Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/6/eaav7848/DC1

# Supplementary Materials for

## Metals likely promoted protometabolism in early ocean alkaline hydrothermal systems

Norio Kitadai*, Ryuhei Nakamura, Masahiro Yamamoto, Ken Takai, Naohiro Yoshida, Yoshi Oono

*Corresponding author. Email: nkitadai@elsi.jp

Published 19 June 2019, *Sci. Adv.* **5**, eaav7848 (2019)
DOI: 10.1126/sciadv.aav7848

**This PDF file includes:**

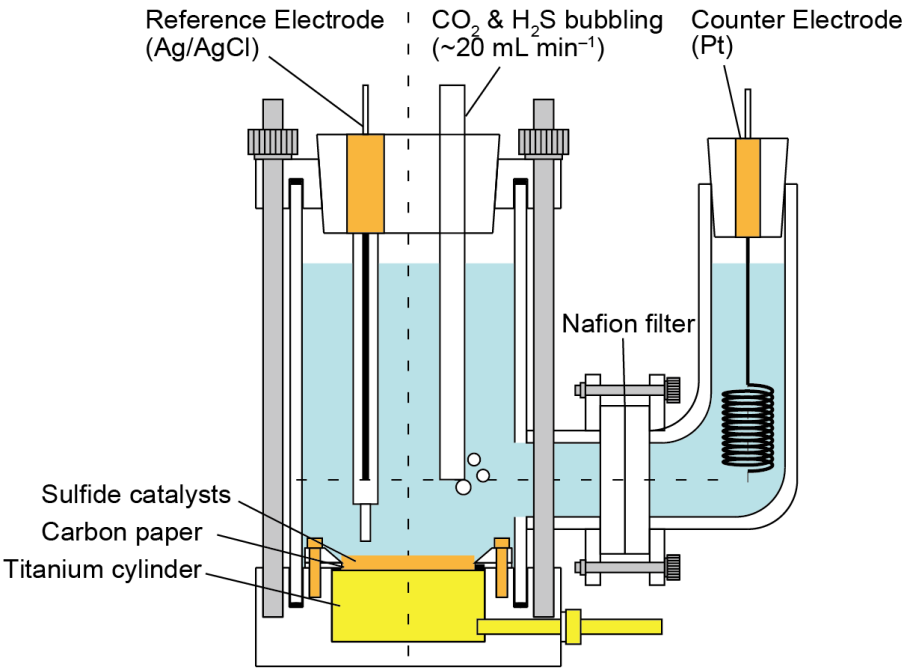


**Fig. S1. A schematic of the electrochemical cell.** The cell is made of a Pyrex glass tube sandwiched between a polyoxymethylene (POM) cap and basement that were tightened together with stainless screws and knurled nuts. The cell has two compartments: a large working electrode side (~100 mL) and a small counter electrode side (~15 mL) that are separated by a proton exchange membrane (Nafion 117; DuPont). On the working electrode side, a titanium cylinder (purity; 99.5%) is placed at the center of the POM basement, and is coated with carbon paper (5.7 $cm^2$) with a silicon and POM packings. An Ag/AgCl electrode (in saturated KCl) is used as the reference, and is fixed at a distance of less than 0.5 cm from the working electrode to reduce solution resistance. On the counter side, a platinum coil is inserted into the glass tube, and is used as the counter electrode.

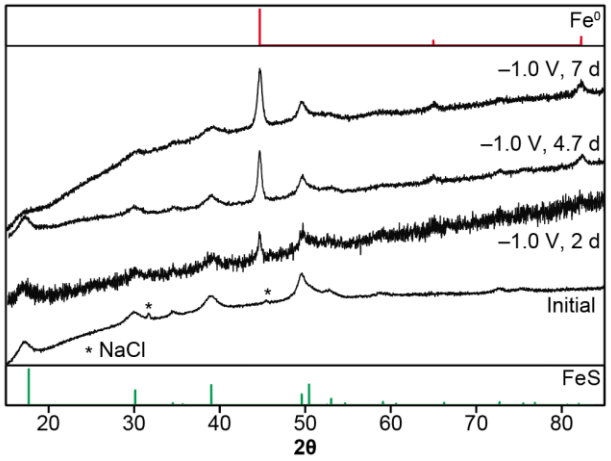


**Fig. S2. XRD patterns of FeS electrolyzed at –1.0 V versus SHE for different durations.**

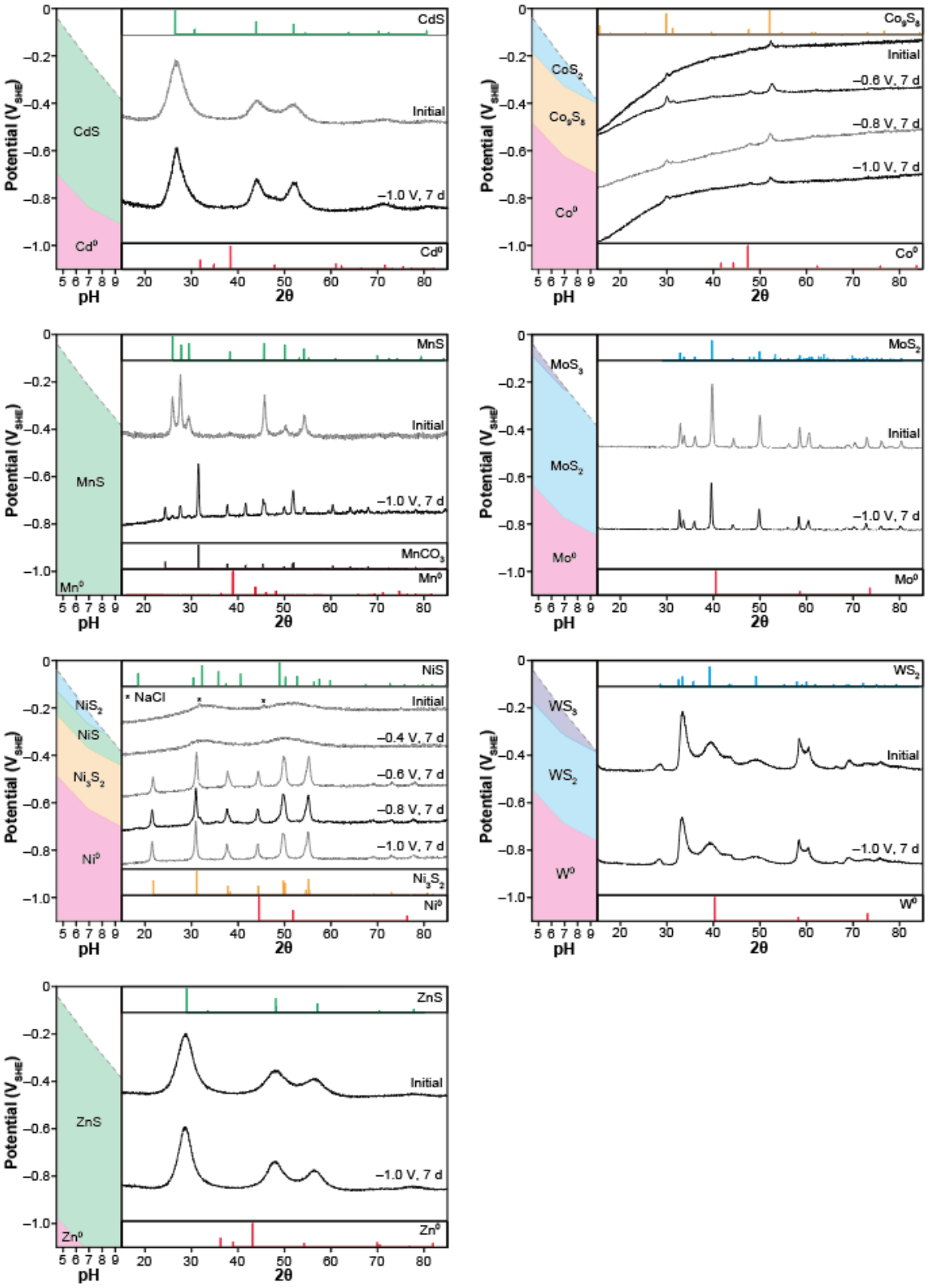


**Fig. S3. XRD patterns of metal sulfides before and after the 7-day electrolysis.** The color convention for the left columns is the same as that for the left columns in Fig 1B–E.

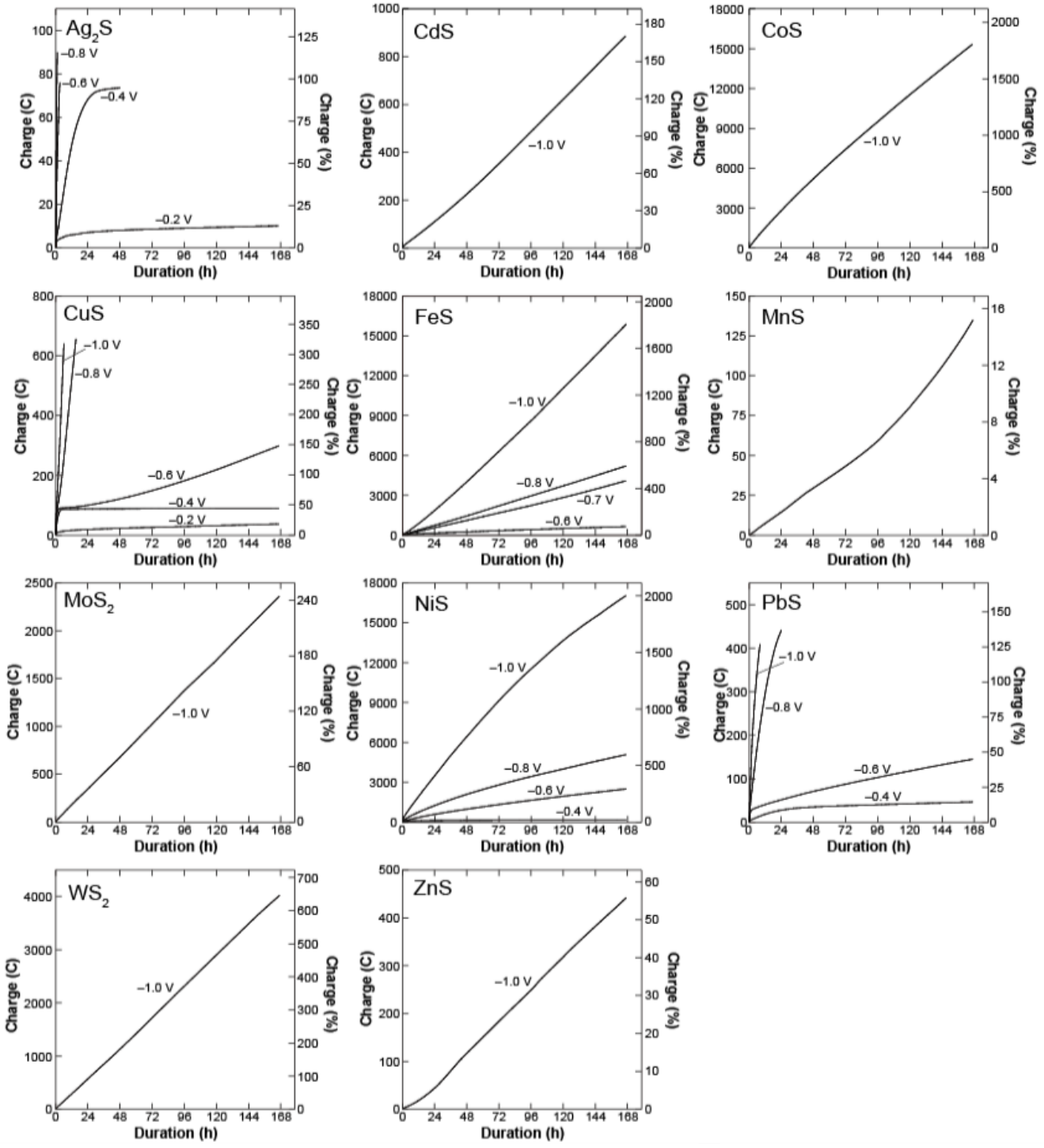


**Fig. S4. Total charges build up during the electrolysis.** The percentages shown at the right axis were calculated relative to the charge required for the complete sulfide-to-metal electroreduction for each reaction condition.

