Increasing the methane evolution rate of methanogenic archaea: a study of *Methanothermobacter marburgensis*

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Objective

- Increasing methane formation rate by adapting agitation speed and optimize medium to gas ratio in batch cultures of *M.marburgensis*

Experiment setup

- 6 stirred flasks (120 mL) and 1 unstirred as control flask per batch (3 h incubation at 63 °C)
- Every flask was filled with the same medium to gas volume per batch and fully grown with *M.marburgensis*
- Before incubation the flasks were gased with a 4:1 H_2:CO_2 gas mixture
- Pressure was measured before and after incubation

Methods and calculation

- Fluorescence microscopy (Exc. 420 nm, Emi. 460 nm)
- Pressure measurement with digital manometer and data logging
- Gas analysis with gas chromatography (TCD, Argon)
- Methane evolution rate (MER) by calculation

$$p_{CH4} = \frac{p_1 - p_2}{4} \qquad n_{CH4} = \frac{p_{CH4} * V_{gas}}{R * T} * 1000$$

$$MER = \frac{n_{CH4}}{dt * V_{reactor}}$$

Results and discussion

- Microorganisms were vital with a stable fluorescence emission
- Highest MER about 3,3 mmol/l*h at 800 rpm and 60 ml medium
- Striking MER drop at 60 ml and 1000 rpm; stirring induced energy may result in this decrease as the mixing is higher compared to 100 ml (stirring energy per medium volume)
- No significant MER increase at 100 ml with increased stirring speed which could result from the high pressure reduction as the flask gas volume is much lower
- Gas analysis showed a significant increase in methane concentration of about 30 Vol.-%
- The increase in hydrogen concentration is due to the decrease of carbon dioxide as it dissolves in the medium

Conclusion

- Future experiments will further optimize the method regarding media and microorganism settings
- The logged data from the 100 ml batches will be analyzed regarding a shorter batch time yielding a higher MER
- The developed methods and calculations for these experiments were proven suitable and will be published soon

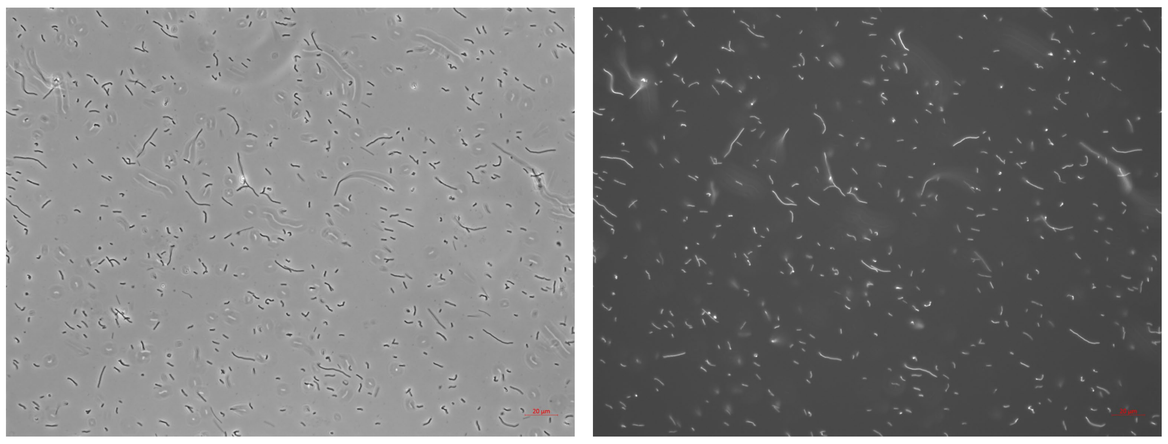

Fig 1.: Transmitted light (left) and fluorescence microscope (right) images from *M. marburgensis* after incubation

