**High throughput screening of methanogens under high pressure**

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**Highlights**

* Biological methane production (BMP).
* Simultaneous bioreactor system (SBRS).
* CO2-hydrogenotrophic methanogenic strains.

**1. Introduction**

The biological methane production (BMP) is a promising technology for CO2 neutral energy production and storage. Usually methanogens, an archaeal group of microorganisms, are applied in biotechnological process to produce methane (CH4), water and biomass from hydrogen (H2) and carbon dioxide (CO2) according to (1). There are two ways to perform the process, either in continuous or discontinuous mode. A further possibility is to use pure or industrial emission waste gas for the biomethanation [1-4].

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|  | CO2 + 4 H2 -> CH4 + 2 H2O |  (1) |

Primarily the bioprocess is limited by the gas-liquid mass transfer step [4‑5]. This is also influenced by different factors like the geometry and velocity of the stirrer, the sparger system or gas flow rates. The prevailing partial pressure in the system is an additional coefficient. Therefore, we developed the simultaneous bioreactor system (SBRS), which operates at elevated pressure to increase the dissolved concentration of H2 and CO2 during the reaction. Different CO2‑hydrogenotrophic methanogenic strains were cultivated in the SBRS at a pressure of 10 and 50 bar relative.

**2. Methods**

The SBRS has four identical reactors. Each of them has a total volume of 160 mL, which can be used for screening of methanogens in closed batch cultivation mode at pressure up to 50 bar relative. Five different methanogenic strains are tested in the SBRS at 10 and 50 bars.

* *Methanothermobacter marburgensis* DSM 2133
* *Methanobacterium thermaggregans* DSM 3266
* *Methanobacterium palustre* DSM 3108
* *Methanobacterium subterraneum* DSM 11074
* *Methanocaldococcus villosus* DSM 22612

The microorganisms are obtained from “Deutsche Stammsammlung für Mikroorganismen und Zellkulturen”. In addition, five hyperthermophilic strains will be tested. The following key aspect were used for the experiments: they were performed with a H2:CO2 (4:1) substrate gas mixture, a simplified medium composition for each strain (60 mL) was used, a Na2S $∙$ 9 H2O solution (1 v/v%) was injected and the SBRS is mounted on a laboratory shaker instead of using a stirrer.

At the end of each experiment, gas- and liquid samples were taken through the respective valves. The obtained gas samples were measured with a gas chromatograph to determine the methane off-gas concentration, while the liquid samples get reconditioned for scanning electron microscope (SEM) pictures. In addition, the biomass samples were taken, after releasing the overpressure from the SBRS. The biomass is washed by centrifugation with water and finally dried over night (105°C) to determine the dry weight.

**3. Results and discussion**

To describe the CO2-BMP process different parameters like the turnover rate, the methane evolution rate (MER) and the maximum conversion rate, were selected. Calculating the turnover rate is an alternative way to determine the CH4 productivity indirectly [6]. *M. villosus* showed an increased conversion rate, turnover rate, and MER results at 50 bar compared to 10 bar.

Through the online measurement of the pressure, it can be shown that the methanogenic CH4 production leads to a pressure drop. The production of CH4 was also checked by GC measurements for each experiment.

**4. Conclusions**

Our developed SBRS is a suitable high throughput bioreactor system for fast characterization of methanogens and gas converting microorganisms at 10 and 50 bar. All strains were successfully cultivated at 10 bar and until now also the two strains *M. Marburgensis* and *M. Villosus* were successfully cultivated at 50 bar. Further experiments with the remaining strains are planned. It has been demonstrated that gas conversion and biomethane production can be achieved in each experiment.

**References**

1. L.-M. Mauerhofer, B. Reischl, T. Schmider, B. Schupp, K. Nagy, P. Pappenreiter, S. Zwirtmayr, B. Schuster, C. Paulik, S. K.-M. R. Rittmann, Appl. Microbiol. Biotechnol. 102(17) (2018) 7643-7656.
2. S. K.-M. R. Rittmann, A. H. Seifert, A. Krajete, BIOspektrum 20(7) (2014), 816-817.
3. B. Lecker, L. Illi, A. Lemmer, H. Oechsner, Bioresource Technology 245 (2107) 1220-1228.
4. E. Inkeri, T. Tynjälä, A. Laari, T. Hyppänen, Applied Energy 209 (2018) 95-107.
5. S. Bernacchi, A. Krajete, C. Herwig, AIMS Microbiology 2(3) (2016), 262-277.
6. R.S. Taubner, P. Pappenreiter, J. Zwicker, D. Smrzka, C. Pruckner, P. Kolar, Nature Communications 9 (2018), 748.