Supporting information

Bio-energy conversion with carbon capture and utilization (BECCU): Integrated biomass fermentation and chemo-catalytic CO_2 hydrogenation for bioethanol and formic acid co-production

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Material and Methods

General

All reagents and solvents were purchased by commercial suppliers. For synthesis, the chemicals were degassed and dried before use. Otherwise, the reagents were used without further purification.

Yeast cultivation

To mimic the industrial process and keep the medium-catalyst interactions assessable, wild type strain *S. cerevisiae* S288C was cultivated in glucose rich Verduyn minimal salt medium for anaerobic growth (VfA).[1] The medium contained, if not otherwise specifically mentioned, 200 g/L glucose, 10 g/L potassium hydrogen phthalate, 5 g/L $(NH_4)_2SO_4$, 3 g/L, KH_2PO_4, 0,5 g/L $MgSO_4$ 7 H_2O, as well as 10 mL/L 100x trace elements, 1 mL/L of 1000x vitamin solution and 1 mL/L of anaerobic growth solution. The vitamin solution contained 0.05 g/L D-biotin, 1 g/L calcium D pantothenate, 1 g/L nicotinic acid, 25 g/L myo-inositol, 1 g/L thiamine hydrochloride, 1 g/L pyridoxine hydrochloride and 0.2 g/L p-aminobenzoic acid. The trace element solution consisted of 1.5 g/L EDTA, 0.45 g/L $ZnSO_4 \cdot 7\ H_2O$, 0.1 g/L $MnCl_2 \cdot 4\ H_2O$, 0.03 g/L $CoCl_2 \cdot 7\ H_2O$, 0.03 g/L $CuSO_4 \cdot 5\ H_2O$, 0.04 g/L $NaMoO_4 \cdot 2\ H_2O$, 0.45 g/L $CaCl_2 \cdot 2\ H_2O$, 0.3 g/L $FeSO_4 \cdot 7\ H_2O$, 0.1 g/L H_3BO_3 and 0.01 g/L KI. The anaerobic growth solution contained ethanol and Tween80 in a 1:1 ratio with 15 g/L ergosterol. The pH was adjusted to 5 after sterile filtration.

Precultures were performed on solid medium containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar. The plates were inoculated with 10 µl of a frozen stock and incubated at 30 °C for 24. Just before each experiment, 3 mL VfA medium was pipetted on the plate and the cells were scraped off with an inoculation loop. The liquid had typically an $OD_{600\ nm}$ of around 70.

Cell solution

For the cell solution used in the experiments, VfA was inoculated with the preculture liquid to an $OD_{600\ nm}$ of 1.0 and then split into the respective number of high pressure reactors or Hungate type anaerobic (16 · 125 mm). While reactors were filled with 6 mL, Hungate type culture tubes were filled with 1 mL to prevent explosions due to the pressure-increase from the fermentation. The tubes and high pressure reactors were subsequently sealed and gas-flushed with nitrogen or argon before reaction at 30°C for 24 or 48 h. While the Hungate tubes were shaken at 300 rpm, the high pressure reactors were stirred with a cross-shaped, Teflon-coated magnetic stir-bar at 1000 rpm. After the reaction, all cell samples were chilled to 8 °C to inhibit further growth until cell counting.

High pressure reactions

High pressure reactions were carried out in stainless steel reactors built and maintained by the mechanical workshop of the *Institute für Technische und Makromolekulare Chemie* of RWTH Aachen University (see Figure 1). The high pressure reactors were equipped with a glass inlet and a magnetic, cross-shaped stir bar. The total inner volume with equipment was determined as 12.5 mL.
To exclude oxygen from the system, in preparation for the experiments vacuum was applied ($< 1 \times 10^{-2}$ mbar) followed by flushing with argon. This procedure was repeated at least three times. Cell solution and catalyst solution were then added in argon counterflow. Hydrogen pressure was applied carefully at about 10 bar s^{-1} if not mentioned otherwise. The high pressure reactors embedded in specially designed aluminium cones were heated and stirred on heating-plates (IKA-Werke GmbH & Co. KG, Staufen, Germany).

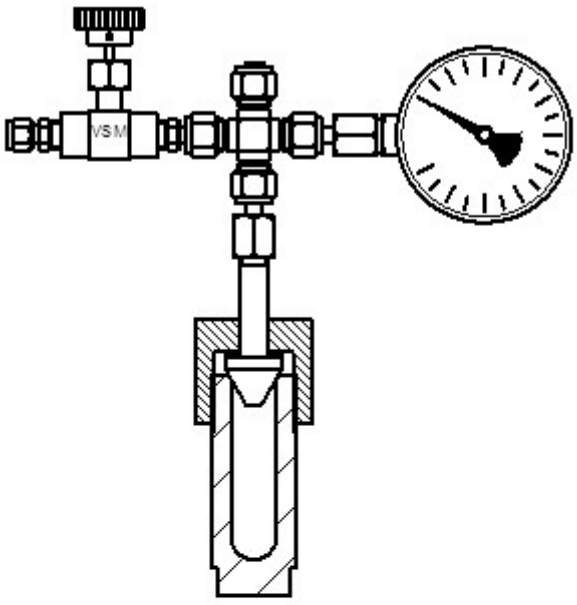


Figure 1: Schematics of the used high pressure reactors (digital manometers were used to record the pressure-curves).

Cell counting with the Ampha Z32

Cells were counted using an Ampha Z32 (Amphasys AG, Root, Switzerland). As the organic phase influences the measurement, the samples were washed with 0.5-fold concentrated phosphate buffered saline (PBS). Samples were diluted 1:50 up to 1:200 depending on the cell density with 0.5-fold concentrated PBS and filtered with a mesh size > 0.1 mm. The chip C00546 (50 µm) was used with 80 rpm pump speed and following setting: frequency 1 20 MHz, frequency 2 12 MHz, stop count cells 10,000, trigger level 0.008 V, modification 5 a.u., amplification 4 a.u. and demolition 1 a.u.. Gating between life and dead cells was set with a sample heated to 90 °C for 10 minutes.

