Supporting Information

Native iron reduces CO_2 to intermediates and end-products of the acetyl CoA pathway

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Content

General information

All reactions were carried out in stainless-steel Parr pressure reactors in 1.5 mL vials with pierced PTFA-lined caps under CO_2 atmosphere, unless otherwise noted.

^{1}H NMR spectra were recorded on a Bruker Avance300 (300 MHz) spectrometer at ambient temperature in a H_2O:D_2O mixture (6:1) as solvent, with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS-Na) as the internal standard (CH_3 peak at 0 ppm). Solvent suppression was achieved through excitation sculpting, using the Bruker ZGESGP pulse program adjusted for the water resonance. 32 scans were acquired for each sample. Relaxation delay D1 was set to 87 s, with time domain size TD = 32768 and sweep width SWH = 4789.27 Hz (11.963 ppm), to allow for quantitative measurements. Integration was performed using *MestReNova v6.0.2* software.

GC-MS analysis was performed on a GC System 7820A (G4320) using an Agilent High Resolution Gas Chromatography Column (PN 19091S – 433UI, HP – 5MS UI, 28 m×0.250 mm, 0.25 Micron, SN USD 489634H). The system was connected to an MSD block 5977E (G7036A). Hydrogen (99.999 % purity) was used as carrier gas at a constant flow rate of 1.5 mL min^{-1}. The analysis was carried out in a splitless mode with 1 µL injection volume, at the injection port temperature of 250 °C. The column was maintained at 60 °C for 1 min, then ramped at 30 °C min−1 to 310 °C with 3 min hold, and the total running time was 12.33 min. The mass spectrometer was turned on after a 2-min solvent delay, and was operated at the electron ionization (EI) mode with quadrupole temperature of 150 °C. Data was acquired in the full-scan mode (50-500 amu).

Materials

All reagents were purchased from commercial suppliers, and were of a grade presented below in Table S1.

Table S1 Specifications of materials used

No.	Reagent	CAS	Supplier
1	Mn powder, 325 mesh, ≥99% trace	7439-96-5	Sigma Aldrich
2	Fe powder (fine), ≥ 99%, reduced	7439-89-6	Sigma Aldrich
3	Co powder, 2 µm particle size, 99.8% trace metal basis	7440-48-4	Sigma Aldrich
4	Ni powder, <150 µm, 99.99% trace metal basis	7440-02-0	Sigma Aldrich
5	Mo powder, 1-5 µm, ≥ 99.9% trace metal basis	7439-98-7	Sigma Aldrich
6	W powder, 12 µm, 99.9% trace metal basis	7440-33-7	Sigma Aldrich

All reagents were tested for the presence of trace acetate and/or formate impurities prior to use – see the "Analytical methods: B. Starting material control experiments" section below. Water was obtained from a Milli-Q purification system (18 MΩcm).

Analytical methods

A. Product identification

All compounds detected in this study were analysed using the Bruker ZGESGP 1D pulse sequence with water suppression, as described in the General information section above. Sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS-Na) was used as the internal standard (Figure S1). Acetate, pyruvate, methanol, formate and lactate were identified based on their chemical shifts compared to authentic samples (Figure S2).

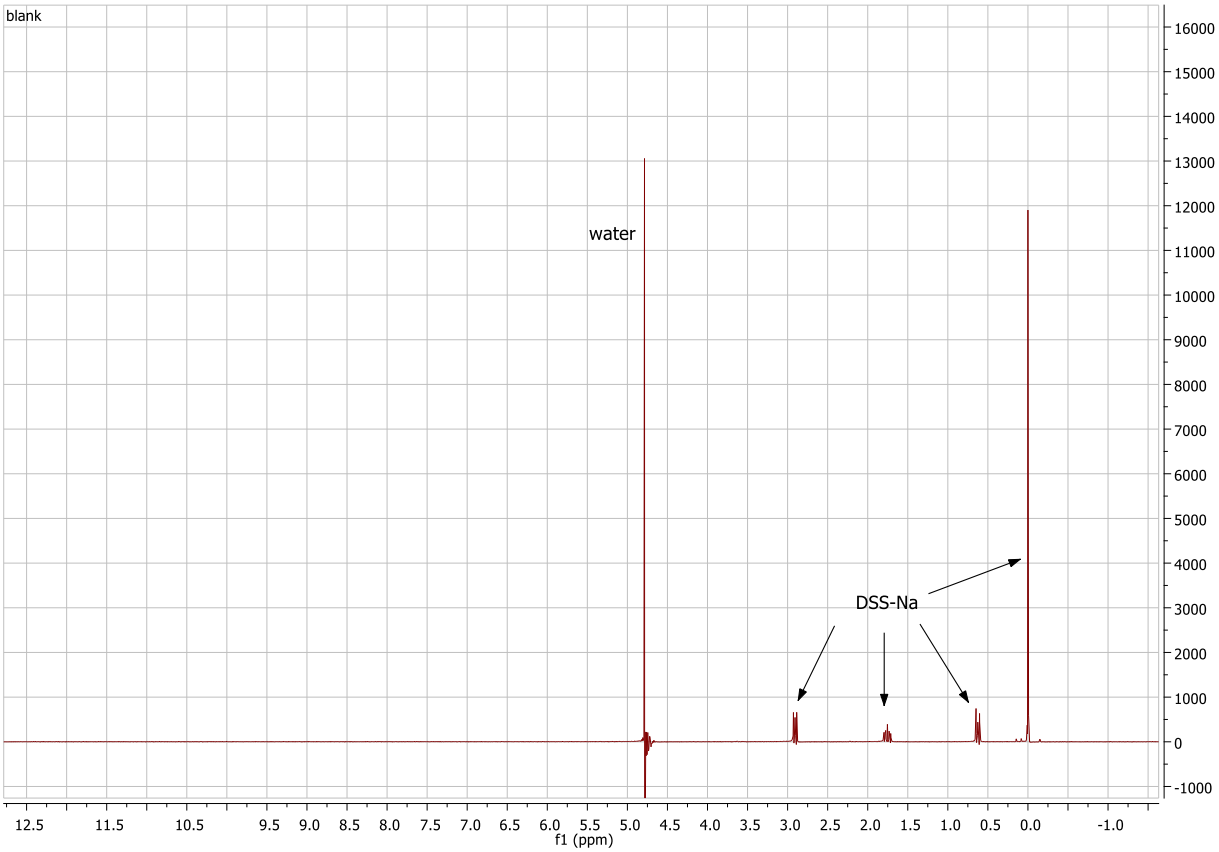


Figure S1 ^{1}H NMR spectrum of sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS-Na) in H_2O : D_2O (6 : 1).

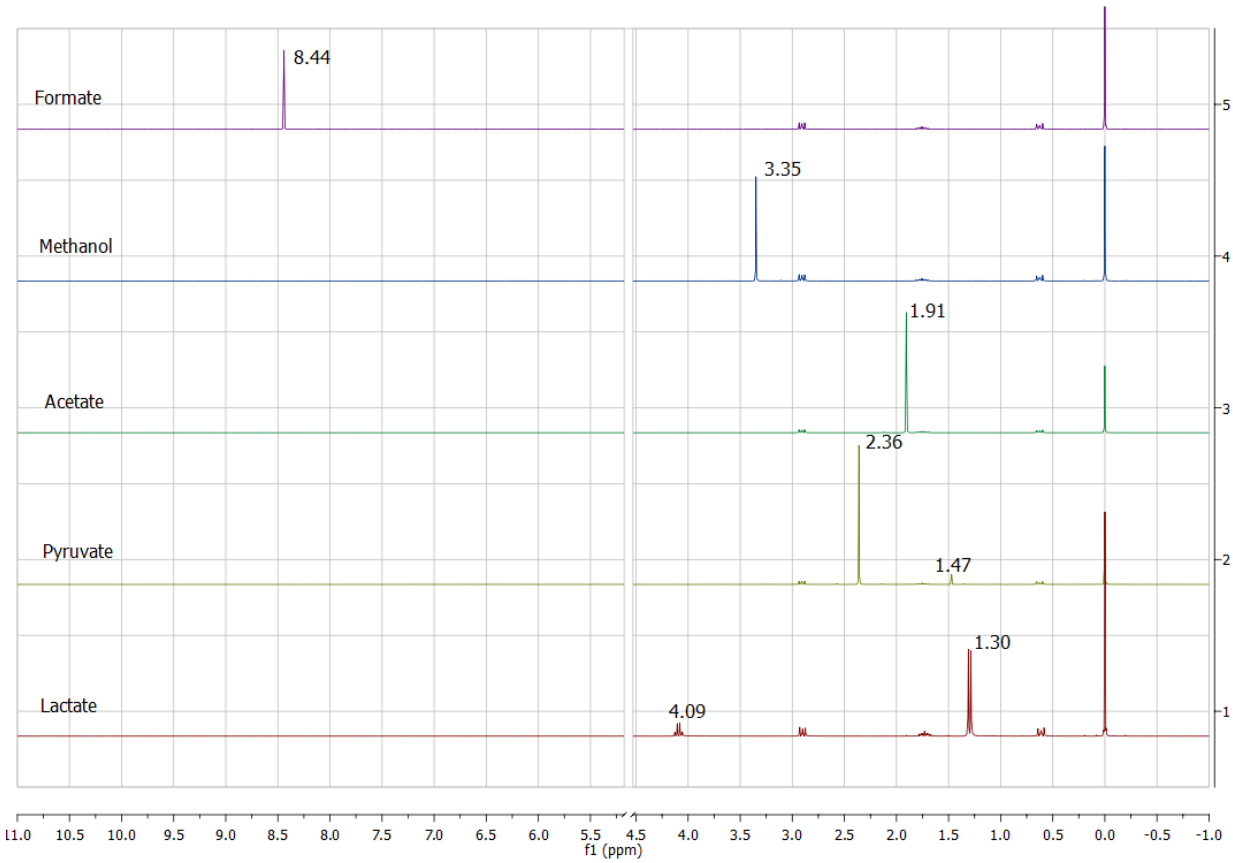


Figure S2 ^{1}H NMR spectra of products detected in this study – formate, methanol, acetate, pyruvate and lactate – with DSS-Na standard in H_2O:D_2O (6:1). The residual suppressed water peak was omitted for clarity.

B. Starting material control experiments

Control experiments were carried out to test for possible trace contamination of organics in the inorganic starting materials and to exclude false positive results.

An aliquot of 1 mmol of the reagent in question (metal powders: 55 mg Mn, or 56 mg Fe, or 59 mg Ni, or 59 mg Co, or 96 mg Mo, or 184 mg W; salts: 59 mg NaCl, 75 mg KCl, 95 mg $MgCl_2$, 111 mg $CaCl_2$) was suspended in 1 mL Milli-Q H_2O, basified using ca. 300 mg sodium hydroxide, vortexed for 1 min, and then centrifuged. 0.6 mL of the supernatant was added to an NMR tube with 0.1 mL D_2O, containing 0.05 M of the internal standard (DSS-Na). This was subjected to NMR spectroscopy. A stack of the control spectra is shown below (Figure S3).

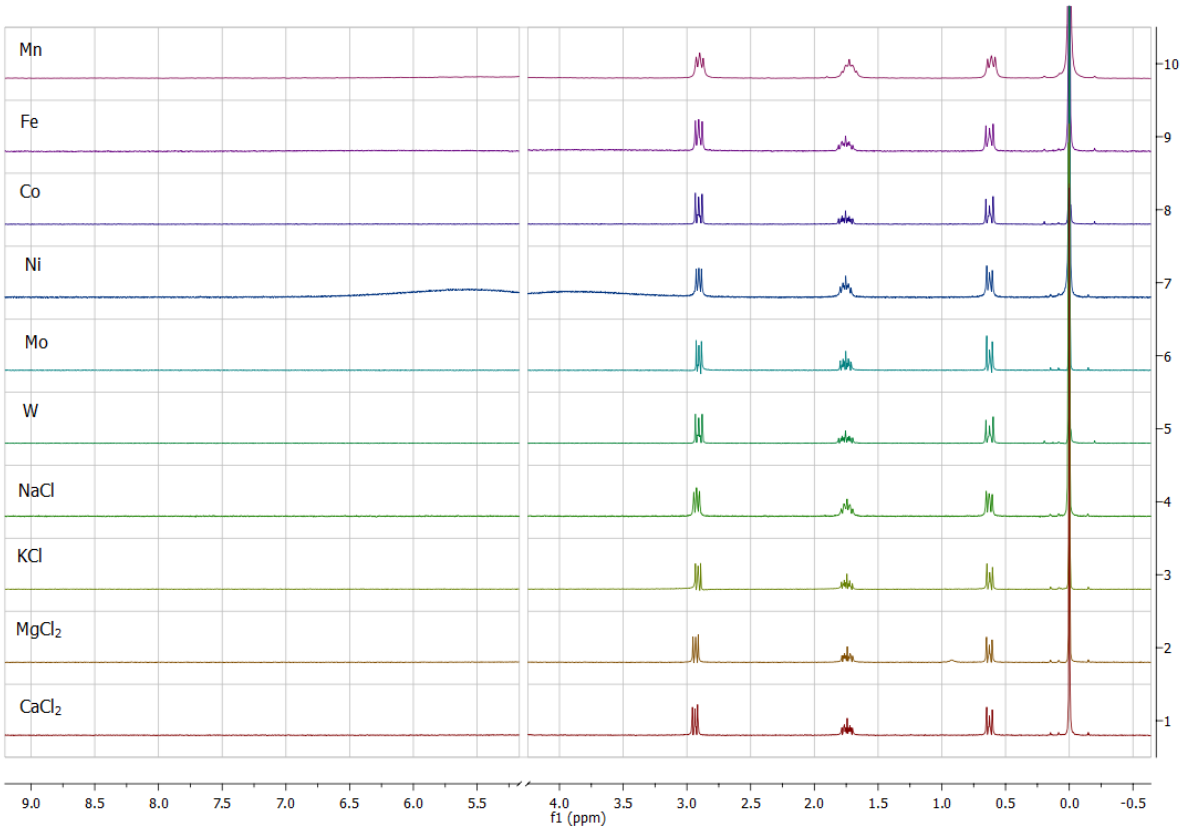


Figure S3 ^{1}H NMR spectra obtained from control experiments with starting materials used in this study: metal powders: Mn, Fe, Ni, Co, Mo, W; salts: NaCl, KCl, $MgCl_2$, $CaCl_2$. DSS-Na was used as standard in H_2O : D_2O (6 : 1). The residual suppressed water peak was deleted for clarity.

C. NMR sample preparation

To the reaction mixture was added ~300 mg of solid KOH ($NaHS{\cdot}xH_2O$ in the case of Mo-promoted reactions) in order to precipitate out any metal ions as their hydroxides (or sulfides in the case of Mo). This was mixed thoroughly. The resulting thick suspension was transferred to a 1.5 mL plastic microtube and centrifuged at 10 000 rpm for 20 minutes. To 600 µL of the supernatant was added 100 µL of 0.05 M solution of internal standard (DSS-Na in D_2O). The resulting solution was analysed by NMR using the Bruker ZGESGP pulse program, as described in the General Information section.

D. Confirmation of formate, acetate and pyruvate by GC-MS

The identities of formate, acetate and pyruvate detected in the reaction mixtures in this study were also confirmed by GC-MS. Formate and acetate were detected as their amides of *N*-methylphenylethylamine. To facilitate detection, pyruvate was reduced to lactate, since lactate esters have a much better response than pyruvate esters on our GC-MS system.[1] Methanol cannot be detected using either GC-MS method.

a. Confirmation of formate and acetate in the reaction mixture by GC-MS

To a 100 µL aliquot of a reaction mixture were added sequentially: 50 µL of 0.12 M solution of 1-hydroxybenzonetriazole in H_2O, 75 µL of 0.08 M 1-ethyl-3-(3-dimethyl-

aminopropyl)carbodiimide solution (EDC) in acetonitrile/H_2O (1:1) and 75 μL of 0.06 M *N*-methylphenylethylamine (MPEA) acetonitrile. The resulting mixture was vortexed for 30 s and incubated at 60 ^oC for 45 min. Subsequently, 200 μL of $CHCl_3$ was added to the reaction mixture and vortexed for 30 s. The $CHCl_3$ layer was then removed, dried over anhydrous $MgSO_4$ and from this 50 μL was added to 150 μL of EtOAc and analysed by the GC-MS (Figure S4, Figure S5 and Figure S6).

b. Confirmation of pyruvate in the reaction mixture by GC-MS

Four individual 1 mL reactions of pyruvate-containing reaction mixture (Fe/KCl/H_2O, 100^oC, 16 h; see Synthetic procedures below) were combined in a centrifugation tube, basified with KOH (see Analytical methods: C. NMR sample preparation), vortexed for 1 min and centrifuged at 10 000 rpm for 20 minutes. The supernatant was transferred to another centrifugation tube, which was then immersed in liquid nitrogen for 15 min. Water was then removed by lyophilization over 12 hrs. The white residue was dissolved in 400 μL of Milli-Q water and to this excess $NaBH_4$ (*ca.* 20 mg) was added. The mixture was vortexed for 1 min and left to react for ~1 h at ambient temperature. After this time, the mixture was derivatized with ethyl chloroformate and ethanol, following the procedure we previously reported.[1] GC-MS detected the presence of ethyl esters of several lactate adducts (Figure S7), whose retention times and mass spectra agreed with those obtained for an authentic sodium pyruvate solution in Milli-Q water, reduced and derivatized analogously (Figure S8).

