

Co-Fermenting Pyrolysis Aqueous Condensate and Pyrolysis Syngas with Anaerobic Microbial Communities Enables L-Malate Production in a Secondary Fermentative Stage

Alberto Robazza ¹, Claudia Welter ¹, Christin Kubisch ¹, Flávio César Freire Baleeiro ^{1,2}, Katrin Ochsenreither ¹, Anke Neumann ^{1,*}

¹ Institute of Process Engineering in Life Sciences 2: Technical Biology, Karlsruhe Institute of Technology –KIT, 76131 Karlsruhe, Germany

² Department of Environmental Microbiology, Helmholtz Centre for Environmental Research –UFZ, 04318 Leipzig, Germany

* Correspondence: anke.neumann@kit.edu

Table of Content

Table S1. Conversion factors for carbon and electron balances.....	2
Table S2. Productivities (mM/d) of selected metabolites calculated at 39 days EFT for bottles of the control experiments M-CTRL, T-CTRL, M-BES, T-BES.	2
Table S3. Acetate, propionate and n-butyrate productivities for all bottles of M-PAC and T-PAC experiments.....	3
Table S4. Productivities of CO, CH ₄ , H ₂ CO ₂ and VFAs in mM/d at increasing PAC concentrations and different temperatures. Negative productivity indicates consumption.	4
Figure S1. Cumulative CO uptake in mmol for experiments M-BES, M-CTRL and M-PAC.	5
Figure S2. Cumulative H ₂ uptake in mmol for experiments M-BES, M-CTRL and M-PAC.....	5
Figure S3. Cumulative CO ₂ production in mmol for experiments M-BES, M-CTRL and M-PAC. Negative values mean consumption.	6
Figure S4. Cumulative CH ₄ production in mmol for experiments M-BES, M-CTRL and M-PAC. ..	Error!
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Figure S5. Cumulative CO uptake in mmol for experiments T-BES, T-CTRL and T-PAC.	Error!
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Figure S6. Cumulative H ₂ uptake in mmol for experiments T-BES, T-CTRL and T-PAC. Negative values mean consumption.	Error! Bookmark not defined.
Figure S7. Cumulative CO ₂ production in mmol for experiments T-BES, T-CTRL and T-PAC.	Error!
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Figure S8. Cumulative CH ₄ production in mmol for experiments T-BES, T-CTRL and T-PAC.	8

1. Analytical methods and calculations

Table S1. Conversion factors for carbon and electron balances.

Compound	Chemical Formula	Molecular Weight (g/mol)	mol C/mol	mol e ⁻ /mol
Acetate	C ₂ H ₄ O ₂	60.0	2.0	8.0
Propionate	C ₃ H ₆ O ₂	74.0	3.0	14.0
Lactate	C ₃ H ₆ O ₃	90.0	3.0	12.0
L-Malate	C ₄ H ₆ O ₅	134.1	4.0	12.0
<i>n</i> -Butyrate	C ₄ H ₈ O ₂	88.0	4.0	20.0
<i>n</i> -Valerate	C ₅ H ₁₀ O ₂	102.1	5.0	26.0
<i>n</i> -Caproate	C ₆ H ₁₂ O ₂	116.1	6.0	32.0
Heptanoate	C ₇ H ₁₄ O ₂	130.2	7.0	38.0
Ethanol	C ₂ H ₆ O	46.0	2.0	12.0
Propanol	C ₃ H ₈ O	60.1	3.0	18.0
Butanol	C ₄ H ₁₀ O	74.0	4.0	24.0
Hydrogen	H ₂	2.0	0.0	2.0
Carbon Monoxide	CO	28.0	1.0	2.0
Carbon Dioxide	CO ₂	44.0	1.0	0.0
Methane	CH ₄	16.0	1.0	8.0

2. Mesophilic and thermophilic anaerobic mixed microbial cultures grown on pyrolysis synthetic syngas

Table S2. Productivities (mM/d) of selected metabolites calculated at 39 days EFT for bottles of the control experiments M-CTRL, T-CTRL, M-BES, T-BES. Gas productivities are the mean value of the productivities calculated between each sampling interval. C2-C6 metabolites productivities were calculated by dividing the net metabolite accumulation (mM) at the end of the fermentation by the total fermentative time. Not detected (nd).