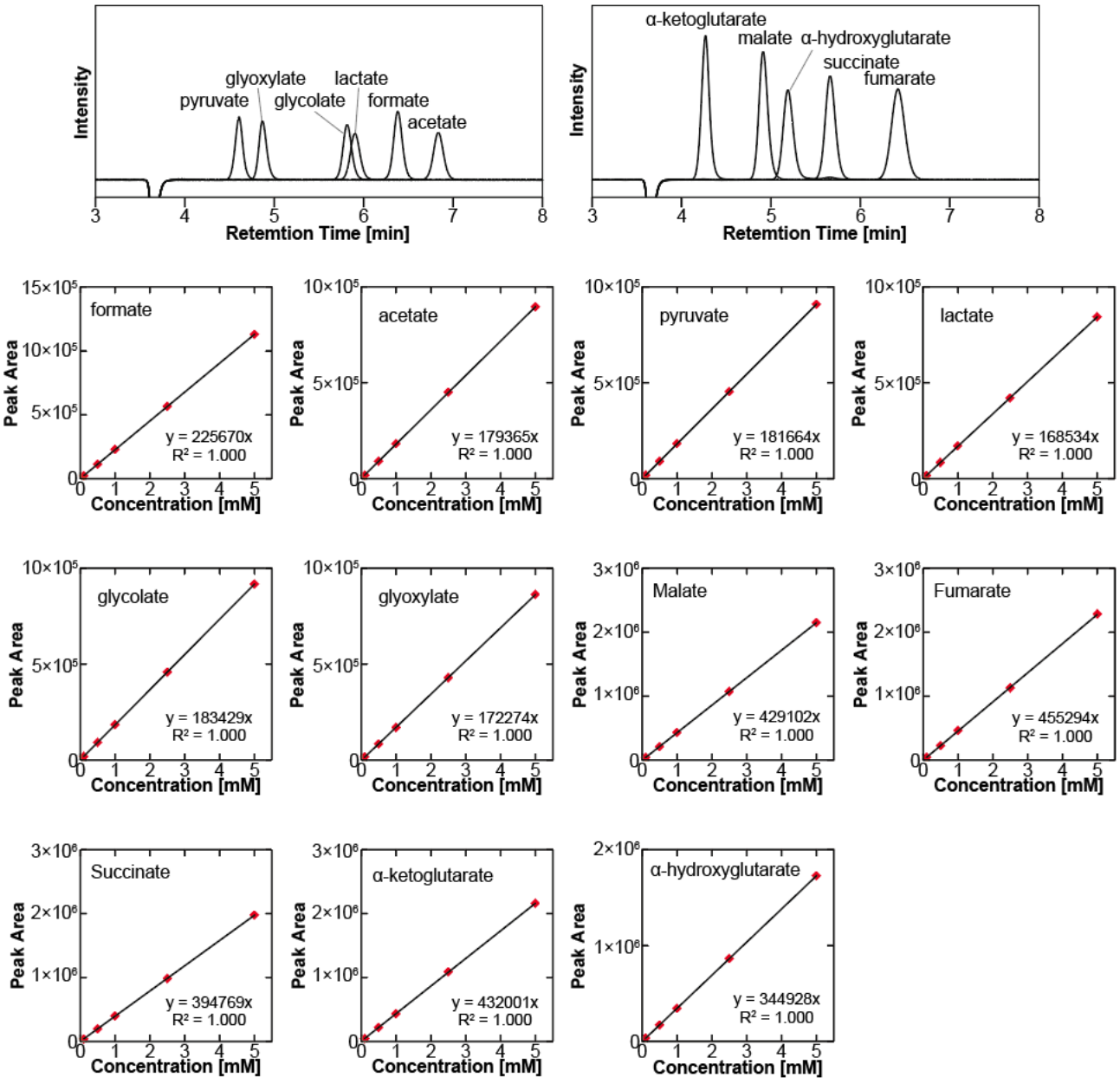


**Fig. S5. Calibration curves for organic acids by the LC-electric conductivity detector system.**

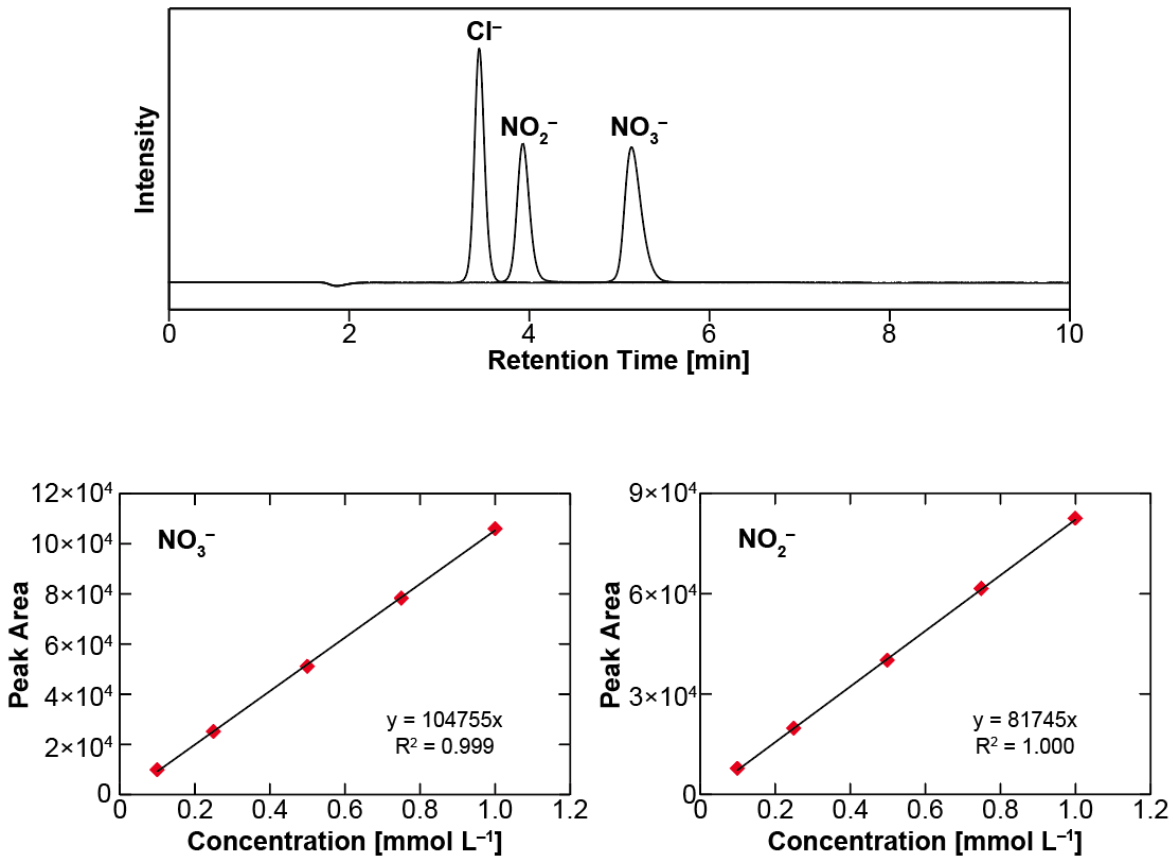


**Fig. S6. Calibration curves for $NO_3^-$ and $NO_2^-$ by the LC-electric conductivity detector system.**

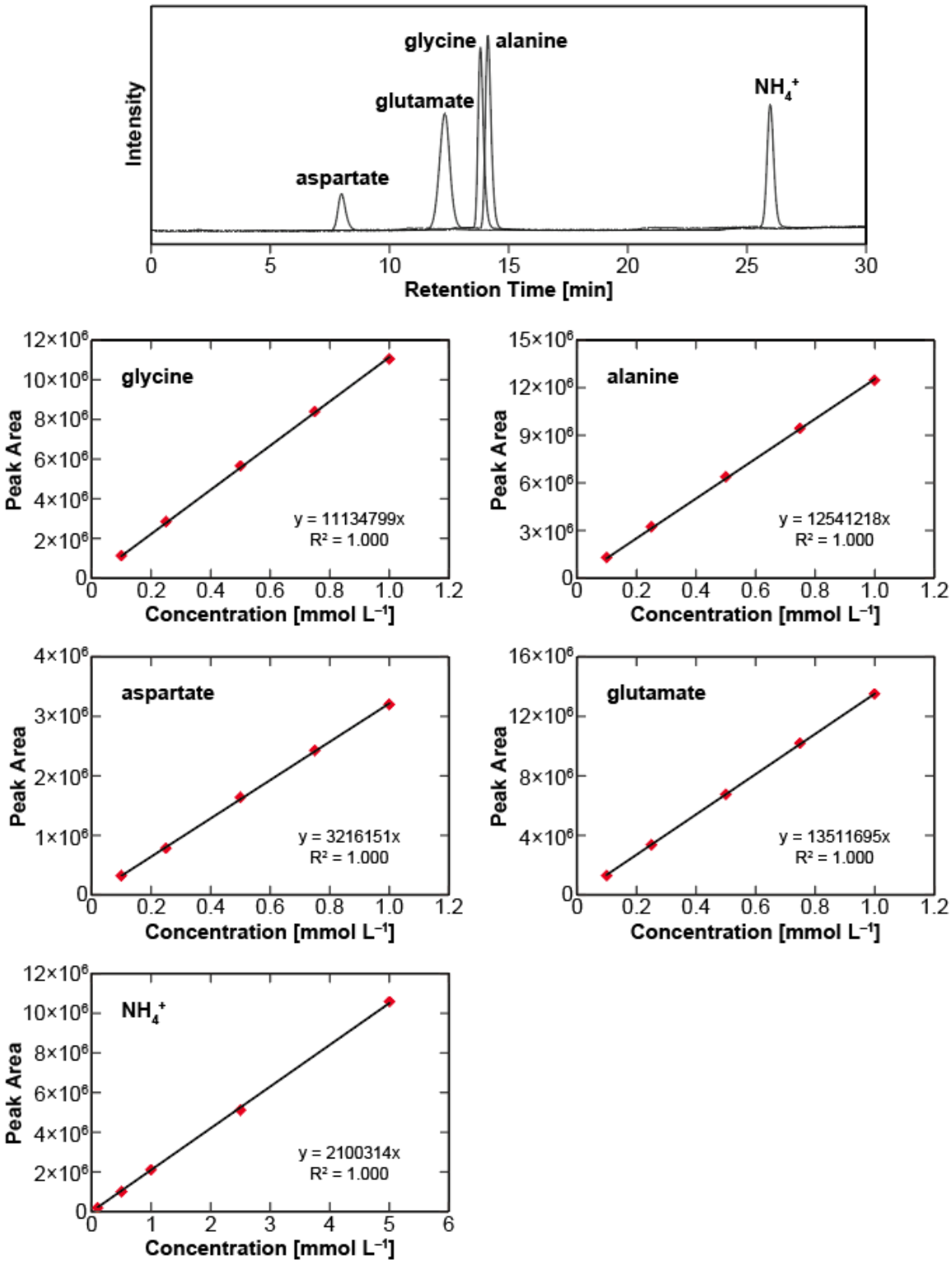


**Fig. S7. Calibration curves for amino acids and ammonia by the LC-fluorescence detector system.**

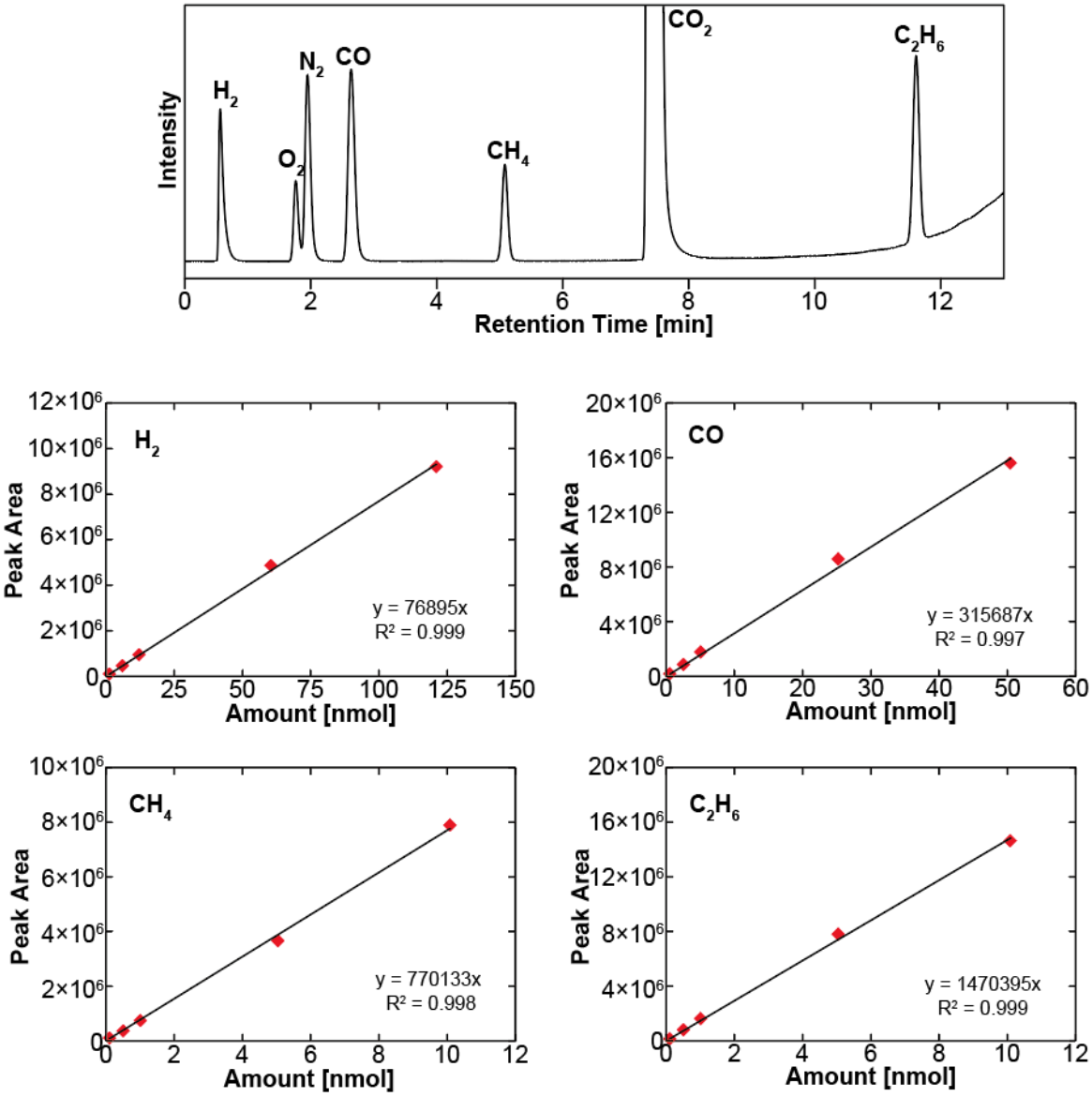


**Fig. S8. Calibration curves for $H_2$, CO, $CH_4$, and $C_2H_6$ by the GC-BID (Barrier Ionization Discharge) detector system.**

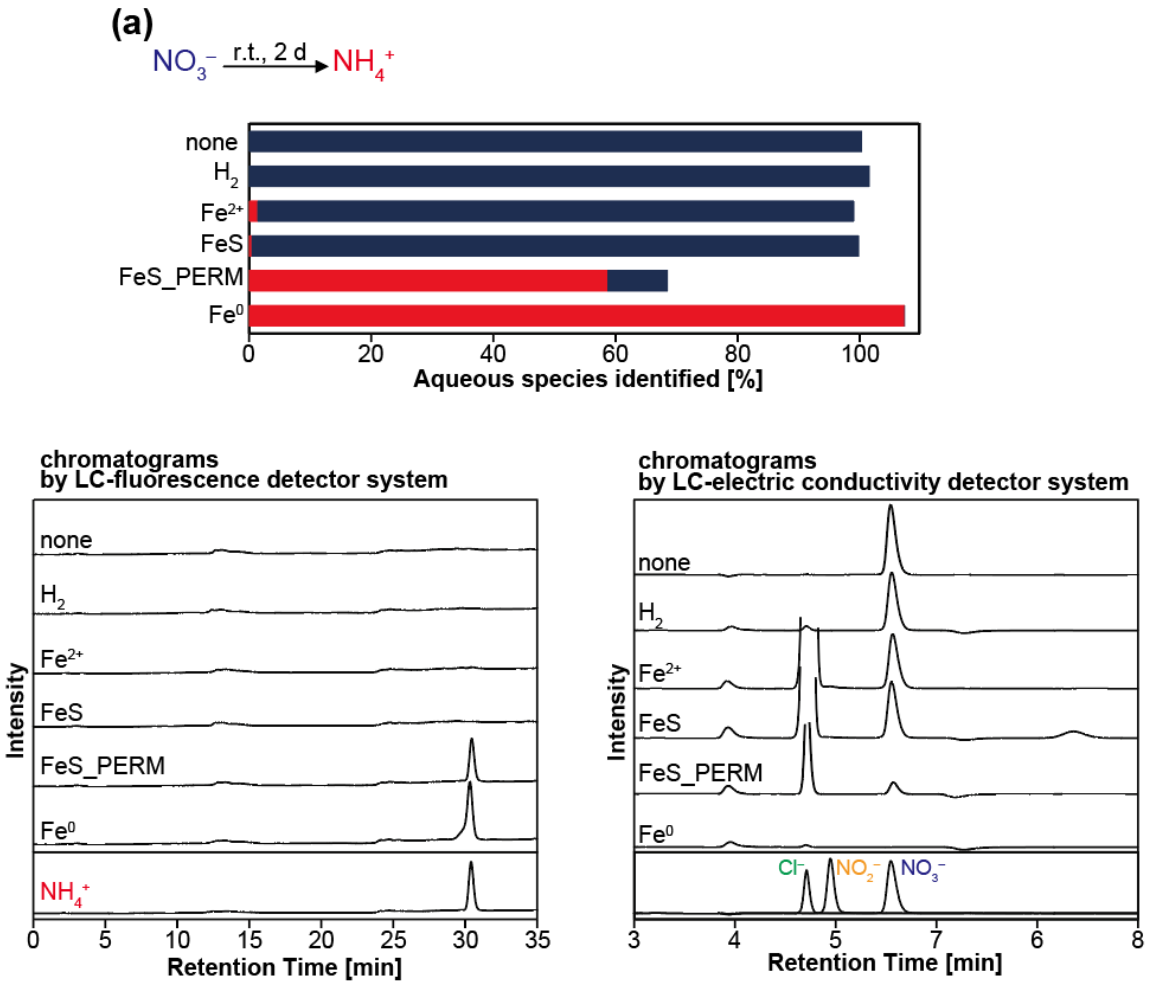


**Fig. S9. Nonenzymatic reactions in the presence of pure $H_2$ gas, $FeCl_2$, FeS, FeS_PERM, and $Fe^0$ and those examined in the absence of reductant.** $^1$H NMR spectra and chromatograms of sample products are shown with those of the standards relevant to the respective reactions. For clarity, $^1$H NMR spectra are presented after deleting residual suppressed water signal around 4.8 ppm. An intense $^1$H NMR signal around 4.2 ppm seen in the spectra of glyoxylate samples (**d**) are due to a glyoxylate oligomer formed in the presence of ammonia catalyst at alkaline pH (A. Hoefnagel, H. van Bekkum, J. A. Peters, The reaction of glyoxylic acid with ammonia revisited. *J. Org. Chem.* **57**, 3916–3921 (1992)).

