Metagenomic analysis on hydrogen assisted carbon dioxide fixation for biomethane production

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In 2022, Europe experienced one of the most significant energy crises, with the price of fossil natural gas dramatically increasing. At this point, renewable gas is taking on more significance. Increasing interest in biogas and biomethane (the upgraded form of biogas) is not a coincidence, as both are recognized as efficient and flexible renewable gas sources and contribute to reducing carbon dioxide emissions. Biogas is a gas mixture composed of about 50-70% methane (CH_4) and 30-50% carbon dioxide (CO_2), with trace amounts of other gases (Angelidaki et al., 2018). The presence of all the other gasses decreases the calorific value of the biogas (Angelidaki et al., 2018). Biomethane, on the other hand, is almost pure methane, typically more than 95% CH_4, that has a much higher calorific value than biogas (Sun et al., 2015). Commercial upgrading technologies are available, and most of them are considered technologically mature. In the latest years, biological biogas upgrade has attracted the attention of many researchers worldwide since it is a sustainable and cost-effective alternative to conventional biogas upgrading methods (Khan et al., 2021). Trickle Bed Reactors (TBRs) belong to the most attractive reactor configurations for biomethanation. In TBRs, microorganisms are immobilized in packing materials and form biofilms, resulting in an interfacial area for the mass transfer of H_2 (Jensen et al., 2021).

The aim of this study was to investigate the effect of different packing materials on the biomethanation efficiency of TBR. For this reason, two distinct packing materials (one organic and one inorganic) were selected and the methanation performance was evaluated. Furthermore, metagenomic analysis was conducted to examine the impact of the packing material on the microbial communities established in both biofilm and liquid phases.

A setup of two trickle bed reactors (R1 and R2) with a working volume of 1L was settled. The reactors were filled with two different packing materials: the organic carbon pellets for R1 and the inorganic raschig rings for R2. Reactors were operated at 55±1^{o}C (thermophilic conditions). Prior to the inoculation, water was trickled into reactors to wet the packing materials, allowing a more efficient formation of biofilm. Afterwards, a liquid medium consisting of enriched hydrogenotrophic inoculum and degassed digestate (as nutrient broth) was constantly provided with a peristaltic pump from vessels to the top of the TBRs and trickled to the packing materials. Additionally, a mix of H_2 and CO_2, in a ratio of 4 to 1, was injected continuously at the top of the reactors using a peristaltic pump to achieve a concurrent parallel gas and trickling flow. The reactors were operated for four distinct periods in which gas retention time (GRT) was decreased: Period 1 (GRT=6h), Period 2 (GRT=4h), Period 3 (GRT=2.25h), and Period 4 (GRT=1.5h). In the latest period, genomic DNA was extracted from the formed biofilm in the upper and lower parts of the reactors, as well as from the liquid media. Additionally, a sample of the initial microbial community of the inoculum was also analyzed to determine the change in the microbial community over the course of the experiment. The process indicators methane concentration, pH, and volatile fatty acids (VFA) were monitored during the entire experimental period.

A gradual increase in gas inflow rate allowed the determination of the limits in which H_2 and CO_2 were entirely converted into CH_4. The strategy of progressively raising the flow rate is the most appropriate when the goal is to operate the reactor at the shortest GRT with the maximum conversion of the limiting substrate. The performance of the TBRs during the four operational periods is shown in Fig. 1, and their effectiveness was assessed in terms of output gas composition (%CH_4, %CO_2 and %H_2). Overall, the

obtained results showed a superiority of the carbon pellet as packing material against raschig rings. Both reactors reached a CH4 concentration higher of 90% within the first week, indicating a quick adaptation of the microbial community in both. While R1 showed a great performance reaching almost the maximum value of CH_4 productivity for Periods 1-3, R2 showed several points of instability during Period 3. For this reason, during this period, the gas inflow was stopped in R2, and a flooding strategy was implemented in an effort to improve the wetting of the trickling media with the nutrient solution and, subsequently, the biofilm formation. Flooding benefited the performance of R2 since, after that showed an increase in CH_4 conversion. At the beginning of Period 4, the concentration of CH_4 dropped dramatically in both reactors. In the following two days, R1 showed an enhancement in methane productivity. Until the end of the experiment, the CH_4 concentration in the output gas exceeded 90%, indicating that the drop in the reactor performance had been temporary. In contrast, TBR2 was negatively affected by the increase in the gas influent rate since the CH_4 content declined throughout Period 4.

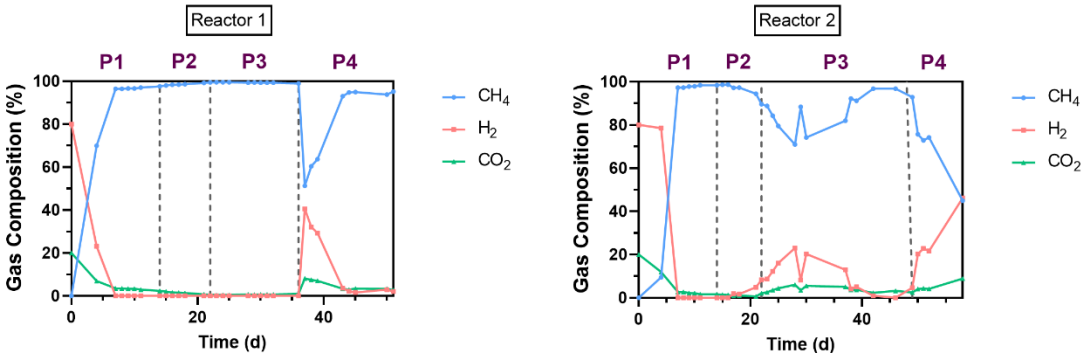

Figure 1. Output gas composition (%) of CH_4, CO_2, and H_2 of reactor R1 and R2 during the different experimental periods. Grey sashed lines indicate the end of each period.

Metagenomic analysis revealed 156 Metagenome Assembled Genomes (MAGs). In both reactors, Bacteria was the dominant Domain. However, it is worth mentioning, that in all samples the most abundant microorganism was the methanogen *Methanothermobacter thermautotrophicus*. According to ordination analysis, samples from the biofilm showed a greater separation compared to samples from the liquid phase, indicating that the biofilm had a higher level of diversity. Additionally, hierarchical cluster analysis (HCA) showed the biofilm in R1 exhibited a stronger separation between lower and upper part compared to R2. There was a higher methanogenic population in the biofilm of the upper part of the reactor compared to the lower part, indicating that the growth was more preferable near the point where influent gas was provided.

This study demonstrated the effect of different packing materials in biological methanation. The trickle bed reactor filled with carbon pellets as packing material was characterized by a more stable performance and achieved higher methane concentration in the output gas throughout all phases of operation. Furthermore, the microbial analysis revealed that biofilm formation can be significantly affected by the flux of influent gas from the top of the reactor to the bottom.

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