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 H₂:CO₂ 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

\[
\text{MER} = \frac{n_{\text{CH}_4}}{d_t \cdot V_{\text{reactor}}} \times 1000
\]

\[
p_{\text{CH}_4} = \frac{p_1 - p_2}{4}
\]

\[
n_{\text{CH}_4} = \frac{p_{\text{CH}_4} \cdot V_{\text{gas}}}{R \cdot T} \times 1000
\]

**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

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