

Article

Performance Evaluation and Optimization of Ex Situ Hydrogen Biomethanation in a Mesophilic Fed-Batch Reactor

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Abstract

Ex situ hydrogen biomethanation represents a promising approach to converting surplus renewable electricity into CH₄-rich gas through the biological reduction in CO₂ with H₂, mediated by hydrogenotrophic methanogens. In this study, an ex situ H₂ biomethanation process was investigated in a lab-scale mesophilic anaerobic reactor operated in fed-batch mode. The system followed a cyclic operational strategy comprising sequential gas feeding, reaction, and discharge phases. Hydrogen was supplied through a pressure-controlled feeding strategy, whereas CO₂ injection maintained dissolved CO₂ concentrations at 25, 17, and 2 mg L⁻¹ during the initial, intermediate, and final stages, respectively. During early operation, volatile fatty acids (VFAs) temporarily accumulated to 3 g L⁻¹, accompanied by a decrease in pH. Progressively lowering the dissolved CO₂ target restored process stability, reduced the VFA concentration to 618 mg L⁻¹, and increased the pH to 7.6. Under stable final-stage conditions, the reactor achieved an average CH₄ concentration of 93.3%, a hydrogen utilization efficiency of 99%, and a methane evolution rate (MER) of 3.95 NL CH₄ L_{VR}⁻¹ d⁻¹. These results show that combining pressure-controlled hydrogen injection with dissolved CO₂ regulation enhances methane production and maintains stable ex situ biomethanation.

Keywords: renewable energy storage; pressure-controlled hydrogen injection; dissolved CO₂ regulation; hydrogenotrophic methanogenesis; methane evolution rate

1. Introduction

Renewable energy capacity has grown rapidly in response to climate change and increasing global energy demand. However, the intermittent nature of renewable electricity remains a major limitation, as production from wind and solar fluctuates depending on weather conditions and temporal availability [1,2]. The Power-to-Gas (P2G) concept offers a route to store surplus renewable electricity by converting it into gaseous energy carriers, such as H₂ or CH₄, which can be integrated with existing energy infrastructure [3,4]. Among these options, Power-to-Methane (PtM) is particularly attractive because H₂ can be biologically or catalytically converted with CO₂ into CH₄, a gaseous fuel with higher volumetric energy density than hydrogen and wider compatibility with existing gas systems [5].

However, it presents significant challenges due to limited infrastructure for H₂ storage and transportation. Consequently, the most promising approach for H₂ utilization and storage is the Power-to-Methane (PtM) method, in which H₂ is combined with CO₂ to



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produce CH₄ via the Sabatier reaction (Equation (1)). The methane produced can be injected directly into the natural gas distribution grid to substitute fossil fuels, providing a calorific value (CH₄ ≈ 35.8 kJ NL^{−1}) higher than (H₂ ≈ 10.8 kJ NL^{−1}) on a volume basis [5].

PtM technologies are categorized into two main pathways: catalytic and biological methanation. In the catalytic methanation pathway, typically, methane is synthesized from CO₂ and H₂ using catalysts such as nickel or ruthenium [6]. Catalytic methanation is characterized by high reaction rates, compact reactor design, and high methane selectivity. However, this process operates at elevated temperatures (200–600 °C) and pressures (up to 40 bar), leading to high energy consumption and degradation of catalyst stability over time [7,8]. In contrast, biological methanation occurs in mesophilic (38 °C) or thermophilic (50–65 °C) conditions and atmospheric pressure, facilitated by methanogenic microorganisms, making it a more energy-efficient and sustainable alternative. Nevertheless, its performance can be limited by gas–liquid H₂ mass transfer, lower volumetric productivity, and sensitivity to changes in microbial operating conditions [9].

Biological methanation can be implemented through two configurations: in situ and ex situ processes, both aimed at increasing the CH₄ content above 90% [10,11]. In in situ biomethanation, H₂ is directly injected into the anaerobic digester, where methanogens convert CO₂ to CH₄ while degrading organic substrates. In contrast, in ex situ biomethanation, H₂ and CO₂ react in a separate reactor by hydrogenotrophic methanogens; however, the additional bioreactor increases capital costs and may limit economic feasibility [12,13].

A key advantage of the ex situ configuration is the improved control over H₂ injection, as hydrogen is supplied to a dedicated reactor rather than directly into the anaerobic digester [13]. This allows better regulation of H₂ partial pressure, minimizing disturbances to syntrophic microbial interactions and limiting changes in the CO₂/HCO₃[−] equilibrium and pH, thereby enhancing process stability and controllability compared to in situ systems [14]. Furthermore, ex situ systems favor the hydrogenotrophic pathway (Equation (1)), which provides a higher Gibbs free energy yield for methane formation than acetolactic methanogenesis (Equation (2)) [15].



In recent years, invaluable efforts have been made to better understand the performance of ex situ biomethanation to improve CH₄ production, highlighting that operational parameters such as temperature, pH, pressure, and reactor configuration critically affect microbial activity and overall methane yield [13,16]. Among these factors, temperature and pH are particularly important because they influence the balance between hydrogenotrophic methanogenesis and competing pathways such as homoacetogenesis, in which homoacetogenic bacteria convert H₂ and CO₂ into acetate [17]. This pathway competes directly with hydrogenotrophic methanogens for the same substrates, potentially reducing methane production efficiency and promoting volatile fatty acid accumulation, which can affect pH stability and overall reactor performance [18].

Temperature plays a key role in shaping the competition between hydrogenotrophic methanogens (HM) and homoacetogens (HAC). Laguillaumie et al. reported that at 35 °C, acetate formation via HACs was not detected, with *Methanobacterium* species dominating, whereas at 25 °C, HACs prevailed under favorable mass-transfer conditions [18]. Other studies have shown that mesophilic conditions can support hydrogenotrophic methanogenic activity by maintaining favorable microbial kinetics and enabling effective H₂ consumption under stable pH conditions [19]. In contrast, lower temperatures may slow methanogenic activity and create conditions in which HACs can compete more

successfully for H_2 and CO_2 , leading to acetate formation [20,21]. Similarly, pH directly affects microbial activity, enzyme function, and pathway stability. Most methanogenic microorganisms exhibit optimal growth within the pH range of 6 to 8, which ensures maximal enzymatic activity and methane production. pH values outside this range may reduce methanogenic activity, alter microbial community structure, and promote the buildup of volatile fatty acids (VFAs), all of which can destabilize the process [22].

Beyond these physicochemical factors, reactor configuration plays a crucial role in biomethanation. The performance of different types of reactors has been investigated, including continuous stirred tank reactors (CSTRs), plug-flow, bubble column, and immobilized bed bioreactors (IBBR) [23,24]. Previous studies indicate that fed-batch operation can be advantageous because it improves process control and gas–liquid transfer efficiency, while avoiding the high energy demand typically associated with CSTRs [25,26]. However, gas–liquid mass-transfer resistance, particularly the limited dissolution of H_2 in the liquid phase, remains a key rate-limiting factor in ex situ biomethanation and highlights the need for pressure-based control strategies. Nevertheless, fed-batch operation under controlled mesophilic and pressurized conditions remains insufficiently investigated.

