Impact of Operational Conditions on Methane Yield and Microbial Community Composition during Biological Methanation in a Hybrid Reactor System

Radziah Wahid
Norwegian University of Life Sciences
Svein Jarle Horn (svein.horn@nmbu.no)
Norwegian University of Life Sciences https://orcid.org/0000-0002-1590-9001

Research Article

Keywords: Biological methanation, in-situ, hybrid, hydrogenotrophic methanogens, CH4 yield.

Posted Date: June 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-567358/v1

Version of Record: A version of this preprint was published at Biotechnology for Biofuels on August 21st, 2021. See the published version at https://doi.org/10.1186/s13068-021-02019-4.

Abstract

Background: Biogas can be upgraded to methane biologically by adding hydrogen to biogas reactors. The process is called biological methanation (BM) and can be done *in-situ* in a regular biogas reactor or the biogas can be transferred to a separate *ex-situ* upgrading reactor. The hybrid BM concept, a combination of *in-situ* and *ex-situ* BM, has received little attention, and only a few studies have been reported. The hybrid BM has the advantage of resolving the issue of pH increment during *in-situ* BM, while the size of the *ex-situ* BM reactor could be reduced.

Results: In this study, the efficiency of *in-situ* and *hybrid* biological methanation (BM) for upgrading raw biogas was investigated. The *hybrid* BM system achieved a CH_4 yield of 257 mL g_{VS}^{-1} when degrading a feedstock blend of manure and cheese waste. This represented an increase in methane yield of 76% when compared to the control reactor with no H_2 addition. A 2:1 H_2:CO_2 ratio resulted in stable reactor performance, while a 4:1 ratio resulted in a high accumulation of volatile fatty acids. H_2 consumption rate was improved when a low manure-cheese waste ratio (90%:10%) was applied. Furthermore, feeding less frequently (every 48 hours) resulted in a higher CH_4 production from CO_2 and H_2. *Methanothermobacter* was found to dominate the archaeal community in the *in-situ* BM reactor, and its relative abundance increased over the experimental time. *Methanosarcina* abundance was negatively affected by H_2 addition and was nearly non-existent at the end of the experiment.

Conclusions: Our results show that *hybrid* BM outperforms *in-situ* BM in terms of total CH_4 production and content of CH_4 in the biogas. The application of *hybrid* BM increased CH_4 yield up to 42%. Furthermore, addition of H_2 at 2:1 H_2:CO_2 ratio in *in-situ* BM resulted in stable reactor operation.

Background

Renewable electricity from photovoltaics and wind turbines could play a significant role in the future European electricity system [1]. However, wind and solar are intermittent energy sources, necessitating long-term and large-scale storage in order to store renewable electricity during excess and supply electricity during shortage [2]. One solution is to store electricity in batteries, but it has its own disadvantages, including high cost of manufacture, low storage capacity and use of rare minerals [3]. Another storage alternative is to use excess electricity from wind or solar energy to generate H_2 via water electrolysis [4]. However, the use of H_2 as a renewable energy carrier presents significant challenges that have not yet been addressed, linked to its low density requiring a high storage capacity infrastructure, while the direct use of H_2 as transport fuel is still under development [5, 6]. However, H_2 may be combined with CO_2 produced in existing biogas plants and converted to CH_4, for which large scale infrastructure and applications are in place [4]. This concept of converting electrical into chemical energy is known as Power-to-Methane (PtM) [7].

PtM can be achieved in two ways, either by thermochemical methanation (TM) or BM [1]. Both methods are based on the Sabatier reaction (Eq. 1), in which four moles of H_2 react with one mole of CO_2 to produce one mole of CH_4 and two moles of H_2O [8]:

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O\ \Delta G^{0} = -130\ kJ\ mol^{-1}$ (Eq. 1)

Metal catalysts such as Ni and Al_2O_3 are used in TM, which operates at high temperatures (between 200 and 500^{o}C) and pressures (up to 100 bar). The metal catalyst is sensitive to contaminants such as hydrogen sulphide (H_2S), so high purity of the reactant gases is required [2]. BM, on the other hand, uses a biological catalyst (methanogenic archaea) and operates at mild temperatures (35 to 65^{o}C) and pressures (< 15 bar). In addition, as opposed to TM, the process tolerates impurities such as H_2S [2]. At present, BM is gaining more attention as a result of its advantages, and a growing number of studies have been dedicated to it [9–11]. Previous research has reported three types of BM concepts: *in-situ* [11, 12], *ex-situ* [13, 14], and *hybrid* [15].

In-situ BM is attractive since biogas is upgraded directly in the biogas reactor without incurring additional costs for a secondary reactor. However, some technical challenges have been reported in previous studies [11, 12] such as increased pH (> 8.5) due to bicarbonate removal to CH_4 and high H_2 partial pressure (exogenous H_2), which inhibits the activity of specific bacteria and methanogens. Furthermore, the low H_2 gas-liquid mass transfer rate limits methanogen uptake of H_2 for CO_2 to CH_4 conversion, which is a key challenge for both *in-situ* and *ex-situ* BM [16]. *Ex-situ* BM involves the injection of CO_2 from biogas (or other sources) and H_2 into a separate reactor containing hydrogenotrophic methanogens (pure or enriched culture) for CH_4 conversion [6]. The *hybrid* BM concept (combination of *in-situ* and *ex-situ*), on the other hand, has received little attention, and only a few studies have been conducted. The *hybrid* BM has the advantage of addressing the issue of pH increment during *in-situ* BM, while smaller reactor size could be the option for *ex-situ* BM [6]. Corbellini et al., [15] used a two-stage thermophilic reactor to investigate the performance of *hybrid* BM and obtained final CH_4 concentrations of more than 95% in some experiments. The *hybrid* concept was also proposed by Voelklein et al., [17] for full scale application as an alternative to conventional upgrading systems.

The goal of this study was to assess the performance of a *hybrid* BM system in terms of substrate conversion efficiency and biogas quality using a 10L continuous-stirred tank reactor (CSTR) (*in-situ*) and a 2L reactor with packing materials (*ex-situ*). A similar CSTR reactor without H_2 addition was used as a control. Furthermore, the performance of *in-situ* and *hybrid* systems was compared in order to evaluate the capability of *hybrid* BM in resolving technical challenges associated with *in-situ*, such as pH increment and low H_2 gas-liquid mass-transfer rate. This work also investigated parameters (e.g. H_2:CO_2 ratio, stirring speed, and feeding frequency) that affect the efficiency of *in-situ* BM and the composition and dynamics of the microbial populations. Parameters such as pH, total ammonium nitrogen (TAN), volatile fatty acids (VFA), and methane yield and content were closely monitored during the experiment.

Results And Discussion

Process performance and biogas upgrading of in-situ BM

The characteristics of the inoculum and the applied substrates are given in Table 1. Operating parameters and performance data for the 10 L control and upgrading reactors (CR, UR) under steady-state conditions are summarized in Table 2 and 3, respectively. The experiment was conducted for 172 days and divided into six phases. Figures 2 and 3 illustrate the changes in methane yield, pH, and VFAs over the experimental period for upgrading and control reactors.

Phase I: Initial phase – without H_2 addition

In this phase, the two reactors were operated identically and showed very similar performance in terms of biogas production (241-245 mL g^{-1}_{VS}) and CH_4 yield (144-145 mL g^{-1}_{VS}) (Table 3). The average CH_4 content of the reactors (58 to 59%) and the pH (7.9) were also similar. The total VFA content was around 18 mM, with acetic acid (AA) accounting for more than 60% of the total VFAs. The ratio of propionic acid (PA) to AA of both reactors was below 1.4, indicating a stable AD process according to [18]. The TAN concentration was around 2.5 g L^{-1}. The values align well with those obtained by [19], who observed that a TAN value of 2.5g L^{-1} (pH 7.9) resulted in stable biogas production during thermophilic (55°C) anaerobic digestion of cow manure.

