The physiology and biotechnology of pure culture biological methane production from H_2 and CO_2

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Archaea Physiology & Biotechnology Group

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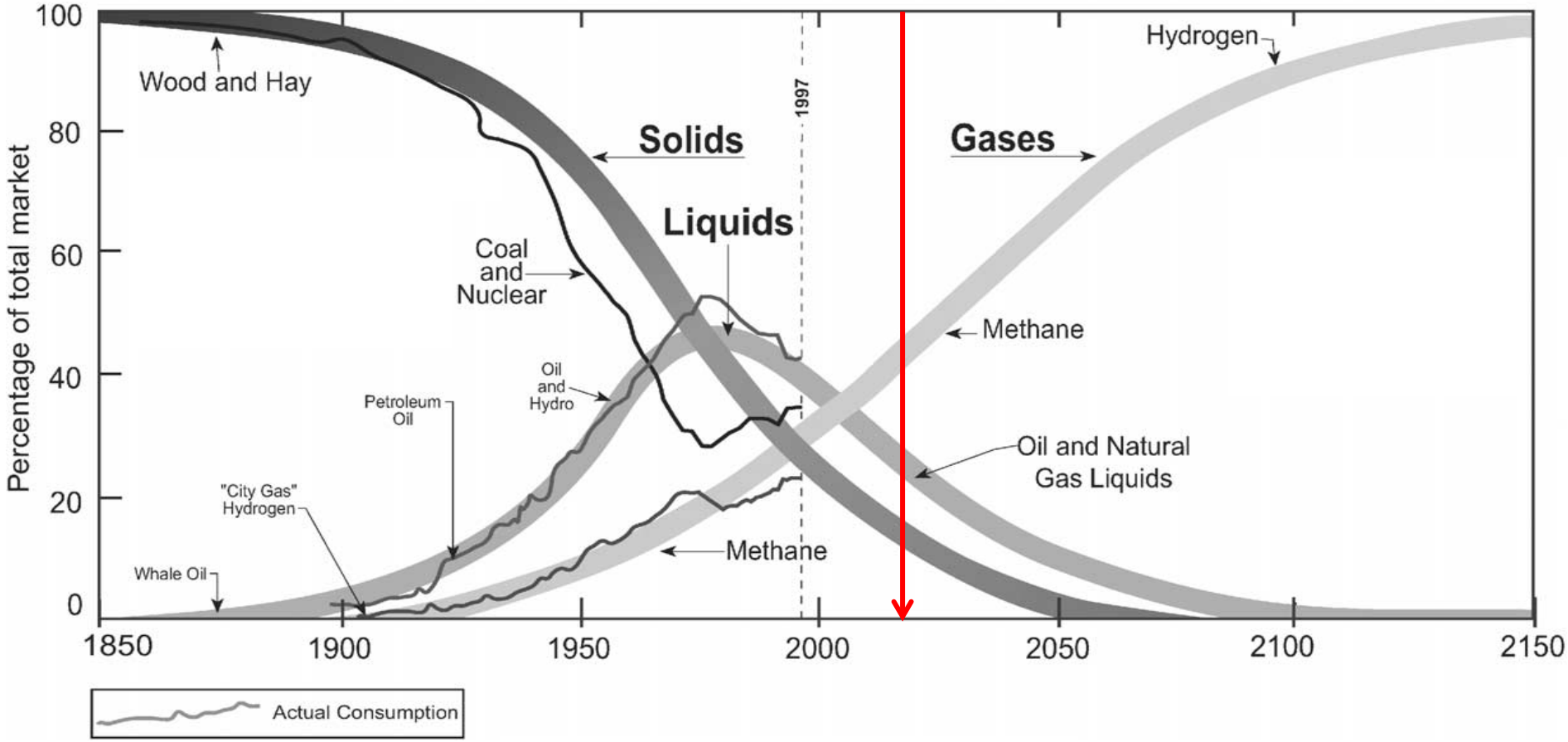
Department of Ecogenomics and Systems Biology

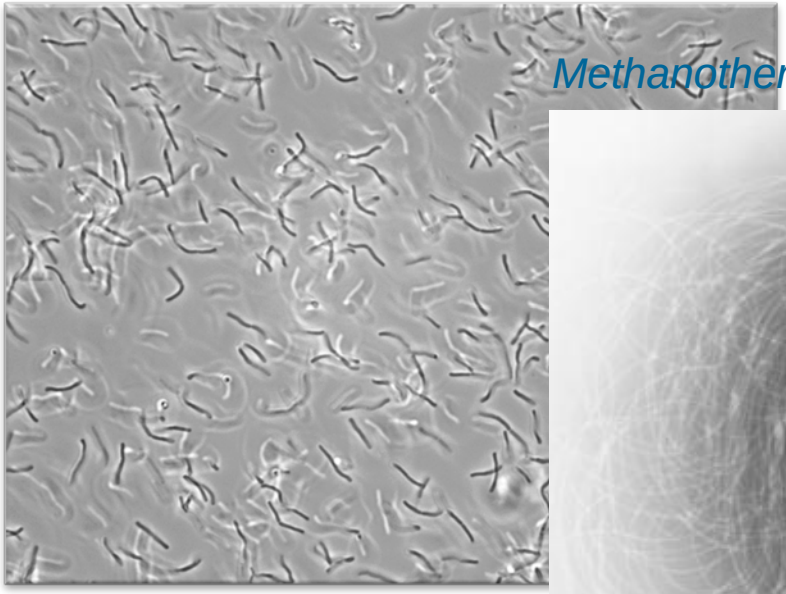
Biofuels – motivation



Winter, 2000

Methanogens

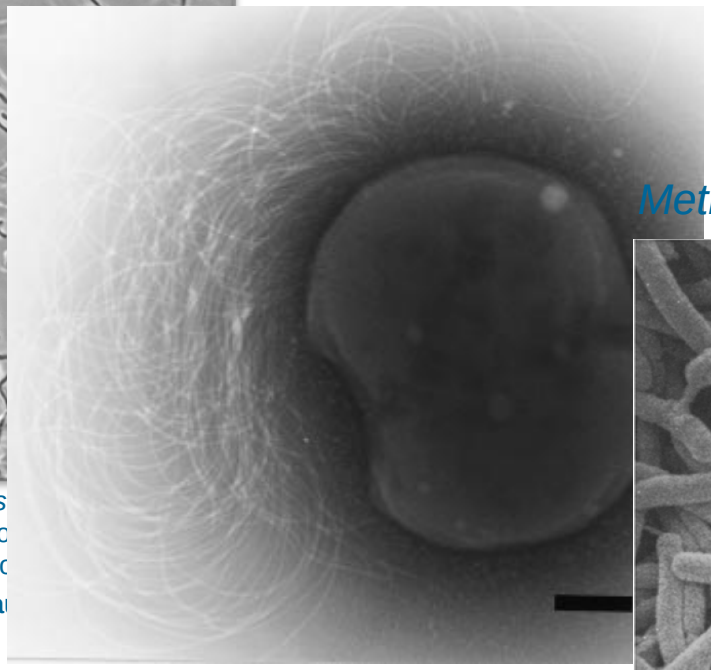
Methanothermobacter marburgensis



Phase contrast image of *M. marburgensis* obtained in late exponential phase fro fermentation. The cells are rod shaped and elo