HPLC analytic

HPLC measurements were carried out on a Jasco HPLC (Jasco Deutschland GmbH, Pfungstadt, Germany) equipped with a 300 mm x 8.0 mm Organic-Acid Resin column with a 5 mM aqueous sulfuric acid solution as the eluent at 20 °C. The flow rate was set to 1.0 mL min^{-1} and a sample volume of 10 µL was injected. Amounts of formic acid, glucose, glycerol, ethanol, and acetate were determined based on calibration curves of the RI detector prepared by dilution series of each single substrate in aqueous solution. Samples containing solid biomass were filtered through CHROMAFIL® Xtra PA-45/25 disposable syringe filters directly after withdrawal from the reactor or Hungate tube.

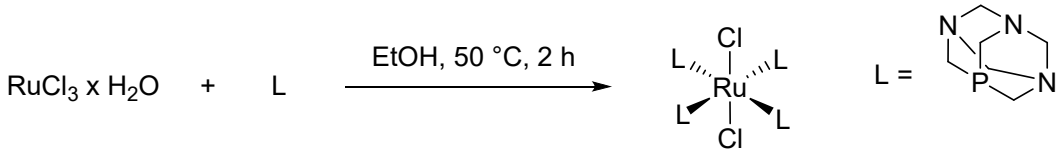
NMR analytic

^{1}H, ^{13}C and ^{31}P NMR measurements were conducted at room temperature on a *Bruker AS 400* (Bruker Corporation, Billerica, MA, USA) spectrometer. The chemical shift δ is given in ppm, the coupling constant J in Hertz. The multiplicities are denoted as s (singlet), br s (broad singlet), d (doublet), t (triplet), m (multiplet). The chemical shift was referenced to the solvent residual signal. Samples that contain biomass were filtered through CHROMAFIL® Xtra PA-45/25 disposable syringe filters before analysis. Quantitative analysis was conducted using maleic acid as internal standard for aqueous solution.

ICP-MS analytic

ICP-MS analytic was carried out on an 8800 ICP-MS Triple Quad by Agilent with water as matrix.

Synthesis of $[RuCl_2PTA_4]$ (Ru-PTA; PTA = 1,3,5-triaza-7-phosphaadamantane)

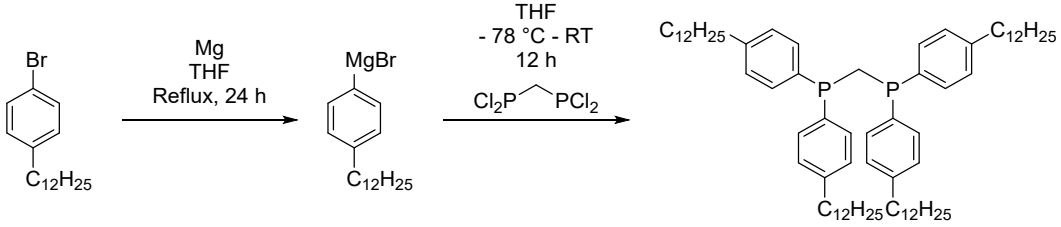


The synthesis was performed as described in literature.[2] To a solution of 1,3,5-triaza-7-phosphaadamantane (307.15 mg, 1.955 mmol, 6.2 eq.) in ethanol (7.5 mL) a solution of ruthenium chloride (71.5 mg, 0.317 mmol, 1 eq.) in ethanol (2.5 mL) was added at 50 °C. The solution was stirred at 50 °C for 2 h and the reaction progress was monitored *via* ^{31}P-NMR. The colour changed from dark brown to a much lighter shade of brown. After completion, the solid product was filtered off and washed with ethanol (10 mL) and acetone (10 mL). After drying *in vacuo,* the product was obtained in quantitative yield as a light orange powder.

$^{1}H\{^{31}P\}$-NMR: **(D_2O, 400,1 MHz)**: δ =4.65 (s, 24H, N-CH_2-N), 4.37 (s, 24H, P-CH_2-N ppm.

$^{31}P\{^{1}H\}$-NMR: **(D_2O, 242.1 MHz):** δ =- 50.25 (s, 4P) ppm.

Synthesis of bis(bis(4-dodecylphenyl)phosphanyl)methane (C_{12}-dppm)



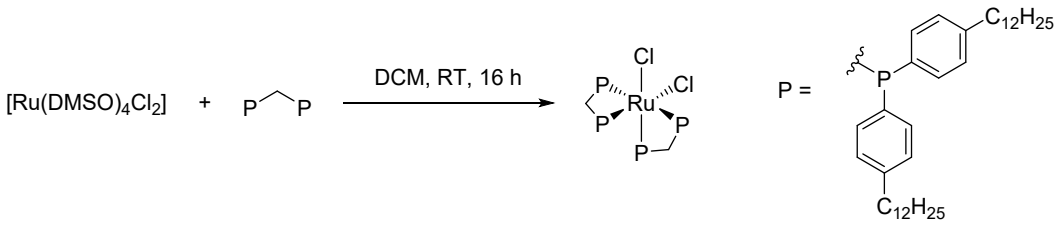
The synthesis was performed similarly to a literature procedure.[3] In a *Schlenk*-flask THF (15 mL) and magnesium turnings (0.370 g, 15.2 mmol, 6.6 eq.) were added and heated to 40 °C. A solution of 1-bromo-4-dodecylbenzene (4.030 g, 12.4 mmol, 5.4 eq.) in THF (10 mL) was added dropwise in a manner that the solution was refluxing self-sustainingly. After complete addition, the solution was refluxed for additional 12 h. The resulting Grignard-solution was filtered and separated from residual magnesium. This solution was cooled to -78 °C and a solution of 1,2-bis(dichlorophosphino)methane (0.500 g, 2.3 mmol, 1.0 eq.) in THF (4.5 mL) was added over 45 minutes. The mixture was then allowed to warm to room temperature and stirred for 12 h. Completion of the reaction was confirmed *via* ^{31}P-NMR. An aqueous, degassed NH_4Cl-solution (20 mL) was then added, the organic phase separated and the aqueous phase extracted with *n*-pentane (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and filtered over Celite®. The solvent was removed *in vacuo* and excess dodecylbenzene was removed at high vacuum (1 × 10^{-3} mbar) and 120 °C. The resulting colourless, viscous oil was obtained (2.07 g, 85 % yield).