Hydrogen availability is a critical constraint in ex situ biomethanation because H_2 must transfer from the gas phase to the liquid phase before becoming available to hydrogenotrophic methanogens. This process is commonly described using Henry's law and the two-film mass-transfer model [27]. According to the solubility form of Henry's law, the equilibrium dissolved hydrogen concentration can be expressed as $C_{H_2}^* = H_{s,H_2} p_{H_2}$; where H_{s,H_2} is the H_2 solubility constant and p_{H_2} is the gas-phase hydrogen partial pressure. The volumetric gas–liquid transfer rate (r_{H_2}) can be described by $r_{H_2} = k_{La}(C_{H_2}^* - C_{H_2})$ where k_{La} is the volumetric gas–liquid mass-transfer coefficient and C_{H_2} is the actual dissolved H_2 concentration in the liquid phase. Therefore, increasing p_{H_2} increases the equilibrium dissolved H_2 concentration and the mass-transfer driving force, whereas k_{La} is primarily governed by hydrodynamic conditions, including mixing intensity, gas flow rate, bubble size, interfacial area, and liquid properties [9,28].

This study evaluated ex situ H_2 biomethanation in an anaerobic, completely mixed fed-batch reactor operated under mesophilic conditions (38 °C) and within a pressure range of 1100–1400 mbar(a). The reactor employed an integrated control strategy in which pressure thresholds regulated H_2 feeding and headspace gas discharge, while CO_2 addition was adjusted based on continuous measurements of dissolved CO_2 in the liquid phase. The pressure-based H_2 control was designed to enhance H_2 dissolution and minimize residual H_2 in the outlet gas, whereas dissolved CO_2 regulation enabled the progressive control of inorganic carbon availability and pH stability. Unlike conventional ex situ systems operated with a fixed gas composition, this approach allowed substrate availability to be adjusted dynamically during reactor operation. Since CO_2 is considerably more soluble in water than H_2 , the system could be operated at relatively low and stable CO_2 partial pressures while maintaining methanation performance.

Previous fed-batch ex situ biomethanation systems have demonstrated promising methane-upgrading performance; however, gas injection and headspace discharge were generally performed at fixed time intervals, independently of process dynamics and substrate consumption rates, as reported by Voelklein et al. [13] and Thapa et al. [29]. In addition, these systems were commonly operated under atmospheric pressure and fixed $H_2:CO_2$ feeding ratios, often with continuous gas recirculation during each batch cycle. In contrast, the present study employed a pressurized, completely mixed fed-batch reactor in which combined pressure-regulated H_2 feeding and gas discharge with dissolved CO_2 regulation allowed substrate availability to be adjusted according to process dynamics rather than fixed feeding conditions.

The novelty of this study therefore lies in integrating pressure-driven H₂ feeding, pressure-triggered gas discharge, dissolved CO₂ regulation, and process-stability assessment under progressively decreasing inorganic carbon availability. It was hypothesized that dissolved CO₂ regulation would support pH stability by maintaining adequate carbonate buffering and thereby limiting excessive VFA accumulation, while pressure-based H₂ control would enhance methane production and hydrogen utilization.

2. Materials and Methods

2.1. Reactor Setup

The ex situ H₂ biomethanation system was designed to operate as a fed-batch anaerobic reactor. It was structured into two main functional compartments: a fully mixed and biologically active liquid phase, and a gaseous phase. The reactor was operated throughout the experimental period under mesophilic conditions (38 ± 1 °C), using an electric resistance heater controlled by a thermostat. A schematic diagram of the reactor setup and its associated laboratory-scale equipment is presented in Figure 1.

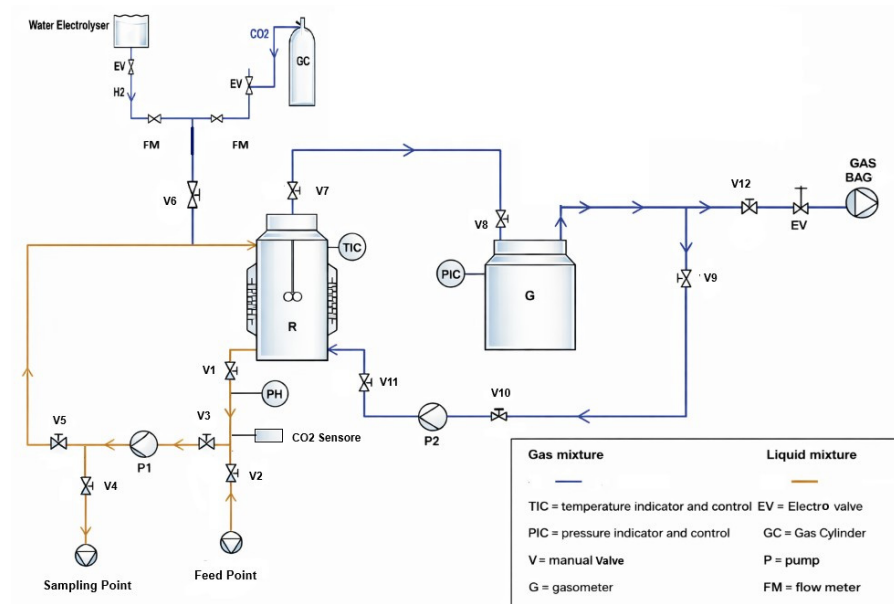


Figure 1. Schematic representation of the ex situ H₂ biomethanation set-up at a laboratory scale.

The anaerobic reactor (R) consisted of a 2.6 L DURAN[®] glass bottle (DWK Life Sciences GmbH (formerly part of the DURAN Group/SCHOTT, Mainz, Germany)) fitted with a GL 80 cap containing five GL 15 ports, with a liquid working volume of 1.4 L. The liquid phase was continuously recirculated through a dedicated tubing circuit using a SEAFLO membrane pump for liquid aspiration and recirculation. This configuration ensured complete and homogeneous mixing of the reactor content. The remaining reactor volume, not occupied by the liquid phase, constituted the headspace, forming part of the gaseous phase. The gas was partially stored in a 0.3 L auxiliary bottle acting as a gasometer (G, Figure 1), which was connected to the main reactor through a tubing system. The gasometer acted as a separation device for removing condensate and foam generated in the digester during the process, while protecting the membrane pump used for gas recirculation. Gas recirculation was ensured by a membrane pump manufactured by KNF (KNF Neuberger GmbH, Freiburg-Munzingen, Germany), operating continuously. This configuration enabled gas transfer and recirculation between the biological reactor and the auxiliary volume, allowing control of the gas-phase composition and ensuring effective gas mixing.