Ta

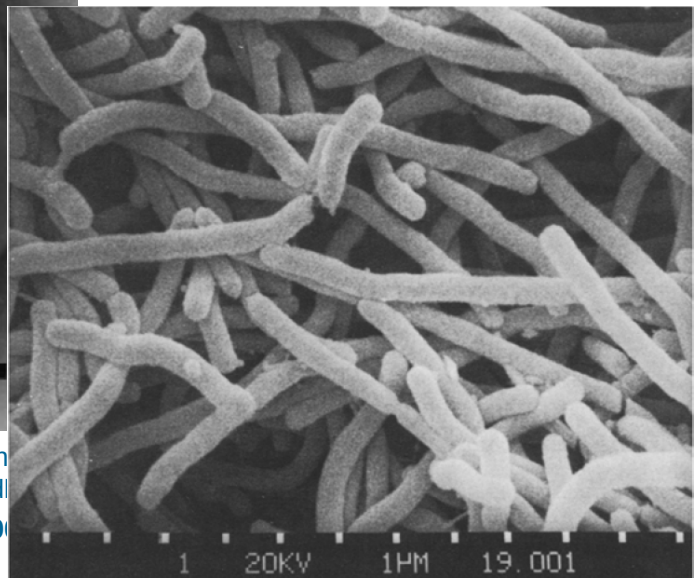
Methanothermococcus okinawensis



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

Takai *et al*., 20

Methanobacterium thermaggregans

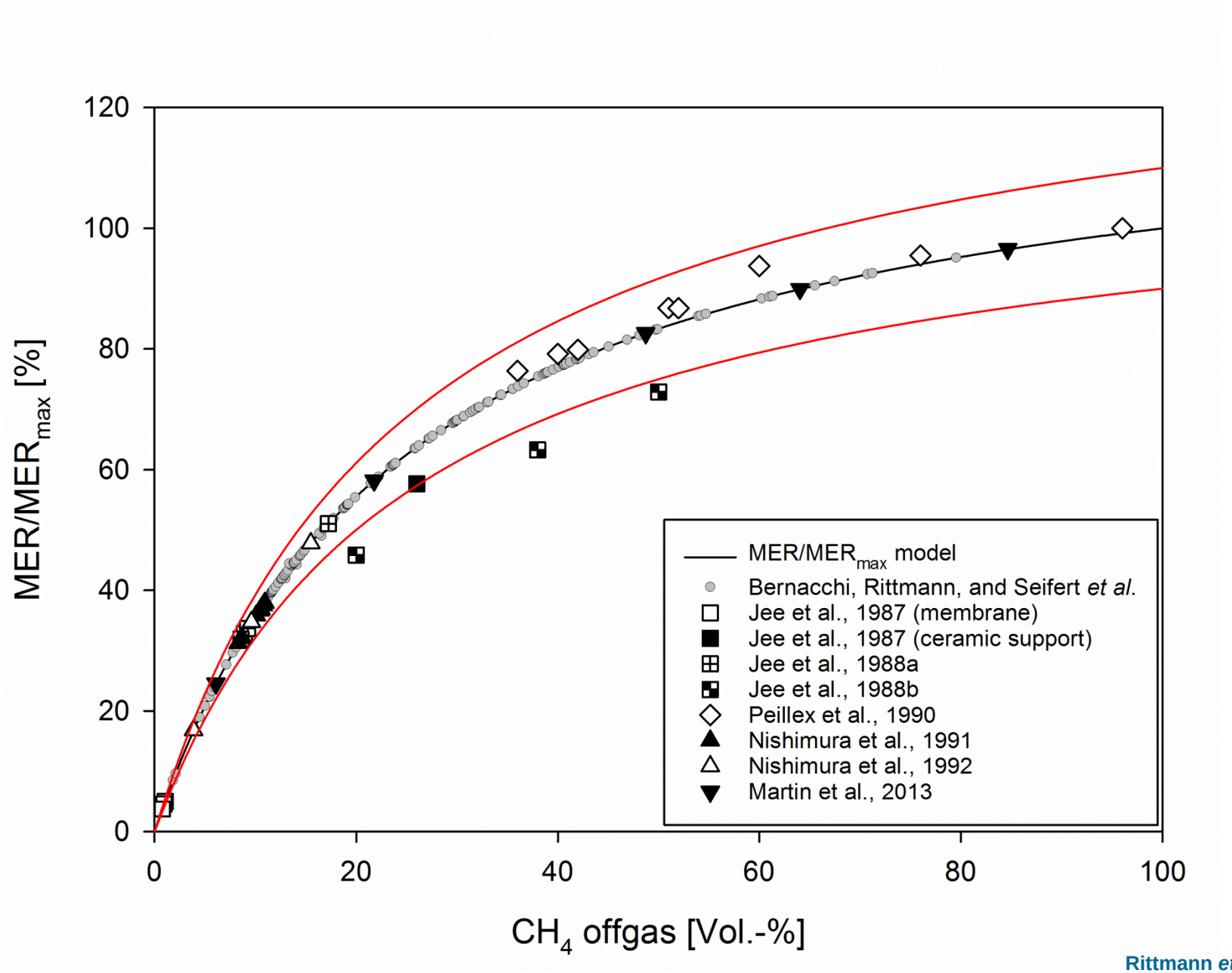


Scanning electron micrograph of Methanobacterium thermaggregans.

Blotevogel and Fischer, 1985

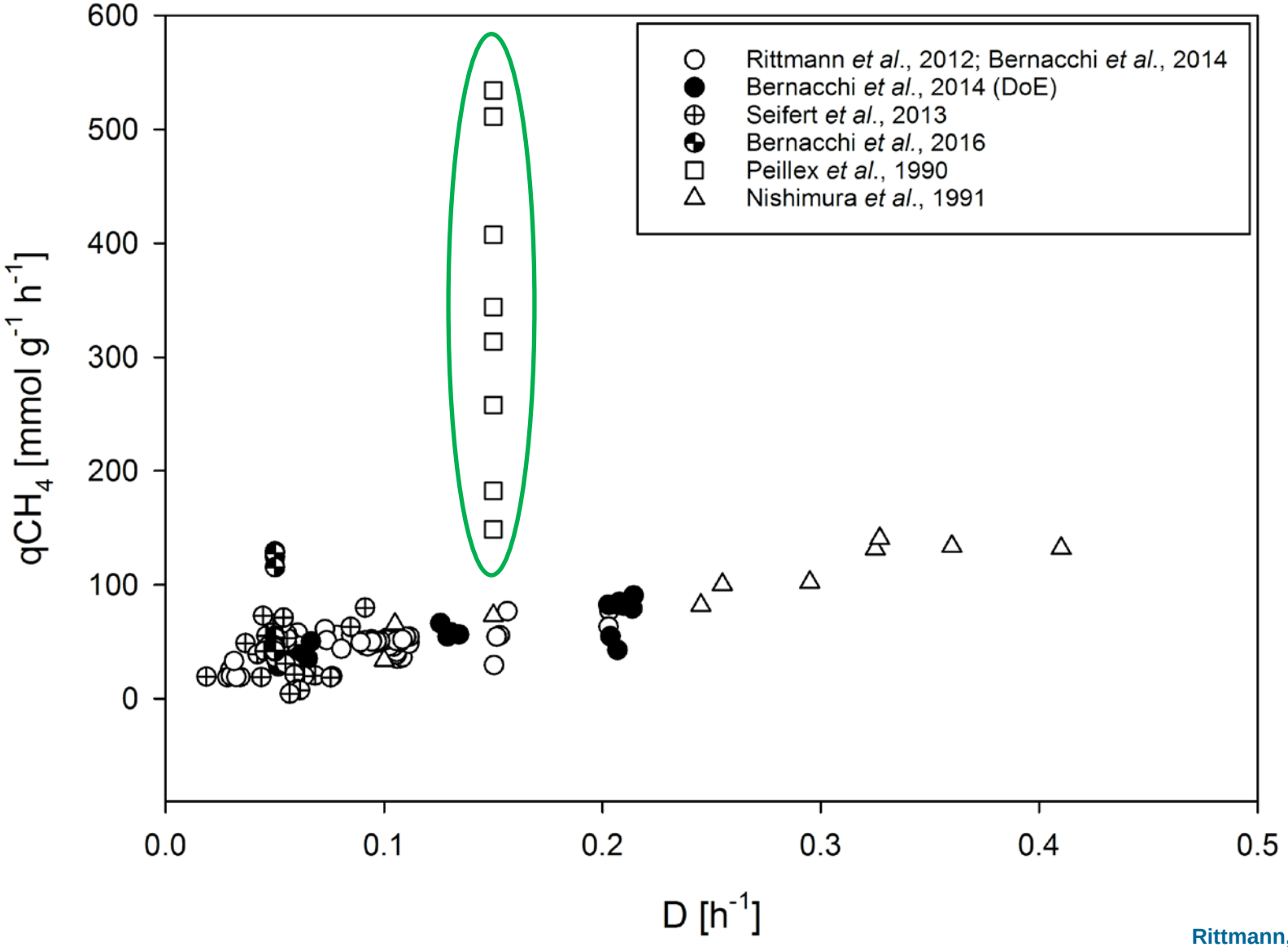
$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

