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Entwicklung eines Hochdruckproduktionsverfahrens für die gekoppelte biologische Wasserstoff- und Methanproduktion

BioHyMe

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2 Introduction

Methane (CH_4) is one of the most important and most used energy gases worldwide. CH_4 is solely produced by strictly anaerobic organisms, referred to as methanogens, which contribute to the global carbon cycle with approximately 1 Gt of CH_4 produced per year. Methanogens are of importance to the global C-cycle and to biological CH_4 production through anaerobic digestion and in pure culture. The carbon and energy metabolism of methanogens is streamlined for the conversion of a restricted number of C1 and C2 substrates, i.e. carbon dioxide (CO_2) and molecular hydrogen (H_2), carbon monoxide (CO) formate, acetate, methanol, methylamines, and methoxylated compounds.

The novelty of the project BioHyMe was to stringently examine growth and productivity of pure culture of methanogens in three different cultivation conditions (closed batch, fed-batch and continuous culture) at 3, 10 and 50 bar. The goal of the project BioHyMe was to prioritize the most productive of methanogens for conversion of pure gases and gas mixtures up to 50 bar.

3 Summary

The goal of the project BioHyMe was to investigate hydrogenotrophic, autotrophic methanogens at three different pressure levels up to 50 bar, and to prioritize the most productive of methanogens for conversion of pure gases and gas mixtures. Screening and cultivation of hydrogenotrophic and autotrophic or carboxydotrophic methanogens and of carboxydotrophic hydrogenogens using closed batch was successfully finished. Cultivation of strains and co-cultures on all gases was successfully finished. No methanogens and no methanogenic co-cultures could be solely grown on CO. An adaption of pure cultures of methanogens to CO/H_2 were successfully performed, but resulted not yet in industrially relevant growth rates and methane (CH_4) production rates. Co-culture cultivations were successful, but no co-culture was prioritized for fed-batch studies, due to the higher industrial relevance of examining H_2/CO_2 converting strains. Quantification of CH_4 evolution rate (MER), specific growth rate (μ) and/or biomass concentration (x) regarding different pressure levels was successfully conducted for 82 methanogenic archaea, however many more methanogens were initially tested. A prioritization of the most promising H_2/CO_2 utilizing strains was thereafter successfully performed by using high frequency gassing (HFG) experiments. Furthermore, in WP1, cultivation and determination of CH_4 productivity of methanogens was successfully performed at pressures >3 bar and even up to 90 bar (Taubner *et al*., 2018). The Simultaneous BioReactor System (SBRS)) was designed, constructed and successfully applied for cultivation of methanogens at pressures beyond 3 bar (Pappenreiter *et al*., 2019). The SBRS allowed simultaneous quantification of gas conversion in the reactor headspace at pressure levels up to 50 bar. H_2/CO_2 utilizing strains were successfully prioritized for fed-batch cultivations in WP3. Hence, WP1 was successfully finished. All milestones and deliverables of WP2 were successfully also achieved. In WP2, the Büchi reactor was successfully adapted to maintain desired stirring rates and high-pressure applications. Furthermore, k_La experiments were successfully performed. Hence, WP2 was successfully finished. All milestones of WP3 were successfully finished. 14 methanogens were attempted to be grown in fed-batch cultivation mode in the Eppendorf bioreactor system at the University of Vienna and in the Büchi reactor system at the JKU Linz. Only 5 of these methanogenic strains could be reproducibly grown in fed-batch cultivation mode (WP3). However, only after an in-depth investigation of the growth kinetics of *Methanobacterium thermaggregans* the strain could be reproducibly cultivated in fed-batch cultivation mode (Mauerhofer *et al*., 2018). Moreover, growth and CH_4 production kinetics of *Methanothermobacter marburgensis* and *Methanothermococcus okinawensis* were successfully optimized and examined (Abdel Azim *et al*., 2017). WP4 was successfully finished. Long-term robustness was achieved in continuous culture (data analysis is still on-going). Project coordination, communication and control were successfully performed in WP5. In total, ten project meetings were held to assure the project progress and several telephone conferences with among the team members assured a vivid communication attitude leading in the successful advancement in the organization of publishing scientific results in SCI-journals and coordinating poster and oral presentations at international conferences. The WP-milestone was successfully accomplished.

Extremely successful dissemination and outreach activities were performed in WP6. In total, 7 SCI-publications were published already during the project period. A highlight was the release of our *Nature Communications* SCI-paper, which was published on the 27th of February 2018 (Taubner *et al*., 2018).

Our *Nature Communications* paper was nationally and internationally very well received and resulted in an enormous digital- and print-media broadcasting echo, especially in the international press. The publication of Taubner *et al*. 2018 *Nature Communications* paper resulted in the accomplishment of milestone of WP6. Another publication, Rittmann *et al*. 2018, was published in the high-ranking SCI-journal *Applied Energy*. Five more publications were published or are accepted for publication in other SCI-journals (Pappenreiter *et al*. 2019, Mauerhofer *et al*. 2019, Abdel Azim *et al*. 2018, Mauerhofer *et al*. 2018, Abdel Azim *et al*. 2017), and two to four papers are currently being drafted. Moreover, two additional non-peer reviewed publications were published during the project period, 14 oral and several posters presentations were given at national or international conferences or workshops, highlighting the FFG and the Klima- und Energiefonds as the funding organization of the BioHyMe project.

4 Results and conclusions

Growth and CH_4 productivity of psychrophilic, mesophilic, thermophilic and hyperthermophilic methanogenic archaea were investigated. In total, 82 methanogenic archaea were screened in closed batch cultivation mode up to 3 bar (2 bar overpressure) in an anaerobic atmosphere consisting of 80 Vol.-% H_2 in CO_2 for autotrophic, hydrogenotrophic methanogens or N_2 (acetoclastic methanogens). To elucidate the CH_4 productivity of the methanogenic archaea at 3 bar, maximal turnover (H_2/CO_2 to CH_4 conversion), CH_4 evolution rate (MER), and maximal CH_4 evolution rate (MER_{max}) were determined. After measuring either OD_{578} or pressure, serum bottles were again pressurized to 3 bar using a H_2/CO_2 gas mixture. Physiological and industrial relevant variables were investigated during cultivation of methanogens in closed batch mode. Moreover, high-throughput screening at 3 bar was performed to prioritize new putative methanogenic cell factories. A key parameter during closed batch cultivation is H_2/CO_2 availability, because in a closed batch cultivation set-up the methanogens can face gas-limiting conditions. To avoid or reduce the gas-limitation through pressure (H_2/CO_2) depletion, high frequency gassing (HFG) experiments were conducted. Respectively to the selection criterion high specific growth rate μ [h^{-1}] and cohesive pressure drop that is reflected in turnover [%] and correlated to MER [mmol L^{-1} h^{-1}], 14 methanogenic archaea were selected for HFG experiments. The HFG experiments were performed with 3 mesophilic, 5 thermophilic, and 6 hyperthermophilic methanogens (Mauerhofer *et al*. manuscript in preparation).

4.1 The physiology of *Methanococcus maripaludis*

The individual and combined effects of copper, zinc, acetate, and propionate on the metabolism of the autotrophic, hydrogenotrophic methanogen *M. maripaludis* S2 were investigated. Copper, zinc, acetate, and propionate may interfere directly and indirectly with the acetyl-CoA synthesis and CH_4 production. We showed that the copper concentration of 1.9 μmol L^{-1} reduced growth of *M. maripaludis*, whereas 4.4 and 6.3 μmol L^{-1} of copper even further retarded biomass production. However, 1.0 mmol L^{-1} of zinc enhanced growth, but at zinc concentrations >2.4 mmol L^{-1} no growth could be observed (**Figure 1**).

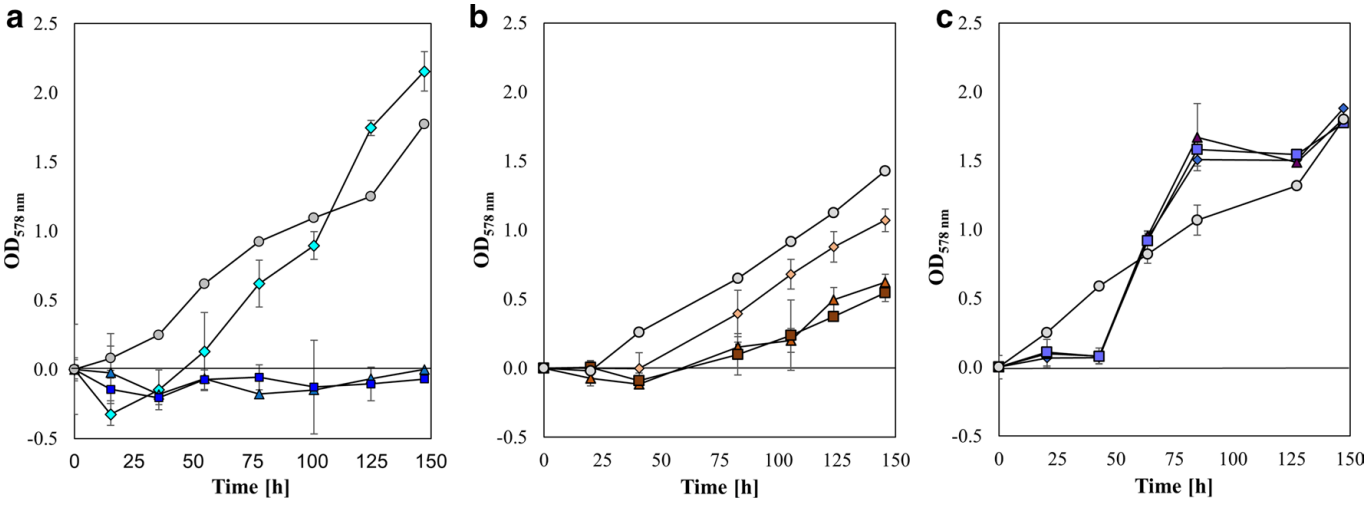


Figure 1 OD_{578} curves of *M. maripaludis* 50 mL culture at 37 °C, 140 rpm, 2.9 bar with Zn, Cu, and Zn and Cu (*n* = 4). This Figure and the associated figure caption were published in Adbel Azim *et al*., 2018 Biotechnology for Biofuels 11:301.

