Supporting information

Pyruvate reactions in the presence of iron oxyhydroxide minerals: All reactions were carried out under anoxic conditions in a N_2-filled glove box to simulate the oxygen-free conditions of the early Earth. Chemical reagents were added to a 120-ml glass vial (iron was added as $FeCl_2 \cdot 4H_2O$ and/or $FeCl_3 \cdot 6H_2O$; ammonia was added as NH_4Cl; pyruvate was added as Na-pyruvate). Milli-Q water was sparged with argon for 30 minutes per 100 mL to remove dissolved oxygen, then all reagents and materials were placed inside the glove box. The argon-purged water was added to the vial containing reagents and stirred to dissolve. 5 ml of 1 M NaOH was slowly added to precipitate with dissolved Fe^{2+} / Fe^{3+}. The solid/liquid mixture was stirred as pH was adjusted with NaOH to the desired value. The magnetic stirrer was removed, the total volume was brought to 100 ml, "t=0" samples were taken, and the precipitate was allowed to settle. The vials were either left at room temperature or placed in a hot water bath programmed to maintain temperature of 50 – 80°C. The ratio of [Fe]:[NH_3] was chosen to be consistent with the experiments of Huber and Wächtershäuser (2003) [1] who also attempted this reaction with ferrous hydroxide (using 50 mM [Fe(II) + Fe(III)]-chloride, and 375 mM NH_4Cl) and the Na-pyruvate concentration was 2.5 mM. Though these concentrations are higher than would have been present in the early oceans, they lead to precipitation of a large enough volume of mineral to drive reactions on short laboratory timescales, and are analogous to conditions within a sediment pile where reactants and reactive minerals could be more concentrated than in the bulk ocean. The vials were not agitated during the reaction and the precipitates were allowed to settle out to form a layer at the bottom of the vial.

Precipitate characterization: Depending on the ratio of Fe(II):Fe(III) in the experiment, the precipitate color varied from blue-green for a purely ferrous hydroxide to red for a purely ferric hydroxide, with darker green / brown colors at intermediate oxidation states (**Figure S1).** Depending on the oxidation state and temperature, all of these experiments likely have a mixed mineral assemblage including various iron oxyhydroxides / oxides. The mineralogy may also change over the course of the experiment and could be affected by the addition of pyruvate / ammonia, and therefore analysis of "pure" minerals in the absence of other reactants is not very relevant. Mineralogical characterization was challenging because all Fe(II)-containing precipitates were extremely redox-sensitive. Oxidation frequently occurred between sample preparation in the N_2-filled glovebox and transport to analytical equipment, even with best efforts at storing and transferring the samples. Selected samples were analyzed with X-ray diffraction and colorimetry, preparing samples by first removing as much supernatant liquid as possible and then lyophilizing under argon to produce a dried solid. Several samples were analyzed, but because oxidation occurred in transport, the mineralogy of those is not accurate and is not reported here.

As an example of a mineral assemblage that may be present in our experiments, we obtained anaerobic XRD analysis on a sample of an iron oxyhydroxide coprecipitated from Fe(II):Fe(III) = 1:1 and NaOH that was reacted with pyruvate and ammonia at 70°C for 48 hours (at pH 10). After sample preparation / drying under argon but before it was sent out for analysis, a portion of the sample was dissolved and the Fe(II):Fe(III) ratio was determined via colorimetry. The XRD pattern of the sample corresponds well to magnetite Fe_3O_4 (RRUFF database, ID: R061111.9). Colorimetry showed that the iron oxyhydroxide sample contained 1:15.2 Fe(II):Fe(III), so there is also some iron oxide present. It is likely that magnetite was

present, but it is also possible that further oxidation occurred during sample preparation and analysis once the experiments were removed from the anoxic glove box.

XRD: X-ray diffraction analysis was obtained on a paid basis from the X-ray Diffraction Laboratory in the Department of Chemistry at Texas A&M University. Samples were analyzed anoxically by being mounted in dry box with a domed sample holder (sealed). The sample was placed in the sample holder of a two-circle goniometer, enclosed in a radiation safety enclosure. The X-ray source was a 2.2kW Cu X-ray tube, maintained at an operating current of 40 kV and 40 mA. The X-ray optics was the standard Bragg-Brentano para-focusing mode with the X-ray diverging from a DS slit (1mm) at the tube to strike the sample and then converging at a position sensitive X-ray Detector (Lynx-Eye, Bruker-AXS). The two-circle 218mm diameter goniometer was computer controlled with independent stepper motors and optical encoders for the θ and 2θ circles with the smallest angular step size of 0.0001° 2θ. The software suit for data collection and evaluation is windows based. Data collection is automated COMMANDER program by employing a DQL file. Data is analyzed by the program EVA.

Colorimetry: In an anaerobic chamber, 300 µl of the mixed mineral/liquid suspension was added to two separate centrifuge tubes ("solid" and "filtrate"). 500 µl of 6 M HCl was added to "solid" and 500 µl of deoxygenated high purity water (HPW) was added to "filtrate". Outside the anaerobic chamber, the filtrate was centrifuged at 13.3 rpm for 60 seconds. 100 µl of the solid and filtrate were diluted separately to 10 mL HPW. For each of these processed samples, two 1-ml aliquots were placed into two separate cryotubes (one for just Fe(II) and one for Fe(total)). 100 µl of HPW was added to the Fe(II) cryotube. 100 µl of 0.8 M ascorbic acid was added to the Fe(total) cryotube (to reduce all Fe(III) into Fe(II)). Then 100 µl 1 M HCl, 100 µl 1 M sodium acetate, and 2 ml of 0.3% 1,10-phenanthroline were added to Fe(II) and Fe(total) vials. Triplicates of 295 µl were placed on a 96 well plate to analyze at 509, 510, 511, and 512 nm. Fe(II) absorbance was subtracted from Fe(total) absorbance to obtain the Fe(III) absorbance.

Figure S1: Iron hydroxide precipitates formed with different Fe(II):Fe(III) ratios. Left to right: 100% Fe(II), 75% Fe(II), 66% Fe(II), 50% Fe(II), 100% Fe(III).