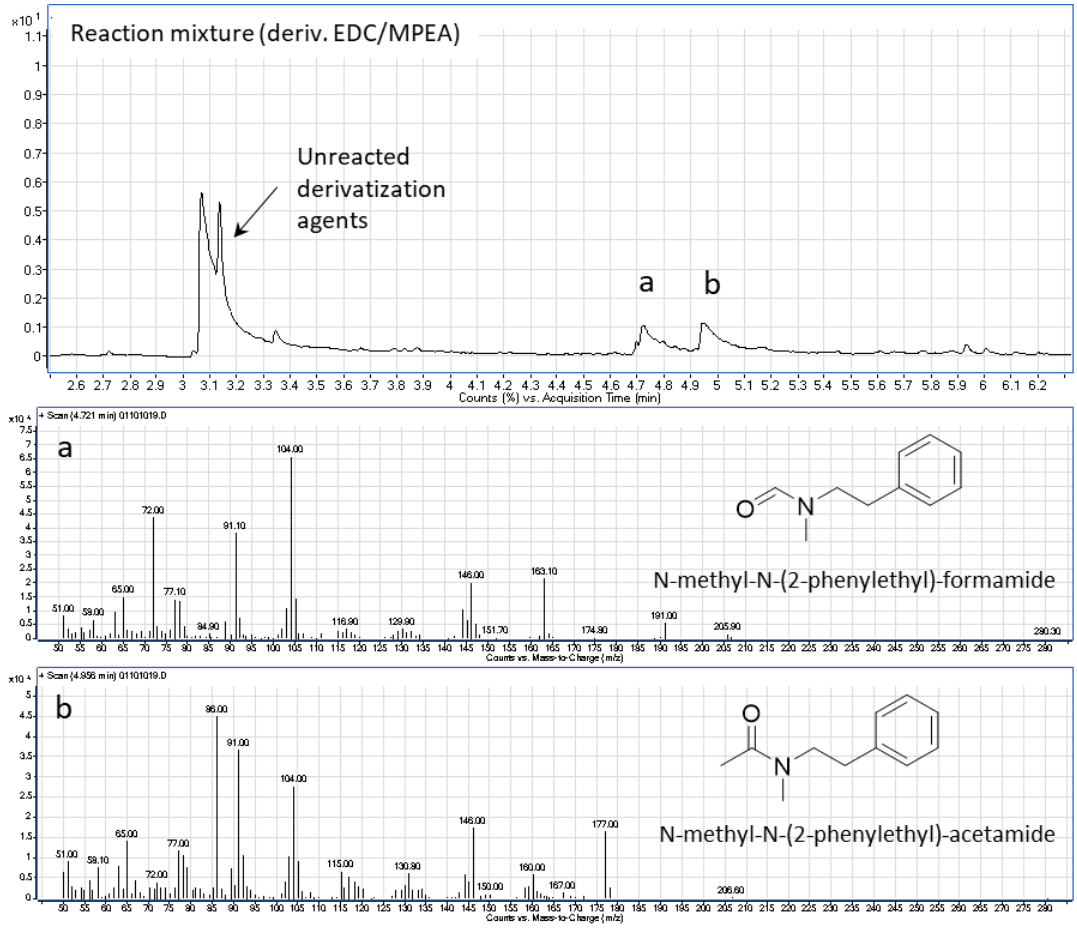


Figure S4 GC trace and mass spectra confirming the presence of formate and acetate, detected as their respective amides, after derivatization of a typical reaction mixture with EDC/MPEA.

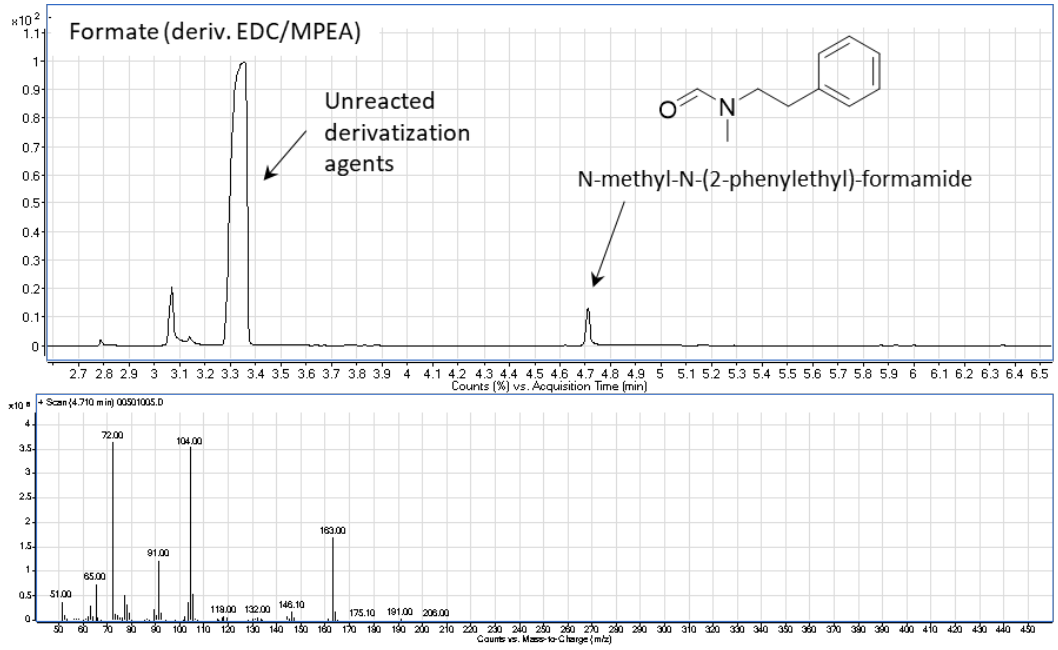


Figure S5 GC trace and mass spectra of authentic formate derivatized with EDC/MPEA.

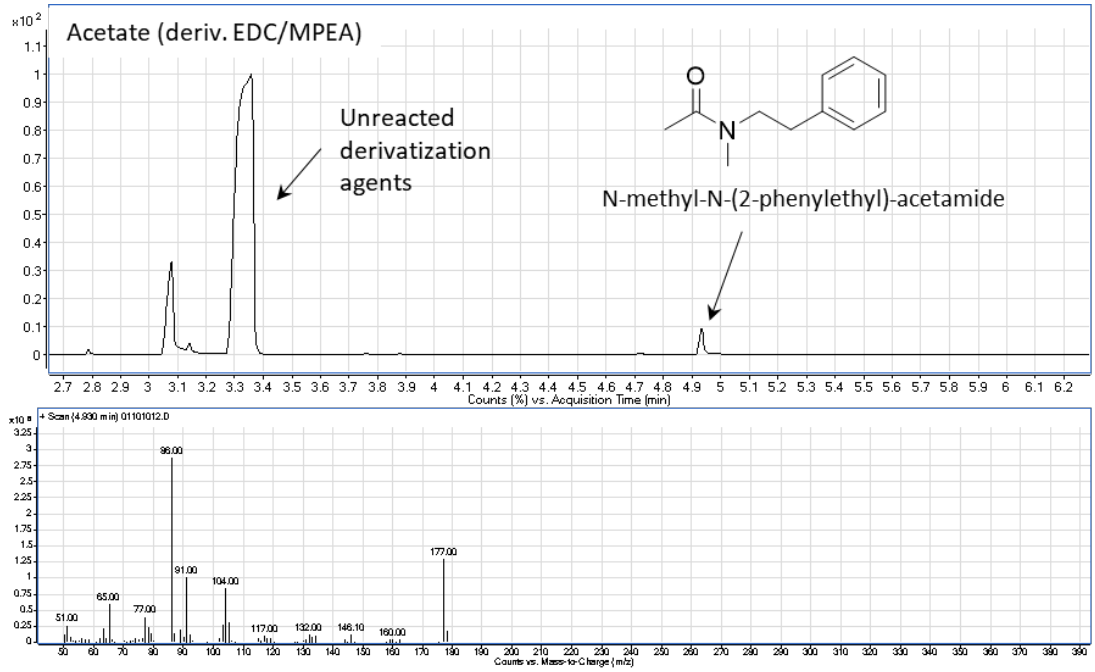


Figure S6 GC trace and mass spectra of authentic acetate derivatized with EDC/MPEA.

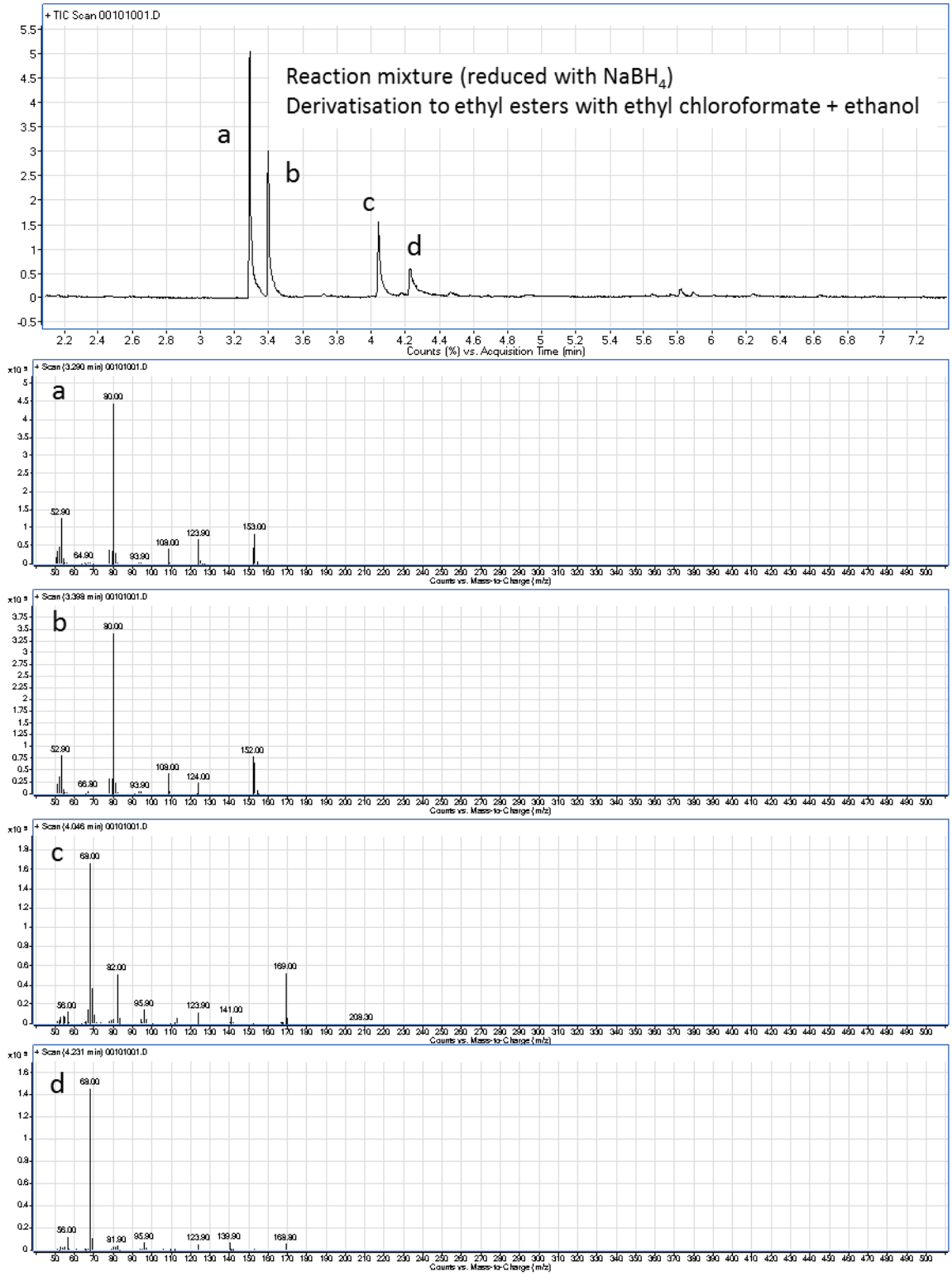


Figure S7 GC trace and mass spectra confirming the presence of pyruvate, detected as lactate after the reduction of a reaction mixture with $NaBH_4$ and subsequent derivatization to lactate ethyl esters with ethyl chloroformate and ethanol.

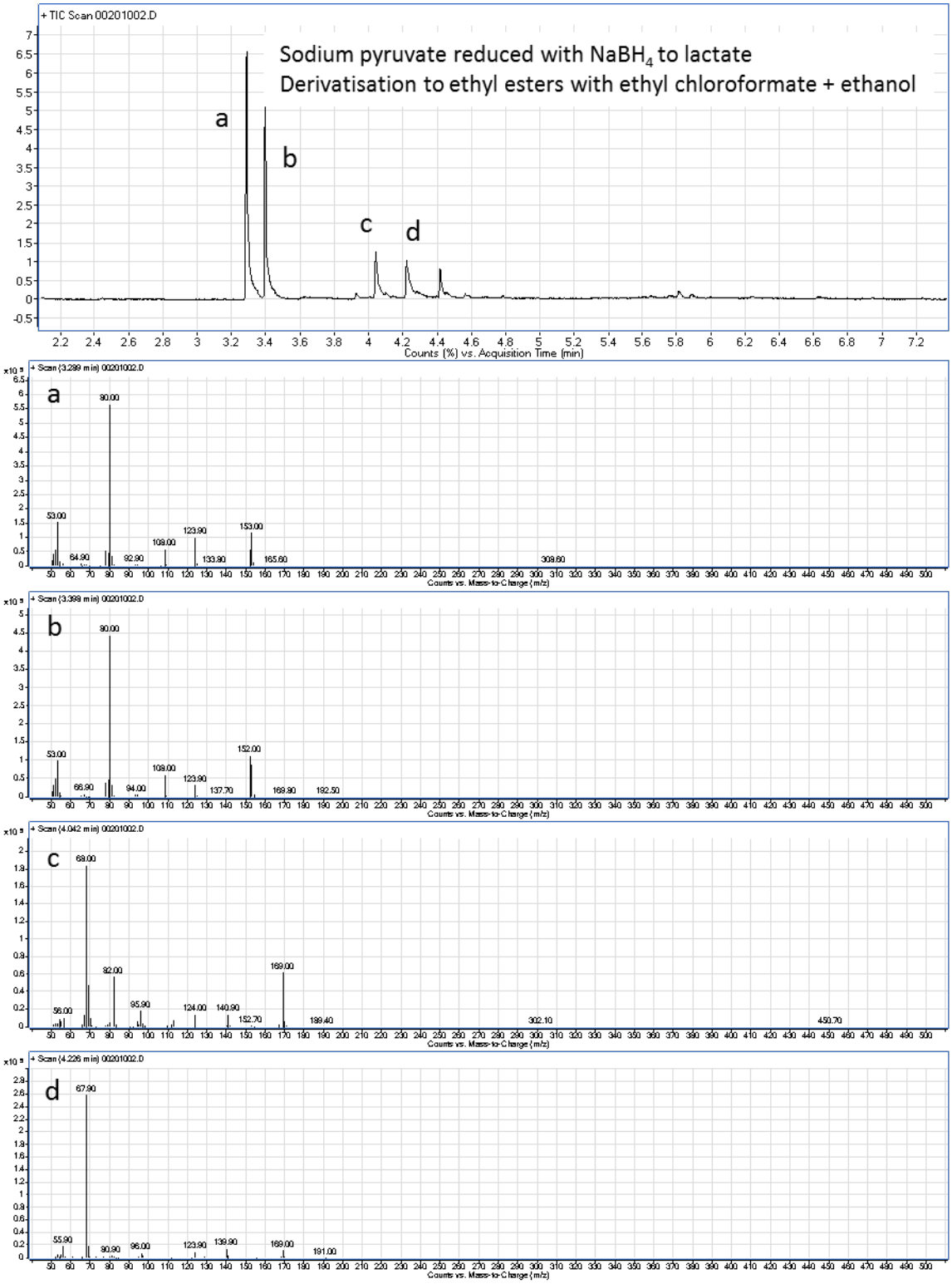


Figure S8 GC trace and mass spectra of authentic pyruvate reduced to lactate with $NaBH_4$ and subsequently derivatized to lactate ethyl esters with ethyl chloroformate and ethanol.