	M-CTRL		T-CTRL		M-BES		T-BES	
	Av.	St.Dev.	Av.	St.Dev.	Av.	St.Dev.	Av.	St.Dev.
CO [mM/d]	-8.17	0.45	-34.30	1.02	-7.68	0.59	-28.70	1.60
CH ₄ [mM/d]	4.03	0.18	11.98	0.36	nd	nd	nd	nd
H ₂ [mM/d]	-7.10	0.54	-12.77	0.40	-0.76	0.18	29.00	1.59
CO ₂ [mM/d]	-1.13	0.06	23.11	0.94	2.16	0.20	27.36	1.38
Lactate [mM/d]	nd	nd	0.01	0.01	0.01	0.00	0.01	0.01
Acetate [mM/d]	0.33	0.05	0.23	0.07	0.95	0.04	0.67	0.09
Propionate [mM/d]	0.03	0.02	0.07	0.03	0.20	0.04	nd	nd
<i>n</i> -Butyrate [mM/d]	0.01	0.01	0.05	0.03	0.18	0.01	0.01	0.01
<i>n</i> -Valerate [mM/d]	nd	nd	nd	nd	0.09	0.03	nd	nd
<i>n</i> -Caproate [mM/d]	nd	nd	nd	nd	0.05	0.01	nd	nd
Ethanol [mM/d]	0.03	0.01	0.04	0.02	0.06	0.02	0.15	0.01
Propanol [mM/d]	nd	nd	nd	nd	0.02	0.016	nd	nd
Butanol [mM/d]	nd	nd	nd	nd	0.02	0.015	nd	nd

3. Co-fermentation of syngas and PAC

Table S3. Acetate, propionate and *n*-butyrate productivities for all bottles of M-PAC and T-PAC experiments. The productivities were calculated by dividing the net metabolite accumulation (mM) at the end of the fermentations by the total fermentative time (39 d).

	M-PAC			T-PAC		
PAC [%]	Acetate [mM/d]	Propionate [mM/d]	<i>n</i> -Butyrate [mM/d]	Acetate [mM/d]	Propionate [mM/d]	<i>n</i> -Butyrate [mM/d]
0.5	0.93	0.18	0.15	0.17	0.08	0.08
1	0.85	0.23	0.06	0.05	0.12	0.05
1.5	0.87	0.22	0.32	0.63	0.13	0.21
2	0.73	0.47	0.24	1.68	0.04	0.04
2.5	0.85	0.18	0.06	1.67	0.10	0.15
3	0.73	0.23	0.31	1.48	0.04	0.01
3.5	0.89	0.11	0.11	2.33	0.08	0.01
4	0.92	0.07	0.13	1.68	0.09	0.07
5	0.90	0.18	0.13	1.17	0.07	0.03
7.5	0.80	0.14	0.03	0.69	0.11	0.00
10	0.25	0.05	0.02	0.23	0.00	0.03
15	0.06	0.00	0.03	0.06	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.02
30	0.03	0.00	0.00	0.00	0.00	0.00

Table S4. Productivities of CO, CH₄, H₂, CO₂ and VFAs in mM/d at increasing PAC concentrations and different temperatures. Negative productivity indicates consumption. Gas productivities are the mean value of the productivities calculated between each sampling interval. The VFAs productivities were calculated by dividing the net metabolite accumulation (mM) at the end of the fermentations by the total fermentative time.