Phase II: Initial H_2 phase

H_2 was added in UR from day 64 at a flow rate of 3 mL min^{-1}, corresponding to a H_2:CO_2 ratio of 2:1. As shown in Figure 2, CH_4 yield increased immediately after H_2 addition and stabilized from day 70. The average CH_4 yield of UR was 185 mL g^{-1}_{VS}, which was approximately 27% higher than the average CH_4 yield of CR (Table 3). A similar observation was reported by Treu et al., [20] where H_2 addition into a CSTR at a 2:1 ratio resulted 13% increase in CH_4 yield. The pH of UR increased from 7.94 to 8.10, while the pH of CR remained the same as in phase 1. BM resulted in a rise in pH due to the removal of CO_2 from the liquid phase. Bicarbonate ions (HCO_3^-) are produced during the AD process when CO_2 reacts with OH in the liquid phase, contributing to the buffering capacity of the reactor. Addition of H_2 to the system resulted in CO_2 consumption and thus loss of buffering capacity [14]. Similar findings have been reported in previous studies [11,20,21]. Total VFA levels in UR rose to more than double the amount in phase I. In contrast to our study, Treu et al., [20] reported relatively low and stable VFA levels after H_2 addition.

In CR, the average AA concentration was 21 mM, while in UR, it was 36 mM. PA levels were slightly higher in both reactors than in phase 1. TAN concentrations were also elevated, with 2.57 g L^{-1} for CR and 2.77 g L^{-1} for UR. The H_2 consumption rate of UR was calculated to be 25%, corresponding to a CH_4 production rate of 0.04 mL L^{-1} d^{-1}.

Phase III: Increased stirring speed

In phase III, the stirring speed of both reactors was increased from 80 to 140 rpm (day 79) in an attempt to improve the transfer of H_2 to the liquid phase in UR. As shown in Figure 2, the CH_4 yield from UR decreased significantly as the stirring speed increased. The CH_4 yield of UR was reduced from 185 (day 78) to 126 mL g^{-1}_{VS} (day 85) for UR. The decrease in CH_4 yield of UR was corroborated by the accumulation of acetate (67 mM on average), which was nearly double of what was measured in phase II (Figure 3b). Besides, the propionate concentration was slightly increased from 9 to 13 mM. These observations could indicate that parts of the microbial community were negatively affected by the higher share forces at 140 rpm.

Regardless of the fact that the total CH_4 yield decreased as the stirring speed increased, the H_2 consumption rate in UR increased from 25% to 46%. This observation was in agreement with our previous study [22]. The rate of CH_4 production from H_2 and CO_2 conversion was increased from 0.04 to 0.08 mL L^{-1} d^{-1}. For the CR, the CH_4 yield was reduced from 143 to 131 mL g^{-1}_{VS}. Ghanimeh et al., [23] observed a decrease in CH_4 yield when stirring speed was increased from 80 to 120 rpm. No AA accumulation was observed in the CR, whereas the PA level was slightly higher than in phase II (12 mM) (Figure 3a and Table 3). The pH in both reactors was higher than in phase II, with pH of 8.15 and 8.28 for CR and UR, respectively. The elevated pH in UR can be attributed to greater CO_2 consumption in the liquid as a result of the increased H_2 gas-liquid mass transfer rate at higher stirring speeds and thus higher BM activity [1].

Phase IV: Change of feedstock blend ratio

On day 86, the stirring speed was again reduced to 80 rpm (return to Phase II conditions), and the CH_4 yield rose significantly until it reached a plateau from day 90 (Figure 2). From day 92 the CW fraction was increased from 10% to 20% on day 93 (Phase IV), resulting in an OLR of 0.78 g_{VS} L^{-1} d^{-1}. The CH_4 yield increased in both reactors, with maximum values being 195 mL g^{-1}_{VS} (CR) and 276 mL g^{-1}_{VS} (UR) (Figure 2). After day 102, however, the CH_4 yield gradually decreased until it reached a stable period around day 111. During the stable period, the average CH_4 yields of CR and UR were 142 mL g^{-1}_{VS} and 204 mL g^{-1}_{VS}, respectively (Table 3). The average CH_4 yield of CR measured in this study was lower than that measured by Comino et al., [24] (similar feedstock blend, 80% CM:20% whey), despite the fact that both studies had comparable CH_4 content (53%). Longer HRT (41 days) and higher OLR (3.33 g_{VS} $L^{-1}d^{-1}$) were used by Comino et al., which may explain the difference in performance. The average CH_4 content of UR was 39%. The H_2 consumption rate was around 17%, which was 31% lower than the consumption rate when CW fraction was set at 10%. The total VFA content of CR was slightly higher towards the end of phase IV (Figure 3a), while the total VFA content of UR was relatively stable (Figure 3b). The pH of both reactors was lower than in phase III, with an average pH of 7.91 for CR and 8.11 for UR. Increased CW ratio to 20% resulted in higher TAN values (both reactors) compared to phase II, suggesting more thorough CW degradation as TAN is a product of protein degradation.

Phase V: Feeding frequency

In phase V, the CW fraction was reduced to 10% and the feeding frequency was changed to once every 48 hours (instead of once per 24 hours). In term of CH_4 yield for CR, no changes were observed, while CH_4 yield for UR was gradually reduced until a stable period was achieved (day 134). The average CH_4 yield for

CR was 139 mL g^{-1}_{VS} and 194 mL g^{-1}_{VS} for UR. The CH_4 yield of UR in phase IV was slightly higher than in phase II (feeding every 24 hours). The H_2 consumption rate was higher than phase II (24 h feeding) when the reactor was fed every 48 hours (25% vs 32%). The increased CH_4 yield and H_2 consumption rate in UR could be attributed to enrichment of hydrogenotrophic methanogens in less frequent feeding. According to Piao et al., [25], reducing feeding frequency tended to increase the abundance of H_2-utilizing methanogens. In the Piao study, the abundance of hydrogenotrophic methanogens increased from 45% to 53% when feeding frequency was reduced from every 24 hours to every 48 hours. The average total VFA content for CR and UR were 26 and 50mM, respectively. The pH of both reactors was slightly lower than in phase II.

Phase VI: Increased H_2:CO_2 ratio

Substrate feeding was changed to once daily starting on day 141, and the H_2 flow rate was increased to 6 mL min^{-1}, equivalent to a 4:1 H_2:CO_2 ratio (Phase VI). The increased H_2:CO_2 ratio initially boosted CH_4 yield in UR with a maximum at day 151. However, the yield fell after day 163. The average CH_4 yield in this period was 165 mL g^{-1}_{VS}, about 11% lower than the value in phase II (H_2:CO_2 ratio = 2:1). Despite the lower CH_4 yield, the H_2 consumption rate was doubled (54%) compared to phase II (25%) due to the increased H_2:CO_2 ratio, which probably stimulated H_2-consuming anaerobic microbes.

AA accumulated toward the end of the phase, reaching a maximum concentration of 84.5 mM. The increase in AA levels may be explained by the inhibition of acetoclastic methanogens (e.g. *Methanosarcina*) caused by high H_2 partial pressure [26] or by the enrichment of particular microbial pathways such as homoacetogenesis (Wood-Ljungdahl pathway) [6]. PA content was also increased from 15 to 18 mM when the H_2:CO_2 ratio was increased. The rise in total VFA content coincided with a drop in pH from 8.01 to 7.91. For CR, the CH_4 yield remained consistent throughout phase VI, with an average of 134 mL g^{-1}_{VS}. The average total VFA concentration was 21 mM, with a pH of 7.82. AA concentration accounted for 58% of the total VFA content. The TAN concentration was 2.65 g L-1, which was similar to the value observed in phase II (2.57 g L-1).

In-situ vs. hybrid configurations

A *hybrid* configuration was tested at the end of the experiment (after day 172). An additional 2 L reactor filled with packing materials was used as an *ex-situ* biogas upgrading reactor (HR) for the biogas from UR (Figure 1b). Initially, the operating parameters of UR were adjusted to the same as in phase II with a H_2:CO_2 ratio of 2:1.

When the *hybrid* setup was used instead of an *in-situ* (phase II), 39% extra CH_4 was obtained (Figure 4). The average CH_4 yield rose from 185 to 257 mL g^{-1}_{VS}. Furthermore, the H_2 consumption rate increased by twofold compared to *in-situ* (phase II), and the average CH_4 content increased from 40% to 63% (Tables 3 & 4). The CH_4 content without considering H_2 from *hybrid* system was around 80%. When compared to the control reactor (Figure 4), the hybrid configuration resulted in a 76% higher CH_4 yield, while *in-situ* configuration resulted in 27% more CH_4 (Figure 4). HR had an average pH of 8.07 and an AA concentration of approximately 4.12 mM. The TAN concentration of HR was around 1.09 g L-1.