The gas supply system consisted of two red-y Vögtlin® mass flow controllers, enabling independent dosing of hydrogen and carbon dioxide, and an electro-valve used for controlled discharge of the methane-rich product gas. Hydrogen was generated by a water electrolyzer (purity grade 4.0, $O_2 < 1$ ppm, according to manufacturer specifications), while carbon dioxide (purity grade 5.0) was supplied from a compressed gas cylinder (SIAD S.p.A, Bergamo, Italy). Both gases were delivered to the reactor at an operating pressure of 2 bars.

Dissolved CO_2 concentration was continuously monitored using a Hamilton CO_2 NTROL RS485 probe (Hamilton Bonaduz AG, Bonaduz, Switzerland), an optical sensor based on mid-infrared absorption, according to the manufacturer's specifications. Both gases were introduced into the system through the liquid-recirculation line, downstream of the mixing pump and the in-line sensors installed along the circuit.

2.2. Substrate and Inoculum Media

The reactor was inoculated with digested sludge obtained from a full-scale anaerobic digester located at the wastewater treatment plant of Castiglione Torinese (SMAT S.p.A., Turin, Italy). The collected sludge was stored at 4 °C before use. Before reactor inoculation, the sludge was centrifuged at 5000 rpm for 15 min to separate the solid and liquid fractions. Subsequently, 50% of the recovered solid fraction was remixed with the corresponding centrifuged liquid to prepare the reactor inoculum, thereby partially retaining active biomass while reducing the solids content to prevent clogging of the recirculation lines. During reactor operation, the system was fed daily with the centrate obtained by centrifugation of digestate produced by a laboratory-scale anaerobic digester, without external additives. The total solids (TS), volatile solids (VS), and other physicochemical characteristics of the digestate used in this study as inoculum and reactor feedstock are summarized in Table 1. The analytical procedures used for inoculum and feedstock characterization are provided in the Supplementary Method S2.

Table 1. Main characteristics of the inoculum and feedstock.

Parameters	Inoculum	Reactor Feedstock
TS (mg L ⁻¹)	12,200 ± 600	1300 ± 400
VS (mg L ⁻¹)	8050 ± 210	610 ± 150
pH	7.96	8.07
FOS/TAC	0.11	0.09
NH ₄ ⁺ -N (mg L ⁻¹)	1311 ± 24	731.7 ± 18.0
VFAs (mg L ⁻¹)	320 ± 14	259 ± 13
tCOD (mg L ⁻¹)	13,360 ± 339	1425 ± 254
sCOD (mg L ⁻¹)	547 ± 32	473 ± 18

2.3. Operating Conditions

At the beginning of the process, after inoculation, the reactor was purged with nitrogen (purity grade 4.0) for 5 min to remove oxygen from the gas phase and establish anaerobic conditions favorable for methanogenic microorganisms. No scheduled purge specifically intended to remove N₂ or other inert gases was performed after reactor start-up. Nevertheless, the pressure-triggered discharge phase partially renewed the headspace during each cycle and removed a fraction of the inert gases potentially present. The system was operated in a sequential fed-batch mode consisting of repeated operational cycles involving gas injection, biological conversion, and gas discharge.

The gas–liquid mixture was continuously recirculated into the reactor and distributed through a ceramic diffuser located at the bottom of the digester. This configuration enhanced hydrogen dissolution and its availability in the liquid phase, thereby improving substrate accessibility for hydrogenotrophic methanogens and promoting the efficient biological conversion of H_2 and CO_2 into methane. Throughout the experiments, the reactor was operated within an absolute pressure range of 1100–1400 mbar(a). This range was selected to enhance gas–liquid mass transfer by increasing the hydrogen partial pressure and, consequently, hydrogen solubility in the liquid phase according to Henry's law, while maintaining moderate energy demand and safe operating conditions. Since the reactor was designed to operate at pressures up to approximately 2 bar(a), the applied pressure range ensured stable and safe performance under laboratory-scale conditions.

The minimum operating pressure of 1100 mbar(a) marked the beginning of each cycle. The control system then initiated hydrogen injection until the maximum pressure of 1400 mbar(a) was reached, after which the H_2 supply was stopped. Meanwhile, CO_2 dosing was dynamically controlled using real-time measurements of dissolved CO_2 in the liquid phase. Specific dissolved CO_2 setpoints (25, 17, and 2 mg L^{-1}) were defined for each operational stage, and a hysteresis-based control strategy was applied to regulate CO_2 injection around the selected operating reference. The setpoints of 25 and 17 mg L^{-1} were adopted as the initial and intermediate operating conditions, respectively, whereas the subsequent reduction to 2 mg L^{-1} was introduced following the VFA accumulation observed during Stage II, to reduce the acidifying contribution of molecular dissolved CO_2 and promote pH recovery.

The relationship between molecular dissolved CO_2 concentration and the corresponding equilibrium gas-phase partial pressure was estimated using the solubility form of Henry's law. Based on a CO_2 solubility constant of 0.0246 mol L^{-1} bar $^{-1}$ at 38 °C, dissolved CO_2 concentrations of 25, 17, and 2 mg L^{-1} correspond to nominal equilibrium CO_2 partial pressures of approximately 23.1, 15.7, and 1.85 mbar, respectively. These values were not selected as universal biological thresholds derived from the literature, but as sequential operating conditions used to evaluate reactor performance under progressively lower molecular dissolved- CO_2 availability. This definition refers specifically to molecular CO_2 (aq) and does not imply limitation of the total inorganic carbon pool, which also includes HCO_3^- and CO_3^{2-} and depends on pH and alkalinity. In addition, regulating dissolved CO_2 concentration enabled indirect control of reactor pH, thereby preventing excessive alkalinization commonly associated with hydrogen injection during ex situ biomethanation [19].

This strategy enabled the reactor response to be evaluated under progressively lower molecular dissolved- CO_2 conditions while limiting excessive fluctuations around the selected operating references. This operating strategy linked gas injection to reactor pressure and gas-consumption dynamics, allowing an H_2 : CO_2 molar ratio close to 4:1 to arise from the process rather than being imposed as a fixed control parameter. This ratio is consistent with the stoichiometric requirements of hydrogenotrophic methanogenesis ($4 \text{ mol } H_2 + 1 \text{ mol } CO_2 \rightarrow 1 \text{ mol } CH_4 + 2 \text{ mol } H_2O$), which defines the theoretical optimum for complete substrate conversion.

Pressure thresholds regulated H_2 injection, whereas CO_2 dosing was controlled independently through the dissolved- CO_2 operating references. This configuration allowed the two gas feeds to respond to different process signals while limiting excessive H_2 accumulation and maintaining the selected dissolved- CO_2 condition. During biomethanation, the consumption of gaseous substrates resulted in a gradual decrease in gas-phase pressure. Since the gas volume remained constant and part of the water vapor condensed due to supersaturation, the pressure decrease was accompanied by a progressive increase in methane

concentration in the gas phase. When the pressure decreased to 1175 mbar(a), corresponding to a 225 mbar(a) reduction from the maximum operating pressure (1400 mbar(a)), the control system initiated the discharge of the produced gas. The discharge valve was subsequently closed once the pressure reached 1100 mbar(a), thereby starting a new operational cycle (Figure 2).