When copper, zinc was added to the medium, growth and CH_4 production could even be observed at the highest tested concentration of copper of 6.3 µmol L^{-1}. Hence, it seems that the addition of 1 mmol L^{-1} of zinc enhanced the ability of *M. maripaludis* to counteract the toxic effect of copper. The physiological effect of rising concentrations of acetate (12.2, 60.9, 121.9 mmol L^{-1}) and/or propionate (10.3, 52.0, 104.1 mmol L^{-1}) were also examined. When instead of acetate 10.3 mmol L^{-1} propionate was provided in the growth medium, *M. maripaludis* could grow without reduction of µ or qCH_4 (**Figure 2**). A combination of inorganic and/or organic compounds resulted in an increase of µ and qCH_4 for copper/zinc and zinc/acetate beyond the values that were observed if only the individual concentrations of copper, zinc, and acetate were used (**Figure 3**). This study sheds light on the physiological effect of organic acids and heavy metals on *M. maripaludis*. Differently from µ and qCH_4, MER was not influenced by the presence of these compounds. This indicated that each of these compounds directly interacted with the C-fixation machinery of *M. maripaludis*. Until now, the uptake of VFAs other than acetate was not considered to enhance growth and CH_4 production of methanogens. The finding of propionate uptake by *M. maripaludis* is important for the interpretation of VFA cycling in anaerobic microenvironments. Due to the importance of methanogens in natural and artificial anaerobic environments, our results help to enhance understanding the physiological and biotechnological importance with respect to anaerobic digestion, anaerobic wastewater treatment, and CO_2 based biological methane production (CO_2-BMP). Finally, we propose a possible mechanism for acetate uptake into *M. maripaludis* supported by in silico analyses. The findings summarized in this paragraph were published in Adbel Azim *et al*., 2018 Biotechnology for Biofuels 11:301.

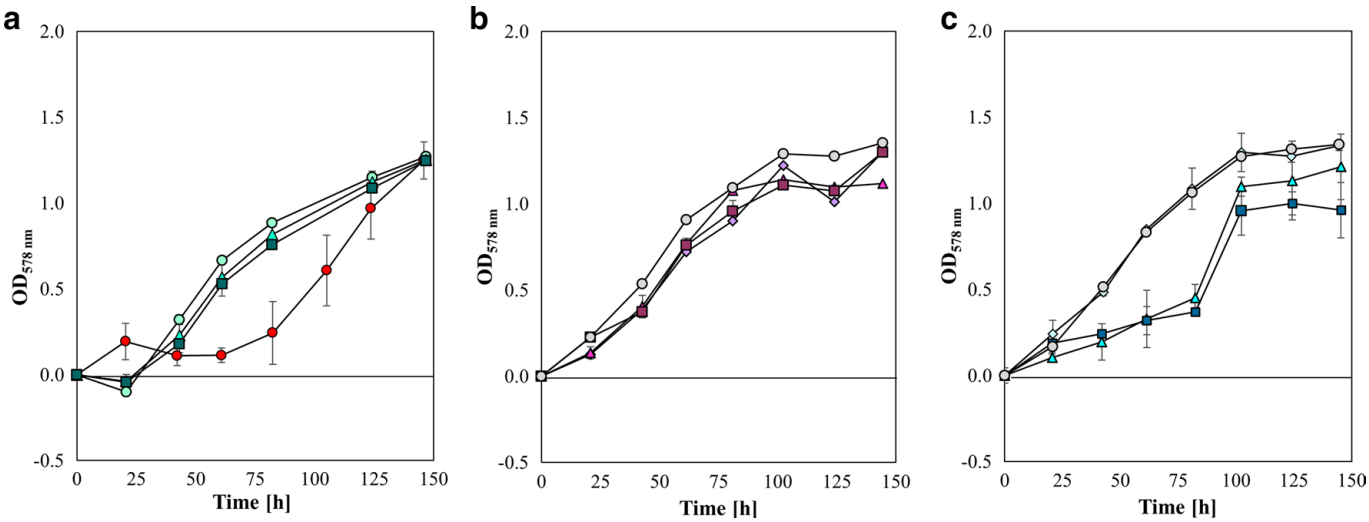


Figure 2: OD_{578} of *M. maripaludis* 50 mL culture at 37 °C, 140 rpm, 2.9 bar with acetate and without acetate, with propionate and acetate, with propionate only (n = 4). This Figure and the associated figure caption were published in Adbel Azim *et al*., 2018 Biotechnology for Biofuels 11:301.

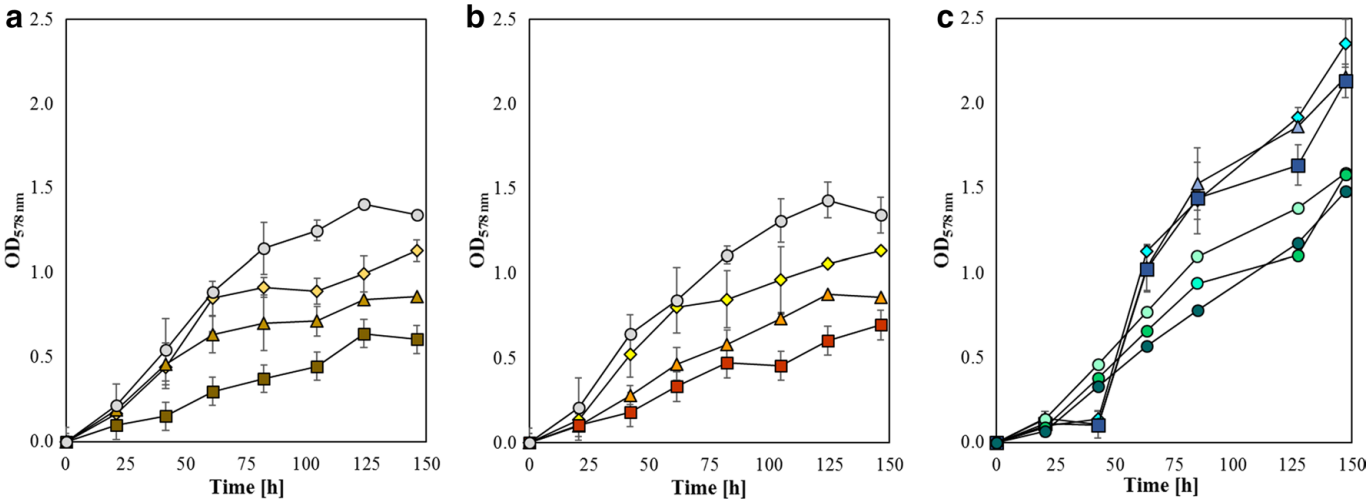


Fig. 4 OD578 nm curves of *M. maripaludis* 50 mL culture at 37 °C, 140 rpm, 2.9 bar with acetate combined with Cu and Zn, respectively (n = 4). This Figure and the associated figure caption were published in Adbel Azim *et al*., 2018 Biotechnology for Biofuels 11:301.

4.2 Comparative physiology of *M. marburgensis* and *M. okinawensis*

Trace element (TE) requirements of *M. okinawensis* and *M. marburgensis* were investigated *in silico* and also using closed batch and fed-batch cultivation experiments. *In silico* analysis showed genomic differences among the transport systems and enzymes related to the archaeal Wood-Ljungdahl pathway of these two methanogens. *M. okinawensis* reacted to rising concentrations of TE by increasing µ and MER during closed batch cultivation, and showed to be able to grow and produce CH_4 during fed-batch cultivation. *M. marburgensis* showed higher µ and MER during fed-batch cultivation and was therefore prioritized for subsequent optimization of CO_2-BMP. Multiple-parameter cultivation dependency of growth and productivity of *M. marburgensis* was eventually examined using exponential fed-batch cultivation at different medium-, TE- and sulphide dilution rates, and different gas inflow rates. MER of 476 mmol L^{-1} h^{-1} and µ of 0.69 h^{-1} were eventually obtained during exponential fed-batch cultivations employing *M. marburgensis*. The findings were published in Abdel Azim *et al*., 2017, Bioresource Technology 241:775–786.

