Differentiating biotic from abiotic methane genesis in hydrothermally active planetary surfaces

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Molecular hydrogen (H2) is derived from the hydrothermal alteration of olivine-rich planetary crust. Biotic and abiotic processes consume H2 to produce methane (CH4); however, the extent of either process is unknown. Here, we assess the temporal dependence and limit of abiotic CH4 related to the presence and formation of mineral catalysts during olivine hydrolysis (i.e., serpentinization) at 200 °C and 0.03 gigapascal (GPa). Results indicate that the rate of CH4 production increases to a maximum value related to magnetite catalysis. By identifying the dynamics of CH4 production, we kinetically model how the H2 to CH4 ratio may be used to assess the origin of CH4 in deep subsurface serpentinization systems on Earth and Mars. Based on our model and available field data, low H2/CH4 ratios (less than approximately 40) indicate that life is likely present and active.

Olivine hydrolysis (i.e., serpentinization) is a major pathway related to the near-surface synthesis of molecular hydrogen (H2) on Earth, Mars, and, potentially, other planets and satellites (1–4). Molecular hydrogen from serpentinization can chemically reduce carbon (e.g., HCOO−, HCOOH, CO, CO2) to form CH4 or be converted to CH4 via microbial processes (i.e., methanogenesis) (1, 3, 5–12). Although CH4 formation is thermodynamically favorable over a range of pressure (P) and temperature (T) (13, 14), CH4 production via Fisher–Tropsch type (FTT) reactions is slow without the aid of mineral catalysts (3, 9, 15, 16). To understand the limits of abiotic CH4 production, it is imperative to assess the role of mineral catalysts, both those initially present in the system and those formed during the process of serpentinization.

Chromite (FeCr2O4), a primary mineral common in serpentinization systems, has been shown to enhance FTT synthesis reactions (17). Additionally, secondary minerals such as magnetite (Fe3O4) and/or awaruite (Ni,Fe) are produced during olivine hydrolysis, providing increased opportunities for FTT catalysis (13, 15, 18–21). Here, we evaluate the rate of abiotic CH4 production for systems catalyzed both by chromite and secondary minerals (magnetite and awaruite) in experimental systems undergoing serpentinization at 200 °C and 0.03 gigapascal (GPa); conditions similar to hydrothermally altered peridotite in mid-ocean ridge environments on Earth or at approximately 5 km depth in the Martian subsurface. By measuring H2 and CH4 formed during olivine hydrolysis, mineral-catalyzed CH4 production can be differentiated from biogenic CH4 production through a consideration of the H2–CH4 kinetic balance.

Experimental Procedures
Serpentization experiments were carried out in the Water—Rock Interaction Laboratory (US Geological Survey, Menlo Park, CA) using the parameters listed in Table 1. Olivine [(Fe0.12,Mg0.88)Si2O5 or Fo38 with 2.510 mg kg−1 of Ni], chromite [(Fe2.02,Mg0.76),(Cr0.59,Mg0.41)2], and synthetic seawater were prepared and characterized using methods outlined in Materials and Methods and by Jones et al. (22). The amount of chrome (approximately 1 wt.%) added was analogous to the typical content of oceanic peridotites (23), but the surface area of the chromite was greater than in natural systems because of moderate powdering. Despite best practices to control and account for carbon, olivine and chromite [<0.04% total/organic carbon (TEOCO)] and N2-purged synthetic seawater provided residual sources of carbon for CH4 generation that, in total, were below carbonate saturation [see McCollom and Seewald (13) and Jones et al. (22) for discussions of carbon sources, thermodynamic routes of CH4 formation, and the impact of carbon species in serpentinization systems]. Samples of the experimental fluid were periodically withdrawn and analyzed for pH, H2, and CH4. For both experiments, the pH values increased from 6 to 11 during the first 200 h and then remained constant.