E. Yield determination and error analysis

700 µL aqueous solutions of potassium acetate, sodium methoxide, sodium pyruvate and sodium formate at different concentrations (0.71, 1.78, 3.57, 5.35, 7.14, 8.92 and 10.71 mM) were prepared by diluting their respective stock solutions (50 mM in Milli-Q water) with Milli-Q water to 600 µL and adding to each an aliquot of 100 µL of 50 mM solution of the standard compound (DSS-Na) in D_2O. Each of the samples was prepared in two replicas by two researchers and subjected to NMR spectroscopy (^1H, ZGESGP water suppression, as described in the General information section). For each of these, three 32-scan spectra were acquired, to account for the instrumental errors. The data from these six measurements for every concentration allowed us to obtain seven-point calibration plots for formate, methanol, acetate and pyruvate, correlating the substrate-to-standard ratios of peaks (8.45 ppm for formate, 3.34 ppm for methanol, 2.36 for pyruvate or 2.08 ppm for acetate, 0 ppm for the methyl peak of the standard DSS-Na) with the product concentration (Figure S9).

The data points were subjected to least-squares fitting (intercept = 0), from which the calibration line equation was obtained. Detection thresholds were estimated for each analyzed compound by integrating across the baseline in these regions of NMR spectra where no peaks were present, and were thus established to be 0.007 mM for acetate and pyruvate, 0.0016 mM for formate and 0.0026 mM for methanol. We note these values are much below the concentrations of acetate, pyruvate, methanol and formate detected in this study (Tables Table **S2** Table S19).

Error bars on the *calibration graphs* correspond to ± standard deviation for each data point. The yields of the *CO_2 fixation experiments* were calculated using the calibration coefficient corresponding to the slope of each calibration line. All yields of the *CO_2 fixation experiments* reported in this study are an average of at least two independent runs, with an error corresponding to ± mean absolute deviation.

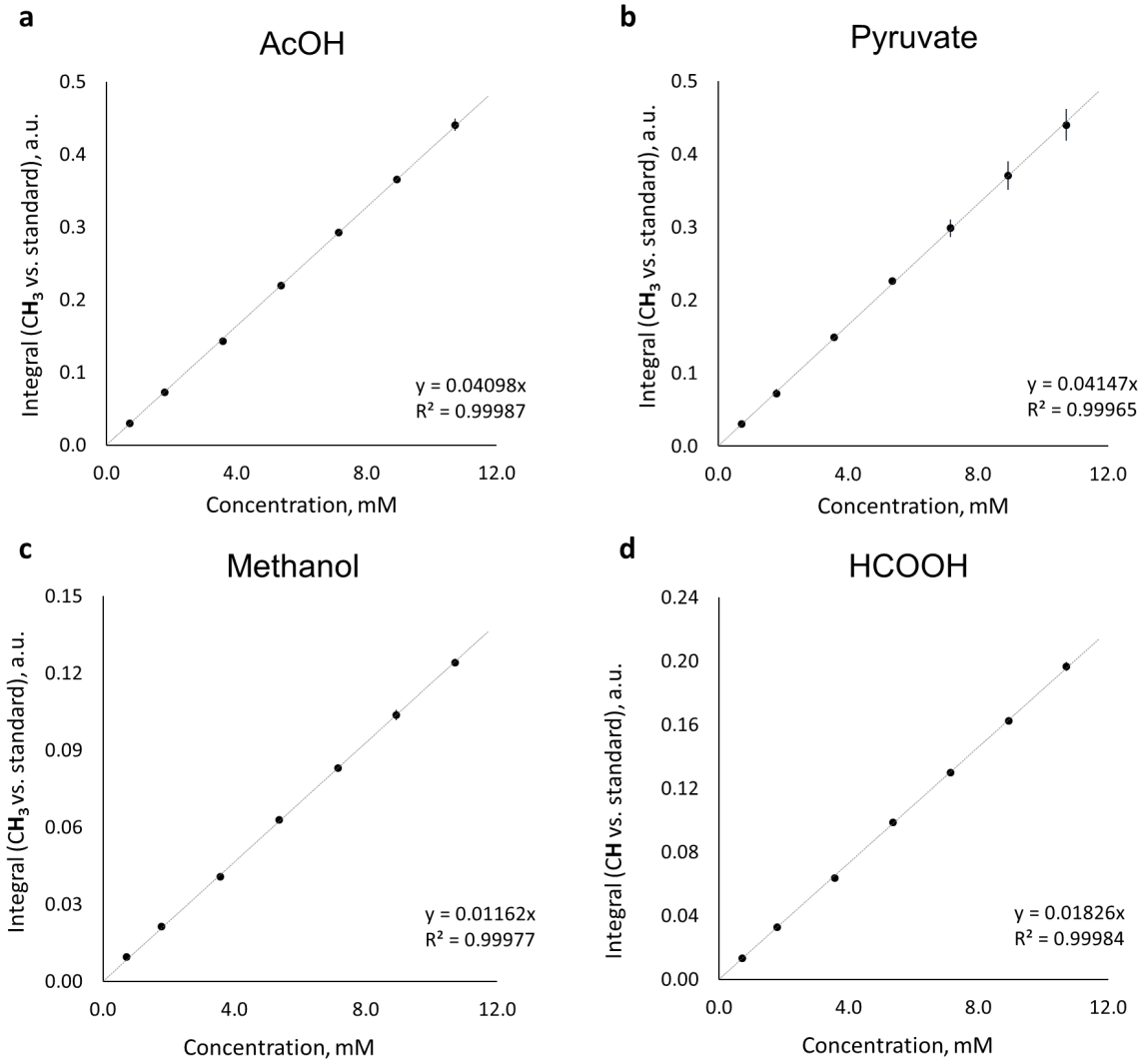


Figure S9 Correlation between the concentration of an aqueous solution of (a) acetate and the ratio of the methyl peak (1.91 ppm), (b) pyruvate and the ratio of the methyl peak (2.36 ppm), (c) methanol and the ratio of the methyl peak (3.35 ppm), and (d) formate and the ratio of the formyl hydrogen peak (8.45 ppm), to the methyl peak of the standard (DSS-Na, 0 ppm). In the cases where error bars are not visible, the error is small enough to be contained within the data point marker.

Synthetic procedures

A. Synthetic procedure A (metal-promoted CO_2 fixing reactions)

To a 1.5 mL glass vial with a PTFE-coated stir-bar was added 1 mmol of each tested reagent (56 mg Fe, or 58 mg Co, or 58 mg Ni, or 55 mg Mn, or 96 mg Mo, or 184 mg W, and/or 58 mg NaCl, and/or 75 mg KCl, and/or 95 mg $MgCl_2$, and/or 111 mg $CaCl_2$) and 1 mL of Milli-Q water. The initial pH was adjusted to the desired value with HCl or NaOH (pH screens). To prevent cross-contamination, the vials were closed with caps with punctured PTFE septa. After placing the vials in a stainless-steel Parr pressure reactor, it was flushed with *ca.* 5 bar CO_2, pressurized to a final value of 35 bar CO_2 (unless noted otherwise), and stirred at the desired temperature (an external heating mantle was used where needed) for 16 h.

B. Synthetic procedure B (rTCA cycle reaction sequences compatible with metal-promoted CO_2 fixation)

To a 1.5 mL glass vial with a PTFE-coated stir-bar were added carboxylic acid substrate (oxaloacetic acid (0.03 mmol, 4 mg) or triethyl oxalosuccinate[a] (0.03 mmol, ~8 μL)), Fe^0 powder (1.0 mmol, 56 mg), KCl (1.0 mmol, 75 mg) and $Cr_2(SO_4)_3 \cdot 12H_2O$ (1 equiv., 0.03 mmol, 18 mg). This was followed by the addition of 1 mL of 0.24 M HCl in H_2O (2 μL conc. HCl in 1 mL MilliQ H_2O), which corresponds to an initial pH = ~ 0.6. To prevent cross-contamination, the vial was closed with a cap with a punctured PTFE septum. After placing the vial in a stainless-steel Parr pressure reactor, it was flushed with *ca.* 5 bar CO_2, pressurized to a final value 35 bar CO_2, and stirred at 140 ^oC (an external heating mantle was used) for 16 h.

C. Synthetic procedure C (reductive amination compatible with metal-promoted CO_2 fixation)

To a 1.5-mL glass vial with a PTFE-coated stir-bar was added sodium pyruvate (1 equiv, 0.03 mmol, 3 mg) in 1 mL MilliQ H_2O, hydrazine monohydrate (2 equiv, 0.06 mmol, ~3 μL), KCl (1.0 mmol, 75 mg), followed by Fe^0 powder (1.0 mmol, 56 mg). To prevent cross-contamination, the vial was closed with a cap with a punctured PTFE septum. After placing the vial in a stainless-steel Parr pressure reactor, it was flushed with *ca.* 5 bar CO_2, pressurized to a final value 35 bar CO_2, and stirred at 140 ^oC (an external heating mantle was used) for 16 h.

[a] As previously reported,**Error! Bookmark not defined.**[1] due to high instability and difficult storage oxalosuccinate was obtained by in situ hydrolysis of triethyl oxalosuccinate under the reaction conditions.

Experimental data

A. Control reactions

Two control reactions were carried out according to general procedure A, in the absence of metals:

a) 1 mL 1 M KCl solution in H_2O at 100 ^oC under 35 bar CO_2 over 16 h

b) 1 mL H_2O at 100 ^oC under 35 bar CO_2 over 16 h

Next, NMR samples were prepared using the method described in Analytical methods: C. NMR sample preparation. Results showing that carbon fixation products are not produced in the absence of metals are presented in the ^1H NMR spectra stack below (Figure S10).

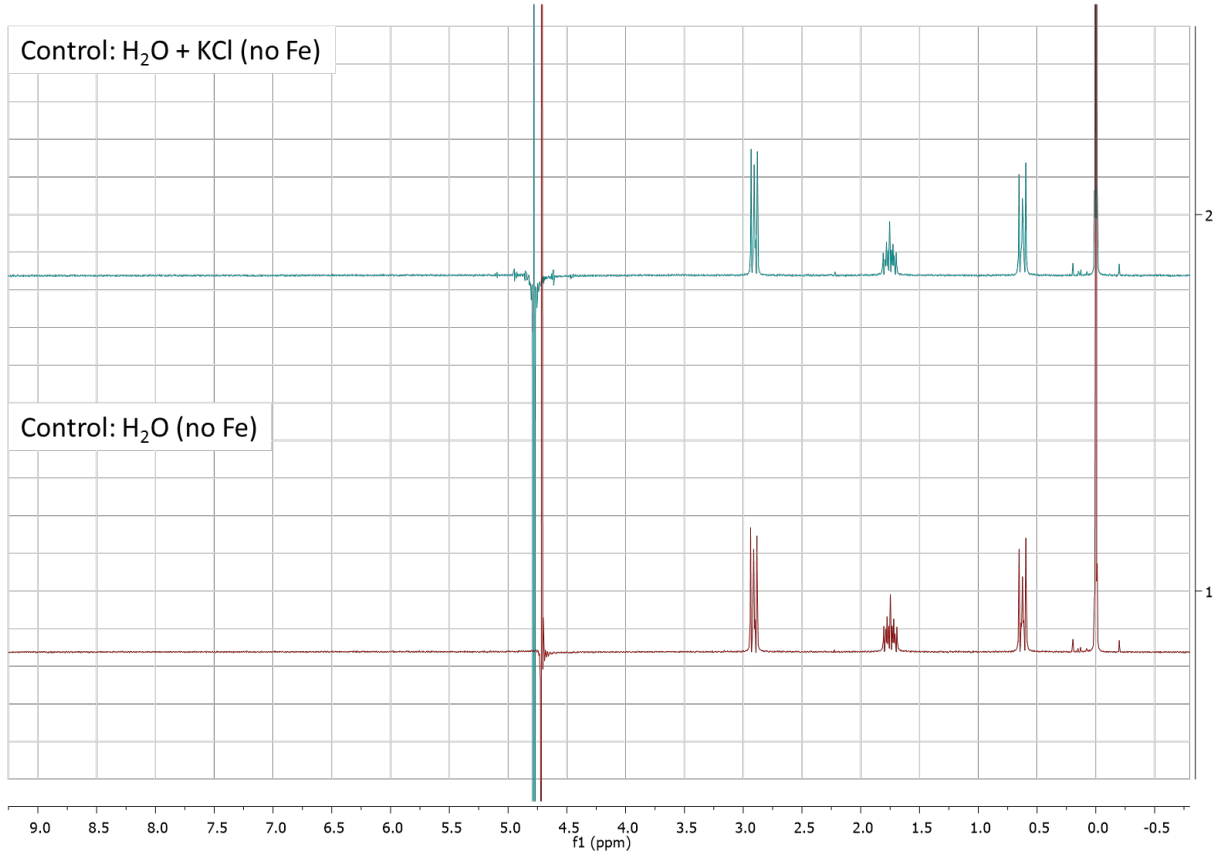


Figure S10 ^1H NMR spectra showing the result of control reactions in the absence of metals (6 : 1 H_2O : D_2O with DSS-Na as standard).

Another control reaction was carried out according to general procedure A, in the absence of CO_2: 1 mL 1 M KCl solution in H_2O at 100 ^oC under 1 bar or argon, over 16 h.

Next, an NMR sample was prepared using the method described in Analytical methods: C. NMR sample preparation. Results proving no carbon fixation products in the absence of CO_2 are presented in the ^1H NMR spectrum below (Figure S11).

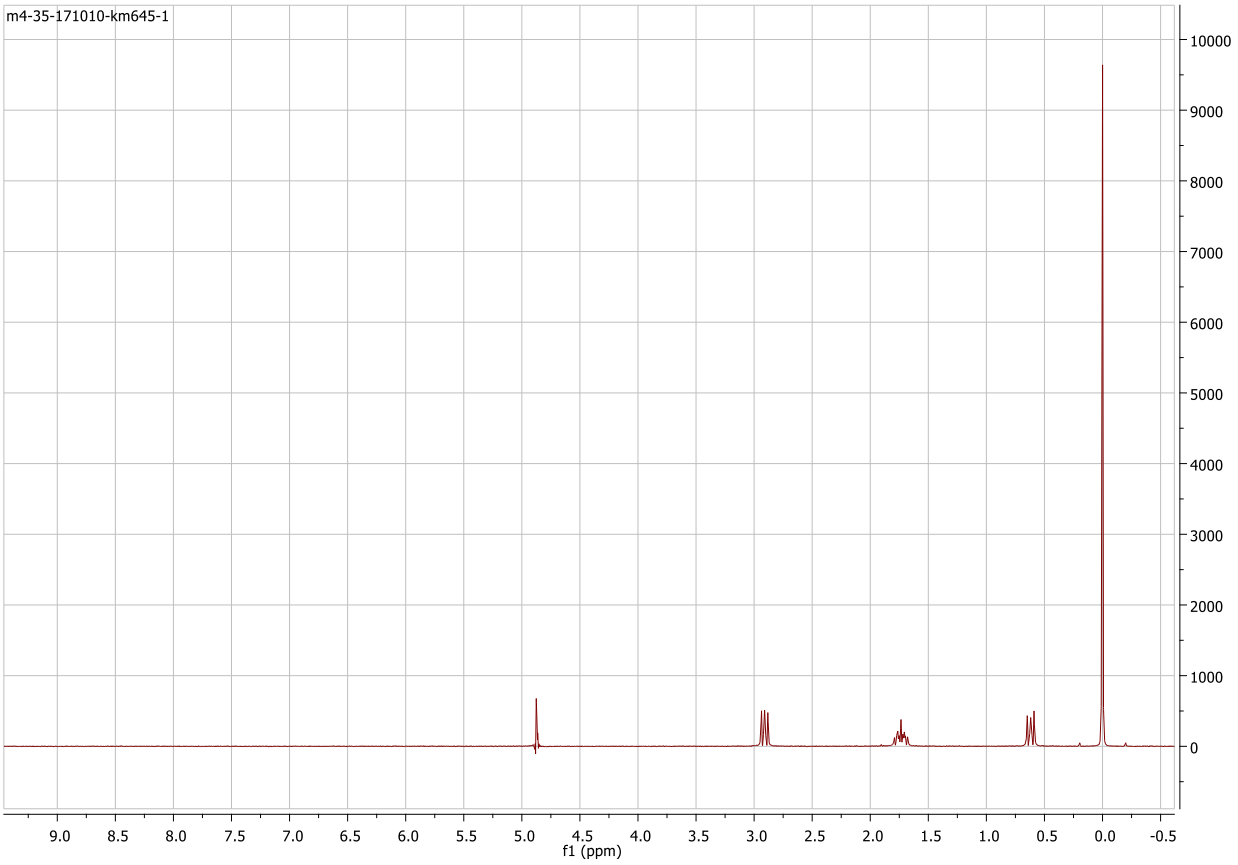


Figure S11 ^1H NMR spectra showing the result of control reactions in the absence of CO_2 (6 : 1 H_2O : D_2O with DSS-Na as standard).

