenic conditions. Of particular relevance to the goals of this study are implications of such changes for 1) the mobility of metal(oids) in reduced environments under elevated CO₂ conditions and, 2) carbon cycling as it pertains to CO₂ storage in reduced geologic formations.

4.2.1. Metal(loid) mobility. Observed shifts in aqueous pH and Eh reflected two key changes of relevance to deciphering the effects of hydrogenotrophic methanogenesis on metal(loid) mobility in reduced environments. These are the capacity for hydrogenotrophic methanogenesis to 1) decrease H⁺ activity and, 2) increase electron activity. Decreasing H⁺ activity dictates that acid-induced dissolution or desorption was less likely to control metal mobility. Instead, precipitation of oxide/carbonate phases and desorption of metalloid species via hydroxyl ligand exchange would be favored. Increasing electron activity would dictate that reductive processes (e.g. reductive dissolution), rather than oxidative processes, would control mobility of redox-sensitive species (e.g. Fe and As) but the potential for anaerobic oxidation of the sulfide minerals cannot be ignored (Bach and Edwards, 2003; Jost et al., 2010).

The comparatively lower dissolved Fe concentrations in methanogenic reactors indicated an overall sequestering of Fe. In contrast, higher dissolved As indicated a net release of As with methanogenesis. With the methanogenesis-induced changes in pH and Eh a decrease in pyrite and arsenopyrite dissolution would be anticipated based on the fact that the acid-induced and oxidative dissolution would be less favored. Such scenarios could plausibly account for lowered aqueous Fe but not the increased aqueous As. Contributions of redox-driven dissolution mechanism(s) could not be eliminated but several lines of evidence pointed to an initial acidification-induced mobilization and subsequent H⁺- or redox-driven precipitation of Fe as the

primary pathways controlling Fe and As mobility in our experiments. Firstly, differences in aqueous Fe in day 1 pyrite (40-45 μM) and arsenopyrite (4-7 μM) reactors indicated a mineral origin for the Fe in solution. Secondly, the decrease in aqueous Fe in the pyrite-containing, methanogenic treatments to ≤ 21 μM by day 3 suggested the likely precipitation of the solubilized Fe as methanogenesis progressed (Figure 4a). Thirdly, arsenopyrite dissolution would be accompanied by a co-release of As and Fe. Therefore, without precipitation, a similar Fe: As ratio is expected in methanogenic as non-methanogenic reactors but was not the case (Figure 4b). Instead, Fe: As ratios were significantly higher in non-methanogenic (2.08 ± 0.63) than methanogenic (1.00 ± 0.08) treatments. The lower Fe: As in methanogenic treatments was consistent with a preferential removal of Fe, compared to As, from solution as would be expected with precipitation of Fe oxides or carbonates. Sorption of Fe^{2+} to microbial cells was also considered but was ruled out as a significant contributor, given the lack of effect on Pb^{2+} or other readily adsorbed cations (e.g. Ca^{2+} or Mg^{2+}). Finally, changes in the relative concentrations of dissolved Fe and As across treatments (C_{HM}/C_{non-HM}) show strong direct links to methanogenesis-induced shifts in H^+ and e^- activity (Figure 6). Strong positive linear relationships ($r^2 \geq 0.87$) between C_{HM}/C_{non-HM} for aqueous Fe and $\frac{1}{(BH_{HM}^+)^n}$ or $\frac{1}{(Be_{HM}^-)^n}$ (where $n=2$ or 0.5 for pyrite- and arsenopyrite-containing samples respectively) pointed to the same methanogenesis-induced process controlling Fe mobility. The magnitude of n reflected the relative impact on the different mineral systems, with the higher n being indicative of methanogenesis having a greater effect on reducing Fe mobility in pyrite- than arsenopyrite-containing samples. Similarities in the absolute values of the slopes in Figure 6b suggested that any precipitation of Fe would occur concomitantly with the release of As.