The H_2:CO_2 ratio was increased to 4:1 after a stable condition was observed. The average CH_4 yield fell from 257 mL g^{-1}_{VS} to 234 mL g^{-1}_{VS} (approximately 9% less CH_4). The average CH_4 content was reduced from 63 to 51%. Nonetheless, the H_2 consumption rate (62%) was slightly higher than at the 2:1 H_2:CO_2 ratio (60%), indicating that acetate-oxidizing bacteria had the capacity to consume more H_2 to produce acetate, as observed in phase VI. Compared to *in-situ* configuration (phase VI), about 42% extra CH_4 was measured and approximately 75% more CH_4 was produced when compared to control (Figure 4). The concentrations of AA and TAN were equivalent to those found at a 2:1 H_2:CO_2 ratio.

Compared to Corbellini et al., [15] our study resulted in lower upgraded CH_4 content of *in-situ* BM. This may be attributed to differences in reactor working volume, as a larger working volume (6L) was used in the present study compared to 3L in [15]. Our findings were more comparable to those of [17], who used a 9L working volume for *in-situ* testing. Furthermore, when a 4:1 H_2:CO_2 ratio was added to UR in our study, AA accumulation (> 4 g L^{-1}) was observed, leading to a decrease in pH, while VFA level observed in [15] was maintained at 2 g L^{-1}.

To prevent process instability in *in-situ* BM reactor, we propose that the amount of H_2 added to the *in-situ* reactor should be kept at a relatively low H_2:CO_2 ratio (e.g. 2:1). This will minimize the increase in pH caused by bicarbonate removal as well as the possible inhibition of some anaerobic bacteria that are sensitive to high H_2 partial pressure. Our study discovered residual H_2 in the in-situ and hybrid BM reactors, indicating that further optimization is required. A pressurized reactor may be a solution. Increased operating pressure enhances the solubility of gases and decreases bubble size. Smaller bubble size is beneficial since it maximizes the contact area between bacteria and gaseous substrates while slowing gas upflow through the reactor [1,27]. Previous research found that increasing reactor pressure during *in-situ* and *ex-situ* BM resulted in improved conversion efficiency [28,29]. A very high CH_4 concentration (> 98%) in the biogas was reported when reactor pressure was set between 5 and 15 bars for a 5 m^3 *ex-situ* CSTR [30]. Additionally, the design of the *ex-situ* reactor used in our study can be improved, for example, by using a long column design like trickle-bed reactor.

Microbial community composition

Microbial analysis of the reactor feed (80% CM:20% CW) showed that *Firmicutes* and *Proteobacteria* were the two dominant bacterial phyla, accounting for approximately 50 and 18 % of the abundance, respectively (Figure 5a). Other phyla present in the feed included *Actinobacteria* (9%) and *Bacteriodetes* (8 %). Analysis of the inoculum microbiology showed that *Firmicutes* was the dominating phylum (71%), followed by *Synergistetes* (7%), *Actinobacteria*, and *Euryarchaeota* (both phyla accounted 3% abundance) (Figure 5b). *Atribacteria* and *Thermotogae* were also detected in the inoculum, but they were not found in the feed sample.

The taxonomic classification of the microbial community revealed that *Firmicutes* were the most abundant phyla n the reactors, accounting for 57 to 72% of relative abundance depending on the time points (Figure 5c). This is in agreement with the findings of [31] where *Firmicutes* dominated a thermophilic biogas reactor digesting cow manure. *Firmicutes* engages in a variety of metabolic processes for carbohydrate and fatty acid degradation, including the Wood–Ljungdahl pathway (homoacetogenesis) and syntrophic acetate oxidation, which explains their abundance in the reactors [11]. *Clostridia*, which belong to the *Firmicutes*, was the most abundant class (representing more than 33% of all bacterial sequences). Other bacterial phyla, such as *Synergistetes* and *Bacteriodetes,* were present in both reactors at first, but their numbers declined over time. In terms of methanogenic population, the abundance of *Euryarchaeota* varied over time, between 13 – 33% for CR, and 18 – 38% for UR (Figure 5c).

Some bacteria, such as *HAW-R60*, an *Atribacteria* phyla, was clearly negatively affected by H_2 addition (Figure 6a). Their abundance declined over time and was nearly non-existent in phase VI.*Atribacteria* have been found previously in thermophilic biogas reactors and are involved in hydrolysis of polysaccharides [32]. Another hydrolytic bacterium, *Halocella*, behaved differently, reaching highest abundance when the H_2:CO_2 ratio was increased to 4:1 (phase VI) (Figure 6b). Their abundance in UR increased from 6.7 (without H_2 addition) to 14.6%. The increase in stirring speed in phase II (day 79-85) seemed to negatively affect *Halocella*, with decreased abundance in both CR and UR. The cellulolytic bacteria *Halocella* belong to the class *Clostridia* and is responsible for cellulose degradation and produces ethanol and H_2 from lignocellulosic substrates [33]. *Halocella* have mainly been found in manure-based samples and their presence in thermophilic biogas reactor has been reported previously [34].

Within the domain archaea, *Methanosarcina* was the only detected methanogen capable of acetoclastic methanogenesis, although it can also carry out hydrogenotrophic methanogenesis [35]. *Methanosarcina* was clearly negatively affected by H_2 addition and disappeared from UR after 108 days (Figure 6c). High H_2 partial pressure has previously been shown to be detrimental to *Methanosarcina* [36]. Furthermore, the observed accumulation of AA in UR (Figure 3b) is in agreement with inhibition of *Methanosarcina*.

In contrast to *Methanosarcina*, the hydrogenotrophic methanogen *Methanothermobacter* increased in abundance over time and responded positively to H_2 addition. *Methanothermobacter* are typical hydrogenotrophic methanogens that are commonly found in thermophilic biogas reactors [37]. As shown in Figure 6d, their abundance in UR got higher than the abundance in CR over time, suggesting that they were enriched as a result of H_2 addition. The high abundance of *Methanothermobacter* found in this study is consistent with previous research that found this genus to be dominant in thermophilic biogas upgrading systems [6,14,38]. According to [39], *Methanothermobacter* expand rapidly when H_2 is abundant and are adaptable to different concentrations of dissolved H_2.

Syntrophaceticus abundance *increased rapidly* in UR when H_2-supplementation was initiated but was greatly reduced after day 140 when the 48h feeding regime was introduced (Figure 6e). *Syntrophaceticus* is a well-known syntrophic acetate-oxidizing (SAO) bacterium that was discovered in a biogas reactor that relied on the energy from acetate oxidation to produce H_2 and CO_2 [15,34]. SAO bacteria, which are syntrophic with hydrogenotrophic methanogens (*Methanothermobacter* in our case), can be inhibited by short or long-term H_2 addition to their living atmosphere [20,35]. Increased H_2 partial pressure can inhibit SAO from a thermodynamic perspective because syntrophic sustainability is dependent on the H_2/formate concentration, which is usually kept low by the methanogenic partners [40]. Interestingly, our study revealed that H_2 addition at an H_2:CO_2 ratio of 2:1 promotes the growth of *Syntrophaceticus* while increasing the H_2:CO_2 ratio to 4:1 significantly reduces their abundance. In addition, the abundance of *Syntrophaceticus* of was maximum when the CW ratio was increased from 10 to 20%.

Similar to *Halocella*, *f_Hydrogenisporaceae_OTU_28*, was also affected by the increased stirring speed, seen as reduced abundance after 64 h in both reactors (Figure 6f). *f_Hydrogenisporaceae_OTU_28,* a member of the *OPB54* class, have previously been reported to be involved in the fermentation of carbohydrates to produce acetate and H_2 [41].

Our findings revealed that the H_2:CO_2 ratio, stirring speed, CM:CW ratio, and feeding frequency all had an effect on *in-situ* BM, either on overall CH_4 production or on CH_4 production from H_2 and CO_2 conversion. However, it was only the H_2:CO_2 ratio and stirring speed that strongly affected the microbial community profile of the reactors.

