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Comparative Study of Mesophilic Biomethane Production in Ex Situ Trickling Bed and Bubble Reactors

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Abstract: Biomethane production via biogas upgrading is regarded as a future renewable gas, further boosting the biogas economy. Moreover, when upgrading is realized by the biogas CO_2 conversion to CH_4 using surplus renewable energy, the process of upgrading becomes a renewable energy storage method. This conversion can be carried out via microorganisms, and has attracted scientific attention, especially under thermophilic conditions. In this study, mesophilic conditions were imposed using a previously developed enriched culture. The enriched culture consisted of the hydrogenotrophic *Methanobrevibacter* (97% of the Archaea species and 60% of the overall population). Biogas upgrading took place in three lab-scale bioreactors: (a) a 1.2 L bubble reactor (BR), (b) a 2 L trickling bed reactor (TBR) filled with plastic supporting material (TBR-P), and (c) a 1.2 L TBR filled with sintered glass balls (TBR-S). The gas fed into the reactors was a mixture of synthetic biogas and hydrogen, with the H_2 to biogas CO_2 ratio being 3.7:1, lower than the stoichiometric ratio (4:1). Therefore, the feeding gas mixture did not make it possible for the CH_4 content in the biomethane to be more than 97%. The results showed that the BR produced biomethane with a CH_4 content of 91.15 $\pm$ 1.01% under a gas retention time (GRT) of 12.7 h, while the TBR-P operation resulted in a CH_4 content of 90.92 $\pm$ 2.15% under a GRT of 6 h. The TBR-S operated at a lower GRT (4 h), yielding an effluent gas richer in CH_4 (93.08 $\pm$ 0.39%). Lowering the GRT further deteriorated the efficiency but did not influence the metabolic pathway, since no trace of volatile fatty acids was detected. These findings are essential indicators of the process stability under mesophilic conditions.

Keywords: biogas upgrading; bio-trickling bed reactor; bubble reactor; mesophilic; hydrogenotrophic methanogenesis; biomethane

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1. Introduction

The European Commission's Energy Roadmap 2050 aims for 75% of the gross final energy consumption to come from renewable sources by 2050, including the transportation sector [1]. Solar and wind power generation fluctuates daily and seasonally, leading to energy waste when production exceeds demand [2]. To address this issue, power-to-gas conversion is being explored as a solution for storing excess renewable energy [3]. This process involves converting excess power into H_2 through electrolysis, with the potential to store up to 20% by volume with infrastructure modifications, although it is not fully interchangeable with CH_4 [4]. Another option involves using CO_2 as an additional carbon source for energy storage, requiring a reducing agent such as renewable H_2 to convert it to CH_4. This approach is linked to carbon capture and utilization (CCU) technologies and additionally utilizes all biomass carbon. It is crucial to integrate CCU technologies with renewable energy production, as relying solely on renewable energy cannot achieve a carbon-neutral world [5]. Furthermore, biomass is not unlimited. The competition with food production for land use and the decreasing willingness to offer feed-in tariffs make it essential to utilize available biomass efficiently and ensure the sustainability of the biogas sector [6]. Therefore, biogas CO_2 reduction with an external

source of hydrogen gas produced using excess renewable electrical power has recently attracted significant attention.

The reduction of CO_2 to CH_4 requires a catalyst, which can be either chemical or biological. In the case of a biological catalyst, the process is referred to as biomethanation, and it offers several advantages over traditional thermochemical processes. Biomethanation occurs at mild temperatures (30–60 °C) and atmospheric pressure conditions. This process is carried out by hydrogenotrophic methanogens, which are plentiful in anaerobic digesters [5].

Biogas upgrading through biomethanation can be achieved either in situ or ex situ. In the in situ process, H_2 is injected into the anaerobic digestion reactor, enhancing hydrogen methanogenic conversion, which is already a part of the biogas production process. However, this approach increases the pH due to CO_2 consumption, and in the presence of proteinaceous compounds in the feedstocks (such as manures), it raises the concentration of the inhibiting free ammonia in the digester [7]. If the introduced H_2 is not rapidly consumed, the acetogenesis is not favored thermodynamically, and volatile fatty acids (VFAs) accumulate. Under these conditions, homoacetogenesis also takes place. In either case, methanogenesis is inhibited [8]. Even under inhibitory conditions, thoughtful engineering design can enhance the process. For instance, pulsed H_2 injection appears to promote the symbiotic growth of microorganisms necessary to maintain non-inhibitory levels of hydrogen during the anaerobic digestion process in the in situ biogas upgrading [9]. In the ex situ method, H_2 and biogas containing CO_2 are supplied to a separate reactor. Separating the two processes seems to enhance their stability and efficiency because it allows the establishment of optimal conditions for both. Furthermore, ex situ upgrading is not solely applied to biogas and can be flexible regarding the source of CO_2 [10]. Ex situ upgrading results in higher methane productivity compared to the in situ process. The methane content is over 95%, drawing increasing interest over the past decade [11].

The main bottleneck in biological biogas upgrading is the limited transfer of hydrogen due to its low solubility in water [12]. Bioreactors have been designed to enhance mass transfer [13] by focusing on efficient diffusers [14], stirring [15], gas recirculation [16], support medium [17], pressure [18], and hydrodynamic cavitation [19], and have yielded promising results. However, some of these methods may be energy-intensive (such as gas recirculation) or incur additional costs (like unique gas distributors).

Another bioreactor type, the trickle bed reactor (TBR), operates on a different concept with significant advantages [11]. In a TBR, the aqueous phase percolates through a column filled with inert material where microorganisms grow. This design provides ample space for gas, allowing it to diffuse through the thin aqueous layer of the biofilm to reach the microorganisms. The TBR has proven to operate efficiently at low gas retention times (GRTs) in comparison with bubble reactors (BR) [20].

In previous studies, research on TBRs has primarily focused on factors such as the choice of packing carriers [21], the incoming CO_2/H_2 ratio [20], nutrient dosing [22], intermittent operation [23], and the inoculation strategy [24]. Most of the research on TBRs has been carried out under high temperatures (thermophilic conditions) due to the faster process rates, while less attention has been given to TBR operations at lower temperatures (mesophilic conditions) [25,26].

However, operating at lower temperatures may have energy efficiency benefits. Therefore, the present study thoroughly examined biogas upgrading in TBR under mesophilic conditions, focusing on two supporting carriers: polyethylene rings and sintered glass balls. Additionally, a bubble reactor was used as a control to assess the potential higher biogas upgrading efficiency of TBRs compared to a suspended growth bioreactor. In this study, a different source of inoculum was used. Typically, the inoculum comes from anaerobic digestate from biogas plants, and the relative abundance of hydrogenotrophic methanogens increases during the bioreactor's operation. To further enrich the consortium with hydrogenotrophic methanogens and exclude non-participating microorganisms, successive dilution cycles were applied in a bubble reactor under mesophilic conditions [27]. This

strategy resulted in obtaining a microbial culture, in which *Methanobrevibacter* sp., a type of hydrogenotrophic methanogen, dominated at 97.9% among the Archaea and constituted 60% of the total population. This is the highest relative abundance of the phylum Euryarchaeota recorded in biomethane bioreactors. Consequently, all bioreactors in this study were inoculated with the enriched culture, which appeared as an off-white powdered solid mixture tending to attach rapidly to the bioreactor walls macroscopically. This observation led to the hypothesis that TBRs inoculated with an enriched consortium would operate efficiently under high loading rates in mesophilic conditions. Therefore, the objective of this study was to demonstrate the potential of the TBR to be used for biogas upgrading at low GRTs under mesophilic conditions, starting from a highly enriched inoculum in the *Methanobrevibacter* sp.