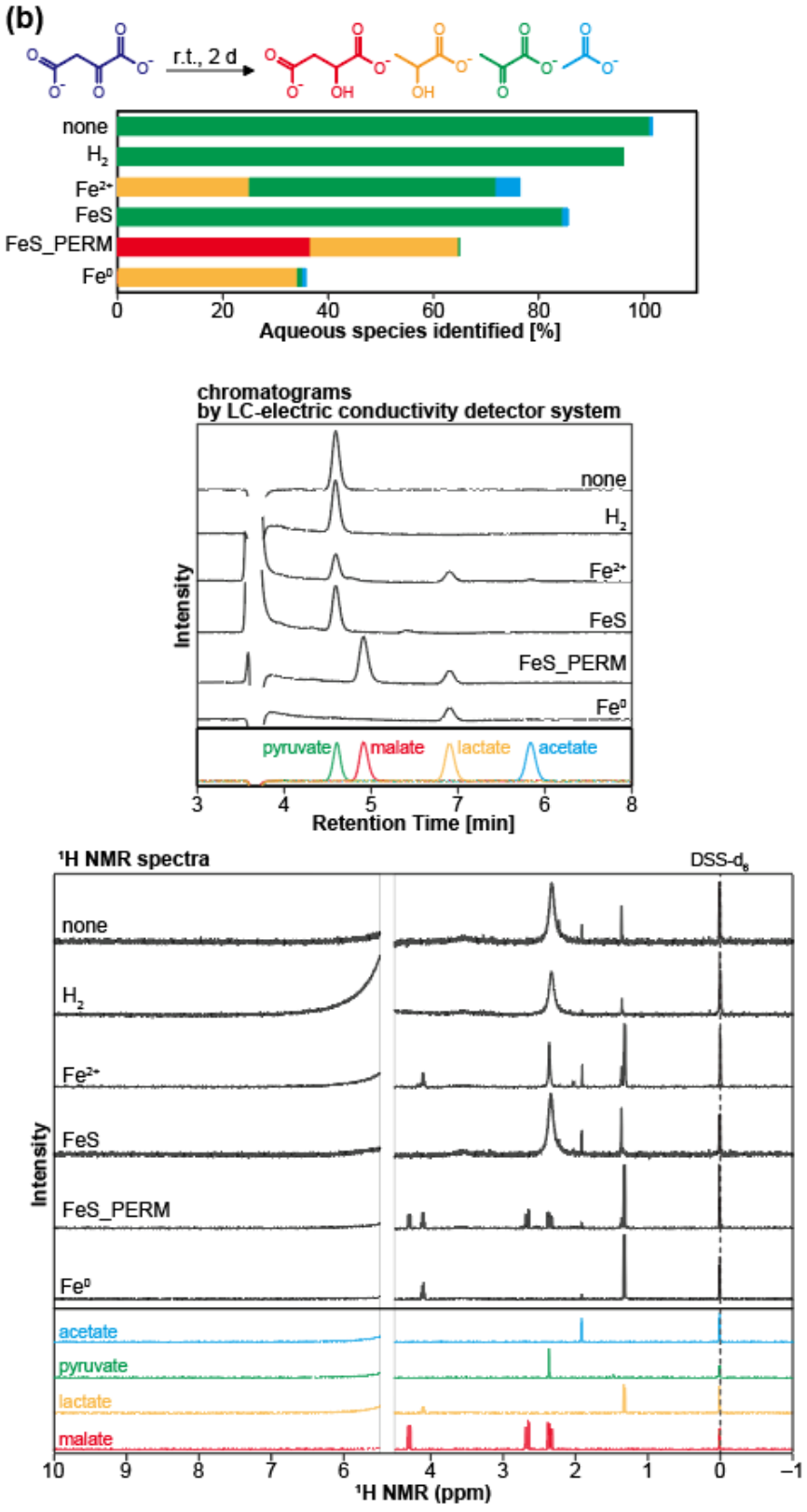


**Fig. S9.** (continued)

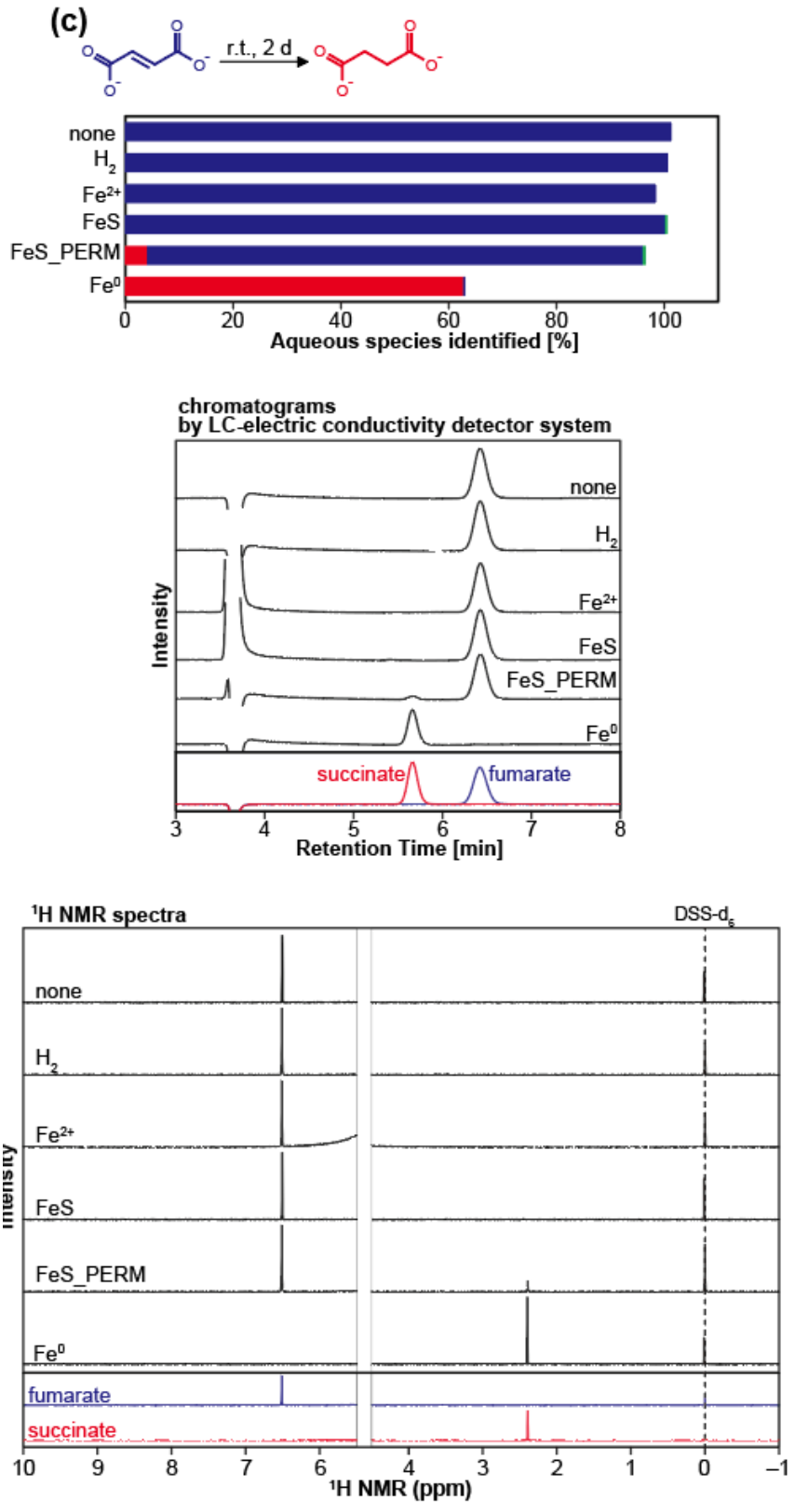


**Fig. S9.** (continued)

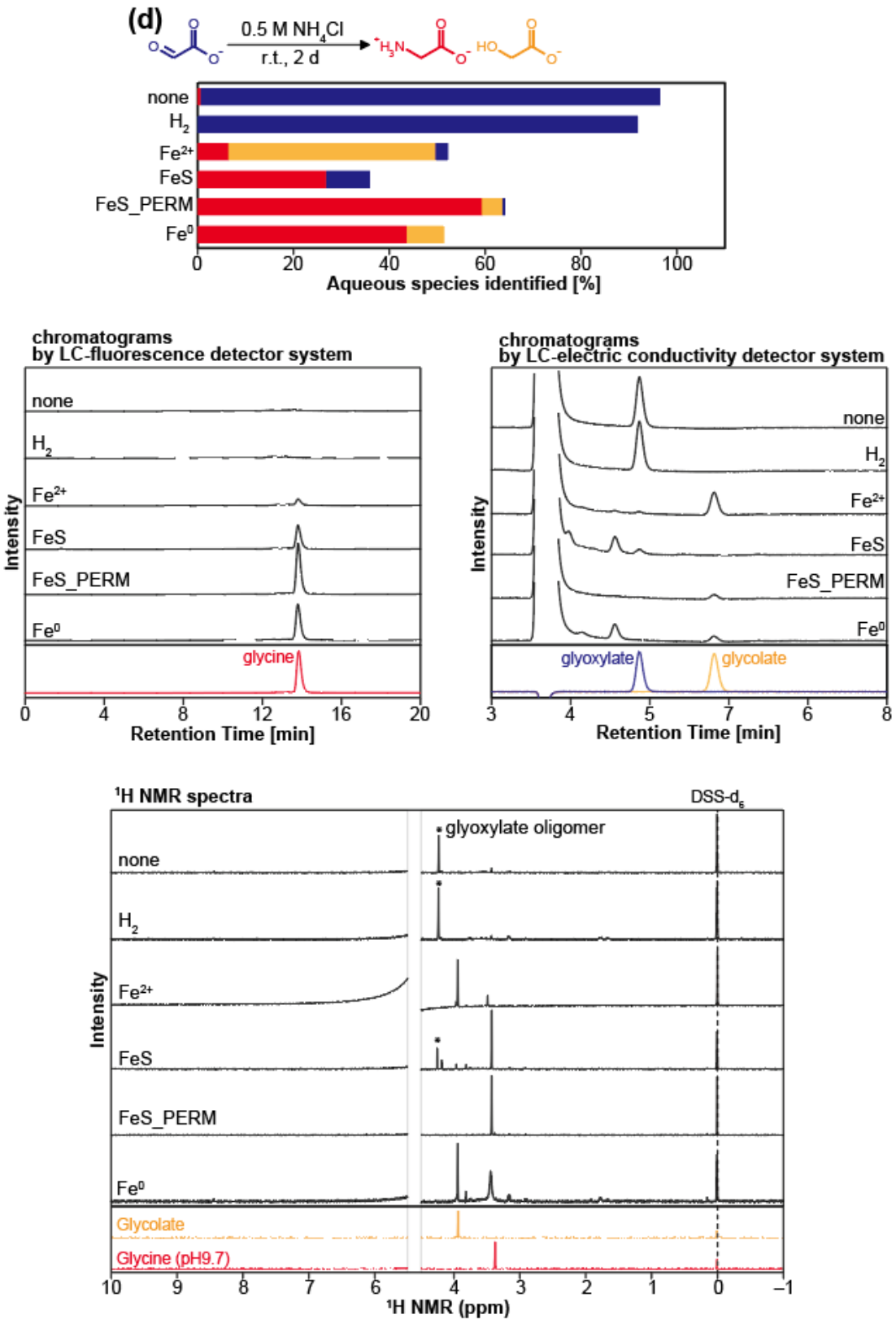


**Fig. S9.** (continued)

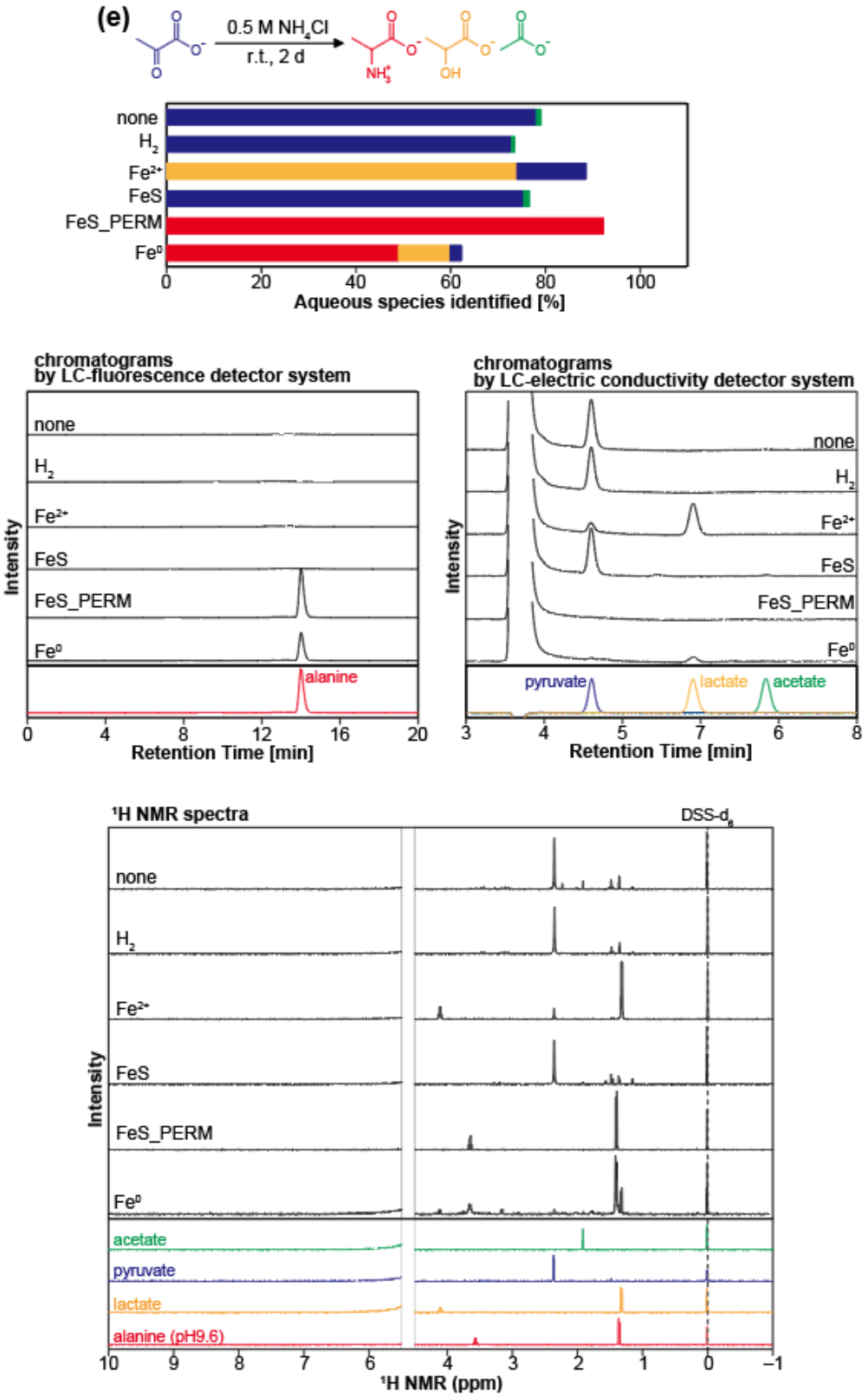


**Fig. S9.** (continued)

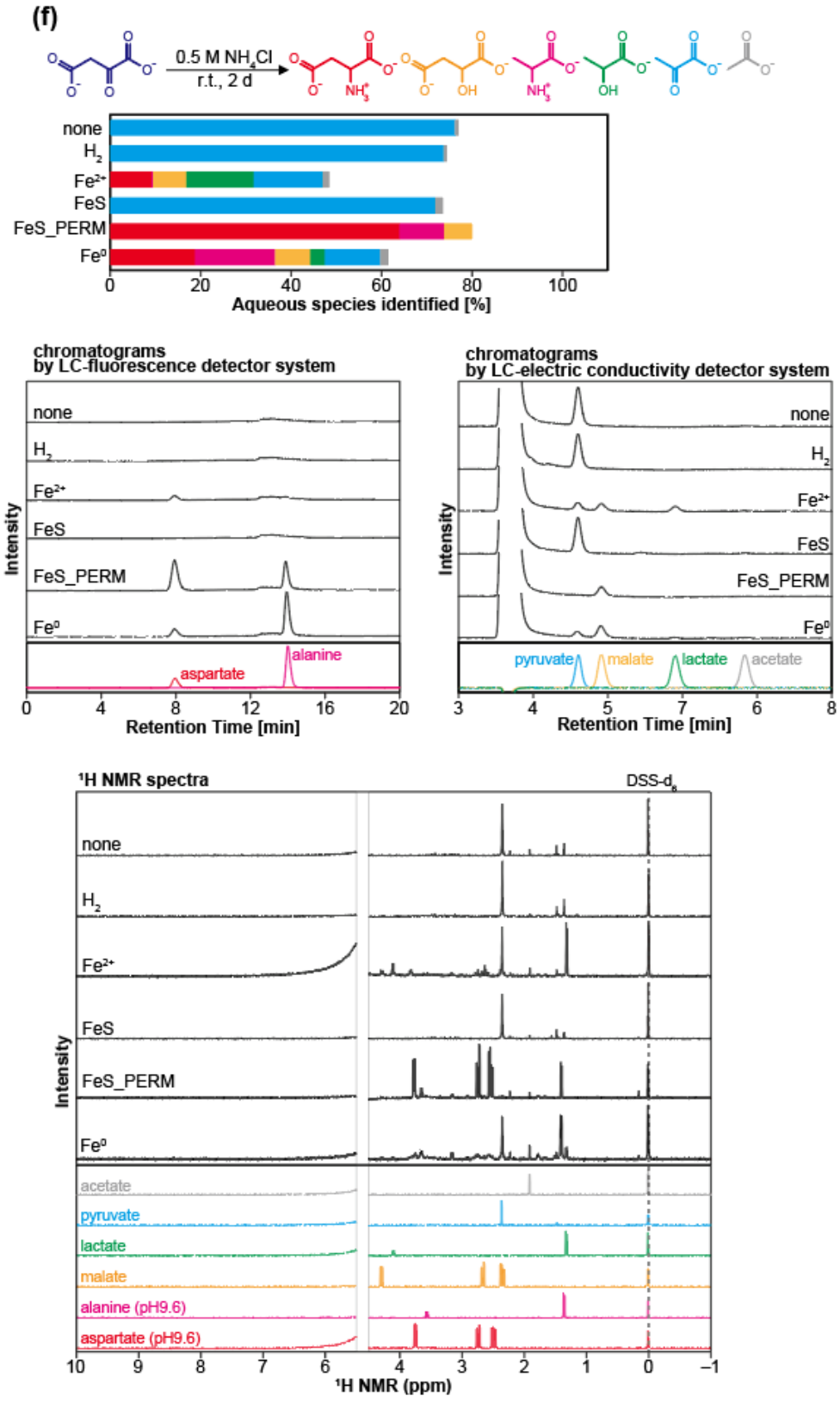


**Fig. S9.** (continued)

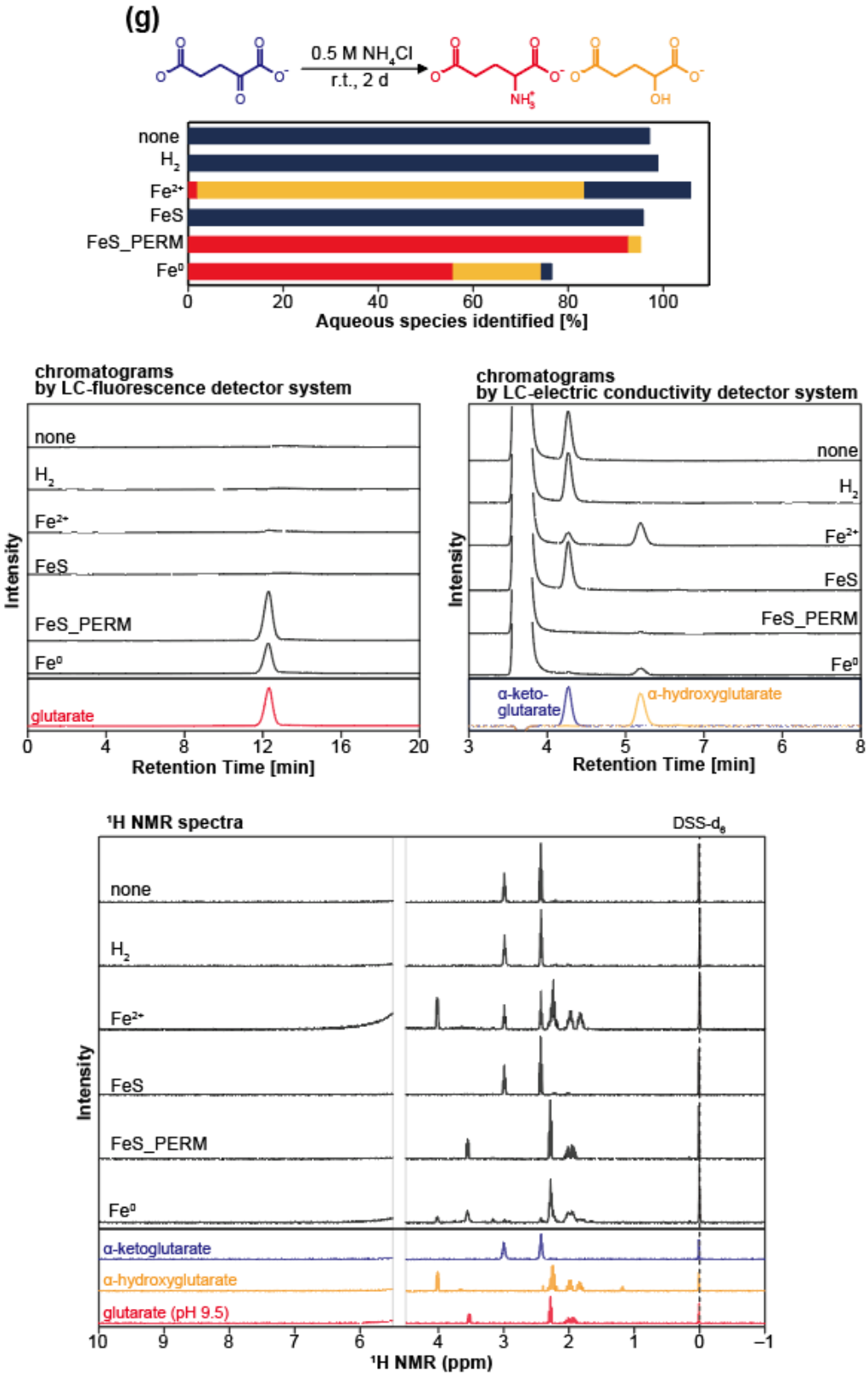


**Fig. S9.** (continued)

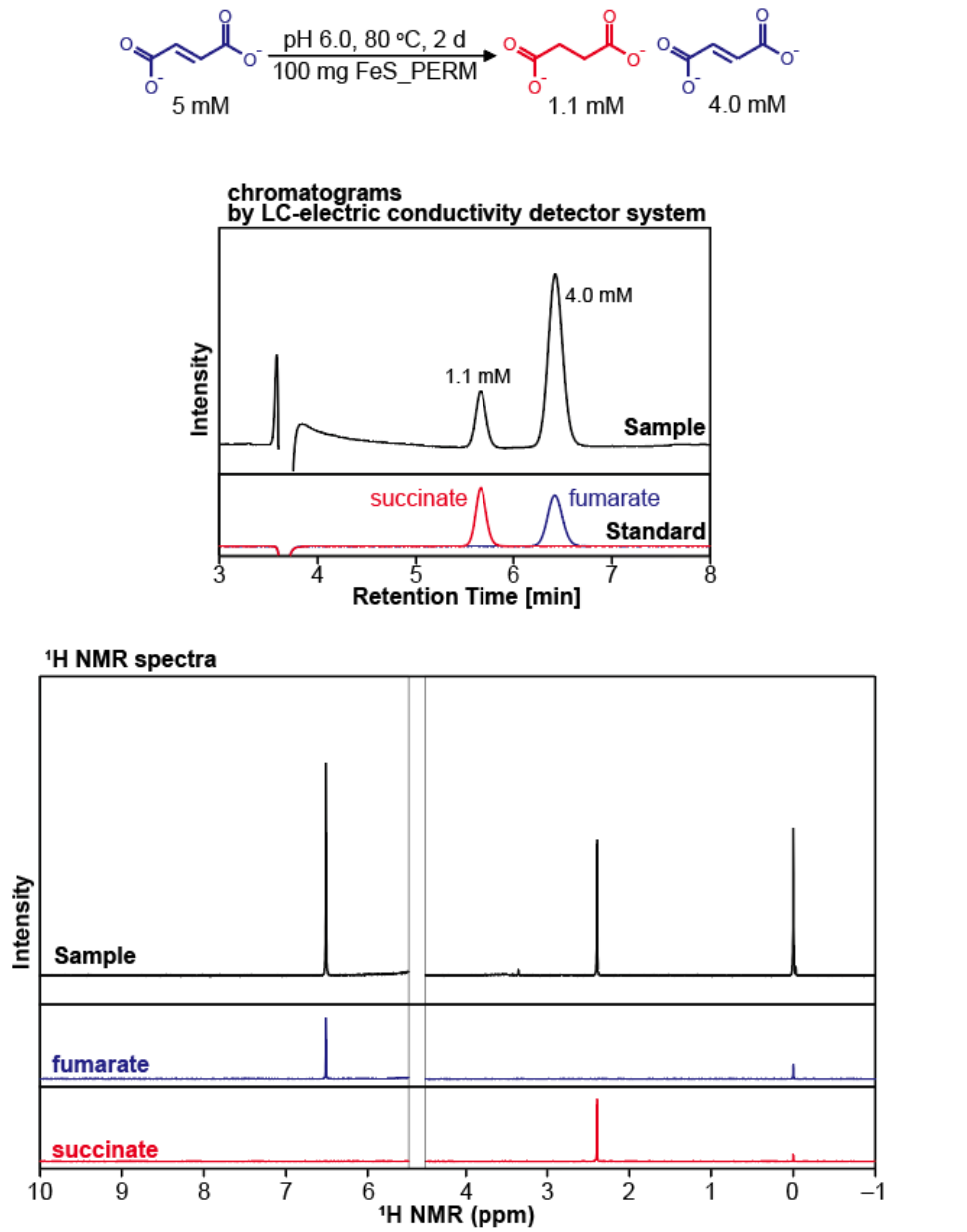


**Fig. S10. Analytical results of fumarate (5 mM, 1.5 ml) incubated with the FeS_PERM (100 mg) at 80°C for 2 days.**

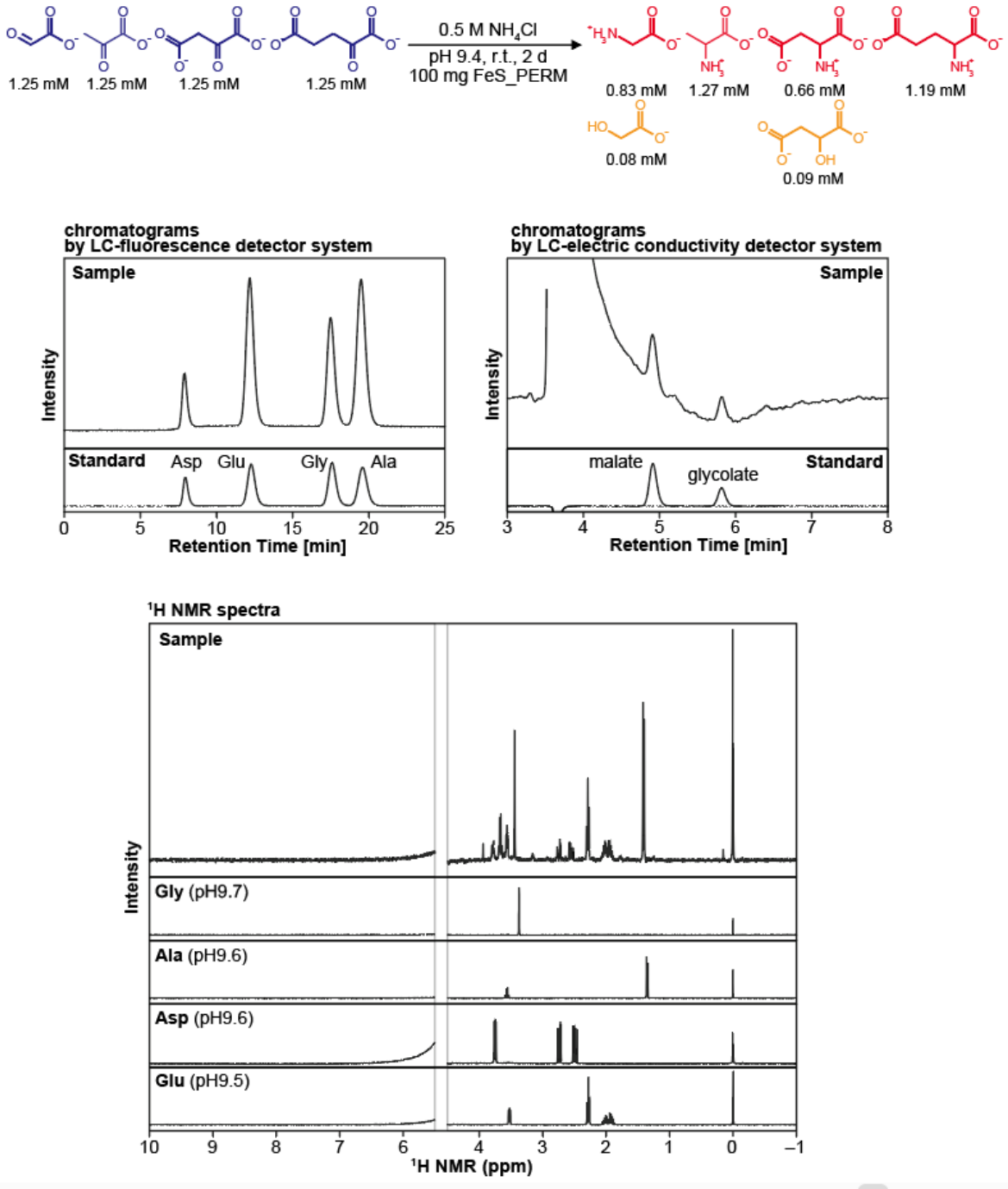


**Fig. S11. Reductive amination of four keto acids promoted by the FeS_PERM in one serum bottle.**

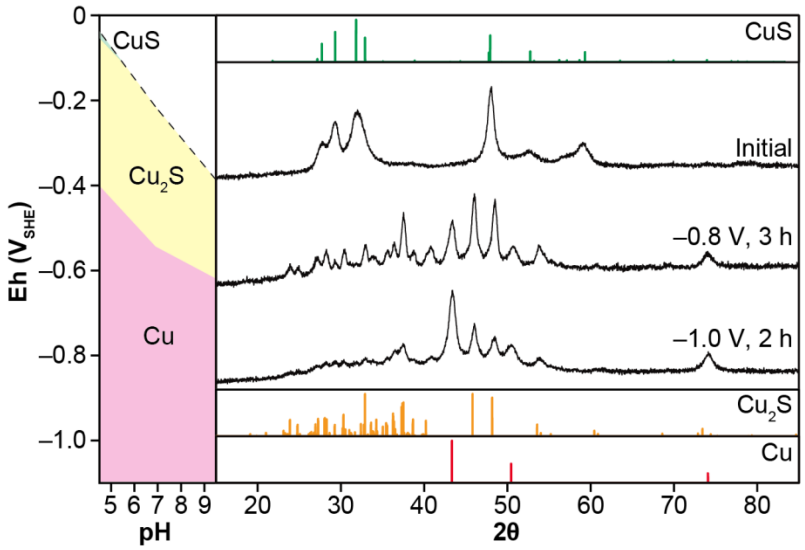


**Fig. S12. XRD patterns of CuS electrolyzed at –0.8 and –1.0 V (versus SHE) for short durations.**

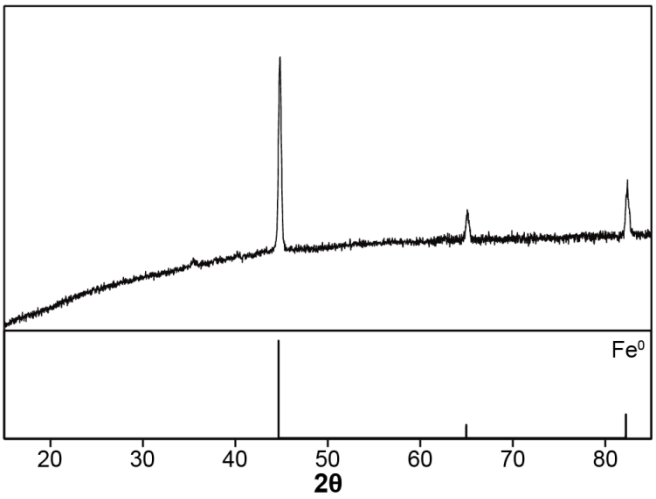


**Fig. S13. XRD patterns of pure $Fe^0$ used in the present study.**

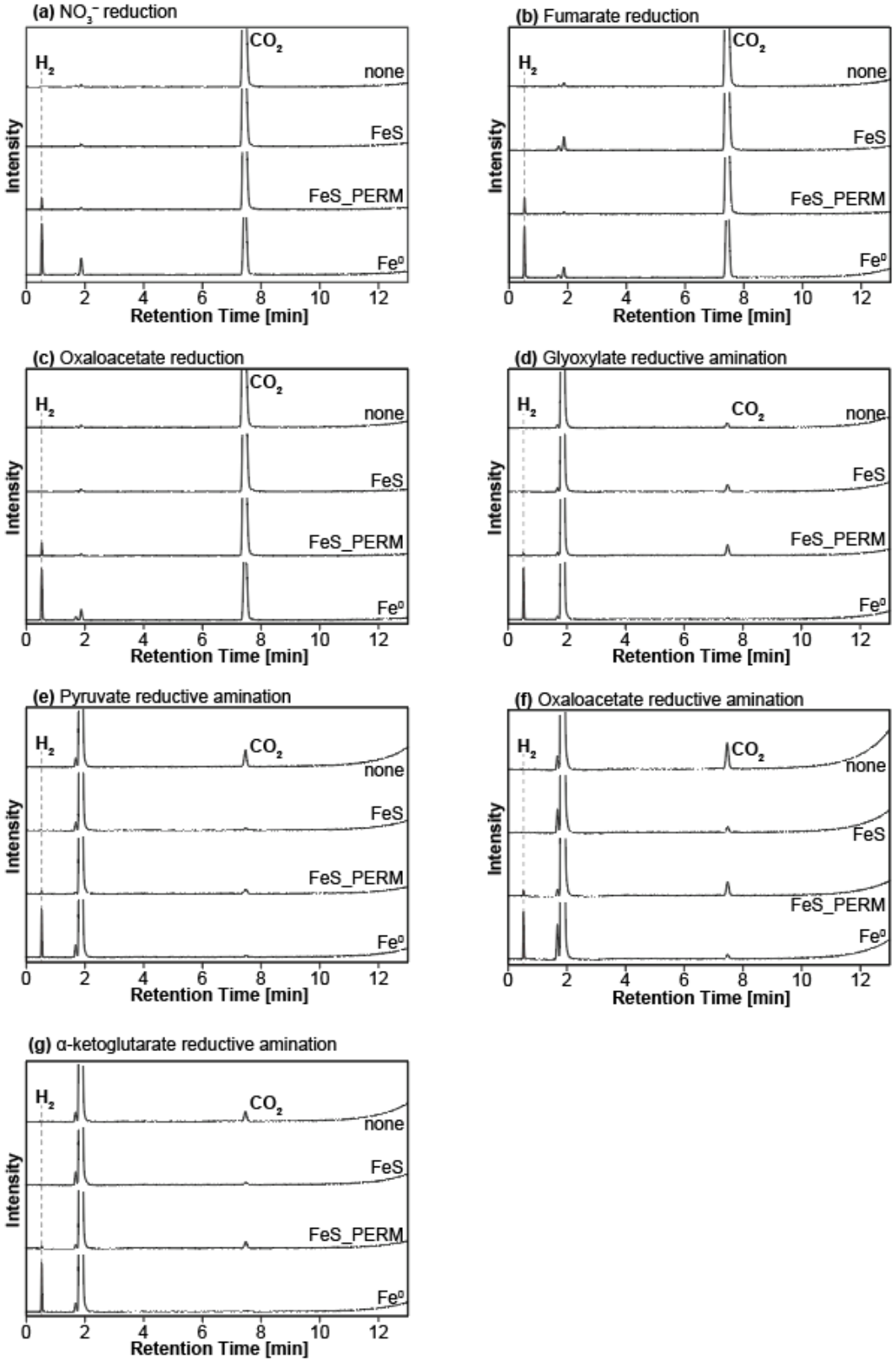