B. Reaction parameter screens.

Reaction parameters such as temperature, CO_2 pressure and reaction time were probed to study their effect on metal-promoted CO_2 fixation reaction yields.

1) Temperature screen

Reactions were carried out according to general procedure A with 1 mmol (56 mg) of Fe powder and 1 mmol (75 mg) of KCl in 1 mL H_2O at various temperature values (30 °C, 50 °C, 100 °C and 140 °C), under 35 bar CO_2 over 16 h.

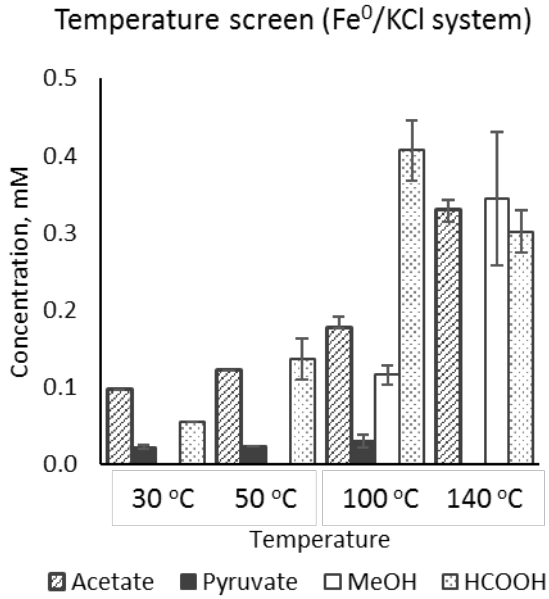


Figure S12 Temperature screen for the Fe^0/KCl system under 35 bar CO_2

Table S2 Temperature screen for the Fe^0/KCl system under 35 bar CO_2

Temperature, °C		30	50	100	140
Products, mM	Acetate	0.10 ± 0.00	0.12 ± 0.00	0.18 ± 0.01	0.33 ± 0.01
	Pyruvate	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.00 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.01	0.34 ± 0.09
	Formate	0.06 ± 0.00	0.14 ± 0.03	0.41 ± 0.04	0.30 ± 0.03

2) Pressure screen

Reactions were carried out according to general procedure A with 1 mmol (56 mg) of Fe powder and 1 mmol (75 mg) of KCl in 1 mL H_2O under final reaction pressures 1, 10, 20, 30 and 40 bar at 30 ^oC, over 16 h.

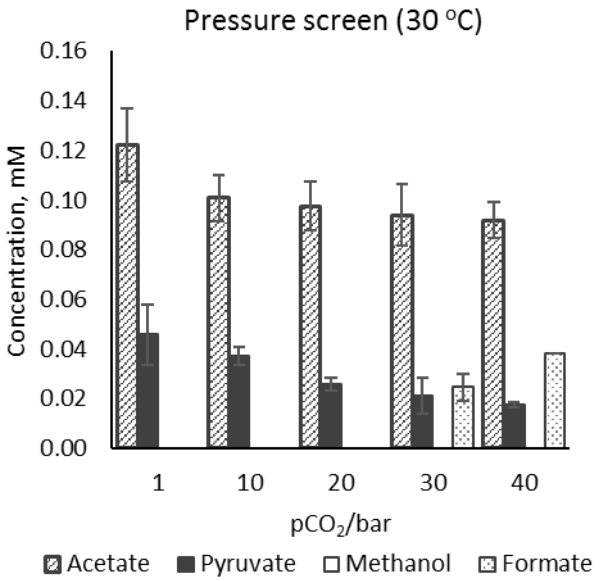


Figure S13 Pressure screen for the Fe^0/KCl system at 30 ^oC

Table S3 Pressure screen for Fe/KCl system at 30 ^oC

Pressure, bar		1	1[a]	10	20	30	40
Products, mM	Acetate	0.12 ± 0.01	0.07 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
	Pyruvate	0.05 ± 0.01	0.00 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.04 ± 0.00

[a] Reaction in pure H_2O without KCl added

3) The effect of time on the yield of CO_2 fixation products.

Reactions were carried out according to general procedure A with 1 mmol (56 mg) of Fe powder and 1 mmol (75 mg) of KCl in 1 mL H_2O at 100 °C under 35 bar CO_2 over 1.5 h, 3 h, 6 h, 16 h, 60 h and 85 h.

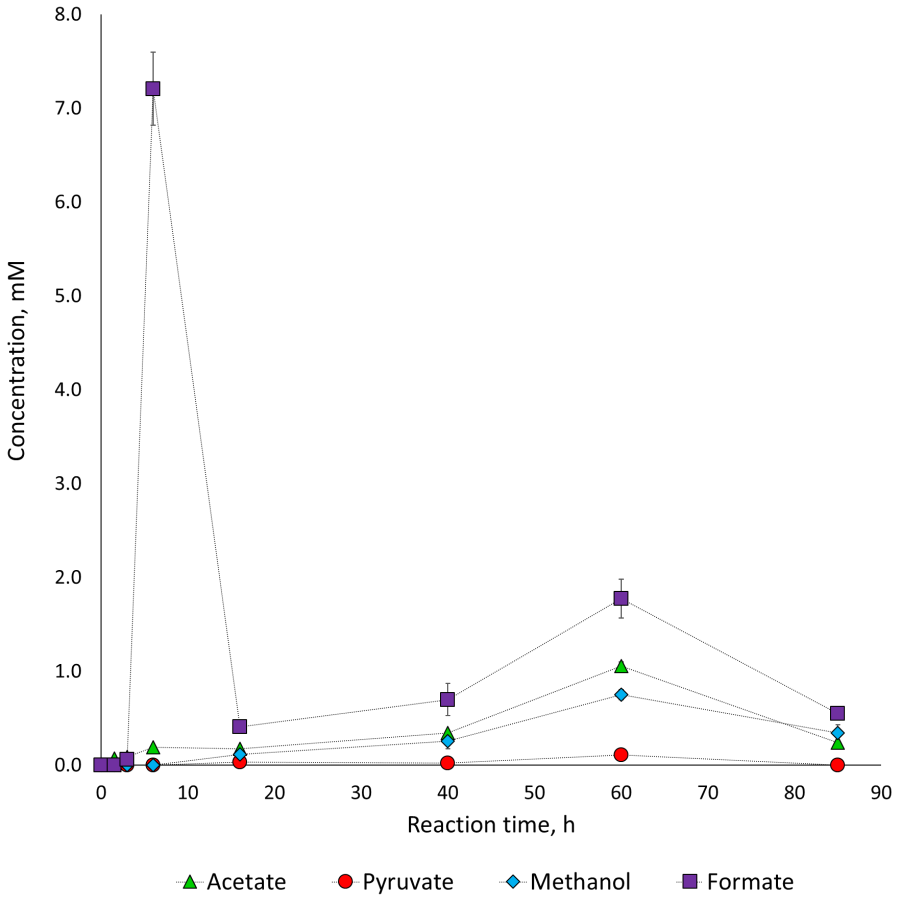


Figure S14 Reaction time screen for the Fe^0/KCl system at 100 °C under 35 bar CO_2

Table S4 Reaction time screen for the Fe^0/KCl system at 100 °C under 35 bar CO_2

Reaction time, h		1.5	3	6	16	60	85
Products, mM	Acetate	0.07 ± 0.00	0.08 ± 0.01	0.19 ± 0.002	0.18 ± 0.01	1.06 ± 0.04	0.24 ± 0.02
	Pyruvate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.11 ± 0.01	0.00 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.01	0.75 ± 0.05	0.34 ± 0.09
	Formate	0.00 ± 0.00	0.06 ± 0.01	7.21 ± 0.39	0.41 ± 0.04	1.77 ± 0.21	0.55 ± 0.04

Contribution of electrons available from iron towards detected CO_2 fixation products

In order to estimate the contribution of electrons available from iron (1 mmol in 1 mL H_2O) towards the formation of CO_2 fixation products (formate - 2ē product; methanol - 6ē product; acetate - 8ē product; pyruvate - 10ē product), the yields of each species (concentration in mM) detected after 16 h reaction time were multiplied by the number of electrons the product required to form from CO_2. These numbers were divided by 2000, with the assumption that each Fe^0 atom would furnish a maximum of 2 electrons upon oxidation to Fe^{2+} (therefore the maximum available electron "concentration" would equal 2000 mM in each reaction mixture). The distribution of these electrons between carbon fixation products, expressed as percentage of all Fe^0-sourced electrons available for reaction, is shown below (Table S5).

Table S5 Percentage contribution of electrons available from iron towards detected CO_2 fixation products.

Entry	Conditions				Formate (2ē product), %	Methanol (6ē product), %	Acetate (8ē product), %	Pyruvate (10ē product), %
	Fe (1 mmol)	KCl (1 mmol)	Temperature, °C	Pressure, bar				
1	yes	yes	30	1 (CO_2)	-	-	0.048 ± 0.006	0.023 ± 0.006
2	yes	yes	30	35 (CO_2)	0.006 ± 0.000	-	0.039 ± 0.000	0.012 ± 0.002
3	yes	no	30	35 (CO_2)	0.012 ± 0.000	-	0.075 ± 0.014	-
4	yes	no	30	1 (CO_2)	-	-	0.029 ± 0.000	-
5	yes	no	100	35 (CO_2)	0.014 ± 0.003	0.026 ± 0.000	0.054 ± 0.005	0.012 ± 0.000
6	yes	yes	100	35 (CO_2)	0.041 ± 0.005	0.035 ± 0.005	0.071 ± 0.006	0.015 ± 0.005
7[a]	yes	yes	100	35 (CO_2)	0.177 ± 0.021	0.225 ± 0.016	0.422 ± 0.017	0.053 ± 0.007
8	yes	yes	100	1 (Ar)	-	-	-	-
9	no	yes	100	35 (CO_2)	-	-	-	-

[a] Reaction time 60 h.

Detection of ethanol in the reaction mixture after 85 h (Fe, KCl, 35 bar CO_2, 100 ^oC)

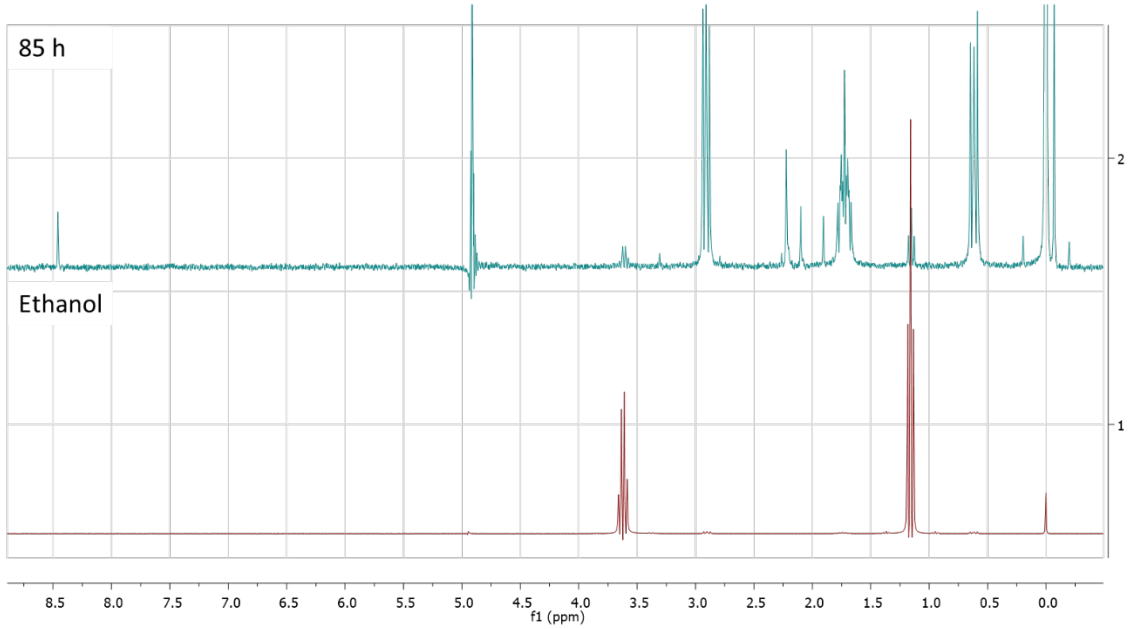


Figure S15 ^1H NMR spectrum of the crude reaction mixture, stacked with a spectrum of an authentic sample of ethanol (6 : 1 H_2O : D_2O with DSS-Na as standard), confirming the presence of ethanol after 85 hrs reaction time.

C. Metal screens in CO_2 fixation reactions

1) Manganese

a. pH screen at 100 °C and 30 °C

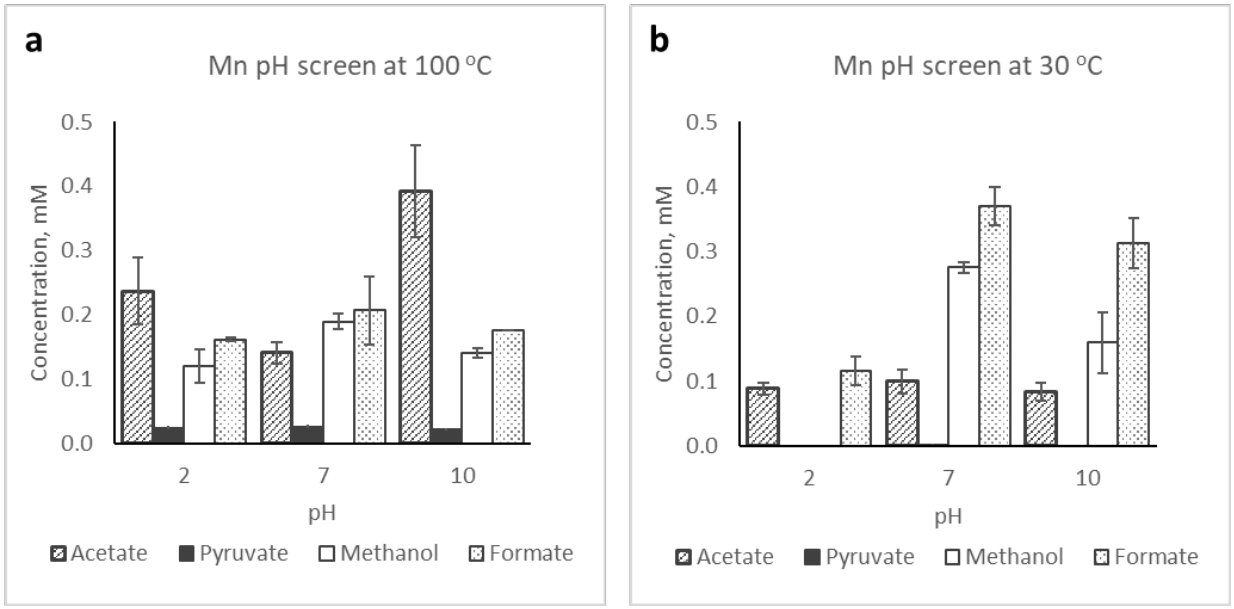


Figure S16 pH screen for manganese at (a) 100 °C and (b) 30 °C

Table S6 pH screen for manganese at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.24 ± 0.05	0.14 ± 0.02	0.39 ± 0.07	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.01
	Pyruvate	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
	Methanol	0.12 ± 0.03	0.19 ± 0.01	0.14 ± 0.01	0.00 ± 0.00	0.28 ± 0.01	0.16 ± 0.05
	Formate	0.16 ± 0.00	0.21 ± 0.05	0.18 ± 0.00	0.12 ± 0.02	0.37 ± 0.03	0.31 ± 0.04

b. Salt screen at 100 °C and 30 °C

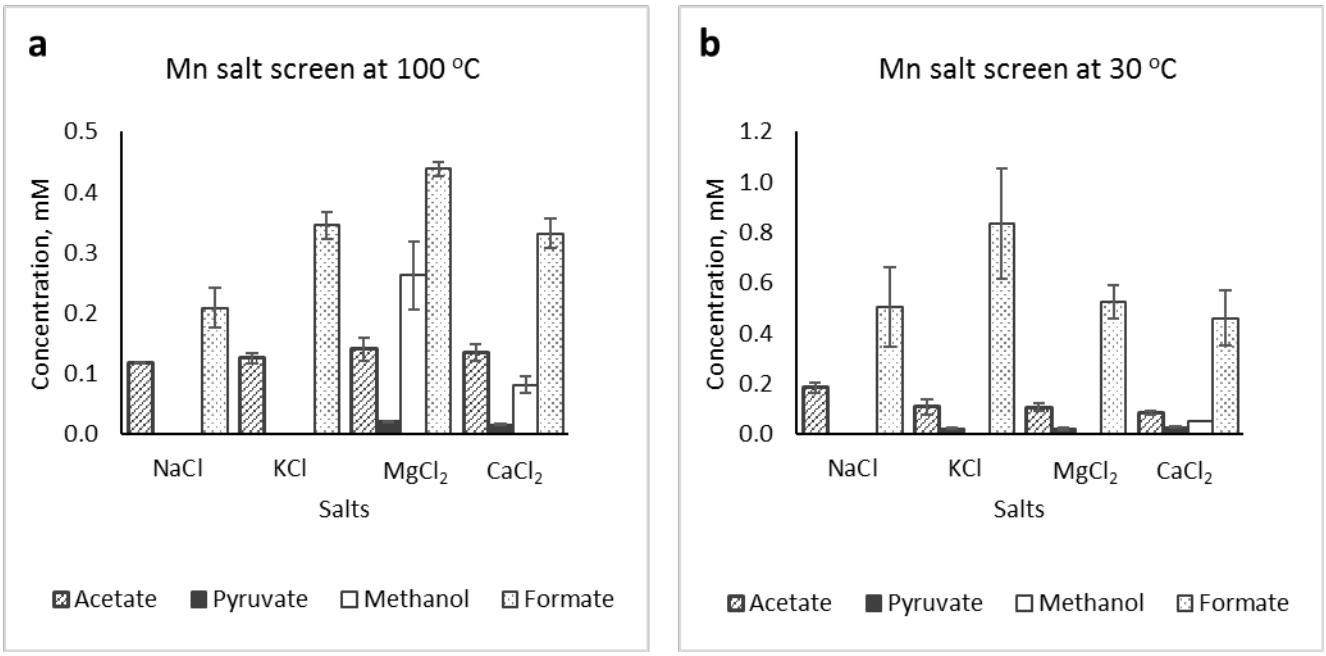


Figure S17 Salt screen for manganese at (a) 100 °C and (b) 30 °C

Table S7 Salt screen for manganese at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.12 ± 0.00	0.12 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.18 ± 0.02	0.11 ± 0.03	0.11 ± 0.01	0.09 ± 0.01
	Pyruvate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.06	0.08 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.00
	Formate	0.21 ± 0.03	0.34 ± 0.02	0.44 ± 0.01	0.33 ± 0.02	0.50 ± 0.16	0.84 ± 0.22	0.53 ± 0.07	0.46 ± 0.11

