The physiology and biotechnology of pure culture biological methane production from $\text{H}_2$ and $\text{CO}_2$

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Biofuels – motivation
**Methanogens**

**Methanothermobacter marburgensis**

Phase contrast image of *M. marburgensis*. Specimen was obtained in late exponential phase from a fed-batch fermentation. The cells are rod shaped and elongated. 

Taubner et al., 2015

**Methanobacterium thermaggregans**

Scanning electron micrograph of *M. thermaggregans*. Blotevogel and Fischer, 1985

**Methanothermococcus okinawensis**

Electron micrograph of negatively stained cells in mid-exponential phase of growth. The polar bundle of flagella is observed. Bar 0.4 µm. 

Takai et al., 2002

\[4H_2 + CO_2 \rightarrow CH_4 + 2H_2O\]
Continuous culture – MER

The graph shows the relationship between MER/MER_max (%) and CH_4 offgas (Vol.-%). The data points represent various studies with different symbols indicating different datasets.

- MER/MER_max model
- Bernacchi, Rittmann, and Seifert et al.
- Jee et al., 1987 (membrane)
- Jee et al., 1987 (ceramic support)
- Jee et al., 1988a
- Jee et al., 1988b
- Peillex et al., 1990
- Nishimura et al., 1991
- Nishimura et al., 1992
- Martin et al., 2013

Rittmann et al., 2018
Continuous culture – qCH$_4$

![Graph showing continuous culture relationship between qCH$_4$ and D](image)

- Rittmann et al., 2012; Bernacchi et al., 2014
- Bernacchi et al., 2014 (DoE)
- Seifert et al., 2013
- Bernacchi et al., 2016
- Peillex et al., 1990
- Nishimura et al., 1991

Rittmann, unpublished
Continuous culture

n = 197

MLR model only for MER

MLR models for MER, $r(x)$, $Y_{(x/CH_4)}$ and CH$_4$ offgas

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<tr>
<th>#</th>
<th>parameter/variable</th>
<th>n = 159</th>
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<td>$H_2$ CO$_2$ in (vvm)</td>
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<td>12</td>
<td>$r(x)$</td>
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<td>14</td>
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<td>15</td>
<td>$\mu$</td>
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<td>16</td>
<td>qCH$_4$</td>
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<td>17</td>
<td>$Y_{CH_4}$</td>
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<tr>
<td>18</td>
<td>$Y_{(x/CH_4)}$</td>
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Rittmann et al., 2018
# Continuous culture

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<td>Y(CH₄)</td>
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<td>Y(CH₄/xCH₄)</td>
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n = 159

Rittmann et al., 2018
Continuous culture – MER/r$_{(x)}$
Continuous culture – $q_{CH_4}$

Rittmann et al., 2018
Continuous culture – qCH$_4$
Growth (OD$_{578}$ nm) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates, respectively.

Abdel Azim et al., 2017
Methane evolution rate (MER) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates, respectively.

*Abdel Azim et al., 2017*
Fed-batch
Comparison of cumulative CH$_4$ production (cum. CH$_4$ production), max. CH$_4$ evolution rate (MER$_{\text{max}}$), max. biomass production rate ($r_{(x),\text{max}}$), and maximum biomass concentration ($x_{\text{max}}$) of *M. thermaggregans* and *M. marburgensis*. The gray bars indicate the performance of *M. marburgensis* at 65 °C, a pH of 7.0, and with exponential feed. The black bars show *M. thermaggregans* cultivated at 60 °C, a pH of 7.0, and with exponential feed. Both strains were cultivated within 1.5 L of MM medium and continuously gassed with H$_2$/CO$_2$ (80 Vol.-% H$_2$ in CO$_2$) at atmospheric pressure. H$_2$/CO$_2$ and DS were exponentially fed to the suspension. The exponential feeding experiments were performed in triplicates. Striped bars show the results from *M. thermaggregans*, observed at the following conditions (optimal DoE runs): cum. CH$_4$ production (G–N: 60 °C and 7.0 pH), MER$_{\text{max}}$ (U, V: 65 °C and 7.4 pH), and $r_{(x),\text{max}}$ and $x_{\text{max}}$ (O: 60 °C and 7.8 pH).

Mauerhofer et al., 2018
Closed batch

- flush and purge
- weigh
- incubate
- weigh
- determine pressure

\[ 4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \]

Taubner and Rittmann, 2016
Closed batch

\[ n_{\text{CH}_4} \rightarrow \text{MER}_{\Delta p} \]

\[ \Delta p \]

\[ x, n_{\text{CH}_4} \rightarrow \text{MER}_{\Delta p + \text{OD} + \text{GC}} \]

\[ x, n_{\text{CH}_4} \rightarrow \text{MER}_{\Delta p + \text{OD}} \]

GC

OD

\[ \text{incubation} \]

\[ \Delta m \]

\[ \Delta m \]

\[ \text{gassing} \]

\[ \text{WER} \]
**M. okinawensis** produces CH₄ up to 90 bar

![Graph showing pressure drop over time under different pressure conditions.](image)

![Bar chart showing conversion and time until conversion under different conditions.](image)

