

Optimized Biomethanation of Hydrogen and Carbon Dioxide via Inoculum Selection and Pre-processing in a Thermophilic Trickle-Bed Reactor

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Abstract

To improve the energy efficiency of biological methanation of CO₂ and H₂, this study evaluates the performance of a thermophilic (55 °C) anaerobic trickle-bed reactor (700 ml). A novel inoculation strategy is presented, involving the use of unfiltered and uncentrifuged anaerobic sludge from a municipal wastewater treatment plant (WWTP) as a mixed microbial culture for methanation. The results demonstrate rapid, stable, and complete conversion of H₂ and CO₂, achieving methane (CH₄) concentrations exceeding 95%. This level of purity enables direct injection into existing natural gas infrastructure without the need for extensive biogas upgrading. Furthermore, a high specific methane production rate of 10 Nm³/m³·day was achieved, indicating strong potential for system scale-up.

Keywords: Biomethanation, trickle bed reactor, inoculum pre-processing.

1. Introduction

With the growing use of renewable energy sources, which produce power intermittently, there is an increasing need for energy systems that can store and convert energy efficiently. One promising solution is to convert hydrogen (H₂) and carbon dioxide (CO₂) into methane (CH₄), which can be stored and used in the existing natural gas grid. The direct biological conversion of CO₂ and H₂ to methane is a well-established process (Burkhardt & Busch, 2013). However, challenges remain, particularly in achieving efficient gas-liquid mass transfer. Trickle bed reactors (TBRs) offer a promising solution, as they enable effective conversion of both liquid and gaseous substrates with enhanced mass transfer characteristics, leading to high methane yields (Feickert Fenske et al., 2023). Nonetheless, biofilm formation within TBRs remains a critical bottleneck, often limiting long-term stability and reactor performance.

This study aims to evaluate the performance and stability of a thermophilic TBR for the conversion of CO₂ and H₂ to CH₄. A novel inoculation strategy was applied, focusing on processing the inoculum in a way that facilitates biofilm

formation without disrupting particle-associated microbial communities. The goal was to assess whether this approach improves reactor performance and supports a scalable efficient biomethanation process.

2. Materials and Methods

A 700 mL thermophilic trickle bed reactor, similar to the configuration described by Ali et al. (2024), was operated at 55 ± 1 °C for a period of fifty days. The reactor was continuously supplied with a gas mixture of H₂ and CO₂ at a molar ratio of 4:1, with flow rates of 20 NmL/min for H₂ and 5 NmL/min for CO₂. Anaerobic sludge obtained from the Ejby Mølle wastewater treatment plant (WWTP) in Odense, Denmark, was used both as inoculum and as the source of nutrients. To prepare the inoculum, the sludge was sieved through a 0.25 mm mesh to remove large particles, preserving the microbial communities associated with smaller particles. For nutrient medium preparation, the sieved sludge was centrifuged at 4800 rpm for 10 minutes and subsequently filtered using a 0.125 mm mesh sieve. The reactor performance was monitored daily by measuring the gas composition using a gas chromatograph. Additionally, liquid and biofilm samples were collected weekly for analysis of volatile fatty acids (VFAs) and microbial analysis to evaluate metabolic activity and stability.

3. Results and Discussion

The reactor showed rapid startup behavior, with near-complete conversion of CO₂ and H₂ achieved within the first four days of operation. By day 4, conversion efficiencies reached 98% for H₂ and 94% for CO₂, peaking early in the experimental period. From that point onward, the reactor maintained stable performance with average conversion rates of approximately 100% for H₂ and 95% for CO₂. The performance of the reactor over time is illustrated in Figure 1. A slight decrease in gas conversion and methane production was observed at the end of each week. This was attributed to the nutrient limitation as a

new nutrient medium was supplied at the beginning of every week.

The average methane production rate during the experimental period was approximately $9.3 \text{ Nm}^3/\text{m}^3\cdot\text{day}$. This performance is comparable to the findings of Strübing et al. (2017), who reported similar results under comparable thermophilic trickle-bed reactor conditions. Furthermore, the achieved CH_4 production and CO_2 conversion rates exceed those observed in other studies

operating under similar temperature and gas loading conditions, including Feickert Fenske et al. (2023), Ullrich et al. (2018), and Thema et al. (2021).

