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1. Supplementary Discussion

Calculation of the energetic feasibility of H_2 formation and consumption during butane oxidation

Literature data[1]

Compound		$\Delta_f G°_{298K}$ (kJ mol^{-1})	$\Delta_f H_{298K}$ (kJ mol^{-1})
H_2 (g)		0	0
H^+ (aq)		0	0
pH = 7	($\Delta_f G°'$)	–40.0	0
H_2O (lq)		–237.18	–285.83
n-C_4H_{10} (g)		–17.20	–125.60
CO_2 (g)		–394.36	–393.51
H_2S (aq)		–27.87	–39.8
SO_4^{2-} (aq)		–744.6	–909.3

[1]From Dean, J.A., Lange's Handbook of Chemistry. McGraw-Hill, New York (1992); and Thauer, R.K., Jungermann, K., Decker, K., Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41, 100–180 (1977).

a) Conversion of butane to hydrogen

$$C_4H_{10}\,(g) + 8\,H_2O\,(lq) \rightarrow 4\,CO_2\,(g) + 13\,H_2\,(g) \qquad (1)$$

At 298.15 K, and standard activities/fugacities:

$$\Delta G°_{298K} = +337.2\ \text{kJ mol}^{-1}{}_{\text{Butane}}$$

$$\Delta H_{298K} = +838.2\ \text{kJ mol}^{-1}{}_{\text{Butane}}$$

At 323.15 K (50 °C), but otherwise standard activities/fugacities:

$$\Delta G^{o}_{323K} = \frac{323}{298}\left(\Delta G^{o}_{298K} - \Delta H^{o}\right) + \Delta H^{o} \qquad (2)$$

(Derived from $\Delta G = \Delta H - T\Delta S$, and the assumption that ΔH and ΔS are the same at the lower and higher temperature.)

$$\Delta G^{o}_{323K} = +295.2\ \text{kJ mol}^{-1}{}_{\text{Butane}} \qquad (3)$$

Free energy depends on activity/fugacity according to:

$$\Delta G = \Delta G° + R\,T \ln \frac{\{CO_2\}^4\{H_2\}^{13}}{\{\text{Butane}\}\{H_2O\}^8} \qquad (4)$$

With the above ΔG^{o}_{323K}, $R = 8.314 \cdot 10^{-3}$ kJ mol^{-1} K^{-1}, $T = 323$ K and assuming for convenience $\{\text{Butane}\} = 1$, $\{H_2O\} = 1$, $\{CO_2\} = 0.3 = 10^{-0.523}$, the free energy (in kJ) depends on H_2 fugacity according to

$$\Delta G = 295.2 + 6.18 \log\left(10^{-2.09} \cdot \{H_2\}^{13}\right) \qquad (5)$$

$$\Delta G = 282.3 + 80.34 \log\{H_2\} \qquad (6)$$

If, as a convenient approximation, H_2 fugacity is expressed via H_2 partial pressure in Pa:

$$\Delta G = 282.3 + 80.34 \log \frac{p_{H_2}}{10^{5.004}\ \text{Pa}} \quad (7)$$

$$\Delta G = -119.7 + 80.34 \log \frac{p_{H_2}}{1\ \text{Pa}} \quad \text{(kJ per mol butane)} \quad (8)$$

b) Consumption of hydrogen by sulfate reduction

$$4\ H_2\ (g) + SO_4^{2-}\ (aq) + 2\ H^+\ (aq) \rightarrow\ H_2S\ (aq) + 4\ H_2O\ (lq) \quad (9)$$

At 298.15 K, and standard activities/fugacities:

$\Delta G° = -232.0$ kJ mol$^{-1}_{\text{Sulfate}}$ (formal value for hypothetical $\{H^+\} = 1$, i.e. pH = 0)

