CO₂ reduction driven by a pH gradient

Reuben Hudson¹,²,³,⁴, Ruvan de Graaf⁵, Mari Strandoo Rodin⁶, Aya Ohno⁵, Nick Lane⁶, Shawn E. McGlynn⁷,⁸,⁹, Yoichi M. A. Yamada⁴,¹⁰, Ryuheki Nakamura¹⁠,²,¹⁰, Laura M. Bargé⁵, Dieter Braun⁷, and Victor Sojo⁵,⁸,¹¹,¹²

¹Department of Chemistry, College of the Atlantic, Bar Harbor, ME 04609; ²Department of Chemistry, Colby College, Waterville, ME 04901; ³RIKEN Center for Sustainable Resource Science, 351-0198 Saitama, Japan; ⁴Department of Genetics, Evolution and Environment, University College London, WC1E 6BT London, United Kingdom; ⁵Earth-Life Science Institute, Tokyo Institute of Technology, 152-8550 Tokyo, Japan; ⁶Blue Marble Space Institute of Science, Seattle, WA 98154; ⁷NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109; ⁸Department of Physics, Center for Nanoscience, Ludwig-Maximilians-Universität München, 80799 Munich, Germany; and ⁹Institute for Comparative Genomics and Richard Gilder Graduate School, American Museum of Natural History, New York, NY 10024

All life on Earth is built of organic molecules, so the primordial sources of reduced carbon remain a major open question in studies of the origin of life. A variant of the alkaline-hydrothermal-vent theory for life’s emergence suggests that organics could have been produced by the reduction of CO₂ via H₂ oxidation, facilitated by geologically sustained pH gradients. The process would be an abiotic analog—and proposed evolutionary predecessor—of the Wood–Ljungdahl acetyl-CoA pathway of modern archaea and bacteria. The first energetic bottleneck of the pathway involves the endergonic reduction of CO₂ with H₂ to formate (HCOO⁻), which has proven elusive in mild abiotic settings. Here we show the reduction of CO₂ with H₂ at room temperature under moderate pressures (1.5 bar), driven by microfluidic pH gradients across inorganic Fe(Ni)S precipitates. Isotopic labeling with ¹³C confirmed formate production. Separately, deuterium (¹H) labeling indicated that electron transfer to CO₂ does not occur via direct hydrogenation with H₂ but instead, freshly deposited Fe(Ni)S precipitates appear to facilitate electron transfer in an electrochemical-cell mechanism with two distinct half-reactions. Decreasing the pH gradient significantly, removing H₂, or eliminating the precipitate yielded no detectable product. Our work demonstrates the feasibility of spatially separated yet electrically coupled geochemical reactions as drivers of otherwise endergonic processes. Beyond corroborating the ability of early-Earth alkaline hydrothermal systems to couple carbon reduction to hydrogen oxidation through biologically relevant mechanisms, these results may also be of significance for industrial and environmental applications, where other redox reactions could be facilitated using similarly mild approaches.

Significance

Biology is built of organic molecules, which originate primarily from the reduction of CO₂ through several carbon-fixation pathways. Only one of these—the Wood–Ljungdahl acetyl-CoA pathway—is energetically profitable overall and present in both archaea and bacteria, making it relevant to studies of the origin of life. We used geochemically pertinent, life-like microfluidic pH gradients across freshly deposited Fe(Ni)S precipitates to demonstrate the first step of this pathway: the otherwise unfavorable production of formate (HCOO⁻) from CO₂ and H₂. By separating CO₂ and H₂ into acidic and alkaline conditions—as would have been in early-Earth alkaline hydrothermal vents—we demonstrate a mild indirect electrochemical mechanism of pH-driven carbon fixation relevant to life’s emergence, industry, and environmental chemistry.