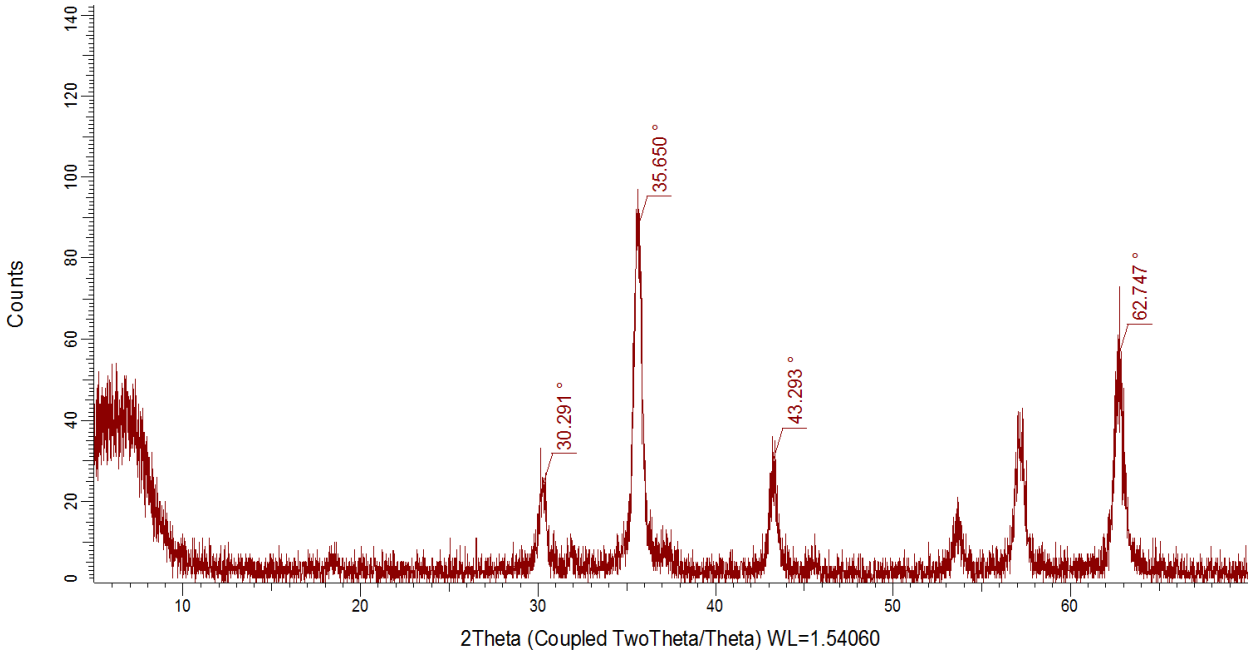

Figure S2: XRD pattern of iron hydroxide precipitate made with (Fe(II):Fe(III) = 1:1) after it was reacted with pyruvate and ammonia at 70°C, pH 10 for 48 hours. The peaks correspond well to magnetite.

Surface area and particle size: BET surface area and particle size (by electrical sensing zone (ESZ) method) were determined on a pay per sample basis (Particle Testing Authority). For particle size measurements, an experiment (70°C, pH 10, t=72 hours, Fe(II):Fe(III) = 1:1, containing 2.5 mM pyruvate) was conducted without agitation. 60 ml of the supernatant was removed and the remaining solution was transferred to a falcon tube and purged with argon. A small amount of this solid/liquid suspension was dispersed in 20 mL of filtered 2% NaCl and sonicated for 3 minutes. The mean particle size was 1.341 µm; median 1.183 µm (detection limit 0.7 µm; only 10% of particles were finer than 1.271 µm). For BET measurements, two experiments (70°C, pH 10, t=72 hours, Fe(II):Fe(III) = 1:1, containing 2.5 mM pyruvate) were conducted without agitating. 85 ml of supernatant was removed from each bottle (leaving the solids) and the remaining suspensions were transferred to falcon tubes and purged with argon. The samples were then vacuum-filtered, the solid residue scraped into a new falcon tube, and the solid was then frozen using liquid nitrogen and lyophilized under argon. 0.5762 g of the dried sample was analyzed under anaerobic conditions at ambient temperature. The BET surface area determined was 50.8352 m^2/g.

Mass estimates for sediment and chimney: Experiments were conducted to estimate and compare the mass of a typical sediment and chimney precipitate. (For mass determination: The chimney experiment ocean simulant contained 0.4 M Fe [Fe(II):Fe(III) = 2:1] and 0.3 M NH_4Cl in 60 mL; and hydrothermal simulant syringe contained 0.4 M NaOH + 20 mM Na-pyruvate in 30 mL; chimney experiment was conducted at room temperature with injection rate 1.5 ml/hour. The sediment experiment conditions were 70°C, Fe(II):Fe(III) = 1:1, pH 10, mass measured after 20 hours.) For the sediment experiment, the vial was weighed prior to making the solutions, then after doing the experiment, 85 ml of the supernatant was removed and then the bottle was left in a desiccator, weighing daily until the mass did not change any further. For the chimney, the vial

and injection apparatus were weighed prior to the experiment, and then after the experiment the "ocean" simulant was removed leaving only the solid chimney behind. The chimney was desiccated for 72 hours and weighed. The estimated mass of the sediment in the vial was ~2.84g; the estimated mass of the chimney was ~0.21g.

Combustion: Combustion analysis to detect C in the solids was done on an experiment conducted at pH 10, 70°C, Fe(II):Fe(III) = 1:1, unagitated, on the 72 hour sample. (We estimated based on the concentrations of pyruvate and iron in our experiments that the expected C in the solid phase would be at trace levels, below 0.5 weight %.) After the experiment was complete, 85 ml of supernatant was removed from the bottle. The remaining solution was transferred to another tube and centrifuged, and the rest of the supernatant removed, leaving only the solid. Combustion analysis was performed on a pay per sample basis (Galbraith Laboratories). Samples were handled under a nitrogen atmosphere. Analysis was done using a LECO SC-632 Carbon/Sulfur Determinator which covered the range of 0.05 – 1.7 mg of carbon. The sample was combusted at 1450 ± 50°C in an atmosphere of pure oxygen using Thermolite as a combustion aid; the resulting CO_2 was determined by non-dispersive infrared detection. The C detected in the solid was at 0.368% (wt/wt).

NMR sampling and analysis procedure for pyruvate reaction products. Samples were taken at t=0, 24, 48, and 72 hours (and occasionally at other time points) by agitating the vial to evenly distribute the solid/liquid and withdrawing a constant volume each time. (In one experiment, the vial was unagitated until sampled at 72 hours to test the effect of agitation on the results.) At every sampling point, five 1-mL aliquots of the liquid/solid mixture were transferred to clean microcentrifuge tubes (Fisherbrand, 1.5 mL graduated tube with flat cap, sterilized), removed from the glove box, and centrifuged at 10 x 1000 min^{-1} for two minutes to separate the solid and liquid. The supernatant was transferred to clean centrifuge tubes and the solid was discarded. 1 M NaOH was added slowly to precipitate out any remaining dissolved iron, and the samples were centrifuged again; this process was repeated until no precipitate remained. Of the five samples for each time point, three were analyzed directly and one was spiked with alanine and one with lactate (or sometimes pyruvate) to aid in peak identification. Liquid samples were transferred to clean NMR tubes and analyzed with liquid ^1H NMR after addition of 10% D_2O containing the water soluble chemical shift reference material DSS (so that the DSS peak at 0 ppm equals a 1 mM concentration). The samples were run on a Bruker AV III HD 400 with a Prodigy liquid nitrogen temperature cryoprobe. A standard Bruker pulse sequence for a 1-dimensional NOESY experiment (noesygppr1d) was employed with a standard Bruker parameter set, WATERSUP, supplied by Bruker as part of the Topspin 3.5.7 software release. The number of scans per spectrum was set at 64; all other parameters were left at their default values. The key default values were a sweep width of 21 ppm centered at the nominal chemical shift of water (4.7 ppm), acquisition time 1.95 seconds, delay of 2 seconds between scans.

The resulting data was analyzed in the NMR processing program MestReNova. The baseline and reference were adjusted to place the peak of the DSS/D_2O standard at zero ppm. Manual integration was performed to find the area of all peaks relative to the standard DSS peak. In the methyl region, the dominant peaks were pyruvate at ~2.23 and 2.16 ppm, a lactate doublet at ~1.3 ppm, and an alanine doublet at ~1.2 ppm. (The 2.23 pyruvate peak is broadened due to the very high pH of the samples produced by the iron removal process.) A peak at 1.91 ppm is present in acetate controls as well as a decarboxylation product in pyruvate controls (determined

by spiking a pyruvate sample with sodium acetate and stacking spectra to compare peaks), but a small peak at 1.91 also appears in control spectra of NH_4Cl. We attribute the 1.91 peak to acetate in our summary but note that there may be some uncertainty in the acetate concentration calculations. The relative concentrations of pyruvate, alanine, lactate, and acetate in the liquid phase were calculated as each of their respective methyl peak sum areas divided by the total area for [pyr + acetate + ala + lac]. For a given experiment, the areas of the individual products were plotted individually without averaging, except where specified. Data were analyzed using GraphPad Prism (v. 7.00, GraphPad Software, Inc.). With the variations in iron redox state and pH in this work, different volumes of NaOH had to be added to samples in order to remove all of the dissolved iron before they were prepared for NMR analysis. This inconsistent dilution means that total organic concentration in mM was unable to be determined precisely, though it should be around the 2.5 mM concentration of pyruvate initially added. We report relative concentrations in the liquid phase, but there is also an incomplete mass balance of pyruvate that may be in the solid phase. In control experiments where alanine or lactate was reacted with iron oxyhydroxide precipitates for 24 – 72h, no significant adsorption or incorporation into the solid phase of alanine or lactate was observed. Thus we conclude that most or all of the alanine or lactate that forms in the experiment is detected in our liquid samples. The "missing" pyruvate may therefore be in the form of intermediates, adsorbed unreacted pyruvate, or perhaps other undetected products.