$\Delta H° = -273.8$ kJ mol$^{-1}_{\text{Sulfate}}$

At 323.15 K (50 °C), but otherwise standard activities/fugacities, via eqn. 2:

$$\Delta G^{\circ}_{323K} = -228.5\ \text{mol}^{-1}_{\text{Sulfate}}$$

Free energy depends on activity/fugacity according to:

$$\Delta G = \Delta G° + R\ T \ln \frac{\{H_2S\}\ \{H_2O\}^4}{\{H_2\}^4 \{SO_4^{2-}\}\ \{H^+\}^2} \quad (10)$$

With the above ΔG°_{323K}, $R = 8.314 \cdot 10^{-3}$ kJ mol^{-1} K^{-1}, $T = 323$ K and assuming for convenience $\{SO_4^{2-}\} = 0.0023 = 10^{-2.64}$ (from 0.023 M sulfate present after 50 days of incubation, and activity coefficient of approx. 0.1 M^{-1}), $\{H^+\} = 10^{-7}$, $\{H_2S\} = 0.005 = 10^{-2.3}$ (from 0.005 M sulfide formed after 50 days of incubation), and $\{H_2O\} = 1$, the free energy (in kJ) depends on H_2 fugacity according to (calculation analogous to eqn. 5):

$$\Delta G = -139.9 - 24.72 \log\{H_2\} \quad \text{(kJ per mol sulfate)} \quad (11)$$

If, as a convenient approximation, H_2 fugacity is expressed via H_2 partial pressure in Pa:

$$\Delta G = -139.9 - 24.72 \log \frac{p_{H_2}}{10^{5.004}\ \text{Pa}} \quad \text{(kJ per mol sulfate)} \quad (12)$$

$$\Delta G = -16.2 - 24.72 \log \frac{p_{H_2}}{1\ \text{Pa}} \quad \text{(kJ per mol sulfate)} \quad (13)$$

Per mol butane, 3.25 mol sulfate are reduced with 13 mol H_2, yielding the free energy change

$$\Delta G = -454.7 - 80.34 \log\{H_2\} \quad \text{(kJ per 3.25 mol sulfate)} \quad (14)$$

or with H_2 pressure in Pa

$$\Delta G = -52.7 - 80.34 \log \frac{p_{H_2}}{1\ \text{Pa}} \quad \text{(kJ per 3.25 mol sulfate)} \quad (15)$$

ΔG vs. p_{H_2} plots are shown in Supplementary Figure 2.

2. Supplementary Tables

Supplementary Table 1. Electron balance of the *n*-butane degradation coupled to sulfate reduction to sulfide by the thermophilic enrichment culture.

n-Butane, sulfate, electrons (mmol l^{-1})	Butane50 + 7% *n*-butane (v/v in headspace)	Butane50 + 12% *n*-butane (v/v in headspace)	Butane50 – *n*-butane	Abiotic control + 7% *n*-butane (v/v in headspace)
n-Butane supplied	2.1	3.2	-	2.1
n-Butane consumed	2.0	3.1	-	0.15
Electrons from *n*-butane [a]	48.1	76.7	-	-
Sulfate supplied	28.0	28.0	28.0	-
Sulfate consumed	7.8	11.8	1.2	-
Electrons for sulfate reduction [c]	52.8	84.8	-	-
Electron balance[d]	1.09	1.10	-	-

Quantitative growth experiments were carried out in serum bottles with 100 ml culture volume and different starting amounts of *n*-butane. The cultures were incubated for 155 days.

[a] Electrons from consumed *n*-butane were calculated considering the complete oxidation reaction: $C_4H_{10} + 8H_2O \rightarrow 4CO_2 + 26H^+ + 26e^-$. The amount of *n*-butane consumed was corrected for the amount of *n*-butane disappearing in the abiotic control.

[b] The amount of sulfate consumed was determined by quantification of produced sulfide, corrected for the concentration of sulfide at the start of the incubation experiments.

[c] Electrons for sulfate reduction were calculated considering: $SO_4^{2-} + 8e^- + 9H^+ \rightarrow HS^- + 4H_2O$. The sulfide produced in cultures with *n*-butane was corrected for the sulfide produced in *n*-butane-free bottles.