Evidence from SEM-EDS analysis showing the presence of a Fe-rich precipitate on the surface of the 14 day arsenopyrite samples from methanogenic experiments was also supportive of a precipitation driven decrease in aqueous Fe (Figure 7). The presence of carbon and oxygen in the EDS spectrum in Figure 7b suggested an iron-carbonate phase but efforts to ascertain its identity via x-ray and electron diffraction were inconclusive. Thermodynamically, siderite (FeCO_3) was the most favored Fe-carbonate phase with saturation conditions increasing with time and always higher in methanogenic than non-methanogenesis (Table 2). While siderite precipitation was highly doubtful in our experiments, given its high required SI (>2.75) and slow precipitation kinetics, the microbially-mediated precipitation of hydrated $\text{FeCO}_3 \cdot 6\text{H}_2\text{O}$ as an amorphous siderite precursor would explain both the sequestering of Fe and the discrepancy in the CO_2 - CH_4 balance observed in our methanogenic samples (Jimenez-Lopez and Romanek, 2004; Radha and Navrotsky, 2013; Sel et al., 2012).

4.2.2. Geologic Carbon sequestration. Our results highlighted three key considerations regarding how hydrogenotrophic methanogenesis could impact carbon sequestration potential in geologic formations; 1) the enhanced dissolution of CO_2 , 2) the increased potential for carbonate precipitation and, 3) the decoupling of CO_2 dissolution from HCO_3^- reduction..

Enhanced aqueous dissolution of CO_2 in methanogenic reactors pointed to the potential for methanogenesis to enhance solubility trapping of CO_2 in geologic formations. The large difference in headspace CO_2 in methanogenic versus non-methanogenic reactors suggest that

1 solubility trapping of CO₂ is likely to be orders magnitude higher with methanogenesis than
2 without. Such observations have significant implications for predicting and modeling solubility
3 trapping in geologic CO₂ reservoirs (e.g. coal-bed formations, exploited oil reservoirs, basalts or
4 any formations with active serpentinization) where methanogenesis have been shown to be
5 significant. Enhanced solubility trapping has been reported for various non-methanogenic
6 microorganism suggesting that this was not unique to the methanogen in our study but a
7 generalized trait for a range of microorganisms. Zhao et al. (2014) and Mitchell et al. (2010)
8 observed increased CO₂ dissolution with microbial growth being consistent with surface
9 complexation of dissolved CO₂ species, by the microbial surface/biofilm, being the main driver
10 of enhanced CO₂ partitioning from the gas to solution phase.

11 Higher saturation index values coupled with evidence for precipitation of iron-carbonate
12 in our methanogenic treatments highlighted the potential for methanogenesis to enhance mineral
13 trapping of CO₂. As with solubility trapping, enhanced mineral trapping capabilities in microbial
14 treatments are not unique to our experiments (or to methanogens) but is consistent with
15 laboratory and field studies examining the role of microorganisms in carbonate formation. A
16 broad range of microorganisms including sulfate-reducers, sulfate-oxidizers, iron-reducers, urea
17 degraders, methanogens and methanotrophs are capable of mediating biogenic carbonate
18 formation in much shorter time scale and through unique mechanisms than would be expected in
19 abiotic systems. For example, Roberts et al. (2004) found that a consortium of methanogens and
20 fermenters mediated the formation of iron-rich dolomite on pre-sterilized Columbia River Basalt
21 within 3 months of its suspension into a reduced fresh water aquifer. The formation of the
22 dolomite was attributed to enhanced microbially-mediated weathering of basalt, coupled to
23 hydrogenotrophic methanogenesis with crystal nucleation occurring on the cell walls of the

methanogens. Recent research points to mineral templating on cell walls and excreted exopolymeric substances (EPS) as the primary modes for enhanced heterogeneous carbonate nucleation/precipitation observed in microbial systems (Braissant et al., 2007; Krause et al., 2012; Stephenson et al., 2008). Cell walls and EPS are thought to enhance carbonate formation via the preferential concentration of component species at the cell wall-solution or EPS-solution interface (Krause et al., 2012).