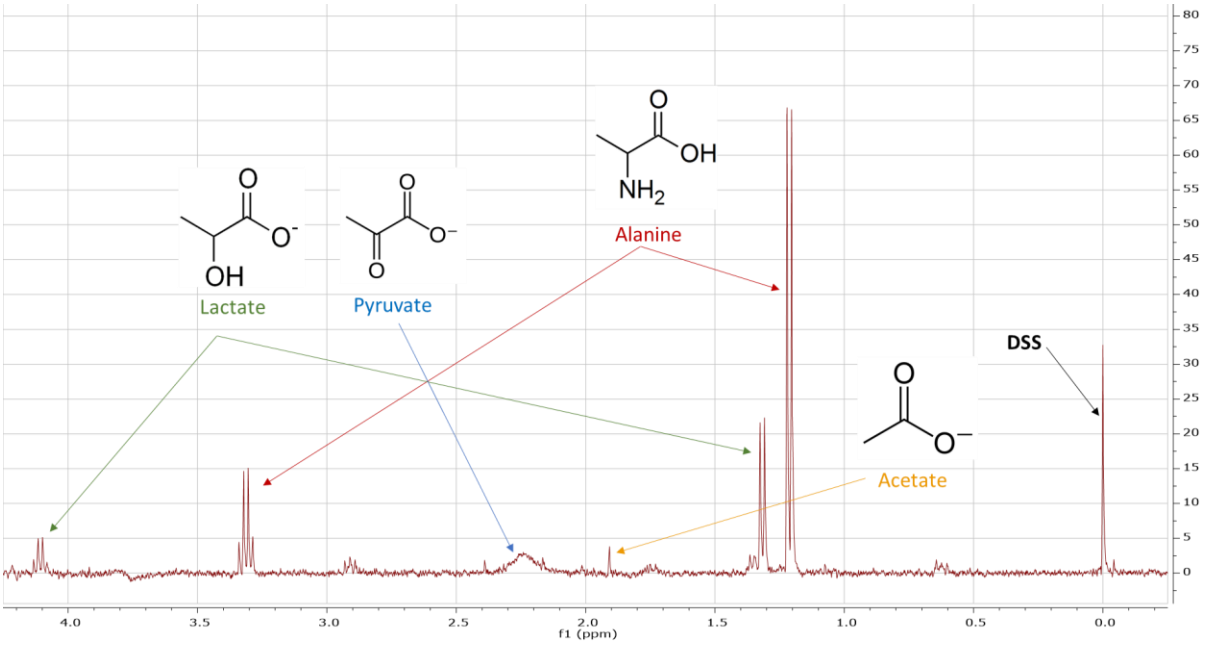

Figure S3: Example of a corrected ^{1}H NMR spectrum showing DSS, lactate, pyruvate, acetate, and alanine peaks. This sample was from an experiment with 66% Fe(II) in the precipitate, at pH 10, 70°C, after 24 hours.

Sampling and analysis procedure for high-resolution mass spectrometry. The identity of alanine, lactate, and pyruvate in a representative reaction mixture [Fe(II):Fe(III) = 1:1, pH=10, T=70°C, t=72h] was confirmed by high-resolution mass spectrometry (**Figure S4**).

Materials: The InfinityLab deactivator and API-TOF reference mass solution kit were purchased from Agilent Technologies (Santa Clara, CA). Ammonium acetate (LC-MS grade)

and ammonia solution (25%) were purchased from Sigma-Aldrich (St. Louis, MO). HPLC-Grade water was purchased from Spectrum Chemical (Gardena, CA) and HPLC-grade acetonitrile from VWR (Radnor, PA).

High resolution mass spectrometry: Aqueous reaction mixtures were analyzed by liquid chromatography tandem quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The sample was thawed at room temperature for 30 min and an aliquot (200 µL) transferred into a 2 mL HPLC vial, followed by dilution with acetonitrile (200 µL). The sample was analyzed by LC-QTOF-MS under the following conditions. The sample (1 µL injection volume) was injected into a series 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA) consisting of a model DEAEW03066 quaternary pump, a DEAEM02325 column compartment, and a DEAGX00258 multisampler operating at 0.25 mL min-1 interfaced to a 6545 QTOF MS (Agilent Technologies) with a model G1958-65268 Dual AJS electrospray ionization source. An Agilent InfinityLab Poroshell 120 HILIC-Z column (2.1 × 100 mm; 2.7 µm) controlled at 30°C was the stationary phase. The following HPLC method was used: (A, 10 mM ammonium acetate, pH 9.0 in water + 5 µM deactivator additive; B, 10 mM ammonium acetate, pH 9.0 in 90 % ACN + 5 µM deactivator additive): 0.00 min 100% B; 6 min ramp from 0:100 A:B to 40:60 A:B; 2 min hold at 40:60 A:B; 0.1 min ramp from 40:60 A:B to 0:100 A:B; 4 min hold at 0:100 A:B and 0.5 mL min-1 for column re-equilibration resulting in a total run time of 12.1 min with alanine retention time of 6.82 min, pyruvate retention time of 2.78 min, and lactate retention time of 4.98 min. The following mass spectrometry parameters were used in MS (scan) mode: Ionization mode, ESI negative; gas temperature, 250 °C; gas flow, 10 L min-1; nebulizer, 45 psi; sheath gas temperature, 350°C; sheath gas flow rate, 11 L min-1; fragmentor, 70 V; capillary voltage, 3500 V; nozzle voltage, 0 V.

The data were analyzed using the Agilent MassHunter Qualitative Analysis Navigator (Version B.08.00, build 8.0.8208.0) Formula Generation tool. The mass and isotope model predictions were generated using the Agilent Isotope Distribution Calculator software (Version 8.0.8208.0).

