**Development of a high pressure bioreactor system for the production of biomethane from CO2 using an axenic methanogenic culture as biocatalyst**

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**Highlights**

* Gas to gas (GtoG) converting bioprocess
* CO2-BMP, an axenic unsterile methanogenic archaea-based process using a fully defined mineral medium for growth
* Gas limitation and liquid limitation in gas converting bioprocesses
* Continuous and intermittent bioprocessing of industrially sampled H2/CO2 gas mixtures

**1. Introduction**

Over recent years the interest in new biofuel generations, based on converting gaseous substrate(s) such as carbon dioxide (CO2), carbon monoxide (CO) or hydrogen (H2) to gaseous product(s), arose. An example for such a gas converting bioprocess is the biological methane production process using CO2 as sole carbon source (CO2-BMP) [1]. Axenic cultures of *Methanothermobacter marburgensis* grown in a defined mineral medium already proved that high conversion rates of CO2 and H2 to methane (CH4) can be reached [2]. However, this bioprocess was often described in literature as a gas transfer limited bioprocess [3]. Therefore, the kinetic limitation towards an increased methane productivity cannot be overcome solely by the growth of more biomass during continuous operation. More important is the development of a suitable bioreactor system that allows reaching a high mass transfer of the limiting gaseous substrate (H2) in the liquid phase once an appropriate feeding strategy is applied to maintain sufficient biocatalyst in suspension for converting all the dissolved reactive gases. This work will present development steps [4], [5], methods [6], [7] as well as the applied bioprocess control approach [8] that enabled to construct and operate a custom designed and manufactured high pressure 20 L bioreactor system to overcome the so far existing performance limitations. In this setup, a methane evolution rate (MER) higher than 1.4 molCH4 Lbroth-1 h-1 was reached using an axenic chemostat culture of *Methanothermobacter marburgensis* grown on a defined mineral medium at pressures up to 16 bar while using solely CO2 as carbon source. The application of this feed forward control strategy enabled to predict and control biomass growth during operation which in return allowed to convert more than 99% of the applied 2.9 vvm [NLgas Lbroth-1 min-1] of H2-CO2 into a high purity bio-CH4 (>95 Vol.% CH4 in the raw wet gas).

**2. Methods**

The integrated modular development workflow that will be presented consists of studying the biomethanation process from different angles and using different “levels” of pressurized H2 and CO2 gas mixtures to unscramble both, the potential physiologic limitation that could arise on one side, and the benefits of high pressure on the reaction kinetic on the other side. The following aspects have been investigated: pressure tolerance, media demands, feed strategy development for fermentation and validation runs in continuous culture to reach performances above 20 kgCH4 m-3 h-1 (MER > 1250 mmolCH4 Lbroth-1h-1) in steady state production in order to support process simulation and tecno-economic assessment.

**3. Results and discussion**

A reference culture of *Methanothermobacter marburgensis* was used to validate the application of the feed forward strategy concept and demonstrate the possibility to control gas transfer limited bioprocesses. This allowed reaching the desired stability for operations as well as predicting media demand when elaborating specific feed strategies for the different operational profiles. An example of a dynamic intermittent operation is shown in **Figure 1**.

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**Figure 1.** Feed forward strategy application to a CO2-BMP intermittent operation allowed uncoupling biomass growth control from methane production by varying the biocatalyst performances.

The feed strategy used to predict and control operations has been validated in pressure less conditions and further used for designing the cultivation medium for high pressure experiments where pseudo-steady state conditions had to be applied. After that, the know-how on the bioprocess was used for engineering, designing and building a custom-made pressure resistant CSTR that allowed to transfer axenic cultures to an elevated pressure environment (up to 16 barg). The latter allowed reaching unprecedent productivities using solely CO2 as carbon source.

**4. Conclusions**

The application of an interdisciplinary modular development approach [9] to the CO2-BMP process allowed on one side assisting the prediction works for tecno-economic analysis [10] and process scale up while on the other hand elaborating a control strategy for the bioprocess suited for a vast range of operational regimes. This resulted in reaching high performances (methane evolution rate (MER) higher than 1.4 molCH4 Lbroth-1 h-1) with sufficient methane (CH4 > 95 Vol.% for the raw wet gas) that allow to reach most of EU countries gas grid specifications after a final purification step. Greatly acknowledge is the funding from the European Union's Horizon 2020 research and innovation program under grant agreement number 679050 (project: CELBICON).

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