4.3 The physiology and productivity of *Methanobacterium thermaggregans*

The physiology and productivity of *M. thermaggregans* was examined in fed-batch cultivation mode in lab-scale bioreactors. It was shown that *M. thermaggregans* can be reproducibly adapted to high agitation speeds for an improved CH_4 productivity (**Figure 4**). Moreover, inoculum volume, sulfide feeding, pH, and temperature were optimized. Optimization of growth and CH_4 productivity revealed that *M. thermaggregans* is a slightly alkaliphilic and thermophilic methanogen. Hitherto, it was only possible to grow seven autotrophic, hydrogenotrophic methanogenic strains in fed-batch cultivation mode.

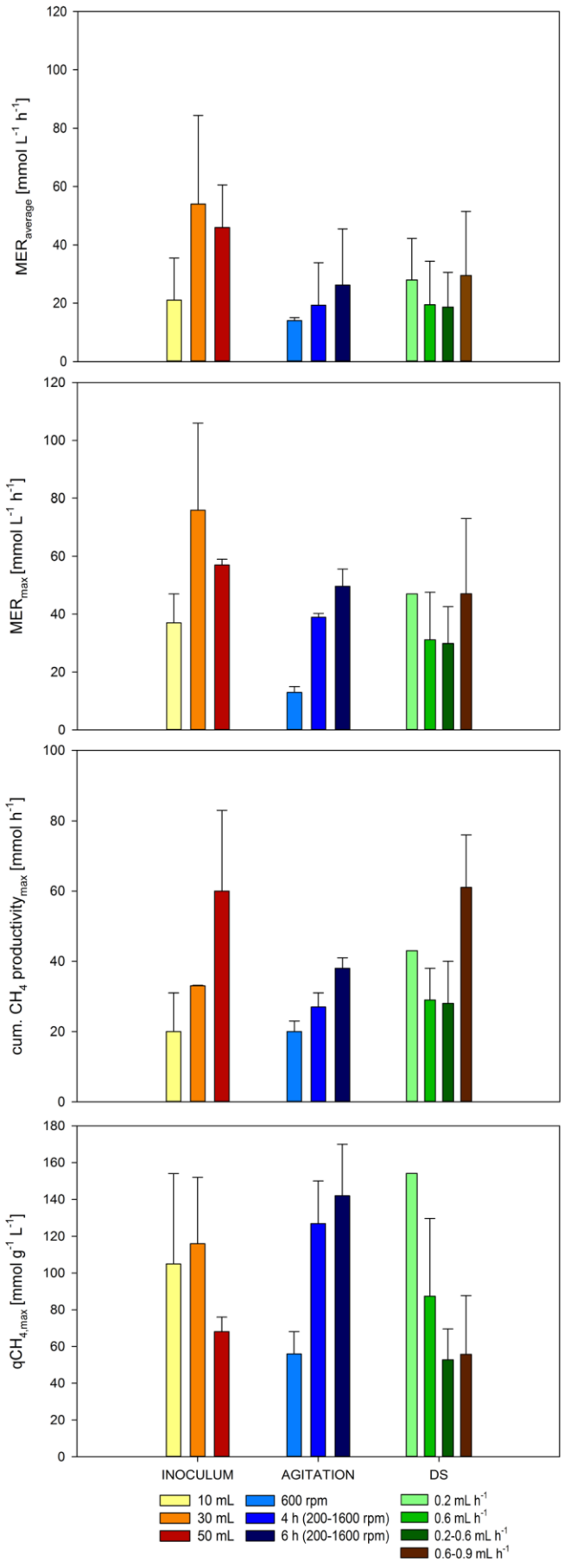


Figure 4: Results of average and max. CH_4 evolution rate ($MER_{average}$ and MER_{max}), max. cumulative CH4 production (cum. CH_4 productivity,max), and max. specific CH4 production rate ($qCH_{4,max}$) for different conditions of inoculation volume, agitation speed, and DS during fed-batch cultivations of *M. thermaggregans* are illustrated. The results of tested inoculation volumes, described as inoculum in the figure, are shown by yellow, orange, and red bars. The tested agitation speed and the two agitation ramps mentioned as agitation in the figure are shown by blue coloured bars. Green and brown bars indicate the results of tested DS. All fed-batch cultivation were performed at 65 °C, within 1.5 L of specific medium, and continuously gassed with 0.5 vvm H_2/CO_2 (80 Vol.-% H_2 in CO_2) at atmospheric pressure. This Figure and the associated figure caption were published in Mauerhofer *et al*., 2018 Applied Microbiology and Biotechnology 102:7643–7656.

We show that after a series of optimization and growth improvement attempts another methanogen, *M. thermaggregans* could be adapted to be grown in fed-batch cultivation mode to cell densities of up to 1.56 g L^{-1}. Moreover, MER of *M. thermaggregans* was compared to *M. marburgensis*, the CO_2-BMP model organism (**Figure 5**).

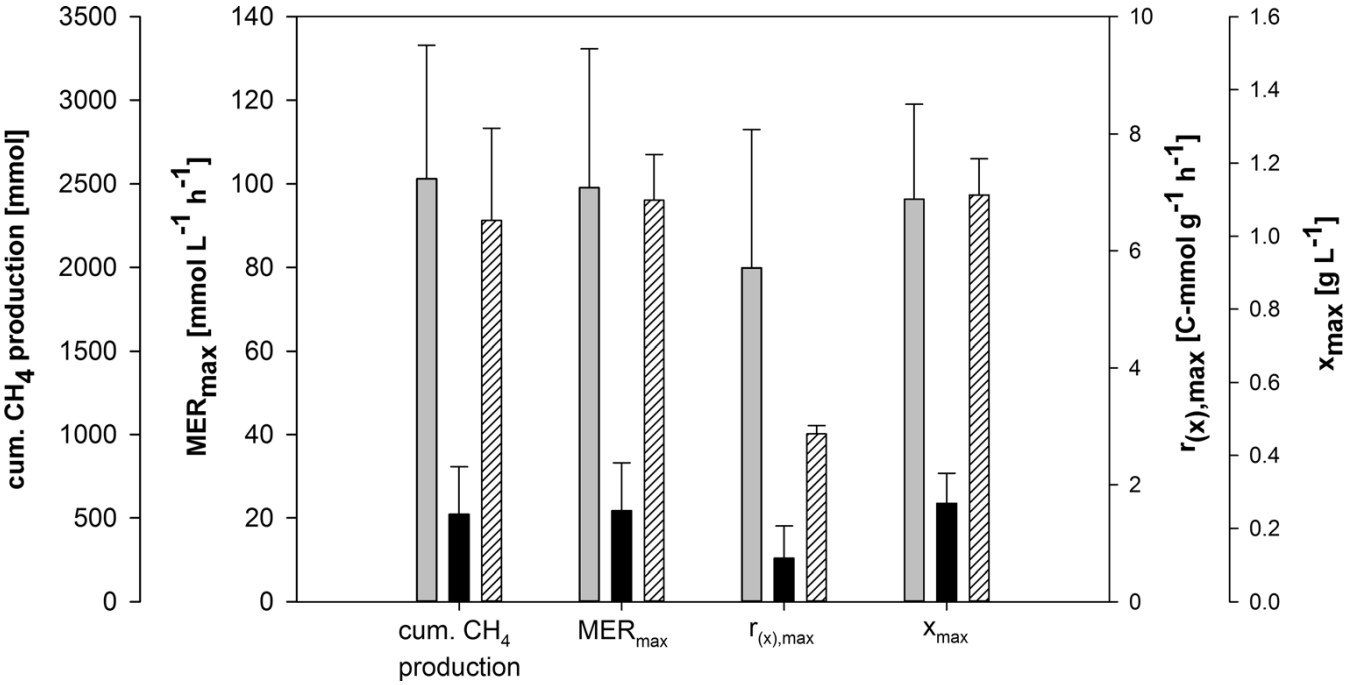


Figure 5: Comparison of cumulative CH_4 production (cum. CH_4 production), max. CH_4 evolution rate (MER_{max}), max. biomass production rate ($r_{(x),max}$), and maximum biomass concentration (x_{max}) of *M. thermaggregans* and *M. marburgensis*. The grey bars indicate the performance of *M. marburgensis* 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 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). This Figure and the associated figure caption were published in Mauerhofer *et al.*, 2018 Applied Microbiology and Biotechnology 102:7643–7656.

Under optimized cultivation conditions (**Figure 6**), a maximum MER of 96.1±10.9 mmol L^{-1} h^{-1} was obtained with *M. thermaggregans* (97% of the maximum MER that was obtained utilizing *M. marburgensis* in the reference experiment). The findings in this paragraph were published in Mauerhofer *et al.*, 2018 Applied Microbiology and Biotechnology 102:7643–7656.