2. Materials and Methods

2.1. Experimental Setup and Operation of Biomethane Reactors

Three distinct bioreactor configurations were utilized in the study: a bubble reactor (BR), a trickle bed reactor packed with polyethylene rings (TBR-P), and a trickle bed reactor filled with sintered glass balls (TBR-S). The specific surface areas of the polyethylene rings (Kaldnes K1) and sintered glass balls (Aquael Bioceramax) were recorded as 800 m^2 m^{-3} and 1600 m^2 m^{-3}, respectively (Figure 1). All bioreactors were fabricated from borosilicate glass. The working capacity of the BR was 2 L, while the operational volumes (packed bed) of the TBR-P and the TBR-S were 1.25 L and 2 L, respectively.

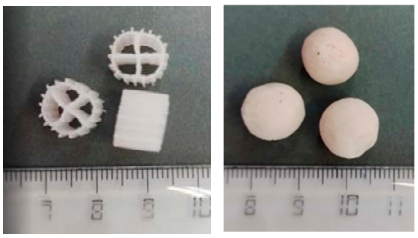


Figure 1. Packing material used in the TBRs: polyethylene rings (**left**) and sintered glass balls (**right**).

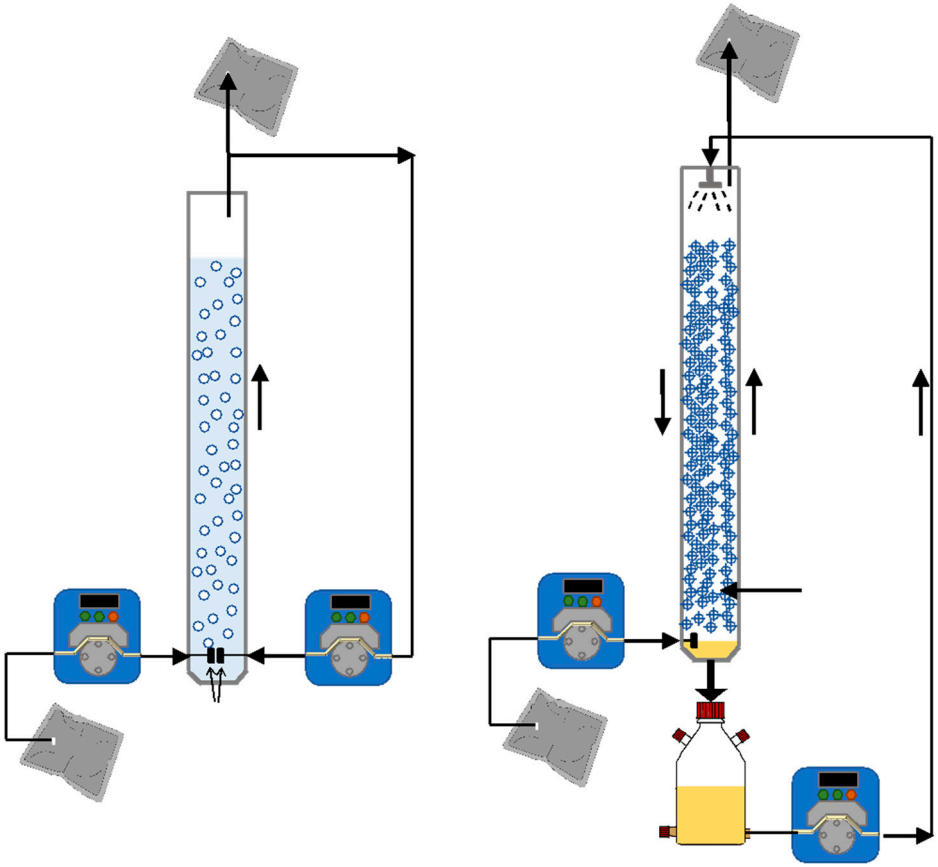
The BR was established and inoculated with a hydrogenotrophic consortium, enriched from a mesophilic biomethane reactor [26]. The BR operated for 367 days. On the 200th day, 250 mL was taken from the BR to inoculate the TBR-P. On the 367th day, the BR was emptied, and the TBR-S was inoculated. In each case, the inoculum was mixed with a nutrient medium (protocol ATCC 2601—Table A1) of up to 2 L for the BR and TBT-S and 1.25 L for the TBR-S. The columns intended to function as TBRs were initially filled with the inoculum and the nutrient medium and operated without the packing material to allow for an increase in volatile suspended solids (VSS) to around 270 mg L^{-1}. Then, the packing material was added, and the TBRs were flushed with nitrogen and operated for three days as packed-bed reactors before switching to TBR operation. The characteristics of the aqueous phase of all reactors at start-up are shown in Table 1. The synthetic medium replenished the volume, which was reduced due to sampling or evaporation.

Table 1. Characteristics of the aqueous phase at the start-up of the bioreactors.

	Value		
Parameters	**BR**	**TBR-P**	**TBR-S**
pH	7.82	8.01	8.21
Conductivity (mS cm^{-1} @25 °C)	3.8	5.62	3.92
VSS (g L^{-1})	0.27	0.289	0.238

The gas mixture used for all bioreactors consisted of 59.7% v/v H_2, 24.2% v/v CH_4, and 16.1% v/v CO_2. The ratio of H_2 to CO_2 was 3.7:1, which is slightly lower than the

stoichiometric ratio of 4:1 to improve the conversion of H_2. The ratio of CH_4 to CO_2 was similar to that of biogas (60:40). The gas mixture was stored in gas sampling bags made of inert multi-layer foil (Supel®, Sigma-Aldrich Co, Merck KGaA, Burlington, MA, USA). It was fed to the bioreactors using commercial gas diffusers and peristaltic pumps. The biomethane produced was collected in gas aluminum bags. Gas recirculation was only applied to the BR at a rate of 4 L L_R^{-1} h^{-1}. Additionally, the nutrient medium in TBRs was collected in a 2 L borosilicate bottle, mixed, and recirculated at a rate of 4 L L_R^{-1} h^{-1} (Figure 2). Both TBRs were operated in a countercurrent mode, allowing the feeding gas to move upwards. All three bioreactors and the bottles containing the nutrient medium were wrapped with heating tapes to maintain the temperature at 39 ± 1 °C via a temperature controller.



(**a**) (**b**)

Figure 2. Schematic depiction of the experimental setup for (**a**) the BR and (**b**) TBR.

The bioreactors were started at a high GRT (16–23 h) and this was decreased after a steady state was achieved. When the bioreactor performance deteriorated upon GRT decrease, the GRT was increased again, especially if the GRT decrease was considered abrupt, and the GRT decrease started at a lower step size. When TBRs had to operate under a very low loading rate for 25 d to cope with practical difficulties in running the experiments, the countdown of the GRT started again. Bioreactor operation was stopped if the CH_4 content in the output gas was below 80%. Table A2 summarizes the GRT variation during the experiments.

2.2. Analytical Methods and Calculations

Samples from the liquid phase of each system were collected twice a week (40 mL) and replenished with fresh nutrient medium. The pH and electrical conductivity were measured using a digital pH and electrical conductivity (EC) meter from HANNA Instruments Hellas (HI 83141, Athens, Greece). To determine the composition, gas sampling occurred three

times a week using a gas chromatograph (SHIMADZU–GC 2014, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). Argon was utilized as a carrier gas to ensure precision in hydrogen measurement. The temperature at the injector and the detector ports was maintained at 210 °C, and the oven was set at a constant temperature of 100 °C. Gas volume was calculated based on the displacement of an equivalent acidified aqueous volume at 20 °C, the ambient temperature during the experiment. Volatile fatty acids (VFAs) were analyzed using a gas chromatograph (SHIMADZU–GC 2014) equipped with a flame ionization detector (FID) and a capillary column. All VFA samples were filtered through 0.22 µm nylon membrane filters and acidified with 6 M hydrochloric acid solution to reduce the pH below 1. The oven temperature ranged from 50 °C to 200 °C at a rate of 10 °C min^{-1}, and helium was used as the carrier gas. The temperature at the detector and injector was set at 260 °C and 210 °C, respectively.