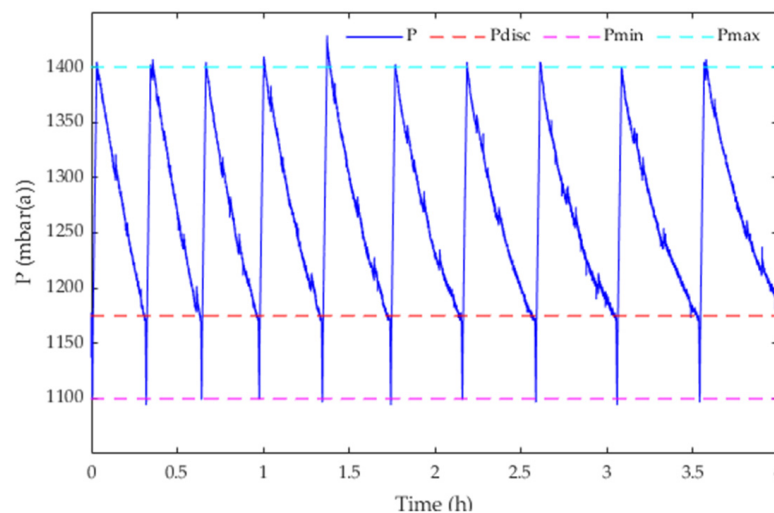


Figure 2. Trend of pressure (mbar(a)) over time.

The produced gas was collected in 10 L Tedlar[®] bags. Daily gas production was calculated as the sum of the gas volume recovered in the bags and the residual gas volume present in the gasometer at the end of each measurement interval. To ensure uninterrupted operation over weekends, three 10 L bags were installed every Friday to accommodate the expected gas production. Starting from Monday, daily measurements of the produced gas volume and its volumetric composition were carried out for all cycles occurring during that day.

Regarding liquid-phase operation, the reactor was operated in a sequential fed-batch regime with an average hydraulic retention time (HRT) of 10 days to minimize biomass washout. Digestate withdrawal and replenishment were adjusted during weekdays to maintain a constant working volume throughout the experiment. Although liquid feeding was temporarily interrupted during weekends, continuous gas supply and recirculation were maintained. The potential influence of this discontinuous feeding regime on reactor stability and VFA accumulation was considered during data interpretation. Both the feed hopper and the digestate discharge valve were integrated into the liquid recirculation circuit, which also housed the sensors for pH and dissolved CO₂ monitoring.

All process parameters were monitored and controlled through an integrated automation system. Sensors for the online measurement of temperature, dissolved CO₂ concentration, and pH were directly connected to a PC via the Modbus RS-485 communication protocol. The analog pressure sensor and gas-discharge valve were connected to and controlled through an Arduino board, which was also interfaced with the same PC.

The mass flow controllers used for hydrogen and carbon dioxide injections were also connected to the PC through the Modbus RS-485 communication protocol. The entire monitoring and process control system, including the Arduino-based hardware, was managed by a MATLAB (R2024a)-based control script that ran continuously in a loop to enable real-time supervision and automatic regulation of operating conditions. All measured data were recorded, and the control script is provided as Supplementary Materials.

The MATLAB code provided as Supplementary Method S1 corresponds to the control script used during Stage III. The same control structure was used during Stages I and II, with the dissolved-CO₂ reference and the corresponding switching thresholds adjusted to 25 and 17 mg L⁻¹, respectively. The numerical interval implemented around the 2 mg L⁻¹ reference represented a switching condition within the control logic and should not be interpreted as the analytical accuracy or as a validated control tolerance. According to the manufacturer's specifications, the sensor measurement range is 5–1000 mbar CO₂, corresponding to approximately 7.5–1500 mg L⁻¹ in water at 25 °C and 101.3 kPa. The nominal accuracy at 25 °C is ±5 mbar within the range of 5–100 mbar and ±5% at values above 100 mbar. The Hamilton CO₂NTROL RS485 sensor was calibrated once per week according to the manufacturer's instructions, and the dissolved-CO₂ signal was acquired by the control system at a frequency of 1 Hz. Because the 2 mg L⁻¹ reference was below the stated lower measurement range, this value was treated as a nominal control reference defining a low-dissolved-CO₂ operating regime rather than as an accurately quantified concentration. The operating conditions used throughout the experiment are reported in Table 2.

Table 2. Summary of operational conditions of the reactor.

Reactor Configuration	Temp (°C)	Inoculum	Injected Gas	Working Volume (L)	HRT (d)	Pressure Range (mbar(a))
FB-STR	38	Digestate Sludge	H ₂ + CO ₂	1.4	10	1400–1100

FB-STR = Fed-batch stirred-tank reactor.

The experimental period was structured into three operational stages to evaluate reactor performance under progressively lower molecular dissolved-CO₂ operating references. This stepwise strategy avoided abrupt changes in the control reference and allowed the response of the reactor to decreasing molecular dissolved-CO₂ availability to be assessed. Stage I (Days 1–18), with the dissolved CO₂ setpoint maintained at 25 mg L⁻¹, represented the start-up period and focused on activating and acclimating the hydrogenotrophic methanogenic community. Stage II (Days 19–38), with the CO₂ setpoint lowered to 17 mg L⁻¹, served as the adaptation stage, during which the system response to dynamic H₂/CO₂ feeding and pressure-based control was evaluated. Stage III (Days 39–58), operated using a nominal dissolved-CO₂ reference of 2 mg L⁻¹, corresponded to the stable operation stage and allowed the assessment of methane-production stability.

2.4. Gas and Liquid Analysis

At the end of each feeding–conversion cycle, the decrease in reactor pressure to the discharge threshold triggered the release of the produced gas. Because discharge was controlled by total pressure rather than by confirmed complete H₂ depletion, residual hydrogen could remain in the headspace and discharged gas.

The produced gas was then discharged, and fresh process gases were introduced to start the next cycle. Total daily gas production was calculated from the sum of the volume collected in the gas bag and the volume remaining in the gasometer. All measured volumes were normalized to standard temperature and pressure conditions (273.15 K, 1 atm). Daily gas samples were analyzed in duplicate to ensure accuracy and reproducibility. The composition of the produced gas was analyzed using a micro gas chromatograph (Fusion micro-GC system with three columns). The system was equipped with a Backflush 1.0 µL Rt-Molsieve 5A column (10 m) for the quantification of H₂, O₂, N₂, CH₄, and CO, and an Rt-Q-Bond column (12 m) for CO₂ analysis.

Liquid-phase samples were collected daily to measure VFAs and alkalinity (expressed as mg CaCO₃ eq/L) using the Fos/Tac method (HACH LANGE). These parameters were monitored daily to ensure effective reactor operation and process control. The pH was measured daily using a pH meter (Thermo Orion 4 Star, Beverly and Waltham, MA, USA).

All parameters mentioned above were measured in duplicate.