To our knowledge, the distinctive lag observed in our experiments between the gas-to-solution removal of CO_2 from the headspace and HCO_3^- reduction has not been previously reported. Such occurrence and the kinetics of methanogenesis would regulate the extent to which methanogenesis could be considered beneficial to geologic CO_2 sequestration. For example, the strong reliance of solubility-trapping, methanogenesis and mineral-trapping on HCO_3^- and differences in their comparative rates of reaction (solubility-trapping > methanogenesis > mineral-trapping) dictate that methanogenesis on one hand would control the longevity of any benefits from solubility trapping and on the other, the limits on mineral-trapping. Our results suggest that HCO_3^- to CH_4 reduction could reverse as much as 76% of solubility-trapping and limit mineral-trapping plus effective solubility-trapping to a maximum of 24% of the initially sequestered CO_2 -C. These values are really extremes of what could be expected in the subsurface but nonetheless, provide an upper limit on the effects methanogenesis could have on geologic CO_2 sequestration. The lower rates of HCO_3^- to CH_4 reduction and lower ϕ_{HM} in natural environments dictate that the outcome would be skewed towards a higher proportion of solubility- plus mineral-trapping in these systems.

5. CONCLUDING REMARKS

1 Significant knowledge gaps exist with regards to CO₂-utilizing processes and their role
2 and impact in assessing/ predicting geochemical response to, and environmental implications of,
3 elevated CO₂ levels in the subsurface. As a first foray into closing these knowledge gaps, we
4 investigated links between CO₂ utilization (via hydrogenotrophic methanogenesis) and
5 geochemical response in model mineral system enriched with CO₂ gas. Our results indicated
6 that: 1) the direction and magnitude of process-induced (in our case methanogenesis-induced)
7 shifts in H⁺ and e⁻ activity, and 2) the order in which processes occur are central to determining
8 how CO₂-utilizing processes will alter geochemical trajectories with respect to key outcomes
9 such as carbon sequestration and contaminant mobility.

10 Intra- and inter-site heterogeneities dictate that the quantitative impact of CO₂ utilizing
11 processes will vary across systems. Nevertheless, as shown experimentally and illustrated in
12 figure 8, some general trajectories are definable by considering changes in thermodynamically
13 favored species with process-induced shifts in pH and Eh. Without methanogenesis, elevated
14 CO₂ levels favor carbonate and Fe-oxide dissolution and consequently, the mobilization of
15 structural (e.g. Ca²⁺, HCO₃⁻ and FeHCO₃⁺; Figure 8a and c) and potentially harmful non-
16 structural components (e.g. As, Pb, Cd, Ni, Zn, Cr, V and U) associated with these minerals
17 through sorption or co-precipitation (Kharaka et al., 2010; Lu et al., 2010). Increases in HCO₃⁻
18 and FeHCO₃⁺ concentration also favor the mobilization of harmful oxyanionic (e.g. As, U and Cr
19 – in the case of HCO₃⁻) and metallic (in the case of FeHCO₃⁺) species via their desorption from
20 the surfaces of minerals and aquifer sediments (Anawar et al., 2003; Mason et al., 1997;
21 Saalfield and Bostick, 2010).

22 With methanogenesis, the precipitation (rather than dissolution) of carbonate minerals is
23 thermodynamically favored (Figure 8a and c). In addition to increasing permanency of CO₂

sequestration (through mineralization), the potential for (re)immobilization of structural and non-structural species (via co-precipitation or incorporation) is therefore enhanced under methanogenic conditions. Increased consumption of HCO_3^- during methanogenesis and that of FeHCO_3^+ during associated carbonate mineralization would also reduce the likelihood for mobilization of potential contaminants via bicarbonate-driven desorption. Unlike carbonates, the reprecipitation of Fe(III) oxides is not favorable under methanogenic conditions. Consequently, re-immobilization of contaminants via sorption to Fe oxide surfaces would not be expected under methanogenic conditions.