Continuous culture – MER



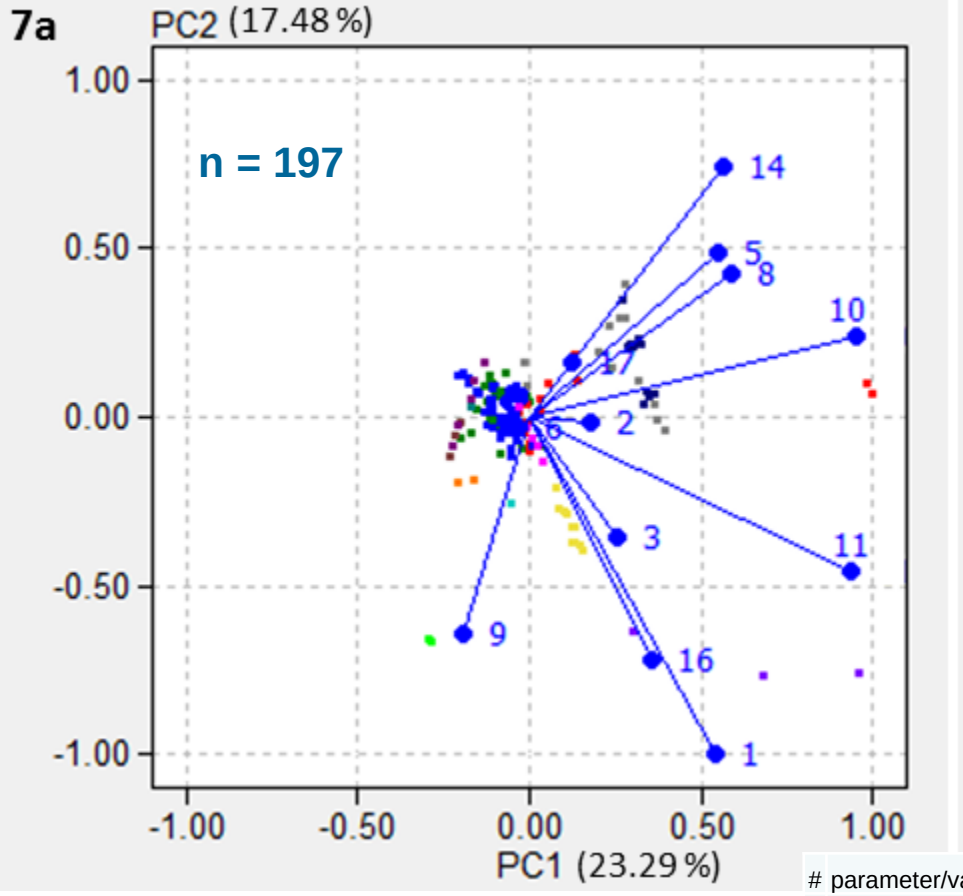
Rittmann *et al.*, 2018

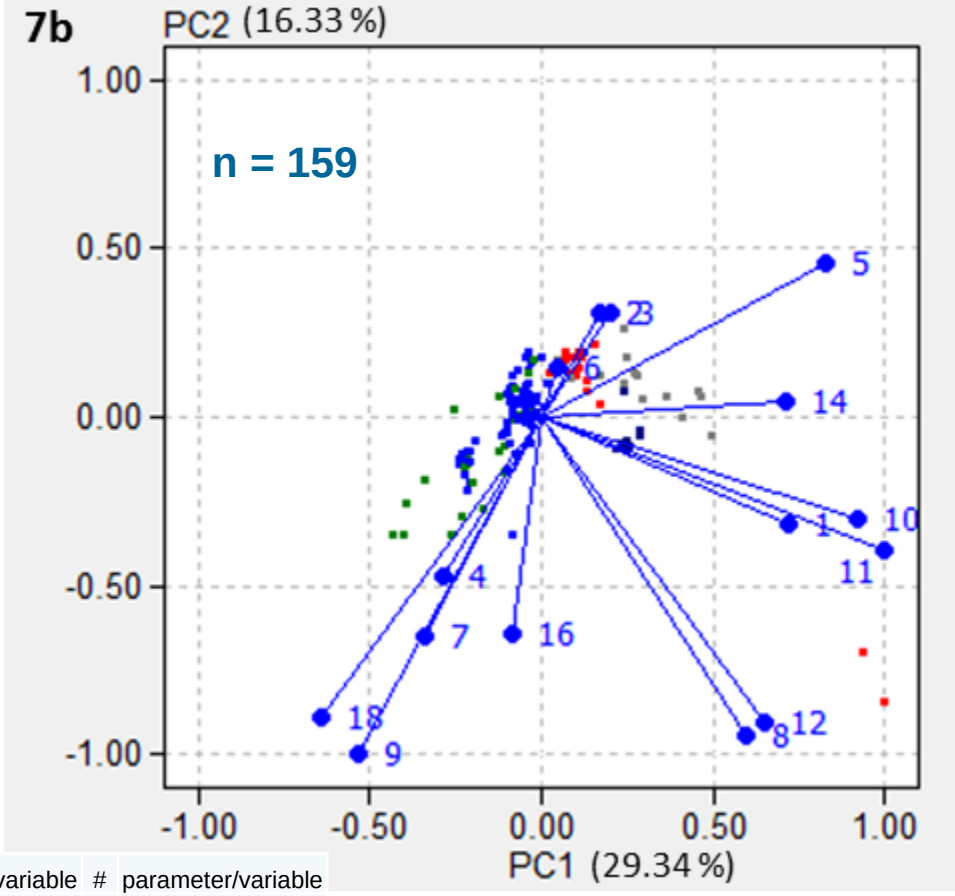
Continuous culture – qCH_4



Rittmann, unpublished

Continuous culture





#	parameter/variable	#	parameter/variable
1	H_2 CO_2 in (vvm)	10	pressure
2	temperature	11	MER
3	pH	12	$r_{(x)}$
4	ORP	13	x
5	agitation	14	CH_4 offgas
6	gassing ratio	15	μ
7	DS	16	qCH_4
8	TE	17	Y_{CH4}
9	D	18	$Y_{(x/CH4)}$