VSS concentration was measured according to standard methods [28] and via a spectrophotometer at 600 nm based on a calibration curve (CV). The CV was calculated based on the absorbance at 600 nm of samples of known VSS concentration taken from the bioreactor (Figure A1). The CV was frequently checked against samples of known VSS concentration.

The hydrogen loading rate (HLR; L L_R^{-1} d^{-1}) was defined as hydrogen volume entering the bioreactors per operating reactor volume (R) per time. It was calculated based on the total gas loading rate (GLR) and the proportion of H_2 (%) in the feeding gas mixture ($H_{2,in}$), as shown in Equation (1).

$$HLR = H_{2in} \cdot GLR \quad (1)$$

The net methane production rate (Net MPR; L L_R^{-1} d^{-1}) was estimated according to Equation (2), where MPR (L L_R^{-1} d^{-1}) and MIR (L L_R^{-1} d^{-1}) are the methane production and influent rates, respectively.

$$NetMPR = MPR - MIR \quad (2)$$

The H_2 utilization efficiency (%) was determined based on Equation (3), where HIR (L L_R^{-1} d^{-1}) and HER (L L_R^{-1} d^{-1}) are the hydrogen influent and effluent rates, respectively.

$$\eta_{H_2} = \frac{HIR - HER}{HIR} \cdot 100 \quad (3)$$

The utilization efficiency of CO_2 (%) was calculated similarly according to Equation (4), where CIR (L L_R^{-1} d^{-1}) and CER (L L_R^{-1} d^{-1}) are the CO_2 influent and effluent rates, respectively.

$$\eta_{CO_2} = \frac{CIR - CER}{CIR} \cdot 100 \quad (4)$$

The average values and standard deviations of the process parameters were calculated based on at least three successive values at steady state.

3. Results

3.1. Overview of Bubble Reactor Performance

The BR underwent three different GRTs before the CH_4 content of the biomethane dropped below 80%. Initially, the operation started with a GRT of 14.4 h, but it resulted in a CH_4 content lower than 90%. Subsequently, the GRT was increased to 23 h, and when the CH_4 content surpassed 90%, the GRT was reduced to 16.4 h. The BR operated under this GRT for 233 days. During this period, improvements were made to the operation of auxiliary equipment (pumps and gas bags) and the bioreactor architecture (feeding and recirculation tubes) to prevent clogging and gas leakage, despite the VSS concentration being below 0.5 g L^{-1} in the BR (Figure A2); all surfaces (column internal wall, tubes in the BR and diffuser) were covered in the off-white fine particles of the hydrogenotrophic enriched consortium [27]. As a result, the BR was emptied for cleaning, and measures were taken to protect the tubing and diffuser from clogging. Samples of 250 mL were taken on

days 154 and 200, causing a drop in the VSS concentration (Figure A2), which then quickly built up again. Most of the 16.4 h phase of the experiment focused on achieving smooth operation before reducing the GRT to 10.4 h and finally to 7.2 h. The CH_4 content in the effluent gas mixture was as follows: 92.24 ± 0.36%, 94.63 ± 0.15%, 91.15 ± 1.01%, and 65.01% ± 7.05 under the decreasing GRT values (Figure A3a). The operation under the last GRT proved detrimental, as evidenced by the rapid decrease in CH_4 content.

Given that the H_2/CO_2 ratio in the feed gas was lower than the stoichiometric ratio, it was anticipated that the CH_4 content would not exceed 97%. As a result, we contrasted the net CH_4 production with the maximum value, determined as the HLR (per Equation (1)) divided by 4 (the stoichiometric ratio of H_2/CH_4). Figure 3 illustrates that the net CH_4 production rate closely approached the maximum level for all GRTs tested, except for the last one. Similarly, the H_2 utilization efficiency reached almost 100% for GRTs of 23 and 16.4 h and was notably high for a GRT of 12.7 h. However, reducing the GRT to 7.2 h resulted in a substantial decline in efficiency, as evidenced by the accumulation of H_2 (Figure A3a).

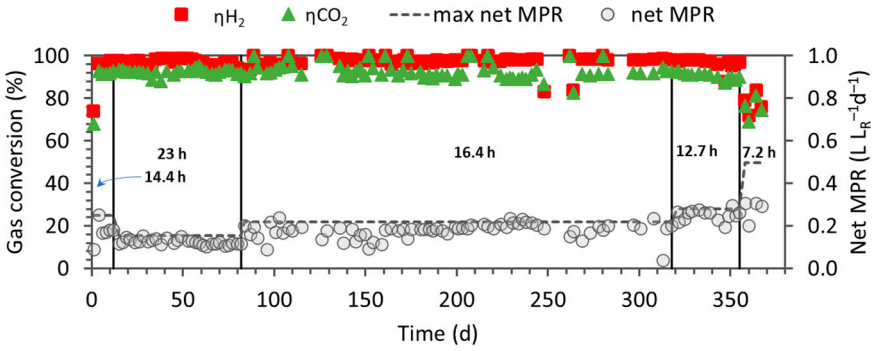


Figure 3. Utilization efficiencies of the reacting gases and the net methane production compared to the maximum level in the BR under different GRTs.

Throughout the operation of the bioreactor, acetate emerged as the predominant volatile fatty acid (VFA), consistently maintaining a level below 0.5 g L^{-1} (as depicted in Figure A4a). It was initially generated during the early phase of the operation, then reduced to insignificant levels after operation under a GRT of 16.4 h and remained at a low concentration during the reduction in the GRT to 12.7 h. However, upon further reduction in the GRT to 7.2 h, acetate exhibited rapid accumulation, reaching a concentration of 1.5 g L^{-1}, with an evident inclination to increase further. This surge in acetate concentration caused a decline in pH to 6.19, prompting attempts at pH correction through the injection of $NaCO_3$ and NaOH 1N. Despite the efforts, the pH failed to recover due to the highly volatile fatty acid concentration, and the experiment stopped.

Moreover, the escalation in conductivity was found to be aligned to the concentration of acetate ions. Notably, the addition of nutrient medium was undertaken to maintain a consistent liquid volume while ensuring regular sample acquisition. Consequently, the recurrent provision of nutrients and trace metals was implemented without measurement of their levels, particularly under elevated loadings. Conductivity may serve to indicate the presence of high concentrations of ions, such as ammonium. The decline in conductivity from the 250th to the 350th day is indicative of possible ammonium deficiency, while the subsequent upsurge in conductivity aligned with the elevation of volatile fatty acids.

3.2. Effect of Packing Material on TBR Efficiency

Two TBRs with different packing materials (TBR-P with polyethylene rings and TBR-S with sintered glass balls) were seeded from the BR. Initially, both TBRs operated with their packing material flooded in the inoculum–nutrient medium. After three days, the liquid phase trickled through the bed. TBR-P was initiated first under a high GRT (Table A1). The GRT decreased stepwise; under a GRT of 4 h, the CH_4 content dropped to 76.27% (from

94.84%). TBR-P returned to a higher GRT to recover. Then, a smoother transition from a GRT of 7.2 h downwards was attempted. It was decreased to 6 h, which seemed tolerable for TBR-P (90.92% CH_4 at GRT 6 h, from 93.48% at GRT 7.2 h). Afterward, there was a 25-day period when TBR-P had to be operated at a minimum flow rate due to practical difficulties. Subsequently, TBR-P started operating under decreasing GRTs for a third time, and the performance was repeatable until the GRT of 7.2 h. Decreasing the GRT to 5.5 h resulted in even lower CH_4 content (83.68%) than the CH_4 content recorded at the GRT of 6 h (90.92%). The experiment ended at a GRT of 4 h, under which the CH_4 content was 65.52%. Figure A3b depicts the variation in the biomethane composition.