2) Iron

a. pH screen at 100 °C and 30 °C

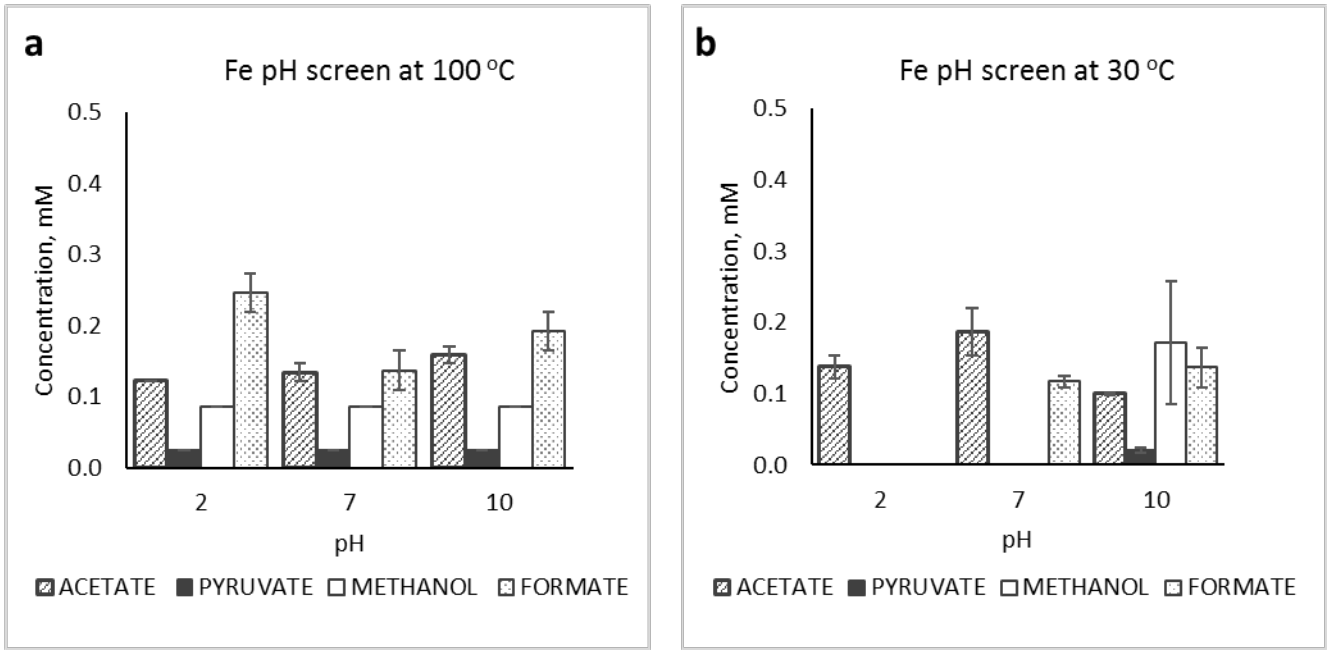


Figure S18 pH screen for iron at (a) 100 °C and (b) 30 °C

Table S8 pH screen for iron at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.12 ± 0.00	0.13 ± 0.01	0.16 ± 0.01	0.14 ± 0.02	0.19 ± 0.03	0.10 ± 0.00
	Pyruvate	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
	Methanol	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.09
	Formate	0.25 ± 0.03	0.14 ± 0.03	0.19 ± 0.03	0.00 ± 0.00	0.12 ± 0.01	0.14 ± 0.03

b. Salt screen at 100 °C and 30 °C

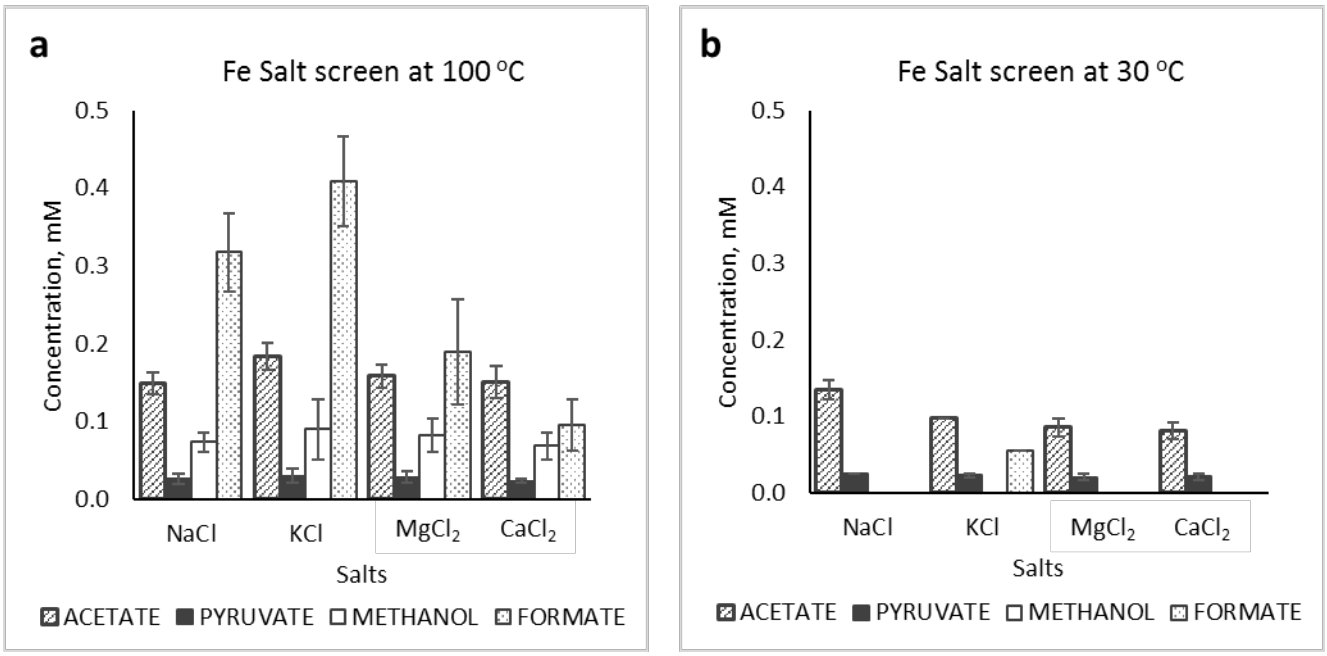


Figure S19 Salt screen for iron at (a) 100 °C and (b) 30 °C

Table S9 Salt screen for iron at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.15 ± 0.02	0.18 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	0.10 ± 0.00	0.08 ± 0.01	0.08 ± 0.01
	Pyruvate	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.07 ± 0.01	0.12 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.32 ± 0.05	0.41 ± 0.04	0.16 ± 0.03	0.10 ± 0.03	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

3) Cobalt

a. pH screen at 100 °C and 30 °C

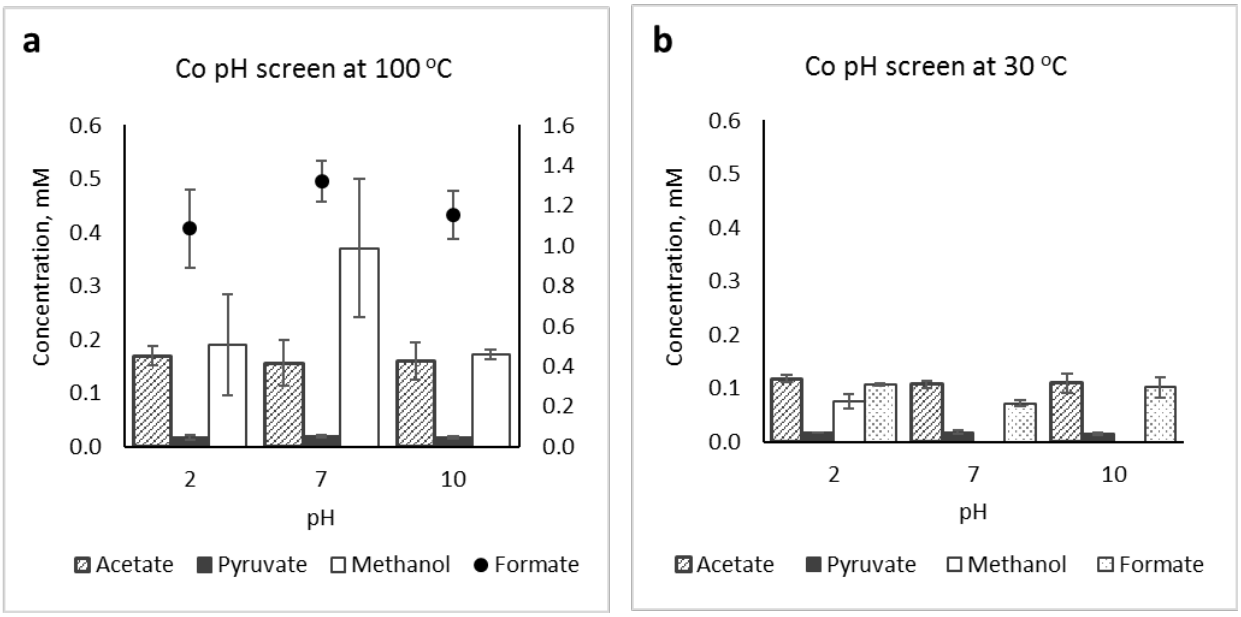


Figure S20 pH screen for cobalt at (a) 100 °C and (b) 30 °C

Table S10 pH screen for cobalt at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.17 ± 0.02	0.16 ± 0.04	0.16 ± 0.04	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.02
	Pyruvate	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.19 ± 0.10	0.37 ± 0.13	0.17 ± 0.01	0.08 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	Formate	1.09 ± 0.19	1.32 ± 0.10	1.16 ± 0.12	0.11 ± 0.00	0.07 ± 0.00	0.10 ± 0.02

b. Salt screen at 100 °C and 30 °C

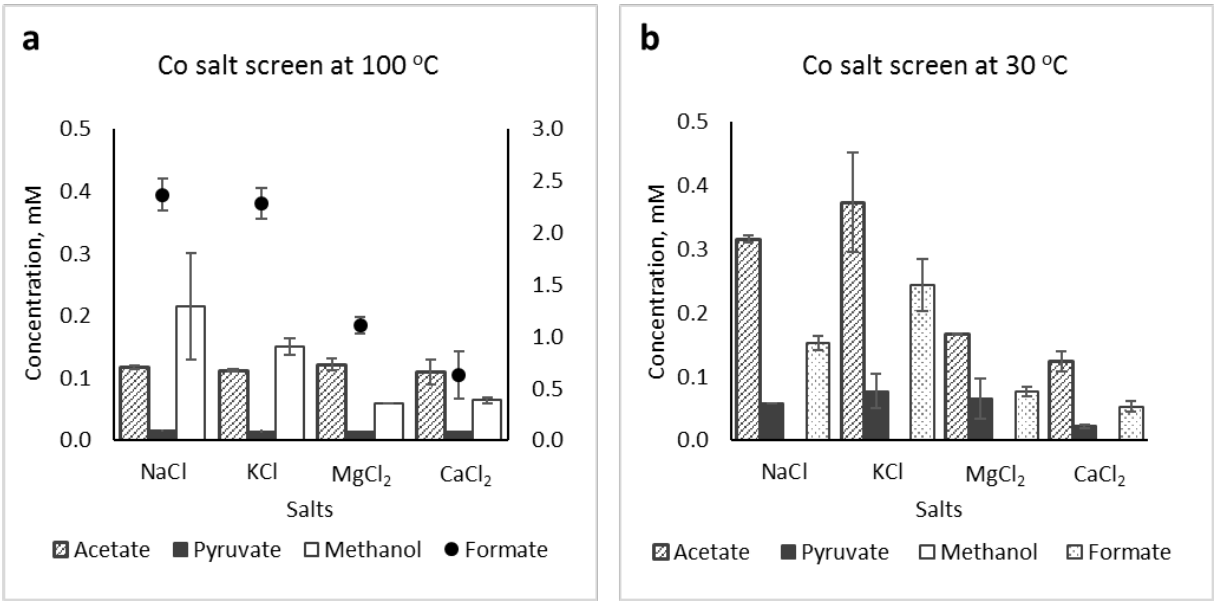


Figure S21 Salt screen for cobalt at (a) 100 °C and (b) 30 °C

Table S11 Salt screen for cobalt at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.11 ± 0.02	0.32 ± 0.01	0.37 ± 0.08	0.17 ± 0.00	0.12 ± 0.02
	Pyruvate	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.06 ± 0.00	0.08 ± 0.03	0.07 ± 0.03	0.02 ± 0.00
	Methanol	0.22 ± 0.09	0.15 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	2.37 ± 0.16	2.28 ± 0.15	1.11 ± 0.08	0.63 ± 0.23	0.15 ± 0.01	0.24 ± 0.04	0.08 ± 0.01	0.05 ± 0.01