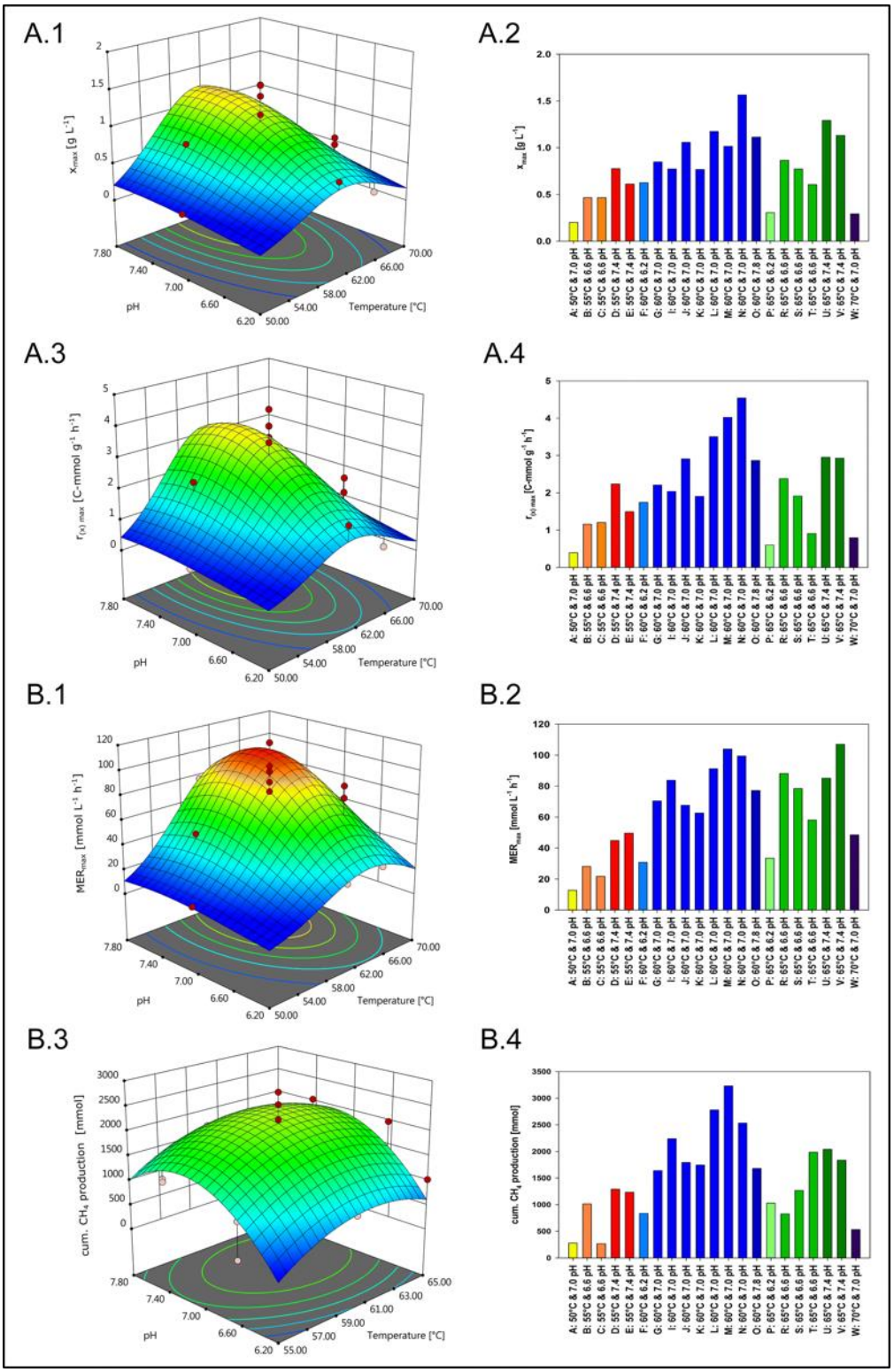


Figure 6: Response surface plots and individual results of growth and CH_4 productivity of *M. thermaggregans* are shown as functions of temperature (50–70 °C) and pH (6.2–7.8). In **A.1**, **A.3**, **B.1**, and **B.3**, four surface response plots are shown for maximum biomass concentration (x_{max}), maximum biomass production rate ($r_{(x),max}$), maximum CH_4evolution rate (MER_{max}), and cumulative CH_4 production (cum. CH_4 production), respectively. In **A.2**, **A.4**, **B.2**, and **B.4**, the individual results corresponding to the response surface plots for x_{max}, $r_{(x),max}$, MER_{max}, and cum. CH_4 production are illustrated. *M. thermaggregans* was cultivated within 1.5 L of MM medium and continuously gassed with 1 vvm H_2/CO_2 (80 Vol.-% H_2 in CO_2) at atmospheric pressure. In addition, 0.5 mol L^{-1} $Na_2S{\cdot}9H_2O$ was continuously added with a DS of 0.3 mL h^{-1}. Experiments indicated with yellow (A: 50 °C and 7.0 pH), light blue (F: 60 °C and 6.2 pH), dark blue (O: 60 °C and 7.8 pH), light green (P: 65 °C and 6.2 pH), and violet (W: 70 °C and 7.0 pH) bars were performed once. Experiments illustrated with orange (B, C: 55 °C and 6.6 pH), red (D, F: 55 °C and 7.4 pH), green (R, S: 65 °C and 6.6 pH), and dark green (U, V: 65 °C and 7.4 pH) bars were performed twice. Experimental results shown with green bars (R, S, T: 65 °C and 6.6 pH) were performed in triplicates. The center point indicated with blue bars (G–N: 60 °C and 7.0 pH) was examined in octuplicates. This Figure and the associated figure caption were published in Mauerhofer *et al.*, 2018 Applied Microbiology and Biotechnology 102:7643–7656.

4.4 Cultivation of methanogens under high pressure

Cultivation of methanogens under high pressure offers the opportunity to improve the gas to liquid transfer of gaseous substrate(s) in the cultivation broth. We describe a newly developed Simultaneous BioReactor System (SBRS) consisting of four interconnected cultivation vessels suited for the investigation of biological gas conversion kinetic and physiological studies at pressures up to 50 bar (**Figure 7**) (Pappenreiter *et al*. 2019, Engineering in Life Science).

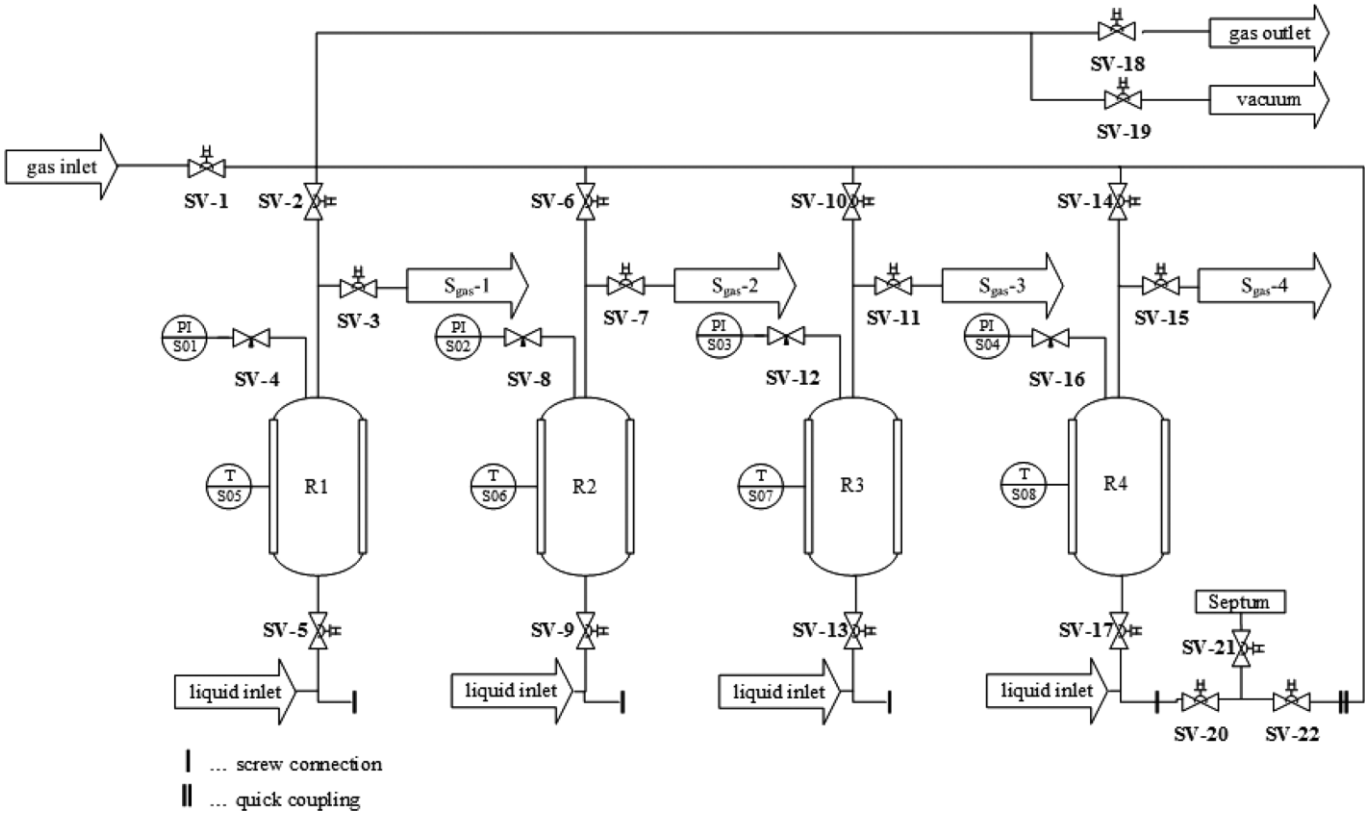


Figure 7: Piping and instrumentation diagram (DIN EN ISO 10628) of the SBRS. Each vessel (R1-R4) is equipped with instrumentations such as pressure sensors (S01-S04), heating jackets (S05-S08), and gas lines including valves. The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).