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Once produced, and depending on local conditions, formate would have had ample abiotic chemical potential. For example, forml groups have been shown to yield intermediates of both the reverse citric acid (Kreb’s) cycle (17) and the Wood–Ljungdahl (WL) reductive acetyl-CoA pathway (18), offering a possible entry route into early biological metabolism. Alternatively, on heating in the presence of ammonia—itself also a predicted component of alkaline-vent effluents—formate yields formamide [HC(O)NH₂], a highly reactive molecule central to a separate major theory for the emergence of life (19). Further reaction of this latter mixture yields hydrogen cyanide (HCN), which is also at the core of a major alternative origin-of-life scenario (20). In turn, dehydration of formate yields carbon monoxide (CO), which features prominently in a separate hydrothermal-vent model for the origin of life (21).

While there were multiple sources of reduced carbon on the early Earth and multiple plausible environments that would have hosted rich chemistries (17–25), the alkaline hydrothermal scenario described above can be especially appealing because of its resemblance to the WL pathway of carbon fixation (SI Appendix, Fig. S1) (2, 26). Highlighting its potential relevance to studies of the emergence of life, the WL process is the only one of six known biological carbon-fixation pathways that releases energy overall instead of consuming it, and variations of it are present in extant members of both Archaea (methanogens) and Bacteria.

碳化物 | 生命起源 | 膜 | 催化 | 电化学

A dependable supply of reduced organic molecules is essential for any scenario of the origin of life. On the early Earth, one way in which this supply could have been attained was by reduction of CO₂ with H₂ (1–6). Geological studies indicate that CO₂ was at comparatively high concentrations in the ocean during the Hadean eon, whereas H₂ was the product of multiple processes in the Earth’s crust and would have emanated as part of the efflux of alkaline hydrothermal vents (3, 7–11). The model suggests that on meeting at the vent–ocean interface, the reaction between the two dissolved gases would have produced hydrocarbons, which would in turn take roles in the transition from geochemistry to biochemistry (2, 12). Under standard conditions (1 atm, 25 °C, pH 7), the reaction between CO₂ and H₂ to produce formate (HCOO⁻) is thermodynamically disfavored, with ΔG° = +3.5 kJ mol⁻¹ (13, 14). In ancient alkaline vents, however (Fig. L4), H₂ was present in the OH⁻ rich effluent of the vent, which would have favored its oxidation to water (1). Conversely, CO₂ would have been dissolved in the relatively acidic ocean, which facilitated protonation in its reduction to HCOO⁻ (1). Assisted by Fe(Ni)S minerals precipitated at the interface, a pH gradient of more than three units should have been enough to increase the viability of the reaction by ~180 mV, thereby rendering it favorable (15, 16).
(acetogens) (2, 4, 6, 12, 13, 27). The first step in the pathway is the reduction of CO₂ with H₂ to produce formate (HCOO⁻), and its dehydrated electronic equivalent, CO. This reaction is endergonic, so multiple members of both Archaea and Bacteria use its dehydrated electronic equivalent, CO). This electrochemical system enables the overall reaction between H₂ and CO₂, which is not observed under standard vent conditions, the oxidation of H₂ (Left) is favored by the alkaline pH due to the presence of hydroxide ions (OH⁻) that react exergonically in the production of water. Released electrons would travel across the micrometers- to centimeters-thick Fe(Ni)S network (53) (Center) to the more oxidizing acidic solution on the ocean side. There they meet dissolved CO₂ and a relatively high concentration of protons (H⁺), favoring the production of formic acid (HCOOH) or formate (HCOO⁻). This reaction is endothermic so multiple members of both Archaea and Bacteria use its dehydrated electronic equivalent, CO). This electrochemical system enables the overall reaction between H₂ and CO₂, which is not observed under standard reaction conditions. (B) Diagram of the reactor, with embedded micrograph of a reaction run with precipitate at the interface. Further details are provided in the main text and SI Appendix, Fig. S2.