$^{1}H\{^{31}P\}$-NMR: **($CDCl_3$, 400,1 MHz)**: δ =7,33 (d, $^{3}J_{H,H}$ = 7,83 Hz, 8H, Ar-*H*), 7,12 (d, $^{3}J_{H,H}$ = 7,83 Hz, 8H, *H*-Ar), 2,77z (s, 2H, P-CH_2-P), 2,57 (t, $^{3}J_{H,H}$ = 7,66 Hz, 8H, Ar-CH_2-CH_2-) 1,59 (m, 6H, Ar-CH_2-CH_2) 1,30 (m, 40H, -CH_2-(CH_2)$_5$-CH_3), 0,88 (t, $^{3}J_{H,H}$ = 6,57 Hz, 12H, -CH_2-(CH_2)$_5$- CH_3) ppm.

$^{13}C\{^{1}H\}$-NMR: **($CDCl_3$, 100.1 MHz):** δ =143,1 (s, Ar-*C*q), 136,0 (t, $^{2}J_{P,C}$ = 3,0 Hz, P-*C*q), 132,9 (t, $^{3}J_{P,C}$ = 10,6 Hz, Ar-*C*), 128,5 (t, $^{4}J_{P,C}$ = 3,6 Hz, Ar-*C*), 35,9 (s, P-*C*H_2-P), 32,0 (s, Ar-*C*H_2- *C*H_2), 31,5 (s, Ar-CH_2-*C*H_2), 29,9-22,8 (-*C*H_2-), 14,2 (s, CH_2-*C*H_3) ppm.

$^{31}P\{^{1}H\}$-NMR: **($CDCl_3$, 242.1 MHz):** δ =-25.0 (s, 2P, *P*Ph) ppm.

Synthesis of *cis*-[$RuCl_2(C_{12}$-dppm)$_2$] (Ru-C_{12}-dppm)



The synthesis was performed similarly to a literature procedure.[3] In a *Schlenk*-tube a suspension of [$Ru(DMSO)_4Cl_2$] (0.41 g, 0.84 mmol, 1 eq.) and C_{12}-dppm (1.78 g, 1.68 mmol, 2 eq.) in CH_2Cl_2 (20 mL) was stirred at room temperature for 16 h. During this time, the yellow suspension became a clear solution changing the colour from colourless over pale yellow to orange. The progress of the reaction was monitored with ^{31}P-NMR. After completion, the solvent was removed *in vacuo*, the residue dissolved in *n*-decane (15 mL) and washed with methanol (3 × 10 mL) to remove DMSO. The emulsion of methanol and *n*-decane was separated *via* centrifugation. After removal of *n*-decane *in vacuo*, a resinous, orange solid was obtained (1.73 g, 90 % yield).

^{1}H{^{31}P}-NMR: **($CDCl_3$, 400,1 MHz):** δ = 8,07 (d, $^3J_{H,H}$ = 8,03 Hz, 4H, Ar-H), 7,80z (d, $^3J_{H,H}$ = 8,03 Hz, 4H, Ar-H), 7,15 (d, $^3J_{H,H}$ = 8,03 Hz, 4H, Ar-H), 6,88 (d, $^3J_{H,H}$ = 8,03 Hz, 4H, Ar-H) 6,72 (d, $^3J_{H,H}$ = 8,20 Hz, 4H, Ar-H), 6,75 (d, $^3J_{H,H}$ = 8,20 Hz, 4H, Ar-H), 6,45 (d, $^3J_{H,H}$ = 8,05 Hz, 4H, Ar-H), 6,25 (d, $^3J_{H,H}$ = 8,05 Hz, 4H, Ar-H), 4,68 (d, $^2J_{H,H}$ = 15,27 Hz, 2H,P-CH_2-P), 4,39 (d, $^2J_{H,H}$ = 15,27 Hz, 2H,P-CH_2-P), 2,56-2,28 (m, 8H, Ar-CH_2-CH_2), 1,59-1,37 (m, 8H, Ar-CH_2-CH_2), 1,29-1,05 (m, 144H, -CH_2-), 0,84-0,75 (m, 24H, -CH_3) ppm.

^{13}C{^{1}H}-NMR: **($CDCl_3$, 100,1 MHz):** δ = 144,6 (s, Ar-*C*q), 144,4 (s, Ar-*C*q), 143,7 (s, Ar-*C*), 143,4 (s, Ar- Cq), 134,1 (m, Ar-*C*), 133,1 (m, Ar-*C*), 132,2 (m, Ar-*C*), 130,7 (m, Ar-*C*), 128,8 (m, Ar- C), 128,6 (m, Ar-*C*), 127,7 (m, Ar-*C*), 126,7 (m, Ar-*C*), 36,0 (m, Ar-*C*H_2-CH_2), 32,1(m, Ar-CH_2-*C*H_2), 31,3-22,8 (-*C*H_2-), 14,2 (m, *C*H_3) ppm.

^{31}P{^{1}H}-NMR: **($CDCl_3$, 242,1 MHz):** δ = -1,7 (t, 2P, $^3J_{P,P}$ = 37,2 Hz *P*cisPh) -28,9 (t, 2P, $^3J_{P,P}$ = 37,2 Hz *P*cisPh) ppm.

Supplementary results

Influence of hydrogen pressure on cell proliferation

A cell-solution (6 mL) was added into the high pressure reactor in argon counterflow, the high pressure reactor was closed and pressurised with H_2 (60 bar) at different speeds (Figure 2, left) or at different pressures at about 10 bar per second (Figure 2, right). After stirring at 1000 rpm for 24 h at 30 °C, the pressure was released, the resulting aqueous suspension withdrawn and cooled down to 8 °C and the cells were counted with the Ampha Z32 as described above.

