

# Biomethanation of Syngas with a Focus on CO Conversion Using Mixed Microbial Cultures in a Thermophilic Trickle bed Reactor

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Abstract Biomethanation of syngas offers a sustainable route for renewable methane production. However, COrich gas streams pose challenges due to microbial inhibition. This study evaluated a thermophilic trickle bed reactor (TBR) operating for 180 days with mixed microbial cultures and varying syngas compositions. Peak methane production of 6.8 Nml/ml·d and high elimination capacities of 85% H<sub>2</sub>, 60% CO, 99% CO<sub>2</sub> were achieved during syngas feeding, while CO-only phases showed reduced performance. The findings highlight TBR as a viable system for syngas upgrading and provide insight into optimizing CO biomethanation.

**Keywords:** Trickle bed reactor, syngas, biomethane, carbon monoxide, conversion, inoculum.

#### 1. Introduction

The global shift from fossil fuels to renewable energy has heigh interest in sustainable technologies like biomass gasification, which converts organic waste into syngas for biofuel production (Ren et al., 2020). Conventional syngas methanation employs metal catalysts (e.g., Ni/Al<sub>2</sub>O<sub>3</sub>), but these face deactivation by sulfur and tar, requiring expensive purification (Riani et al., 2023).

Biomethanation, which utilizes microorganisms to convert syngas components into methane under anaerobic conditions, presents a viable alternative. However, syngas biomethanation faces several challenges, particularly when CO is used as a substrate. Elevated CO partial pressures can inhibit microbial activity, affecting both methanogens and syntrophic bacteria. Additionally, low syngas component solubility, mass transfer limitations, and potential microbial pathway competition can reduce methane yields and process stability (Laguillaumie et al., 2022).

This study investigates the biomethanation potential of syngas using mixed microbial cultures in a thermophilic TBR. Key objectives include evaluating reactor performance under varying syngas compositions, assessing CO conversion and methane production

efficiency, and identifying inhibitory effects associated with CO-rich substrates.

**Table 1.** Experimental design and operating conditions during the experiment.

Phase	Period [d]	Gas flow [Nml/min]	Gas composition		Inoculum and media
-	0.25	17.5	**	7.40/	use
I	0-25	17.5	$H_2$	74%	Inc 1+ SM
			$CO_2$	9%	
			CO	14%	
			$N_2$	3%	
II	25-109	17.5	$H_2$	74%	Inc1 + SM
			$CO_2$	9%	
			CO	14%	
			$N_2$	3%	
III	110-	14.5	$H_2$	54%	Inc 1+ SM
	124		CO	17%	
			$N_2$	31%	
IV	124-	14.5	CO	17%	Inc1 + SM
	137		$N_2$	83%	
V	138-	14.5	CO	17%	Inc 2
	180		$N_2$	83%	

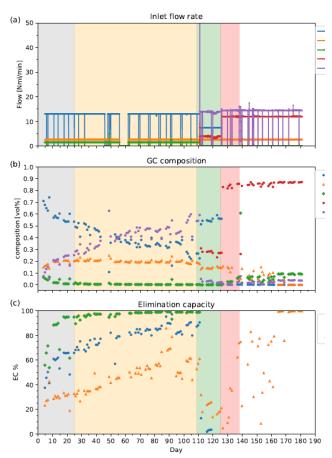
## 2. Material and methods

A 697.1 mL thermophilic TBR was operated at  $52 \pm 2$  °C and pH 7.5–8.5 for 180 days, the reactor configuration is described elsewhere (Ali et al., 2024). Two inocula were used: anaerobic digestate (Inc 1) from a biogas plant and anaerobic sludge (Inc 2) from a wastewater treatment plant. The experiment was divided into six phases with varying syngas compositions; in the final two phases, CO was supplied as the sole substrate to assess its methanation potential. Design and operating conditions are provided in Table 2. Synthetic nutrient media (SM) was used in all but the final phase to support microbial activity. Reactor performance was evaluated via flow rates, gas composition, elimination capacity (EC), and methane production capacity (PC), while weekly liquid samples

were analyzed for volatile fatty acids and alcohols. Microbial communities in the inocula and reactor biofilms were characterized at stabilization using 16S/18S rRNA gene sequencing.

#### 3. Results and discussion

The 180-day performance of TBR is shown in Figure 2. The reactor maintained stable syngas conversion throughout the continuous operation, achieving methane production rates comparable to other biomethanation systems (Asimakopoulos et al., 2020). Initial CO<sub>2</sub> consumption reached 99% within 25 days, while CO and H<sub>2</sub> showed slower but steady conversions, 60% and 85%, respectively. Methane production reached a maximum of



**Figure 1.** Time series of key parameters over the 180-day operation of the TBR: (a) inlet and total syngas flow rates, (b) outlet gas composition (vol%) for H<sub>2</sub>, CO, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>, and (c) elimination capacity (EC%) of syngas components. Colored background regions represent experimental phases: grey = Phase I, yellow = Phase II, green = Phase III, red = Phase IV, and white = Phases V and VI.

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6.8 Nml/ml·d during Phase II, demonstrating strong biomethanation performance (Thapa et al., 2022). Notably, residual CO<sub>2</sub> detected after its supply ceased in Phase III suggests continued microbial CO-to-CO<sub>2</sub> conversion via water-gas shift activity.

Carbon balance analysis confirmed a close match between estimated and measured methane output, supporting the reliability of the monitoring methodology. Metabolic profiling showed rapid depletion of volatile fatty acids and alcohols, suggesting their key role as intermediates in methanogenic pathways. These results demonstrate the potential of TBR systems for biogas upgrading while highlighting key areas for optimization in scaling syngas biomethanation.

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