

## Supplementary Materials

# **Biomethanation of Carbon Monoxide by Hyperthermophilic Artificial Archaeal Co-Cultures**

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**Table S1:** Chemical composition of medium A\*

Chemical	Concentration	L <sup>-1</sup>
NaCl		25.00 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O		0.14 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O		3.40 g
MgCl <sub>2</sub> ·6H <sub>2</sub> O		4.10 g
KCl		0.33 g
KH <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O		0.18 g
NiCl <sub>2</sub> ·6H <sub>2</sub> O		0.0005 g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O		0.0005 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O		0.01 g
Yeast extract		1.5 g
S <sub>0</sub> *		10.00 g
141 Trace element solution		10.00 mL
Balch's Vitamin solution (1)		10.00 mL
L-Cysteine-HCl·H <sub>2</sub> O	0.16 mol L <sup>-1</sup>	20 mL
NaHCO <sub>3</sub>	0.6 mol L <sup>-1</sup>	20.00 mL
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5 mol L <sup>-1</sup>	4.00 mL

\*S<sub>0</sub> was only augmented when *T. onnurineus* was inoculated.

**Table S2:** Chemical composition of medium B\*

Chemical	Concentration	L <sup>-1</sup>
NaCl		25.00 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O		0.40 g
NH <sub>4</sub> Cl		0.30 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O		3.90 g
Na <sub>2</sub> HPO <sub>4</sub>		0.15 g
Na <sub>2</sub> SiO <sub>3</sub>		0.03 g
KCl		0.70 g
Yeast extract		1.00 g
S <sub>0</sub> *		10.00 g
Holden's Trace element solution		1.00 mL
Balch's Vitamin solution (1)		1.00 mL
L-Cysteine-HCl·H <sub>2</sub> O	0.16 mol L <sup>-1</sup>	20 mL
NaHCO <sub>3</sub>	0.6 mol L <sup>-1</sup>	10.00 mL
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5 mol L <sup>-1</sup>	4.00 mL

\*S<sub>0</sub> was only augmented when *T. onnurineus* was inoculated.

**Table S3:** 141 trace element solution modified from DSMZ

Chemical	L <sup>-1</sup>
Nitrilotriacetic acid	1.50 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.00 g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.586 g
NaCl	1.00 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.10 g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.10 g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18 g
CuSO <sub>4</sub>	0.007 g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02 g
H <sub>3</sub> BO <sub>3</sub>	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01 g
NiCl <sub>2</sub> ·2H <sub>2</sub> O	0.03 g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.0003 g
Na <sub>5</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.0004 g

**Table S4:** Holden's trace element solution 2

Chemical	L <sup>-1</sup>
Nitrilotriacetic acid	4.5 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	1.54 g
CuSO <sub>4</sub>	0.00627 g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.005 g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.10 g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.10 g
KBr	0.05 g
KI	0.05 g
H <sub>3</sub> BO <sub>3</sub>	0.10 g
NaF	0.05 g
LiCl	0.05 g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.0693 g
NiCl <sub>2</sub> ·12H <sub>2</sub> O	0.01 g
VO <sub>2</sub> SO <sub>4</sub> ·H <sub>2</sub> O	0.0042 g
Na <sub>5</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.0059 g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.0069 g
SrCl	0.005 g
BaCO <sub>3</sub>	0.0047 g

**Table S5:** The physiological maximal and mean values of CH<sub>4</sub> production and growth kinetics of *M. marburgensis* and *M. thermautotrophicus*

	Substrate	Turnover (max)	MER <sub>max</sub> / mmol L <sup>-1</sup> h <sup>-1</sup>	MER <sub>mean</sub> / mmol L <sup>-1</sup> h <sup>-1</sup>	qCH <sub>4,max</sub> / mmol g <sup>-1</sup> h <sup>-1</sup>	qCH <sub>4,mean</sub> / mmol g <sup>-1</sup> h <sup>-1</sup>	μ <sub>max</sub> / h <sup>-1</sup>	μ <sub>mean</sub> / h <sup>-1</sup>
<i>M. marburgensis</i>	H <sub>2</sub> :CO	93.93 %	1.37	0.947	13.8	6.12	0.013	0.004
	H <sub>2</sub> :CO	30.60 %	0.111	0.043	5.53	1.81	0.001	0.001
	H <sub>2</sub> :CO	16.15 %	0.036	0.007	1.62	0.406	0.002	0.001
	CO	-	-	-	-	-	-	-
<i>M. thermautotrophicus</i>	H <sub>2</sub> :CO	80.09 %	1.36	0.845	16.3	6.59	0.013	0.005
	H <sub>2</sub> :CO	30.35 %	0.114	0.017	8.79	1.45	0.002	0.001
	CO	-	-	-	-	-	0.003	0.000