**Fig. S14. GC chromatograms of the gas headspaces of serum bottles measured after the reduction experiments of organic/inorganic compounds.** All chromatograms show the $O_2$, $N_2$, and $CO_2$ signals (partly) due to the intrusion of air into the GC system at the sample injections

**Table S1. Summary of the reduction experiments of organic/inorganic compounds.**

(a) $NO_3^-$ reduction ($[NO_3^-]_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **$NH_4^+$ [mmol $L^{-1}$]** | $NO_3^-$ [mmol $L^{-1}$] |
|---|---|---|---|
| none | 4.7 | **<0.01** | 5.03 ± 0.11 |
| $H_2$ | 3.0 | **<0.01** | 5.09 ± 0.25 |
| $FeCl_2$ | 4.5 | **0.07 ± 0.00** | 4.90 ± 0.24 |
| FeS | 6.7 | **0.02 ± 0.02** | 4.98 ± 0.01 |
| FeS_PERM | 6.5 | **2.94 ± 0.06** | 0.49 ± 0.49 |
| $Fe^0$ | 6.5 | **5.38 ± 0.01** | <0.01 |

(b) Oxaloacetate reduction ($[Oxaloacetate]_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Malate [mmol $L^{-1}$]** | Lactate [mmol $L^{-1}$] | Pyruvate [mmol $L^{-1}$] | Acetate [mmol $L^{-1}$] |
|---|---|---|---|---|---|