2.5. Analytical Methods and Calculations

2.5.1. Methane Evolution Rate

The MER quantifies the volume of methane produced over a specified timeframe relative to the reactor volume and is calculated using Equation (3).

$$\text{MER} = \frac{Q_{\text{gas}} \times \text{CH}_4}{V_R \times 100} \quad (3)$$

where Q_{gas} is the total daily normalized biogas volume expressed daily (NL d⁻¹), CH₄ is the methane concentration in the produced gas (% v/v), and V_R is the liquid reactor volume (L).

2.5.2. H₂ Utilization Efficiency

H₂ utilization efficiency was calculated as follows:

$$\eta_h = \left(1 - \frac{R_{H \text{ out}}}{R_{H \text{ in}}}\right) \times 100 \quad (4)$$

η_h : Hydrogen Utilization efficiency (%), $R_{H \text{ in}}$: Total hydrogen supplied to the system (mL/L_{VR}/d), $R_{H \text{ out}}$: Discharged hydrogen (mL/L_{VR}/d).

3. Results and Discussion

Reactor performance and stability were evaluated throughout the experimental period by monitoring the MER (NLCH₄ L_{VR}⁻¹ d⁻¹) and outlet gas composition, shown in Figure 3. The operational cycle profile presented in Figure 2 showed cycle durations ranging from approximately 0.5 to 1 h, depending on microbial activity and gas consumption rates. Each cycle consisted of three sequential phases: (i) gas injection, (ii) biological conversion under sealed conditions, and (iii) gas release. Variations in cycle duration reflected changes in the net rate of gas consumption and were therefore used as an operational indicator of reactor activity rather than as a pathway-specific measure of microbial conversion efficiency.

3.1. Stage I—Start-Up Phase (Microbial Acclimation)

The start-up phase was conducted at a dissolved CO₂ setpoint of 25 mg L⁻¹ to support the initial microbial acclimation and establish stable biomethanation activity. During Days 1–7, the reactor showed unstable performance, characterized by variable hydrogen feeding and limited methane production, as expected under start-up conditions. This behavior is consistent with a start-up period during which stable H₂-consuming methanogenic activity had not yet been established. Therefore, data from the first seven days were excluded from the subsequent performance analysis.

After approximately day 8, the reactor performance began to stabilize, with methane concentration increasing to around 75% and the MER reaching approximately 2 NLCH₄ L_{VR}⁻¹ d⁻¹. During this transitional period, MER still exhibited noticeable fluctuations, ranging between 2 and 4.6 NLCH₄ L_{VR}⁻¹ d⁻¹ (Figure 3A). The observed fluctuations in MER and the residual H₂ concentration in the outlet gas are consistent with incomplete stabilization of H₂ conversion during the early operational period, resulting in lower methane concentrations and higher residual H₂ in the discharged gas [26]. Although the observed increase in

MER was temporary, methane production progressively stabilized during the following operational period. By the end of the stage, methane concentration in the outlet gas stabilized at approximately 83%. Residual hydrogen accounted for about 12% of the outlet gas composition, corresponding to an overall hydrogen utilization efficiency of approximately 97% (Figure 3B). Applying Equation (4) to the cumulative supplied and discharged H_2 volumes of 249.78 and 8.20 NL, respectively, yielded a hydrogen utilization efficiency of 96.72%. The 12% residual H_2 represents the instantaneous volumetric composition of the discharged gas and is therefore not directly complementary to the hydrogen utilization efficiency, which is based on the cumulative supplied and discharged H_2 volumes. At this relatively high dissolved CO_2 level (25 mg L^{-1}), the pH and alkalinity profiles did not indicate severe inorganic-carbon limitation or loss of buffering capacity during the acclimation period.

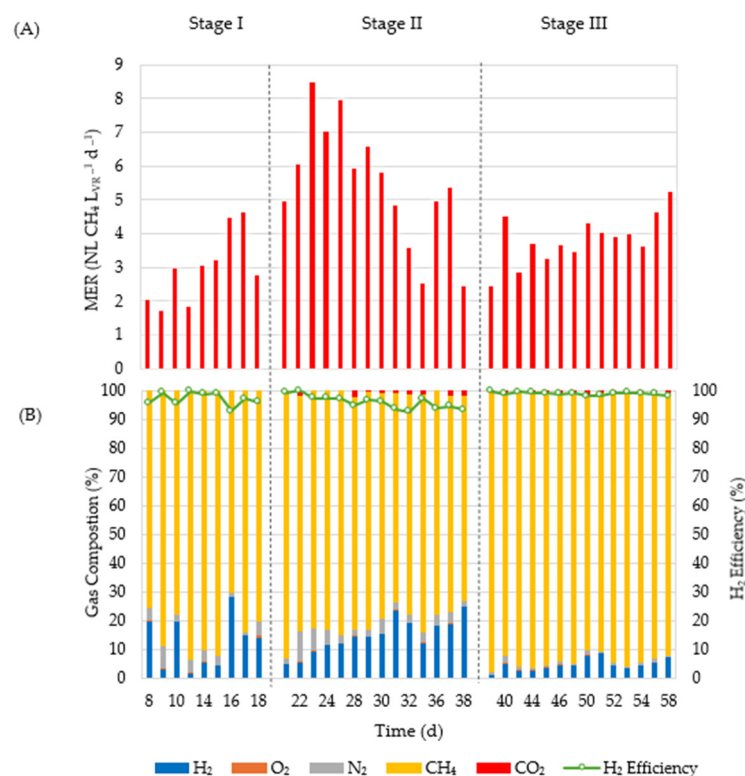


Figure 3. Methane evolution rate (A), Gas concentration, and H_2 utilization efficiency (B).

During this stage, the total VFA concentration remained relatively low, averaging $412 \pm 222 \text{ mg L}^{-1}$. This moderate level indicated that start-up was not accompanied by the excessive accumulation of organic intermediates and supported the overall stability of the reactor [22,30]. Acetic acid was the dominant VFA, accounting for approximately 56% of the total concentration (229 mg L^{-1}), followed by propionic acid at 24% (97 mg L^{-1}) and isovaleric acid at 15% (62 mg L^{-1}) (Figure 4). The average pH during this stage was approximately 7.31 (Figure 5C), which remained within a suitable range for methanogenic activity.

As shown in Figure 6, total alkalinity (TAC) decreased progressively, accompanied by a decline in bicarbonate concentration. This pattern indicated gradual depletion in the bicarbonate-buffering capacity, potentially because of biological CO_2 conversion and changes associated with microbial acclimation. Consequently, the FOS/TAC ratio gradually increased but generally remained below 1.0, suggesting that sufficient alkalinity and buffering capacity were still maintained despite moderate VFA accumulation and temporary fluctuations in the methanogenic activity. The relatively stable pH observed

throughout this period further confirms that bicarbonate alkalinity effectively prevented severe acidification.