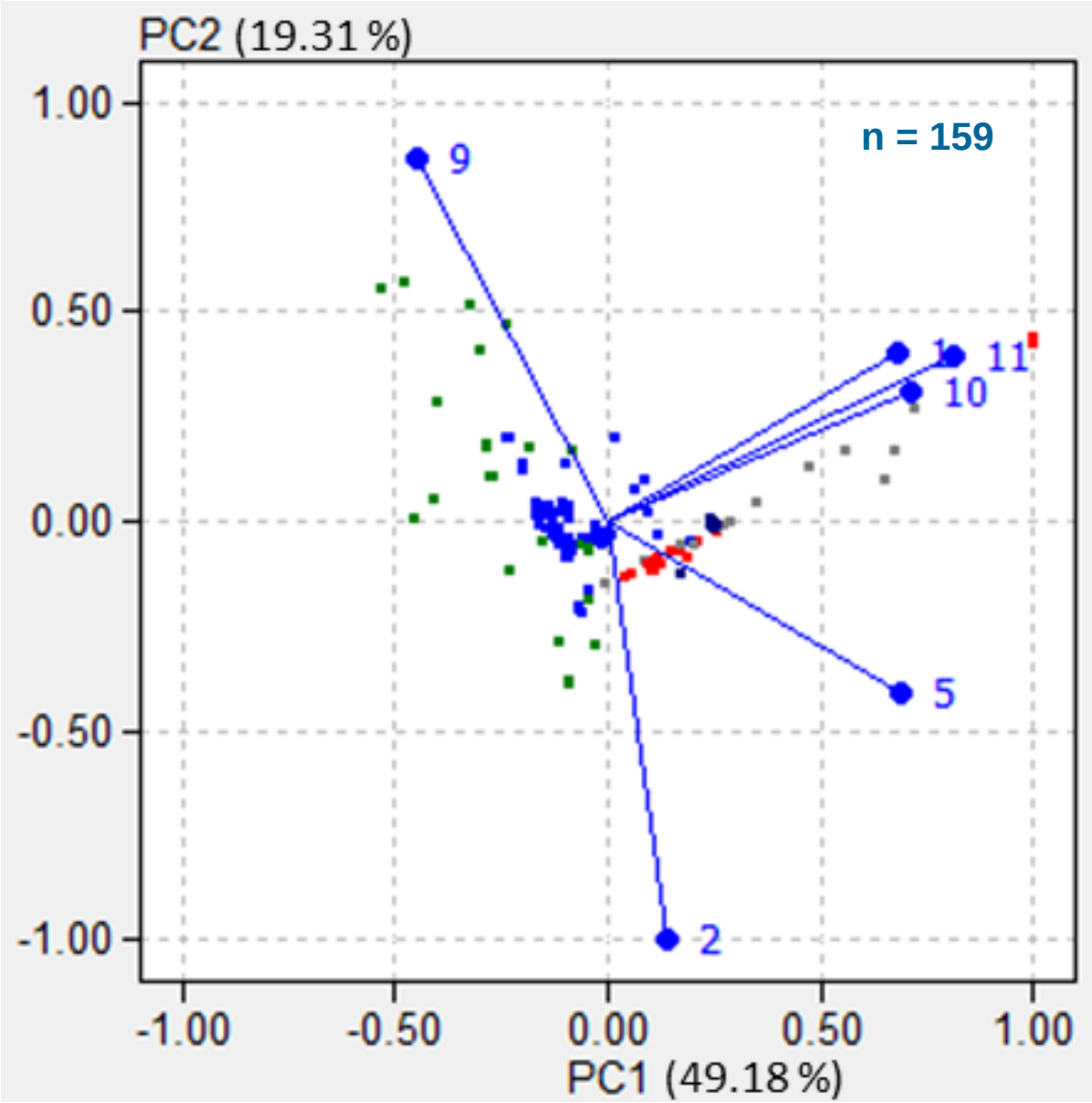
MLR model only for MER

MLR models for MER, $r_{(x)}$, $Y_{(x/CH4)}$ and CH_4 offgas

Rittmann *et al.*, 2018

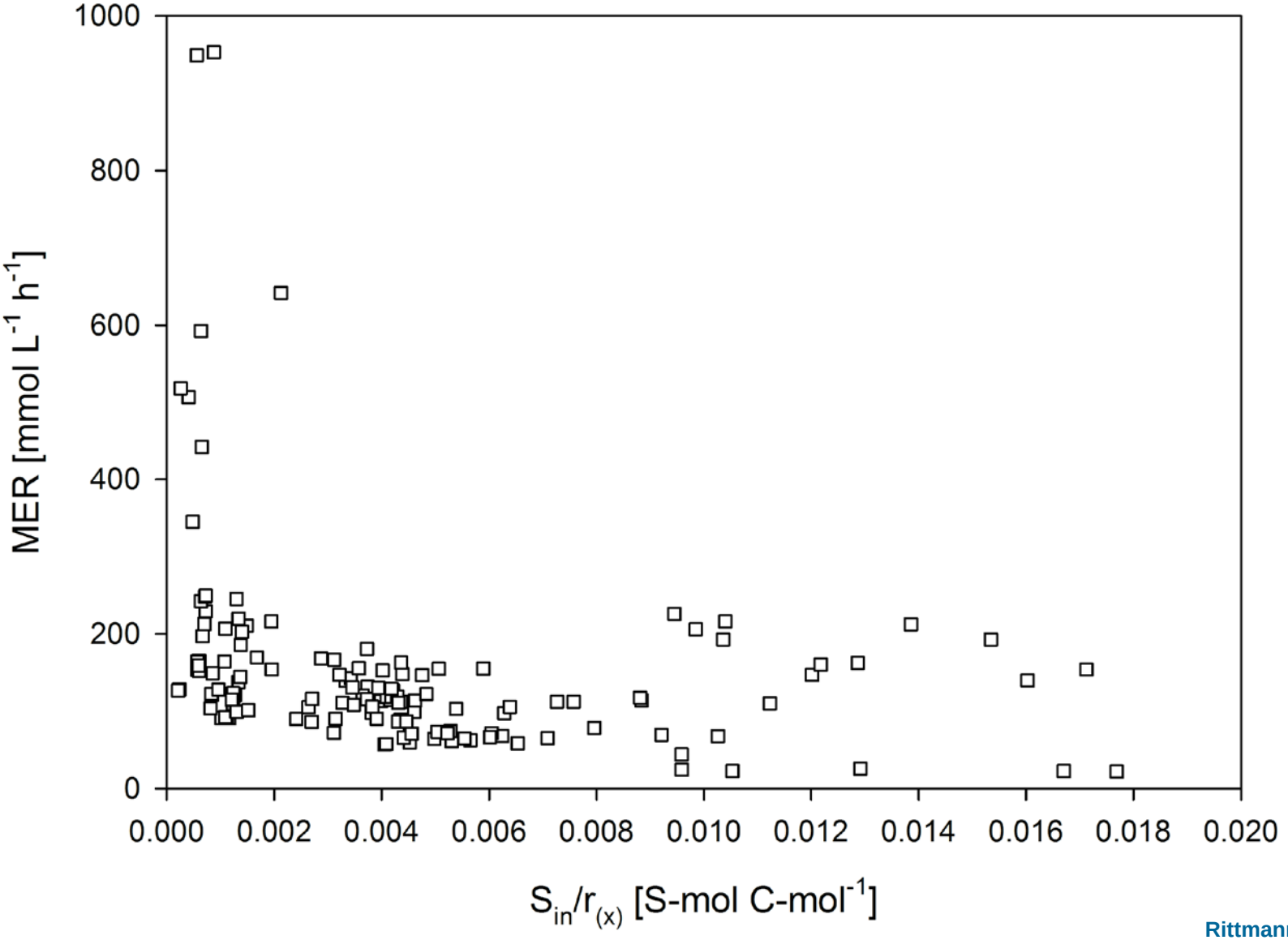
Continuous culture

#	parameter/variable
1	H_2 CO_2 in (vvm)
2	temperature
3	pH
4	ORP
5	agitation
6	gassing ratio
7	DS
8	TE
9	D
10	pressure
11	MER
12	$r_{(x)}$
13	x
14	CH_4 offgas
15	μ
16	qCH_4
17	Y_{CH4}
18	$Y_{(x/CH4)}$