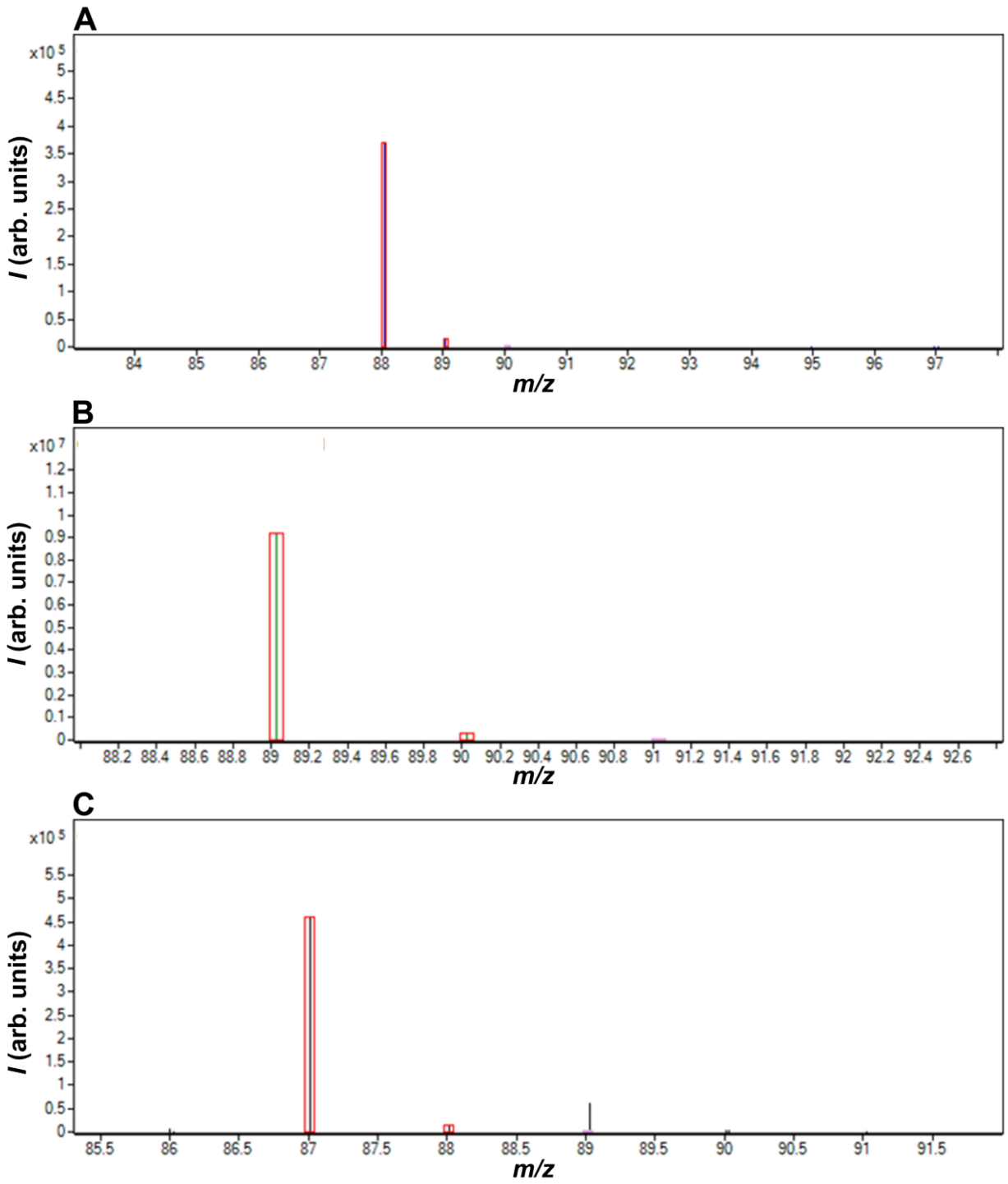

Figure S4. High resolution mass spectra of representative reaction mixture components showing the molecular ion isotope envelope. Lines correspond to the observed ions; red outlines correspond to predicted ions. A) Alanine (retention time 6.82 min) HRMS-ESI (*m/z*): $[M - H]^-$ calcd for $C_3H_6NO_2$, 88.0404; found, 88.0405. B) Lactate (retention time 4.98 min) HRMS-ESI (*m/z*): $[M - H]^-$ calcd for $C_3H_6O_3$, 89.0244; found, 89.0246. C) Pyruvate (retention time 2.78 min) HRMS-ESI (*m/z*): $[M - H]^-$ calcd for $C_3H_4O_3$, 87.0088; found, 87.0088.

Results from reacting pyruvate with iron oxyhydroxides. Sediment experiments were conducted at various pH between 7-11, temperature between 25 - 80°C, and using different %Fe(II) relative to Fe(III) in the precipitate. All experiments contained 2.5 mM pyruvate, 50 mM Fe(total) and 0.375 M ammonia as described above and were sampled using the same procedure. Pyruvate reacted to form alanine and lactate under certain conditions; alanine yield was generally higher at more alkaline conditions and around 50% Fe(II). Specific results are described in the main text and in **Figures S5-S9**; the results of all conditions tested are summarized in **Table SI-1.** In general, experiments conducted at 70°C were reproducible, though there was some variation in measured concentrations of pyruvate and its products between repeats of the same condition. We conducted tests to determine whether these variances in product concentration were the result of agitation during the experiment. **Figure S6** shows an experiment sampled at t=0, 0.5, 1, 2, 4, 8, and 24 hours, compared to an experiment at the same conditions sampled only at 24, 48, and 72 hours; the 24-hour concentrations of products are similar. **Figure S7** shows a comparison between an experiment agitated every 24 hours and an unagitated experiment both sampled at 72 hours; the product concentrations are similar. Thus we concluded that agitation was not a big factor in our results. It is most likely that the differences in relative yield measured by ^{1}H NMR are due to issues with peak measuring, including the broadening of the pyruvate peak due to high pH in the iron removal process, and also perhaps due to water peak suppression; the differences in yield could also be due to unpredictable factors such as changing surface area or particle size of the precipitate at different conditions and timesteps. Results from experiments conducted at lower temperatures were generally even less reproducible and there was often quite a bit of scatter in the data; this may be because the reactions occur more slowly at lower temperature.