[d] Electrons consumed by sulfate reduction divided by electrons from *n*-butane consumed .

Supplementary Table 2. Basic information of the metagenome and the metatranscriptome of the Butane50 culture

Metagenome	**Mate-pair library**	Number of raw Illumina reads	21,182,518
		Number of reads after trimming	12,333,536
		Reads in metagenomic contigs	7,483,911
	Paired-end library	Number of raw Illumina reads	4,460,548
		Number of reads after trimming	4,187,678
		Reads in metagenomic contigs	2,172,844
	Bulk assembly	Reads from both libraries	9,656,755
		Assembled metagenome size (Mbp)	35.9
		N50 (bp)	3,014
		Maximum scaffold size (bp)	941,878
		Number of scaffolds	17,436
Metatranscriptome		Number of raw Illumina reads	48,444,528
		Number of reads after trimming	46,198,928

Supplementary Table 3. Pairwise comparison of nucleotide sequences and whole genome identity of the HotSeep-1 bin from the Butane50 culture vs. *Ca.* D. auxilii

	HotSeep-1 bin/*Ca.* D. auxilii			***Ca.* D. auxilii/HotSeep-1 bin**		
	Identity	Coverage	e-value	Identity	Coverage	e-value
16S RNA gene	99	100	0.00	99	100	0.00
23S RNA gene	99	100	0.00	99	100	0.00
Sat	99	100	0.00	99	100	0.00
***apr* alpha**	99	100	0.00	99	100	0.00
***apr* beta**	99	100	5e-113	99	100	5e-113
***dsr* alpha**	100	97	0.00	97	86	0.00
ITS	99	97	4e-144	99	100	4e-144

	Average Nucleotide Identity (Blast)	**Average Nucleotide Identity (MUMmer)**	**Tetranucleotide frequency**
HotSeep-1 bin/*Ca.* D. auxilii	98.38	98.45	99.48
Ca. D. auxilii/HotSeep-1 bin	98.48	98.24	99.48

sat, sulfate adenylyltransferase; *apr,* adenylylsulfate reductase; *dsr*, dissimilatory sulfite reductase; ITS, internal transcribed spacer

Supplementary Table 4. Accession numbers of published McrA sequences that have been included in the phylogenetic analyses of McrA subunits of *Ca.* Syntrophoarchaeum (Figure 3, Supplementary Figure 1).

P12971	CAE46369	YP_004004295	KT387808*
ABN56311	WP_011171503	YP_004520866	KT387809*
ABD41854	P07962	YP_003707758	KT387811*
ABN07237	NP_633264	WP_023845930	KT387813*
ABN07725	NP_988679	YP_007250108	KT387816*
CAJ37024	1MRO_D	YP_001549157	KT387817*
AAB98063	1E6Y_D	YP_003541782	KT387818*
AAB98851	1E6V_D	YP_008075839	
ABK14360	ZP_01702953	YP_007248437	
AAB85618	YP_001046528	WP_008513191	
AAB85653	YP_567018	WP_004079635	
ABE53268	CBH39484	YP_001403743	
CAA50044	AIJ05353	WP_004039455	
AAM01870	AHY86395	YP_006545377	
AAA73439	AHY86394	WP_004037742	
AAZ69867	AIJ05899	YP_004743325	
AAM07885	YP_007713068	YP_004291467	
AAM30936	YP_005919503	YP_006545160	
AAB02003	YP_005380187	YP_003457854	
AAQ63481	YP_007489939	YP_003424666	
ABO34334	YP_003355571	YP_008916519	
CAF31115	YP_004484839	WP_007044524	
AAA72598	YP_003246499	WP_004031277	
ABC56731	YP_686530	YP_004484066	
CAA30633	YP_004383383	WP_008514009	
CAE48306	YP_003615915	YP_001273475	
CAA30639	YP_003128256	WP_019264905	
AAA73445	YP_003458728	WP_018154763	
AAA72197	YP_006922405	WP_004035797	
AAQ63476	YP_002467317	YP_003895179	
ZP_01799689	WP_007043982	YP_004289577	
ZP_01799496	YP_007313393	YP_003895599	
CAA50044	WP_017981119	YP_003247534	
S43897	WP_018153522	YP_008072226	
AAU84252	YP_003726594	WP_019176774	
AAU83782	YP_003850404	Q58256.1	
AAU83544	YP_004004345	O27232.1	
AAU83007	YP_004576704	P11558.1	
AAU82960	YP_008916650	KT387810	
AAU82711	YP_004615938	KT387805	
AAU82276	WP_004029250	KT387806	
AAB98063			