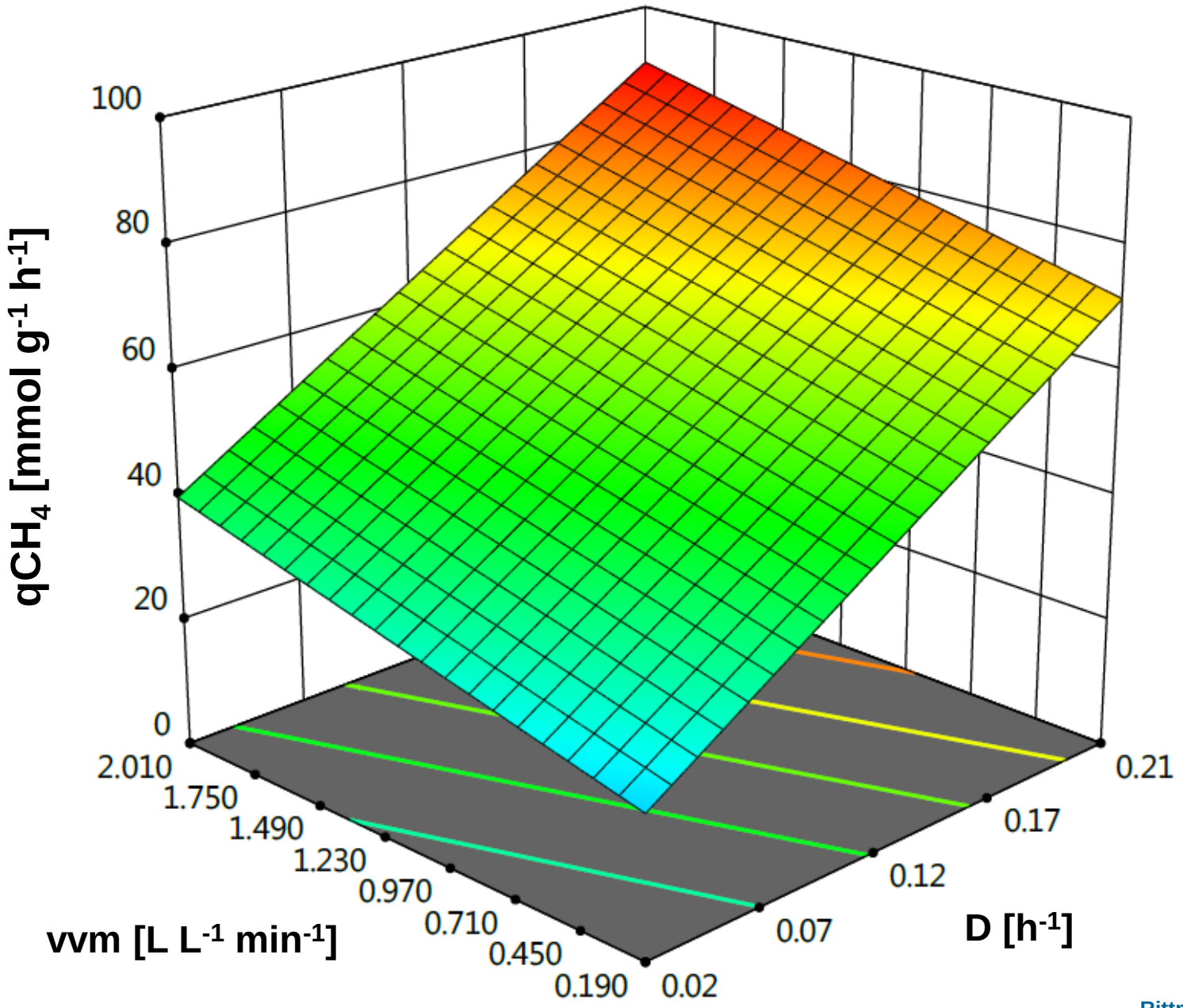
Rittmann *et al.*, 2018

Continuous culture – MER/$r_{(x)}$



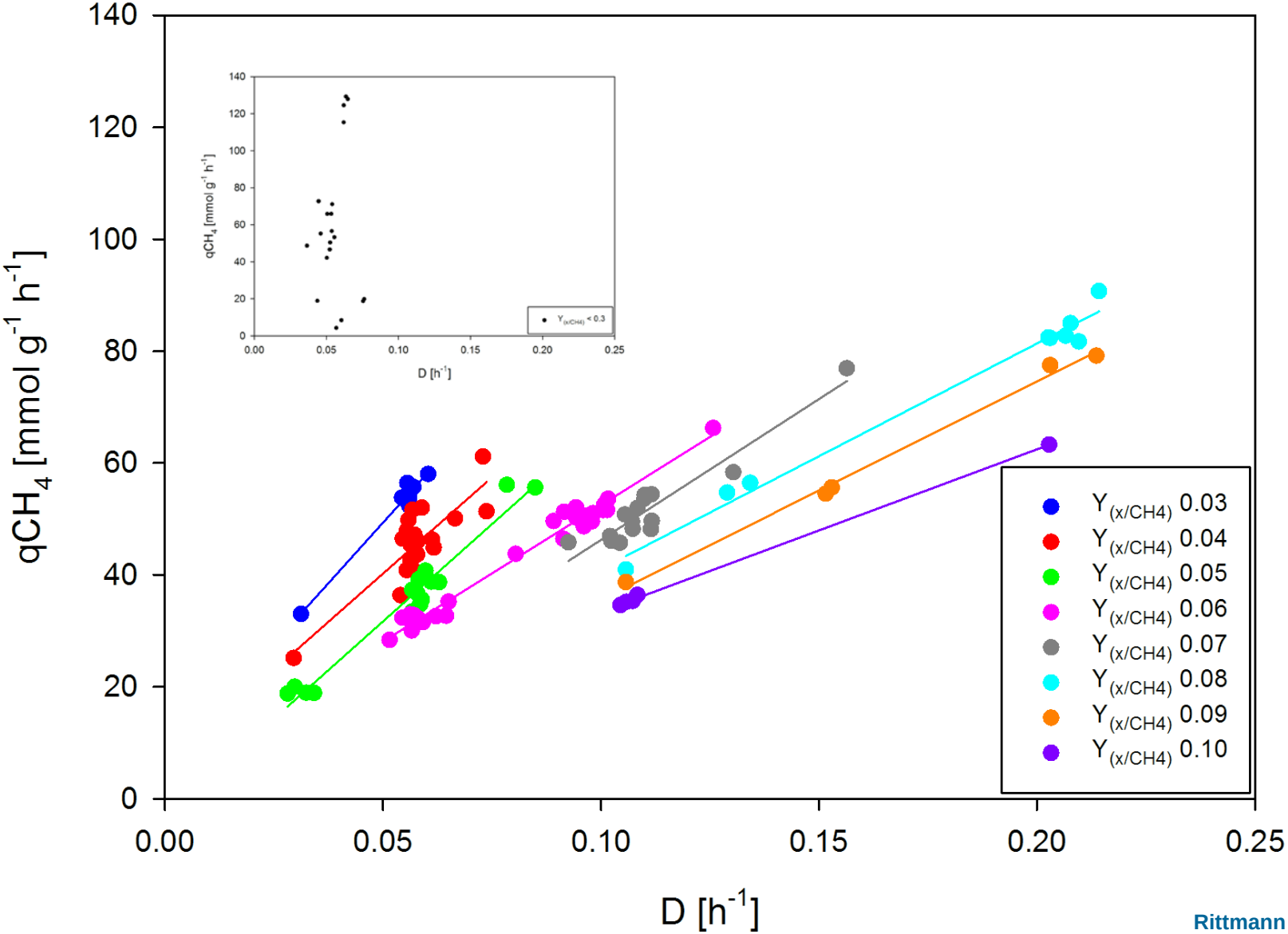
Rittmann *et al.*, 2018

Continuous culture – qCH_4



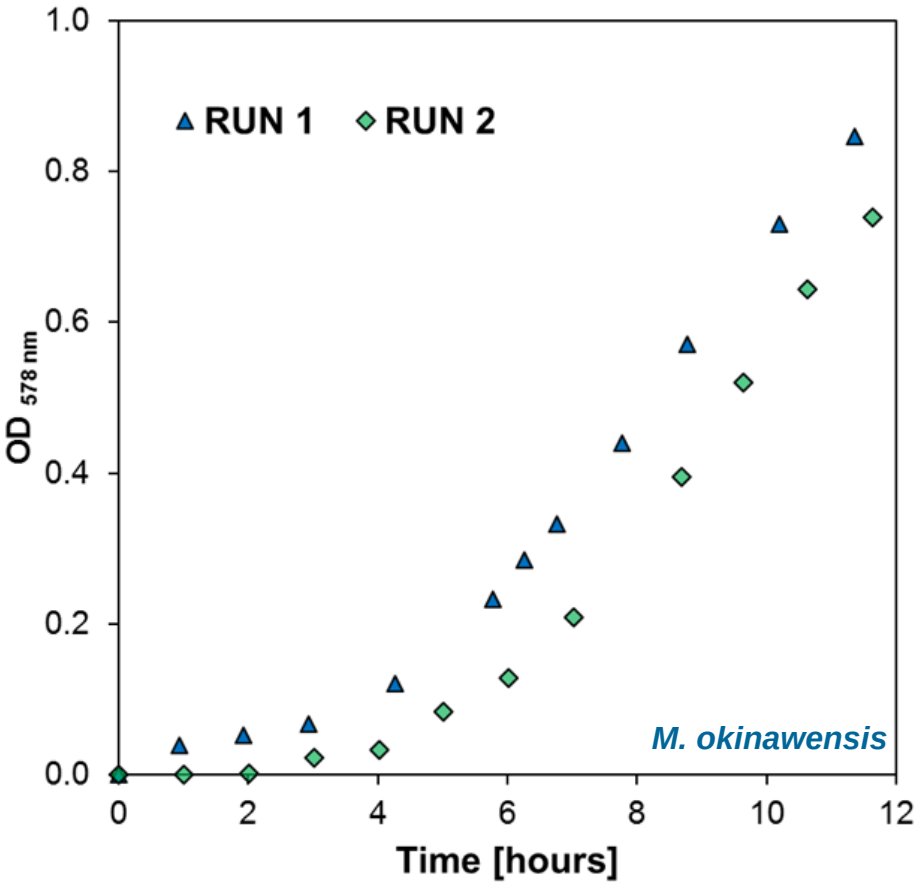
Rittmann *et al.*, 2018

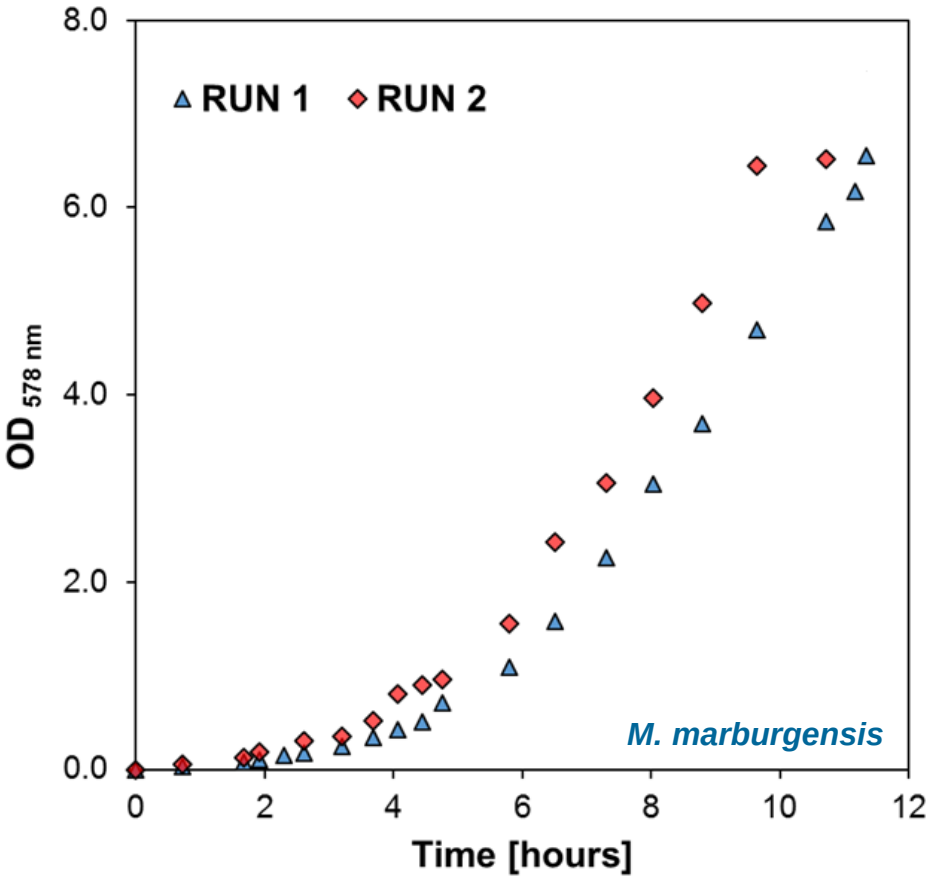
Continuous culture – qCH_4



Rittmann *et al.*, 2018

Fed-batch

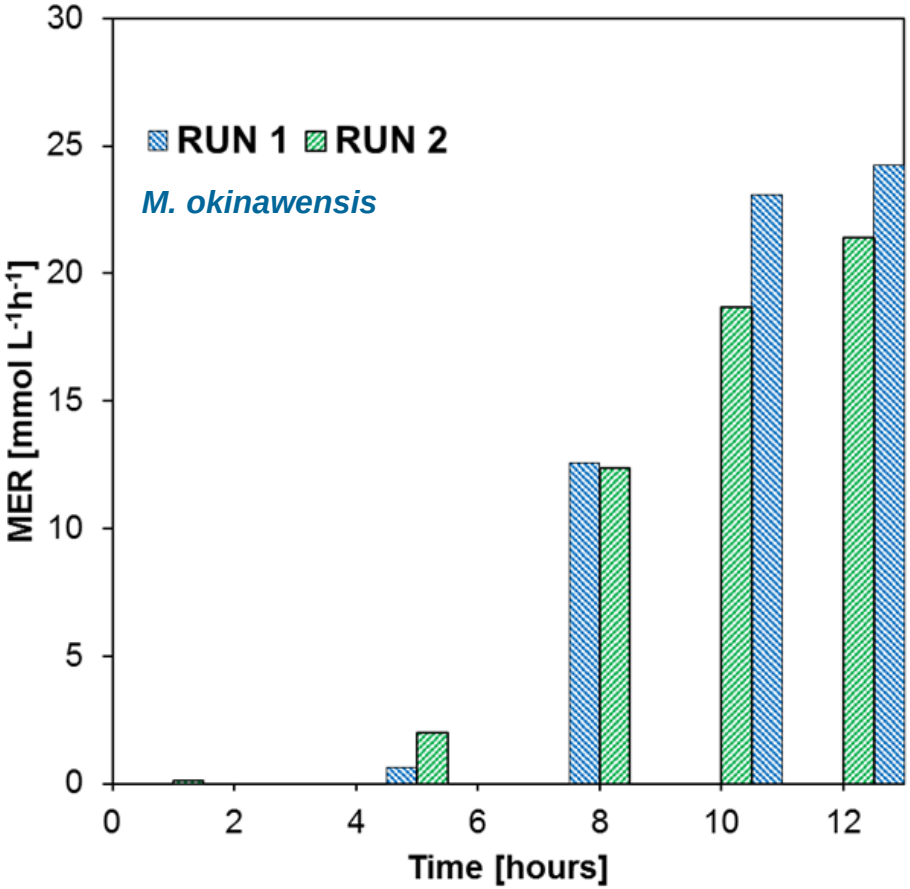


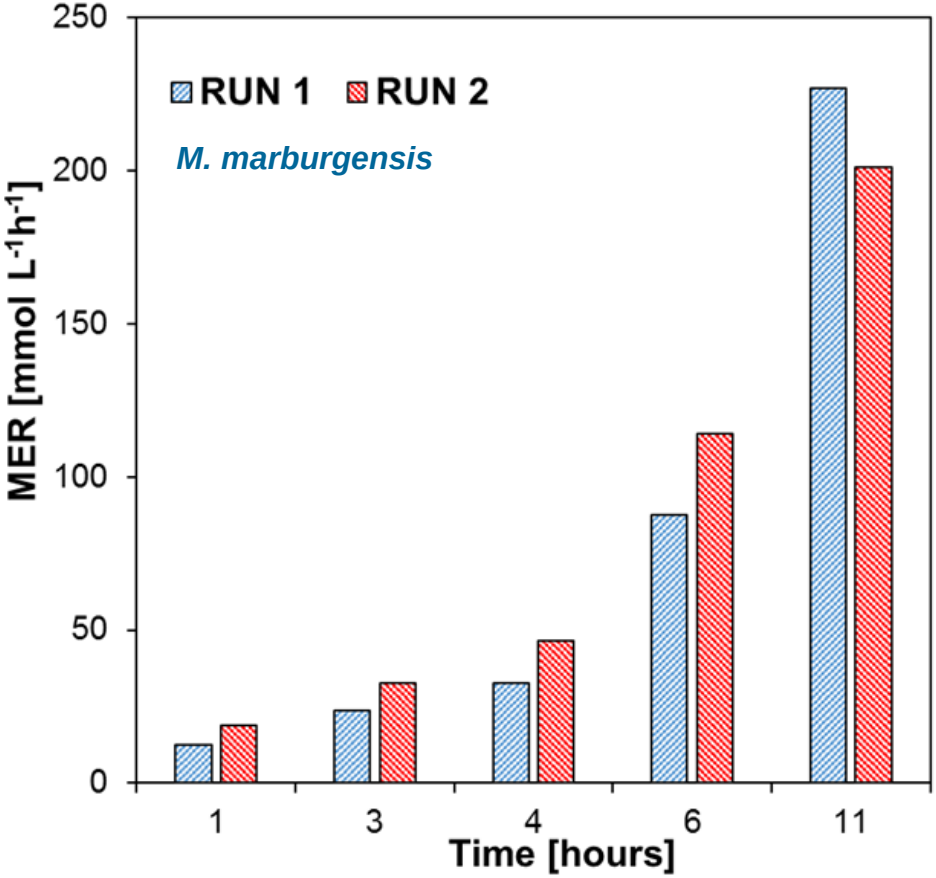


Growth (OD_{578nm}) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates, respectively.