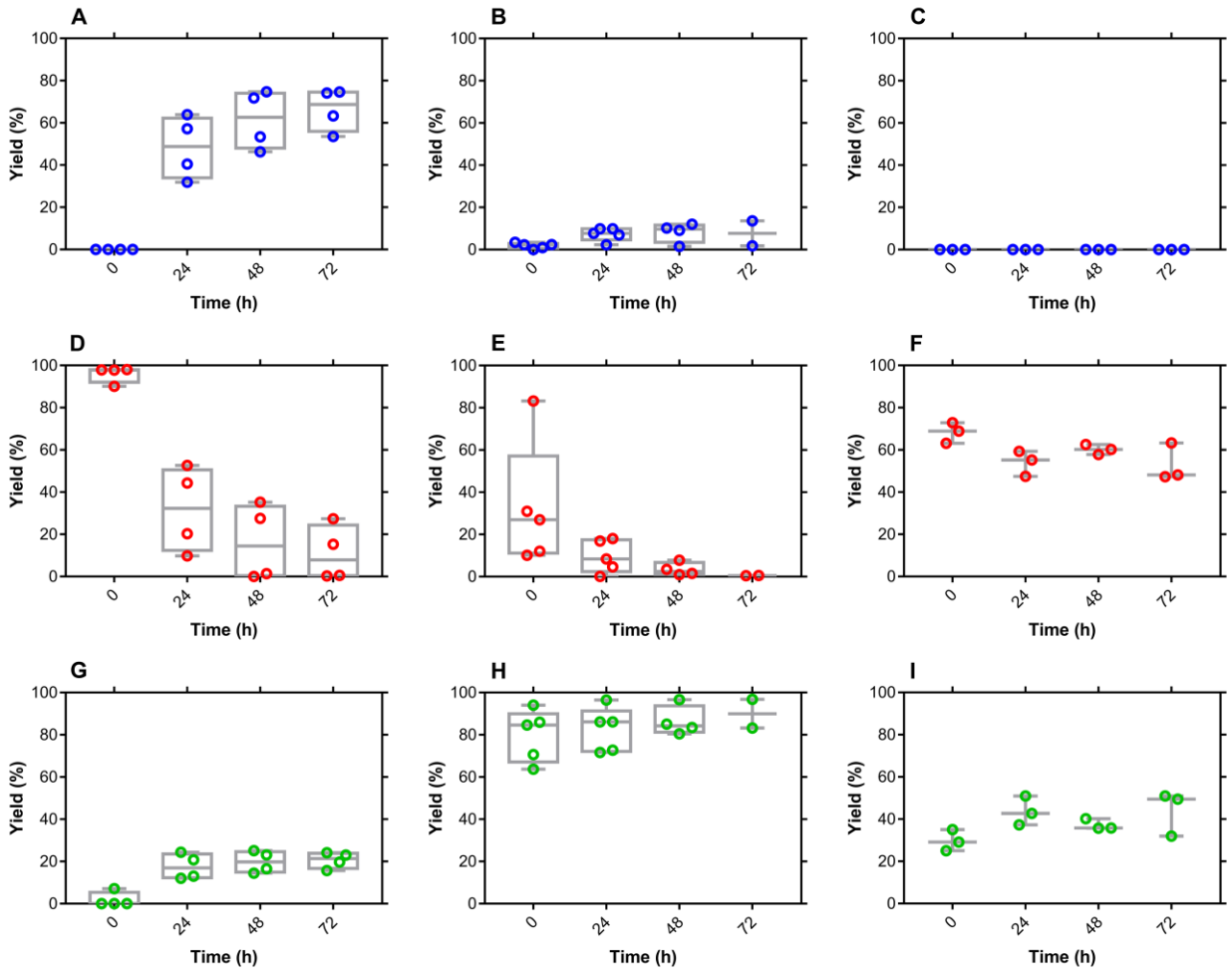

Figure S5: Pyruvate reactions in the presence of ammonia and freshly precipitated iron hydroxides as a function of time. Experiments conducted at 70°C are shown; pH and Fe(II) mole fraction in the mineral were varied. Plots of analyte reaction yields with values from individual experiments superimposed (A-I); blue = alanine; red = pyruvate; green = lactate. The box extends from the 25th to 75th percentiles, with the horizontal line in the box representing the median; whiskers represent the lowest and highest datum. (A, D, G): 66% Fe^{2+}, pH 10. (B, E, H): 90% Fe^{2+}, pH 9.2. (C, F, I): 75% Fe^{2+}, pH 7.

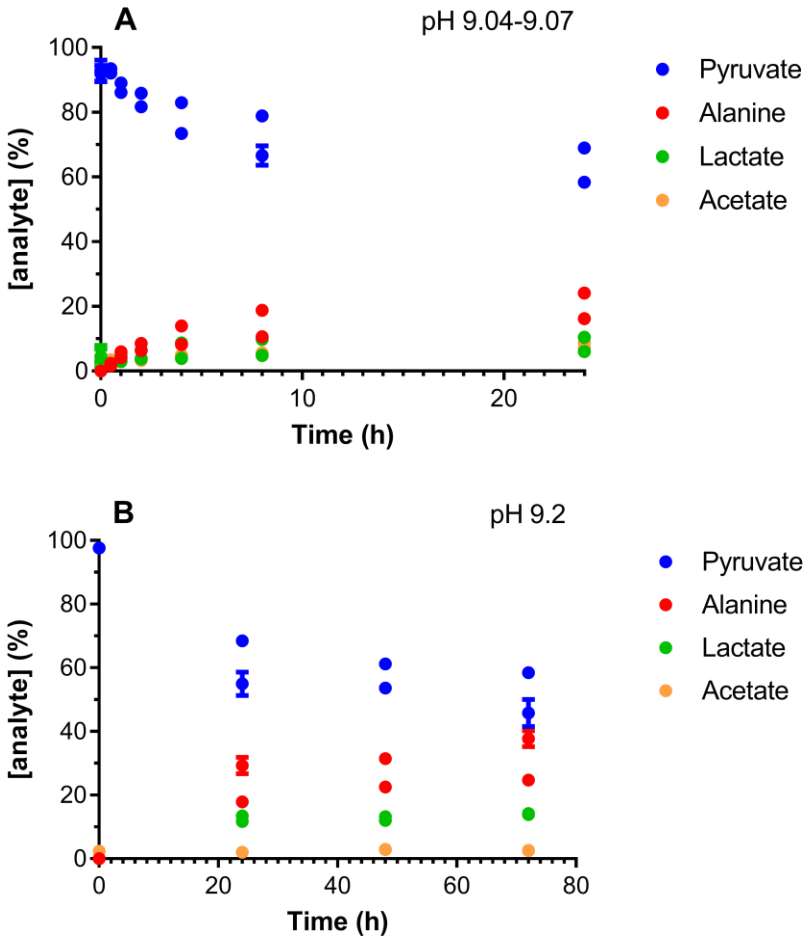

Figure S6: Pyruvate reactions sampled at different timesteps. Experiments conducted at 70°C, Fe(III):Fe(II) = 1:2, pH 9 - 9.2 are shown. A) Samples were taken at t = 0, 0.5, 1, 2, 4, 8, and 24 hours; B) Samples were taken at t = 24, 48, and 72 hours. The 24 hour values of pyruvate, alanine, and lactate in both plots are similar, indicating that sampling frequency does not affect reaction yield.

