SUPPORTING INFORMATION

Direct and indirect effect of increased CO_2 partial pressure on the bioenergetics of syntrophic propionate and butyrate conversion

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Role of inoculum origin on pCO_2 response

The anaerobic conversion of propionate in batch reactors at 1 bar pCO_2 was monitored for nine days for inocula A, B, C and the results are presented in **Figure S1**. Inoculum C showed the fastest propionate conversion. Inoculum B showed an unusually low level of activity and was discarded for additional testing. Propionate conversion by inoculum A seemed to be halted after two days of incubation, which hindered further assessment of conversion rates. The main differences between inoculum A and C, in terms of the physicochemical characteristics, were associated with the initial alkalinity as well as the NH_4^+ concentration (**Table S1**). Bacterial and archaeal communities differed among inoculum A and C, with the latter showing a higher abundance of uncultured microorganisms and hydrogenotrophic methanogens (**Figure S2**). This preliminary data led to the selection of C as the working inoculum for further experiments.

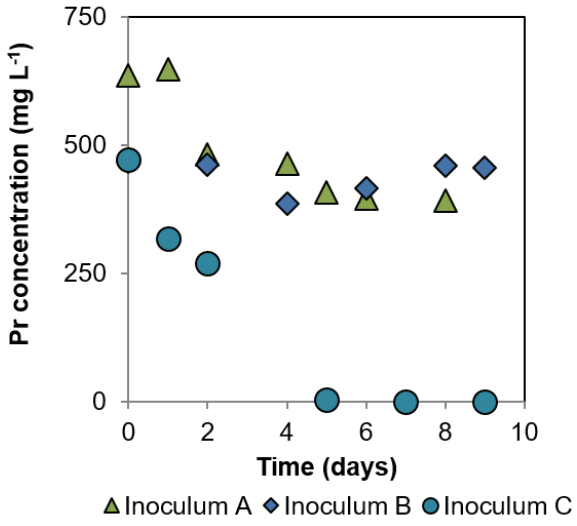

Figure S1. Evolution of propionate concentration (mg L^{-1}) for the experiments of anaerobic substrate oxidation under moderate pCO_2 (1 bar) using three different inocula (A, B and C). Data points represent experimental data.

Table S1. Physicochemical characteristics of the three inocula initially selected for the study of propionate oxidation activity at pCO_2=1 bar. SD=Standard Deviation.

Parameter	Inoculum A	SD	Inoculum B	SD	Inoculum C	SD	Units
COD	34.63	0.19	38.99	2.33	22.22	0.52	g L^{-1}
sCOD	0.64	0.01	5.37	0.08	1.92	0.04	g L^{-1}
P	0.25	0.00	1.06	0.01	0.11	0.00	mg L^{-1}
N-total	1.93	0.03	2.08	0.08	ND	ND	mg L^{-1}
$N\text{-}NH_4^+$	1.08	8.66	0.93	0.01	0.11	0.00	mg L^{-1}
Alkalinity	120.10	0.01	182.22	0.52	36.29	0.50	meq L^{-1}
VSS	21.47	0.02	30.42	0.15	13.62	0.01	g L^{-1}
TSS	28.88	0.06	57.42	0.60	15.87	0.07	g L^{-1}
VSS/TSS	74.35		52.97		85.80		%
pH	7.10		7.25		7.30		-

Microbial community analysis

DNA extraction

Biomass pellets stored at -80°C, were thawed and DNA was extracted according to the instructions included in the DNeasy Ultra- Clean Microbial kit (Qiagen, Germany). Quality and quantity of the obtained DNA was checked by means of Qubit 3.0 DNA detection (Qubit® dsDNA HS Assay Kit, Life Technologies, U.S).