**H₂/CO₂ conversion of M. okinawensis under high pressure conditions.** a, decreasing pressure over time as indirect evidence for stoichiometric H₂/CO₂ to CH₄ conversion. If 50 Vol.-% N₂ is added to the gas phase, the time until conversion from CO₂ to CH₄ is even shorter (as seen for the experiments at 20 bar). b, the conversion (or turnover) was examined using the observed pressure drop according to equation (2) without the division by Δt.
The dual nature of the qCH$_4$ dependence from D/vvm can originate from both, the variable capacity to transfer the gas limiting substrate or from physiological variations that influence $Y_{(x/CH4)}$

MER of 945 mmol L$^{-1}$ h$^{-1}$ could already be achieved at low $S_{in}/r_{(x)}$ ratios in continuous culture with $M. marburgensis$

Physiological characteristics and MER of $M. marburgensis$ are superior compared to $M. okinawensis$ and $M. thermaggegans$ when cultivated in fed-batch cultivation mode

Exponential fed-batch cultivations of $M. marburgensis$ resulted in a MER of 476 mmol L$^{-1}$ h$^{-1}$ and the highest ever reported $\mu$ of 0.69 h$^{-1}$, and closed batch at high pressure demonstrate the versatility of methanogens for gas conversion processes

We need to go beyond biotechnology and statistics to understand the physiology of methanogens
CO$_2$-BMP – an example

- $17,800 \text{ kWh a}^{-1}$ (100 m$^2$, 3 persons) Statistik Austria  $ightarrow 2,032 \text{ kWh h}^{-1}$

- Biological CH$_4$ production bioreactor produces $950 \text{ mmol L}^{-1} \text{ h}^{-1} = 212.294 \text{ kWh m}^{-3} \text{ h}^{-1}$

➢ A ~10L (C)STR would be sufficient to supply three people living a 100 m$^2$ flat with bioenergy!
Thank you for your attention!
Further reading

Quantitative analysis of media dilution rate effects on *Methanothermobacter marburgensis* grown in continuous culture on H₂ and CO₂

S. Rittmann, A. Seifert, C. Herwig

*Biomass & Bioenergy, 2012*

Short Communication

Method for assessing the impact of emission gasses on physiology and productivity in biological methanogenesis

A.H. Seifert 1, S. Rittmann 1, S. Bernacchi, C. Herwig

*Vienna University of Technology, Institute of Chemical Engineering, Research Area Biochemical Engineering, Gumpendorferstraße 11a, 1060 Vienna, Austria*

*Bioresource Technology, 2013*

Analysis of process related factors to increase volumetric productivity and quality of biomethane with *Methanothermobacter marburgensis*

A.H. Seifert, S. Rittmann, C. Herwig

*Bioresource Technology, 2013*

Rapid extraction of total RNA from an anaerobic sludge biocenosis

Simon Rittmann • Peter Holubar

*Folia Microbiologica, 2014*

**REVIEW ARTICLE**

Essential prerequisites for successful bioprocess development of biological CH₄ production from CO₂ and H₂

Simon Rittmann*¹, Arne Seifert*², and Christoph Herwig

*Critical Reviews in Biotechnology, 2015*

**A Critical Assessment of Microbiological Biogas to Biomethane Upgrading Systems**

**Advances in Biochemical Engineering/Biotechnology, 2015**

Simon K.-M.R. Rittmann

Review

Assessing the Ecophysiology of Methanogens in the Context of Recent Astrobiological and Planetological Studies

Ruth-Sophie Taubner ¹,², Christa Schleper ³, Maria G. Firneis ¹,² and Simon K.-M. R. Rittmann ³

*Life, 2015*

**Method for Indirect Quantification of CH₄ Production via H₂O Production Using Hydrogenotrophic Methanogens**

Ruth-Sophie Taubner ¹,²,³ and Simon K.-M. R. Rittmann ³

*Frontiers in Microbiology, 2016*
Further reading

The physiology of trace elements in biological methane production

Bioreource Technology, 2017

Biological methane production under putative Enceladus-like conditions

Nature Communications, 2018

Evidence for archaeal methanogenesis within veins at the onshore serpentinite-hosted Chimaera seeps, Turkey

Chemical Geology, 2018

Intact polar lipid and core lipid inventory of the hydrothermal vent methanogens Methanocaldococcus villosus and Methanothermococcus okinawensis
Lydia M.F. Baumann, Ruth-Sophie Taubner, Thorsten Bauersachs, Michael Steiner, Christa Schleper, Jörn Peckmann, Simon K.-M.R. Rittmann, Daniel Birgel

Organic Geochemistry, 2018

Physiology and methane productivity of Methanobacterium thermagogrenans

Applied Microbiology and Biotechnology, 2018

The physiological effect of heavy metals and volatile fatty acids on Methanococcus maripaludis S2
Annalisa Abdel Azim, Simon K.-M. R. Rittmann, Debora Fino and Günther Bochmann

Biotechnology for Biofuels, 2018

Methods for quantification of growth and productivity in anaerobic microbiology and biotechnology

Folia Microbiologica, 2018

Kinetics, multivariate statistical modelling, and physiology of CO₂-based biological methane production
Simon K.-M.R. Rittmann, Arne H. Seifert, Sébastien Bernacchi

Applied Energy, 2018