Our results also indicated that whereas methanogenesis would increase CO_2 sequestration via enhanced solubility trapping of CO_2 and carbonate mineralization in CO_2 -impacted environments, the extent to which such benefits are realized depends largely on the extent and kinetics of HCO_3^- reduction step. For example, the comparative rates of reaction (CO_2 dissolution > HCO_3^- reduction > mineralization) and the strong reliance of all three steps on HCO_3^- availability creates the scenario where HCO_3^- reduction controls on one hand, the longevity of any benefits from solubility trapping and on the other, the limits on CO_2 mineralization. In light of our findings, we concluded that CO_2 -utilizing processes (including, but not limited to methanogenesis) are central to predicting long-term outcomes of elevated CO_2 levels in the subsurface. Minimally, their modulating effects on geochemical outcome should be considered and incorporated into assessment and strategic efforts to improve current understanding of CO_2 behavior as it relates to geologic CO_2 storage or potential alterations of environmental quality.

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LIST OF FIGURES

Figure 1. Concentration of (a) CO₂ and (b) CH₄ measured in the headspace of methanogenic (filled circles), non-methanogenic (unfilled circles) and gas blank (squares) batch reactors. Error bars show standard deviation for $n = 9, 6$ or 3 samples for methanogenic, non-methanogenic and gas blank treatments, respectively.

Figure 2. Calculated aqueous HCO₃⁻ concentration in methanogenic reactors and associated kinetic model. Error bars show standard deviation for $n = 9$ samples.

Figure 3. Aqueous pH measured in methanogenic (filled circles), non-methanogenic (unfilled circles) and gas blank (squares) reactors. Error bars show standard deviation for $n = 9, 6$ or 3 samples for methanogenic, non-methanogenic and gas blank treatments, respectively.

Figure 4. Aqueous (a) Fe from pyrite, (b) Fe and As from arsenopyrite and (c) Pb from galena measured in methanogenic (filled symbols) and non-methanogen (unfilled symbols) reactors. Error bars show standard deviation for $n = 3$ or 2 replicates from methanogenic and non-methanogenic treatments, respectively.

Figure 5. Relationship between methanogenesis-induced shift in electron activity (Be_{HM}^-) and changes in free energy ($\Delta G_e - \Delta G$) for initial Eh of $-0.26, -0.30$ and $-0.35V$. Unfilled symbols indicate estimated limits for natural environments and closed symbols, the values for our experiments.

Figure 6. Relationship between methanogenesis-induced shifts in electron activity ($X = Be_{HM}^-$), H⁺ activity ($X = BH_{HM}^+$), aqueous Fe (filled symbols) and aqueous As (unfilled symbols) concentration ratios (C_{HM}/C_{non-HM}) in methanogenic: non-methanogenic treatments.

Figure 7. (a,c) Scanning electron microscopy image and (b,d) energy dispersive spectra (EDS) showing evidence of methanogenesis-induced precipitation of iron carbonate neophase on the surface of arsenopyrite. Grey shaded areas on EDS indicate the presence of oxygen and lower Fe/C ratio in spectrum 1 (the neophase) compared to spectrum 3 (the clean arsenopyrite surface). Sample was incubated under methanogenic conditions for 14 days.

Figure 8. Pourbaix (Eh-pH) diagrams showing thermodynamically-stable (a) Ca, (b)Pb, (c)Fe and (d)As species expected in a typical reduced groundwater within the contiguous U.S. Points on the diagrams are pH-Eh signatures for median US groundwater composition (star), gas blanks (square), non-methanogenic samples (unfilled circles) and methanogenic samples (filled circles). Data for median groundwater composition were from Birkholzer et al.(2008). Remaining data points are average pH and Eh values across pyrite-, arsenopyrite- and galena-containing reactors after 1, 3, 5, 7 and 14 days of incubation.

