Impact of organic loading rate on hydrogen consumption rate during in-situ biomethanation

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Impact of organic loading rate on hydrogen consumption rate during *in-situ* biomethanation

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Abstract: *In-situ* biomethanation shows significant potential for enhancing biogas quality. The efficiency of this process relies on various factors, including organic loading rate and hydrogen injection frequency. Experiments were carried out to evaluate the impact of different organic loading rates on hydrogen consumption rate during *in-situ* biomethanation. Results indicate that higher organic loading rates resulted in increased hydrogen consumption rates, with the highest value (57 mg COD/L/h) being observed at the highest OLR tested (2.75 g VS/L/d). In addition, stable hydrogen kinetics were maintained after a 12-hour hydrogen starvation period. These results provide valuable insights that can contribute to the understanding and optimisation of *in-situ* biomethanation.

Keywords: Anaerobic digestion; *In-situ* biomethanation; power to gas; biogas upgrading

Introduction

In-situ biomethanation is a technique consisting of the direct injection of hydrogen into an anaerobic digester to increase the methane content in the biogas via hydrogenotrophic CO_2 consumption (Agneessens et al., 2018). *Ex-situ* biomethanation is an alternative technique in which hydrogen is injected into a separate reactor (Kozak et al., 2022).

Organic loading rate (OLR) is a crucial operational parameter affecting AD performance. High values are desired to increase treatment capacities, but excessive OLRs can potentially result in AD failure due to reactor acidification (Duan et al., 2019). Despite the interest of *in-situ* biomethanation, hydrogen injection in the digester can increase the risk of acidification by inhibiting the degradation of volatile fatty acids (VFAs) due to thermodynamic constraints (Jiang et al., 2021). Previously, over a small range of OLRs from 0.5 to 2.0 g VS/L/d, a faster hydrogen consumption rate was observed for higher OLRs, (Agneessens et al., 2018). Furthermore, biomethanation reactors, whether i*n-situ* or *ex-situ*, may be operated via intermittent H_2 injection, leading to fluctuating periods of starvation, which could potentially impact the efficiency of overall process (Braga Nan et al., 2022).

The objective of this study was to assess the impact of a wide range of OLRs on hydrogen consumption kinetics during food waste AD. The orientation of metabolic pathways during *in-situ* biomethanation was also assessed, as well as the impact of a 12-hour starvation period on the hydrogen consumption rates.

Material and Methods

A 10-liter semi-continuous AD reactor, fed with food waste (Capson-Tojo et al., 2017) and operated at mesophilic temperature (35°C) and a hydraulic retention time (HRT) of 21 days served as source of adapted inoculum. OLR was incrementally increased to 0.75, 1.25, 1.75, 2.25, and 2.75 g VS/L/d. At each OLR (after stabilisation), samples were collected for biomethanation tests in 1-liter Schott-flask reactors, with a working volume of 220 mL. These reactors were run in triplicate for four conditions: *in-situ* biomethanation, *ex-situ* biomethanation, AD, and endogenous control (no substrate added). Food waste was added to the *in-situ* biomethanation and AD reactor at an equivalent OLR to that in the semi-continuous reactor, and pure H_2 and H_2/CO_2 (80:20% v:v) were introduced into the reactors to reach pressures of 1.20-1.25 bar in the *in-situ* and *ex-situ* biomethanation reactors. After feeding, all the reactors were flushed with N_2 to establish anaerobic conditions. After equilibration at mesophilic temperatures, the reactors were placed on a shaking table, and gas composition in the headspace was analysed every hour. At the beginning and end of the cycle, liquid samples were collected for VFA measurement and for 16S rRNA sequencing and qPCR analysis. A second cycle was carried out after 12 hours following the same procedure.

Results and Conclusions

Increasing the OLR resulted in higher hydrogen consumption rates in most cases (Figure 1), with a statistically significant difference observed between OLR pairs of (0.75, 1.25) and (1.75, 2.25, and 2.75 g VS/L/d) based on ANOVA and Tuckey tests. This observation may be attributed to the increase in concentration of available microorganisms when the OLR is raised, as supported by Owamah and Izinyon (2015). This hypothesis will be validated by qPCR results. Furthermore, Agneessens et al. (2018) noted faster hydrogen consumption with an increase in OLR from 0.5 to 2.0 g VS/L/d. Comparing the results between the first and second pulses of hydrogen injection (Figure 1), no difference was observed in terms of hydrogen consumption rate. This implies that the hydrogen consumption rate was stable after a 12-hour period of hydrogen starvation. This result is in line with the findings of Braga Nan et al. (2022), where methanogens were able to tolerate a one-week starvation period. Moreover, it can be seen that there is no difference between *in-situ* and *ex-situ* biomethanation reactors with regards to hydrogen consumption rate (Figure 1), suggesting that there is no benefit to build an external reactor to perform ex-situ biomethanation. This might be due to similar microbial communities and operational conditions among both reactors (de Jonge et al., 2020; Figeac et al., 2020).

Figure 2 depicts the results of methane production for the 5 OLRs tested, and during the two H_2 injections. Interestingly, the increase in OLR resulted in higher amounts of methane produced in all reactors, without observing VFA accumulation in any condition. However, it is worth noting that at OLR levels of 0.75, 2.25, and 2.75 g VS/L/d, there was no significant differences between the methane produced in i*n-situ* biomethanation and AD, despite H_2 being consumed during biomethanation. This phenomenon could be attributed to the inhibition of substrate degradation resulting from H_2 injection, as confirmed by Yellezuome et al. (2023). Furthermore, at high OLRs, the methane production in endogenous control approached that of *ex-situ* biomethanation. Considering that H_2 was consumed during the *ex-situ* tests, H_2 injection resulted again in a slower degradation of the remaining substrate present in the used inoculum.