Amplicon libraries construction

Library construction and sequencing were performed at the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. Their internal procedure summarizes as follows: Approximately 1ng of DNA was used for amplification with the bacterial 16s V3_F357_V4-R805 and archaeal 349F-806R primers in the Fluidigm Access Array. The barcoded amplicons generated from each sample were harvested, transferred to a 96 well plate, quantified on a Qubit fluorometer (ThermoFisher, CA) and the average size of the amplicons was determined on a Fragment Analyzer (AATI, IA). All amplicons were pooled in equimolar concentration, size selected on a 2% agarose Ex-gel (Thermofisher) to remove primer dimers and extracted from the isolated gel slice with a Qiagen gel extraction kit (Qiagen). Cleaned size selected product was quantitated and run on a Fragment Analyzer again to confirm appropriate profile and for determination of average size. The pool was diluted to 5nM and further quantitated by qPCR on a CFX Connect Real-Time qPCR system (Biorad, Hercules, CA) for maximization of number of clusters in the flowcell.

Sequencing on an Illumina MiSeq

The pool was denatured and spiked with 20% non-indexed PhiX V3 control library provided by Illumina and loaded onto the MiSeq V2 flowcell at a concentration of 8 pM for cluster formation and sequencing. The PhiX control library provides a balanced genome for calculation of matrix, phasing and prephasing, which are essential for accurate basecalling. The libraries were sequenced from both ends of the molecules to a total read length of 250nt from each end. The run generated .bcl files which were converted into demultiplexed fastq files using bcl2fastq 2.20 (Illumina, CA).

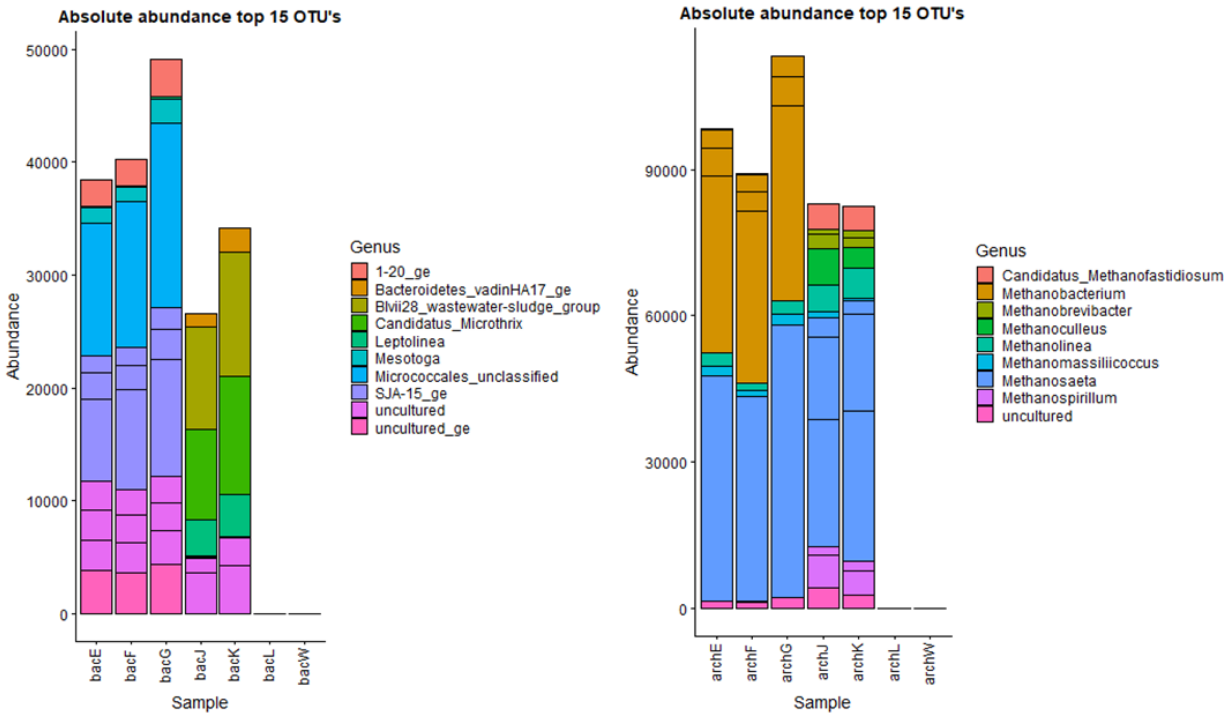

Figure S2. Absolute abundance of different genera for bacteria and archaea. Samples E, F and G correspond to starting inoculum C. Samples J and K correspond to starting inoculum A. Samples L and W correspond to negative controls.