**Table S6:** HER of *T. onnurineus* after 7h of incubation\*

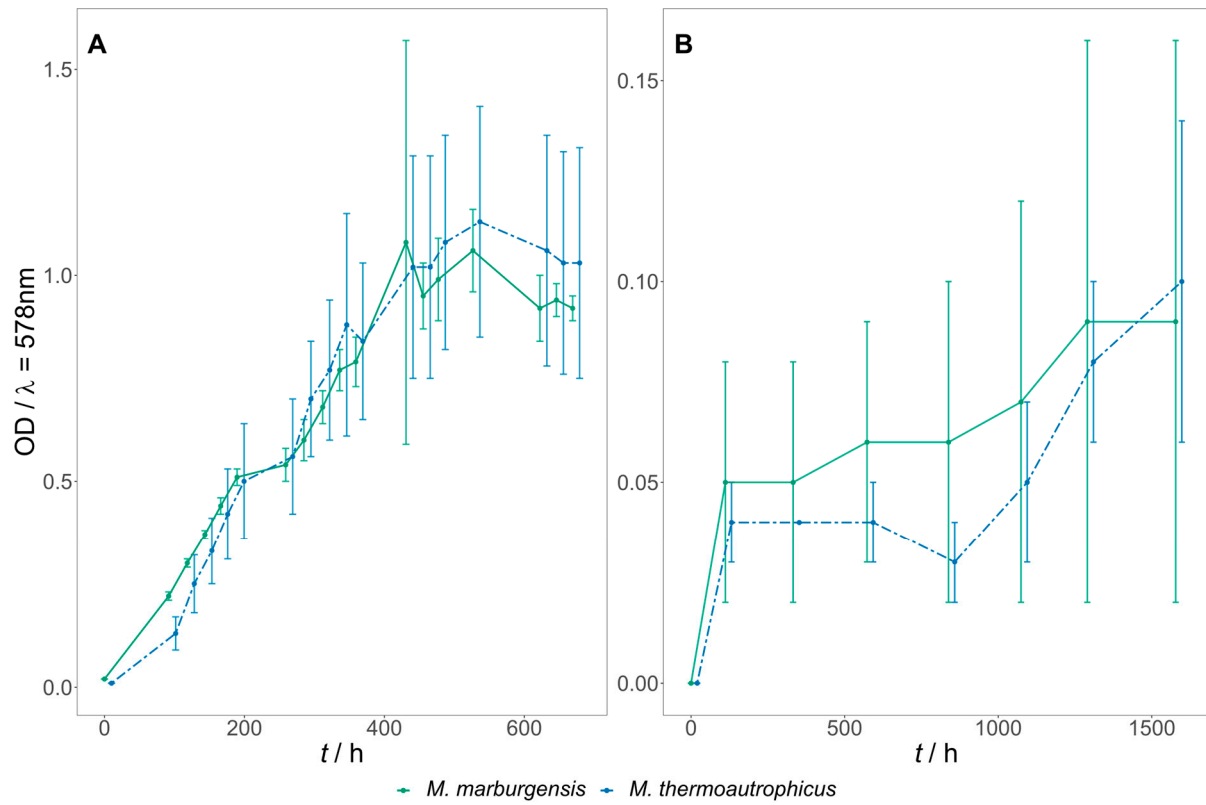
Medium	HER / mmol L <sup>-1</sup> h <sup>-1</sup>
A	6.7±0.6
B	4.8±0.4
B without vitamin solution	4.6±1.0

\*An initial pressure of 1 barg CO was used. Values are shown with standard deviation. (N = 1, n = 3).