* indicates partial sequences only used in some of the phylogenetic calculations (see Methods)

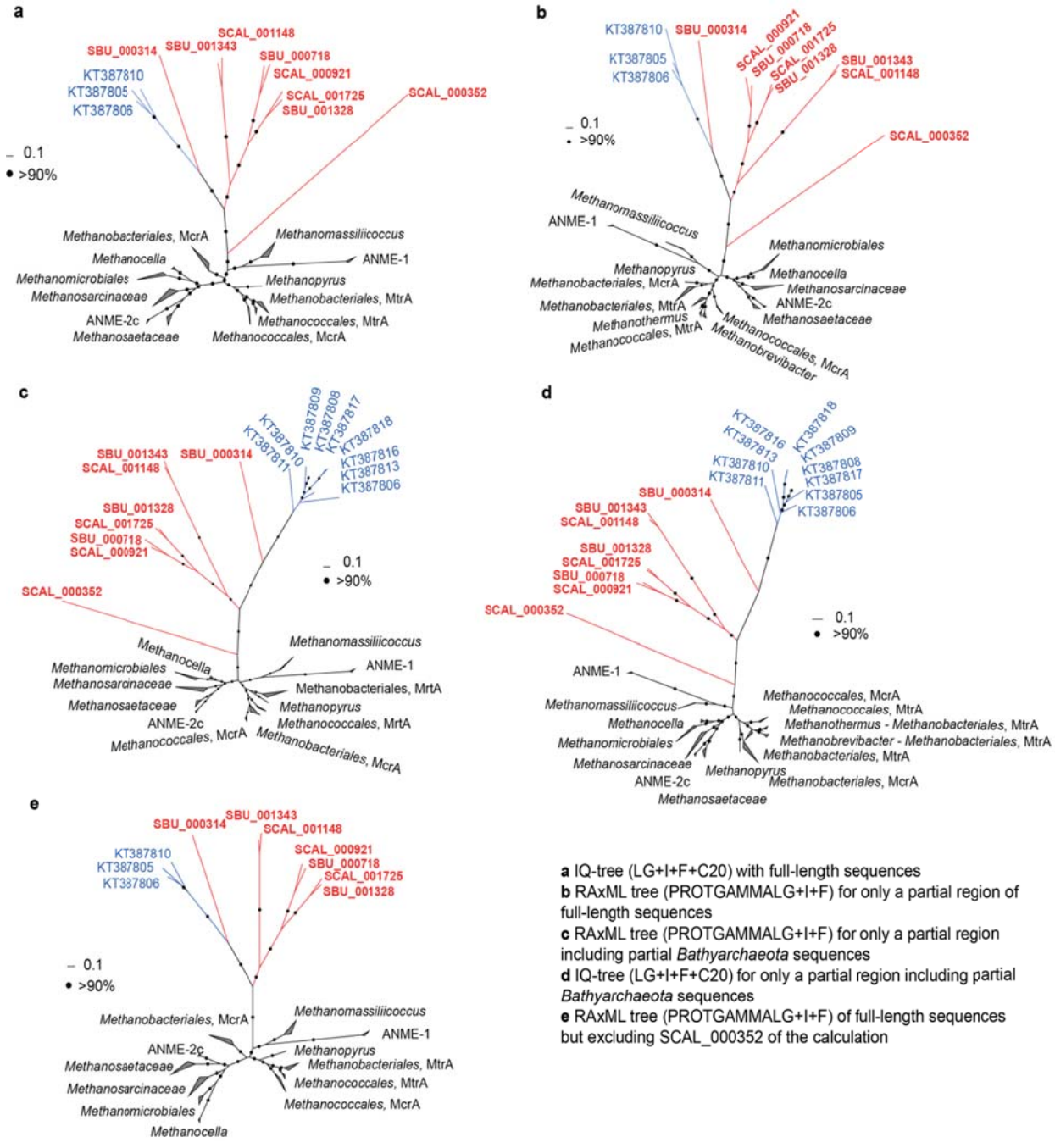
Supplementary Table 5. Primer sequences and PCR conditions used for amplification of *mcrA* genes from Butane50 enrichment.

