Supplementary Information Tables

Table S1. Composition of mineral medium (Zinder, 1998)

Components	Per (L) of distilled water
Zinder medium	NH_4Cl 0.5 g K_2HPO_4 0.4 g $MgCl_2.6H_2O$ 0.1 g $CaCl_2.2H_2O$ 0.05 g trace metal solution 10 mL resazurin (1 g/L) 100 µL • Medium was flushed with 70 % N_2 / 30 % CO_2 for 45 min • Sealed with Teflon coated stoppers and crimped • Autoclaved (121°C, 40 min) Amendments added post autoclave per 50 mL medium: $NaHCO_3$ (10 %) 0.5 mL Na_2S (5 %) 0.25 mL Vitamins 10X 0.5 mL
Vitamin solution	Per L: biotin 20 mg folic acid 20 mg pyridoxine hydrochloride 100 mg thiamine hydrochloride 50 mg riboflavin 50 mg nicotinic acid 50 mg DL-calcium pantothenate 50 mg Vitamin B12 20 mg *p*-aminobenzoic acid 50 mg lipoic acid 50 mg Note: • nitrogen flushed • sterile filtered in glovebox
Trace solution	Per L: nitriloacetic acid 4.5 g $FeSO_4.7H_2O$ 556 mg

	$MnSO_4 . H_2O$	86 mg
	$CoCl_2 .6H_2O$	170 mg
	$ZnSO_4.7H_2O$	210 mg
	H_3BO_3	19 mg
	$NiCl_2.6H_2O$	20 mg
	$Na_2MoO_4.2H_2O$	10 mg
	pH adjusted to 7.0 with 10 M KOH	

At least 600 µM added in (0.5 µL neat TCE)

Gas mix in chamber N_2 (95%) + H_2 (5%)

Table S2. Overview of microcosm replicates used in the study. Each temperature treatment (10–60°C) included five active TCE-amended replicates (A, B, C, F, G), two sterile controls (D, E; autoclaved and TCE-amended), and one anaerobic control (ANA; not amended with TCE). This setup enabled evaluation of both biological activity and abiotic controls across temperature regimes.

Temperature	Active replicates	Sterile controls	Anaerobic control
Incubation	TCE added	- Autoclave sterilisation -TCE added	No TCE added
10°C	1A, 1B, 1C, 1F, 1G	1D, 1E	1ANA
20°C	2A, 2B, 2C, 2F, 2G	2D, 2E	2ANA
30°C	3A, 3B, 3C, 3F, 3G	3D, 3E	3ANA
40°C	4A, 4B, 4C, 4F, 4G	4D, 4E	4ANA
50°C	5A, 5B, 5C, 5F, 5G	5D, 5E	5ANA
60°C	6A, 6B, 6C, 6F, 6G	6D, 6E	6ANA

Table S3. Replicate sequencing information, including if replicates where analysed, how many sequence reads were derived. Abbreviations: ENA sample ID= the European Nucleotide Archive (ENA) identifier used for archive sequence submission, Y= Yes, N= No, mcrA: methanogen specific primer mcrA, targeting the methyl coenzyme M reductase gene, TCE= TCE amended replicate, ANA= non TCE amended replicate, see Table S3 for explanation of the replicates

Replicate	Sequenced for 16S rDNA	Sequence reads	ENA sample ID	Sequenced for mcrA	Sequence reads	ENA sample ID	Replicate	Sequenced for 16S rDNA	Sequence reads	ENA sample ID	Sequenced for mcrA	Sequence reads	ENA sample ID
TCE 1A	Y	46883	S0	Y	93062	S36	TCE 4A	Y	80846	S18	Y	51018	S42
TCE 1B	Y	60171	S1	N			TCE 4B	Y	70019	S19	N		
TCE 1C	Y	70120	S2	N			TCE 4C	Y	69442	S20	N		
TCE 1D	N	-		N			TCE 4D	N	-		N		
TCE 1E	N	-		N			TCE 4E	N	-		N		
TCE 1F	Y	100879	S3	N			TCE 4F	Y	81024	S21	N		S43
TCE 1G	Y	98215	S4	N			TCE 4G	Y	88258	S22	N		
TCE 1ANA	Y	81074	S5	Y	69695	S37	TCE 4 ANA	Y	63974	S23	Y	113905	
TCE 2A	Y	104396	S6	Y	81608	S38	TCE 5A	Y	71001	S24	N		
TCE 2B	Y	42205	S7	N			TCE 5B	Y	47505	S25	N		
TCE 2C	Y	86218	S8	N			TCE 5C	Y	80966	S26	N		
TCE 2D	N	-		N			TCE 5D	N			N		
TCE 2E	N	-		N			TCE 5E	N			N		
TCE 2F	Y	89551	S9	N			TCE 5F	Y	89982	S27	N		
TCE 2G	Y	76786	S10	N			TCE5G	Y	69688	S28	N		
TCE 2 ANA	Y	87631	S11	Y	77117	S39	TCE 5ANA	Y	64204	S29	N		
TCE 3A	Y	56660	S12	Y	55810	S40	TCE 6A	Y	83382	S30	N		
TCE 3B	Y	46136	S13	N			TCE 6B	Y	67500	S31	N		
TCE 3C	Y	71947	S14	N			TCE 6C	Y	74574	S32	N		
TCE 3D	N	-		N			TCE 6D	N			N		
TCE 3E	N	-		N			TCE 6E	N			N		
TCE 3F	Y	77523	S15	N			TCE 6F	Y	74784	S33	N		
TCE 3G	Y	51973	S16	N			TCE 6G	Y	42211	S34	N		
TCE 3 ANA	Y	82805	S17	Y	63779	S41	TCE 6ANA	Y	72670	S35	N		