Abdel Azim *et al*., 2017

Fed-batch



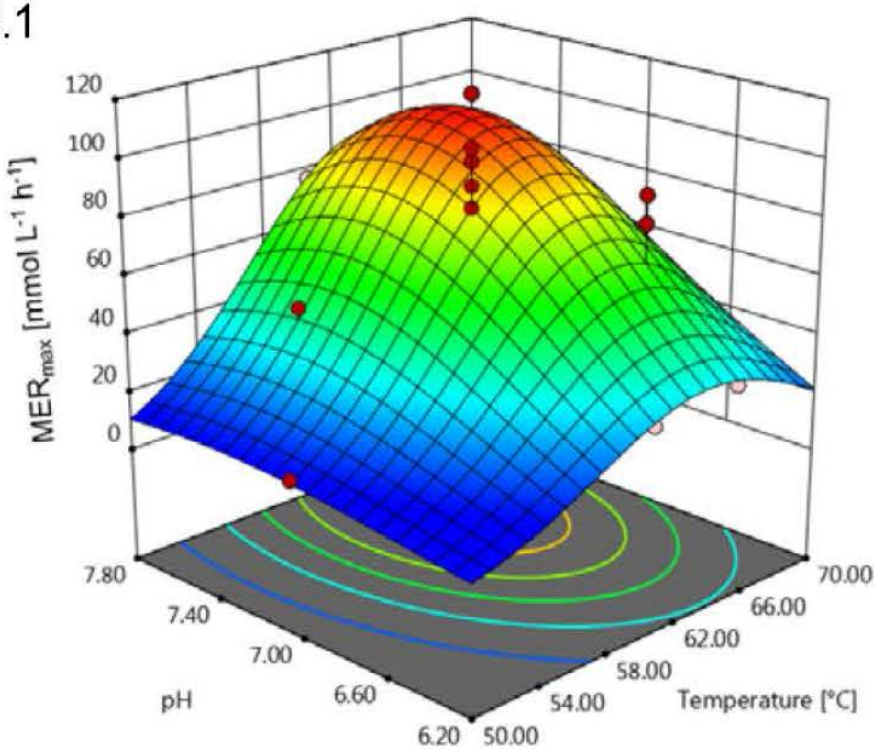


Methane evolution rate (MER) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates, respectively.