4) Nickel

a. pH screen at 100 °C and 30 °C

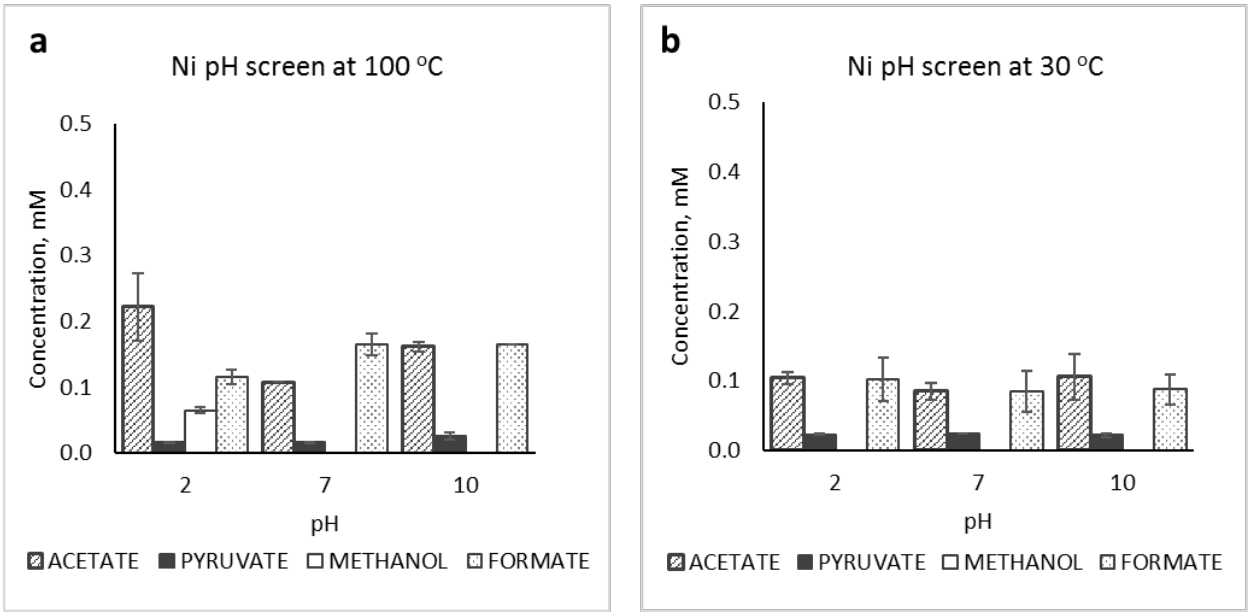


Figure S22 pH screen for nickel at (a) 100 °C and (b) 30 °C

Table S12 pH screen for nickel at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.22 ± 0.05	0.11 ± 0.00	0.16 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.03
	Pyruvate	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.12 ± 0.01	0.16 ± 0.02	0.16 ± 0.00	0.10 ± 0.03	0.08 ± 0.03	0.09 ± 0.02

b. Salt screen at 100 °C and 30 °C

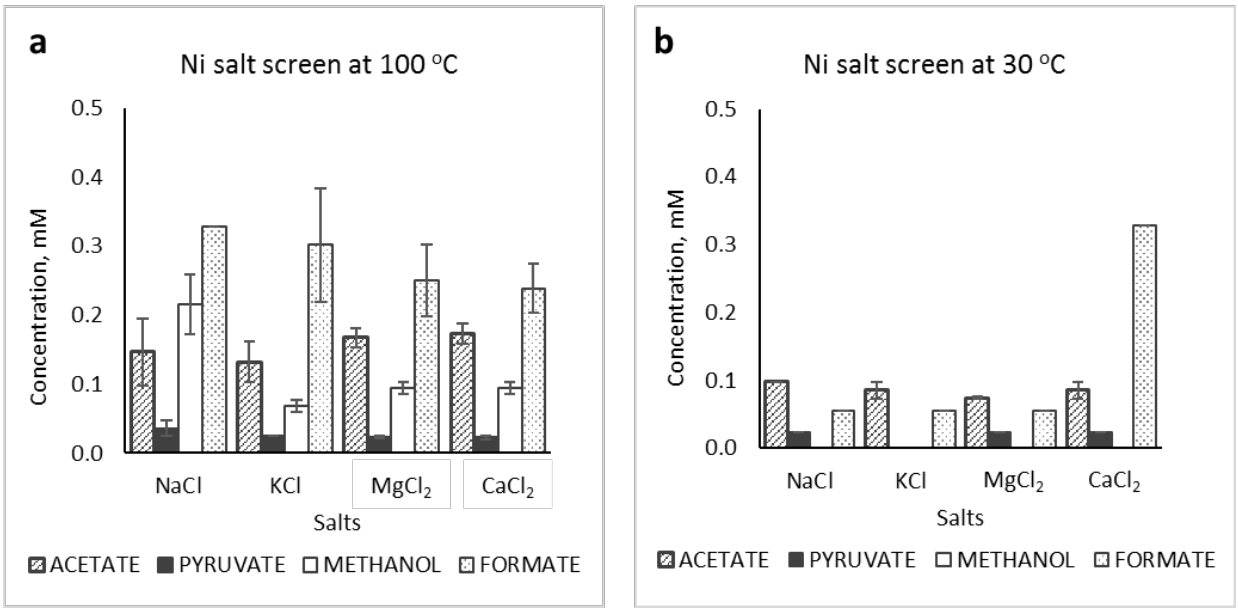


Figure S23 Salt screen for nickel at (a) 100 °C and (b) 30 °C

Table S13 Salt screen for nickel at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.15 ± 0.05	0.13 ± 0.03	0.17 ± 0.01	0.17 ± 0.02	0.10 ± 0.00	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.01
	Pyruvate	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.22 ± 0.64	0.07 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.33 ± 0.04	0.30 ± 0.08	0.25 ± 0.05	0.24 ± 0.04	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.33 ± 0.00

5) Molybdenum

a. pH screen at 100 °C and 30 °C

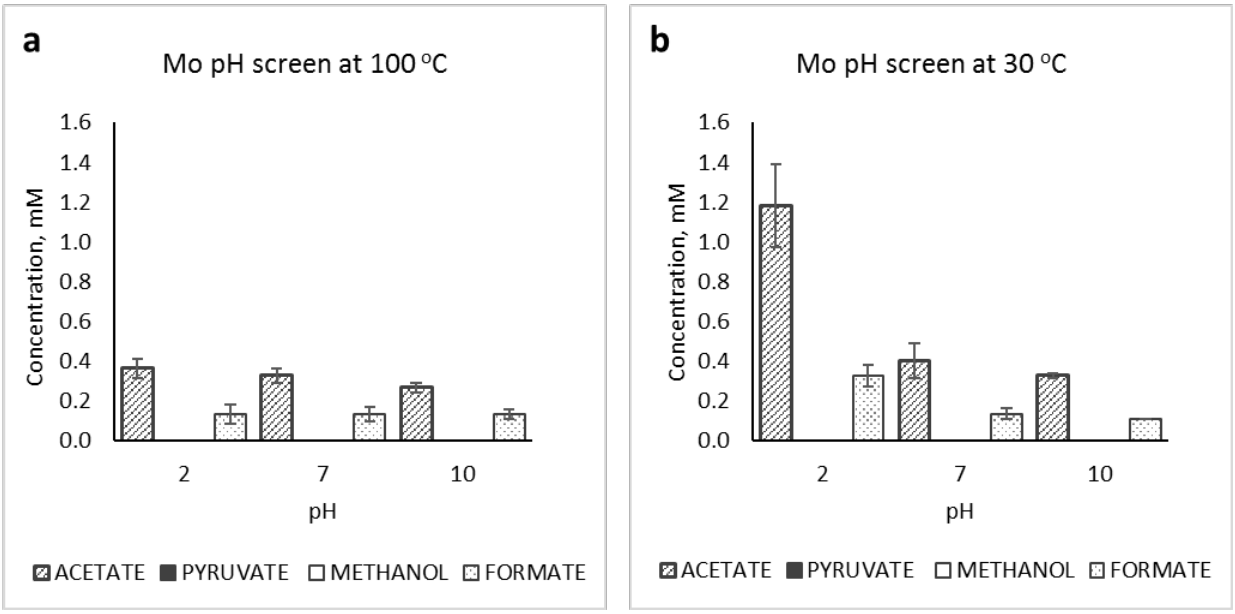


Figure S24 pH screen for molybdenum at (a) 100 °C and (b) 30 °C

Table S14 pH screen for molybdenum at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.37 ± 0.05	0.33 ± 0.04	0.27 ± 0.02	1.18 ± 0.21	0.40 ± 0.08	0.33 ± 0.01
	Pyruvate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.14 ± 0.03	0.14 ± 0.03	0.14 ± 0.03	0.33 ± 0.06	0.14 ± 0.03	0.11 ± 0.00

b. Salt screen at 100 °C and 30 °C

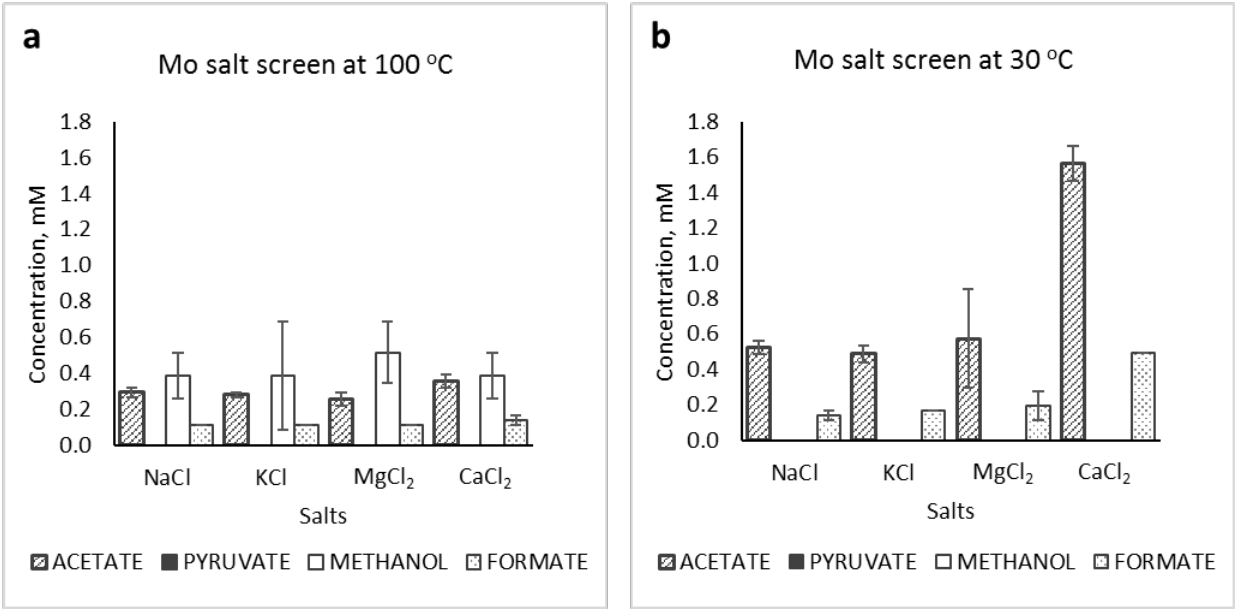


Figure S25 Salt screen for molybdenum at (a) 100 °C and (b) 30 °C

Table S15 Salt screen for molybdenum at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.29 ± 0.02	0.28 ± 0.01	0.26 ± 0.04	0.35 ± 0.04	0.53 ± 0.04	0.49 ± 0.05	0.57 ± 0.28	1.56 ± 0.10
	Pyruvate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Methanol	0.39 ± 0.13	0.39 ± 0.30	0.52 ± 0.17	0.39 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.14 ± 0.03	0.14 ± 0.02	0.16 ± 0.00	0.19 ± 0.08	0.49 ± 0.00

6) Tungsten

a. pH screen at 100 °C and 30 °C

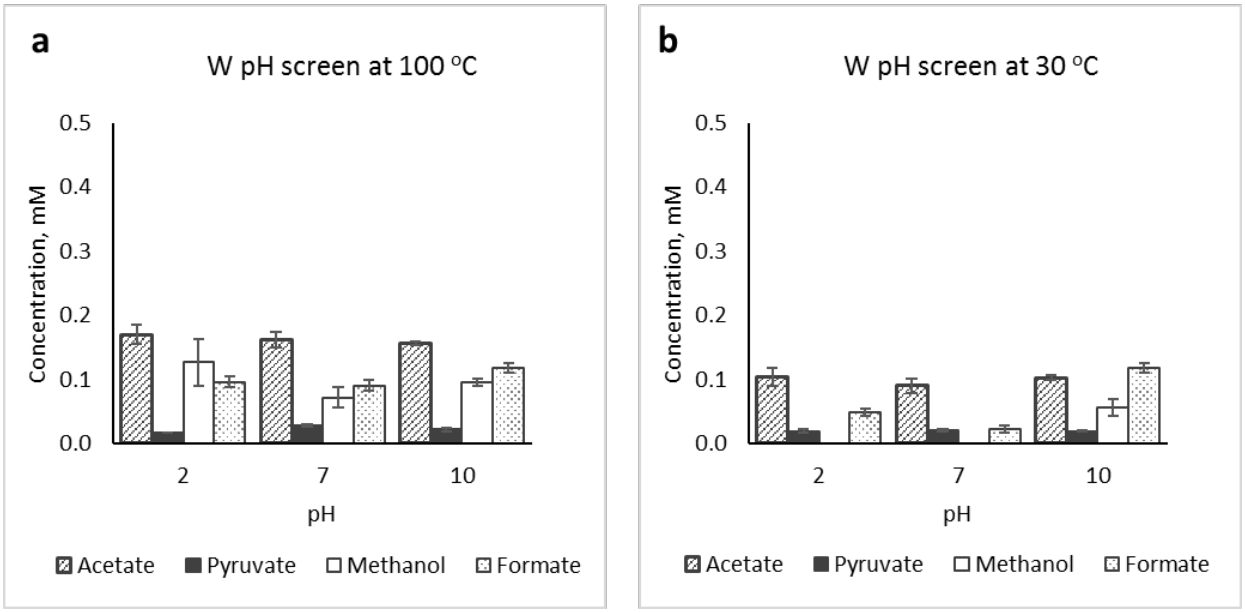


Figure S26 pH screen for tungsten at (a) 100 °C and (b) 30 °C

Table S16 pH screen for tungsten at 100 °C and 30 °C

Temperature		100 °C			30 °C		
pH		2	7	10	2	7	10
Products, mM	Acetate	0.17 ± 0.02	0.16 ± 0.01	0.16 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.00
	Pyruvate	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.13 ± 0.04	0.07 ± 0.02	0.10 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.01
	Formate	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.05 ± 0.00	0.02 ± 0.00	0.12 ± 0.01

b. Salt screen at 100 °C and 30 °C

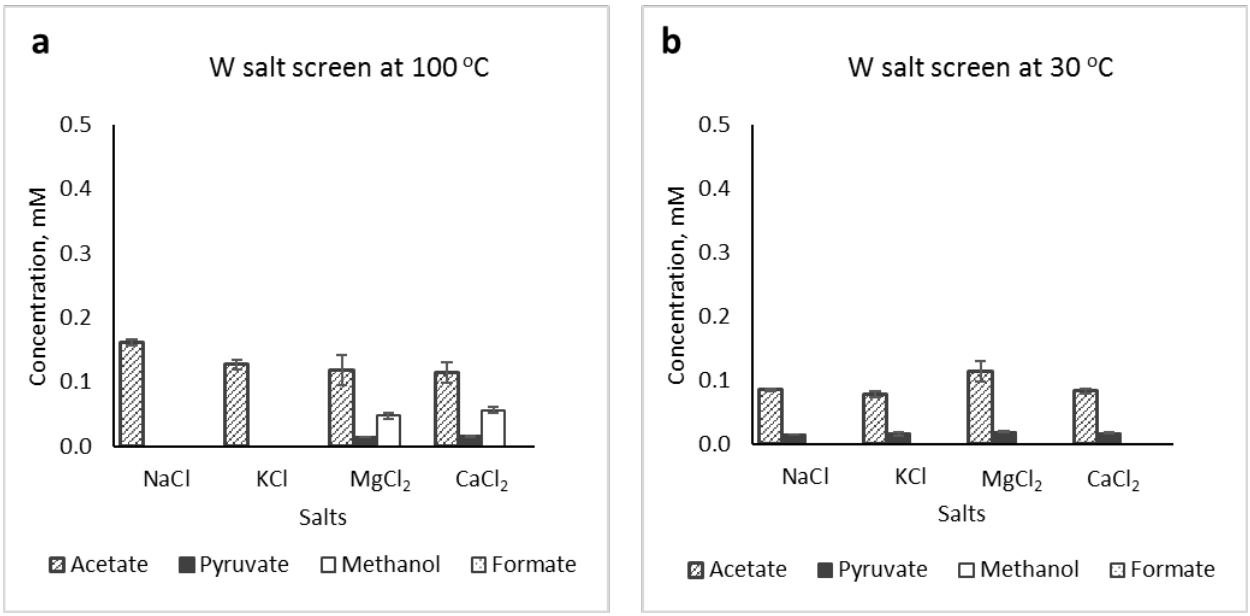


Figure S27 Salt screen for tungsten at (a) 100 °C and (b) 30 °C

Table S17 Salt screen for tungsten at 100 °C and 30 °C

Temperature		100 °C				30 °C			
Salts		NaCl	KCl	$MgCl_2$	$CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$
Products, mM	Acetate	0.16 ± 0.00	0.13 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.08 ± 0.00	0.08 ± 0.00	0.11 ± 0.02	0.08 ± 0.00
	Pyruvate	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Formate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

7) The effect of salt concentration on reaction yields

Reactions were carried out according to general procedure A with 1 mmol of Fe powder and with varying quantity of KCl in 1 mL H_2O (0.1, 0.5, 1.0 and 2.0 mmol), at 100 °C over 16 h.