In Figure 4, the removal efficiency of the reactants is shown. The CO_2 utilization is lower than that of H_2, as there was an excess of CO_2. When the GRT was 6 h, the removal of H_2 remained high, but at 5.5 h, it decreased significantly. A comparison of the maximum net MPR with the actual MPR indicates that the hydrogenotrophic biomass, which was attached to the plastic supporting medium of the TBR-P, could achieve productivity close to the maximum level up to a GRT of 6 h.

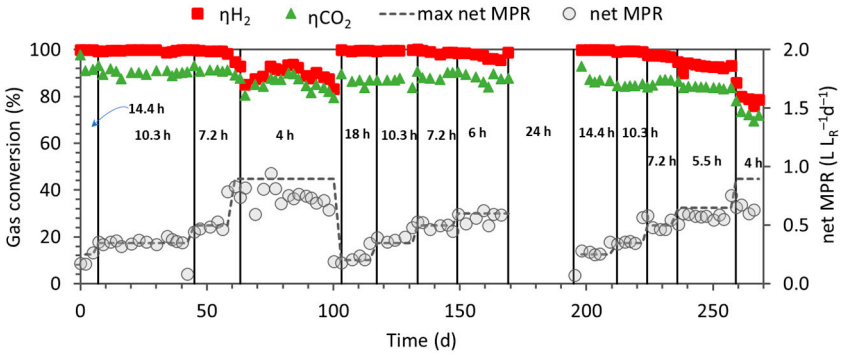


Figure 4. Utilization efficiencies of the reacting gases and the net methane production compared to the maximum level in the TBR-P under different GRTs.

An interesting finding was observed in this experiment. As the transition from the GRT 7.2 h to 4 h occurred, VFAs accumulated, with acetate being the most prevalent (see Figure A4b). Furthermore, conductivity increased during this period, possibly due to the rise of acetate and propionate anions, and NaOH and Na_2CO_3, which were introduced to maintain the pH above 7. However, this pattern did not repeat during the last transition to 4 h. Despite declining efficiency, there was no production of VFAs, and the pH and conductivity remained stable.

TBR-S was started later than TBR-P. After the necessary increase in the GRT to 29 h to maintain feeding at a low rate due to technical issues (as encountered in TBR-P), TBR-S operated concurrently with TBR-P under the same GRTs. Initially, both reactors had similar efficiency in terms of CH_4 content in the biomethane (93–96%) and H_2 conversion (above 99%). However, reducing the GRT to 7.2 h resulted in a slight decrease in TBR-P, but not in TBR-S, which maintained the CH_4 content of the effluent gas above 95% (see Figure A3c). Further reduction in the GRT to 5.5 and 4 h led to a significant decline in the CH_4 content of the effluent gas and the H_2 conversion efficiency of TBR-P, while TBR-S maintained the CH_4 content of gas effluent above 93%, and the H_2 conversion remained at 98% (see Figure 5). The decline in the CH_4 content and the H_2 conversion of TBR-S was noticeable at a GRT of 2.8 h (88.86 $\pm$ 0.43% and 94.91 $\pm$ 0.29%, respectively) and even more so at 2 h (71.93 $\pm$ 0.36% and 83.90 $\pm$ 3.41%, respectively).

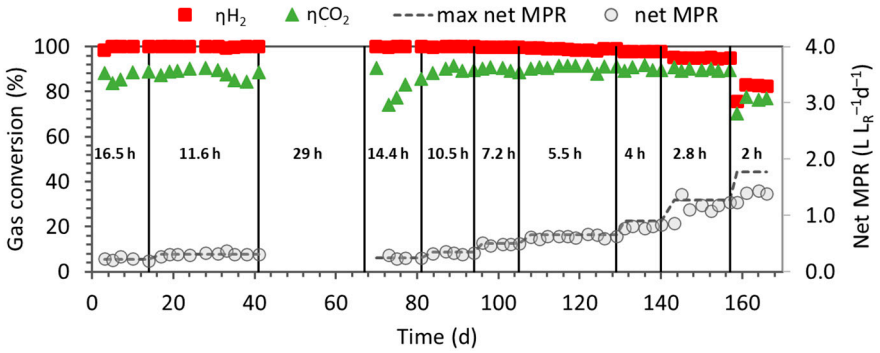


Figure 5. Utilization efficiencies of the reacting gases and the net methane production compared to the maximum level in the TBR-S under different GRTs.

It is important to note that there was no accumulation of volatile fatty acids (VFAs), even with the higher loading (GRT 2 h). This is aligned with the stable pH level that was maintained without the need for NaOH or Na_2CO_3 addition, and the conductivity, which remained at 5.7 ± 0.14 (Figure A4c).

4. Discussion

The table below presents a summary of the performance of all three bioreactors with respect to GRT. It is evident that, as the loading increased, each system reached its operational limits at distinct GRT thresholds. BR demonstrated satisfactory operation at higher GRT, whereas TBR-S exhibited efficient performance at lower GRT levels. Upon column-wise examination of Table 2, it becomes apparent that TBR-S displayed the most favorable performance at identical GRT values. It is imperative to note that certain GRT values were applied multiple times during the experimental period. The parameter values provided in Table 2 represent the averages of all repetitions. For example, a GRT of 7.2 h was enforced for TBR-P during the time intervals of days 46 to 63, days 134 to 149, and days 225 to 236 (Table A1). The process consistently yielded similar performance across all occurrences of the same GRT, underscoring its replicability and stability. Similarly, Figure 6 shows that the net CH_4 production rate is proportional to the HLR for all bioreactors, except for the cases in which the substrate conversion is low. It is evident that the TBR-S exhibited the best performance under the high HLRs, while the performance of all systems coincided under low HLRs.

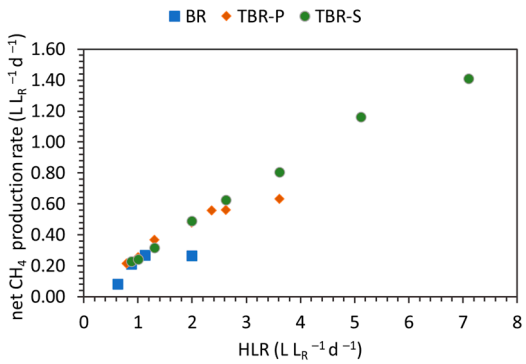


Figure 6. Comparison of the net MPR of all bioreactors versus the HLR.

Table 2. Summary of the efficiency of biogas upgrading in different configurations: BR, TBR-P, and TBR-S.