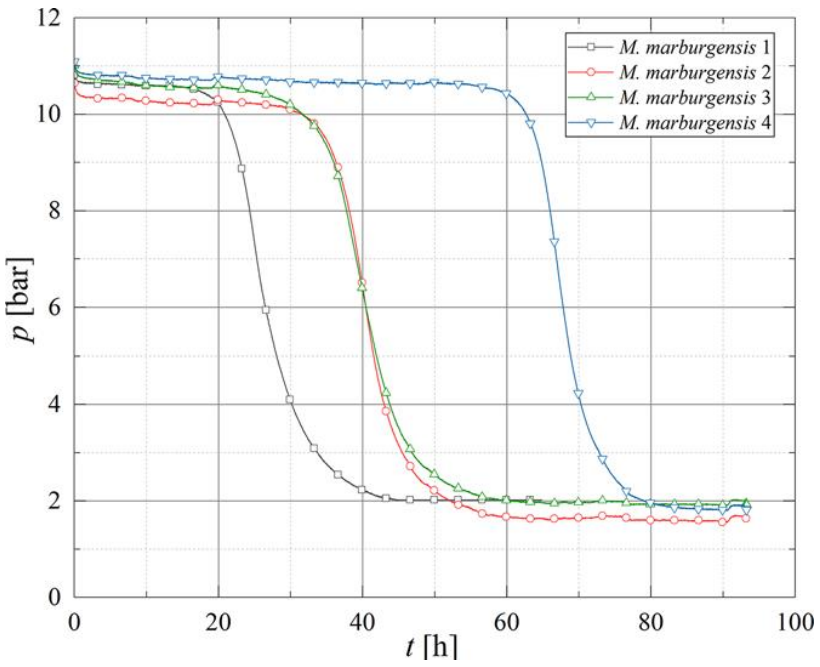
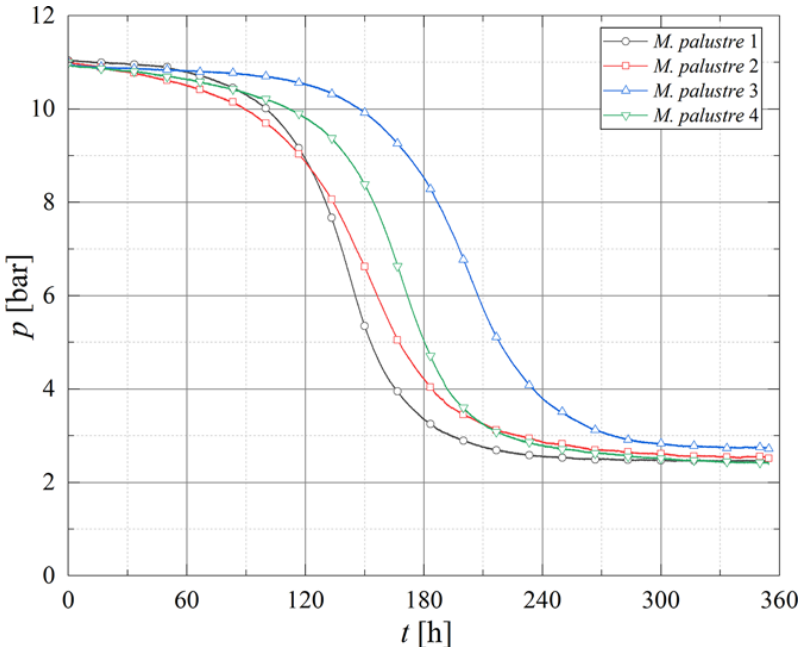


Figure 8: Pressure curves obtained during the gas conversion experiments with MM10 in the SBRS. The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).



Experiments at 10 and 50 bar using *M. marburgensis* (**Figure 8**) *Methanobacterium palustre* (**Figure 9**), and *M. thermaggregans* (**Figure 10**) were performed to evaluate the reproducibility of the system as well as to test the productivity of these strains.

Figure 9: Pressure curves obtained during the gas conversion experiments with MP10. The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).

The strains were compared with respect to gas conversion (%), MER, turnover rate (h^{-1}), and maximum conversion rate (k_{min}) (bar h^{-1}). A pressure drop that can be explained by the reaction stoichiometry showed that all tested strains were active under pressurized conditions (**Figure 11**). Our study sheds light on the production kinetics of methanogenic strains under high-pressure conditions. In addition, the SBRS is a suitable system for a first step screening analysing the substrate uptake and/or gas production and conversion kinetics for barophilic or barotolerant microbes. The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).

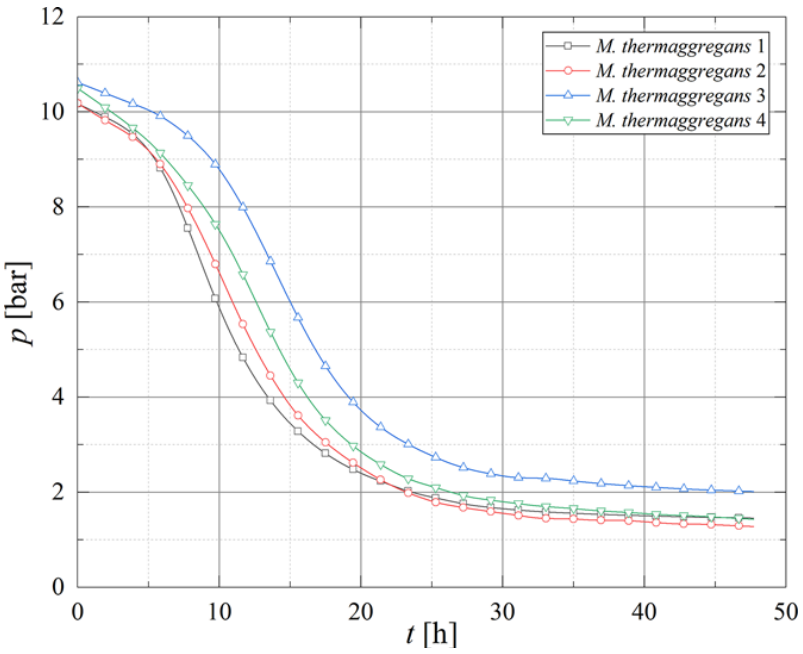


Figure 10: Pressure curves obtained during the gas conversion experiments with MTa10 The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).