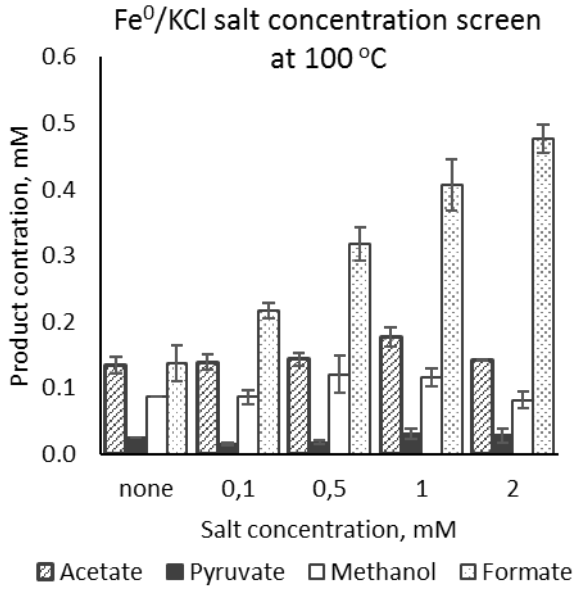


Figure S28 KCl concentration screen for the Fe^0 system at 100 °C

Table S18 KCl concentration screen for the Fe^0 system at 100 °C

KCl. mM		0.1	0.5	1.0	2.0
Products, mM	Acetate	0.14 ± 0.01	0.14 ± 0.01	0.18 ± 0.01	0.14 ± 0.00
	Pyruvate	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
	Methanol	0.09 ± 0.01	0.12 ± 0.03	0.12 ± 0.01	0.08 ± 0.01
	Formate	0.22 ± 0.01	0.32 ± 0.03	0.41 ± 0.04	0.48 ± 0.02

D. **Miscellaneous experiments**

1) The effect of KOH workup

Two reactions were carried out in parallel according to general procedure A with 1 mmol of Fe powder in 1 mL H_2O at 100 ^oC under 35 bar CO_2 over 16 h. Next, NMR samples were prepared using the method described in Analytical methods: C. NMR sample preparation, except in one of the cases where the KOH quenching step was omitted. Compared results are presented in the ^1H NMR spectra stack below (Figure S29)

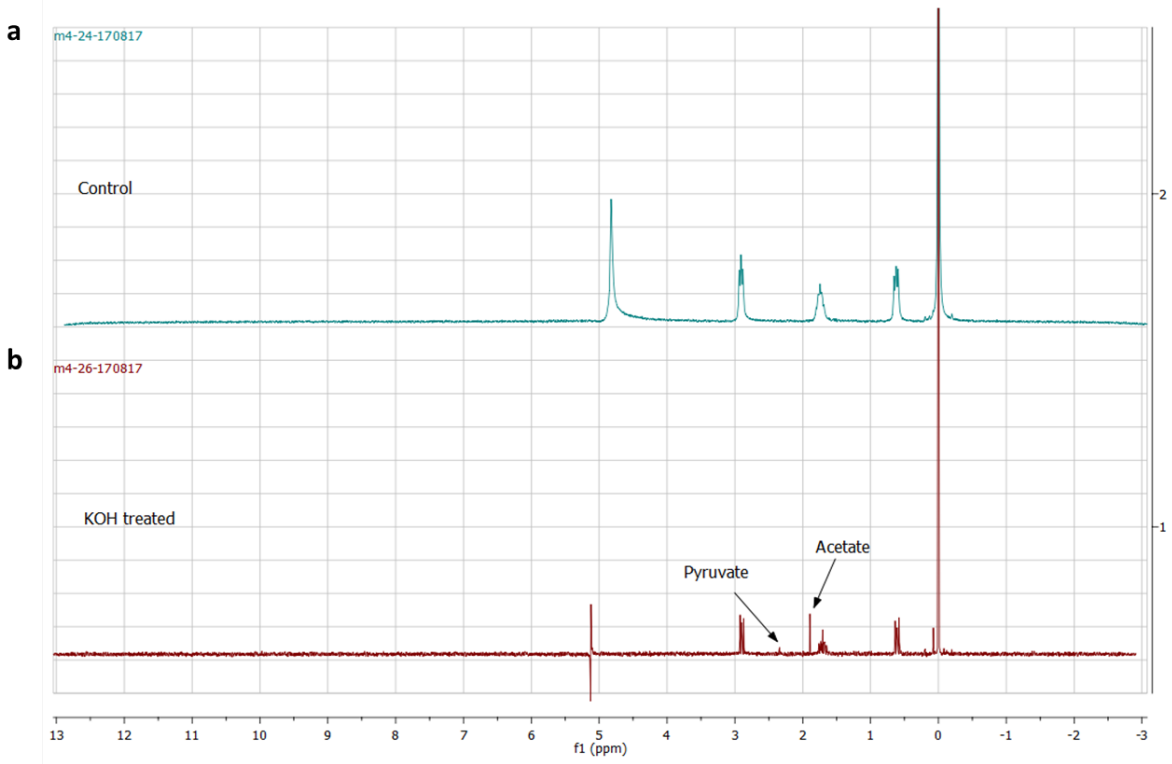


Figure S29 ^1H NMR of the reaction mixture (a) not treated with KOH during sample preparation and (b) treated with KOH during sample preparation (6 : 1 H_2O : D_2O with DSS-Na as standard).

The addition of KOH during the NMR sample preparation allows for the separation of all analysed products otherwise bound to the metal species present in solution, thus permitting us to acquire quantitative ^1H NMR spectra.

2) Iron-promoted CO_2-fixation in the presence of each of its products as the starting material.

Reactions (A)-(D) (Figure S30) were carried out according to general procedure A with 1 mmol of Fe powder and 1 mmol of KCl in 1 mL H_2O, except to each reaction vial additional CO_2-fixation products were added as starting materials: (A) sodium formate, (B) sodium methoxide, (C) potassium acetate, and (D) sodium pyruvate. The reactions were stirred at 100 ^oC under 35 bar CO_2 over 16 h.

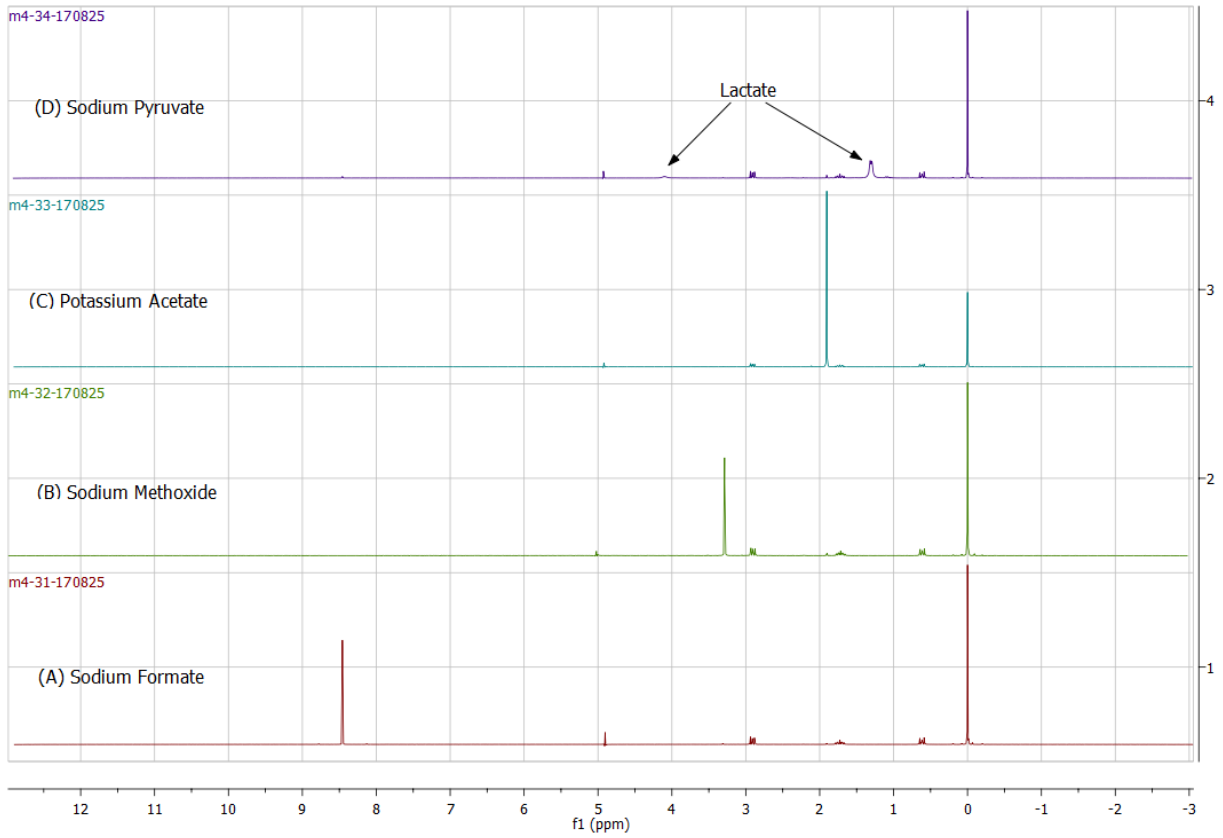


Figure S30 ^1H NMR spectra showing the result of iron-promoted CO_2-fixation reactions in the presence of each if its products the starting material (6 : 1 H_2O : D_2O with DSS-Na as standard).

The fact that reaction (D), with pyruvate present as the starting material, yielded only its reduction product (lactate) under normal reaction conditions points towards the role of a surface bound chemistry during the course of reaction (see main text).

3) Iron-promoted CO fixation

Two reactions were carried out according to general procedure A with 1 mmol of Fe powder and 1 mmol of KCl in 1 mL H_2O:

a) at 30 °C under 1 bar CO over 16 h
b) at 100 °C under 35 bar CO over 16 h

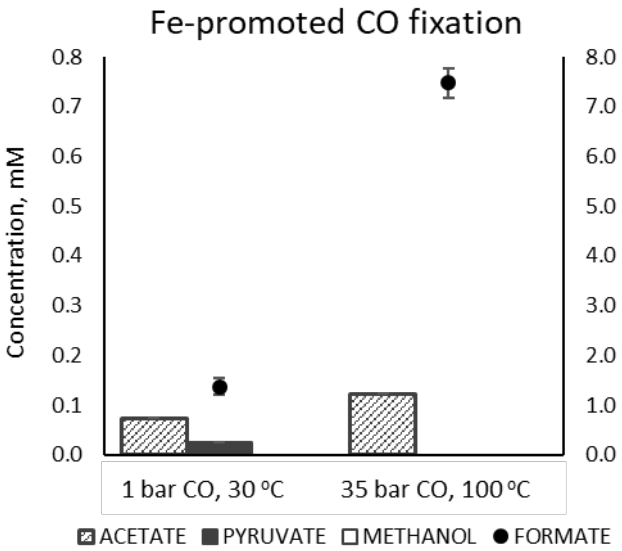


Figure S31 CO fixation reactions using the Fe^0/KCl system at 30 °C under 1 bar CO and at 100 °C under 35 bar CO.

Table S19 CO fixation reactions using the Fe^0/KCl system at 30 °C under 1 bar CO and at 100 °C under 35 bar CO.

Conditions		1 bar CO 30 °C	35 bar CO 100 °C
Products	Acetate	0.07 ± 0.00	0.12 ± 0.00
	Pyruvate	0.02 ± 0.00	0.00 ± 0.00
	Methanol	0.00 ± 0.00	0.00 ± 0.00
	Formate	1.37 ± 0.16	7.48 ± 0.30

E. Compatibility of typical CO_2 fixation conditions with 3-reaction sequences of the rTCA cycle

Two rTCA cycle reaction sequences were carried out in two replicas each, according to general procedure B following which the mixtures were subjected to a KOH workup. In the case of the oxaloacetate-malate-fumarate-succinate sequence, NMR spectra were acquired (as described in the Analytical methods section). NMR spectra (Figure S32) allowed only for a qualitative detection of acetate, formate, malate, fumarate, succinate, lactate and levulinate (side product), due to high complexity and low concentration of the reaction mixture. In the case of the oxalosuccinate-isocitrate-aconitate-citrate sequence, the obtained spectrum of a crude reaction mixture was too complex to allow for unambiguous product determination. Therefore, the presence of all rTCA cycle products in both sequences was confirmed additionally by GC-MS. 200 μL of the basified reaction mixture was derivatized with ethyl chloroformate/ethanol or methyl chloroformate/methanol to convert the carboxylic acid products to their respective ethyl (or methyl) esters, using a well-established literature procedure.[1] The obtained ester mixtures were subjected to GC-MS measurements (Figure S33 and Figure S34). Note that acetate, formate and methanol cannot be detected using this method. The identity of the detected species was confirmed by the comparison with ^{1}H NMR spectra of authentic samples (for the oxaloacetate-malate-fumarate-succinate sequence), as well as by the

obtained mass spectra (Figure S32, Figure S33 and Figure S34), which agree with the data we reported previously.[1]

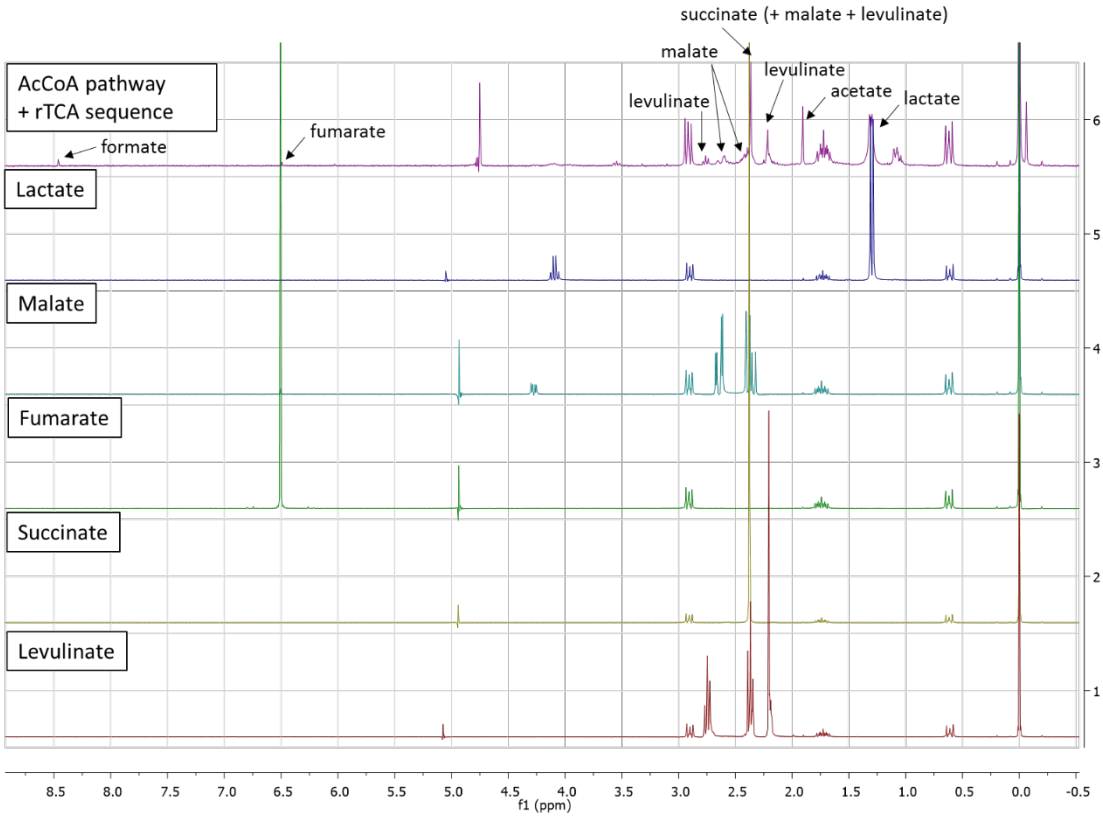


Figure S32 ^{1}H NMR spectra of the rTCA cycle (3-step sequence) reaction mixture, stacked with analogous spectra of authentic samples of rTCA cycle intermediates and levulinate (6 : 1 H_2O : D_2O with DSS-Na as standard).