Table 1. Calculated alkalinity and mineral saturation indices for selected carbonate and Fe-oxide minerals under non-methanogenic versus methanogenic conditions. With exception of SI for iron-bearing minerals, all values are average ($\pm$ standard deviation) across pyrite, arsenopyrite and galena containing samples.

Solution Parameters	Day 1		Day 3		Day 5		Day 7		Day 14		ΔSI^c
	Non-methanogenic	Methanogenic	Non-methanogenic	Methanogenic	Non-methanogenic	Methanogenic	Non-methanogenic	Methanogenic	Non-methanogenic	Methanogenic	
Carbonate Alkalinity (mg L ⁻¹)	$1.71(\pm 0.36) \times 10^3$	$1.72(\pm 0.36) \times 10^3$	$1.96(\pm 0.27) \times 10^3$	$2.51(\pm 0.38) \times 10^3$	$2.27(\pm 0.68) \times 10^3$	$2.02(\pm 0.69) \times 10^3$	$2.26(\pm 0.32) \times 10^3$	$1.92(\pm 0.57) \times 10^3$	$2.11(\pm 0.39) \times 10^3$	$1.85(\pm 0.30) \times 10^3$	
Mineral Saturation Index, SI^a											
<i>Carbonates (non-Fe bearing)</i>											
Dolomite	2.76 $\pm$ 0.3	2.85 $\pm$ 0.29	3.00 $\pm$ 0.12	5.21 $\pm$ 0.72	3.28 $\pm$ 0.34	4.65 $\pm$ 0.21	3.30 $\pm$ 0.28	5.03 $\pm$ 0.21	3.1 $\pm$ 0.42	4.88 $\pm$ 0.29	1.77 $\pm$ 0.34
Huntite	2.19 $\pm$ 0.63	2.35 $\pm$ 0.59	2.66 $\pm$ 0.27	7.13 $\pm$ 1.47	3.20 $\pm$ 0.49	5.96 $\pm$ 0.26	3.26 $\pm$ 0.55	6.63 $\pm$ 0.52	2.87 $\pm$ 0.82	6.43 $\pm$ 0.61	3.54 $\pm$ 0.71
Magnesite	1.24 $\pm$ 0.16	1.28 $\pm$ 0.15	1.35 $\pm$ 0.08	2.49 $\pm$ 0.37	1.48 $\pm$ 0.19	2.18 $\pm$ 0.08	1.51 $\pm$ 0.14	2.37 $\pm$ 0.11	1.41 $\pm$ 0.2	2.30 $\pm$ 0.16	0.90 $\pm$ 0.18
Rhodochrosite	0.35 $\pm$ 0.16	0.42 $\pm$ 0.17	0.48 $\pm$ 0.06	1.66 $\pm$ 0.42	0.63 $\pm$ 0.13	1.31 $\pm$ 0.09	0.61 $\pm$ 0.14	1.47 $\pm$ 0.13	0.52 $\pm$ 0.2	1.36 $\pm$ 0.21	0.89 $\pm$ 0.21
Calcite	-0.04 $\pm$ 0.14	0.02 $\pm$ 0.14	0.10 $\pm$ 0.05	1.16 $\pm$ 0.35	0.24 $\pm$ 0.06	0.92 $\pm$ 0.11	0.23 $\pm$ 0.14	1.1 $\pm$ 0.11	0.14 $\pm$ 0.22	1.03 $\pm$ 0.14	0.88 $\pm$ 0.16
Hydromagnesite				3.57 $\pm$ 1.12	-2.81 $\pm$ 0.65	1.51 $\pm$ 0.12	-2.52 $\pm$ 0.71	2.45 $\pm$ 0.66	-2.14 $\pm$ 0.4	2.59 $\pm$ 0.58	5.05 $\pm$ 0.80
<i>Fe-bearing minerals^b</i>											