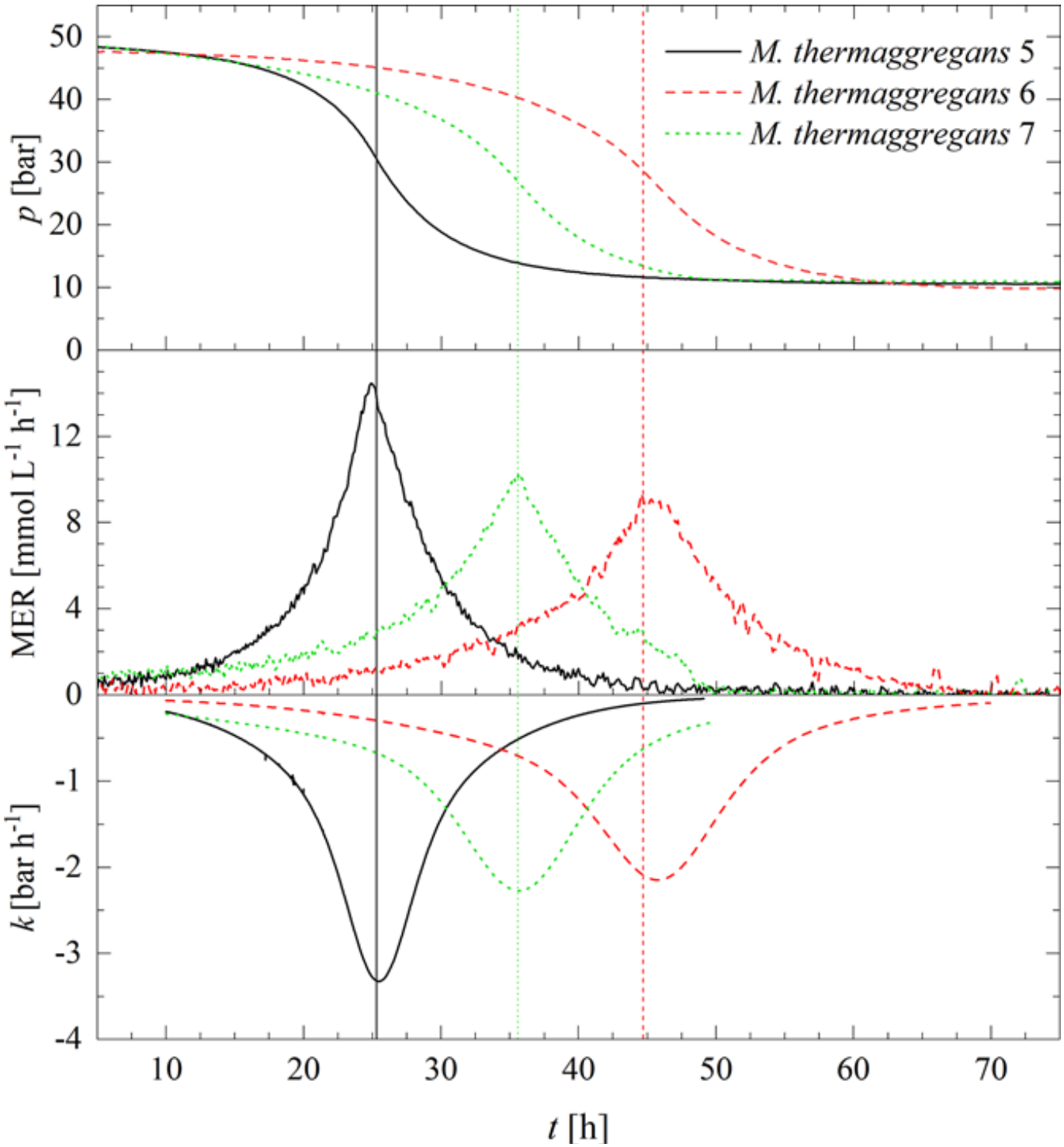


Figure 11: Pressure curves of MTa50 and the corresponding calculated MER and k values over time, where MER_{max} and k_{min} are determined by the peak maxima and peak minima respectively. Data is not shown for the replicate *M. thermaggregans* 8, because no pressure drop was detected). The results of this study were published in Pappenreiter *et al*., 2019 Engineering in Life Sciences (article in press).

4.5 Multivariate statistical analysis of CO_2-BMP

The inherent challenges faced in analysing CO_2-BMP process for energy conversion and storage were investigated (**Figures 12-14**) and discussed. A comprehensive assessment of key process parameters on several CO_2-BMP process variables was conducted (**Figure 12**).

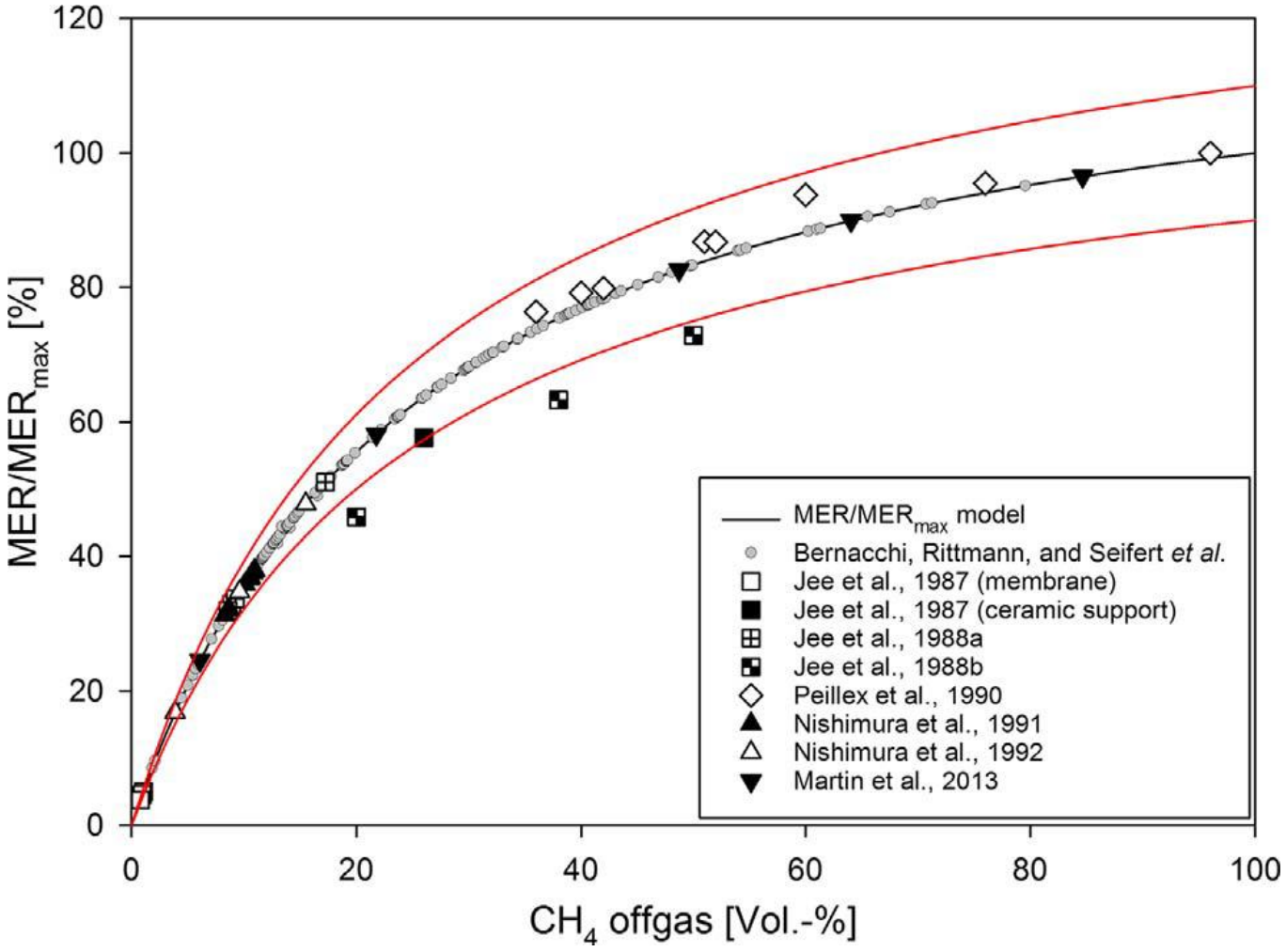


Figure 12: Data on CO_2-BMP is shown as a function of MER/MER$_{max}$ to CH_4 offgas. The MER/MER$_{max}$ model follows the continuous graph. The continuous red lines denote a 10% deviation from the MER/MER$_{max}$ model. As indicated in the legend, the individual data points were directly extracted from literature or calculated from literature data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) The findings presented in this work could were published in Rittmann *et al.*, 2018 Applied Energy 216:751-760.

It was found that literature data often misses important information and/or the required accuracy for the resolution of underlying mechanistic effects, especially when modelling reactor dependent variables. Multivariate dependencies inherently attributed to gas-to-gas conversion bioprocesses are particularly illustrated with respect to the CO_2-BMP process. It is concluded that CO_2-BMP process modelling requires the application of appropriate process analytical technology and experimental strategies. The

understanding of the CO_2-BMP process mechanistic aspects is discussed to assist modelling works also of other gas-to-gas converting bioprocesses. The findings presented in this work were published in Rittmann *et al*., 2018 Applied Energy 216:751-760.