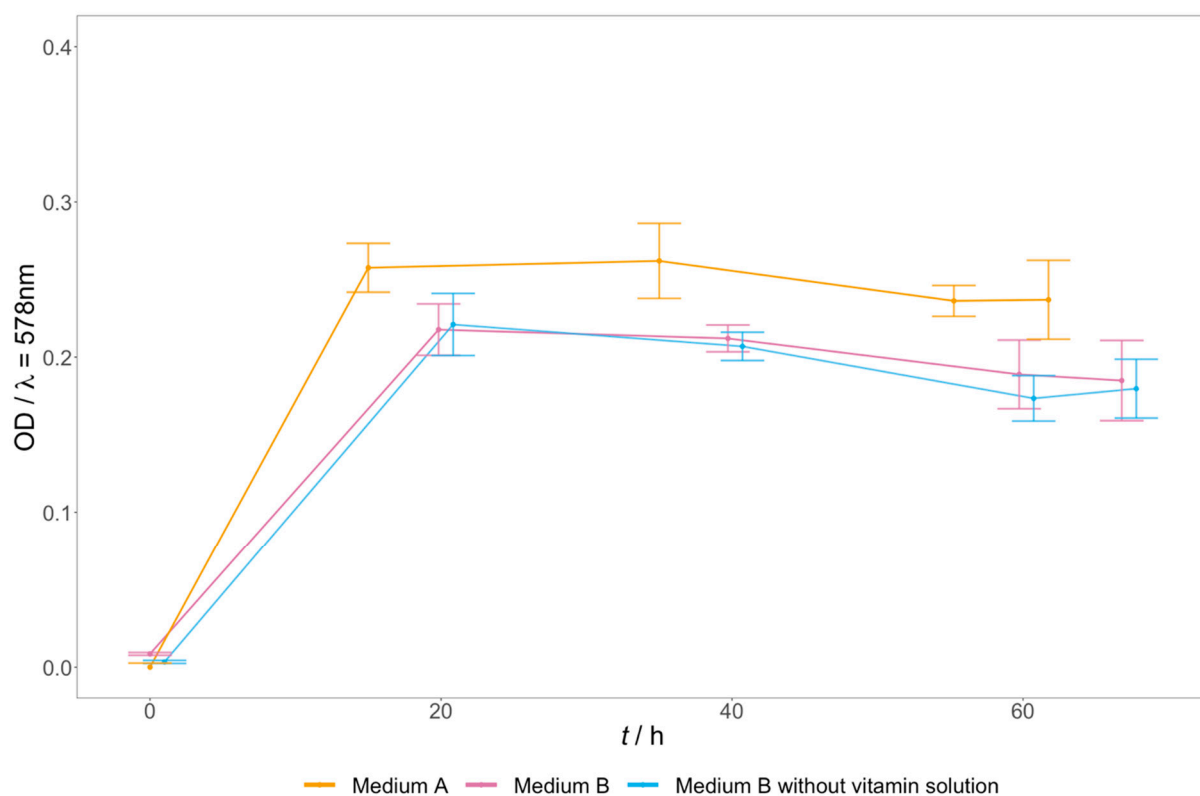
**Table S7:** Relative mean molar gas composition of the co-culture's headspace after a 7 h incubation period\*

Co-culture	Gas	CO / mol-%	CH <sub>4</sub> / mol%	H <sub>2</sub> / mol%	CO <sub>2</sub> / mol%	N <sub>2</sub> / mol%
<i>M. villosus</i> + <i>T. onnurineus</i>	CO	51.4	10.5	0.3	37.8	0
<i>T. onnurineus</i> + <i>M. villosus</i>	CO	71.7	6.7	0.1	21.5	0
<i>M. villosus</i> + <i>T. onnurineus</i>	H <sub>2</sub> :CO	39.0	7.1	53.8	0.1	0
<i>T. onnurineus</i> + <i>M. villosus</i>	H <sub>2</sub> :CO	40.0	7.3	52.7	0.1	0
<i>M. villosus</i> + <i>T. onnurineus</i>	Art. syngas	30.3	24.9	5.8	22.3	16.7
<i>T. onnurineus</i> + <i>M. villosus</i>	Art. syngas	30.7	22.5	7.1	22.9	16.7

\*(N = 1, n = 6)



**Figure S1:** Growth of *M. marburgensis* and *M. thermoautotrophicus* in defined medium. A: growth under  $\text{H}_2:\text{CO}_2$ . B: growth under  $\text{H}_2\text{CO}$ . No growth was observed while gassing with pure CO (data not shown). Error bars show the standard deviation.



**Figure S2:** Growth of *T. onnurineus* at 1 barg CO. Error bars show the standard deviation. N = 1, n = 3.

## References

1. Balch WE, Wolfe R. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl Environ Microbiol.* 1976;32(6):781–91.