Phase	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
GRT	23	18	16.4	14.4	12.7	11.6–10.3	7.2	6	5.5	4	2.8	2
HLR	0.62	0.79	0.88	1	1.13	1.24–1.4	1.99	2.36	2.62	3.61	5.11	7.1
						BR						
CH_4	92.24 (0.36)		94.63 (0.15)		91.15 (1.01)		65.01 (7.05)					
H_2	4.92 (0.34)		2.38 (0.16)		4.93 (1.08)		27.1 (5.61)					
CO_2	2.84 (0.08)		2.99 (0.06)		3.92 (0.31)		7.88 (1.46)					
η_{H2}	94.81 (1.9)	-	98.53 (0.2)	-	96.53 (0.88)	-	77.57 (5.96)	-	-	-	-	-
η_{CO2}	92.49 (1.04)		93.11 (1.37)		89.83 (0.7)		75.88 (5.9)					
MPR	0.37 (0)		0.57 (0.03)		0.73 (0.02)		1.07 (0.06)					
Net MPR	0.08 (0.04)		0.21 (0.03)		0.27 (0.02)		0.27 (0.06)					
						TBR-P						
CH_4		94.2 (0.42)		94.4 (0.11)		94.29 (1.17)	93.03 (1.98)	90.92 (2.15)	83.68 (0.51)	70.89 (6.4)		
H_2		0.5 (0.09)		0.32 (0.04)		0.66 (0.41)	2.68 (1.29)	4.6 (2.4)	10.24 (0.52)	21.51 (4.9)		
CO_2		5.31 (0.48)		5.28 (0.11)		5.05 (0.91)	4.3 (0.75)	4.48 (0.44)	6.08 (0.03)	7.59 (1.52)		
η_{H2}	-	99.65 (0.05)	-	99.78 (0.02)	-	99.54 (0.29)	98.18 (0.9)	96.77 (1.73)	92.53 (0.5)	82.03 (5.08)	-	-
η_{CO2}		86.05 (1.87)		86.73 (0.46)		87.14 (2.6)	89.19 (2)	88.47 (1.2)	83.57 (0.34)	76.55 (6.03)		
MPR		0.54 (0.02)		0.66 (0.01)		0.93 (0.02)	1.29 (0.02)	1.53 (0.06)	1.61 (0.02)	2.11 (0.04)		
Net MPR		0.22 (0.02)		0.26 (0.01)		0.37 (0.02)	0.48 (0.02)	0.56 (0.06)	0.56 (0.02)	0.63 (0.04)		
						TBR-S						
CH_4			95.24 (0.47)	92.99 (1.64)		95.18 (1.19)	95.55 (0.44)		94.28 (1.47)	93.08 (0.39)	88.86 (0.43)	71.93 (0.36)
H_2			0.02 (0.04)	0.04 (0.02)		0.15 (0.17)	0.33 (0.05)		1.87 (0.82)	3.1 (0.09)	7.26 (0.34)	20.7 (0.24)
CO_2			4.74 (0.5)	6.97 (1.62)		4.68 (1.08)	4.12 (0.39)		3.85 (0.65)	3.82 (0.47)	3.88 (0.09)	7.37 (0.18)
η_{H2}	-	-	99.99 (0.03)	74.98 (0.01)	-	99.96 (0.01)	99.78 (0.04)	-	98.75 (0.58)	97.91 (0.02)	94.91 (0.29)	83.06 (0.25)
η_{CO2}			88 (1.95)	82.52 (4.24)		90.49 (1.12)	89.77 (1.1)		90.49 (1.86)	90.46 (1.37)	89.9 (0.36)	77.68 (0.56)
MPR			0.58 (0.04)	0.64 (0.01)		0.89 (0.01)	1.31 (0.01)		1.69 (0.03)	2.31 (0.04)	3.25 (0.07)	4.29 (0.01)
Net MPR			0.23 (0.04)	0.24 (0.01)		0.34 (0.01)	0.5 (0.01)		0.63 (0.03)	0.84 (0.04)	1.18 (0.07)	1.41 (0.01)

The GRT and HLR are expressed in h and L L_R^{-1} d^{-1}, respectively, CH_4, H_2, and CO_2 are the percentage of the gases in the effluent biomethane mixture (%), η_{H2} and η_{CO2}, have been defined in Equations (3) and (4), respectively (%), and MPR and Net MPR have been defined in Equation (1), respectively (L L_R^{-1} d^{-1}). The values are the average of three at the end of each phase. The values in parentheses are the standard deviations.

BR technology offers the advantage of having the biomass suspended and in direct contact with the reactants, depending on their solubility in water. Researchers have been focusing on techniques to enhance the low solubility of hydrogen in water, such as efficient diffusers [14], stirring [15], gas recirculation [16], supporting medium [17], pressure [18], and hydrodynamic cavitation [19]. However, the major disadvantage is that all these techniques are energy-intensive. In the present work, without any special diffusion device for H_2 dissipation to the liquid phase, the performance at GRTs higher than 10 h was similar to the results of other studies. Bassani et al. [16], also operating a BR but under thermophilic conditions, reported a net MPR of 0.21 L_{CH4} L_R^{-1} d^{-1} at a GRT of 15 h (equivalent to a yield of 0.24 L_{CH4} $L_{H2,fed}^{-1}$). In the present work, the yield at GRT 16.4 and 14.4 h was similar (0.24 L_{CH4} $L_{H2,fed}^{-1}$), but dropped severely to 0.13 L_{CH4} $L_{H2,fed}^{-1}$ when the GRT was decreased to 7 h. On the contrary, Bassani et al. [16] reported a higher yield (0.19 L_{CH4} $L_{H2,fed}^{-1}$) than the present work but much lower than the yield recorded at 15 h. However, the study of Bessani et al. was conducted under thermophilic conditions and at a higher gas recirculation rate than applied in the present work (4 L L_R^{-1} d^{-1}), of 5.75 L L_R^{-1} d^{-1}. When they applied a low gas recirculation rate (2.88 L L_R^{-1} d^{-1}) under the GRT of 15 h, the yield decreased dramatically, proving the importance of mixing. Similar results were demonstrated by Kougias et al. [8], who also operated a BR under thermophilic conditions. At a GRT of 8 h and gas recirculation rate of 4 L L_R^{-1} d^{-1}, they reported an H_2 conversion efficiency of about 80%, which is comparable with the results of this work. To improve the performance of the reactor, they had to apply energy-intensive techniques, resulting in an H_2 conversion efficiency of 100% after a three-fold increase in the gas recirculation rate from 4 to 12 L_R^{-1} d^{-1}.

Both TBRs performed significantly better than the BR (Figure 6). Specifically, TBR-P and TBR-S demonstrated high efficiency when the GRTs were lower than 7 h, while the performance of the BR degraded under similar conditions. This is in line with the findings of the existing literature, as most TBRs can operate effectively with GRTs lower than 2 h, particularly under thermophilic conditions [8,14,21,29,30]. Using TBR technology instead of BR or other suspended growth systems enhances the gas–liquid mass transfer of H_2. The liquid phase in the TBR is a thin layer containing the microorganisms which grow in a biofilm attached to the supporting material. The H_2 can be easily transferred through the thin layer to the microorganisms and be converted, without the need to be dispersed in the bulky liquid phase of the BR [11]. In our study, TBR-S was more efficient than TBR-P and achieved a GRT of 4 h with high H_2 removal (98%) and a CH_4 content of 93.1% in the biomethane. When the GRT was reduced to 2.8 h, the H_2 removal remained high (95%) but lower than at 4 h, and the CH_4 content also decreased to 89%. However, due to the lower H_2:CO_2 ratio (3.7:1) compared to the stoichiometric ratio (4), the achievable CH_4 content could reach up to 97%, indicating that, at a GRT of 2.8 h, the CH_4 content of the biomethane was 98% and 92% of the maximum at GRTs of 4 and 2.8 h, respectively. Furthermore, the feeding gas in our study consisted of 59.7% H_2, 24.2% CH_4, and 16.1% CO_2, resulting in an HLR of 3.6 LH_2 L_R^{-1} d^{-1} and a net MPR as high as 0.8 LCH_4 L_R^{-1} d^{-1} (methane yield 0.22 LCH_4 $LH_{2,fed}^{-1}$) under a GRT of 4 h. This is an important consideration when comparing with other studies (Table 3) that have utilized binary mixtures of CH_4 and CO_2, as the partial pressure of the reacting gases is higher, leading to increased mass transfer rates [26].