Table 1. Reactants and experimental parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivine (Fo38)</td>
<td>52.0 g</td>
<td>67.7 g</td>
</tr>
<tr>
<td>Chromite</td>
<td>—</td>
<td>0.354 g</td>
</tr>
<tr>
<td>Evolved seawater</td>
<td>132 g</td>
<td>175 g</td>
</tr>
<tr>
<td>Water:rock</td>
<td>2:5:1</td>
<td>2:5:1</td>
</tr>
<tr>
<td>Pressure</td>
<td>0.030 ± 0.002 GPa</td>
<td>0.030 ± 0.004 GPa</td>
</tr>
<tr>
<td>Temperature</td>
<td>200 ± 0.8 °C</td>
<td>200 ± 1.1 °C</td>
</tr>
</tbody>
</table>

Results and Discussion
The quantity of H2 and CH4 as well as the H2/CH4 ratios are shown as a function of time (h) in Fig. 1 for Exp. 1 (no chromite) and 2 (chromite added). The duration of Exp. 1 was 525 h, while Exp. 2 ran for 861 h. In Exp. 1, H2 and CH4 measurements were not collected after 525 h because of a leak in the autoclave. Rates of H2 and CH4 production are calculated via least square linear regression and are given with their respective standard deviations in Fig. 1. Hydrogen production rates in Exp. 1 and 2 are 11 ± 2 and 1 ± 1 μmol kg−1 h−1, respectively. Methane production rates in both experiments are similar for the first 500 h: 0.09 ± 0.02 (Exp. 1) and 0.08 ± 0.01 (Exp. 2) μmol kg−1 h−1. While Exp. 1 gas measurements were terminated at 525 h because of a slow gas leak, the rate of CH4 production in Exp. 2 appears to increase to 0.19 ± 0.07 μmol kg−1 h−1 after 500 h. Assuming the CH4 production rate was constant, the overall average rate of CH4 production for Exp. 2 is 0.15 ± 0.02 μmol kg−1 h−1.

The evolution of the ratio of total H2 to total CH4 (Fig. 1) shows the relationship between H2 generated through olivine hydrolysis and CH4 generated through carbon reduction. If the rates of each process change during the course of the experiment,


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The $H_2/CH_4$ ratio will reflect these changes. If the rate of $H_2$ production increases more rapidly than the rate of $CH_4$ formation, then the ratio of $H_2/CH_4$ will increase as a function of time. Alternatively, if the rate of $CH_4$ formation increases more rapidly than the rate of $H_2$ production, then the ratio of $H_2/CH_4$ will decrease as a function of time. In both experiments, the $H_2/CH_4$ ratio increases during the first 500 h to a maximum value of approximately 100. In Exp. 2, the $H_2/CH_4$ ratio decreases during the subsequent 500 h. This decrease is indicative of a 2.5-fold increase in the rate of $CH_4$ production during the latter part of the experiment, providing support that $CH_4$ production increased after 500 h.

Examining $H_2$ and $CH_4$ production rates provides an opportunity to evaluate the role of FTT mineral catalysts in such systems. Olivine hydrolysis results in the formation of chrysotile [Mg$_3$Si$_2$O$_7$(OH)$_4$], brucite [Mg(OH)$_2$], magnetite, and $H_2$ as shown in the reaction,

$$\text{Mg}_0.88\text{Fe}_{0.12}2\text{SiO}_4 + 1.34\text{H}_2\text{O} = 0.5\text{Mg}_3\text{Si}_2\text{O}_7(\text{OH})_4 + 0.08\text{Fe}_3\text{O}_4 + 0.26\text{Mg(OH)}_2 + 0.08\text{H}_2.$$ [I]

where the FTT catalyst magnetite is initially absent and increases in abundance over time. Note that a small amount of Fe(II) may substitute into chrysotile and brucite. Based on textural comparisons in residual solids, magnetite is expected to be a better catalyst than chromite because of the greater surface area of the fine-grained serpentinization-generated magnetite (Fig. 2). Further, a larger quantity of magnetite (>4 wt.% magnetite noted in X-ray diffraction (XRD) analyses of residual solids) is generated, greater than the amount of initial chromite (0.5 wt.%). Awaruite was not detected via XRD or SEM despite the abundance of Ni in the olivine.