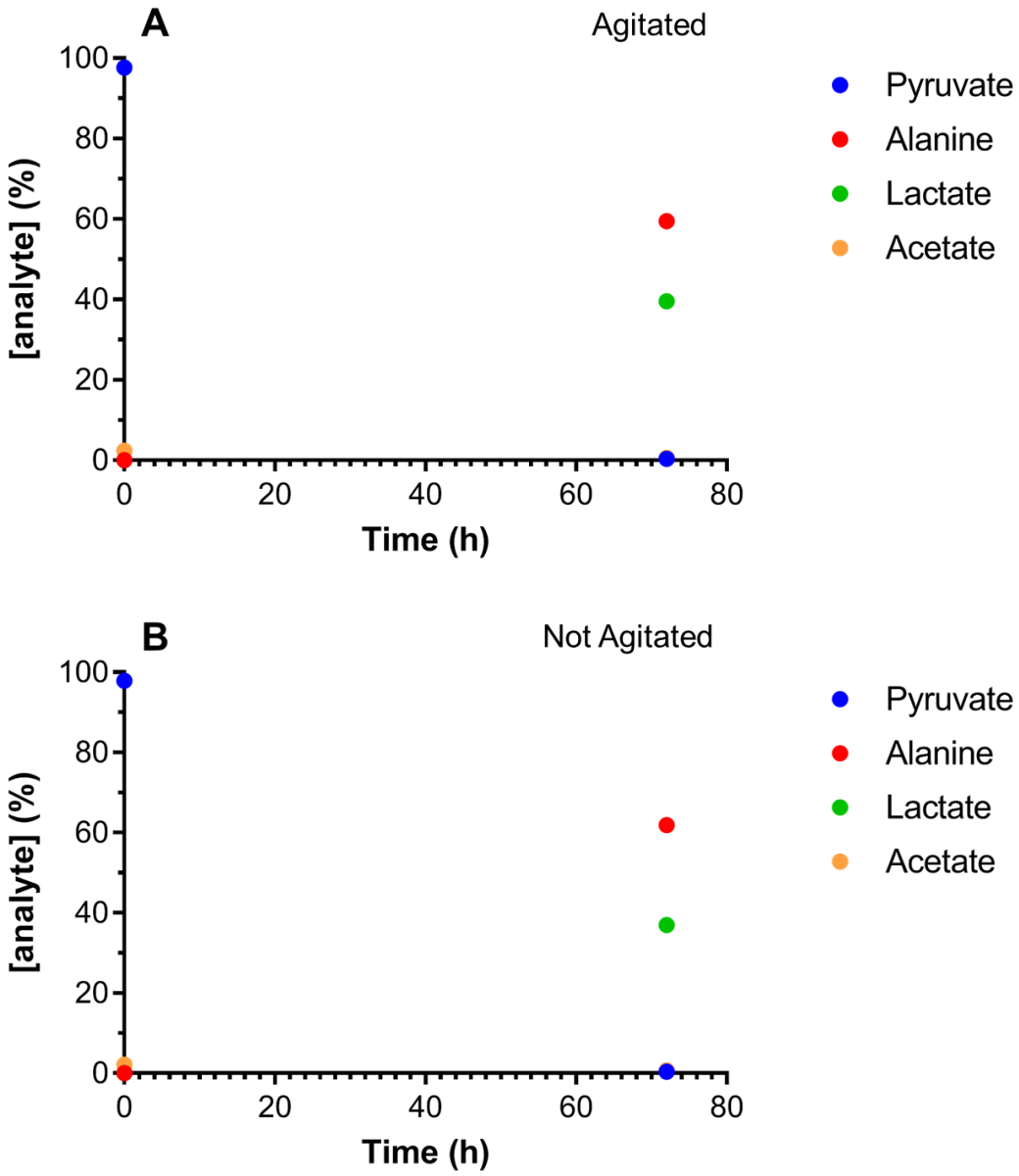

Figure S7: Agitated and not-agitated pyruvate experiments. Experiments conducted at 70°C, Fe(III):Fe(II) = 1:1, pH 10 are shown. A) An experiment was agitated when sampled every 24 hours. B) An experiment was not agitated at all for 72 hours, then sampled. The relative yields of products are similar, indicating that agitation does not affect product yield.

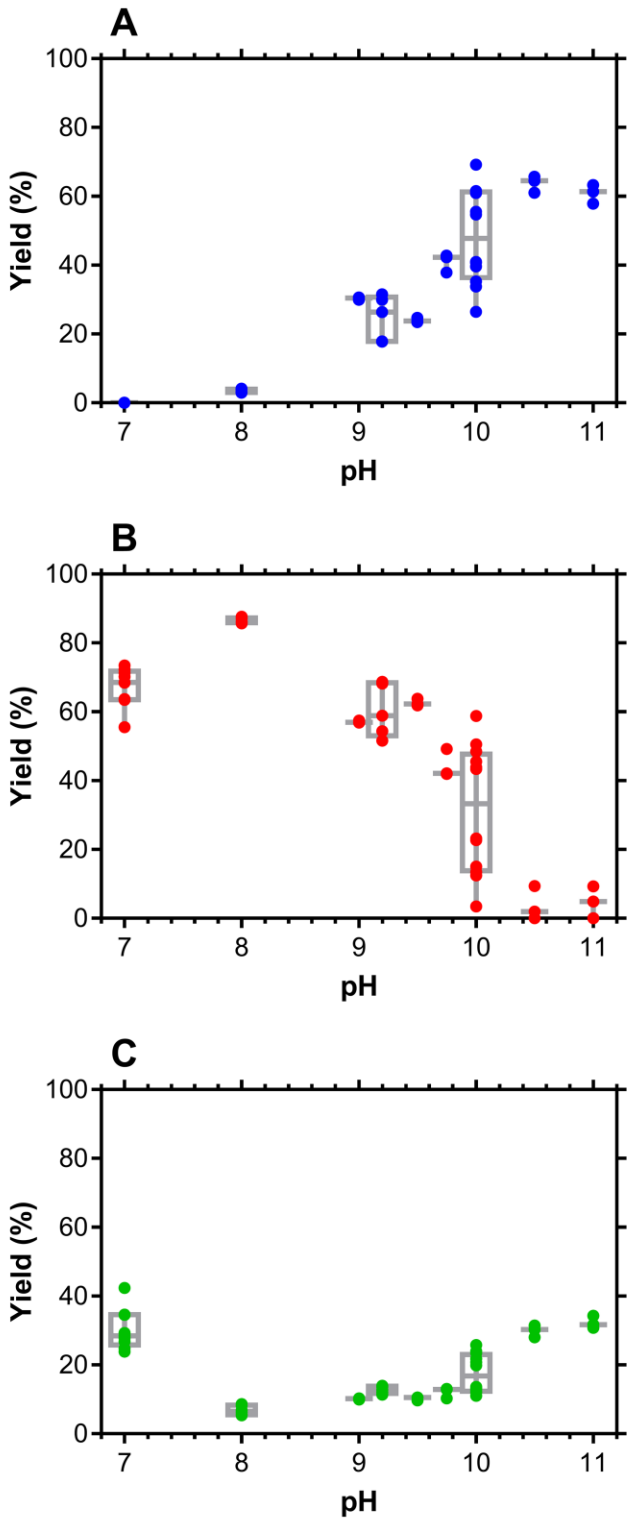

Figure S8: Pyruvate reactions as a function of pH after 24 hours with 66% Fe(II) in the iron hydroxide precipitate and at 70°C. A) Alanine yield, B) Pyruvate yield, C) Lactate yield.

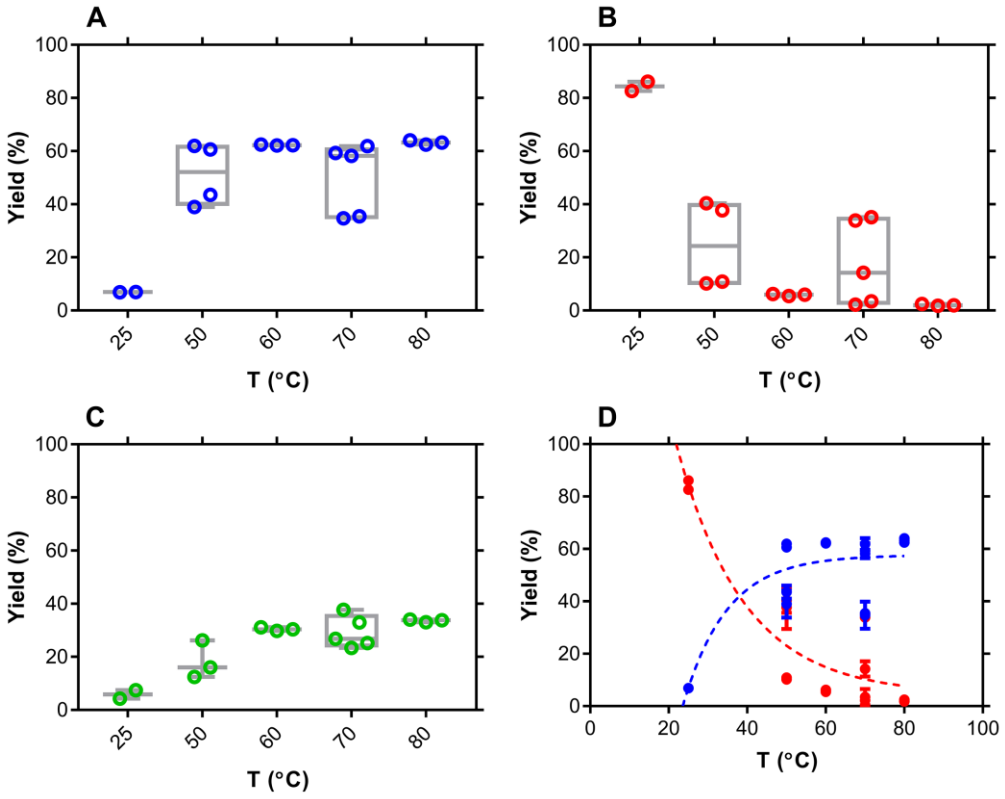

Figure S9: Pyruvate reactions as a function of temperature after 72 hours at pH 9.2 with 50% Fe(II) in the iron hydroxide precipitate. A) Alanine yield; B) Pyruvate yield; C) Lactate yield; D) Means and SEMs for pyruvate (red) and alanine (blue) superimposed, the dotted line represents a least squares (ordinary) fit using a one phase decay model.