PAC [%]	0.5	1	1.5	2	2.5	3	3.5	4	5	7.5	10	15	20	30
PAC availability [gCOD/gVSS]	0.103	0.206	0.309	0.413	0.516	0.619	0.722	0.825	1.032	1.547	2.063	3.095	4.127	6.190
Mesophilic PAC Fermentations (M-PAC)														
CO productivity [mM/d]	-23.59	-17.77	-15.33	-4.43	-1.87	-1.22	-1.24	-1.56	-0.94	-1.35	-0.39	-0.40	-0.38	-0.34
CH ₄ productivity [mM/d]	5.73	3.97	2.09	0.13	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H ₂ productivity [mM/d]	-8.52	-6.08	-6.12	-0.80	-0.21	-0.11	-0.47	-0.29	-0.14	-0.21	-0.11	-0.11	-0.09	-0.07
CO ₂ productivity [mM/d]	8.81	6.50	3.95	1.65	0.88	0.62	0.65	0.67	0.72	0.76	0.28	0.27	0.22	0.35
VFAs productivity [mM/d]	1.26	1.15	1.40	1.44	1.10	1.27	1.11	1.12	1.21	0.97	0.32	0.10	0.00	0.033
CO uptake norm. to M-CTRL [%]	231.5	151.3	141.1	50.15	23.91	16.03	15.74	18.95	15.74	8.45	4.37	4.37	4.96	3.79
C-mol balance [%]	76.7	82.5	75.0	131.6	246.1	293.2	246.2	207.7	270.4	265.4	297.1	163.2	146.4	151.4
e-mol balance [%]	94.5	99.5	93.1	173.7	384.6	477.0	290.0	286.8	473.6	292.2	308.6	124.6	-	-
Thermophilic PAC Fermentations (T-PAC)														
CO productivity [mM/d]	-33.57	-34.85	-36.05	-14.74	-12.21	-12.33	-9.10	-9.02	-3.83	-0.59	-0.36	-0.36	-0.38	-0.15
CH ₄ productivity [mM/d]	10.37	12.59	12.33	2.03	0.12	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H ₂ productivity [mM/d]	-12.44	-12.66	-12.68	4.99	4.79	13.09	8.22	9.18	3.82	0.44	0.28	0.20	0.13	0.05
CO ₂ productivity [mM/d]	22.18	24.51	25.10	10.01	8.50	12.69	8.31	8.71	3.83	0.94	0.53	0.42	0.38	0.16
VFAs productivity [mM/d]	0.33	0.22	0.96	1.76	1.91	1.53	2.42	1.84	1.27	0.80	0.26	0.06	0.02	0.00
CO uptake norm. to T-CTRL [%]	97.56	101.3	104.8	36.70	34.80	35.48	26.00	27.15	15.03	1.56	1.02	0.81	1.22	0.41
C-mol [%]	100.0	108.3	109.9	109.7	102.2	122.5	140.3	134.5	142.4	436.0	279.4	147.8	111.6	113.5
e-mol balance [%]	96.1	109.7	115.3	121.3	89.5	126.8	141.4	138.3	189.1	618.5	365.2	122.8	-	-