Primer sets	Primer sequences (5' - 3')	Specificity	Polymerases[$]	Annealing-temp. [°C]	Expected amplicon length*	PCR product
McrA_1706F / McrA_1706R	CTTGACGACTTCAAGCGAATA/ CCCCTCCGGTGTAATTGGA	SCAL_001725	Phusion	52	1645	+
McrA_354F2 / McrA_354R	CGTGGAAGATGTACGCGAA/ ACGCTCACCTGCGGGCAT	SCAL_000352	TaKaRa Taq	52	1638	+
McrA_445F / McrA_445_1470R	GCCAGCGGGAGATGTACAA/ CGCTCACCKGCAGGCTCA	SBU_000314	TaKaRa Taq	54	1674	+
McrA_850_1455_F2 / McrA_850_915_1455R	GGAGATTTTCRGGGAGGA/ ACCTCCCWGGYTTTATYG	SBU_000718	Phusion	50	1632	+
McrA_915F / McrA_850_915_1455R	ACGATGCAACACGTGAGTA/ ACCTCCCWGGYTTTATYG	SCAL_000921	TaKaRa Taq	48	1614	+
McrA_1135F / McrA_1135R	AACATGGTGGAACACGCCA/ CTCTCACCCGCAGGCTCA	SCAL_001148	TaKaRa Taq & Phusion	52	1572	-
McrA_1455F / McrA_850_915_1455R	TGATCCCACCCGGAGAAA/ ACCTCCCWGGYTTTATYG	SBU_001328	TaKaRa Taq	48	1613	+
McrA_1470F / McrA_445_1470R	GACAGGACAAAGGAGCATAT / CGCTCACCKGCAGGCTCA	SBU_001343	TaKaRa Taq	52	1654	+

*according to *mcrA* genes retrieved from *Ca.* S. butanivorans and *Ca.* S. caldarius genomes; [$] Phusion: Phusion High-Fidelity DNA Polymerase (Thermo Fischer Scientific, Germany); TaKaRa Taq: TaKaRa Taq DNA Polymerase (TaKaRa Bio Europe, France)