Conclusions

The current work demonstrates the feasibility of the *hybrid* biogas upgrading concept and identifies some challenges that must be tackled for future process improvement. When *hybrid* BM was used instead of *in-situ* BM, it resulted in a 39% increase in CH_4 yield. Furthermore, maximum H_2 utilization (62%) was observed during hybrid BM. The co-digestion of CM and AC aided in keeping the pH of the reactor below 8.1 during *in-situ* BM. The addition of H_2 at a H_2:CO_2 ratio of 2:1 resulted in stable operation of the *in-situ* reactor system, while at higher ratio VFAs started to accumulate resulting in pH drop. The microbial analysis revealed that *Methanothermobacter*, a hydrogenotrophic methanogen, dominates both the control and the H_2 reactors, with a higher abundance in the H_2 reactor. The main factors affecting the microbial community composition were H_2 addition and stirrer speed. The findings of our study may be useful to other researchers or biogas plant operators in developing processes for enhancing BM performance and methane yields.

Materials And Methods

Inoculum and substrate

Thermophilic inoculum was obtained from two 10L CSTRs digesting cow manure (CM) collected from a cow farm in Ås, Norway. Both reactors were operated at 55^{o}C and 20 days of hydraulic retention time. The same CM was also used as a model substrate for the present study. To limit pH increment during in-situ BM, the CM was co-digested with acidic cheese obtained from the Food pilot plant at Norwegian University of Life Sciences (NMBU). The cheese was

produced only for experimental purposes [42] and discarded once the experiment was completed. The cheese waste (CW) was collected and was stored at 4^{o}C until further usage. Table 1 lists the characteristics of the inoculum and substrates used in this study.

In-situ BM setup

The setup comprised of two 10L CSTRs (Control reactor, CR, and *in-situ* upgrading reactor, UR), each with 6L working volume. The temperature of both reactors was maintained at thermophilic condition (55^{o}C). Three-blade Elephant Ear impeller operated in the down-pumping mode was used for mixing at 80 rpm. Approximately 300 g of substrate (90% CM: 10% CW) were fed into the reactors every 24 hours after the same amount of effluent had been discharged. Initially, the organic loading rate was kept at 0.83 g_{VS} L^{-1} d^{-1}. Starting day 64, H_2 was injected into UR using a stainless-steel Mott sparger with a pore size of 2 µm, which was mounted at the bottom of the reactor. The sparger measured 12 cm in length and had a 12 mm outer diameter. The flow rate of H_2 was initially set to 3 mL min^{-1} (H_2:CO_2 ratio = 2:1). To increase the contact time between anaerobic microbes and H_2, gas recirculation was introduced from day 64. A peristaltic pump was used to recirculate the output gas at gas recirculation rates of 7.63 mL min^{-1}.

Experimental parameters

Stirring speed (80 vs 120 rpm), CM:CW ratio (90%:10% vs. 80%:20%), feeding frequency (24h vs. 48h), and H_2:CO_2 ratio (2:1 vs. 4:1) were varied from day 79 to 172 to examine how these factors influenced the process performance of the two reactors. The experiment was divided into 6 different phases (I – VI) and Table 2 provides an overview of the corresponding parameter-settings.

Hybrid BM setup.

A *hybrid* BM set-up where the *in-situ* reactor (UR) was combined with *ex-situ* reactor (HR) was tested at the end of the experiment (day 173 to 203). The CR was not included in this experiment. The *ex-situ* upgrading reactor was established using a 2 L bottle filled with 800 mL filtered and degassed inoculum (digestate from UR) and 108 g polyethylene packing materials (Hel-X biocarriers, HXF13KLL+, Christian Stöhr GmbH & Co). Once a week, 50 mL of the filtered and pasteurized CM was added to HR (nutrient supply) after the same amount of effluent had been discharged. A peristaltic pump was used to transfer the outlet biogas from the UR to the 2L bottle and inject it at the bottom via a diffuser. Figure 1a & 1b depicts the *in-situ* and *hybrid* configurations.

Sample analysis

Gas chromatography (GC) (SRI 8160C) with a Flame Ionization Detector and N_2 as the carrier gas was used to measure the gas composition (CH_4, CO_2, and H_2). A standard biogas mixture (64% CH_4 and 36% CO_2) and a 10% H_2 gas mixture (with 90% N_2) (AGA Norway) were used for GC calibration on a regular basis. A digital pH meter (Thermo Scientific Orion Dual Star, USA) was used to measure pH of the digestate. pH measurement was performed immediately after the digestate was discharged from the reactors to avoid CO_2 removal from liquid phase.

Digestates from the reactors were collected regularly for total solid (TS), volatile solid (VS), TAN and VFA analysis. TS, VS and TAN were measured according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). VFA samples were prepared following [22]. VFA concentration was determined using a high performance liquid chromatography (Dionex, Sunnyvale, CA, USA) with Aminex column as described previously [22].

Microbial analysis

DNA sampling and extraction

The liquid effluent from each reactor was collected regularly and stored at -80^{o}C until DNA analysis. DNA extraction and sequencing were performed by DNASense (Aalborg, Denmark). The template DNA was extracted using the FastDNA Spin kit for Soil (MP Biomedicals, USA). The DNA extraction was performed following the manufacturer protocol except that samples were subjected to bead beating at 6 m/s for 4x40s [43]. DNA quantity and quality were assessed using gel electrophoresis with Tapestation 2200 and Genomic DNA screentapes (Agilent, USA). The Qubit dsDNA HS/BR Assay kit was used to determine the concentration of DNA (Thermo Fisher Scientific, USA).

Sequencing analysis

Microbial community profiles were determined using 16S rRNA gene variable region V4 with primers [515FB] GTGYCAGCMGCCGCGGTAA and [806RB] GGACTACNVGGGTWTCTAAT [44]. The 25 µL PCR reactions contained (12.5µL) PCRBIO Ultra mix, 400 nM primers and up to 10 ng of extracted DNA. The PCR thermal cycling consisted of a hot start step at 95^{o}C for 2 min, followed by 30 cycles of 95^{o}C for 15 s, 55^{o}C for 15 s, 72^{o}C for 50 s, and then a final 72^{o}C extension step for 5 min. For each sample, duplicate PCR reactions were performed, and the duplicates were pooled following PCR. The obtained amplicon libraries were purified using the standard protocol for CleanPCR SPRI beads (CleanNA, NL) with a bead to sample ratio of 4:5. The DNA concentration was quantified using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA) and the quality was confirmed by gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA). The purified libraries were pooled in equimolar concentrations and spiked with > 10 % PhiX control. The denatured library was sequenced on a MiSeq (Illumina, USA) using the Miseq Reagent kit V3.

Bioinformatics

The sequenced amplicon libraries were trimmed for quality using trimmomatic v. 0.32 and merged [45,46]. The reads were dereplicated and formatted for in the UPARSE workflow [47].Taxonomy was assigned using the RDP classifier as implemented in the script in QIIME and the SILVA database [48–50]. Bioinformatic processing was conducted by RStudio IDE (1.2.1335) (version 4.0.2) [43,51,52].

Abbreviations

AA, acetic acid; BM, biological methanation; CM, cow manure; CR, control reactor; CSTR, continuous stirred tank reactor; CW, cheese waste; GC, gas chromatography; HR, *ex-situ* upgrading reactor; PA, propionic acid; PtM, Power-to-Methane; TAN, total ammonium nitrogen; TM, thermochemical methanation; TS, total solid; UR, in-situ upgrading reactor; VFA, volatile fatty acids; VS, volatile solid.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The nucleotide sequence dataset used this study is available in the European Nucleotide Archive (ENA).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Research Council of Norway through grants 270038 (NorBioLab) and 257622 (Bio4Fuels).

Authors' contributions

RW and SJH conceived the idea for the study. RW set up and operated the reactors, as well as collected samples and process data. RW was responsible for sample analyses and interpretation of the experimental data. RW wrote the first draft of the manuscript, and SJH reviewed and edited subsequent drafts. Both authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank Michal Sposob and Hege Bergheim for their assistance during the experiments. We thank Sara M. Gaber for providing cheese waste that used as substrate in this study. We also thank Live Heldal Hagen for her assistance in uploading sequences in ENA.