Table S2. Sampling strategy for the triplicate incubation carried out for the experiments of syntrophic propionate and butyrate conversion under elevated pCO_2.

pCO_2 (bar)[1]	Propionate				Butyrate			
	Time (days)	Replicate	Liquid (1.5 mL)	Gas (5 mL)	Time (days)	Replicate	Liquid (1.5 mL)	Gas (5 mL)
0.3, 1, 3, 5, 8	0, 10, 13	1	x	x	0, 5, 12	1	x	x
		2	x	x		2	x	x
		3	x	x		3	x	x
	Other	1	x		Other	1	x	
		2		x		2		x
		3	-	-		3	-	-

[1] This value corresponds to initial pressure considering a headspace of >99% CO_2 that will equilibrate with the liquid medium described in the Materials and Methods section.

Table S3. Summary of input parameters for the bioenergetics scenario analysis of syntrophic propionate and butyrate conversion. The initial concentrations of propionate, butyrate and acetate were fixed at 10, 7 and 0.1 mM, respectively. Temperature was fixed at mesophilic conditions, 308 K. The employed bicarbonate concentration corresponded to the initial amount of buffer supplied to the system, 100 mM as HCO_3^-. The initial partial pressure was selected at 0.001 bar for CH_4 and $1x10^{-5}$ bar for H_2.

Scenario	Initial pCO_2 (bar) [1]	pH	Scenario	pCO_2 (bar)	pH[2]	Scenario	pCO_2 (bar)	pH
A	0.1	7.0	B	0.3	7.9	C	0.1	7.9
	1				6.9		1	6.9
	3				6.4		3	6.4
	5				6.2		5	6.2
	8				5.9		8	5.9
	10				5.8		10	5.8
	20				5.5		20	5.5

[1] This value corresponds to initial pressure considering a headspace of >99% CO_2 that will equilibrate with a solution at the conditions previously described.

[2] pH value calculated under the assumption that enough buffer was provided at the initial conditions in order that at the equilibrium point, 100 mM HCO_3^- are still available in the system.

Table S4. ΔG_{Pr-Ox} calculated for different pCO_2 used in Scenario A to evaluate the effect of increased pCO_2 in the thermodynamic feasibility of syntrophic conversion of propionate and butyrate

	pCO_2 (bar)[1]	log pCO_2	ΔG_{Pr-Ox} (kJ mol^{-1})
	0.10	-1.00	-31.0
Experimental range	0.30	-0.52	-28.2
	1.00	0.00	-25.1
	3.00	0.48	-22.3
	5.00	0.70	-21.0
	8.00	0.90	-19.8
	10.00	1.00	-19.2
	20.00	1.30	-17.4

[1] This value corresponds to initial pressure considering a headspace of >99% CO_2 that will equilibrate with the solution at the conditions previously described.