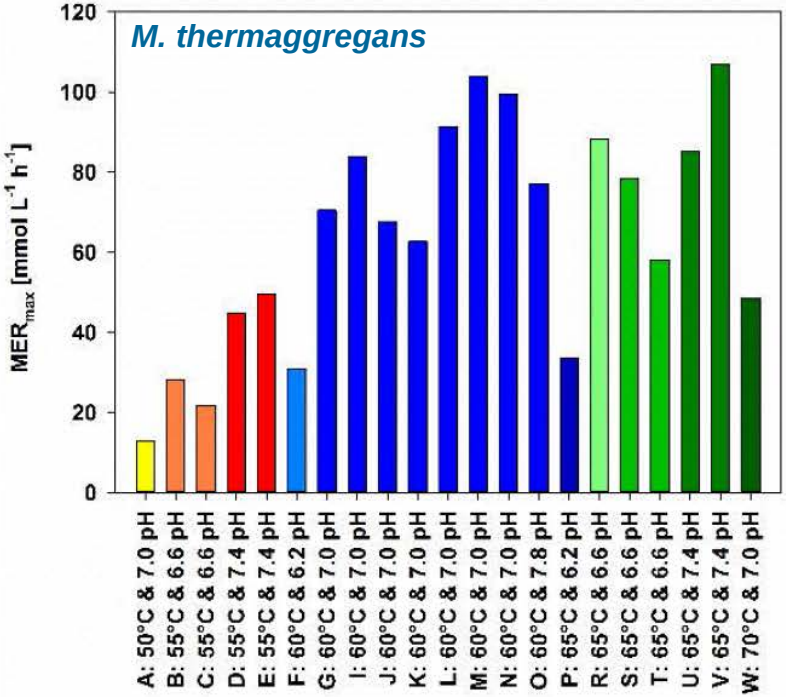
Abdel Azim *et al*., 2017

Fed-batch

B.1

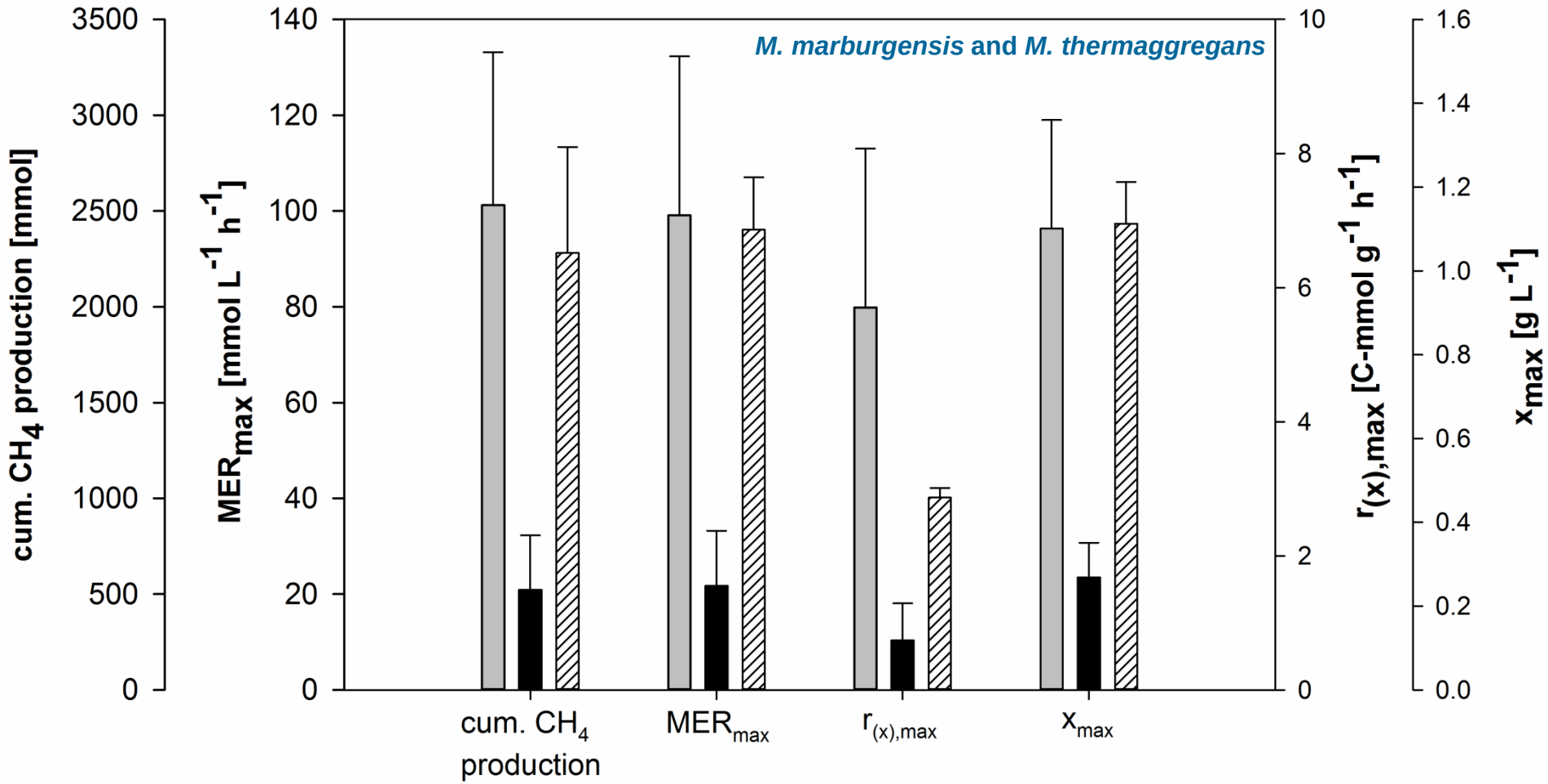


B.2



Mauerhofer *et al*., 2018

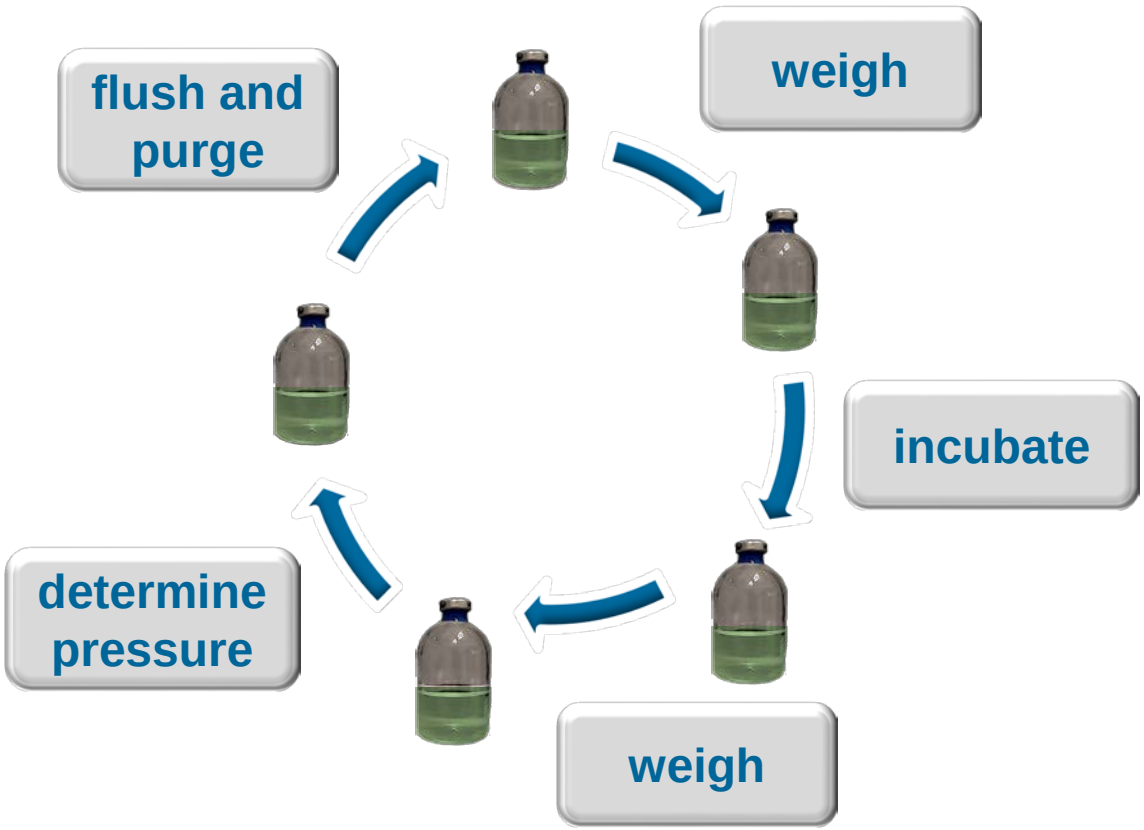
Fed-batch

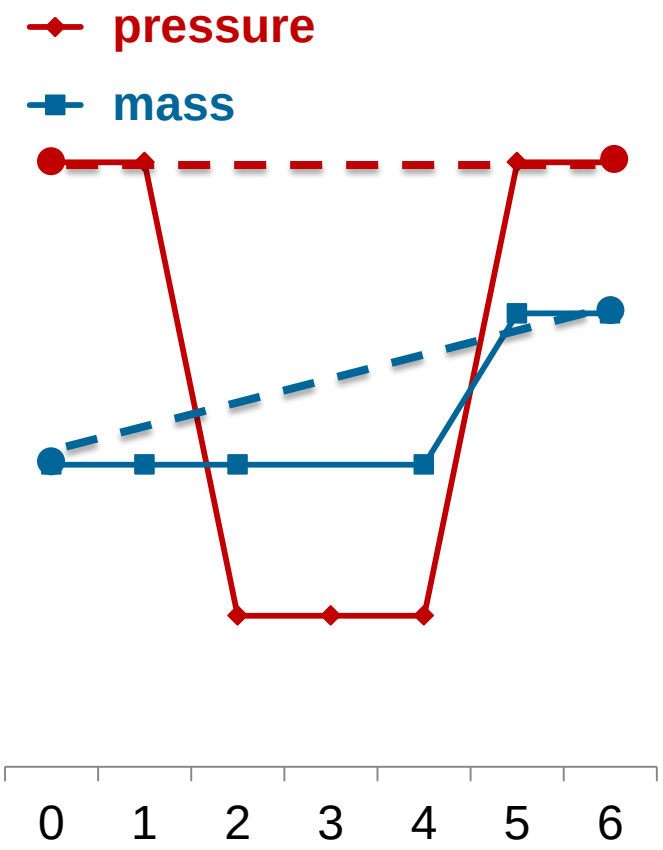


Comparison of cumulative CH_4 production (cum. CH_4 production), max. CH_4 evolution rate (MERmax), max. biomass production rate ($r_{(x),max}$), and maximum biomass concentration (x_{max}) of *M. thermaggregans* and *M. marburgensis*. The gray bars indicate the performance *of M. marburgensis* at 65 °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_{max} (U, V: 65 °C and 7.4 pH), and $r_{(x),max}$ and x_{max} (O: 60 °C and 7.8 pH).