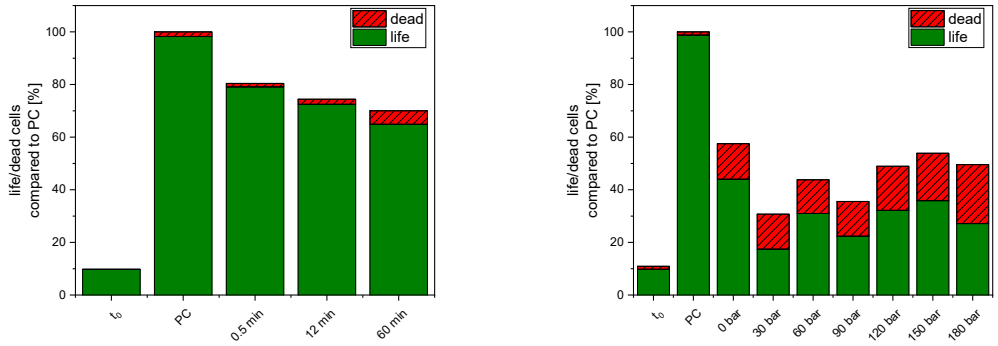


Figure 2: Influence of the speed in which 60 bar H_2 pressure is applied to the inoculated reactor (left). Different pressures added (right).

Influence of the organic phase on the cell proliferation

The preparation of the cell solution was conducted as described above but to a lower $OD_{600\ nm}$ to better monitor the growth behaviour. To an aliquot of this solution (2 mL) in Hungate tubes, the corresponding organic solvent (1 mL) was added. The tubes were shaken at 300 rpm for over 10 h at 30 °C. For sampling, the Hungate tubes were inverted vertically and it was waited for a clear phase separation. The samples were analysed with the Ampha Z32 as described above.

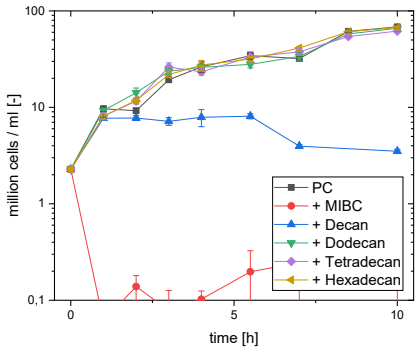


Figure 3: Influence of 1 mL organic solvent on the anaerobic growth of S. cerevisiae S288C in Hungate tubes.

Production of formate in an integrated system by Ru-PTA

The indicated amount of Ru-PTA (see figure 4) was dissolved in the cell solution (6 mL), resulting in a monophasic mixture. This solution was then transferred into the high pressure reactor in argon counterflow, the high pressure reactor was closed and pressurised with H_2 (60 bar). After stirring at 1000 rpm for 24 h at 30 °C, the pressure was released, the resulting aqueous suspension withdrawn, cooled down to 8 °C and formic acid was quantified by ^{1}H-NMR in D_2O. Turnover numbers correspond to the produced molecules of formic acid per catalyst-molecule.

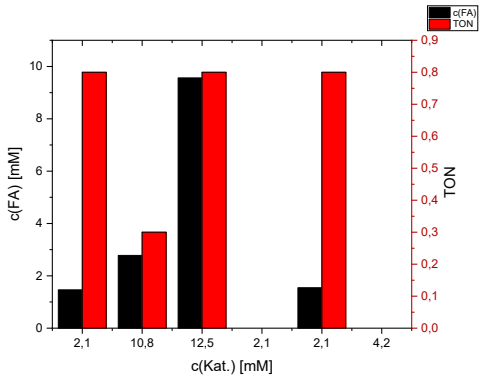


Figure 4: Production of formic acid by Ru-PTA catalyst in presence of S. cerevisiae and H_2 (60 bar) after 24 h in multiple experiments and with different catalyst concentrations.

Catalytic activity of Ru-PTA under model conditions

Ru-PTA (4 µmol) was dissolved in VfA (6 mL) or water (6 mL). This solution was then transferred into the high pressure reactor in argon counterflow, the high pressure reactor was closed and pressurised with CO_2 (5 bar) and H_2 (60 bar). After stirring at 1000 rpm for 24 h at 30 °C, the pressure was released and formic acid was quantified by ^{1}H-NMR in D_2O. Turnover numbers correspond to the produced molecules of formic acid per catalyst-molecule.

Table 1: Formic acid concentration and corresponding TON from model experiments with Ru-PTA in VfA and water with externally pressurized CO_2 instead of fermentation.

Aqueous Medium	c(FA)	TON
	[mM]	
VfA	2.65	4
Water	3.03	4

Influence of hydrogen pressure on formate production

The cell solution (6 mL) and Ru-C_{12}-dppm (4 µmol) dissolved in tetradecane (1 mL) were added to the high pressure reactor in argon counterflow. The high pressure reactor was closed and pressurised with the corresponding H_2-pressure. After stirring at 1000 rpm for 24 h at 30 °C, the pressure was released, the resulting aqueous suspension withdrawn, cooled down to 8 °C and formic acid was quantified by HPLC as described above.

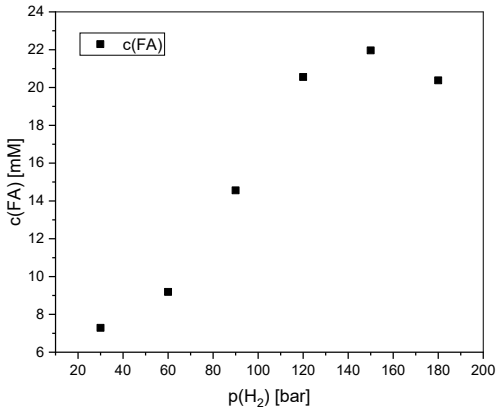


Figure 5: Formate concentration after 24 h reaction at different hydrogen pressure.