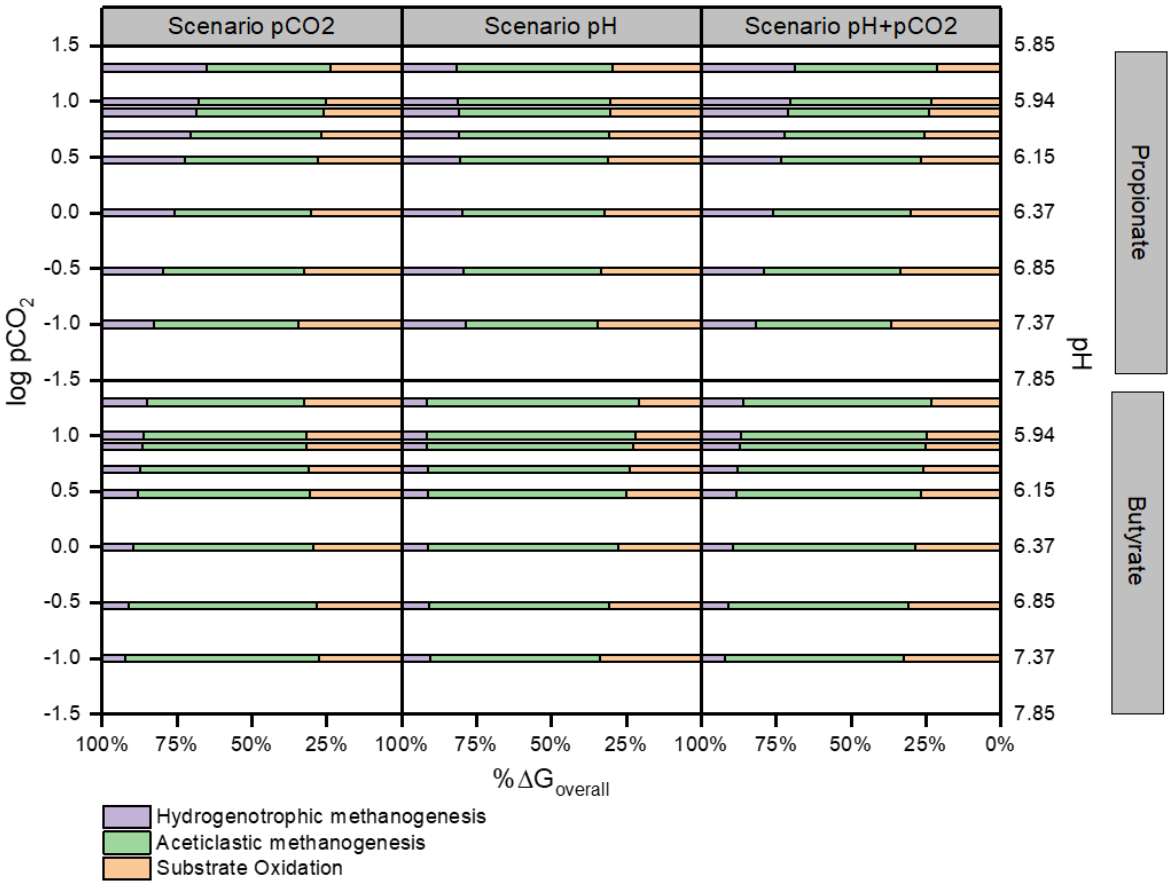

Figure S3. Theoretical share of $\Delta G_{Overall}$ for each of the proposed scenarios for the syntrophic conversion of propionate and butyrate. Scenario A – partial pressure of CO_2 (pCO_2) in propionate and butyrate conversion. Scenario B – pH. Scenario C – concomitant effect of pH and pCO_2. All the scenarios were calculated at T=35°C and pH_2= $1x10^{-5}$ bar. The colored segment of the bar represents the potential biochemical energy share for: hydrogenotrophic methanogenesis (ΔG_{HyM}) in purple, propionate (ΔG_{Pr-Ox}) or butyrate (ΔG_{Bu-Ox}) oxidation in orange, aceticlastic methanogenesis (ΔG_{AcM}) in green.