**Fig. 1.** Proposed mechanism of pH-driven CO₂ reduction with H₂ across a conducting Fe(Ni)S barrier, and schematic of the reactor. (A) Under alkaline-vent conditions, the oxidation of H₂ (Left) is favored by the alkaline pH due to the presence of hydroxide ions (OH⁻) that react exergonically in the production of water. Released electrons would travel across the micrometers- to centimeters-thick Fe(Ni)S network (53) (Center) to the more oxidizing acidic solution on the ocean side. There they meet dissolved CO₂ and a relatively high concentration of protons (H⁺), favoring the production of formic acid (HCOOH) or formate (HCOO⁻). This electrochemical system enables the overall reaction between H₂ and CO₂, which is not observed under standard reaction conditions. (B) Diagram of the reactor, with embedded micrograph of a reaction run with precipitate at the interface. Further details are provided in the main text and SI Appendix, Fig. S2.

Results

**Precipitation of Fe(Ni)S at the Interface in a Gas-Driven Y-Shaped Microfluidic Reactor.** Aiming to coincide with fluid compositions in previous work (5, 30, 31), and as detailed in SI Appendix, Table S1, we prepared an alkaline vent-simulant fluid containing Na₂S (100 mM), K₂HPO₄ (10 mM), and Na₂SiO₃ (10 mM) in deaerated water. The counterpart ocean analog fluid contained FeCl₂ (50 mM) and NiCl₂ (5 mM). The two fluids were introduced at the inlets of a Y-shaped borosilicate microfluidic reactor (Fig. 1B). Ambient pressures of H₂ and CO₂ have proven insufficient to drive CO₂ reduction (31, 32), so instead of attempting to dissolve either gas by bubbling before the reaction, here we used gas pressure-driven microfluidic pumps. The alkaline fluid was pushed with H₂ at 1.5 bar, and the ocean analog was pushed with CO₂ at the same pressure (see reactor schematic in Fig. 1B and photograph in SI Appendix, Fig. S2). We split each reactor run into two consecutive stages, the first for deposition of the Fe(Ni)S precipitates at the interface between the two fluids and the second (referred to as “postprecipitation” below) for attempting the reaction between CO₂ and H₂ (or other reagents, as detailed where relevant). Confluence of the ocean and vent analogs over 15 to 60 s in the precipitation stage yielded a 30- to 60-μm-wide precipitate at the interface between the two fluids, visible under a digital 200x optical microscope (Fig. 1B, Center). Removing metals from the ocean side following precipitation prevented the precipitate from growing to the point of occluding the reactor channel.

**Detection of Formate and Confirmation with ¹³C Isotopic Labeling.** Following formation of the precipitate, and to prevent further precipitation from clogging the microfluidic channels (32), in the second (postprecipitation) stage we switched the ocean fluid to pure deaerated H₂O, pushed by CO₂ (Fig. 1B, Right). The vent simulant was as before, with Na₂S, K₂HPO₄, and Na₂SiO₃, pushed by H₂. Using in-line pH meters, we determined the pH of the ingoing fluids at the point of entry (shortly before entering the reactor; Fig. 1B), finding the ocean simulant at pH 3.9 and the vent simulant at pH 12.3. At a flow rate of 5 μL/min for each input, the residence time of fluids within the central channel was ~3.3 s, so we allowed the system to flow for at least 2 min before collecting output. We then collected a single (i.e., mixed) output of the reactor over the subsequent 50 min and later analyzed it via NMR spectroscopy. Under these conditions, we detected HCOO⁻ by both ¹H and ¹³C NMR (Table 1, experiment 1), at an average concentration of 1.5 μM. Singlet peaks in the ¹H (8.42 ppm; Fig. 2A) and ¹³C (165.8 ppm; SI Appendix, Fig. S3) NMR spectra matched samples of pure (>98%) formic acid (SI Appendix, Fig. S4). Keeping the ocean solution free of ocean-side Fe from the start—i.e., in the absence of the precipitate—gave no detectable product (SI Appendix). Running both the precipitation and reaction stages with isotopically enriched (99% ¹³C) ¹³C CO₂ (Table 1, experiment 2) gave a stronger singlet in the ¹³C spectrum (165.8 ppm; Fig. 2B) and yielded the expected splitting of the formyl singlet into a doublet in the ¹H spectrum (J = 195 Hz) due to ¹H-¹³C coupling in the formyl group (Fig. 2C).