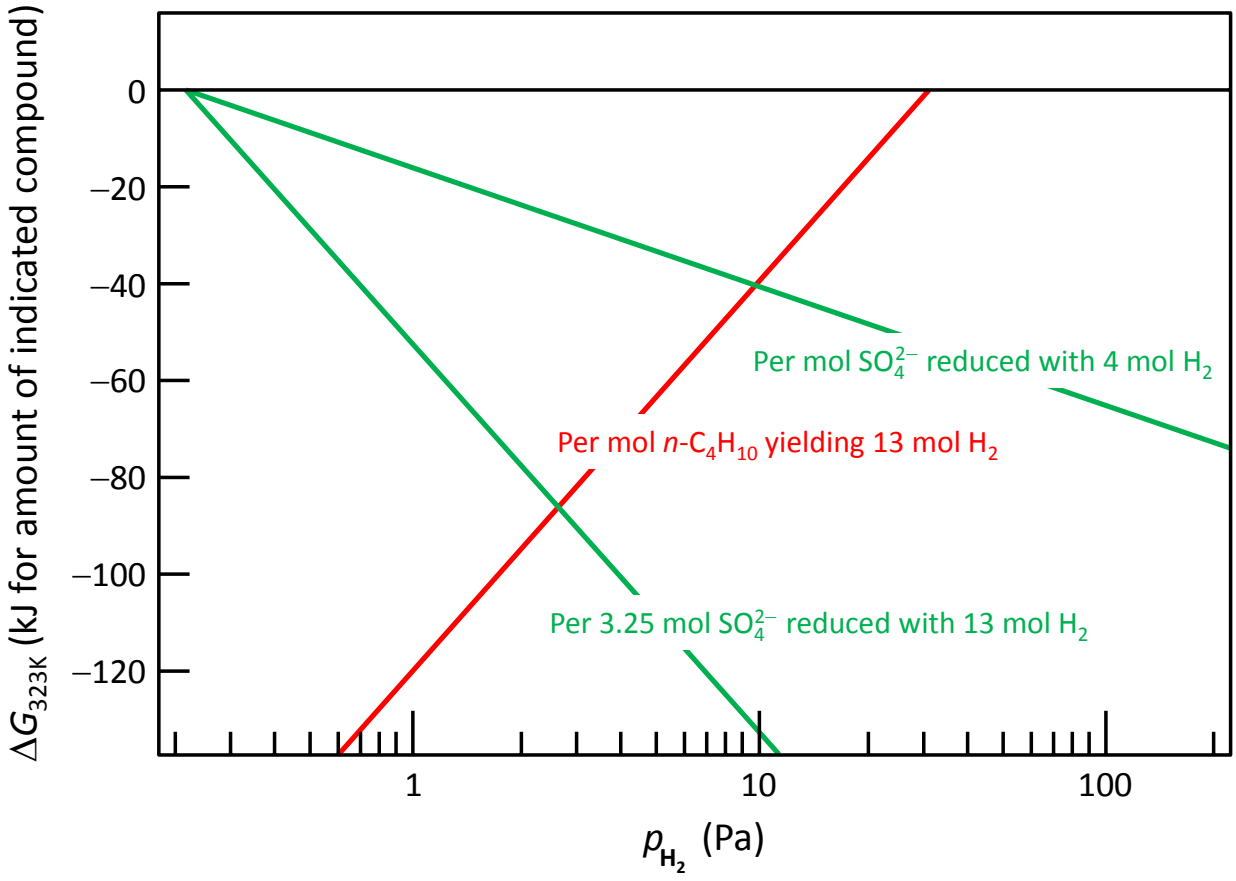
3. Supplementary Figures

Supplementary Figure 1. Affiliation of the amino acid sequences of the *mcrA* genes of *Ca.* Syntrophoarchaeum (in red) obtained from the Butane50 culture. The *Ca.* Syntrophoarchaeum sequences are labelled with their locus tag in the draft genomes (SBU=*Ca.* S. butanivorans; SCAL=*Ca.* S. caldarius). In blue, *Bathyarchaeota* sequences from Evans et al., Science, 2015. Black dots represent bootstrap values >90; scale bar indicates the number of amino acids substitutions per site.