| none | 2.8 | **<0.01** | <0.01 | 5.05 ± 0.41 | 0.03 ± 0.00 |
| $H_2$ | 2.8 | **<0.01** | <0.01 | 4.81 ± 0.24 | <0.01 |
| $FeCl_2$ | 2.8 | **<0.01** | 1.25 ± 0.06 | 2.34 ± 0.12 | 0.23 ± 0.01 |
| FeS | 6.5 | **<0.01** | <0.01 | 4.22 ± 0.15 | <0.01 |
| FeS_PERM | 6.4 | **1.83 ± 0.29** | 1.40 ± 0.19 | 0.01 ± 0.01 | <0.01 |
| $Fe^0$ | 6.5 | **<0.01** | 1.70 ± 0.15 | 0.05 ± 0.01 | 0.04 ± 0.00 |

(c) Fumarate reduction ($[Fumarate]_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Succinate [mmol $L^{-1}$]** | Fumarate [mmol $L^{-1}$] | Malate [mmol $L^{-1}$] |
|---|---|---|---|---|
| none | 3.1 | **<0.01** | 5.06 ± 0.03 | <0.01 |
| $H_2$ | 3.0 | **<0.01** | 5.06 ± 0.25 | <0.01 |
| $FeCl_2$ | 2.9 | **<0.01** | 4.92 ± 0.25 | <0.01 |
| FeS | 6.3 | **<0.01** | 5.01 ± 0.05 | 0.02 ± 0.02 |
| FeS_PERM | 6.4 | **0.21 ± 0.07** | 4.59 ± 0.02 | 0.02 ± 0.02 |
| $Fe^0$ | 6.4 | **3.14 ± 0.03** | 0.02 ± 0.00 | <0.01 |

(d) Glyoxylate reductive amination ($[Glyoxylate]_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Glycine [mmol $L^{-1}$]** | Glycolate [mmol $L^{-1}$] | Glyoxylate [mmol $L^{-1}$] |