Chromite is considered to be relatively inert with low solubility over a range of geologic conditions unless oxidants are present in the system (24, 25). Thus, the process of chromite altering to magnetite requires much longer than the 1,000 h of our experiments to create any appreciable amount of magnetite. If chromite were to serve as an additional route for magnetite formation despite the slow reaction kinetics, it would account for less than 2 wt.% of the total magnetite, assuming it completely converted to magnetite and that the total mass was similar to the starting mass. This is a minor amount of magnetite, compared to that generated by serpentinization, to serve as an alternative/major route for FTT reactions. Additionally, chromite was present in Exp. 2 residual solids [observed via SEM with energy-dispersive X-ray spectroscopy (EDS)] and had an appearance similar to the starting chromite, indicating that it did not undergo significant alteration. Overall, chromite is, at best, a very minor source for magnetite generation and a reticent phase for alteration under these experimental conditions.

Comparing Exps. 1 and 2, the rates of $H_2$ production and the similarity in the residual minerals demonstrate that the addition of chromite did not modify the rate of olivine hydrolysis or serve as an additional source of $H_2$. Consequently, the rate of $H_2$ production is directly related to olivine surface area; powdered olivine will have a much faster $H_2$ production rate than granular olivine. Because of the granular texture of olivine utilized herein, the $H_2$ production rates from Exps. 1 and 2 are likely among the slowest for laboratory serpentinization experiments (e.g., 15, 20). Although it is difficult to compare directly the laboratory $H_2$ production rates with rates in mid-ocean ridge systems, elevated $H_2$ generated primarily via serpentinization has been reported at mid-ocean ridge serpentinization localities at concentrations very similar to our reported values (3, 8, 12, 26).

The rate of $CH_4$ production in Exp. 2, identical to Exp. 1 for the first 500 h, suggests that either the amount of chromite present was insufficient to act as a FTT catalyst or that chromite itself is not an effective catalyst for $CH_4$ generation. Methane produced in tandem with $H_2$ from the onset of each experiment demonstrates that neither olivine nor chromite are sources of $CH_4$, as such a mechanism would result in an initial $CH_4$ spike as it was liberated from the mineral substrate. Additionally, $H_2$ and $CH_4$ increase in lockstep [also demonstrated by Jones et al. (22)], strongly suggesting that $CH_4$ formation is related to $H_2$ production and not released from fluid inclusions (13). Based on the identical increase in $H_2/CH_4$ ratios in Exps. 1 and 2 in these experiments, $CH_4$ production at 0.08 to 0.09 μmol kg$^{-1}$ hr$^{-1}$ re-

*Fig. 1. CH$_4$ (μmol kg$^{-1}$), H$_2$ (μmol kg$^{-1}$), and H$_2$/CH$_4$ collected over time (h) for Exps. 1 and 2 (Table 1) at 200 °C and 0.03 GPa. Each data point represents one analysis per withdrawn sample; calibration and controls constrain uncertainties with errors smaller than the symbol.*

*Fig. 2. SEM image of fine-grained magnetite and chrysotile with scale bar included.*
fects H₂ reacting with carbon in the system unaided by mineral-surface FTT catalysis. The CH₄ rate increase after 500 h for Exp. 2 indicates that a new mechanism for CH₄ formation, most likely FTT catalysis on serpentinization-generated magnetite, begins to become dominant. It appears that it takes approximately 500 h for the amount of magnetite in the system to reach an autocatalytic threshold at which it can significantly influence the production of CH₄. While direct observations of the H₂ and CH₄ values after 500 h for Exp. 1 are lacking, multiple sources of evidence (as described above) support the contention that chromite was not significant to the processes of H₂, CH₄, and magnetite production. Therefore, Exps. 1 and 2 may be treated as identical experiments in which the main mechanism of magnetite generation is serpentinization, and magnetite is the only appreciable FTT catalyst introduced.

