Microbial and phage dynamics associated with gas retention time and mechanical failure in a pilot-scale CO_2 biomethanation system

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Carbon capture and utilization (CCU) is a critical challenge of the 21st century, aimed at resource recovery and reducing environmental pollution, aligning with sustainable development goals. Among CCU strategies, carbon dioxide (CO_2) biomethanation is a key technology for renewable energy recovery from anaerobic digestion of biomass, converting hydrogen (H_2) and CO_2 into methane (CH_4) under anoxic conditions. This process relies on the cooperation between hydrogenotrophic methanogens and syntrophic bacteria, forming highly specific microbial communities. Despite progress, a complete understanding of the "microbial dark matter" involved in these processes remains elusive, limiting full biotechnological exploitation. In particular, mobile genetic elements such as phages play a crucial role in microbial dynamics by facilitating horizontal gene transfer and influencing community structure, e.g. by eliminating microbial species. However, much of the viral diversity in these ecosystems is still uncharacterized, highlighting the need for further research to enhance bioconversion efficiency. In this work, we analysed the composition and dynamics of a microbiome performing CO_2 biomethanation in a pilot-scale trickle-bed reactor (TBR) undergoing gas residence time (GRT) reduction. This setup aimed to assess the microbiome's response and adaptation capability to increasing inlet flow rates of feed gasses, evaluating the stability and production capacity at each achievable GRT.

The system featured a 30L TBR filled with Raschig rings, operating at 55°C. CO_2 served as the primary carbon source, mixed with H_2 in a 1:4 ratio, while digestate provided essential vitamins and cofactors for microbial growth (Figure 1A). Throughout the operational phase, GRT was gradually reduced by increasing the gas feed flow, starting from 4 hours down to 50 minutes, with phase transitions occurring once the output gas composition stabilized above 95% CH_4. Liquid samples were collected at the end of each experimental phase to monitor microbiome changes in response to the increased gas flow rate. Biofilm samples were collected at three levels within the TBR to assess the impact of reduced CO_2 and H_2 partial pressures on biofilm development and microbial competition, while environmental parameters like pH, VFA concentration, and gas composition were continuously monitored as indicators of microbial activity. A comprehensive approach combining genome-centric metagenomics, metatranscriptomics, and community-based metabolic modeling was utilized to investigate the microbial community in the system. In particular, we analyzed the composition of the microbial and viral community in terms of composition, taxonomy, functional potential, and single-nucleotide variants (SNVs), comprehensively characterising biological dynamics.

A total of 113 medium-to-high quality metagenome-assembled genomes (MAGs) were recovered, representing 12 archaeal and 101 bacterial taxa. The composition and functional potential of the microbial community remained largely stable throughout the experimental period between 4-hour and 1-hour GRT, with *Methanothermobacter thermautotrophicus* BIN1 comprising over one third of the community (Figure 1B). However, a decline in CH_4 yield was observed at a 1-hour GRT due to a malfunction in the trickling pump. To restore optimal performance, a bioaugmentation strategy was employed with the introduction of fresh methanogenic culture, which maintained biomethane purity above 95% for about 30 days. Patterns in microbial abundance and replication rates lead us to hypothesise that the mechanical failure triggered a phage induction event (Figure 1B), which was corroborated by analysis of viral genomes. 503 uncultivated viral genomes (UViGs) were identified and clustered into 38 medium-to-high quality species-like units. Consistent with the hypothesis, a peak in viral read depth was observed across the majority of the virosphere at the first 1-hour GRT time point (Figure 1C). This sudden increase was accompanied by the loss of abundant species such as Tediphilus sp. BIN54, Bacillota sp. BIN96 and Anaerolineae sp. BIN41 on the following time points. Moreover, prophages infecting these specific lineages were classified as virulent and were also characterised by a proteolytic genetic makeup. To verify the consistency of a viral shunt with the observed microbial abundances and biomethane yield, we reconstructed genome-scale metabolic models of the microbial community and tested alternative scenarios, finding that cell lysis with consequent release of intracellular nutrients best explained our data. Moreover, the distribution and dynamics of single-nucleotide variants in the population indicate a selection event concerning genes involved in quorum sensing and defence systems and resulting in strain replacement over multiple taxa. Archaeal genes with high non-synonymous SNV accumulation were primarily methanogenesis-related hydrogenases in response to lower GRTs.