3.1. Impact of PAC on the syngas metabolism of an anaerobic mixed culture at mesophilic temperature (37°C)

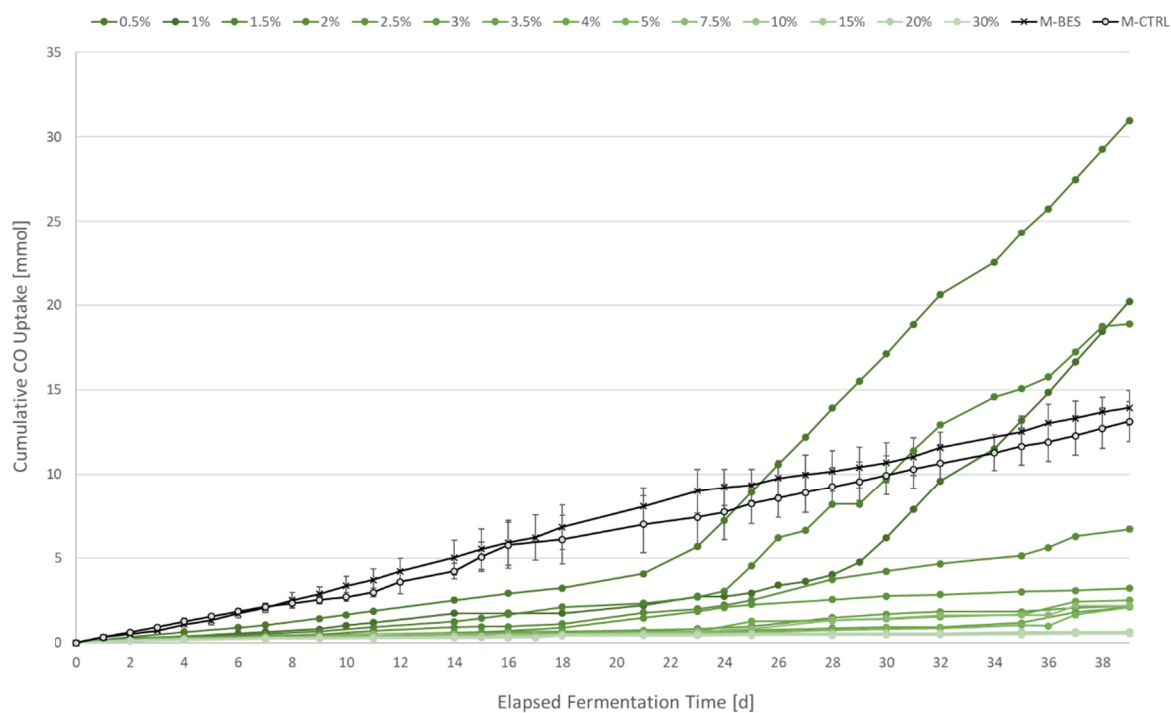


Figure S1. Cumulative CO uptake in mmol for experiments M-BES, M-CTRL and M-PAC.

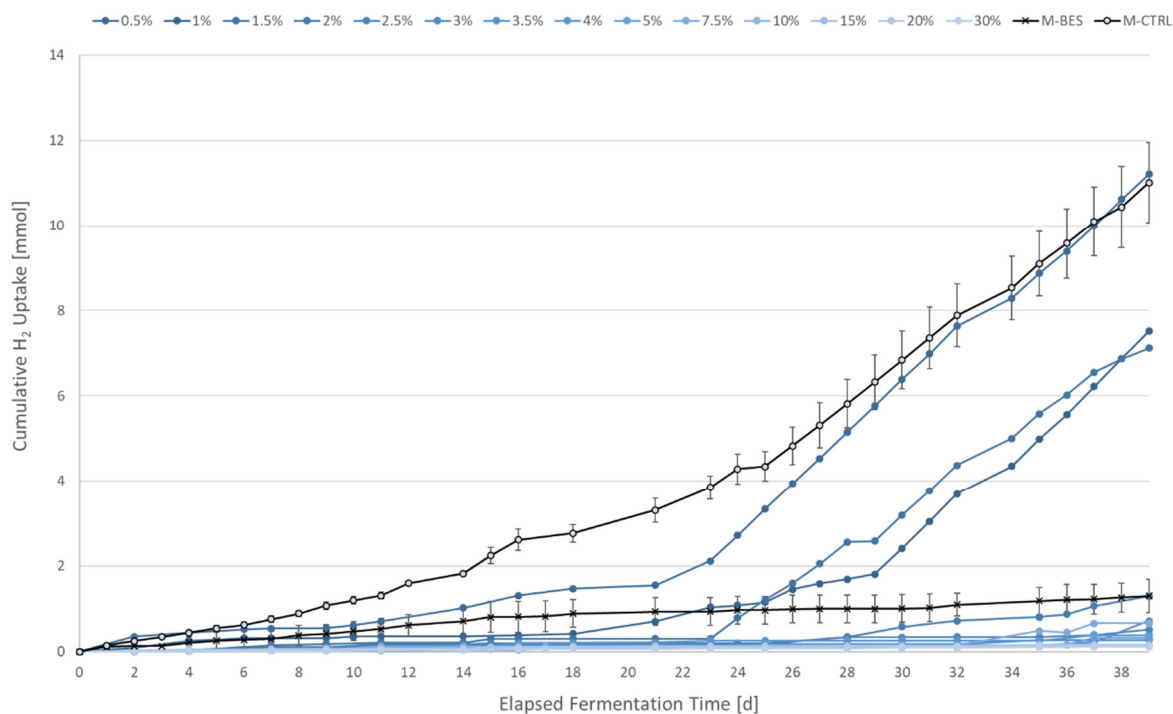


Figure S2. Cumulative H₂ uptake in mmol for experiments M-BES, M-CTRL and M-PAC.