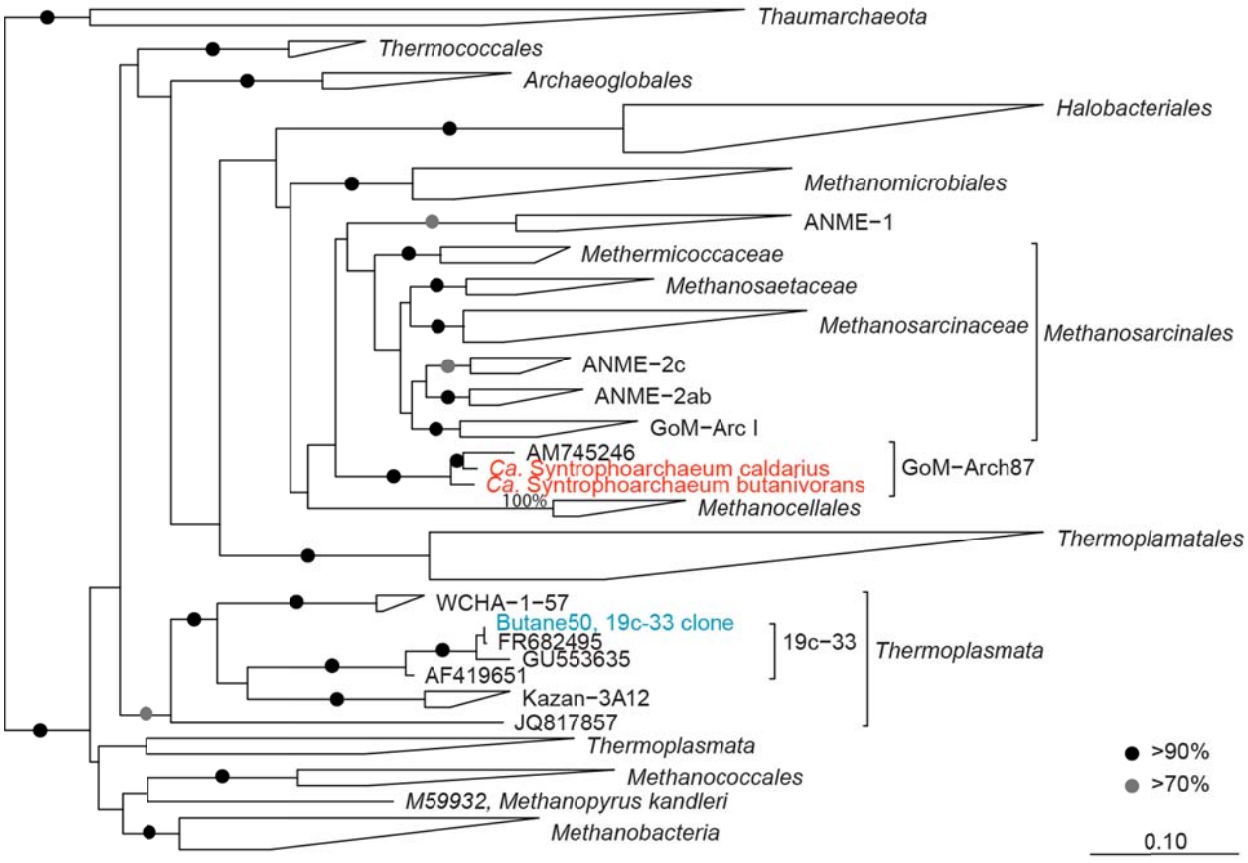


a IQ-tree (LG+I+F+C20) with full-length sequences
b RAxML tree (PROTGAMMALG+I+F) for only a partial region of full-length sequences
c RAxML tree (PROTGAMMALG+I+F) for only a partial region including partial *Bathyarchaeota* sequences
d IQ-tree (LG+I+F+C20) for only a partial region including partial *Bathyarchaeota* sequences
e RAxML tree (PROTGAMMALG+I+F) of full-length sequences but excluding SCAL_000352 of the calculation