Fig. 3 describes how the ratio of H₂ to CH₄ in this system is influenced by H₂ and CH₄ reaction rates and by the introduction of magnetite. At the onset of serpentinization, the H₂ production is fast while CH₄ production is slow because of insufficient magnetite catalyst. This results in H₂ building up faster than it is consumed, causing the H₂/CH₄ ratio to increase. As magnetite is produced, the rate of CH₄ production begins to increase. Hydrogen consumption increases as more CH₄ is produced, and the H₂/CH₄ ratio decreases until CH₄ production reaches its maximum rate. After excess H₂ is consumed, the H₂/CH₄ ratio will reach a near constant value. Ratios of H₂/CH₄ greater than this value represent abiogenic serpentinization systems in which either the rate of H₂ production is very high, excess H₂ has not been consumed, or CH₄ production has not achieved its maximum rate. Systems with extremely high rates of H₂ production may result from high-surface area olivine and would require more time than our experiments to achieve this rate balance. In systems where the H₂/CH₄ ratio is lower than that dictated by our model (i.e., faster CH₄ production than achievable through magnetite-catalyzed FTT processes), methanogenesis can be invoked. Biological organisms such as methanogens convert H₂ to CH₄ at faster rates than achieved by strictly mineral-based FTT processes, as demonstrated by their ability to survive and thrive in their unique biological niche. Consequently, serpentinization systems with a strong biological presence will have significantly lower H₂/CH₄ values than abiogenic systems.

Conclusions

Using the framework described above (Fig. 3) and the rates of H₂ and CH₄ formation from Exps. 1 and 2, we kinetically modeled serpentinization-fueled H₂ production and CH₄ formation (Fig. 4). This figure contains the data points from Exps. 1 and 2 (Fig. 1) as well as a model output (see Materials and Methods) in which the magnetite-catalyzed rate of CH₄ production has been varied. The best fit to the experimental data is a model in which the magnetite-catalyzed rate of CH₄ production is 0.28 μmol kg⁻¹ h⁻¹, leading to a near constant H₂/CH₄ ratio of 42, in relatively good agreement with the data from Fig. 1. This H₂/CH₄ ratio provides a means to separate preliminarily abiogenic (greater than approximately 40) from biotic (less than approximately 40) systems. Because of our limited number of analyses, and to address the potential imprecision of our experimental fit, simulations using CH₄ production rates between 0.60 μmol kg⁻¹ h⁻¹ and 0.19 μmol kg⁻¹ h⁻¹ are also shown in Fig. 3 and result in steady H₂/CH₄ ratios ranging from 20 (for the fastest rate of CH₄ production at 0.60 μmol kg⁻¹ h⁻¹) to 60 (for the slowest rate of CH₄ production at 0.19 μmol kg⁻¹ h⁻¹). Despite significantly changing CH₄ production rates, our model provides a H₂/CH₄ envelope (20–60) that may be used to separate abiogenic and biotic systems.

For comparison, H₂/CH₄ ratios from the scientific literature for both abiogenic serpentinization experiments and field measurements from serpentinization systems where life is present are shown in Fig. 4 (1, 3, 5–7, 13, 15, 17, 20, 27–30). Of all the experiments, results from Horita and Berndt (19) yield the lowest purely abiogenic, laboratory-based H₂/CH₄ ratio of 42, in good agreement with our model and experimental data. Mid-Atlantic Ridge serpentinization systems evaluated by Charlou et al. (3) demonstrate the highest H₂/CH₄ ratio of 33 for naturally occurring systems, serving as an upper bound for natural systems with dominantly abiogenic CH₄ and minimal biological activity. These two studies in conjunction with our experiments and model demonstrate that H₂/CH₄ ratios must be less than approximately 40 (preferably less than 30) to indicate that life is present and active. Overall, ratios that result from abiogenic experiments are equal to or much greater than our (abiogenic) experimental data, while systems in which biological processes may contribute to CH₄ production all have lower H₂/CH₄ ratios (most, less than 10), as our model predicts. This is a significant finding as it demonstrates that the H₂/CH₄ ratio may be used to evaluate if life is present and affecting CH₄ generation in serpentinization systems.