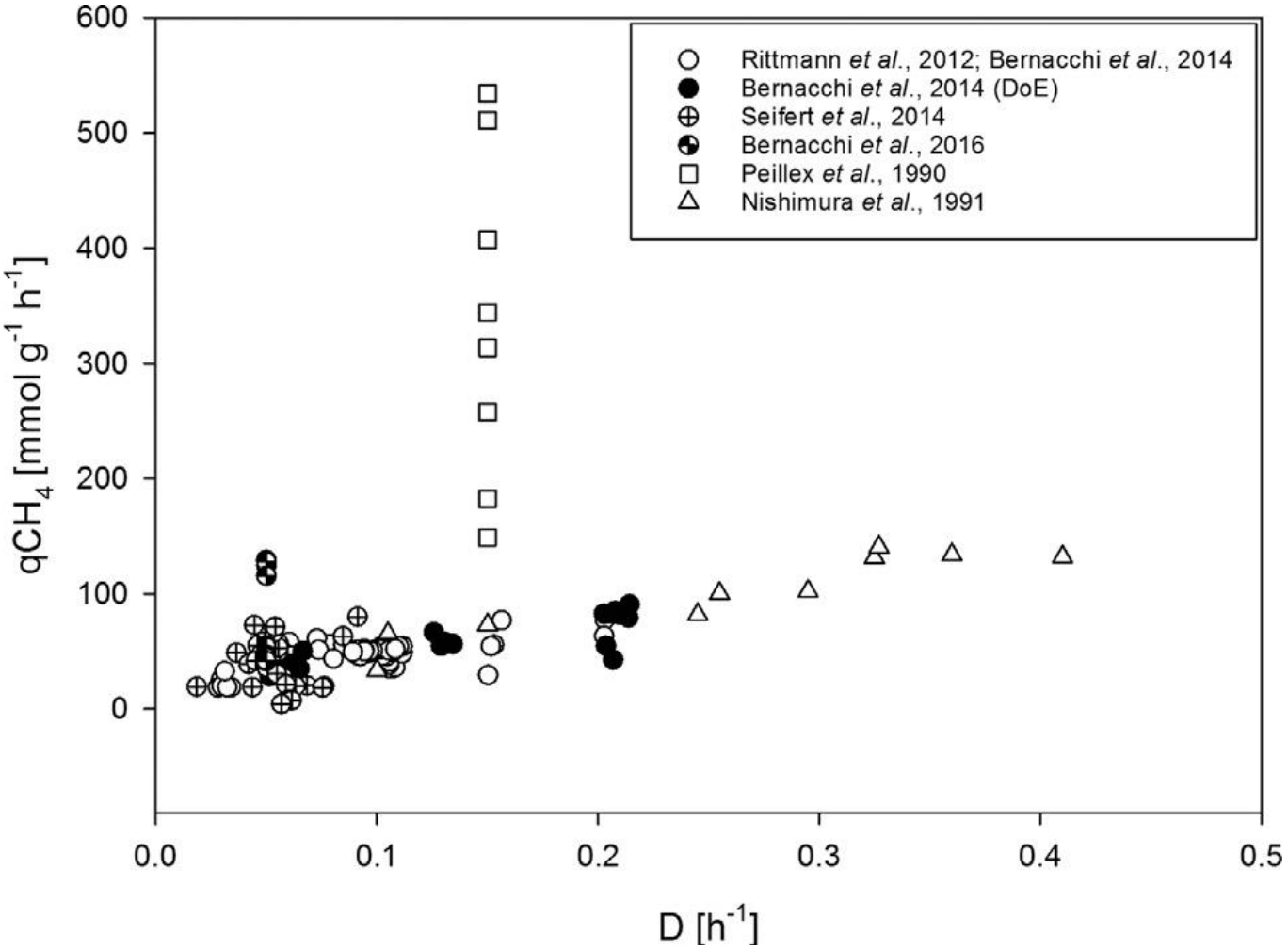


Figure 13: Data on CO_2-BMP is shown as qCH_4 as a function of D. qCH4 data from several publications could almost be fitted by using linear regression. However, qCH_4 data from Peillex *et al*. do not reflect the probable physiological constraints of *M. marburgensis*. The findings presented in this work were published in Rittmann *et al*., 2018 Applied Energy 216:751-760.

Many more methanogens were investigated in fed-batch and continuous culture mode. However, due to the enormous amount of generated data, its analysis is currently still on-going. The results obtained from these analyses and experiments will be published in SCI-journals. During the BioHyMe project, already 7 SCI-publications, incl. our papers in *Nature Communications* and *Applied Energy*, 2 public publications, 18 oral presentations and 4 poster presentation were published or presented at national or international conferences or workshops.

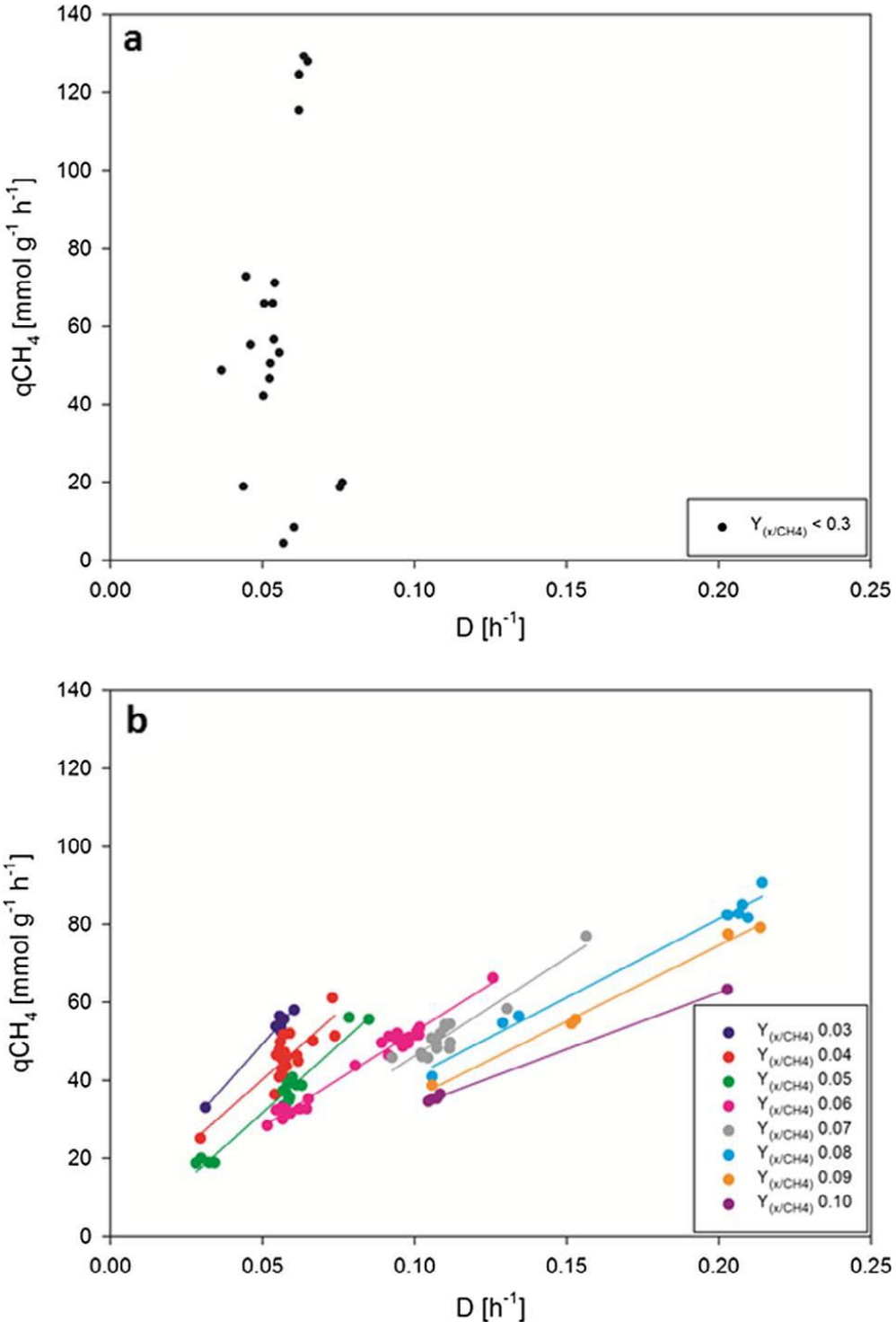


Figure 14. qCH_4 plotted against D for CO_2-BMP continuous cultures under different types of limitations. The $Y_{(x/CH4)}$-range is indicated. (a) liquid- limited cultures at $Y_{(x/CH4)}$<0.3 C-mol mol^{-1}, (b) gas-limited cultures. For gas-limited cultures at different $Y_{(x/CH4)}$ only selected steady states were used. This Figure and the associated figure caption were published in Rittmann *et al*., 2018 Applied Energy 216:751–760.

5 Recommendations and outlook

The results of the literature assessment (Rittmann *et al*., 2018; Mauerhofer *et al*., 2018) show that only few methanogens were prioritized to be optimized in fed-batch or continuous cultivation mode. Examining the physiology and biotechnological characteristics of methanogens in closed batch cultivation mode revealed many new promising physiological and biotechnological features (Abdel Azim *et al*., 2017; Abdel Azim *et al*., 2018; Mauerhofer *et al*., manuscript in preparation). Moreover, up to now, the CH_4 productivity of methanogens grown at high pressure was not investigated on a comparative basis at high-pressure (Pappenreiter *et al*., 2019; Taubner *et al*., 2018) and the prioritization of methanogens from closed batch cultivation mode to fed-batch at atmospheric pressure and/or high-pressure conditions is highly challenging (Mauerhofer *et al*., 2018; Pappenreiter *et al*., manuscript in preparation). However, compared to H_2/CO_2 other gasses or gas mixtures were not investigated. In the project BioHyMe this gap was closed. Nevertheless, many results of this study suggest that the technology breakthrough still requires a strong political will to enable renewable energy production technologies and more efforts with regard to applied basic research and development activities in the area of biological engineering. These research and development activities should be the joint endeavour of national academic and industrial partners from and beyond the project BioHyMe to be able to lift this promising as soon as possible to higher technology readiness level.

6 List of publications and presentations

The following dissemination of SCI publications, (invited) talks, and poster presentations were published:

SCI-Publications

1) Pappenreiter P.A., Zwirtmayr S., Mauerhofer L.-M., Rittmann S.K.-M.R., Paulik C. (2019) Development of a simultaneous bioreactor system for characterization of gas production kinetics of methanogenic archaea at high pressure, **Engineering in Life Sciences**, *in press*

2) Mauerhofer L.-M., Pappenreiter P., Paulik C., Bernacchi S., Seifert A.H., Rittmann S.K.-M.R. (2019) Methods for quantification of growth and productivity in anaerobic microbiology and biotechnology, **Folia Microbiologica**, 64(3):321-360

3) Abdel Azim A., Rittmann S.K.-M.R., Fino D., Bochmann G. (2018) The physiological effect of heavy metals and volatile fatty acids on *Methanococcus maripaludis* S2, **Biotechnology for Biofuels** 11:301

4) Mauerhofer L.-M., Reischl B., Schmider T., Schupp B., Nagy K., Pappenreiter P., Zwirtmayr S., Schuster B., Bernacchi S., Seifert A. H., Paulik C., Rittmann S.K.-M.R. (2018) Physiology and methane productivity of Methanobacterium thermaggregans, **Applied Microbiology and Biotechnology** 102(17):7643-7656

5) Rittmann S.K.-M.R., Seifert A.H., Bernacchi S. (2018) Kinetics, multivariate statistical modelling, and physiology of CO_2-based biological methane production, **Applied Energy**, 216:751-760

6) Taubner R.-S., Pappenreiter P., Zwicker J., Smrzka D., Pruckner C., Kolar P. Bernacchi S., Seifert A.H., Krajete A., Bach W., Peckmann J., Paulik C., Firneis M.G., Schleper C., Rittmann S.K.-M.R. (2018) Biological methane production under putative Enceladus-like conditions, **Nature Communications** 9:748

7) Abdel Azim A., Pruckner C., Kolar P., Taubner R-S., Fino D., Saracco G., Sousa F.L., Rittmann S.K.-M.R. (2017) The physiology of trace elements in biological methane production, **Bioresource Technology** 241:775-786

Two to four more manuscripts are currently being drafted.