References

1. Lecker B, Illi L, Lemmer A, Oechsner H. Biological hydrogen methanation–a review. Bioresour Technol. Elsevier; 2017;245:1220–8.
2. Thema M, Bauer F, Sterner M. Power-to-Gas: Electrolysis and methanation status review. Renew Sustain Energy Rev. Elsevier; 2019;112:775–87.
3. Angelidaki I, Treu L, Tsapekos P, Luo G, Campanaro S, Wenzel H, et al. Biogas upgrading and utilization: Current status and perspectives. Biotechnol Adv [Internet]. Elsevier; 2018;36:452–66. Available from: https://doi.org/10.1016/j.biotechadv.2018.01.011
4. Hidalgo D, Martín-Marroquín JM. Power-to-methane, coupling CO2 capture with fuel production: An overview. Renew Sustain Energy Rev. Elsevier; 2020;132:110057.
5. Díaz I, Pérez C, Alfaro N, Fdz-Polanco F. A feasibility study on the bioconversion of CO2 and H2 to biomethane by gas sparging through polymeric membranes. Bioresour Technol. Elsevier; 2015;185:246–53.
6. Kougias PG, Treu L, Benavente DP, Boe K, Campanaro S, Angelidaki I. Ex-situ biogas upgrading and enhancement in different reactor systems. Bioresour Technol. Elsevier; 2017;225:429–37.
7. Ghaib K, Ben-Fares F-Z. Power-to-Methane: A state-of-the-art review. Renew Sustain Energy Rev. Elsevier; 2018;81:433–46.
8. Vogt C, Monai M, Kramer GJ, Weckhuysen BM. The renaissance of the Sabatier reaction and its applications on Earth and in space. Nat Catal. Nature Publishing Group; 2019;2:188–97.
9. Alfaro N, Fdz-Polanco M, Fdz-Polanco F, Díaz I. H2 addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of sewage sludge. Bioresour Technol. Elsevier; 2019;280:1–8.
10. Bassani I, Kougias PG, Treu L, Porté H, Campanaro S, Angelidaki I. Optimization of hydrogen dispersion in thermophilic up-flow reactors for ex situ biogas upgrading. Bioresour Technol. Elsevier; 2017;234:310–9.
11. Wahid R, Mulat DG, Gaby JC, Horn SJ. Biotechnology for Biofuels Effects of H_2: CO_2 ratio and H_2 supply fluctuation on methane content and microbial community composition during in - situ biological biogas upgrading. Biotechnol Biofuels. BioMed Central; 2019;12:104:1–15.
12. Agneessens LM, Ottosen LDM, Voigt NV, Nielsen JL, de Jonge N, Fischer CH, et al. In-situ biogas upgrading with pulse H_2 additions: the relevance of methanogen adaption and inorganic carbon level. Bioresour Technol. Elsevier; 2017;233:256–63.

13. Rachbauer L, Voitl G, Bochmann G, Fuchs W. Biological biogas upgrading capacity of a hydrogenotrophic community in a trickle-bed reactor. Appl Energy. Elsevier; 2016;180:483–90.
14. Porté H, Kougias PG, Alfaro N, Treu L, Campanaro S, Angelidaki I. Process performance and microbial community structure in thermophilic trickling biofilter reactors for biogas upgrading. Sci Total Environ. Elsevier B.V.; 2019;655:529–38.
15. Corbellini V, Kougias PG, Treu L, Bassani I, Malpei F, Angelidaki I. Hybrid biogas upgrading in a two-stage thermophilic reactor. Energy Convers Manag. Elsevier; 2018;168:1–10.
16. Rafrafi Y, Laguillaumie L, Dumas C. Biological Methanation of H 2 and CO 2 with Mixed Cultures: Current Advances, Hurdles and Challenges. Waste and Biomass Valorization. Springer; 2020;1–24.
17. Voelklein MA, Rusmanis D, Murphy JD. Biological methanation: Strategies for in-situ and ex-situ upgrading in anaerobic digestion. Appl Energy. Elsevier; 2019;235:1061–71.
18. Hill DT. A Comprehensive Dynamic Model for Animal Waste Methanogenesis. 1982;1374–80.
19. Angelidaki I, Ahring BK. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. Appl Microbiol Biotechnol. Springer; 1993;38:560–4.
20. Treu L, Tsapekos P, Peprah M, Campanaro S, Giacomini A, Corich V, et al. Microbial profiling during anaerobic digestion of cheese whey in reactors operated at different conditions. Bioresour Technol. Elsevier; 2019;275:375–85.
21. Luo G, Angelidaki I. Co-digestion of manure and whey for in situ biogas upgrading by the addition of H2: process performance and microbial insights. Appl Microbiol Biotechnol. 2013;97:1373–81.
22. Wahid R, Horn SJ. The effect of mixing rate and gas recirculation on biological CO2 methanation in two-stage CSTR systems. Biomass and Bioenergy. Elsevier; 2021;144:105918.
23. Ghanimeh SA, Al-Sanioura DN, Saikaly PE, El-Fadel M. Correlation between system performance and bacterial composition under varied mixing intensity in thermophilic anaerobic digestion of food waste. J Environ Manage. Elsevier; 2018;206:472–81.
24. Comino E, Riggio VA, Rosso M. Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. Bioresour Technol. Elsevier; 2012;114:46–53.
25. Piao ZH, Lee J, Kim JY. Effect of substrate feeding frequencies on the methane production and microbial communities of laboratory-scale anaerobic digestion reactors. J Mater Cycles Waste Manag. Springer; 2018;20:147–54.
26. Bassani I, Kougias PG, Treu L, Angelidaki I. Biogas upgrading via hydrogenotrophic methanogenesis in two-stage continuous stirred tank reactors at mesophilic and thermophilic conditions. Environ Sci Technol. ACS Publications; 2015;49:12585–93.
27. Sarker S, Lamb JJ, Hjelme DR, Lien KM. Overview of recent progress towards in-situ biogas upgradation techniques. Fuel. Elsevier; 2018;226:686–97.
28. Martin MR, Fornero JJ, Stark R, Mets L, Angenent LT. A single-culture bioprocess of Methanothermobacter thermautotrophicus to upgrade digester biogas by CO2-to-CH4 conversion with H2. Archaea. Hindawi; 2013;2013.
29. Burkhardt M, Jordan I, Heinrich S, Behrens J, Ziesche A, Busch G. Long term and demand-oriented biocatalytic synthesis of highly concentrated methane in a trickle bed reactor. Appl Energy. Elsevier; 2019;240:818–26.
30. IEA - International Energy Agency. Biological methanation demostration plant in Allendorf, Germany - An upgrading facility for biogass. IEA Bioenergy Task 37 [Internet]. 2018; Available from: https://www.ieabioenergy.com/wp-content/uploads/2018/11/Germany-P2G_Case-Story_LAY2.pdf
31. Moset V, Poulsen M, Wahid R, Højberg O, Møller HB. Mesophilic versus thermophilic anaerobic digestion of cattle manure: Methane productivity and microbial ecology. Microb Biotechnol. 2015;8:787–800.
32. Hagen LH, Frank JA, Zamanzadeh M, Eijsink VGH, Pope PB, Horn SJ, et al. Quantitative metaproteomics highlight the metabolic contributions of uncultured phylotypes in a thermophilic anaerobic digester. Appl Environ Microbiol. Am Soc Microbiol; 2017;83.
33. Hassa J, Maus I, Off S, Pühler A, Scherer P, Klocke M, et al. Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. Appl Microbiol Biotechnol. Springer; 2018;102:5045–63.
34. Luo G, Fotidis IA, Angelidaki I. Comparative analysis of taxonomic, functional, and metabolic patterns of microbiomes from 14 full-scale biogas reactors by metagenomic sequencing and radioisotopic analysis. Biotechnol Biofuels. Springer; 2016;9:1–12.
35. Demirel B, Scherer P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev Environ Sci Bio/Technology. Springer; 2008;7:173–90.
36. Ahring BK, Westermann P, Mah RA. Hydrogen inhibition of acetate metabolism and kinetics of hydrogen consumption by Methanosarcina thermophila TM-1. Arch Microbiol. Springer; 1991;157:38–42.
37. Gagliano MC, Braguglia CM, Gianico A, Mininni G, Nakamura K, Rossetti S. Thermophilic anaerobic digestion of thermal pretreated sludge: role of microbial community structure and correlation with process performances. water Res. Elsevier; 2015;68:498–509.
38. Treu L, Kougias PG, de Diego-Díaz B, Campanaro S, Bassani I, Fernández-Rodríguez J, et al. Two-year microbial adaptation during hydrogen-mediated biogas upgrading process in a serial reactor configuration. Bioresour Technol. Elsevier; 2018;264:140–7.
39. Reeve JN, Morgan RM, Nölling J. Environmental and molecular regulation of methanogenesis. Water Sci Technol. Elsevier; 1997;36:1–6.
40. Treu L, Campanaro S, Kougias PG, Sartori C, Bassani I, Angelidaki I. Hydrogen-fueled microbial pathways in biogas upgrading systems revealed by genome-centric metagenomics. Front Microbiol. Frontiers; 2018;9:1079.
41. Huber DH, Chavarria-Palma JE, Espinosa-Solares T. Co-digestion of Dairy Cattle Waste in a Pilot-Scale Thermophilic Digester Adapted to Poultry Litter Feedstock: Stress, Recovery, and Microbiome Response. BioEnergy Res. Springer; 2021;1–11.