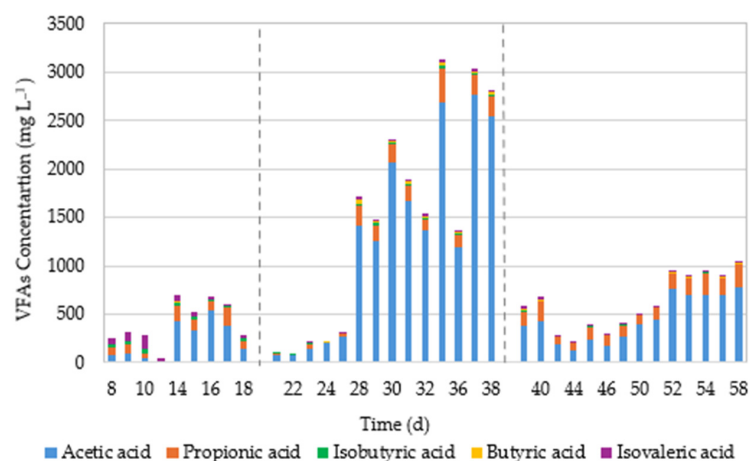


Figure 4. Profile of VFAs over the experimental period.

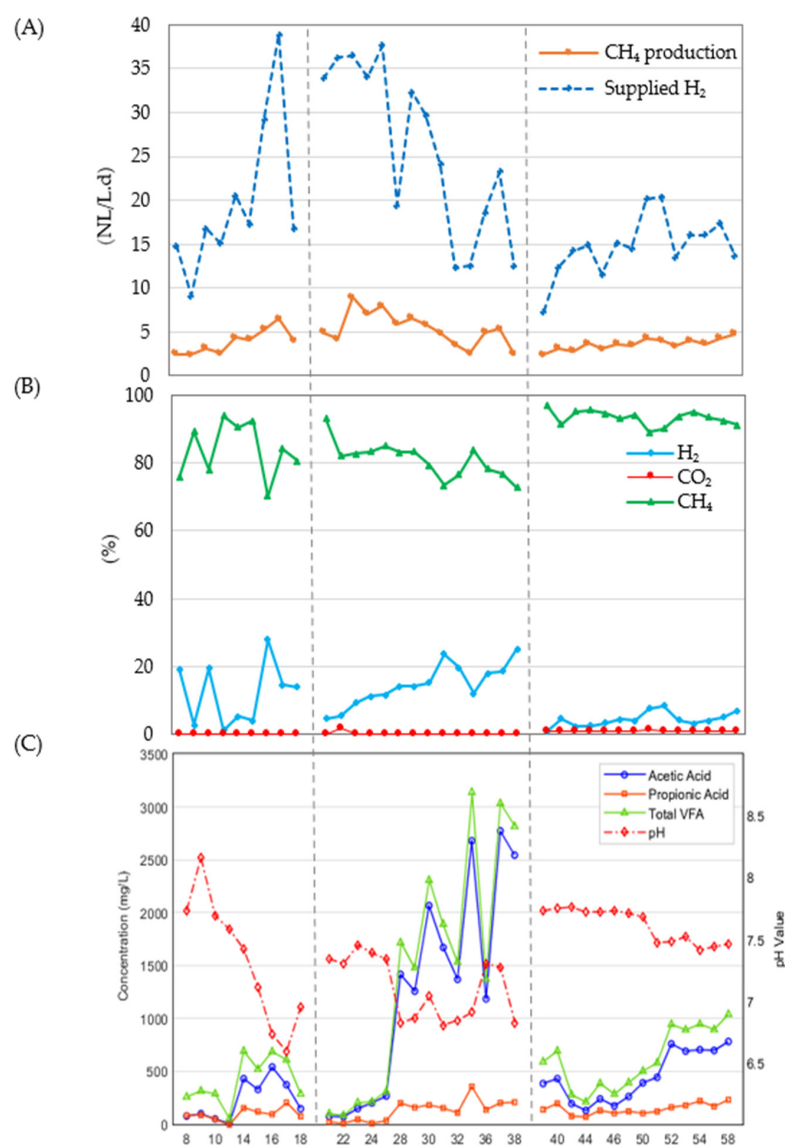


Figure 5. Methane production along with supplied hydrogen (A); Gas composition of CH₄, H₂, and CO₂ (B); Volatile fatty acid content and pH (C).

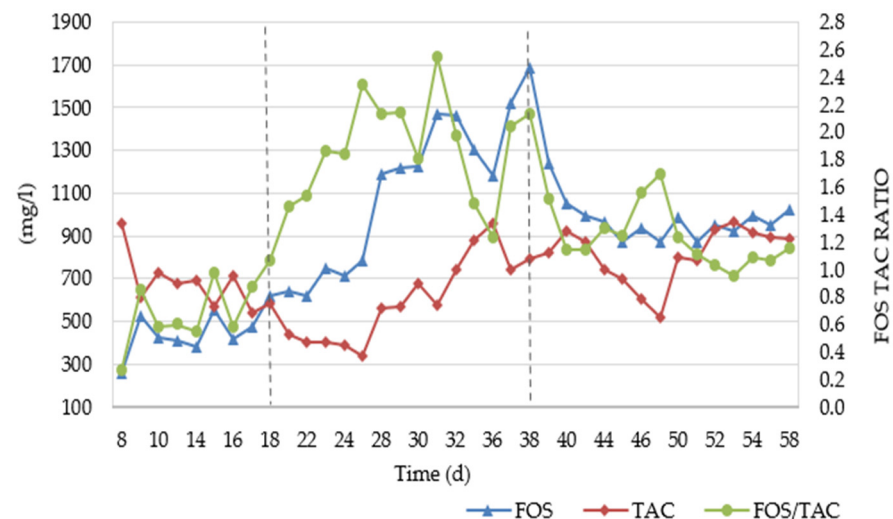


Figure 6. Variation in FOS, TAC, and FOS/TAC ratio in the reactor outflow.

Overall, CH_4 production stabilized during Stage I without evidence of severe VFA accumulation or acidification. Following this acclimation period, dissolved CO_2 concentration was reduced to 17 mg L^{-1} , marking the transition to Stage II reactor operation.

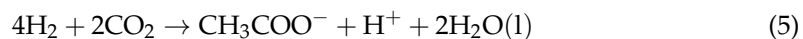
3.2. Stage II—Adaptation Phase

During Stage II (days 19–38), the dissolved CO_2 concentration was maintained at 17 mg L^{-1} . Under this lower setpoint, the MER initially increased as H_2 loading, and CH_4 production rose. As shown in Figure 5A, during Stage II, the H_2 loading rate averaged $29.2 \pm 9.6 \text{ NL H}_2 \text{ L}_{\text{VR}}^{-1} \text{ d}^{-1}$. A peak daily value of $34.5 \text{ NL H}_2 \text{ L}_{\text{VR}}^{-1} \text{ d}^{-1}$ was recorded on Day 23 and coincided with a peak MER of approximately $8.5 \text{ NL CH}_4 \text{ L}_{\text{VR}}^{-1} \text{ d}^{-1}$, while the CH_4 concentration remained around 83%. Following this peak, the H_2 loading rate decreased, while the MER gradually declined and stabilized between 5.5 and $6.0 \text{ NL CH}_4 \text{ L}_{\text{VR}}^{-1} \text{ d}^{-1}$. Although CH_4 productivity remained relatively high, its concentration in the outlet gas decreased progressively to approximately 79.4%, accompanied by a reduction in H_2 utilization efficiency to 96.18% (Figure 3B).