H₂ appears to be necessary for CO₂ reduction. With the vent-side fluid driven by N₂ instead of H₂ (i.e., in the absence of H₂ both during and following precipitation), we observed no reduction products (experiment 3 and Fig. 2E and F).

**Evidence for an Indirect pH-Dependent Electrochemical Mechanism of CO₂ Reduction Driven by H₂ Oxidation.** In search of mechanistic insight, we conducted deuterium (²H, or D)-labeling experiments (Table 1, experiment 4 and 5), using the isotopic variants in previous work (5, 30, 31), and as detailed in SI Appendix. Regardless of whether we used unlabeled H₂ (experiment 1) or D₂ (experiment 4) to drive the vent-side pump, we observed only non-isotopically labeled HCOO⁻ in the efflux, suggesting that CO₂ reduction may be occurring exclusively on the ocean side. Conversely, with D₂O used in place of regular H₂O on the ocean side, and with unlabeled H₂ driving the vent-side pump (experiment 5), we detected only deuterated formate (DCOO⁻), as evidenced by a triplet in ¹³C NMR, J = 33 Hz, and the lack of any other discernible peaks (Fig. 2D). This further confirms that the CO₂ reduction here
matches the isotopic composition of the ocean side, not that of the vent side.

We next explored the role of the inherent pH gradient of the simulated submarine alkaline hydrothermal system. The successful reductions reported in Table 1 proceeded with an initial ocean analog pH of 3.9 and a vent analog pH of 12.3. On mixing, this initial ΔpH of 8.4 units would have inevitably dropped, but, as we have previously shown, pH gradients of multiple units hold successfully over time across microfluidic scales, particularly in the presence of a precipitate at the interface (30). We aimed to determine whether such a pH gradient was required in our reduction system to facilitate the oxidation of H$_2$ on the alkaline side and the reduction of CO$_2$ on the acidic side (Fig. 1A). Following precipitation under the same conditions as above for experiment 1 in Table 1 (repeated in Table 2), we evaluated the effects of various changes to the pH and composition of each of the two fluids (Table 2). Replacing the vent simulant with pure H$_2$O driven by H$_2$ afforded no product (Table 2, experiment 6). Likewise, leaving Na$_2$S, K$_2$HPO$_4$, and Na$_2$Si$_3$O$_7$ in the vent solution and H$_2$ as the driver gas, but acidifying the vent solution with HCl to pH 3.9 and pH 7.0, resulted in no detectable formate production (Table 2, experiments 7 and 8, respectively). Adding 100 mM Na$_2$CO$_3$ to the ocean fluid while still using CO$_2$ as the driving gas (Table 2, experiment 9) raised the ocean-side pH to 9.8, and no product was detected under these conditions. Removing silicate from the vent side after precipitation still yielded formate (Table 2, experiment 10), as did removing both silicate and phosphate to have only Na$_2$S (Table 2, experiment 11). With only K$_2$HPO$_4$ in the vent side postprecipitation, we detected only trace amounts of formate (below our limit of quantification of 0.37 μM; see SI Appendix, Methods and Table S2), possibly due to the insufficiently alkaline pH of 9.1 (Table 2, experiment 12). The more alkaline K$_2$PO$_4$ raised the pH to 12.1 and led to production of considerably more formate (Table 2, experiment 13).