Influence of basic amino acids on cell proliferation

The corresponding amounts of arginine, histidine and lysine were added to the cell solution (2 mL) in Hungate tubes. The tubes were shaken at 300 rpm for 24 h at 30 °C and the cells were counted with the Ampha Z32 as described above. The relative cell-count is referenced to the positive control (cell-solution without additives).

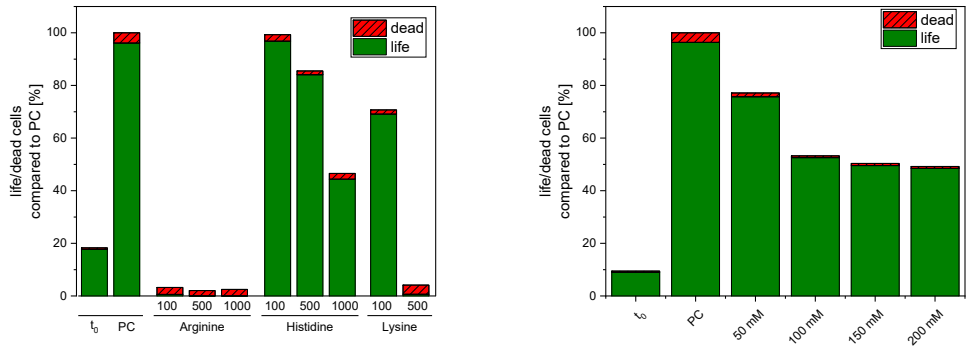


Figure 6: Influence of added basic amino acids on the cell proliferation (left) and a more detailed test on histidine (right). Tests were performed in Hungate tubes at 30 °C for 24 h at the concentrations, all concentrations are in mM.

Production and consumption of histidine by *S. cerevisiae* S288C

Histidine was added to the cell solution (2 mL) in Hungate tubes obtaining a 150 mM solution. The tubes were shaken at 300 rpm for 24 and 48 h at 30 °C. The concentration of histidine at the start (t_0), after 24 h and 48 h was determined by ^{1}H-NMR and is normalized to t_0 = 100 %. The experiments were conducted in triplicates.

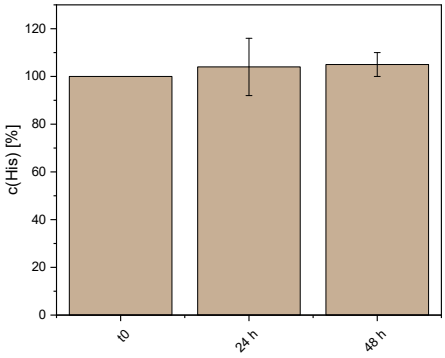


Figure 7: Histidine concentration before and after incubation of S. cerevisiae S288C in VfA spiked with 0.15 M histidine

Influence of formate on *S. cerevisiae* S288C

Potassium formate was added to the cell solution (2 mL) in Hungate tubes, obtaining a 100 mM solution. The tubes were shaken at 300 rpm for 24 h at 30 °C. The concentration of formate at the start (t_0) and after 24 h was determined by HPLC and normalized to t_0 = 100 %. The cells were counted with the Ampha Z32 as described above. The relative cell-count was referenced to the positive control (cell solution without additives). In the positive control, no formic acid was detected.

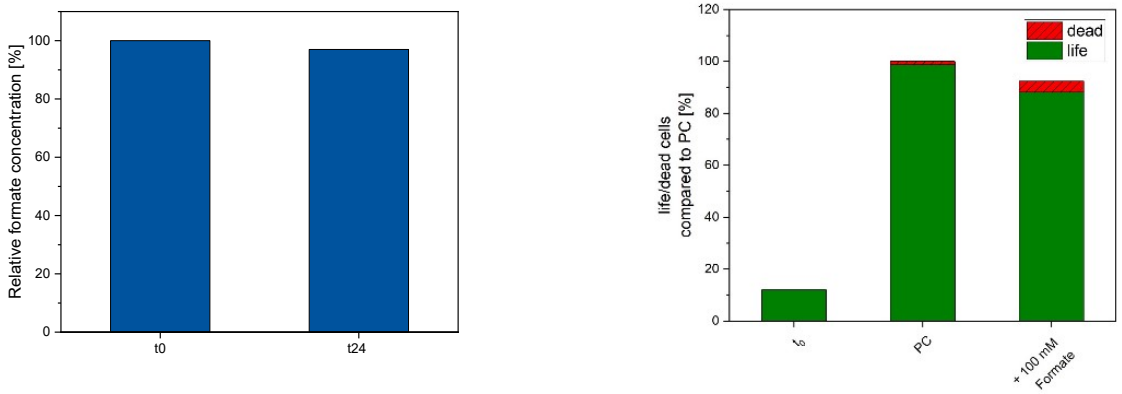


Figure 8: Formate concentration (left) and cell counts (right) before and after 24 h incubation of S. cerevisiae S288C in VfA spiked with HCOOK (0.1 M)

Influence of Histidine on the pH

The cell solution (6 mL) conatining histidine (150 mM) and Ru-C_{12}-dppm (4 µmol) in tetradecane (1 mL) were added to the high pressure reactor in argon counterflow. The high pressure reactor was closed and pressurised with H_2 (60 bar). After stirring at 1000 rpm for 24 h or 48 h respectively at 30 °C, the pressure was released, the resulting aqueous suspension withdrawn, cooled down to 8 °C and the pH was determined roughly by pH-indicators.