Other publications

1) Taubner R.-S, Rittmann S.K.-M.R., Bach W. (2018) Methan auf Enceladus könnte teilweise biologischen Ursprungs sein, Spektrum der Wissenschaft – Sterne und Weltraum 9:16-17

2) Krajete A., Seifert A., Bernacchi S., Sibilla F., Rittmann S. (2016) Krajete® process for CO_2 valorization, Pan Europeans Networks, Science & Technology, 18:38-39, March

Presentations

1) Rittmann S.K.-M.R., Biological methane production under putative Enceladus-like conditions, Christian-Albrechts Universität zu Kiel, 22.21.2018, Kiel, Germany

2) Mauerhofer L.-M., Reischl B., Schmieder T., Schupp B., Nagy K., Pappenreiter P., Zwirtmayr S., Schuster B., Bernacchi S., Seifert A. H., Paulik C., Rittmann S.K.-M.R., H_2/CO_2 fed-batch cultivation of *Methanobacterium thermaggregans*, Workshop on Gas in Biotechnology, January 28-29, 2019, Vienna, Austria

3) Abdel Azim A., Rittmann S.K.-M.R., Fino D., Bochmann G. The physiology of methanogens in the presence of inorganic and organic compounds, Workshop on Gas in Biotechnology, January 28-29, 2019, Vienna, Austria

4) Rittmann S.K.-M.R., Novel insights into the physiology and biotechnology of CO_2 to CH_4 conversion, Johannes Kepler Universität, 09.05.2017, Linz, Austria

5) Mauerhofer L.-M., Pappenreiter P., Reischl B., Schmieder T., Schupp B., Bernacchi S., Seifert A. H., Krajete A., Paulik C., Rittmann S.K.-M.R., Novel insights into the physiology and productivity of a H_2/CO_2 converting methanogen, 7th International Conference on Biological Air Pollution and Control 19th of July to 21st of July 2017, La Coruna, Spain

6) Rittmann S.K.-M.R., Abdel Azim A., Taubner R.-S., Pappenreiter P., Mauerhofer L.-M., Reischl B., Paulik C., Novel insights into the physiology and biotechnology of CO_2 to CH_4 conversion, 10th International Symposium on Anaerobic Microbiology (ISAM 10), 11.07.2017-14.07.2017, Liblice, Czech Republic

7) Taubner R.-S., Baumann L.M.F., Steiner M., Firneis M.G., Schleper C., Peckmann J., Birgel D., Rittmann S.K.-M.R., Lipid Pattern of *Methanothermococcus okinawensis* under Varying Enceladus-like Conditions, EANA 2018, September 24 – 28, 2018, Berlin, Germany

8) Taubner R.-S., Pappenreiter P., Zwicker J., Smrzka D., Pruckner C, Kolar P., Bernacchi S., Seifert A.H., Krajete A., Bach W., Peckmann J., Paulik C., Firneis, M.G., Schleper C., Rittmann S.K.-M.R., Simulating putative Enceladus-like conditions: The possibility of biological methane production on Saturn's icy moon, 30th IAU General Assembly, Symposium 345: Origins: From the Protosun to the First Steps of Life, August 20 – 31, 2018, Vienna, Austria

9) Taubner R.-S., Pappenreiter P., Zwicker J., Smrzka D., Pruckner C., Kolar P., Bernacchi S., Seifert A. H., Krajete A., Bach W., Peckmann J., Paulik C., Firneis M. G., Schleper C., Rittmann S.K.-M.R., 2018, Biological methane production under putative Enceladus-like conditions; Workshop ExoOceans: Space Exploration of the Outer Solar System Icy Moons Oceans at the International Space Science Institute, June 18-22, Bern, Switzerland

10) Taubner R.-S., Baumann L.M.F., Birgel D., Firneis, M.G., Schleper C., Rittmann S.K.-M.R., Lipid pattern of *Methanothermococcus okinawensis* under varying Enceladus-like conditions, Astrobiology 2017: A research meeting by Interantional Astronautical Federation, Coyhaique, Chile

11) Rittmann S.K.-M.R., Seifert A. H., Bernacchi S., Modelling, qualitative and quantitative analysis of pure-culture biological methane production (BMP) from H_2 and CO_2, Workshop on ABE Production and H_2/CO_2 Fermentation, 10th-11th of November 2016, University of Natural Resources, Vienna, Austria

12) Abdel Azim A., Pruckner C., Kolar P., Fino D., Saracco G., Rittmann S.K.-M.R., H_2/CO_2 (fed-batch) fermentation for biological production of CH_4 by (pure culture of) hydrogenotrophic archaea, Workshop on ABE Production and H2/CO2 Fermentation, 10th-11th of November 2016, University of Natural Resources, Vienna, Austria

13) Pappenreiter P., Taubner R.-S., Seifert A.H., Bernacchi S., Paulik C., Schleper C., Firneis M.G., Rittmann S.K.-M.R., Testing the habitability of Enceladus with Methanothermococcus okinawensis, Workshop on ABE Production and H_2/CO_2 Fermentation, 10th-11th of November 2016, University of Natural Resources, Vienna, Austria

14) Taubner R-S, Rittmann S.K.-M.R., A new method for indirect quantification of methane production via water production using hydrogenotrophic methanogens, Workshop on ABE Production and H_2/CO_2 Fermentation, 10th-11th of November 2016, University of Natural Resources, Vienna, Austria

15) Mauerhofer L.-M., Reischl B., Schmieder T., Schupp B., Pappenreiter P., Bernacchi S., Seifert A. H., Krajete A., Paulik C., Rittmann S.K.-M.R., Development of a high-pressure process to couple biological hydrogen and methane production, Workshop on ABE Production and H2/CO2 Fermentation, 10th-11th of November 2016, University of Natural Resources, Vienna, Austria

16) Taubner R.-S., Pappenreiter P., Pruckner C., Zwiscker J., Smrzka D., Firneis M. G., Paulik C., Schleper C., Rittmann S.K.-M.R., An interdisciplinary approach: On the habitability of Saturn's icy moon Enceladus, Astrobiology Seminar of the University of Stockholm, 28th of September 2016, Stockholm, Sweden

17) Taubner R.-S., Pappenreiter P., Pruckner C., Zwiscker J., Smrzka D., Firneis M. G., Paulik C., Schleper C., Rittmann S.K.-M.R., Testing the habitability of Saturn's icy moon Enceladus; 16th European Astrobiology Conference, Athens, Greece, September 27–30.

18) Taubner R.-S., Firneis M. G., Schleper C., Rittmann S.K.-M.R., Cultivation of methanogens in Enceladus-like conditions, Science Day of the Faculty of Life Sciences of the University of Vienna, 1st of July 2016, Wien, Austria

Posters

1) Zwirtmayr S., Pappenreiter P., Mauerhofer L.-M. Rittmann S.K.-M.R., Paulik C., Screening of methanogens in a Simultaneous Bioreactor System (SBRS) with multiple determination under high pressure conditions, MBoA6 Workshop: Molecular Biology of Archaea: from mechanisms to ecology, September 3-5, 2018, Vienna, Austria

2) Pappenreiter P., Paulik C., Mauerhofer L.-M. Rittmann S.K.-M.R., Bernacchi S., Seifert A., Experimental methods for investigations of biomethanation processes at elevated operating pressures, 10th World Congress on Chemical Engineering (WCCE), 1st-5th of October 2017, Barcelona, Spain

3) Zwirtmayr S., Pappenreiter P., Mauerhofer L.-M., Bernacchi S., Seifert A.H., Krajete A., Rittmann S.K.-M.R., Paulik C., Development of a Simultaneous Bioreactor System (SBRS) for high throughput screening and characterization of methanogens at high pressure, 17th Austrian Chemistry Days, 25th-28th of September 2017, Salzburg, Austria

4) Abdel Azim A., Pruckner C., Kolar P., Taubner R.-S., Fino D., Saracco G., Sousa F.L., Rittmann S.K.-M.R., 2017, CO2 bioconversion: trace elements utilization in biological methane production (BMP), 7th International Conference on Biological Air Pollution and Control 19th-21st of July 2017, La Coruna, Spain

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