While we cannot preclude the possibility of precipitate-bound sulfide acting as a reductant in addition to H$_2$, these results simultaneously confirm the role of the pH gradient and show that a continuous supply of aqueous sulfide is not required in our system; this contrasts with the continued requirement for H$_2$ (Table 1, experiment 3). Removing Ni from the ocean precipitation fluid (experiment 14) produced only small amounts of formate (below our limit of quantification of 0.37 μM). Conversely, replacing Fe to leave Ni as the only metal in the ocean precipitation fluid (NiCl$_2$, 55 mM; experiment 15) yielded 1.4 μM formate, pointing toward a crucial role for Ni within the precipitates. Removing both FeCl$_2$ and NiCl$_2$ from the ocean fluid expectedly did not produce a precipitate, and did not result in the production of detectable formate (experiment 16).

NMR spectra and further analyses for all experiments discussed here are presented in SI Appendix, Figs. S3–S20. Before-and-after images of the precipitates in a number of the reactions are provided in SI Appendix, Fig. S21.

**Discussion**

Here we report the abiotic pH-driven reduction of CO$_2$ to formate with H$_2$ in a minimalistic microfluidic replicant of an alkaline hydrothermal system. Removing the pH gradient entirely or significantly reducing it—either by acidifying the vent fluid or alkalizing the ocean fluid—yielded no detectable formate. With the acidic ocean side fixed at pH 3.9, we observed greater formate yields with increasing vent-side pH. In experiments 7, 12, 13, 1, and 11 in Table 2, as the vent-side pH rose (7.0 < pH < 12.1 < 12.3 < 12.6, respectively), so did the average formate concentration measured (undetected < below 0.37 μM < 0.9 μM < 1.5 μM < 1.8 μM).

Removing the precipitate entirely also yielded no product. Without Ni in the ocean precipitation fluid, the yield dropped below our limit of quantification, suggesting that Ni is a crucial

**Table 1. Mechanistic analysis of CO$_2$ reduction**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Vent driving gas</th>
<th>Ocean driving gas</th>
<th>Ocean solvent</th>
<th>Product detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H$_2$</td>
<td>CO$_2$</td>
<td>H$_2$O</td>
<td>HCOO$^-$</td>
</tr>
<tr>
<td>2</td>
<td>H$_2$</td>
<td>$^{13}$CO$_2$</td>
<td>H$_2$O</td>
<td>H$^{13}$COO$^-$</td>
</tr>
<tr>
<td>3</td>
<td>N$_2$</td>
<td>CO$_2$</td>
<td>H$_2$O</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>D$_2$</td>
<td>CO$_2$</td>
<td>D$_2$O</td>
<td>DCOO$^-$</td>
</tr>
<tr>
<td>5</td>
<td>H$_2$</td>
<td>CO$_2$</td>
<td>D$_2$O</td>
<td></td>
</tr>
</tbody>
</table>

n.d., none detected. For precipitation, the ocean fluid contained FeCl$_2$ (50 mM) and NiCl$_2$ (5 mM) in deaerated H$_2$O or D$_2$O (as shown); no solutes other than the driving gas (CO$_2$) were included in the ocean fluid postprecipitation. Vent fluid contained Na$_2$S (100 mM), K$_2$HPO$_4$ (10 mM), and Na$_2$Si$_3$O$_7$ (10 mM) both during and following precipitation. The ocean and vent fluid pumps were driven by the gases shown, each at 1.5 bar and a flow rate of 5 μL/min. For relevant experiments from this table, quantification is listed in Table 2 and was achieved through $^1$H NMR by integrating the formyl singlet compared with a known concentration of internal standard. Most entries in this table could not be quantified in this fashion, because deuterium labeling resulted in loss of the $^1$H signal (experiment 5), the formyl singlet was split into a doublet by the $^{13}$C labeling (experiment 2), or no formate was detected (experiment 3).
part of the precipitate for the reduction mechanism operating here. Given that Ni can typically serve as a more efficient classical and ionic hydrogenation catalyst than Fe (33), the requirement for Ni within the precipitate is not surprising. Although we do not invoke such a direct hydrogenation mechanism here, the indirect electrochemical mechanism that we propose in Fig. 1 would rely on the metal center for similar catalytic functions as those required during classical or ionic hydrogenations.