Burkhardt et al. [25] operated a mesophilic TBR up to a GRT of 4 h. The feed consisted only of H_2 and CO_2 at the stoichiometric ratio, without CH_4 or another gas simulating methane in biogas. As a result, the hydraulic loading rate (HLR) was higher at 4.8 NL_{H2} NL^{-1} d^{-1}) and so was the MPR at 1.2 NL_{CH4} L^{-1} d^{-1} at the same GRT of the present work. Mesophilic biogas upgrading was also studied in a TBR operating for 8 months on real biogas and renewable H_2 provided at a stoichiometric ratio concerning biogas CO_2. The inflow was variable, with GRTs ranging from 30 to less than 5 h. In most cases, the CH_4 content in the outlet gas was more than 95% (average value 96.6 ± 5.91%) [31]. Similarly, high efficiencies of TBR operating on biogas and H_2 under a GRT of 2.3 h (HLR: 7.5 NL_{H2}

NL^{-1} d^{-1}) reached 97.2% CH_4 in the outlet gas [26]. In another work, with a very long mesophilic TBR (7 m of flexible PVC tube, 13 mm internal diameter), extremely high MPRs were recorded at 30 L_{CH4} L^{-1} d^{-1} with 90% CH_4 in the produced biomethane [32]. The high length-to-diameter ratio of this bioreactor imposed a plug flow mode of operation, which favors the reaction extent according to fundamental principles of chemical reactor engineering. This concept of plug flow was also realized in a tubular bioreactor with a foam bed, developed to increase the gas–liquid interface and the bubble gas retention time [33], achieving an MPR of up to 15.1 L_{CH4} L^{-1} d^{-1} under mesophilic conditions.

Operating biogas upgrading reactors under thermophilic conditions has contradictory effects. While the solubility of hydrogen in the liquid phase decreases, the increased biochemical rates (H_2 and CO_2 utilization by microorganisms) efficiently deplete the H_2 concentration in the liquid phase, thereby enhancing the gas–liquid mass transfer rates and compensating for the reduced H_2 solubility. Table 3 provides a comprehensive overview of the efficiency of various TBRs, demonstrating that, under thermophilic conditions, TBRs can achieve higher methane production rates, such as 6.1 L_{H2} L^{-1} d^{-1} at an 800 L pilot scale reactor and 10.6 L_{H2} L^{-1} d^{-1} in a 2000 L pilot scale TBRs [29,30]. Overall, the methanation rate is 2–4 times higher than the mesophilic conditions, making a distinct difference in favor of thermophilic conditions.

Most thermophilic biogas upgrading TBRs showed high concentrations of VFAs, which could inhibit the methanogenesis process. For example, Porte et al. [34] observed VFAs accumulating up to 900 mg COD L^{-1}, while others reported accumulations of up to 2.1 and 8 g L^{-1}, potentially leading to process failure [29,30]. Similarly, Tsapekos et al. [35] recorded VFAs up to 2 g L^{-1} when the pilot TBR was fed on CO_2 and 0.8–1.2 g L^{-1} when fed on real biogas. Additionally, some studies recorded pH values lower than 7, attributed partially to acetogenesis [36]. However, in the present study, no VFAs were detected in TBR-P or TBR-S when operated at the lower GRT range, which aligns with stable pH values from 8 to 8.5. On the other hand, VFAs were present throughout the operation of BR and accumulated when the GRT decreased from 12.7 to 7.2 h. When the BR was used for inoculation of the TBR-P and, later, of the TBR-S, the VFA concentration was decreased and was not detected in the TBRs' operation, even under low GRTs, where the performance declined (Figure A4). The fact that the poor performance under low GRTs was not accompanied by VFA detection suggests that the enriched culture with *Methanobrevibacter* species used as inoculum [27] was further established, excluding homoacetogens.

However, the absence of acetate formation could not ensure high performance at GRTs less than 4 h. Under high hydrogen loading rates, syntrophic interactions between members of mixed consortia can be advantageous [37], while the possible exclusion of homoacetogens in this work may have deprived the mixed culture of the benefit of adapting to increases in the substrate flowrate. Even the limited richness within the archaeal genera dominated by one hydrogenotrophic, the *Methanobrevibacter* [27], could have harmed the reactor performance, while a versatile methanogenic consortium can result in a more robust hydrogenotrophic methanogenic process due to the different tolerance levels of the different methanogens [16]. Moreover, the *Methanobrevibacter* species grow slowly compared to other methanogenic strains [38], which indicates that more adaptation time is needed for these methanogens to increase in concentration and consume the hydrogen while increasing the hydrogen loading rate. Trace elements and nutrients were provided at constant concentration throughout the experiment, but there are indications that some elements (Na, K, Mg, and Ca) are load-dependent [31]. In addition, NH_4^+ was not monitored, and there is no indication whether it sufficed, especially under high loading conditions.

Other reasons for the performance deterioration may be related to mass transfer limitations induced by the experimental configuration. In the literature, the limitation of gas transfer mass in TBRs has been attributed to the liquid recirculation rate and mode (continuous versus intermittent) and the choice of packing material [21,39]. A lower (from 5.4 to 0.84 L L_R^{-1} d^{-1}) and intermittent (once per day) recirculation rate resulted in an increase in the H_2 utilization efficiency up to 96–100% [21]. More dense layered

polyurethane foam packing material was more effective than a loosely structured trickling bed made from the same material [21]. Sposobe [39] concluded that the shape of the packing material (more open structure with few protruded parts to avert the formation of dead zones that favor the growth of unwanted microorganisms, e.g., homoacetogens) is the most crucial factor despite the inner or total specific area. The sintered glass balls used in the present study, though they proved more efficient than Kaldane rings, are not an open-structured packing material, limiting the void volume of the TBR. Measurements on the void volume of the bioreactors filled with the sintered glass balls and the Kaldane rings showed that the TBR-S had 46% empty space, while the TBR-P was much higher (75%). Therefore, the effective reactor volume of the TBR-S was much less than its nominal volume, reducing the useful GRT. Although the sintered glass ball absorbed water at 20% its volume, it is dubious whether H_2 could enter the inner porosity of this packing material. Moreover, the recirculation rate applied to the TBRs is considered high [21]. These aspects could explain why the highly enriched consortium could not perform better than it did in other studies under the mesophilic range.

For upscaling, improvements regarding the packing material, recirculation rate, and nutrient supply are necessary. Moreover, the enrichment procedure could be revised to include more than one methanogen. As can be seen in [27], from which the inoculum was obtained to perform the experiments in the present study, the archaeal population contained *Methanobacterium* sp. and *Methanobrevibacter* sp. at almost equal relative abundance proportions (21.5% and 26%, respectively) initially. Within 15 days of the enrichment, *Methanobacterium* reduced to 12.4%, and reached 2.1% after 170 d. On the contrary, *Methanobrevibacter* increased abruptly to 85.7% within 15 d and finally prevailed at 97.9% after 170 d. Therefore, a feeding pattern different from the fed-batch mode selected to perform the enrichment could impose less stress on the archaeal population and sustain both genera. Despite the higher GRT compared to other studies, the system's stability (in the absence of VFAs) is noteworthy since it is essential for full-scale processes. Moreover, the advantage of performing biological upgrades at mesophilic conditions is that energy savings can be made if there is no thermal energy available from the combined and heat power (CHP) unit (this happens in case the biomethane is not processed in CHP but valorized in other ways). Besides the volume of biogas entering the TBR, one should consider the volume of hydrogen too; the total gas flowrate entering an upgrading unit in a 1 MW biogas plant is approximately 33,000 $m^3\ d^{-1}$ (11,000 $m^3\ d^{-1}$ biogas of 50% CH_4 and 22,000 $m^3\ d^{-1}$ H_2), which needs heating to reach the operation temperature. In the long term, the advantages of a stable process at the mesophilic temperature range may counterbalance the higher CAPEX of a larger bioreactor.

Upscaling should also consider the utilization of real biogas and nutrient mixtures. The presence of H_2S at high levels may inhibit the upgrading process and it would be beneficial to remove it before feeding the biogas to the upgrading bioreactor. Moreover, adding digestate as a source of nutrients would influence the microbial population, the effect of which on the performance needs further investigation.

Table 3. Overview of ex situ biological upgrading of biogas in TBRs under mesophilic or thermophilic conditions.