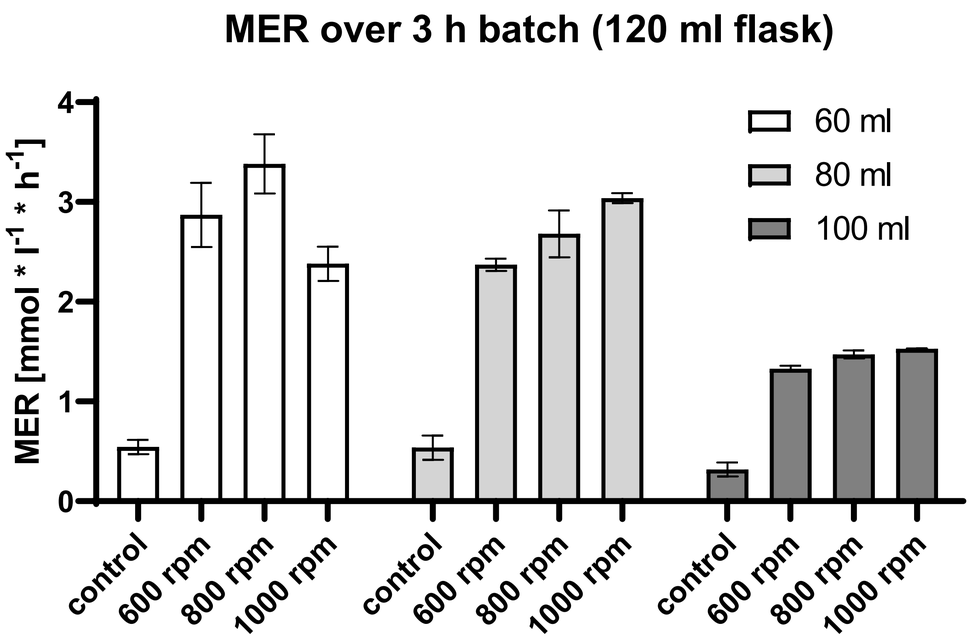

Fig 2.: Calculated methane evolution rate (MER) after 3 h incubation at 63 °C in 120 ml flasks at different stirring speeds (600, 800 and 1000 rpm) and medium to gas volumes (60, 80 and 100 ml medium) as well as control samples for comparison

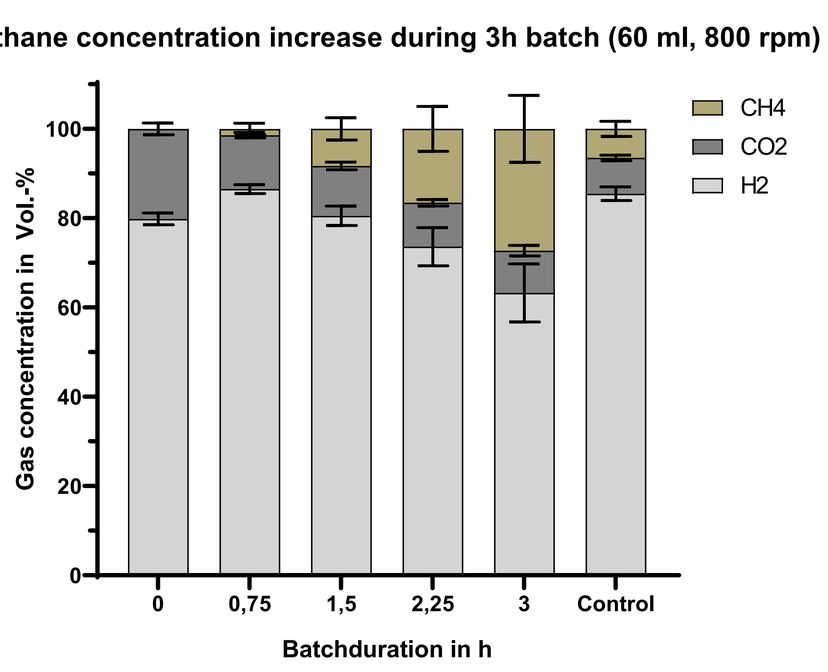

Fig 3.: Increase in methane concentration over the 3 h batch (60 ml, 800 rpm) with control sample as comparison

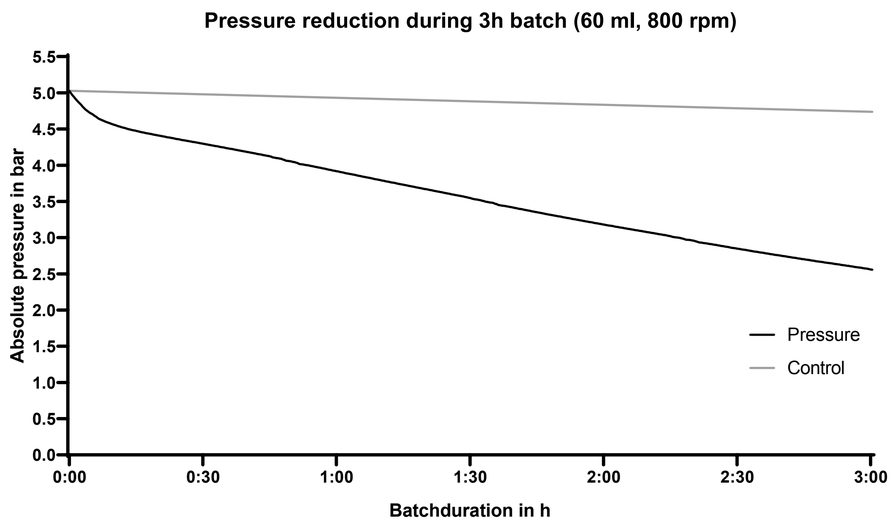

Fig 4.: The pressure reduction over the 3h batch (60 ml, 800 rpm) of the stirred and an unstirred control sample

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