Removing H2 entirely by replacing it with N2 as the vent-driving gas, while still keeping Na2S in the vent fluid, also led to no detectable product (experiment 3). Conversely, we did observe successful formate production with Na2S absent from the vent fluid postprecipitation and H2 as the vent-driving gas (experiment 14). Taken together, these results suggest that H2 is a necessary reagent in our mechanism and that sulfide is insufficient (and might not be required) as an electron donor, that a large pH gradient is necessary for its oxidation, and that reduced carbon (31, 32). In those experiments, the two gases were dissolved into the fluids before the reactions, with inevitable loss by volatilization, particularly of H2. Recent calculations suggest that atmospheric pressures of H2 and CO2 are only marginally insufficient to drive the reaction to formate (32). Therefore, we infer that the positive results reported in this work are most plausibly due to the advantages of the gas-driven system used here, which likely resulted in higher dissolved gas concentrations than the atmospheric pressure bubbling performed previously (31, 32). Chiefly, the ability to keep the gases consistently within the system, as well as to more reliably keep the fluids anoxic, may suffice as an explanation for the positive results observed here compared with previous attempts.

**Alternative Mechanisms for the Reduction of CO2 Linked to H2 Oxidation.** Several CO2-reduction mechanisms are possible for a laminar-flow system such as the one that we have used. We briefly explore a number of them below and discuss why we deem them less likely than the electrochemical mechanism proposed in Fig. 1A. Further details are presented in SI Appendix, Figs. S22–S27.

Perhaps the most chemically intuitive—albeit least biochemically homologous—mechanisms of carbon reduction possible here would be direct hydrogenations (Fig. 3A–C), in which hydrogen from H2 would be transferred directly to CO2 (34–38) either as atomic hydrogen (classical hydrogenation) or as hydride (ionic hydrogenation). Most simply, the product in such a mechanism should match the isotopic signature of the H2/D2 vent gas. Instead, the formate produced here matches only the isotopic makeup of the ocean-side water, regardless of the composition of the vent-side gas or water. In these direct-hydrogenation mechanisms, adsorbed hydrogen species could in principle exchange with the surrounding fluid, such that the original isotopic signature is lost (39). However, any such process would imply the migration of significant amounts of fluid across the precipitate. The substantial mixing of fluids involved should have produced a scrambled H/D formyl signal, which we did not observe, all but ruling out any direct hydrogenation mechanisms.

Alternatively, the hydrogen atoms in the resultant formate might not derive directly from H2. Instead, the mechanism may proceed via redox cycling of an edge or corner Fe or Ni atom (M2+ ⇄ M0), wherein a metal is first reduced by H2 (leaving two electrons on to CO2, with accompanying proton abstraction from the local aqueous environment (Fig. 3C–E)). Noteworthy for any mechanisms that rely on proton transfer, none of our experiments contained acids added to the ocean side. The acidic pH of 3.9 that we report was achieved solely by dissolution of CO2 in water. Thus, any ocean-side protons must derive from the dissociation of carbonic acid via:

\[ \text{H}_2\text{O} + \text{CO}_2 = \text{H}_2\text{CO}_3 = \text{H}^+ + \text{HCO}_3^- \]

When our reaction was conducted with D2O (experiment 5) as the ocean-side solvent, we found only DCOO− in the efflux, suggesting that CO2 reduction did not occur on the vent side.