42. Gaber SM, Johansen A-G, Devold TG, Rukke E-O, Skeie SB. Manufacture and characterization of acid-coagulated fresh cheese made from casein concentrates obtained by acid diafiltration. J Dairy Sci. Elsevier; 2021;
43. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH. Back to basics–the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One. Public Library of Science San Francisco, CA USA; 2015;10:e0132783.
44. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75:129–37.
45. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. Oxford University Press; 2014;30:2114–20.
46. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. Oxford University Press; 2011;27:2957–63.
47. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. Nature Publishing Group; 2013;10:996–8.
48. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. Nature Publishing Group; 2010;7:335–6.
49. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. Oxford University Press; 2012;41:D590–6.
50. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. Am Soc Microbiol; 2007;73:5261–7.
51. Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, et al. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol Monogr. Wiley Online Library; 2014;84:45–67.
52. Hsieh TC, Ma KH, Chao A. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). Methods Ecol Evol. Wiley Online Library; 2016;7:1451–6.

Tables

Table 1

Characteristics of inoculum and substrates

	TS (%)	VS (%)	pH	TAN (g L^{-1})	TVFA (mM)
Inoculum	3.04	1.22	8.07	1.54	4.29
Cow manure	9.35	1.69	7.34	1.08	48.39
Cheese waste	12.64	0.99	4.78	0.14	11.01

TS – Total solid, VS – volatile solid, TAN – total ammonium nitrogen, TVFA – total volatile fatty acids

Table 2

Operating conditions of control- and *in-situ* upgrading reactors at different experimental phases

Parameters	**Unit**	**Phases**											
		I (day 1-64)		**II (day 65-78**		**III (day 79-85)**		**IV (day 93-113)**		**V (day 114-140)**		**VI (day 141-172)**	
		CR	UR	CR	UR	CR	UR	CR	UR	CR	UR	CR	UR
Stirring speed	rpm	80	80	80	80	140	140	80	80	80	80	80	80
CM:CW ratio	%	10	10	10	10	10	10	20	20	10	10	10	10
Feeding frequency	hours	24	24	24	24	24	24	24	24	48	48	24	24
H_2:CO_2 ratio	-	-	-	-	2	-	2	-	2	-	2	-	4

CR – control reactor, UR – *in-situ* upgrading reactor

CM – cow manure, CW – cheese waste

Day 86-92 – same conditions as phase II

Table 3

Performance data for control and upgrading reactors at different experimental phases (mean ± S.D)

Phases	I		II		III		IV		V
Reactor	CR	UR	CR	UR	CR	UR	CR	UR	CR
Biogas yield ($mL\ g^{-1}_{VS}$)	244.72±8.29	241.15±11.01	245.59±4.52	298.11±4.84	232.47±4.16	218.43±9.70	263.18±7.16	349.90±3.81	231.00±4
CH_4 yield ($mL\ g^{-1}_{VS}$)	144.77±2.38	143.50±3.95	146.34±2.25	185.44±1.94	133.52±2.22	132.96±5.20	142.19±1.83	204.15±1.48	141.70±6
Gas compositions (%)									
CH_4	58.24± 1.09	59.14±1.25	59.88±0.66	39.97±0.60	57.57±0.13	40.76±0.45	53.70±0.36	38.69±0.38	58.55±0.5
CO_2	41.76± 1.09	40.86±1.25	40.41±0.68	28.59±0.59	42.43±0.13	26.19±0.14	46.30±0.36	28.04±0.44	41.45±0.5
H_2	-	-	-	31.44±0.15	-	33.05±0.31	-	33.27±0.11	-
H_2 consumption (%)	-	-	-	24.96±0.09	-	45.99±0.36	-	17.35±0.70	-
pH	7.92±0.02	7.94±0.01	7.94±0.01	8.10±0.01	8.15±0.07	8.28±0.03	7.91±0.03	8.11±0.03	7.82±0.06
TVFA (mM)	18.99±5.33	17.12±5.37	30.73±3.68	44.55±0.36	30.56±0.45	66.63±9.85	37.67±2.29	62.18±7.42	40.18±5.0
AA (mM)	12.04±4.02	12.07±4.71	20.58±1.91	35.68	18.32±1.68	53.50±9.05	20.57±2.97	45.83±9.10	25.59±3.6
PA (mM)	6.95±1.31	5.05±0.66	10.15±1.77	8.87±0.36	12.24±1.23	13.13±0.95	17.10±0.67	16.36±1.67	14.58±1.5
TAN ($g\ L^{-1}$)	2.48±0.06	2.52±0.02	2.57±0.01	2.77±0.16	3.32±0.22	2.88±0.14	3.12±0.11	3.17±0.03	2.80±0.15

CR – control reactor, UR – *in-situ* upgrading reactor

TVFA – total volatile fatty acid, AA – acetic acid, PA – propionic acid

Table 4

Performance of hybrid reactor system at different H_2:CO_2 ratios (mean ± S.D)

H_2:CO_2 ratio	pH*	TAN* ($g\ L^{-1}$)	AA* (mM)	CH_4 yield+ ($mL\ gVS^{-1}$)	H_2 consumptions+ (%)	CH_4 content (without considering H_2)+ (%)	Output gas compositions+ (%)		
							CH_4	CO_2	H_2
2:1	8.07	1.09	4.12	257.27±4.28	60.23±0.75	79.89±1.40	63.20±1.44	16.10±1.18	20.70±0.43
4:1	8.06	1.01	4.23	234.15±3.70	62.22±2.63	73.09±2.22	50.58±0.93	18.64±1.75	30.78±0.83

TAN – total ammonium nitrogen, AA – acetic acid

* - Parameters measured in ex-situ upgrading reactor (HR)

+ - Data from hybrid system (*in-situ* (UR) + *ex-situ* (HR))