Packing Material	Feeding H_2:CO_2:CH_4 *1 H_2:CO_2:	Inoculum	Nutrients	Methanogens Identified (Genus Level)	V (L)	T (°)	RR (L L_R^{-1} d^{-1})	GRT (h)	VFAs (g L^{-1})	CH_4 (%)	Net MPR (L_{CH4} L_R^{-1} d^{-1})	Ref
PUF—560–580 m^2 m^{-3}	3.7:1:1 3.7:1	Enriched	Pasteurized cow manure	*Methanobacterium* *Methanoculleus*	0.29	52	50 *2	0.57	n	90%	8.54 *3	[20]
PUF—12 layers	62:15:23 4.12:1	Enriched from [40]	Cow manure digestate	*Methanobacterium*	0.8	54	5.4 5.4 0.84 *4	4 2 2	n 2.17 n	97 52 95	0.83 0.62 1.73	[21]
MBBR PE08 >3200 m^2 m^{-3} 76% void ratio	61.4:16.2:22.4 3.8–3.9:1	Digestate	Synthetic (commercial solutions)	nr	2 × 1000	52–57	16.8	2.1	2	97.4	10.6	[29]
HX09 11.2 m^2 total	4:1:0 4:1	nr	Nr	nr	14.5 *5	40 55	60 *6	2.79 2.85	nr	88 95	8.48 8.59	[41]
RFK 25 L (313 m^2 m^{-3}) and HXF12KLL (859 m^2 m^{-3})	3.78–4:1:0 3.78–4:1	Anaerobic sludge from sewage treatment plant	synthetic	nr	58.1	55	4.13	Nr *7	Nr *8	98.5	15.4	[36]
HXF12KLL	59.1:15.1:25.8 3.9:1	Anaerobic sludge from sewage treatment plant	synthetic	nr	1000	53–56	180 *10	0.7	2.12	98.2	3.96 *9	[30]
PUF	Variable *11 4:1	Enriched	Digested municipal biowaste	*Methanobacter* *Methanothermobacter*	68	52	1.2 *12	5	0.4–1.2	98.5	nr	[35]
Glass rings	62:15:23 4.13:1	Enriched	Digestate	*Methanothermobacter*	1	54	2.38 *13	2	0.3–0.65	94.9–95.1	1.71–1.74	[34]
Hiflo rings 15–7 313 m^2 m^{-3} 91% void ratio	36–42:58–64 *14 3.67–4.15:1	Anaerobic mixed liquor	Synthetic	nr	58	37	6.21	2.3	<0.1	>97	2.52	[26]
Bioflow 40 305 m^2 m^{-3}	4:1:0 4:1	Anaerobic sludge from sewage treatment plant	Synthetic	nr	61	37	6.15	4	nr	98	1.49	[25]
Linpor polyurethane 2000 m^2 m^{-3} 97% void ratio and Xingfeng PE-10 polyethylene 1200 m^2 m^{-3}	55:13.7:31.3 4:1	Anaerobic sludge from sewage treatment plant	Synthetic	*Methanobacter* *Methanobrevibacter*	8	38	5.4	25–5	<0.4	>95	nr	[31]
Kaldnes K1 800 m^2 m^{-3}	59.7:16.1:24.2 3.7:1	Enriched *Methano-brevibacter*	Synthetic	nr	1.25	39	4	7.2	0	90.9	0.56	This study
Sintered glass 1600 m^2 m^{-3}					2	39	4	4	0	93.1	0.84	

PUF—polyurethane foam; RR—recirculation rate of liquid; n—negligible; nr—not reported; *1 or N_2; *2 50 mL per min for 3 min; *3 at standard pressure and temperature conditions; *4 once per day; *5 operating pressure 5 bar; *6 60 L per h for 1 min twice per day; *7 the pH fell below 7; *8 the HLR was 62.1 L_{H2} L_R^{-1} d^{-1}; *9 as suggested by the authors: 6.1/1.54; *10 180 L/h; *11 depending on the biogas composition; *12 1.2 L per min once a day; *13 2.38 L L_R^{-1} d^{-1} for 30 s every 30 min; *14 biogas composition CO_2:CH_4.

5. Conclusions

The inoculum used for this study was enriched under mesophilic conditions in methanogens (*Methanobrevibacter* accounting for 97% of the archaeal population and 60% of the total population) and had a strong tendency to attach to surfaces. The best result for mesophilic biological biogas upgrading was achieved in a TBR filled with sintered glass balls and operated with a GRT of 4 h, resulting in the production of a biomethane mixture with a high CH_4 content of 93.08 ± 0.39%. Based on the feeding mixture, which provided an H_2:CO_2 ratio of 3.7:1, the maximum achievable CH_4 content was 97%. The mesophilic process demonstrated stability with no VFA production even under high loading rates (GRT: 2.2 h), where the performance deteriorated, suggesting that there was no imbalance in the metabolic reactions, but the process could be rather limited by mass transfer, trace metal/nutrient availability, or even the archaeal population of the inoculum, which was not versatile. This finding implies that the performance of the biogas upgrading process under mesophilic conditions can be further improved through proper reactor engineering and enrichment procedures to obtain an inoculum with more than one archaeal genus.

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Appendix A

Table A1. Nutrient medium composition.

Substance	Concentration (mg L^{-1})
NH_4Cl	1000
$MgCl_2 \cdot 6H_2O$	100
$CaCl_2 \cdot 2H_2O$	50
$K_2HPO_4 \cdot 3H_2O$	400
EDTA	5
$FeCl_2$ $4H_2O$	1
H_3BO_3	0.1
$ZnCl_2$	1
$CuCl_2$	0.1
$MnCl_2 \cdot 4H_2O$	5
$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	0.1
$AlCl_3$	0.1
$CoCl_2 \cdot 6H_2O$	1
$NiCl_2$	0.2
H_2SeO_3	0.01
$NaHCO_3$	1.56

Table A2. GRTs during the operation of the bioreactors.

Time (d)	GRT (h)	Comment
	BR	
0–12	14.4	Starting condition
13–82	23	GRT increase to recover from low performance
83–317	16.4	GRT decrease
318–355	12.7	GRT decrease
356–367	7.2	Final GRT decrease—operation termination
	TBR-P	
0–7	14.4	Starting condition
8–45	10.3	GRT decrease
46–63	7.2	GRT decrease
64–103	4	GRT decrease
104–117	18	GRT increase to recover from low performance
118–133	10.3	GRT decrease
134–149	7.2	GRT decrease
150–169	6	GRT decrease
170–195	24	GRT increase due to practical difficulties to operate the bioreactor at low GRT
196–212	14.4	GRT decrease
213–224	10.3	GRT decrease
225–236	7.2	GRT decrease
237–259	5.5	GRT decrease
260–268	4	Final GRT decrease—operation termination
	TBR-S	
0–14	16.5	Starting condition
15–41	11.6	GRT decrease
42–67	29	GRT increase due to practical difficulties to operate the bioreactor at low GRT
68–81	14.4	GRT decrease
82–94	10.5	GRT decrease
95–105	7.2	GRT decrease
106–129	5.5	GRT decrease
130–140	4	GRT decrease
141–157	2.8	GRT decrease
158–166	2	Final GRT decrease—operation termination

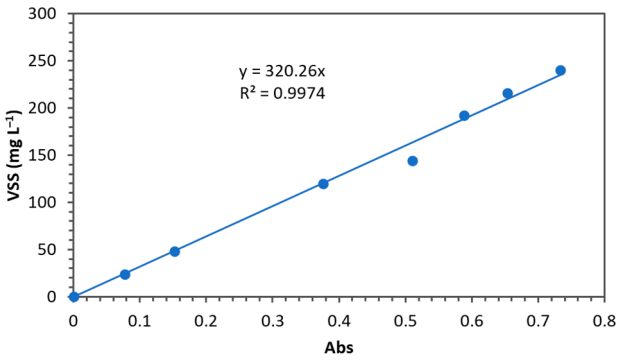

Figure A1. Calibration curve for VSS estimation based on spectrophotometry.