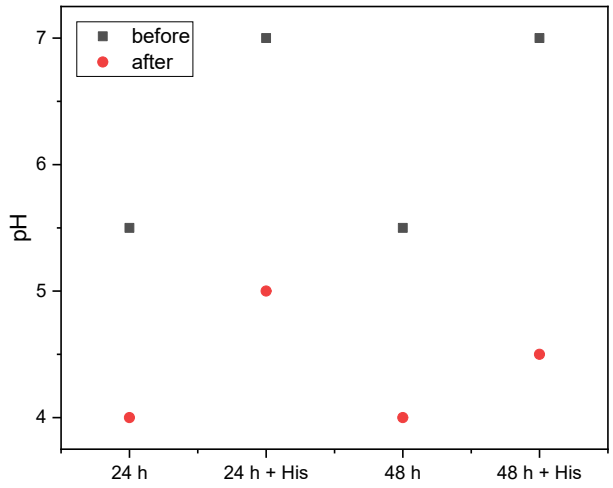


Figure 9: Influence of the addition of 150 mM histidine on the pH of the medium before (black squares) and after (red circles) fermentation.

Production of selected metabolites and pressure curves under optimized conditions

In tables 2-6 and figures 10-17 the results of experiment series conducted under different conditions with and without added histidine are summarised. In particular, the metabolites of the fermentation and the catalytically formed formic acid (all quantified by HPLC) and the resulting C-balance are given. The residual amount of CO_2 in mmol was calculated by the ideal gas law. The C-balance was calculated from the initial amount of glucose considering all quantified metabolites, formate and CO_2. The results are shown with standard deviation (SD) from five-fold determination (six experiments conducted, one outlier eliminated). The experiments were conducted at the optimized H_2 pressure of 120 bar and with a reduced catalyst amount of 2 µmol.

The experiments with histidine were conducted using a cell solution was prepared as described above with additional histidine to reach an end concentration of 150 mM.

The cell solution (6 mL) and Ru-C_{12}-dppm (2 µmol) in tetradecane (1 mL) were added to the high pressure reactor in argon counterflow. The high pressure reactor was closed and pressurised with H_2 (120 bar). After stirring at 1000 rpm for 24 h or 48 h respectively at 30 °C, the pressure was released, the resulting aqueous suspension withdrawn, cooled down to 8 °C and quantified by HPLC as described above.

Table 2: Metabolites after 24 h fermentation.

run	Δ(p)	n(CO_2)	c(FA)	TON	c(Glu)	cEtOH)	c(Glyc)	c(Ac)	C-balance
	[bar]	[mmol]	[mM]	[-]	[mM]	[mM]	[mM]	[mM]	[%]
176-1	0.94	4.0	7.40	23	908	336	22.6	0.0	95.2
176-3	0.96	4.0	9.17	29	899	343	23.1	0.0	94.7
177-1	0.74	3.0	10.17	29	844	467	27.8	0.0	93.2
177-2	1.17	4.9	11.26	37	842	467	27.1	0.0	94.0
177-3	1.16	4.8	12.37	38	842	462	27.7	0.0	93.8
Average	1.0	4.1	10.0	31	867	415	26	0.0	94.0
SD	0.2	0.7	2.0	6	30	62	2.3	0,0	0.3

Conditions: reactor volume = 12.5 mL, H_2 = 120 bar, [Ru] = 2 µmol, tetradecane (1 mL), VfA medium (6 mL) with glucose (20 wt%), 1000 rpm, 30 °C; 24 h.

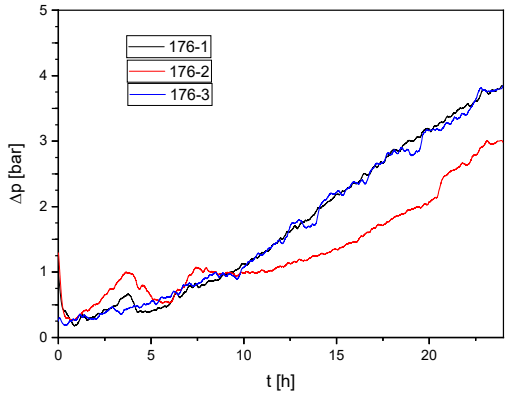


Figure 10: Pressure curves for 24 h fermentation.

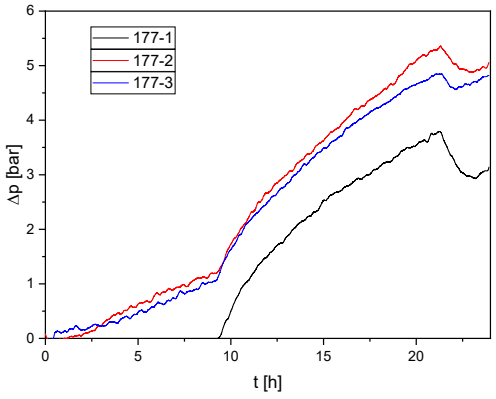


Figure 11: Pressure curves for 24 h fermentation.

Table 3: Metabolites after 48 h fermentation

	Δ(p)	n(CO_2)	c(FA)	TON	c(Glu)	c(EtOH)	c(Glyc)	c(Ac)	C-balance
	[bar]	[mmol]	[mM]	[-]	[mg L^{-1}]	[mM]	[mM]	[mM]	[%]
190-1	7.6	1.87	26.26	86	698	727	33.2	0.0	91.1
190-2	7.5	1.84	24.74	83	688	747	32.7	0.0	90.6
190-3	7.2	1.76	21.34	66	714	701	32.8	0.0	91.2
195-1	6.0	1.53	28.95	93	743	547	31.1	0.0	88.8
195-2	5.6	1.41	25.04	79	770	530	29.7	0.0	90.4
Average	6.8	1.70	25.3	82	723	651	31.9	0.0	90.4
SD	0.8	0.20	2.5	9	30	93	1.3	0.0	0.9

Conditions: reactor volume = 12.5 mL, H_2 = 120 bar, [Ru] = 2 µmol, tetradecane (1 mL), VfA medium (6 mL) with glucose (20 wt%), 1000 rpm, 30 °C; 48 h.

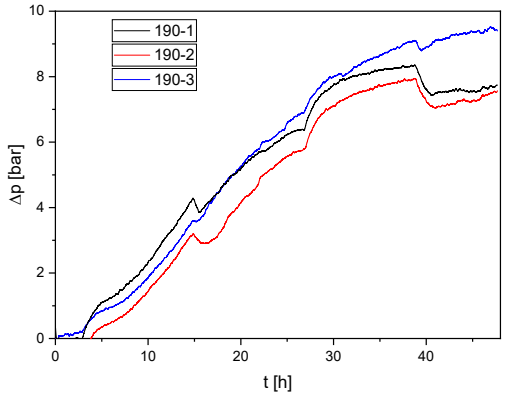


Figure 12: Pressure curves for 48 h fermentation.