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**Table 2. Exploration of the role of the microfluidic pH gradient across the mineral precipitate in CO2 reduction**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ocean-side pH</th>
<th>Ocean-side postprecipitation solute†</th>
<th>Formate concentration, μM‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.3</td>
<td>Na2S/K2HPO4/Na2Si3O7</td>
<td>1.5 (1.66; 1.40)</td>
</tr>
<tr>
<td>6</td>
<td>7.0</td>
<td>None</td>
<td>n.d.</td>
</tr>
<tr>
<td>7†</td>
<td>7.0</td>
<td>Na2S/K2HPO4/Na2Si3O7</td>
<td>n.d.</td>
</tr>
<tr>
<td>8†</td>
<td>3.9</td>
<td>Na2S/K2HPO4/Na2Si3O7</td>
<td>n.d.</td>
</tr>
<tr>
<td>9</td>
<td>12.3</td>
<td>Na2S/K2HPO4/Na2Si3O7</td>
<td>Na2CO3/CO2</td>
</tr>
<tr>
<td>10</td>
<td>12.3</td>
<td>Na2S/K2HPO4</td>
<td>1.5 (1.33; 1.69)</td>
</tr>
<tr>
<td>11</td>
<td>12.6</td>
<td>Na2S</td>
<td>1.8 (2.12; 1.44)</td>
</tr>
<tr>
<td>12</td>
<td>9.1</td>
<td>K2HPO4</td>
<td>(0.37)§</td>
</tr>
<tr>
<td>13</td>
<td>12.1</td>
<td>K2PO4</td>
<td>0.9 (1.04; 0.76)</td>
</tr>
</tbody>
</table>

†Same as in Table 1.

§Peak was observable, but concentration was below the limit of quantification (0.37 μM; SI Appendix, Fig. S16).

n.d., none detected.

*Vent fluid concentrations for precipitation in all reactions were as follows: Na2S, 100 mM; K2HPO4, 10 mM; Na2Si3O7, 10 mM. The same concentrations were used postprecipitation, as relevant. K2HPO4 (100 mM) was used as a reducing agent in our mechanism and that sulfide is insufficient (and might not be required) as an electron donor, that a large pH gradient is necessary for its oxidation, and that reduced carbon (31, 32). In those experiments, the two gases were dissolved into the fluids before the reactions, with inevitable loss by volatilization, particularly of H2. Recent calculations suggest that atmospheric pressures of H2 and CO2 are only marginally insufficient to drive the reaction to formate (32). Therefore, we infer that the positive results reported in this work are most plausibly due to the advantages of the gas-driven system used here, which likely resulted in higher dissolved gas concentrations than the atmospheric pressure bubbling performed previously (31, 32). Chiefly, the ability to keep the gases consistently within the system, as well as to more reliably keep the fluids anoxic, may suffice as an explanation for the positive results observed here compared with previous attempts.
which had normal \( H_2 \) as the feed gas and normal \( H_2O \) as the solvent and thus would have yielded unlabeled HCOO\(^{-} \) as the product. This was confirmed in experiment 4 with \( D_2 \) as the vent-solvent and thus would have yielded unlabeled HCOO\(^{-} \) through precipitate (Table 1) indicates that the formyl H derives from ocean-side water rather than \( H_2 \) from the vent side. (D) Localized redox cycling with \( CO_2 \) and permeability of \( H^+ \) (either through a pore or anhydrously through the precipitate) are both unlikely, because in our fully hydrated system, \( H^+/D^+ \) would exchange with local vent water, giving a mixed or exchanged isotopic signal that we do not observe. \( E \) \( H_2 \) permeability is also an unlikely mechanism here, because \( H_2 \) oxidation is much less favored on the acidic (ocean) side compared with the alkaline (vent) side, as demonstrated by the pH exploration data in Table 2. More details are provided in the text and SI Appendix, Figs. S22–S27.