The decrease in CH_4 concentration coincided with the accumulation of VFAs, particularly acetic and propionic acids, together with a temporary decrease in pH (Figure 5C). These changes suggest that the reactor experienced a temporary imbalance between VFA-forming and methane-forming processes during Stage II [25]. A clear manifestation of this effect occurred during the weekend, when liquid feeding and digestate withdrawal were interrupted, whereas H_2 and CO_2 supply and gas recirculation continued. During this period, the liquid phase therefore operated under batch conditions.

As a result, total VFA concentration increased from 310 mg L^{-1} on Day 25 (Friday) to 1717 mg L^{-1} on Day 28 (Monday), as shown in Figure 5C. Simultaneously, the pH decreased from above 7.3 to 6.9, indicating temporary acidification and reduced buffering capacity. Because no liquid exchange occurred during the weekend, the observed concentration increase may have resulted from both net VFA formation and the absence of VFA dilution and removal. The increase cannot therefore be attributed exclusively to homoacetogenesis. Nevertheless, continued gas feeding, decreasing pH, and possible pH-related inhibition of methane-forming activity may have allowed competing H_2 -consuming pathways, including homoacetogenesis, to contribute to acetate accumulation [31]. This interpretation remains a mechanistic hypothesis because the activities of the relevant microbial groups were not directly measured.

Elevated VFA concentrations persisted throughout stage II, reaching a peak value of 3136 mg L⁻¹ on day 35. The marked increase in acetate concentration is consistent with a possible contribution of homoacetogenic activity, according to the following reaction:



The FOS and TAC profiles also indicated temporary process instability. As VFA accumulation intensified, FOS increased substantially, while TAC decreased or fluctuated, causing the FOS/TAC ratio to exceed 2.0 (Figure 6). This increase closely corresponded with reductions in pH and CH₄ concentration, suggesting that acid formation temporarily exceeded the methanogenic conversion capacity of the reactor. The elevated FOS/TAC ratio therefore indicated stress on the bicarbonate-buffering system and reduced process stability during Stage II.

Although changes in VFA concentration coincided with variations in MER and outlet-gas composition, these temporal relationships do not identify the responsible metabolic pathway. Methane and acetate formation could affect the pressure-controlled cycle differently because of the different stoichiometry and phase partitioning of their products. Methane has low aqueous solubility and rapidly contributes to the gas phase, whereas acetate remains predominantly in the liquid phase under the operating conditions [31].

The corresponding net reduction in the dry-gas inventory is approximately four moles. By contrast, homoacetogenesis consumes four moles of H₂ and two moles of CO₂ and produces acetate and water, both of which remain predominantly in the liquid phase. Its corresponding net reduction in the dry-gas inventory is therefore 6 moles. These calculations assume that consumed dissolved CO₂ is replenished from the gas supply. For an equivalent amount of H₂ consumption, acetate formation could therefore produce a greater decrease in total reactor pressure than methanogenesis [28].

Gas discharge was triggered when the total reactor pressure reached 1175 mbar(a), rather than after confirmed depletion of H₂. A more rapid pressure decrease could therefore cause the discharge threshold to be reached after a smaller amount of H₂ had been consumed, resulting in a higher residual H₂ fraction in the headspace and discharged gas. This mechanism could have contributed to the lower CH₄ concentration and higher residual H₂ fraction observed during Stage II and may also have affected the duration and frequency of the operational cycles. However, it does not independently explain the variation in MER and cannot be considered direct evidence of homoacetogenesis. The increase in MER is therefore reported as an operational observation rather than being mechanistically attributed to acetate formation.

This mechanistic interpretation is consistent with previous studies showing that H₂ partial pressure, gas–liquid mass transfer, and thermodynamic constraints can affect the competition between hydrogenotrophic methanogenesis and homoacetogenesis [13]. However, neither time-resolved gas-phase H₂ partial pressure nor dissolved H₂ concentration was measured in the present study. Dissolved H₂ availability depends not only on gas-phase H₂ partial pressure, but also on gas–liquid mass transfer, the volumetric mass-transfer coefficient (kLa), reactor hydrodynamics, and the biological H₂ uptake rate [9]. Consequently, the Gibbs free energy of the competing pathways could not be determined under the actual reactor conditions. The weekend effect should therefore be interpreted as the possible combined result of continued gas supply, interruption of liquid feeding and digestate withdrawal, absence of VFA dilution and removal, pH decreases and possible pH-related inhibition, microbial kinetics, gas–liquid mass transfer, and competition among H₂-consuming pathways. The individual contributions of these factors could not be resolved from the available data.

Previous studies have reported that when the total VFA concentration exceeds approximately 1500 mg L^{-1} , metabolic pathways may shift toward alternative hydrogen-consuming routes such as homoacetogenesis [32]. In the present system, the rapid increase in acetate concentration coincided with a decrease in pH to about 6.9, slightly below the optimal range for methanogenic activity. This decrease in pH indicates temporary buffering limitations and mild acidification of the liquid phase [30]. At the same time, the CH_4 concentration decreased from approximately 83% to 79%. This pattern was consistent with temporarily reduced methanogenic performance and the accumulation of liquid-phase intermediates. Nevertheless, these measurements do not establish carbon partitioning between the gas and liquid phases or quantify the relative contributions of hydrogenotrophic methanogenesis, homoacetogenesis, and other biological pathways.

To restore pH stability and limit further acetate accumulation, dissolved CO_2 regulation was modified as a corrective operational measure. The nominal setpoint was reduced from 17 to 2 mg L^{-1} , marking the transition from Stage II to the low-dissolved- CO_2 operating conditions of Stage III.

3.3. Stage III—Stable Operation Phase

Following the reduction in the dissolved CO_2 setpoint from 17 to 2 mg L^{-1} , the reactor progressively recovered, as indicated by increases in pH, CH_4 concentration, and H_2 utilization efficiency, together with a decrease in VFA concentration. The 2 mg L^{-1} value should be interpreted as a nominal control reference defining a low-dissolved- CO_2 operating condition. Because this value was below the sensor's validated measurement range, the system could not verify that dissolved CO_2 was accurately maintained at 2 mg L^{-1} during Stage III.

From a stoichiometric and thermodynamic perspective, the reduction in dissolved CO_2 availability may have affected both hydrogenotrophic methanogenesis and homoacetogenesis, because CO_2 is a substrate for both pathways. However, homoacetogenesis requires 2 mol of CO_2 per mole of acetate produced, whereas hydrogenotrophic methanogenesis requires 1 mol of CO_2 per mole of CH_4 produced. On a stoichiometric basis, lower CO_2 availability may therefore have constrained acetate formation more strongly than methane formation. However, this difference alone does not establish the relative thermodynamic competitiveness of the two pathways. This interpretation could not be quantitatively verified because dissolved H_2 concentration was not measured and the Gibbs free energy of the competing reactions could not be calculated under the actual reactor conditions. It cannot therefore be concluded that homoacetogenesis has become thermodynamically unfavorable.