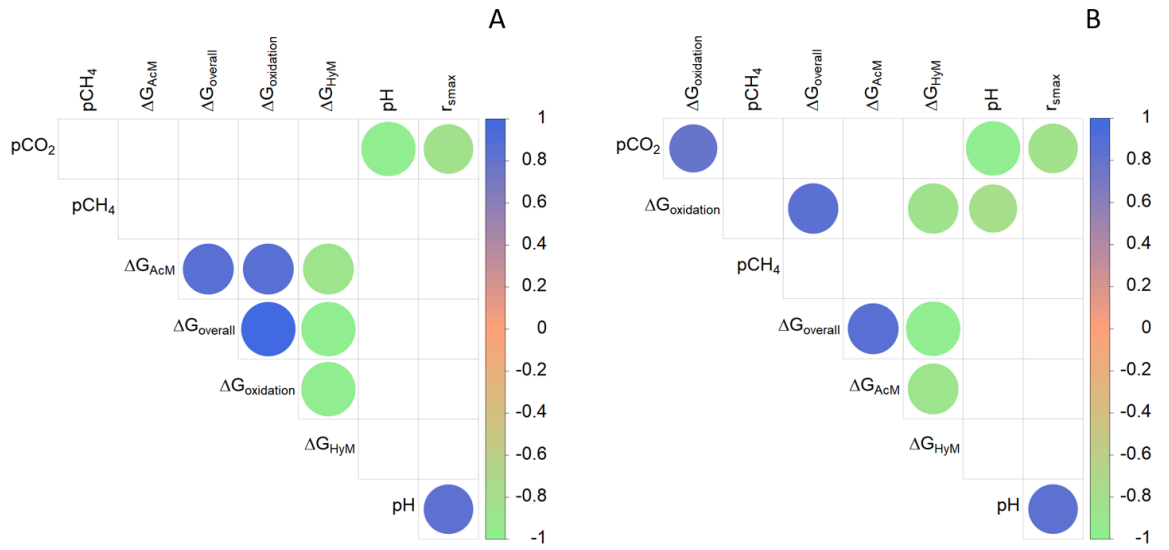

Figure S4. Correlogram for the measured and calculated data from the experiments of syntrophic propionate A) and butyrate B) conversion under conditions of elevated pCO_2 considering two different pH_2 values: $1x10^{-5}$ bar and $6x10^{-4}$ bar. The correlation was calculated using the Spearman correlation coefficient (r_S) and ordered through hierarchical clustering. Circles with increased diameter represent a stronger correlation. According to the scale included to the right of the figure, values closer to 1 (in blue) represent a significant positive correlation, whereas values closer to -1 (in green) represent a significant negative correlation. Abbreviations correspond to the overall Gibbs free energy ($\Delta G_{Overall}$), the Gibbs free energy of the sub-reactions (substrate oxidation: $\Delta G_{Oxidation}$, acetoclastic methanogenesis: ΔG_{AcM}, hydrogenotrophic methanogenesis: ΔG_{HyM}) and to the maximal substrate utilization rate (r_{smax}).

Table S5. Calculation of the dissolved CO_2, undissociated acid concentrations corresponding to the experimental conditions of this study

Initial pressure (bar)					0.3	1	3	5	8
Eq. pCO_2 in the experiments (bar)					0.3	1	1.3	2	3.5
$CO_{2(aq)}$ (g L^{-1})					0.3	1.1	3.8	5.4	8.6
HCO_3^- (meq L^{-1})					100	100	100	100	100
Eq. pH					7.4	6.9	6.8	6.6	6.3
Compound	Exp. Initial Concentration (M)	Exp. Initial Concentration (gCOD L^{-1})	pKa (35°C)	Undisso-ciated specie	Concentration range in the experiments (mg L^{-1})*				
Propionate	0.01	1.1	4.89	HPr	2.3 - 27.3				
Butyrate	0.01	1.2	4.81	HBu	1.6 - 18.9				
Acetate**	0.02	1.0	4.76	HAc	26.9 - 45.1				

*Undissociated acids calculated using carbonate equilibrium constant k_1 corrected by temperature (35°C) = 5.54 x 10^{-7}, Henry's equilibrium constant k_H corrected by temperature (35°C)= 0.0245 mol L^{-1} bar^{-1}

** Included as additional reference value