<i>(in FeS₂-containing samples)</i>											
Siderite	0.48 $\pm$ 0.19	0.72 $\pm$ 0.16	0.96 $\pm$ 0.02	2.10 $\pm$ 0.32	2.13 $\pm$ 0.03	2.85 $\pm$ 0.19	1.21 $\pm$ 0.22	1.91 $\pm$ 0.2	1.3 $\pm$ 0.07	2.00 $\pm$ 0.14	0.81 $\pm$ 0.22
Hematite	-1.34 $\pm$ 0.3	-1.19 $\pm$ 0.28	-0.48 $\pm$ 0.12	3.22 $\pm$ 0.9	1.94 $\pm$ 0.06	4.35 $\pm$ 0.37	0.22 $\pm$ 0.55	2.53 $\pm$ 0.25	0.46 $\pm$ 0.24	2.9 $\pm$ 0.35	2.71 $\pm$ 0.66
Goethite	-1.17 $\pm$ 0.15	-1.1 $\pm$ 0.14	-0.74 $\pm$ 0.06	1.11 $\pm$ 0.45	0.47 $\pm$ 0.03	1.68 $\pm$ 0.19	-0.39 $\pm$ 0.28	0.76 $\pm$ 0.13	-0.27 $\pm$ 0.12	0.95 $\pm$ 0.18	1.36 $\pm$ 0.33
<i>(in AsFeS-containing samples)[!]</i>											
Siderite	-0.07 $\pm$ 0.02	0.03 $\pm$ 0.16	0.11 $\pm$ 0.12	1.25 $\pm$ 0.12	0.6 $\pm$ 0.04	1.86 $\pm$ 0.04	0.43 $\pm$ 0.02	1.04 $\pm$ 0.11	0.32 $\pm$ 0.04	1.08 $\pm$ 0.05	0.95 $\pm$ 0.31
Hematite	-2.39 $\pm$ 0.15	-2.58 $\pm$ 0.31	-2.01 $\pm$ 0.06	1.43 $\pm$ 0.29	-1.06 $\pm$ 0.01	2.57 $\pm$ 0.31	-1.44 $\pm$ 0.07	1.07 $\pm$ 0.21	-1.77 $\pm$ 0.23	0.98 $\pm$ 0.35	3.08 $\pm$ 0.54
Goethite	-1.73 $\pm$ 0.01	-1.79 $\pm$ 0.15	-1.51 $\pm$ 0.03	0.22 $\pm$ 0.15	-1.03 $\pm$ 0.01	0.79 $\pm$ 0.15	-1.22 $\pm$ 0.03	-0.05 $\pm$ 0.21	-1.39 $\pm$ 0.12	-0.01 $\pm$ 0.18	1.52 $\pm$ 0.3

^aSI = log(IAP/K_{sp}) where IAP is ion activity product and K_{sp} is solubility product. Only SI values greater than -3.00 were considered.

^bThere was evidence for precipitation of iron-bearing neophase. Hence, SI for iron-bearing minerals was based on aqueous Fe concentration in non-methanogenic treatment.

^c $\Delta SI = 1/4 \sum [SI(\text{methanogenic}) - SI(\text{non-methanogenic})]$. I.e. average methanogenesis-induced change in SI for days 3, 5, 7 and 14 (the period of observed methanogenesis; hence normalization by 4).

FIGURES

Figure 1a.

Figure 1b.

Figure 2.

1

2 **Figure 3.**

3

1

2 **Figure 4a.**

1

2 **Figure 4b**

Figure 4c

1

2 **Figure 5**

1

2 **Figure 6a**

1

2 **Figure 6b**

Figure 7.

1

2 **Figure 8a.**

3

1

2 **Figure 8b**

1

2 **Figure 8c**

1

2 **Figure 8d**

3

4