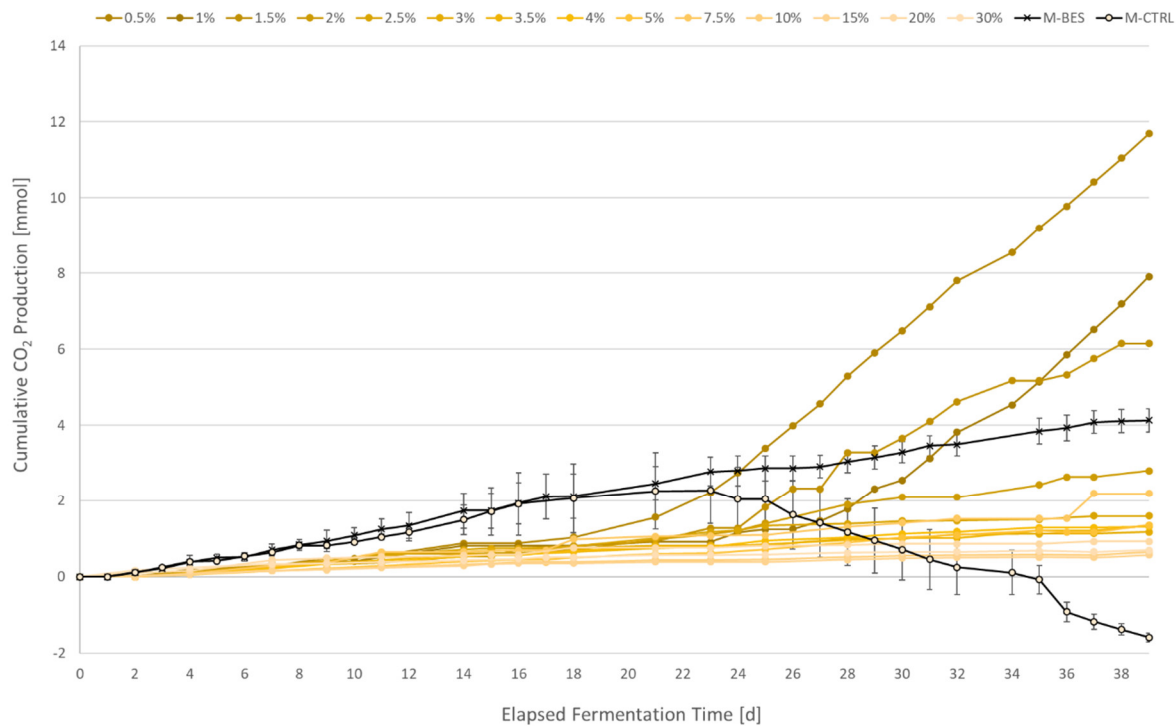


Figure S3. Cumulative CO₂ production in mmol for experiments M-BES, M-CTRL and M-PAC. Negative values mean consumption.