These results support previous assessments based on carbon isotope chemistry that modern Earth’s serpentinization systems have a biogenic CH₄ component (3, 5, 9, 12, 13, 27). More importantly, the presence of life in inaccessible subsurface serpentinization environments may be investigated by evaluating bulk H₂ and CH₄ released at the surface, whether on Earth or other planets. On Mars, ultramafic rocks, serpentine minerals, H₂O, and CH₄ are present (31, 32). A seasonal Martian CH₄ plume with a mean mixing ratio of approximately 33 parts per billion (ppb) has been observed (31). If this CH₄ resulted from the combination of serpentinization and biological processes, the amount of H₂ present should be less than 1,400 ppb, assuming H₂ is entirely derived from serpentinization, and alternative routes of H₂ production (i.e., atmospheric photolysis reactions involving H₂O) and consumption [i.e., Fe(III) reduction] are not significant. However, approximately 10 times that much H₂ is present in the lower Martian atmosphere (33), suggesting that biological processes may not be responsible for the observed CH₄. By monitoring both H₂ and CH₄ flux released at the Martian surface, we may be able to determine whether life (if similar to Earth) is present and active in the subsurface.
Materials and Methods
Olivine, chromite, and synthetic seawater were prepared and characterized as shown in Jones et al. (22). The average composition of olivine was determined to be (Fe$_{0.12}$Mg$_{0.88}$)$_2$SiO$_4$ or Fo$_{88}$ based on electron microprobe microanalysis on an automated JEOL 733A electron microprobe (15-kV accelerating potential and a 15-nA beam current). Additionally, major and minor element chemistry for olivine and chromite were obtained by completely dissolving the minerals using a mixture of hot, concentrated nitric, perchloric, and hydrofluoric acids. Elemental concentrations such as Ni (as shown in Experimental Procedures) were measured in the supernatant using inductively coupled atomic emission spectroscopy. Synthetic seawater was prepared with reagent-grade KCl (0.0747 wt. %), CaCl$_2$ (0.205 wt. %), and NaCl (2.76 wt. %) in deionized water to approximate the typical “evolved” seawater chemistry in mid-ocean ridge and forearc serpentization sites (34). Residual solids following hydrothermal alteration were evaluated using: (i) FEI 600 Quanta field effect gun (FEG) scanning electron microscope with EDS and/or a Hitachi S-4700 field-emission scanning electron microscope (FESEM) with EDS, and (ii) XRD with Bragg–Brentano/monochromator capabilities using a Rigaku diffractometer. For all models presented in Fig. 4, the rate of CH$_4$ production was varied. The model that was used to simulate the H$_2$/CH$_4$ ratios was constructed using Microsoft Excel (Dataset S1). Data points were calculated every 10 h for a total of 2,000 h, based on the various rates of H$_2$ and CH$_4$ generation observed. For all models presented in Fig. 4, the rate of CH$_4$ generation was assumed to increase rapidly from 0.1 μmol kg$^{-1}$ h$^{-1}$ to 10 μmol kg$^{-1}$ h$^{-1}$ and then to remain constant at 10 μmol kg$^{-1}$ h$^{-1}$ for the duration of the experiment. For all models presented in Fig. 4, the rate of CH$_4$ production was assumed to increase from 0.02 μmol kg$^{-1}$ h$^{-1}$ to 0.08 μmol kg$^{-1}$ h$^{-1}$ during the first 250 h of the experiment. The rate of CH$_4$ production was held constant at 0.08 μmol kg$^{-1}$ h$^{-1}$ for 300 h and was then increased after 550 h to a rate that persisted for the remainder of the experiment. In order to simulate different H$_2$/CH$_4$ ratios, only this final rate of CH$_4$ production was varied.

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