%Fe(II)/ [Fe(II)+ Fe(III)]	pH	T (°C)	Time points sampled (h)	No. of repeats	Result
100	9.2	70	0, 24, 48, 72	4	All pyr reacts immediately to form lactate.
90	9.2	70	0, 24, 48, 72	5	65-100% lactate at t=0; trace alanine after 48h
75	7	70	0, 24, 48, 72	3	~30-50% lactate, no alanine
75	9.2	70	0, 24, 48, 72	3	Only ~30% of pyr is consumed; alanine + lactate
75	10	70	0, 24, 48, 72	3	10-30% lactate; 20-50% alanine
75	9.2	50	0, 24, 48, 72	4	Only ~10-30% of pyr is consumed; alanine + lactate
67	7	70	0, 24, 48, 72	3	~30% lactate at t=0; no alanine
67	8	70	0, 24, 48, 72	2	Only ~10% of pyr consumed; alanine + lactate
67	9	70	0, 24, 48, 72	1	~10% lactate, ~40% alanine
67	9.2	70	0, 24, 48, 72	2	~10% lactate, ~20-40% alanine
67	9.5	70	0, 24, 48, 72	1	~15% lactate, ~30% alanine
67	9.75	70	0, 24, 48, 72	1	All pyr is consumed; ~20% lactate, ~50% alanine
67	10	70	0, 24, 48, 72	4	~20% lactate, ~50-70% alanine
67	10.5	70	0, 24, 48, 72	1	All pyr consumed after 24h. ~30% lactate, ~70% alanine
67	11	70	0, 24, 48, 72	1	All pyr consumed after 24h. ~30% lactate, ~70% alanine
67	9.2	50	0,1,3,5,24,48,72	4	Inconsistent data. ~10-15% lactate, minor alanine
50	9	70	0, 24, 48, 72	3	~30-40% lactate, ~50-60% alanine
50	9.2	25	72	2	~5-10% lactate and alanine
50	9.2	50	72	4	~10-30% lactate, ~40-65% alanine
50	9.2	60	72	3	~30% lactate, ~65% alanine
50	9.2	70	0, 24, 48, 72	3	~30% lactate, ~60% alanine
50	9.2	80	72	3	~30% lactate, ~60% alanine
50	10	70	0, 24, 48, 72	3	~30% lactate, ~60-70% alanine
42	9.2	70	0, 24, 48, 72	3	~10-30% lactate, ~40-50% alanine
42	9.2	50	0, 24, 48, 72	4	Only ~30% of pyr consumed, ~10% lactate, ~15% alanine
33	9.2	70	0, 24, 48, 72	4	No reaction, ~96% of pyr remains
0	9.2	70	0, 24, 48, 72	4	No reaction, ~98-100% of pyr remains
37.5% Fe^{2+} + 37.5% Ni^{2+}	9	70	0, 24, 48, 72	1	No reaction, 100% of pyr remains

Table SI-1: Iron hydroxide precipitate experiments with pyruvate and ammonia that were conducted in this study. Table indicates %Fe(II), pH, temperature, and time points sampled. All experiments contained 50 mM Fe, 0.375 M ammonia, and 2.5 mM pyruvate except when otherwise noted.

Control reactions. A control reaction was performed to determine whether the reaction was happening in the aqueous solution surrounding the precipitate or whether the precipitate itself is required. 1 M NaOH was slowly added to a solution of [Fe(II) + Fe(III)] chloride salts dissolved in argon-purged Milli-Q water to precipitate iron hydroxide. The liquid/solid mixture was titrated to pH 9.2, allowed to settle and placed in the hot bath at 70°C for 3 hours. Then the supernatant liquid ("liquid control") was separated from the precipitate ("solid control"). Solutions containing pyruvate and ammonia were added to the liquid and solid control separately, and the mixtures were heated for 24 hours. In the liquid control, the pyruvate did not react, and in the solid control pyruvate reacted to form alanine and minor lactate, thus showing that the precipitate is involved in the reaction. Another control was performed to test whether lactate has any role in alanine synthesis. An experiment was conducted under a typical condition (at pH 9.2, 70°C, 66% Fe(II)) except that Na-lactate was added instead of Na-pyruvate. After 24 hours no reaction was observed (and when pyruvate is used in this condition alanine is synthesized), thus we conclude that lactate is stable against oxidation under these conditions and does not affect alanine synthesis. A control experiment using 100% Fe(II) and no ammonia was run under the same conditions and results showed that all of the pyruvate reacted to make lactate as soon as the solutions are mixed together, at t=0. Another similar experiment was run with no pyruvate, and no reaction occurred.

Hydrothermal chimney simulations. Pyruvate reactions were also attempted in an experiment in which iron hydroxides were precipitated in a simulated hydrothermal chimney structure instead of via coprecipitation / mixing; a chimney experiment produces an experiment that has a gradient within the single vial, instead of the vials of sediment representing points within a gradient. In natural systems, hydrothermal chimneys can form large and self-organized structures, often containing networks of pores and/or catalytic minerals across which the gradients between vent fluid and seawater are focused. An inverted 120-mL glass vial with the base cut off was used as the chimney apparatus (**Figure 1; Figure S10**). A solution representing reactants in the early ocean was placed in the vial, and a solution representing reactants from the hydrothermal fluid was placed in a syringe and slowly injected into the "ocean reservoir" in the vial. The ocean solution was made with Milli-Q water that had been sparged with argon, and the headspace of the chimney experiment was purged with argon throughout the injection period. As in previous work [2] a chemical garden precipitate structure formed at the injection point from the interfacing of the two contrasting fluids. Various ocean and hydrothermal simulant compositions and injection rates / volumes were tested in an attempt to synthesize chimneys which were analogous to the sediment precipitation experiments (in terms of precipitate composition and total precipitate volume) and which would generate observable products of lactate and/or alanine (**Table SI-2**). Variations included: adding the ammonia to either the ocean or the hydrothermal simulant, increasing concentrations of all reactants to precipitate a larger chimney in a short time, injecting a volume of hydrothermal simulant either much smaller than or equal to the ocean volume, and injecting slowly so that chimney formation occurred throughout the reaction period vs. injecting quickly to form a chimney that would provide a constant amount of precipitate throughout the reaction. After the alkaline solution was injected and the reaction was complete, the vial was drained and the ocean simulant and the chimney precipitate were sampled. The ocean simulant, which contained a large amount of dissolved iron, was treated with NaOH to remove iron for ^{1}H NMR analysis. The solid chimney precipitate was dissolved in HCl, treated with NaOH to remove iron, and analyzed with ^{1}H NMR. Chimney

experiments were carried out at room temperature and at 70°C (heating the chimney vessel by partially submerging in a hot water bath). For the heated experiments, no chimney grew when ammonia was added with the alkaline solution, and the precipitate forming settled to the bottom instead. When ammonia was added in with the ocean simulant in a heated experiment, a chimney formed, that (unlike the room temperature chimneys) did not exhibit much branching and did not grow as tall (**Figure S10**).