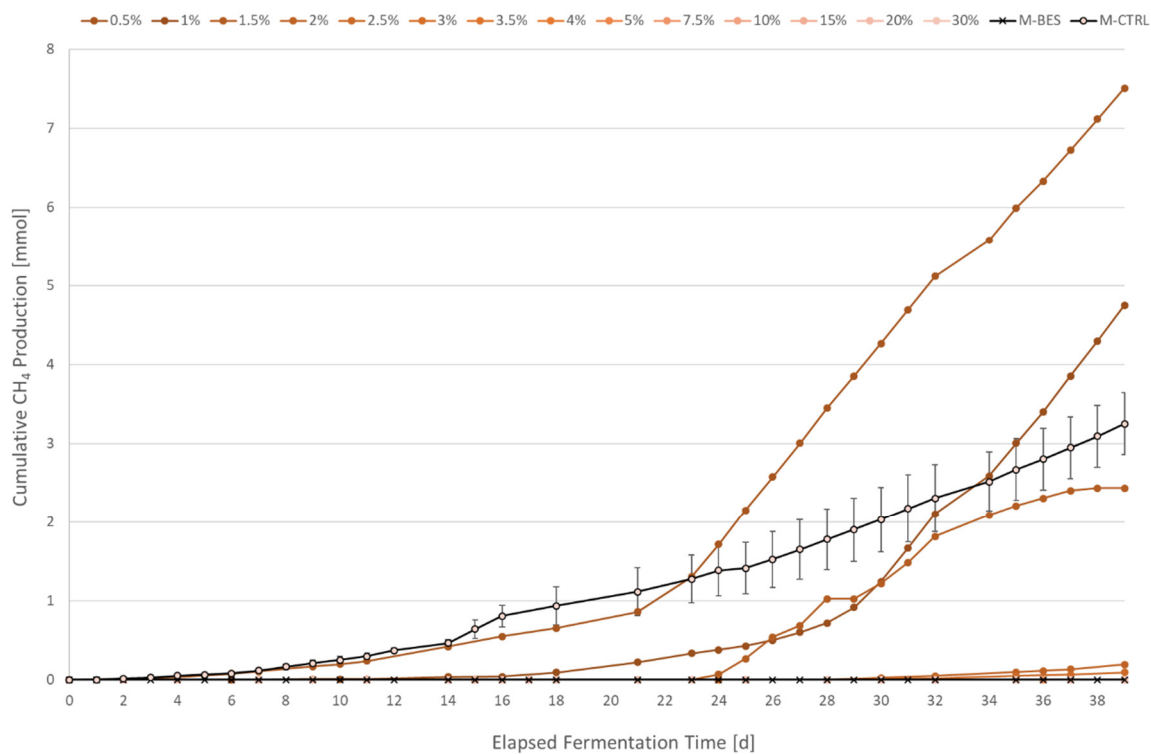


Figure S4. Cumulative CH₄ production in mmol for experiments M-BES, M-CTRL and M-PAC.

3.2. Impact of PAC on the syngas metabolism of an anaerobic mixed culture at thermophilic temperature (55°C)

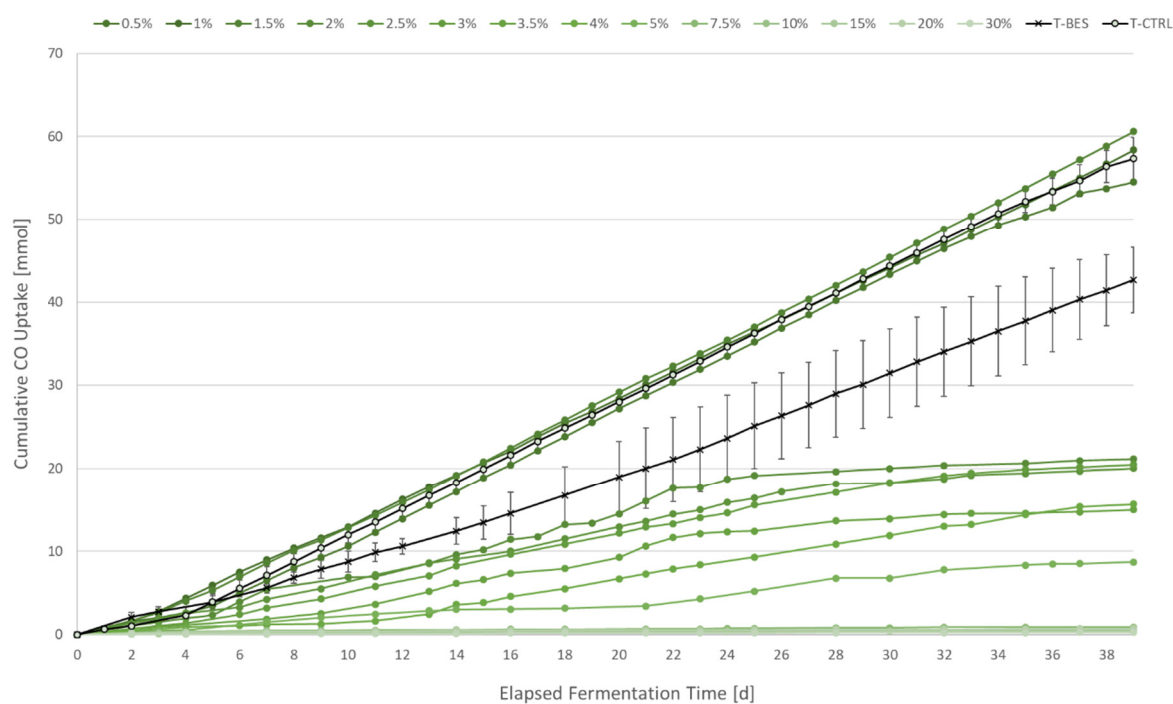


Figure S5. Cumulative CO uptake in mmol for experiments T-BES, T-CTRL and T-PAC.