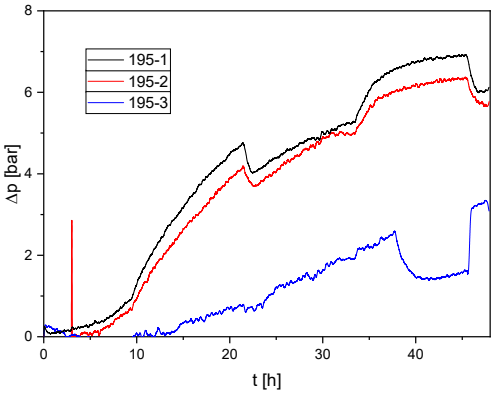


Figure 13: Pressure curves for 48 h fermentation.

Table 4: Metabolites after 24 h fermentation with 150 mM histidine.

	Δ(p)	n(CO_2)	c(FA)	TON	c(Glu)	cEtOH)	c(Glyc)	c(Ac)	C-balance
	[bar]	[mmol]	[mM]	[-]	[mg L^{-1}]	[mM]	[mM]	[mM]	[%]
189-1	1.1	0.47	35.17	116	979	164	24.0	4.8	96.0
189-2	1.3	0.47	27.48	87	976	174	23.6	5.6	95.9
206-1	1.6	0.16	25.32	82	922	128	18.8	10.0	88.8
206-2	2.6	0.16	25.79	83	901	145	21.0	10.8	87.8
206-3	1.9	0.16	25.30	84	899	157	21.4	11.0	87.7
Average	1.8	0.3	25.3	83	936	154	21.4	8.6	91.3
SD.	0.5	0.2	1.5	4	37	16	1.6	2.5	3.9

Conditions: reactor volume = 12.5 mL, H_2 = 120 bar, [Ru] = 2 µmol, tetradecane (1 mL), VfA medium (6 mL) with glucose (20 wt%) and histidine (150 mM), 1000 rpm, 30 °C; 24 h.

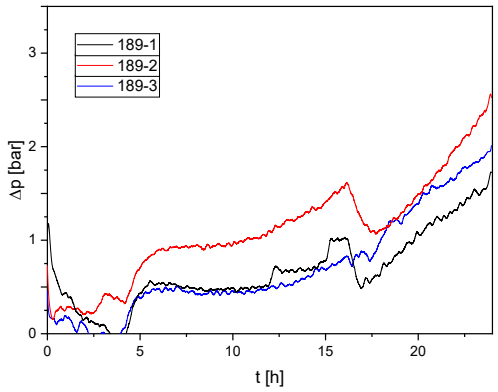


Figure 14: Pressure curves for 24 h fermentation with 150 mM histidine.

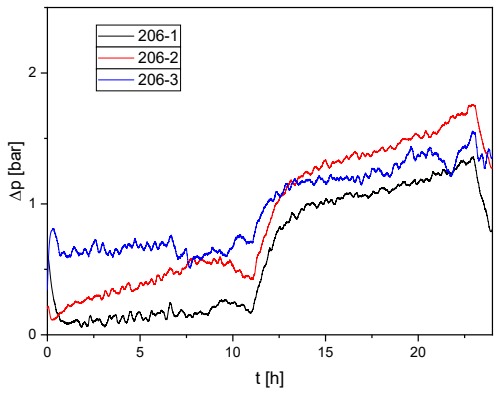


Figure 15: Pressure curves for 24 h fermentation with 150 mM histidine.

Table 5: Metabolites after 48 h fermentation with 150 mM histidine.

	Δ(p)	n(CO_2)	c(FA)	TON	c(Glu)	cEtOH)	c(Glyc)	c(Ac)	C-balance
	[bar]	[mmol]	[mM]	[-]	[mg L^{-1}]	[mM]	[mM]	[mM]	[%]
173-1	2.1	1.40	141.21	414	789	476	47.7	7.96	93.1
173-2	4.1	1.69	113.84	398	746	559	52.6	8.28	92.1
173-3	2.1	1.31	131.58	417	771	505	50.8	7.84	92.2
186-1	1.8	1.23	128.55	401	815	437	46.4	7.77	93.7
186-2	1.9	1.23	125.58	397	809	443	47.9	7.93	93.3
Average	2.4	1.4	128	406	786	484	49.1	8.0	92.9
SD.	0.9	0.2	9	8	25	45	2.3	0.2	0.6

Conditions: reactor volume = 12.5 mL, H_2 = 120 bar, [Ru] = 2 µmol, tetradecane (1 mL), VfA medium (6 mL) with glucose (20 wt%) and histidine (150 mM), 1000 rpm, 30 °C; 48 h

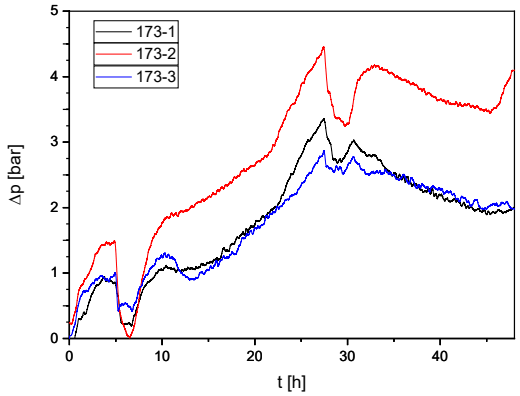


Figure 16: Pressure curves for 48 h fermentation with 150 mM histidine.