Mauerhofer *et al*., 2018

Closed batch

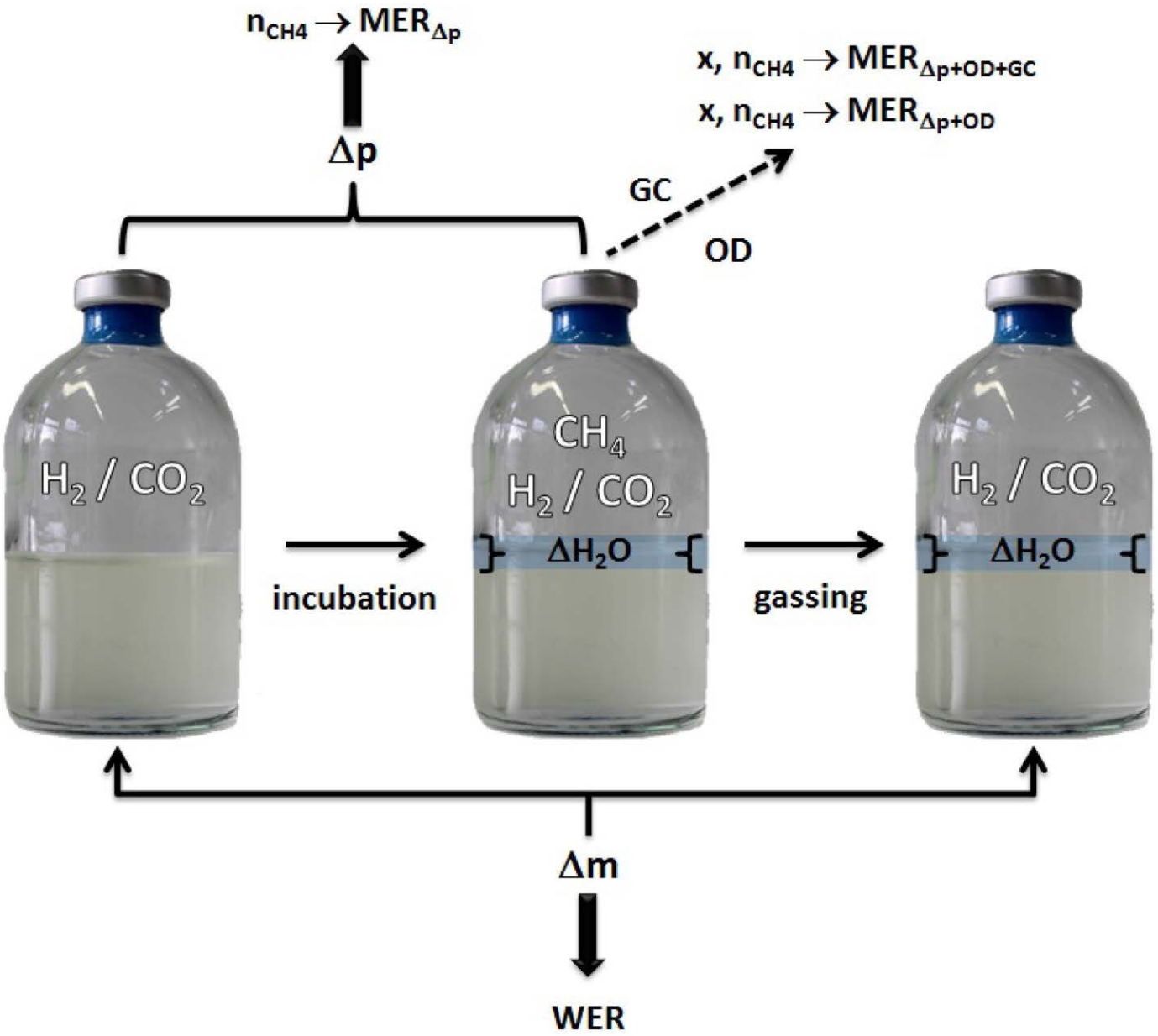




$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

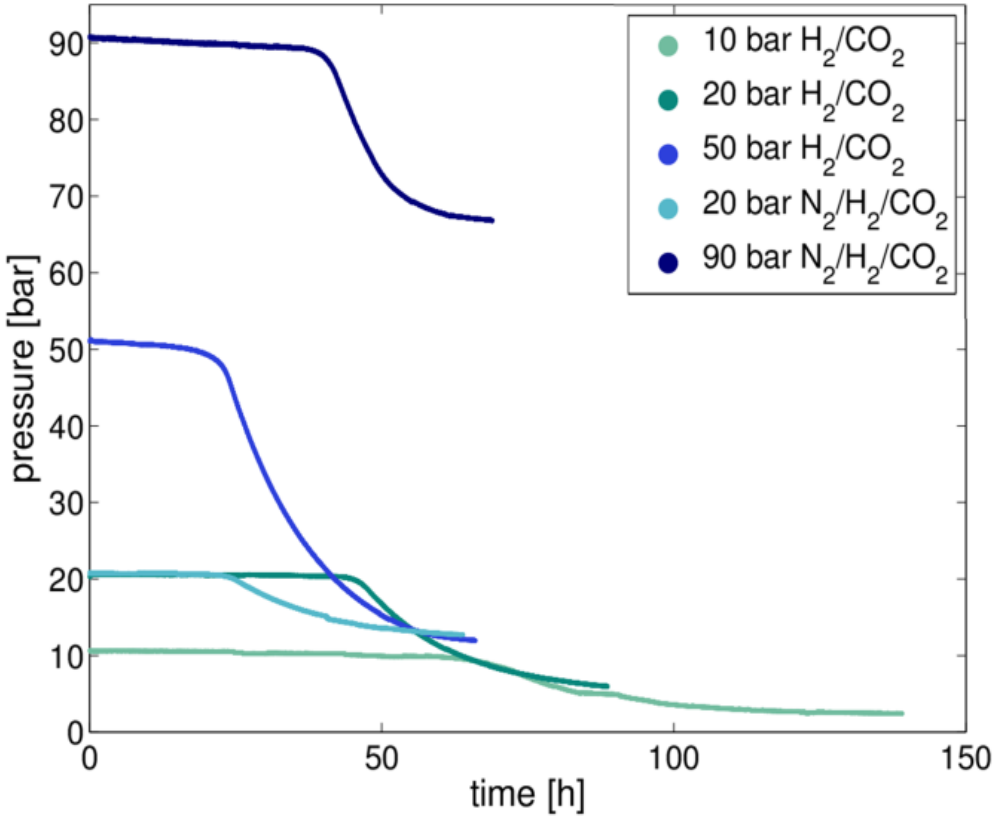
Taubner and Rittmann, 2016

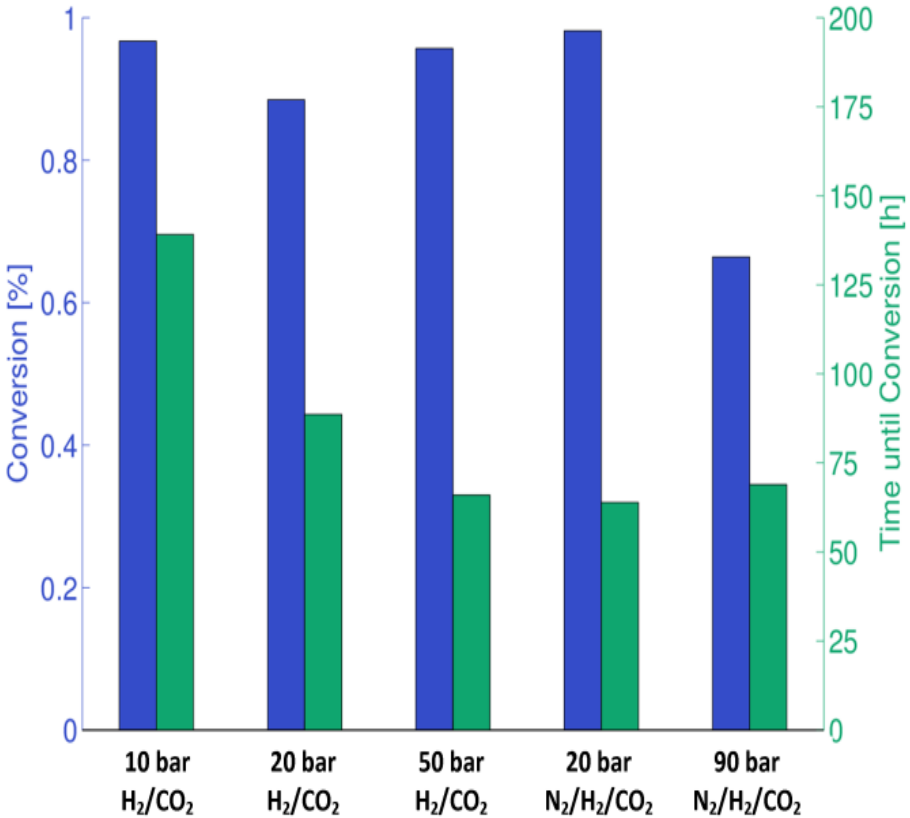
Closed batch



Taubner and Rittmann, 2016

M. okinawensis produces CH_4 up to 90 bar





H_2/CO_2 conversion of *M. okinawensis* under high pressure conditions. a, decreasing pressure over time as indirect evidence for stoichiometric H_2/CO_2 to CH_4 conversion. If 50 Vol.-% N_2 is added to the gas phase, the time until conversion from CO_2 to CH_4 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.

Taubner *et al*., 2018

Summary & Conclusions

- 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 μ 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

- 17800 kWh a^{-1} (100m^2, 3 persons) Statistik Austria → 2.032 kWh h^{-1}

- Biological CH_4 production bioreactor produces 950 mmol L^{-1} h^{-1} = 212.294 kWh m^{-3} 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

universität wien

Quantitative analysis of media dilution rate effects on *Methanothermobacter marburgensis* grown in continuous culture on H_2 and CO_2

Biomass & Bioenergy, 2012

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

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 1a, 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 *

Vienna University of Technology, Institute of Chemical Engineering, Gumpendorferstraße 1a/166-4, 1060 Vienna, Austria

Applied Energy, 2014

Research article

Experimental methods for screening parameters influencing the growth to product yield ($Y_{(x/CH4)}$) of a biological methane production (BMP) process performed with *Methanothermobacter marburgensis*

Sébastien Bernacchi [1], Simon Rittmann [1,2], Arne H. Seifert [1,3], Alexander Krajete [3], Christoph Herwig [1,*]

AIMS Bioengineering, 2014

Rapid extraction of total RNA from an anaerobic sludge biocoenosis

Folia Microbiologica, 2014

Simon Rittmann · Peter Holubar

REVIEW ARTICLE

Essential prerequisites for successful bioprocess development of biological CH_4 production from CO_2 and H_2

Simon Rittmann*†, Arne Seifert*, 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

Life, 2015

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

Method for Indirect Quantification of CH_4 Production via H_2O Production Using Hydrogenotrophic Methanogens

Frontiers in Microbiology, 2016

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

Further reading

The physiology of trace elements in biological methane production

Annalisa Abdel Azim[a,b], Christian Pruckner[a], Philipp Kolar[a], Ruth-Sophie Taubner[a,c], Debora Fino[b], Guido Saracco[b,d], Filipa L. Sousa[a], Simon K.-M.R. Rittmann[a,*]

Bioresource Technology, 2017

Biological methane production under putative Enceladus-like conditions

Ruth-Sophie Taubner[1,2], Patricia Pappenreiter[3], Jennifer Zwicker[4], Daniel Smrzka[4], Christian Pruckner[1], Philipp Kolar[1], Sébastien Bernacchi[5], Arne H. Seifert[5], Alexander Krajete[5], Wolfgang Bach[6], Jörn Peckmann[4,7], Christian Paulik[3], Maria G. Firneis[2], Christa Schleper[1] & Simon K.-M.R. Rittmann[1]

Nature Communications, 2018

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

J. Zwicker[a], D. Birgel[b], W. Bach[c], S. Richoz[d,e], D. Smrzka[a], B. Grasemann[a], S. Gier[a], C. Schleper[f], S.K.-M.R. Rittmann[f], E. Koşun[g], J. Peckmann[a,b,*]

Chemical Geology, 2018

Intact polar lipid and core lipid inventory of the hydrothermal vent methanogens *Methanocaldococcus villosus* and *Methanothermococcus okinawensis*

Lydia M.F. Baumann[a,1], Ruth-Sophie Taubner[b,1], Thorsten Bauersachs[c], Michael Steiner[b], Christa Schleper[b], Jörn Peckmann[a], Simon K.-M.R. Rittmann[b], Daniel Birgel[a,*]

Organic Geochemistry, 2018

Kinetics, multivariate statistical modelling, and physiology of CO_2-based biological methane production

Simon K.-M.R. Rittmann[a,*], Arne H. Seifert[b], Sébastien Bernacchi[b]

Applied Energy, 2018

Physiology and methane productivity *of Methanobacterium thermaggregans*

Lisa-Maria Mauerhofer[1] · Barbara Reischl[1,2] · Tilman Schmider[1] · Benjamin Schupp[1] · Kinga Nagy[1,3] · Patricia Pappenreiter[4] · Sara Zwirtmayr[4] · Bernhard Schuster[3] · Sébastien Bernacchi[2] · Arne H. Seifert[2] · Christian Paulik[4] · Simon K.-M. R. Rittmann[1]

Applied Microbiology and Biotechnology, 2018

RESEARCH Open Access

The physiological effect of heavy metals and volatile fatty acids on *Methanococcus maripaludis* S2

Annalisa Abdel Azim[1,2,3,4], Simon K.-M. R. Rittmann[2*], Debora Fino[3] and Günther Bochmann[1]

Biotechnology for Biofuels, 2018

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

Lisa-Maria Mauerhofer[1] · Patricia Pappenreiter[2] · Christian Paulik[2] · Arne H. Seifert[3] · Sébastien Bernacchi[3] · Simon K.-M. R. Rittmann[1]

Folia Microbiologica, 2018