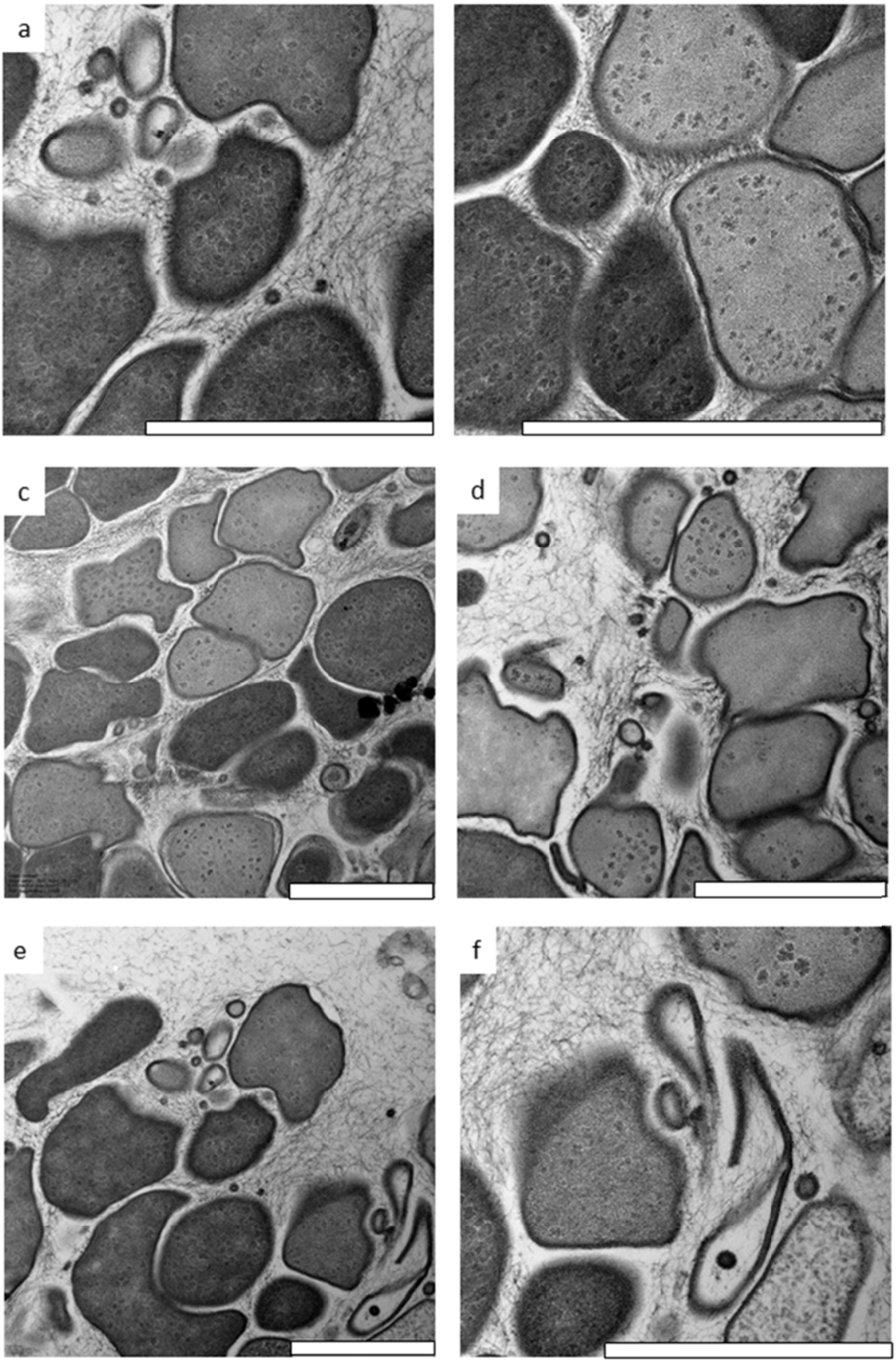
Supplementary Figure 2. Dependence of the free energy of butane oxidation (archaeal partner, red line) and sulfate reduction (bacterial partner, green lines) on the partial pressure of H_2 as an assumed intermediate (see Supplementary Discussion).



Supplementary Figure 3. Phylogenetic affiliation of the 16S rRNA gene sequences from the studied *Ca.* Syntrophoarchaeum strains (in red) and the *Thermoplasmata* sequence (blue) obtained from the Butane50 enrichment within *Euryarchaeota* (outgroup = *Thaumarchaeota*); bar = 10% estimated sequence divergence; bootstrap values > 90% and >70% are indicated by filled black and grey circles, respectively, on corresponding branch.



Supplementary Figure 4. Additional electron micrographs showing the abundant presence of pili-like nanowires in the intercellular space of archaeal bacterial aggregates collected from the Butane50 culture. Bar scales 1 µm.



Continuation Supplementary Figure 4.