|---|---|---|---|---|
| none | 9.6 | **0.03 ± 0.00** | <0.01 | 4.79 ± 0.11 |
| $H_2$ | 9.5 | **<0.01** | <0.01 | 4.59 ± 0.23 |
| $FeCl_2$ | 9.1 | **0.33 ± 0.02** | 2.16 ± 0.11 | 0.13 ± 0.01 |
| FeS | 9.6 | **1.34 ± 0.06** | <0.01 | 0.45 ± 0.07 |
| FeS_PERM | 9.6 | **2.97 ± 0.02** | 0.21± 0.20 | 0.02 ± 0.00 |
| $Fe^0$ | 9.6 | **2.19 ± 0.04** | 0.38 ± 0.13 | <0.01 |

(e) Pyruvate reductive amination ([Pyruvate]$_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Alanine [mmol $L^{-1}$]** | Lactate [mmol $L^{-1}$] | Pyruvate [mmol $L^{-1}$] | Acetate [mmol $L^{-1}$] |
|---|---|---|---|---|---|
| none | 9.6 | **<0.01** | <0.01 | 3.90 ± 0.23 | 0.05 ± 0.02 |
| $H_2$ | 9.5 | **<0.01** | <0.01 | 3.64 ± 0.18 | 0.03 ± 0.00 |
| $FeCl_2$ | 9.1 | **<0.01** | 3.70 ± 0.18 | 0.74 ± 0.04 | <0.01 |
| FeS | 9.6 | **<0.01** | <0.01 | 3.77 ± 0.11 | 0.07 ± 0.03 |
| FeS_PERM | 9.6 | **4.61 ± 0.18** | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 9.6 | **2.45 ± 0.01** | 0.55 ± 0.08 | 0.12 ± 0.00 | <0.01 |