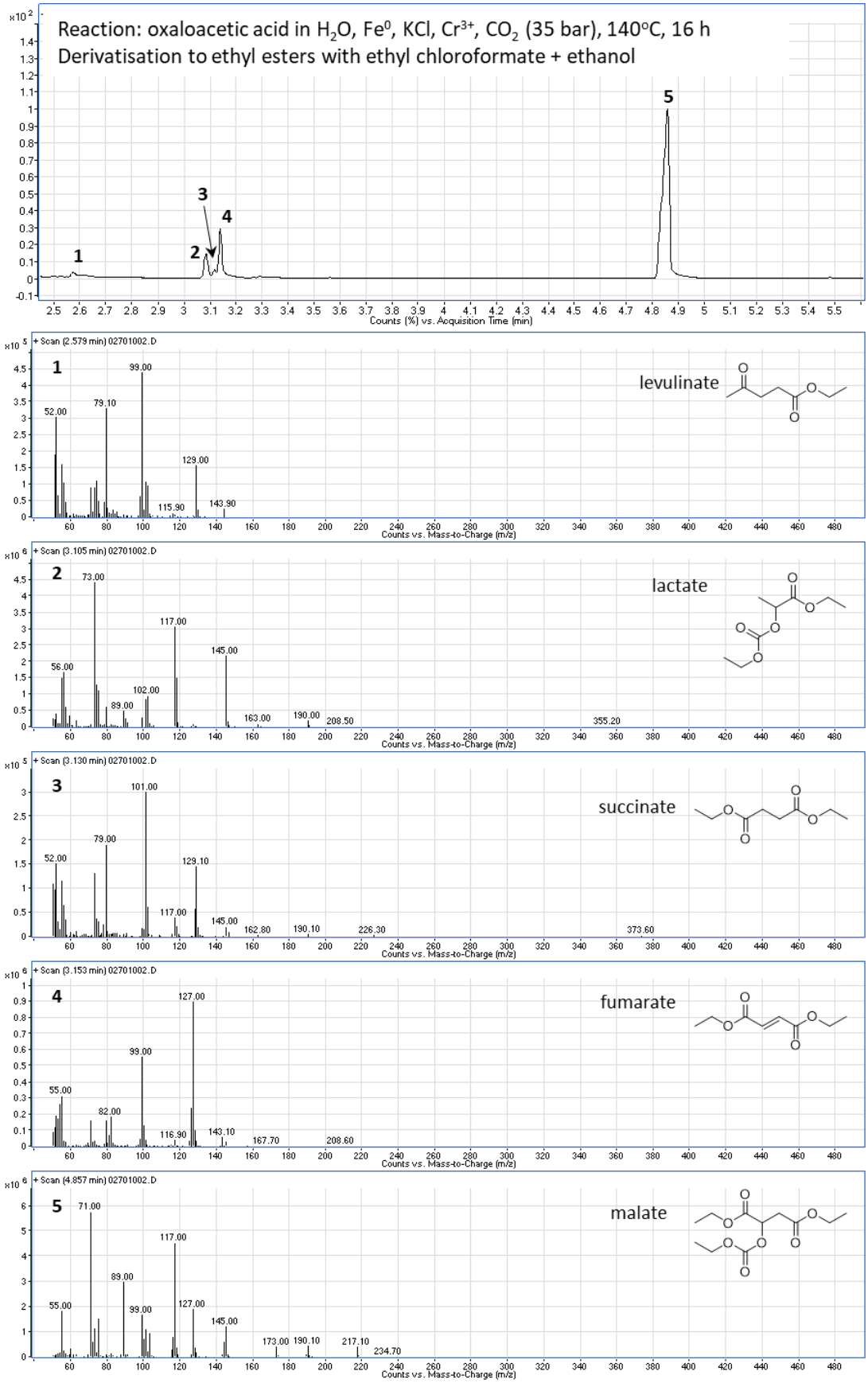
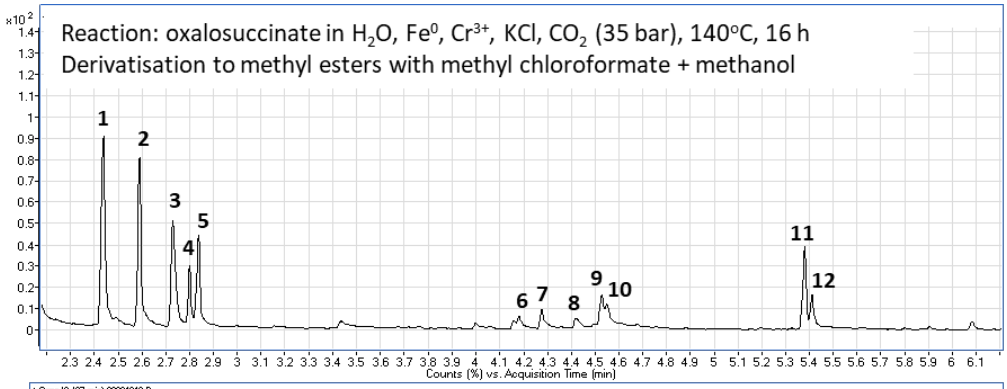
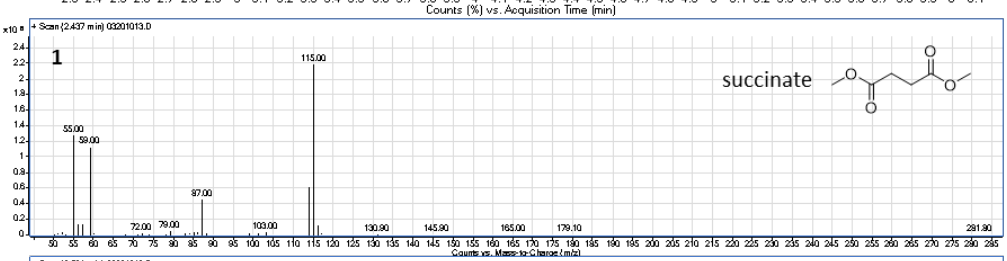
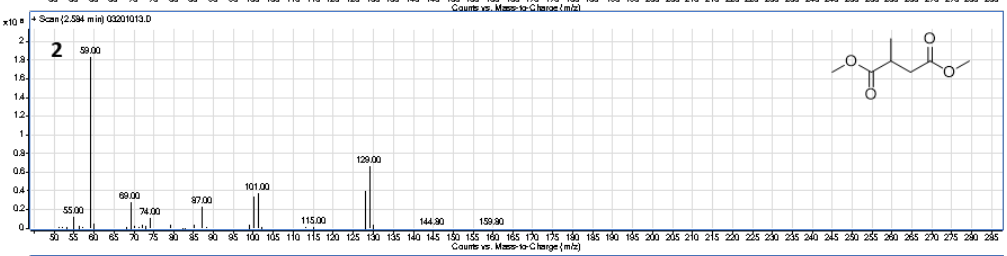
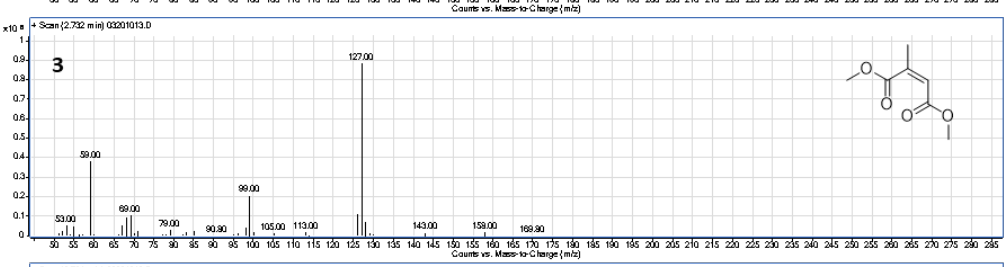


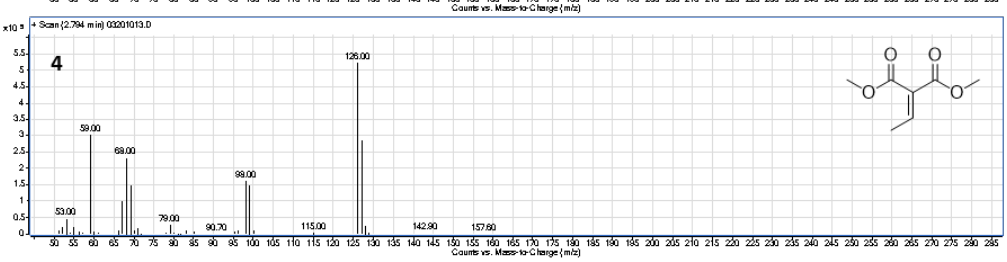
Figure S33 GC traces and mass spectra obtained for the rTCA cycle (3-step sequence: oxaloacetate-malate-fumarate-succinate) reaction mixture, subjected to ethyl chloroformate/ethanol derivatization to ethyl esters.

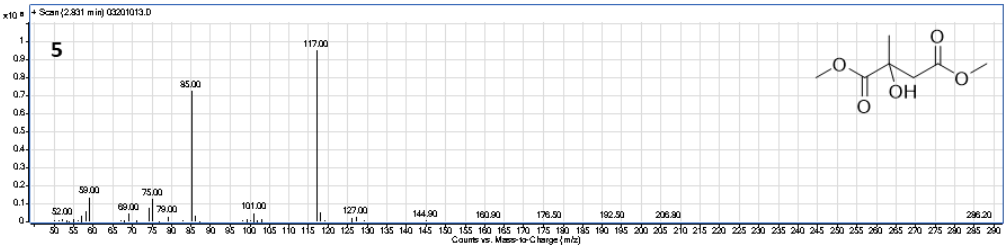

Reaction: oxalosuccinate in H_2O, Fe^0, Cr^{3+}, KCl, CO_2 (35 bar), 140°C, 16 h
Derivatisation to methyl esters with methyl chloroformate + methanol
1
2
3
4
5
6
7
8
9
10
11
12
Counts (%) vs. Acquisition Time (min)

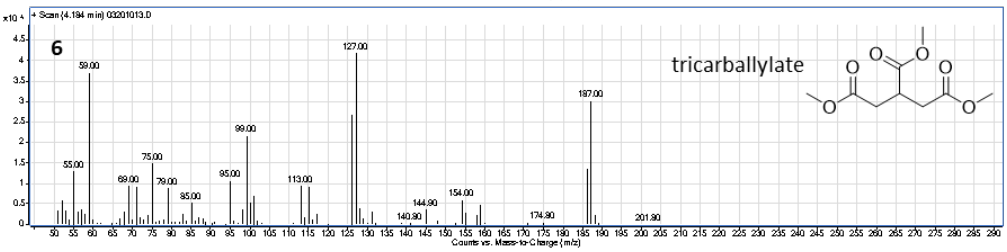

1
succinate
Counts vs. Mass-to-Charge (m/z)


2
Counts vs. Mass-to-Charge (m/z)


3
Counts vs. Mass-to-Charge (m/z)


4
Counts vs. Mass-to-Charge (m/z)


5
OH
Counts vs. Mass-to-Charge (m/z)


6
tricarballylate
Counts vs. Mass-to-Charge (m/z)

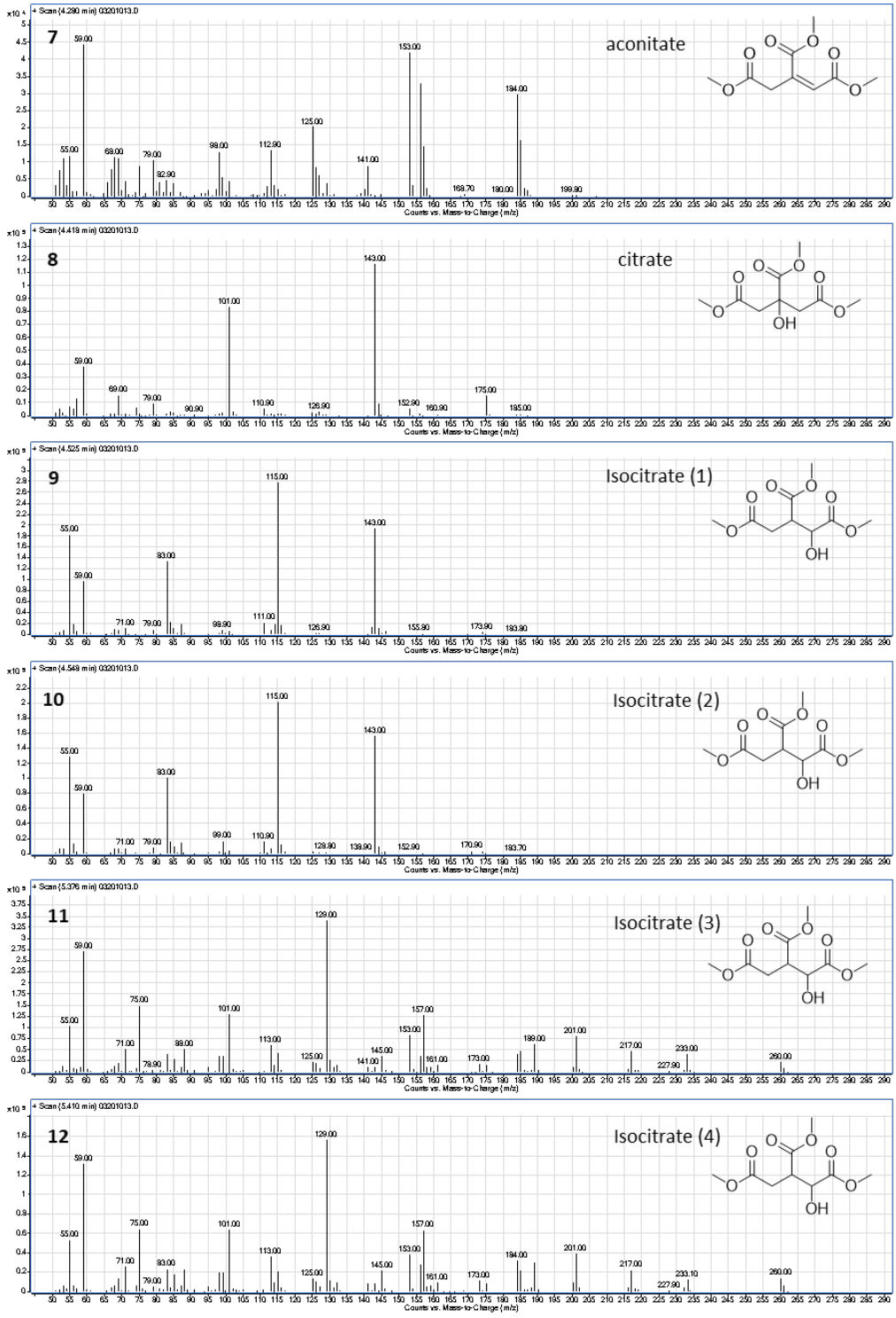


Figure S34 GC traces and mass spectra obtained for the rTCA cycle (3-step sequence: oxalosuccinate-isocitrate-aconitate-citrate) reaction mixture, subjected to methyl chloroformate/methanol derivatization to methyl esters.

F. Compatibility of typical CO_2 fixation conditions with reductive amination of pyruvate

Reductive amination of pyruvate was carried out in two replicas according to general procedure C, following which the mixtures were subjected to a KOH workup. The presence of alanine was confirmed by GC-MS. 200 μL of the basified reaction mixture was derivatized with ethyl chloroformate/ethanol to convert alanine to ethyl (ethoxycarbonyl)alaninate, using a well-established literature procedure.[1] The obtained ester mixtures were subjected to GC-MS measurements. The identity of the detected species was confirmed by the obtained mass spectra (Figure S35), which agree with the data we reported previously.[1]

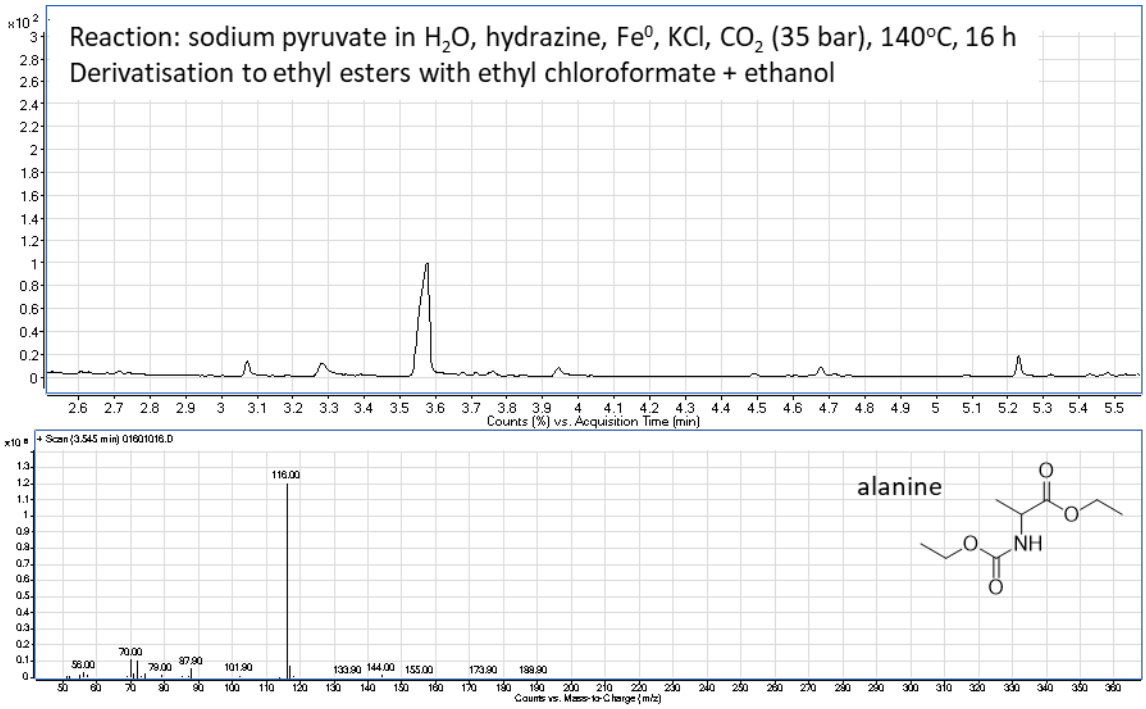


Figure S35 GC traces and mass spectra obtained for the reductive amination reaction mixture, subjected to ethyl chloroformate/ethanol derivatization to ethyl esters.

Supporting references

1. Muchowska, K. B., *et al*. Metals promote sequences of the reverse Krebs cycle. *Nature Ecol. Evol.* **1**, 1716-1721 (2017).