These results demonstrate the technical feasibility and robustness of the applied inoculum strategy in thermophilic trickle bed systems. The high methane production rates and stable performance support the potential for scaling up this configuration for industrial biomethanation applications.

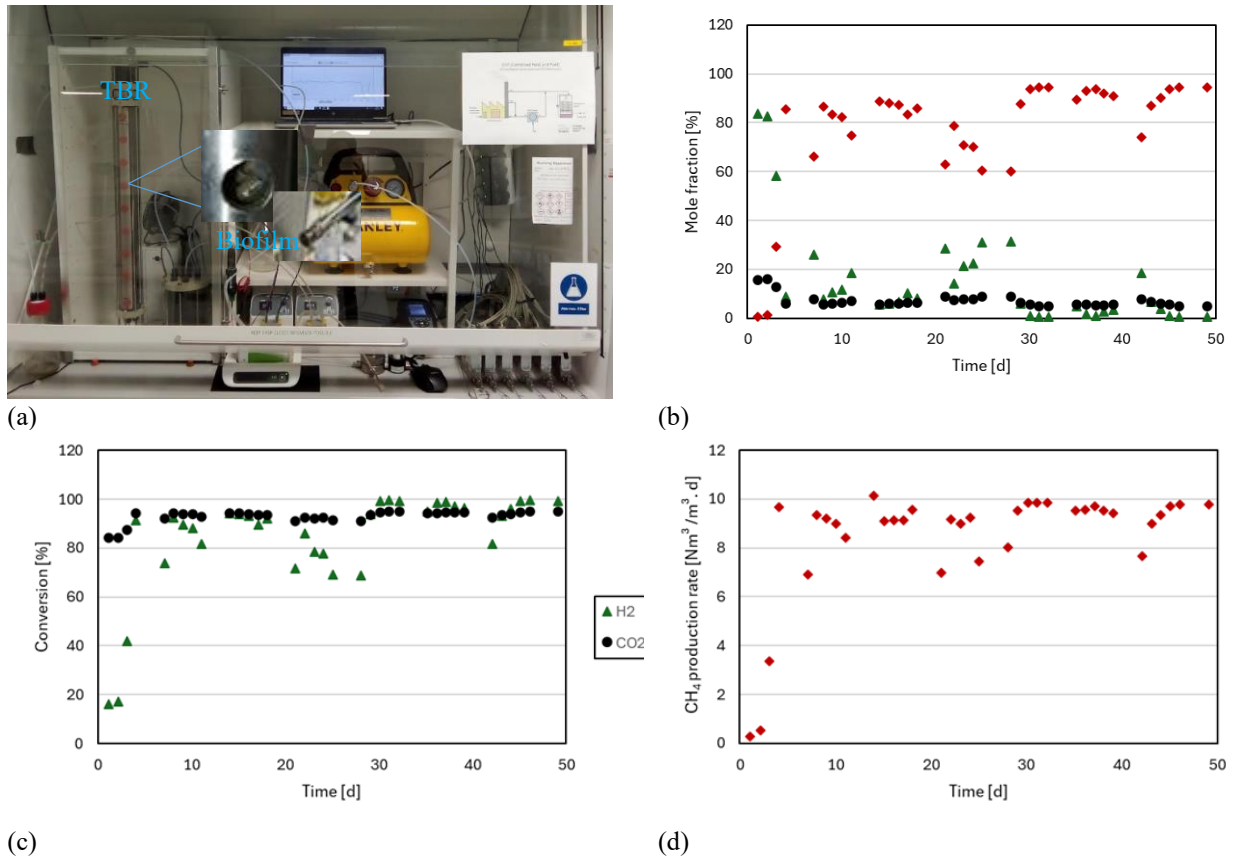


Figure 1. Reactor performance over time: (a) TBR system showing a biofilm sample (b) gas composition (CH_4 , H_2 , CO_2), (c) conversion efficiency of H_2 and CO_2 , and (d) methane production rate ($\text{Nm}^3/\text{m}^3\cdot\text{day}$).

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