Our results provide an overview on microbial adaptation to increasing levels of feed gas at pilot scale and support the hypothesis of a diffuse viral shunt associated with chemico-physical imbalances of the bioreactor at the 1-hour GRT time point, with critical effects on the microbiome composition. Overall, this work demonstrates the potential of simultaneously deciphering microbial and viral dynamics for optimizing CO_2 biomethanation.

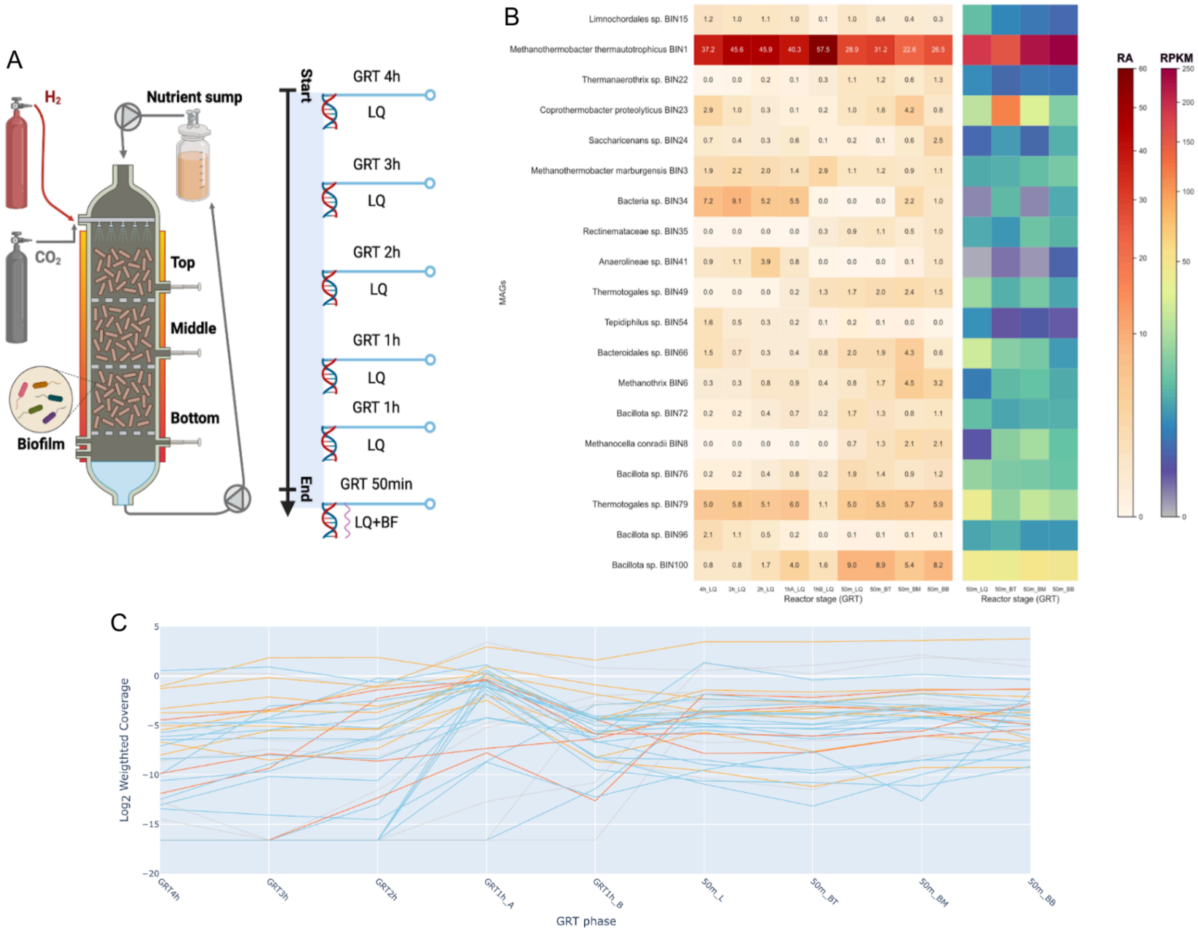

Figure 1. (A) Experimental design and sample collection. (B) Temporal dynamics of microbial abundance and total transcriptomic activity of the dominant archaeal and bacterial community. (C) Temporal dynamics of viral abundance represented as log_2-transformed genome-length-normalized read depth. Viral genomes encoding endolysin, holin genes, and proviruses are highlighted in blue, red, and orange, respectively.