(f) Oxaloacetate reductive amination ([Oxaloacetate]$_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Aspartate [mmol $L^{-1}$]** | Alanine [mmol $L^{-1}$] | Malate [mmol $L^{-1}$] | Lactate [mmol $L^{-1}$] | Pyruvate [mmol $L^{-1}$] | Acetate [mmol $L^{-1}$] |
|---|---|---|---|---|---|---|---|
| none | 9.6 | **<0.01** | <0.01 | <0.01 | <0.01 | 3.81 ± 0.33 | 0.04 ± 0.04 |
| $H_2$ | 9.5 | **<0.01** | <0.01 | <0.01 | <0.01 | 3.69 ± 0.18 | 0.03 ± 0.00 |
| $FeCl_2$ | 9.0 | **0.46 ± 0.02** | 0.02 ± 0.00 | 0.36 ± 0.02 | 0.74 ± 0.04 | 0.76 ± 0.04 | 0.07 ± 0.00 |
| FeS | 9.6 | **<0.01** | <0.01 | <0.01 | <0.01 | 3.60 ± 0.17 | 0.08 ± 0.03 |
| FeS_PERM | 9.6 | **3.20 ± 0.11** | 0.50 ± 0.06 | 0.30 ± 0.19 | <0.01 | <0.01 | 0.01 ± 0.01 |
| $Fe^0$ | 9.6 | **0.94 ± 0.16** | 0.89 ± 0.02 | 0.39 ± 0.23 | 0.16 ± 0.01 | 0.61 ± 0.06 | 0.08 ± 0.00 |

(g) α-ketoglutarate reductive amination ([α-ketoglutarate]$_i$ = 5 mmol $L^{-1}$])

| reductant | pH | **Glutamate [mmol $L^{-1}$]** | α-hydroxyglutarate [mmol $L^{-1}$] | α-ketoglutarate [mmol $L^{-1}$] |
|---|---|---|---|---|
| none | 9.6 | **<0.01** | <0.01 | 4.87 ± 0.00 |
| $H_2$ | 9.5 | **<0.01** | <0.01 | 4.96 ± 0.25 |
| $FeCl_2$ | 9.0 | **0.10 ± 0.02** | 4.09 ± 0.20 | 1.12 ± 0.06 |
| FeS | 9.6 | **<0.01** | <0.01 | 4.81 ± 0.07 |
| FeS_PERM | 9.6 | **4.65 ± 0.06** | 0.13 ± 0.10 | <0.01 |
| $Fe^0$ | 9.6 | **2.80 ± 0.09** | 0.93 ± 0.31 | 0.11 ± 0.01 |

**Table S2. Amounts of $H_2$, CO, $CH_4$, and $C_2H_6$ in the serum bottles (30 ml) after the reduction experiments of organic/inorganic compounds.**

(a) $NO_3^-$ reduction

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 58.8 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 350.6 | <0.01 | 0.02 | <0.01 |

(b) Fumarate reduction

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | 0.2 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 82.8 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 348.1 | <0.01 | 0.02 | <0.01 |

(c) Oxaloacetate reduction

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | 0.1 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 65.4 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 356.2 | <0.01 | 0.02 | <0.01 |

(d) Glyoxylate reductive amination

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | 0.2 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| $FeS_{PEM}$ | 4.7 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 77.9 | <0.01 | <0.01 | <0.01 |

(e) Pyruvate reductive amination

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 3.3 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 63.7 | <0.01 | <0.01 | <0.01 |

(f) Oxaloacetate reductive amination

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 3.3 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 27.4 | <0.01 | <0.01 | <0.01 |

(g) α-ketoglutarate reductive amination

| reductant | $H_2$ [μmol] | CO [μmol] | $CH_4$ [μmol] | $C_2H_6$ [μmol] |
|---|---|---|---|---|
| none | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS | <0.1 | <0.01 | <0.01 | <0.01 |
| FeS_PERM | 2.6 | <0.01 | <0.01 | <0.01 |
| $Fe^0$ | 55.5 | <0.01 | <0.01 | <0.01 |

**Table S3. Thermodynamic data for sulfide minerals.**

| Sulide | $\Delta_f G^o_{P_r,T_r}$ [kJ/mol] | $\Delta_f H^o_{P_r,T_r}$ [kJ/mol] | $S^o_{P_r,T_r}$ [J/mol·K] | $V^o_T$ [$cm^3$/mol] | $C^o_{P_r}$ [J/mol·K][a] | refs. |
|---|---|---|---|---|---|---|
| $Ag_2S$, acanthite | –39.70 | –32.00 | 142.89 | 34.19 | 75.31 (at 298.15 K) | *68* |
| CdS, greenockite | –146.10 | –149.60 | 72.18 | 29.93 | $35.38 + 0.01503 \times T - 533300 \times T^{-2} + 235.7 \times T^{-0.5} - 0.0000024 \times T^2$ | *68* |
| CoS, jaipurite | –96.20 | –95.16 | 58.60 | 16.70[b] | $42.47 + 16.95 \times 10^{-3}T$ | *70* |
| $CoS_2$, cattierite | –145.10 | –150.90 | 74.80 | 25.52 | $65.82 + 0.03032 \times T - 587000 \times T^{-2}$ | *68* |
| $Co_3S_4$, linnaeite | –334.90 | –347.50 | 176.00 | 62.55 | – | *68* |
| $Co_9S_8$, cobaltpentlandite | –834.69[c] | –851.40 | 470.70 | 147.10[d] | $362 + 0.14 \times T$ | *70* |
| CuS, covellite | –55.30 | –54.60 | 67.40 | 20.42 | $43.049 + 0.02023 \times T - 139938 \times T^{-2} + 0.435838 \times T^{-0.5}$ | *68* |
| $Cu_{1.75}S$, anilite | –78.50 | –73.30 | 107.50 | 25.79 | – | *68* |
| $Cu_{1.80}S$, digenite | –78.30 | –73.00 | 109.60 | 25.63 | – | *68* |
| $Cu_{1.95}S$, djurleite | –84.20 | –78.80 | 114.80 | 26.89 | – | *68* |
| $Cu_2S$, chalcocite | –89.20 | –83.90 | 116.20 | 27.48 | 76.84 (at 298.15 K) | *68* |
| FeS, mackinawite | –100.07 | –100.85[e] | 56.52 | 18.20[f] | $44.685 + 0.019037 \times T - 289000 \times T^{-2}$ | *71* |
| FeS, troilite | –102.90 | –102.60 | 60.30 | 18.20 | 50.49 (at 298.15 K) | *68* |
| $Fe_{0.875}S$, pyrrhotite | –98.90 | –97.50 | 60.70 | 17.49 | 49.88 (at 298.15 K) | *68* |
| $Fe_3S_4$, greigite | –311.88 | –320.03 | 182.13 | 72.499[g] | $143.344 + 0.076567 \times T$ | *71* |
| $FeS_2$, pyrite | –160.10 | –171.50 | 52.90 | 23.94 | $-20.32 + 0.0503 \times T - 3200000 \times T^{-2} + 1787 \times T^{-0.5}$ | *68* |
| $FeS_2$, marcasite | –158.40 | –169.50 | 53.90 | 24.58 | $383.2 - 0.3173 \times T + 1676000 \times T^{-2} - 4488 \times T^{-0.5} + 0.0001676 \times T^2$ | *68* |
| MnS, alabandite | –218.70 | –213.90 | 80.30 | 21.46 | $135.3 - 0.05775 \times T + 1169000 \times T^{-2} - 1435 \times T^{-0.5} + 0.00002087 \times T^2$ | *68* |
| $MnS_2$, hauerite | –224.60 | –223.80 | 99.90 | 34.20 | – | *68* |
| $MoS_2$ | –267.14[h] | –276.14 | 62.57 | 31.63[b] | $71.67318 + 7.515343 \times t - 0.044947 \times t^2 + 0.008866 \times t^3 - 0.921212 \times t^{-2}$ | *69* |
| $MoS_3$ | –295.24[h] | –310.00 | 75.30 | 37.97[i] | – | *70* |
| $Mo_2S_3$ | –395.62[h] | –407.10 | 114.98 | 49.50[j] | $110.2911 + 32.96866 \times t + 0.000664 \times t^2 - 0.000106 \times t^3 - 0.962354 \times t^{-2}$ | *69* |
| NiS, millerite | –88.30 | –91.00 | 52.97 | 16.89 | 47.11 (at 298.15 K) | *68* |
| $Ni_3S_2$, heazlewoodite | –210.20 | –216.30 | 133.20 | 40.95 | $1057 - 0.8988 \times T + 8139000 \times T^{-2} - 13880 \times T^{-0.5} + 0.000466 \times T^2$ | *68* |
| $NiS_2$, vaesite | –124.82[k] | –131.38 | 71.96 | 27.688[g] | $64.4336 + 20.76331 \times t - 0.009134 \times t^2 + 0.002569 \times t^3 + 0.000227 \times t^{-2}$ | *69* |
| $Ni_3S_4$, polydemite | –291.78[k] | –301.12 | 186.48 | 64.326[g] | $121.9707 + 143.6585 \times t + 0.025374 \times t^2 - 0.01786 \times t^3 + 0.000434 \times t^{-2}$ | *69* |
| PbS, galena | –96.80 | –98.30 | 91.70 | 31.49 | $44.6 + 0.0164 \times T$ | *68* |
| $WS_2$, tungstenite | –233.00 | –241.60 | 67.80 | 32.07 | $76.33 + 0.0005561 \times T - 1137000 \times T^{-2} + 0.000001408 \times T^2$ | *68* |
| $WS_3$ | –277.68 | –293.00 | 77.40 | 36.85[l] | – | *70* |
| ZnS, sphalerite | –199.60 | –204.10 | 58.70 | 23.83 | $61.51 + 0.0007631 \times T - 79630 \times T^{-2} - 260.4 \times T^{-0.5}$ | *68* |
| ZnS, wurtzite | –199.40 | –203.80 | 58.80 | 23.85 | $41.91 + 0.0007619 \times T - 660300 \times T^{-2} + 157.7 \times T^{-0.5}$ | *68* |

[a] T = temperature [K], $t = T \times 10^{-3}$.

[b] ref. (*73*).

[c] calculated using the values of $\Delta_f H^o$ and $S^o$ listed in the table together with the standard molar entropies of Co and S reported by ref. (*68*).

[d] ref. (*72*).
[e] calculated using the values of $\Delta_f G^o$ and $S^o$ listed in the table together with the standard molar entropies of Fe and S reported by ref. (*68*).
[f] assumed to be the same as that of troilite.
[g] ref. (*75*).
[h] calculated using the values of $\Delta_f H^o$ and $S^o$ listed in the table together with the standard molar entropies of Mo and S reported by ref. (*68*).
[i] calculated using the density value for $MoS_2$ reported by ref. (*73*).
[j] ref. (*74*).
[k] calculated using the values of $\Delta_f H^o$ and $S^o$ listed in the table together with the standard molar entropies of Ni and S reported by ref. (*68*).
[l] calculated using the density value for $WS_2$ reported by ref. (*73*).