Fig. 3. Alternative \( CO_2 \) reduction mechanisms. (A–C) Classical hydrogenations with \( CO_2 \) permeability (A), \( H_2 \) permeability (B), or passage of dissociatively adsorbed atomic H (C) are all unlikely: isotopic labeling (Table 1) indicates that the formyl H derives from ocean-side water rather than \( H_2 \) from the vent side. (D) Localized redox cycling with \( CO_2 \) and permeability of \( H^+ \) (either through a pore or anhydrously through the precipitate) are both unlikely, because in our fully hydrated system, \( H^+/D^+ \) would exchange with local vent water, giving a mixed or exchanged isotopic signal that we do not observe. (E) \( H_2 \) permeability is also an unlikely mechanism here, because \( H_2 \) oxidation is much less favored on the acidic (ocean) side compared with the alkaline (vent) side, as demonstrated by the pH exploration data in Table 2. More details are provided in the text and SI Appendix, Figs. S22–S27.

Venturi Pull Inside Hydrothermal Pores Would Bring Ocean Fluids into the Vent System. We show that production of organics could have occurred on the ocean side of an ancient alkaline hydrothermal-vent system. This raises the question of whether these organics would simply dilute away into the ocean before they could assume any biochemical role (40). It is also conspicuously unlike the WL pathway (32), in which carbon reduction occurs inside the cell. Because the microscopic structure of hydrothermal vents is typically highly porous and reticulated (9, 41), we hypothesize that ocean fluids could have been actively pulled into the microfluidic system due to Venturi-effect suction. Once inside, the ocean’s carbonic fluids could react with electrons being transferred across the Fe(Ni)S precipitates toward \( CO_2 \) on the acidic ocean side (Fig. 1A). There the reacting \( CO_2 \) picks up a proton from the local water, yielding formate with the isotopic signature of the ocean side.

Batch vs. Flow Reactors. An alternative one-pot batch (rather than microfluidic) system for the reduction of \( CO_2 \) with \( H_2 \) has been
reported recently (43), elaborating on previous results (18, 44, 45). Using more highly reduced minerals (Fe,Ni), higher pressures (10 bar H₂), and higher temperatures (100 °C) than those used here, the batch system generates significantly higher concentrations of formate, as well as multiple further reduction products (including acetate, methanol, and pyruvate), all at a formate production rate (5.21 × 10⁻¹⁰ mol/s) four orders of magnitude higher than that reported here (2.5 × 10⁻¹³ mol/s). Several of these reduction products (e.g., acetate, pyruvate) represent an as-yet-achieved benchmark for microfluidic systems: the formation of new C–C bonds.

In a closer comparison to our system, when the mineral Fe₃S₄ was used (43) at a pressure of 1.6 bar H₂ and temperature of 20 °C, the production rate (1.35 × 10⁻¹¹ mol/s) was two orders of magnitude higher than that for our system. The differences between the two systems are crucial. The pH-driven CO₂ reduction demonstrated here, along with the possibility of thermal-driven concentration increases in small pores (46–48), make microfluidic reactors attractive despite the presently lower yields. The two types of systems thus provide complementary compelling evidence for organics arising under anoxic alkaline hydrothermal vent conditions, and both must be explored further as potential sources for life’s first molecules.

Fates of Formate beyond the WL Pathway. While we speculatively favor the continuity between geochemistry and biochemistry offered by the WL pathway of carbon fixation, it is worth noting that formate can readily yield formamide, hydrogen cyanide, and carbon monoxide, each of which is at the core of major alternative scenarios for the origin of life (19–21). Therefore, our results may extend beyond the alkaline hydrothermal vent theory or may link it to other scenarios, a set of possibilities that remain to be explored.

Conclusions

We report the pH-driven reduction of CO₂ with H₂ in the first step of a geochemically plausible analog—and proposed evolutionary predecessor (2)—of the WL pathway of carbon fixation. These results tie in with previous findings that alkaline vent-like pH gradients can be kept at a microscale across Fe(Ni)S precipitates (30), that these pH gradients can provide the necessary pH gradients can be kept at a microscale across Fe(Ni)S pre-

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