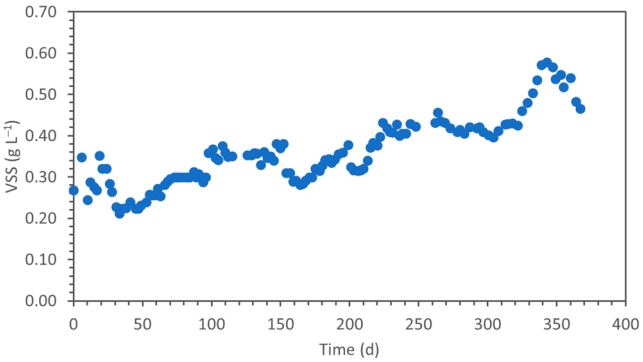

Figure A2. VSS concentration in the BR.

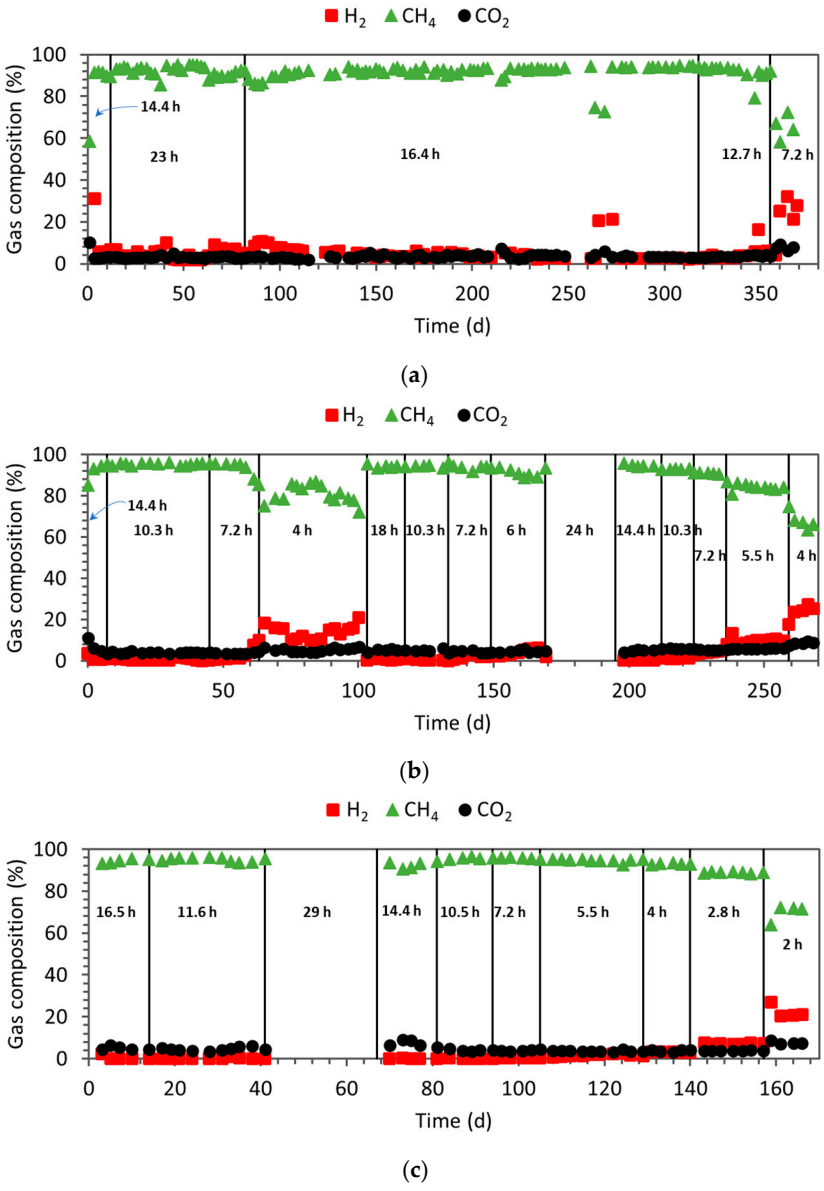

Figure A3. Biomethane gas composition produced from the (**a**) BR, (**b**) TBR-P, and (**c**) TBR-S under different GRTs.

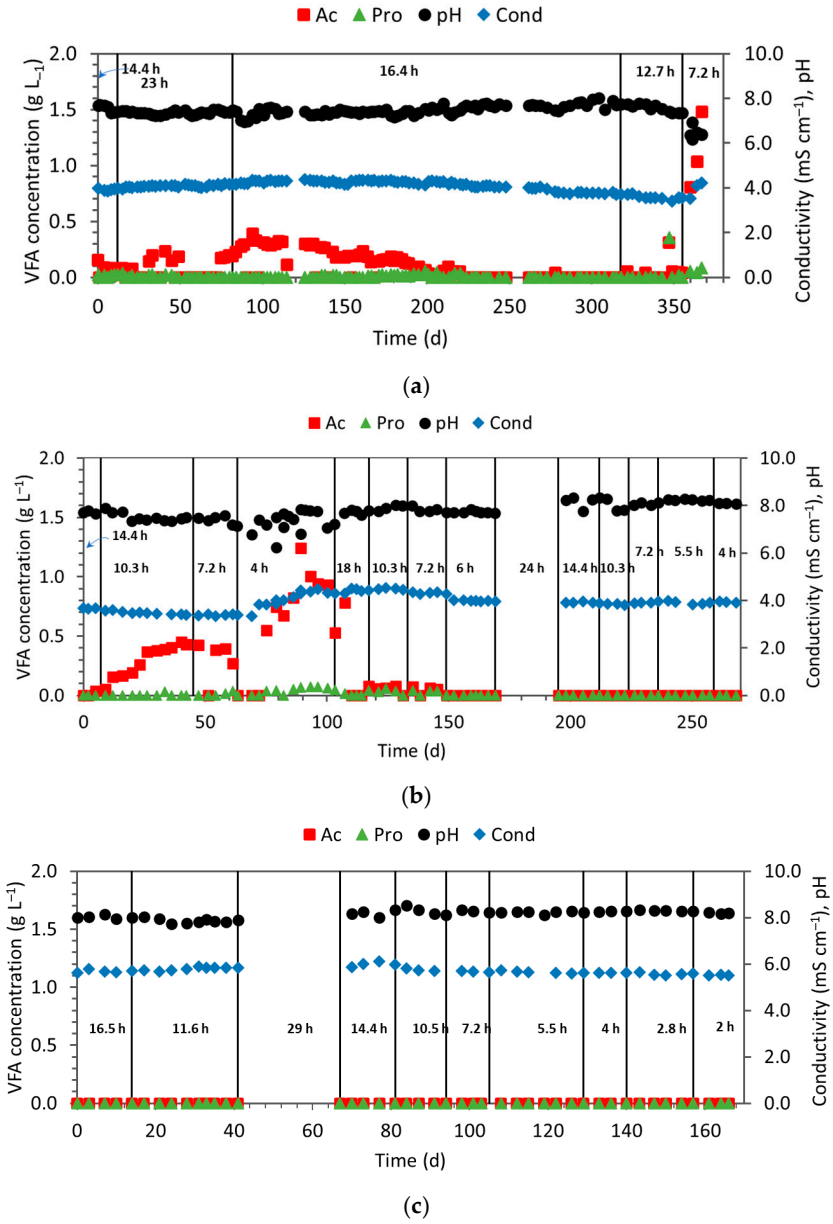

Figure A4. VFA concentration, conductivity, and pH in the (**a**) BR, (**b**) TBR-P, and (**c**) TBR-S under different GRTs.

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