Table S4. Shapiro–Wilk normality test results for ethene and methane concentrations across different temperature treatments (n = 5 per group). The W statistic measures the degree to which the data conform to a normal distribution, with values approaching 1 indicating a higher degree of normality. The corresponding *p*-value determines the statistical significance of this deviation. Normality was assumed for $p > 0.05$. Significant deviations from normality were observed for ethene at 40°C and methane at 50°C ($p < 0.001$)

compound	sample	n	W	p_value	normal
ethene	10 °C	5	0.849173	0.191918	TRUE
ethene	20 °C	5	0.982363	0.946851	TRUE
ethene	30 °C	5	0.918554	0.520655	TRUE
ethene	40 °C	5	0.552394	0.000132	FALSE
ethene	50 °C	5	0.984929	0.959131	TRUE
ethene	60 °C	5	0.864498	0.244852	TRUE
methane	10 °C	5	0.898272	0.400409	TRUE
methane	20 °C	5	0.904646	0.436093	TRUE
methane	30 °C	5	0.922303	0.544903	TRUE
methane	40 °C	5	0.8108	0.098951	TRUE
methane	50 °C	5	0.572077	0.000257	FALSE
methane	60 °C	5	0.795051	0.073802	TRUE

Table S5. Pairwise Wilcoxon rank-sum test results for ethene and methane concentrations across temperature treatments. The table includes the U statistic, raw *p*-values, Benjamini–Hochberg adjusted *p*-values (*p*.adj), and significance calls (*p*.adj < 0.05). These comparisons were conducted to evaluate temperature-dependent differences in product accumulation. Significant differences are indicated as TRUE.

compound	group1	group2	U_statistic	p_value	p_adj	significant
ethene	10 °C	20 °C	0	0.007936508	0.014880952	TRUE
ethene	10 °C	30 °C	0	0.007936508	0.014880952	TRUE
ethene	10 °C	40 °C	20	0.150793651	0.161564626	FALSE
ethene	10 °C	50 °C	25	0.007936508	0.014880952	TRUE
ethene	10 °C	60 °C	25	0.007936508	0.014880952	TRUE
ethene	20 °C	30 °C	22	0.055555556	0.075757576	FALSE
ethene	20 °C	40 °C	24	0.015873016	0.026455026	TRUE
ethene	20 °C	50 °C	25	0.007936508	0.014880952	TRUE
ethene	20 °C	60 °C	25	0.007936508	0.014880952	TRUE
ethene	30 °C	40 °C	23	0.031746032	0.047619048	TRUE
ethene	30 °C	50 °C	25	0.007936508	0.014880952	TRUE
ethene	30 °C	60 °C	25	0.007936508	0.014880952	TRUE
ethene	40 °C	50 °C	5	0.150793651	0.161564626	FALSE
ethene	40 °C	60 °C	5	0.150793651	0.161564626	FALSE
ethene	50 °C	60 °C	15	0.69047619	0.69047619	FALSE
methane	10 °C	20 °C	0	0.007936508	0.011904762	TRUE
methane	10 °C	30 °C	0	0.007936508	0.011904762	TRUE
methane	10 °C	40 °C	0	0.007936508	0.011904762	TRUE
methane	10 °C	50 °C	21	0.095238095	0.119047619	FALSE
methane	10 °C	60 °C	25	0.007936508	0.011904762	TRUE
methane	20 °C	30 °C	11	0.841269841	0.841269841	FALSE
methane	20 °C	40 °C	16	0.547619048	0.586734694	FALSE
methane	20 °C	50 °C	25	0.007936508	0.011904762	TRUE
methane	20 °C	60 °C	25	0.007936508	0.011904762	TRUE
methane	30 °C	40 °C	16	0.547619048	0.586734694	FALSE
methane	30 °C	50 °C	25	0.007936508	0.011904762	TRUE
methane	30 °C	60 °C	25	0.007936508	0.011904762	TRUE
methane	40 °C	50 °C	25	0.007936508	0.011904762	TRUE
methane	40 °C	60 °C	25	0.007936508	0.011904762	TRUE
methane	50 °C	60 °C	23	0.031746032	0.043290043	TRUE

References

Zinder, S. H. (1998). Methanogens (Ch. 5). In R. S. Burlage, R. Atlas, D. Stahl, G. Sayler, & G. Geesey (Eds.), *Techniques in microbial ecology* (pp. 113–116). Oxford University Press.