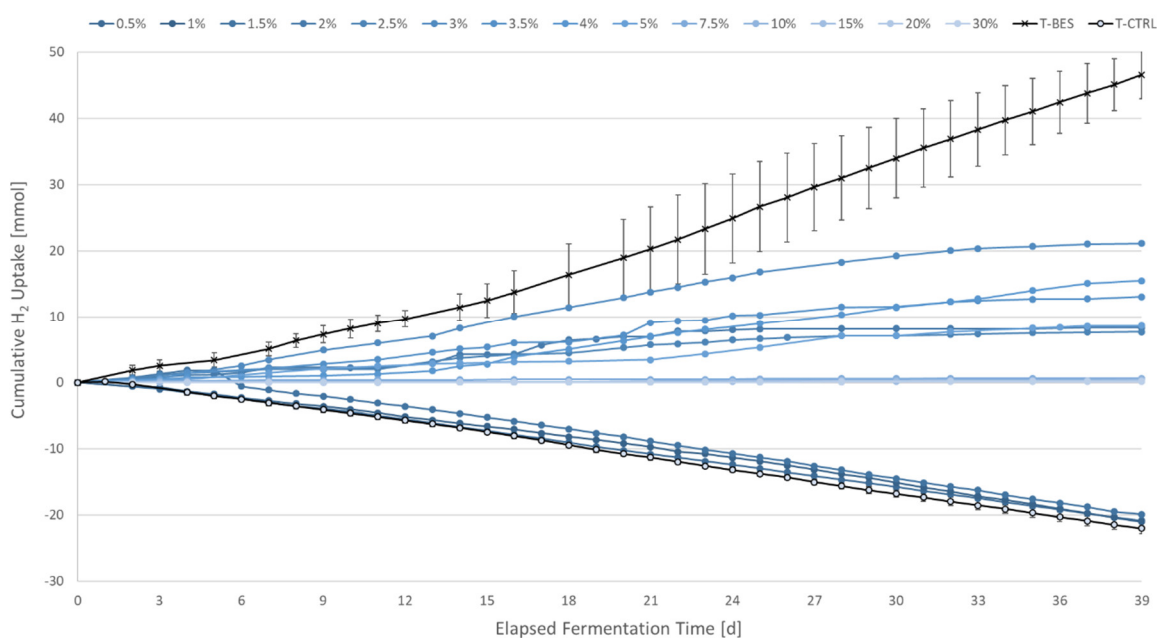


Figure S6. Cumulative H₂ uptake in mmol for experiments T-BES, T-CTRL and T-PAC. Negative values mean consumption.