Microbial community analyses are underway, and will provide the information needed to assess the impact of OLR on archaea abundance (such as Methanomicrobiaceae, *Methanosaetaceae*, and *Methanobacteriaceae*) and community structure in relation to the metabolic pathways identified. The apparent lack of VFAs suggests that H_2 was primarily consumed through the hydrogenotrophic methanogenesis, no via homoacetogenesis. This will be validated by qPCR results.

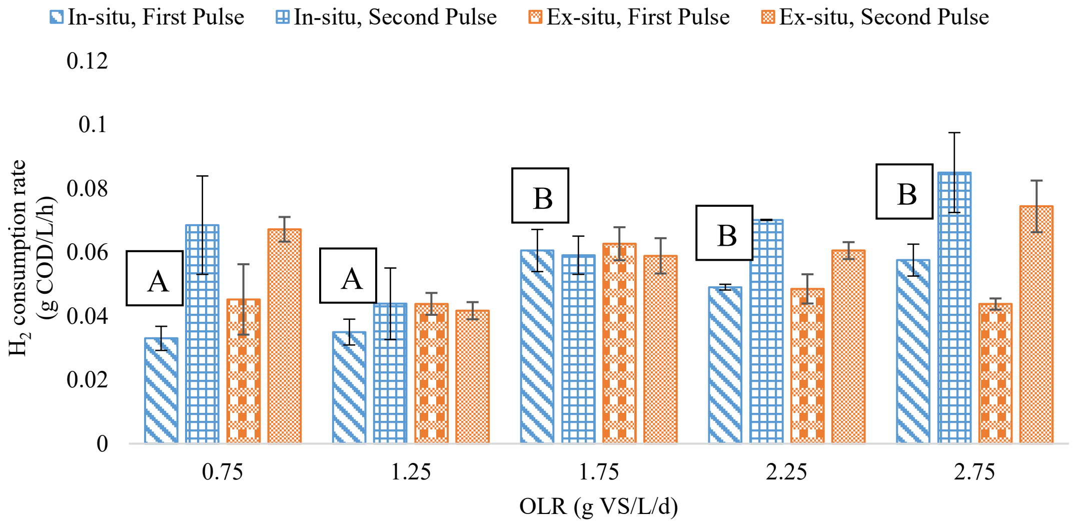

Figure 1. Hydrogen consumption rate during the first and second pulses of hydrogen injection for *in-situ* and *ex-situ* biomethanation at different OLRs (A and B refers to group with statistically similar hydrogen consumption rate)

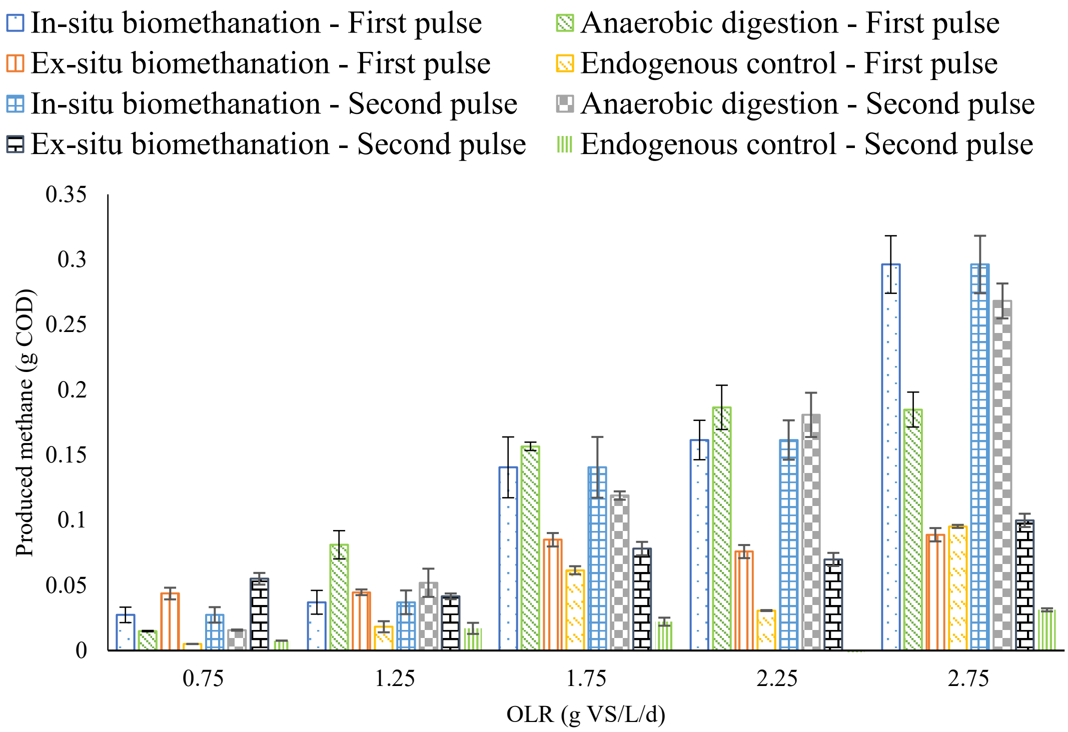

Figure 2. Produced methane during the first and second pulses of hydrogen injection for *in-situ* biomethanation, anaerobic digestion, *ex-situ* biomethanation and endogenous control at different OLRs.

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