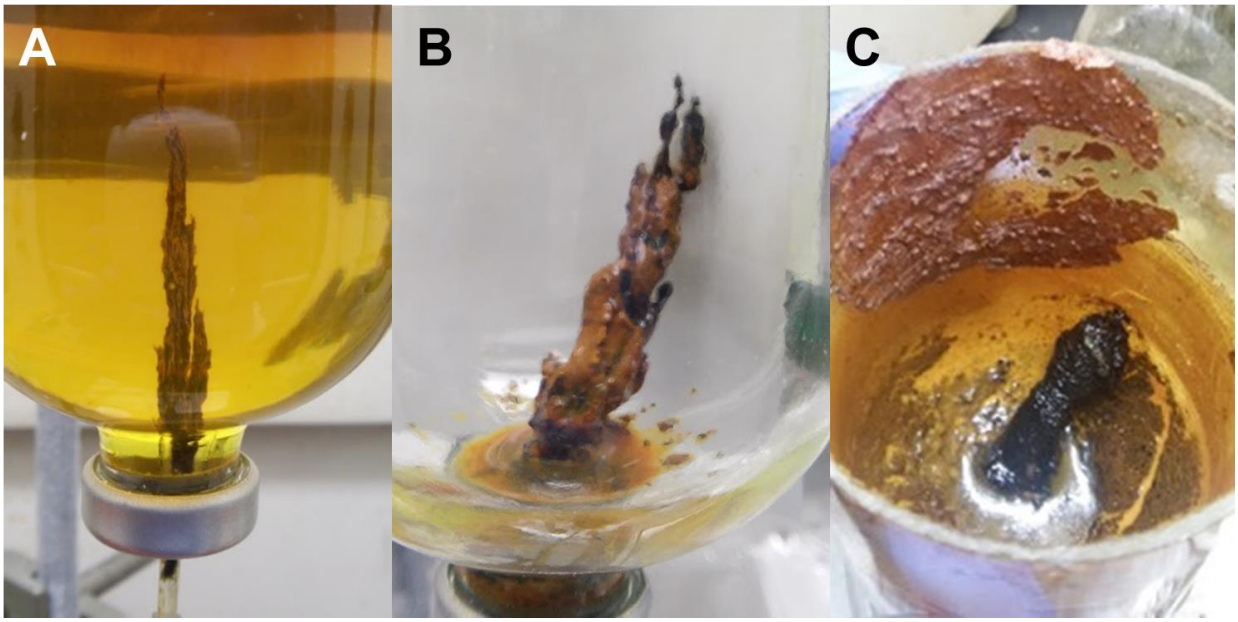

Figure S10: Precipitates resulting from chimney simulation experiments. A) Chimney formed at room temperature after one hour from an alkaline solution containing 20 mM pyruvate and 0.4 M NaOH injected into an ocean simulant containing 0.4 M Fe-chloride (Fe(II):Fe(III) = 2:1) and 3 M NH_4Cl. B) Chimney formed at room temperature after 24 hours from an alkaline solution containing 20 mM pyruvate, 0.4 M NaOH, and 3 M NH_4Cl injected into an ocean simulant containing 0.4 M Fe-chloride (Fe(II):Fe(III) = 3:1), after the ocean simulant had been drained. C) Chimney formed after 24 hours at 70 degrees C by injecting an alkaline solution containing 20 mM pyruvate and 0.4 M NaOH into an ocean simulant containing 0.4 M Fe-chloride (Fe(II):Fe(III) = 3:1), and 3 M NH_4Cl, after the ocean simulant had been drained.

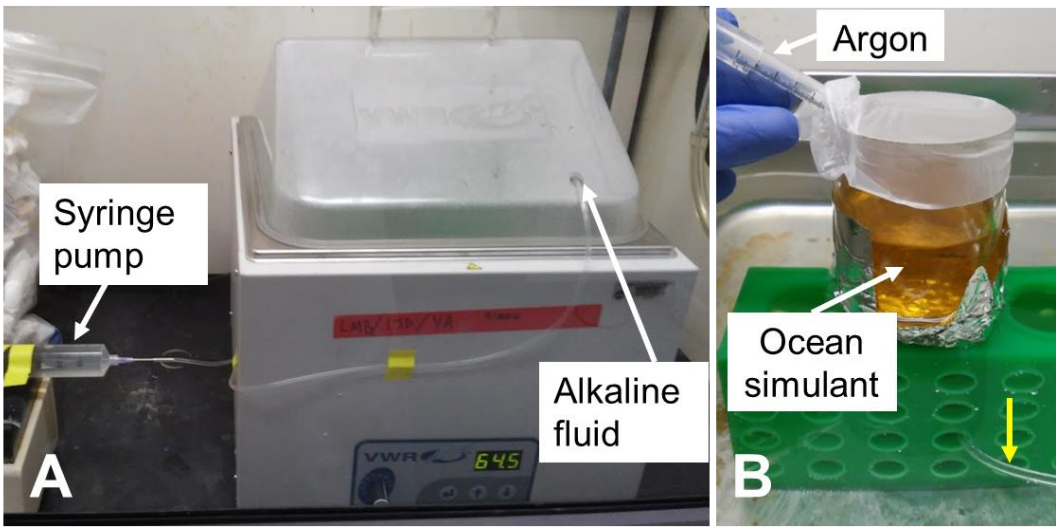

Figure S11: Setup for heated chimney experiments. A) A syringe pump injected simulated hydrothermal solution into a tube that fed into the hot bath. B) The ocean simulant vial shown in Figure S8 was held in place in the hot water bath and the tube of "hydrothermal fluid" fed into it from below. Argon was fed continuously into the ocean simulant headspace to keep experiments anoxic.

Table SI-2: Iron hydroxide chimney experiments with pyruvate and ammonia. "Ocean simulants" refer to the iron-containing solution that was placed in the chimney vessel. "Hydrothermal simulants" refer to the hydrothermal simulant that was placed in the syringe and injected into the ocean simulant. RT = room temperature. Compositions are described as total Fe concentration (split between Fe(II) and Fe(III)).

Experiment		Ocean simulant		Hydrothermal simulant		Injection rate	Results
T (°C)	%Fe(II)/ [Fe(II)+ Fe(III)]	Composition	Vol (mL)	Composition	Vol (mL)		
RT	100	0.2 M Fe 1.5 M NH_4Cl 10 mM Na-pyruvate	75	0.2 M NaOH	25	5 ml / hour	After 24h, pyruvate is consumed, lac is present, no ala.
	75	0.4 M Fe	60	0.4 M NaOH 3.0 M NH_4Cl 20 mM Na-pyruvate	60	2 ml / hour	After 24h pyruvate remains, trace lac present, no ala
	66	0.4 M Fe 3.0 M NH_4Cl	60	0.4 M NaOH 20 mM Na-pyruvate	60	0.05 ml / min	After 48h, pyruvate remains, trace lac present, no ala
	66	75 mM Fe	100	0.1 M NaOH 50 mM NH_4Cl 25 mM Na-pyruvate	10	2.5 ml / hour	No chimney formed after 24h. Pyruvate remains, no lac or ala
70	75	0.4 M Fe	60	0.4 M NaOH 3.0 M NH_4Cl 20 mM Na-pyruvate	60	2 ml / hour	After 24h pyruvate remains, trace lac present, no ala
	75	0.4 M Fe 3.0 M NH_4Cl	60	0.4 M NaOH 20 mM Na-pyruvate	60	0.05 ml / min	Pyruvate remains, no lac or ala
	66	0.4 M Fe	60	0.4 M NaOH 3.0 M NH_4Cl 20 mM Na-pyruvate	60	2 ml / hour	After 24h pyruvate remains, trace lac present, no ala
	66	0.4 M Fe 3.0 M NH_4Cl	100	0.4 M NaOH 20 mM Na-pyruvate	10	2.5 ml / hour	Pyruvate remains, no lac or ala

References:

1. Huber C, Wächtershäuser G (2003) Primordial reductive amination revisited. *Tetrahedron Letters* 44(8):1695-1697.
2. Barge LM et al. (2015) Chemical gardens as flow-through reactors simulating natural hydrothermal systems. *Journal of Visualized Experiments* (105):e53015. doi:10.3791/53015.