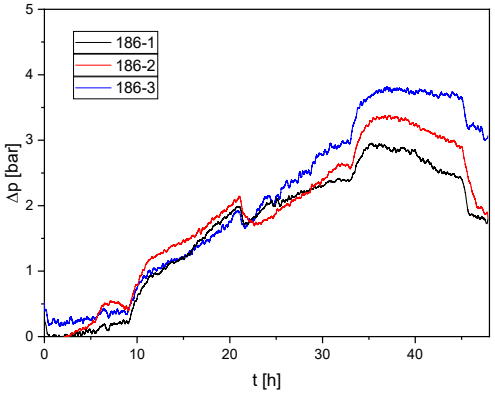


Figure 17: Pressure curves for 48 h fermentation with 150 mM histidine.

The cells were counted with the Ampha Z32 as described above for each of the experiments in tables 2-5 and are shown in Figure 18 (left). The ethanol and formate production is summed up in Figure 18 (right).

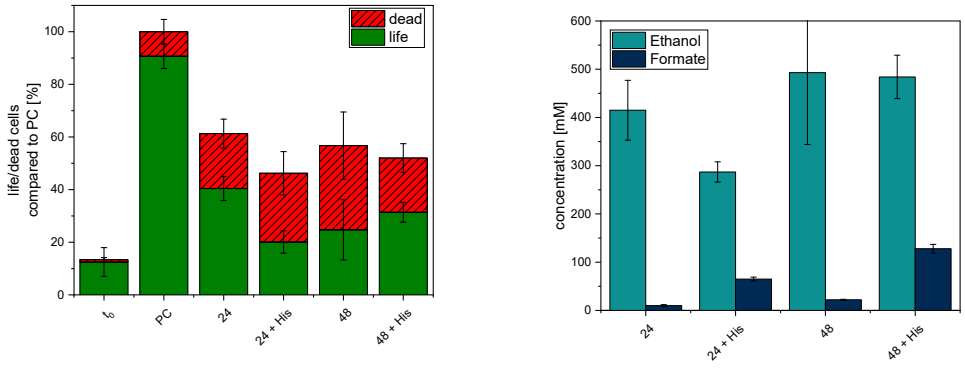


Figure 18: Cell counts as well as ethanol and formic acid production during the cultivations.

Catalyst Recycling

The cell solution was prepared as described above and histidine was added to reach an end concentration of 150 mM. The resulting cell solution (6 mL) and Ru-C_{12}-dppm (2 µmol) in tetradecane (1 mL) were added to the high pressure reactor in argon counterflow. The high pressure reactor was closed and pressurised with H_2 (120 bar). After stirring at 1000 rpm for 24 h or 48 h respectively at 30 °C, the pressure was released by connecting the outlet of the high pressure reactor to a *Schlenk* line with overpressure valve. After the pressure was released, the phases were allowed to settle for 30 minutes. Then, the aqueous solution (~5.5 mL) was taken from the bottom of the high pressure reactor by an argon-flushed syringe under argon counterflow. Although this operation was carefully done, the withdrawn of small amounts of catalyst-phase may still occur, as the phase separation could not be observed visually. The withdrawn aqueous phase was cooled down to 8 °C and was quantified by HPLC as described above (results see table 6).

For the consecutive recycling run, freshly prepared cell-solution was added and the procedure was repeated. Runs 1-3 were performed as triplicates, while run 4 and 5 were a single determination. The concentrations of formic acid and ethanol resulting from 5 consecutive recycling runs are depicted in Figure 20.

Table 6: Metabolites for each recycling-run with 150 mM histidine after 48 h.

run	Δ(p)	n(CO_2)	c(FA)	TON	c(Glu)	cEtOH)	c(Glyc)	c(Ac)	C-balance
	[bar]	[mmol]	[mM]	[-]	[mg L^{-1}]	[mM]	[mM]	[mM]	[%]
1-1		0.90	139.00	492	790	482	47.4	7.54	92.2
1-2	3.3	1.60	133.51	455	766	547	49.5	8.51	93.6
1-3	2.4	1.36	128.21	427	782	507	50.3	7.85	93.3
Average	2.9	1.3	133.60	458	779	512	49.0	8.0	93.0
SD.	0.5	0.3	4.5	27	10	27	1.2	0.4	0.6
2-1		0.67	104.18	364	775	508	49.4	8.07	90.7
2-2	3.80	1.33	74.39	252	767	547	48.1	8.38	92.2
2-3	2.00	1.22	119.65	402	810	440	44.2	7.44	93.0
Average	2.9	1.1	99.4	339	784	498	47.2	8.0	91.9
SD.	0.9	0.3	18.8	64	18	44	2.2	0.4	0.9
3-1		0.28	43.09	153	780	524	52.8	7.95	89.9
3-2	4.30	1.36	61.85	208	744	586	51.2	8.18	91.3
3-3	4.60	1.35	49.06	162	756	589	52.7	7.80	92.3
Average	4.5	1.0	51.3	174	760	566	52.2	8.0	91.2
SD.	0.2	0.5	7.8	24	15	30	0.8	0.2	1.0
4	5.4	49.37	19.53	65	732	535	48.2	18.15	88.1
5	5.6	49.52	16.02	52	672	591	48.3	7.29	84.2

Conditions: reactor volume = 12.5 mL, H_2 = 120 bar, [Ru] = 2 µmol, tetradecane (1 mL), VfA medium (6 mL) with glucose (20 wt%) and histidine (150 mM), 1000 rpm, 30 °C; 48 h.

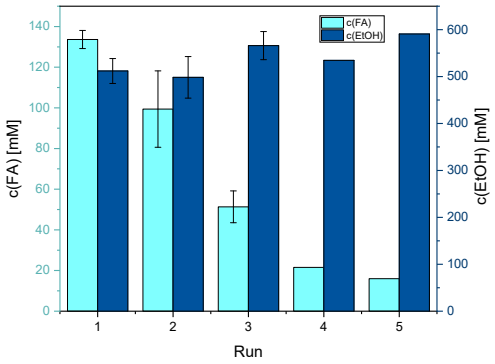


Figure 19: Concentration of formic acid (FA) and Ethanol (EtOH) over five consecutive recycling runs.

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