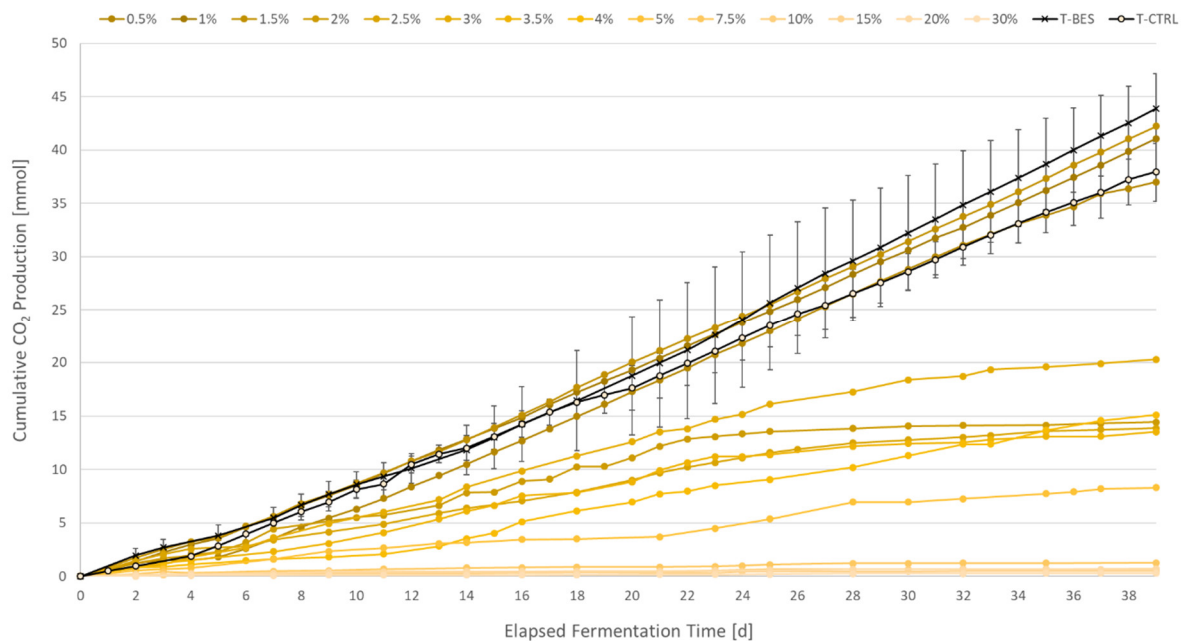


Figure S7. Cumulative CO₂ production in mmol for experiments T-BES, T-CTRL and T-PAC.

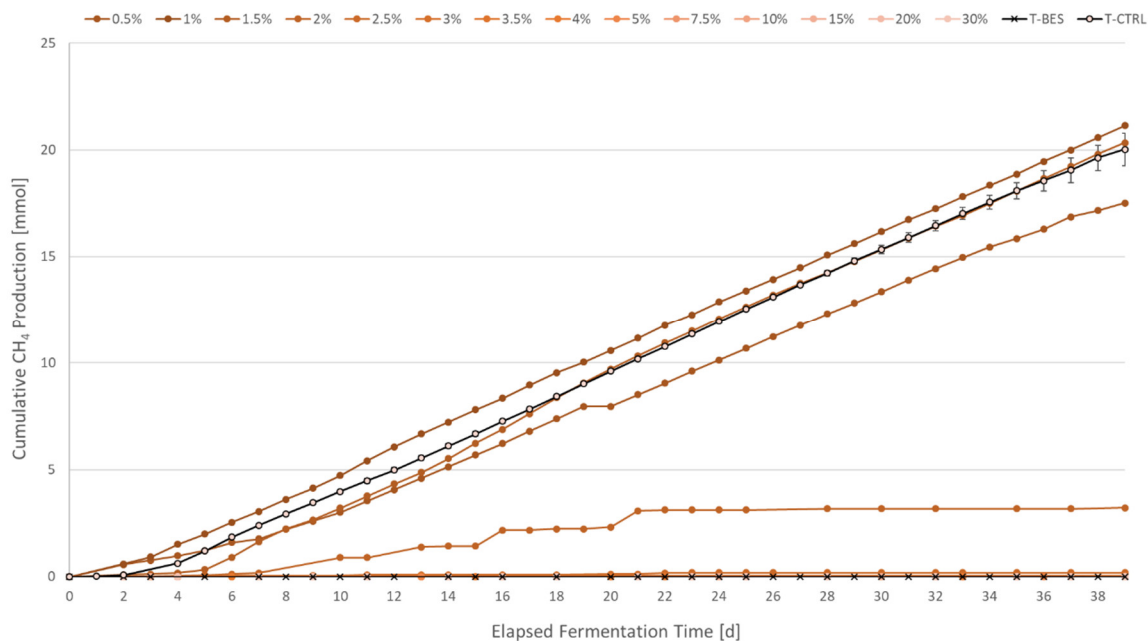


Figure S8. Cumulative CH₄ production in mmol for experiments T-BES, T-CTRL and T-PAC.