Figures

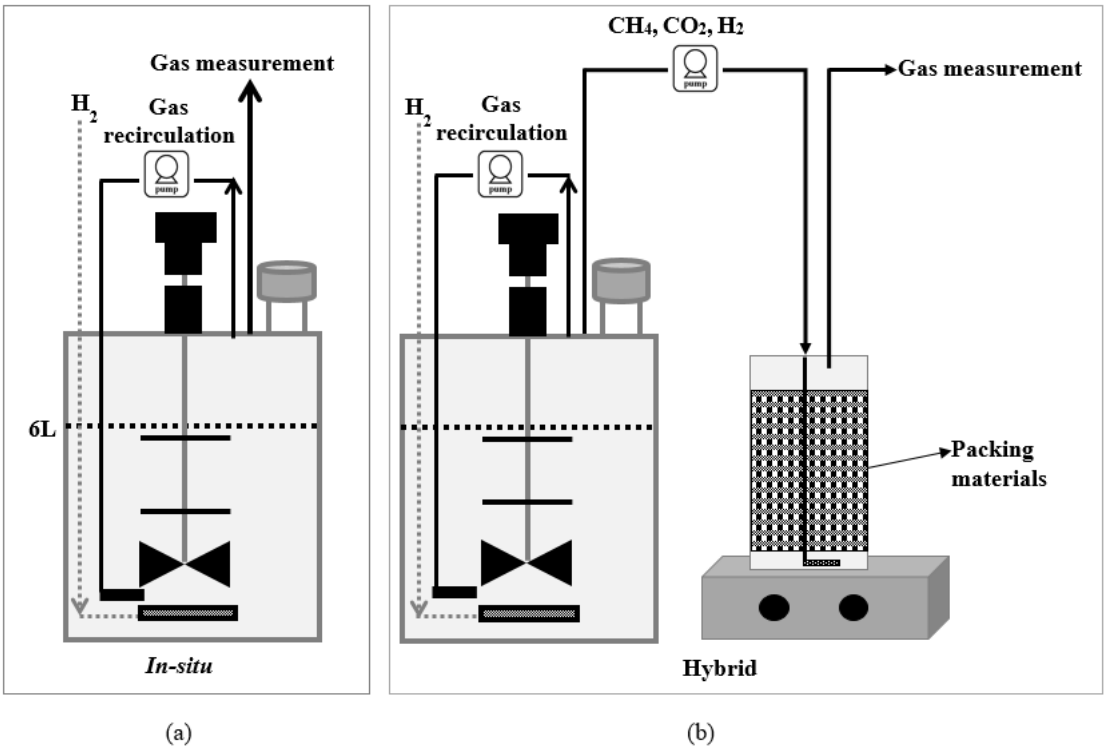

Figure 1

(a) Illustration of in-situ and (b) hybrid reactor setups

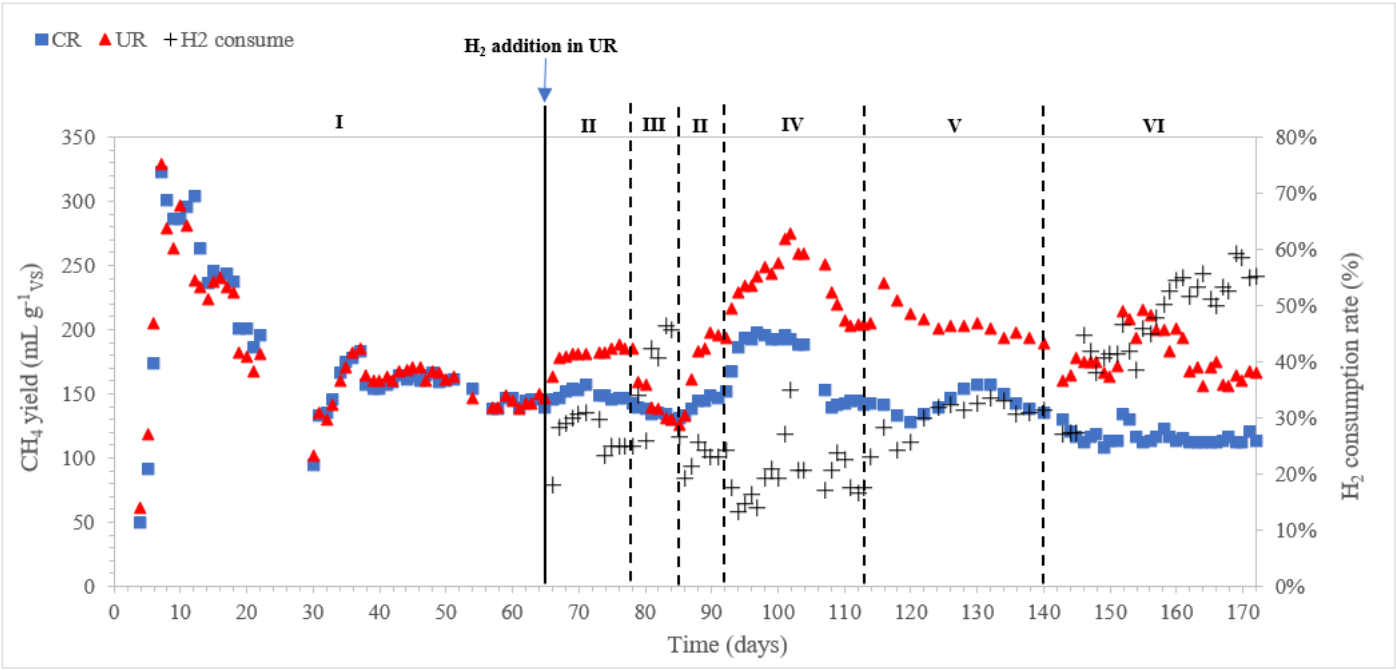

Figure 2

Methane yield and H2 consumption at different experimental phases (I – VI). CR, control reactor; UR, in-situ upgrading reactor; H2 consumed, H2 consumption.

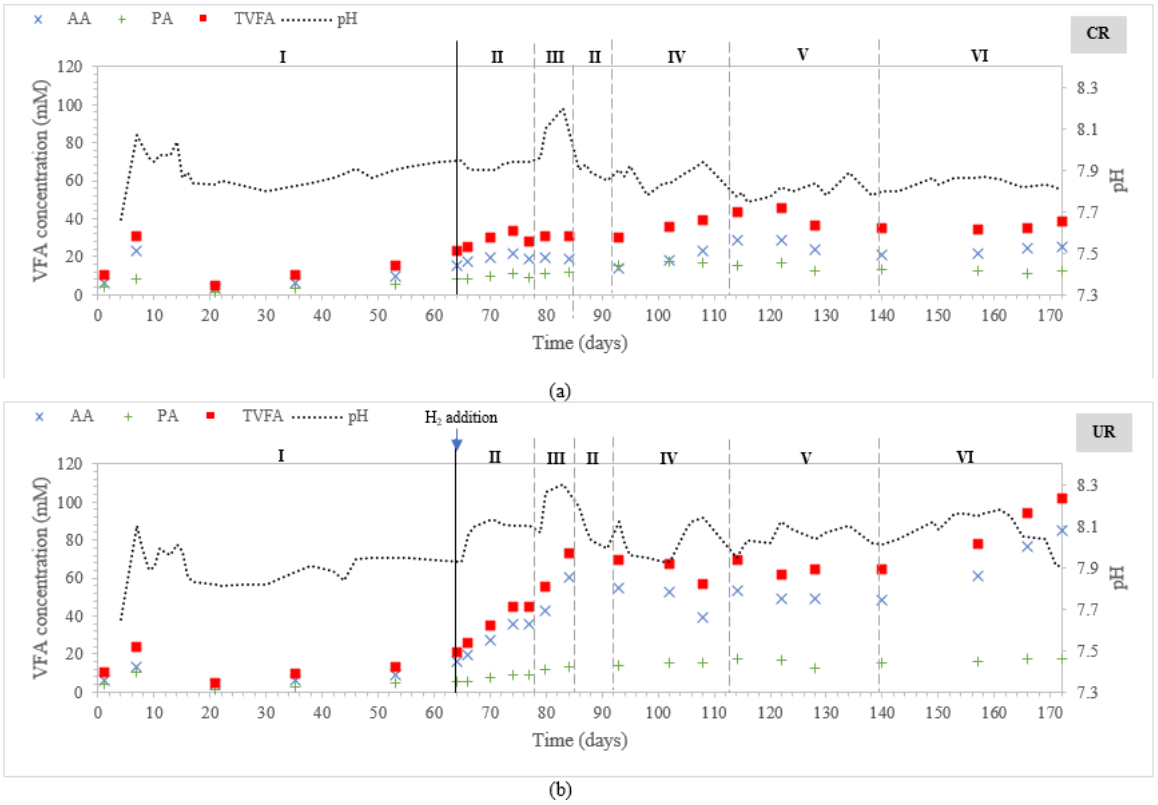

Figure 3

pH and volatile fatty acid concentrations in (a) control reactor (CR) and (b) in-situ upgrading reactor (UR). AA, acetic acid; PA, propionic acid; TVFA, total volatile fatty acids.

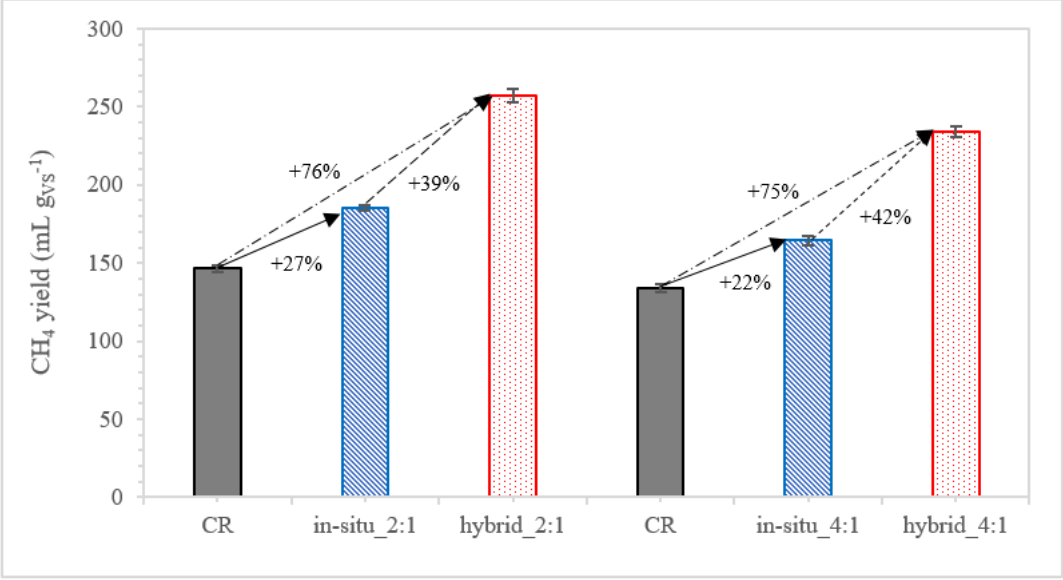

Figure 4

Average methane yield of control reactor (CR), in-situ upgrading reactor (UR) and hybrid upgrading reactor (HR). 2:1 – H2:CO2 ratio of 2:1, 4:1 – H2:CO2 ratio of 4:1

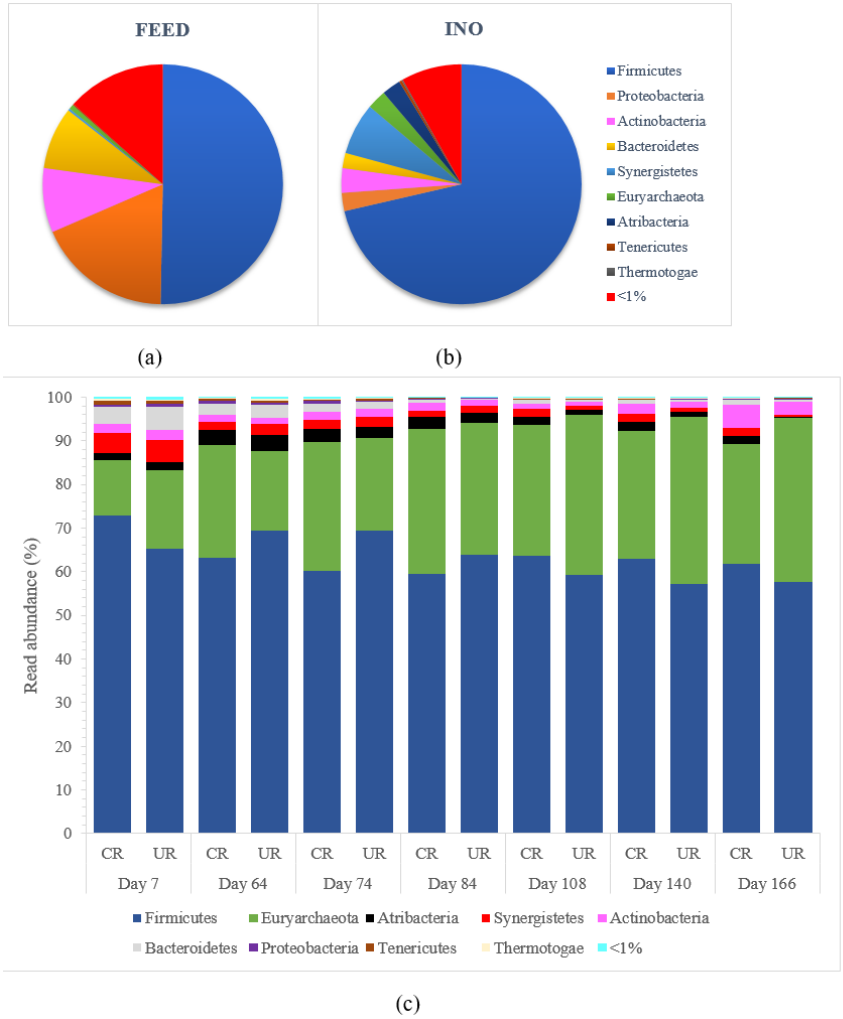

Figure 5

Stacked bar plot of the read abundance (%) of phyla detected in (a) feed, (b) inoculum and (c) control reactor (CR) and in-situ upgrading reactor (UR) over time. Phyla are indicated by the colors displayed in the legend of the figure. Samples with less than 1000 sequences were omitted from the figure. FEED is the biogas substrate used in the study (90%CM:10%CW) and INO indicates the inoculum.

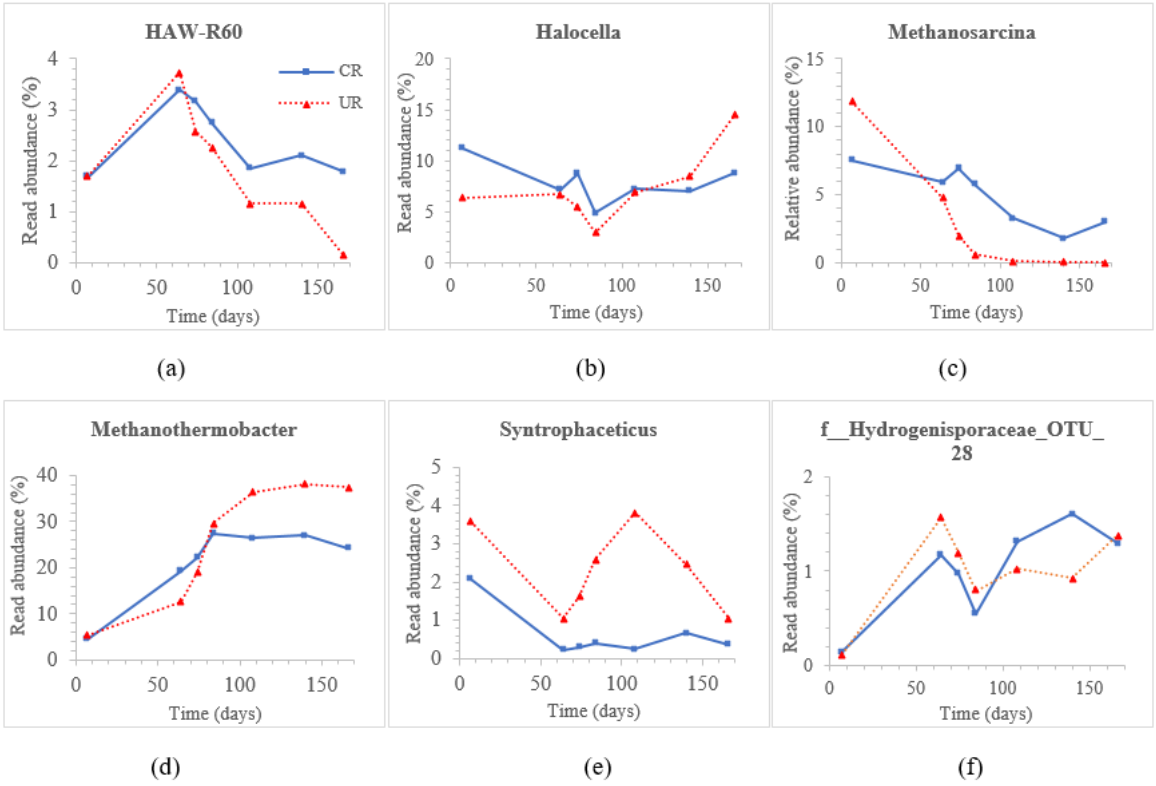

Figure 6

Genus abundance (%) of (a) HAW-R60, (b) Halocella, (c) Methanosarcina, (d) Methanothermobacter, (e) Syntrophaceticus, and (f) f_Hydrogenisporaceae_OTU_28 in the control and in-situ upgrading reactors over time. Samples with less than 1000 sequences were omitted from the figure. CR, control reactor; UR, in-situ upgrading reactor.