The transition to the low-dissolved- CO_2 operating condition was accompanied by a decrease in total VFA concentration from 2800 mg L^{-1} at the end of Stage II to about 618 mg L^{-1} during Stage III (Figure 5C). The average acetic acid concentration decreased to around 450 mg L^{-1} , while propionic acid declined to below 145 mg L^{-1} . At the same time, pH recovered and stabilized at 7.61. Concurrent increases in CH_4 concentration and H_2 utilization efficiency were consistent with improved methanogenic performance.

The observed process recovery likely resulted from the combined effects of lower dissolved CO_2 availability, pH recovery, reduced H_2 loading, consumption or removal of accumulated VFAs, and progressive adaptation of reactor activity. The individual contributions of these factors could not be distinguished using the available experimental data.

Under the stabilized Stage III conditions, hydrogen was supplied at an average loading rate of $15.9 \text{ NL L}_{\text{VR}}^{-1} \text{ d}^{-1}$ (Figure 5A), while the average MER reached $3.95 \text{ NLCH}_4 \text{ L}_{\text{VR}}^{-1} \text{ d}^{-1}$. This methane production rate corresponds to approximately one quarter of the hydrogen supply rate, on the same normalized volumetric basis, close to

the theoretical maximum of 25% defined by hydrogenotrophic methanogenesis. The reactor achieved a methane concentration above 93.3% and an H₂ utilization efficiency of 99.2%, indicating low residual H₂ in the product gas. Although these results demonstrate the production of a CH₄-rich gas, assessment of compliance with gas-grid or vehicle-fuel standards would require characterization of the complete gas-quality profile [13]. The operating conditions and overall reactor performance during the experimental period are summarized in Table 3.

Table 3. Summary of the ex situ H₂ biomethanation performances under three stages.

Parameter	Stage I	Stage II	Stage III
MER (NLCH ₄ L _{VR} ^{−1} d ^{−1})	2.95 ± 1.38	5.49 ± 1.85	3.95 ± 0.89
CH ₄ (%)	83.9 ± 8.2	79.4 ± 5.5	93.3 ± 2.2
H ₂ Feed Rate (NL L _{VR} ^{−1} d ^{−1})	19.8 ± 8.9	29.2 ± 9.6	15.9 ± 3.6
H ₂ Utilization efficiency (%)	97.1 ± 2.28	96.2 ± 2.25	99.2 ± 0.48
Total VFA (mg L ^{−1})	412 ± 222	1700 ± 698	618 ± 285
pH	7.31 ± 0.51	6.9 ± 0.25	7.61 ± 0.13

During this stage, the pH remained within the commonly reported range of 7.4–7.6 for methanogenic activity, supporting consistent CH₄ production [33,34]. The FOS/TAC profile (Figure 6) also indicated improved process conditions compared with Stage II. The ratio declined to an average of 1.2, reflecting partial process recovery. TAC was relatively stable and generally higher than during Stage II, suggesting the partial recovery of bicarbonate-buffering capacity. In parallel, reduced fluctuations in FOS were consistent with lower VFA accumulation and an improved balance between VFA-forming and methane-producing processes. The decrease in the FOS/TAC ratio, together with pH recovery, coincided with the overall improvement observed after transition to the low-dissolved-CO₂ condition. However, the relative effects of CO₂ regulation, reduced H₂ loading, liquid exchange, and reactor adaptation could not be separated.

3.4. Cross-Stage Performance Assessment and Study Limitations

The values reported in Table 3 were obtained from a single reactor and therefore describe temporal variability within the experimental run. Duplicate gas- and liquid-phase analyses were used to assess analytical repeatability and should not be considered independent biological replicates. Accordingly, the reported variability represents temporal and analytical variation rather than between-reactor variability.

MER is reported as the total measured methane evolution rate. Similarly, H₂ utilization efficiency quantifies the fraction of supplied H₂ that was not detected in the discharged gas and should not be interpreted as a pathway-specific H₂-to-CH₄ conversion yield. The overall average gas-phase H₂ utilization efficiency calculated from the daily experimental data was 97.3%. The maximum potential contribution of organic matter supplied with the centrate was estimated using total chemical oxygen demand (tCOD), thereby accounting for VFAs as well as other soluble and particulate organic fractions in the feed. Given a tCOD concentration of 1.425 ± 0.254 g L^{−1}, a reactor working volume of 1.4 L, and an average hydraulic retention time of 10 d, the mean liquid feed rate was 0.14 L d^{−1}. This corresponded to an organic loading rate of 0.200 ± 0.036 g COD d^{−1}. Assuming, as an upper-bound condition, that all supplied tCOD was biodegradable and completely converted into methane, the maximum methane production attributable to the centrate was 0.070 ± 0.012 NL CH₄ d^{−1}. By comparison, the measured methane production rates were 4.13, 7.69, and 5.53 NL CH₄ d^{−1} during Stages I, II, and III, respectively. The theoretical

maximum contribution of centrate-derived organic matter therefore represented 1.7%, 0.9%, and 1.3% of the measured methane production in the corresponding stages. Even under the conservative assumption of complete COD conversion, organic matter supplied with the centrate could therefore account for less than 2% of the measured methane production. Because COD is an electron-equivalent parameter, this calculation also provides an upper-bound estimate of the reducing equivalents introduced with the centrate. This estimate constrains the potential contribution of feed organic matter but does not constitute a closed carbon or electron balance.

No abiotic, inoculum-only, or feedstock-only control reactors were included. Consequently, the individual contributions of hydrogenotrophic methanogenesis, acetoclastic methanogenesis, homoacetogenesis, and endogenous metabolism could not be experimentally resolved. Nevertheless, the COD-based upper-bound estimate indicates that methane potentially derived from centrate organic matter represented only a minor fraction of the total measured production.

Biological methanation is primarily evaluated through MER and CH₄ concentration, which reflect methane productivity and product gas quality and are influenced by reactor type, operating conditions, and substrate characteristics [35]. Previous studies investigating different reactor configurations, including up-flow reactors, bubble column reactors, and continuous stirred tank reactors, have reported outlet methane concentrations typically in the range of 88–96%, as summarized in Table 4. However, systems operating under continuous or semi-continuous gas-feeding regimes, with elevated residual H₂ concentrations and significant gas–liquid mass-transfer limitations, are frequently observed. Moreover, shear forces in these reactor types may inhibit microbial growth, which can adversely affect methane purity and reduce the MER [32,33].

Table 4. Comparison of Reactor Performance: This Study vs. Previous Studies.

Type	Reactor Type	Gas Feed	Temp (°C)	VR (L)	CH ₄ (%)	MER (NLCH ₄ L _{VR} ^{−1} d ^{−1})	Reference
Ex situ	FB-STR	Intermittent cyclic H ₂ /CO ₂ feeding	38	1.4	93.3	3.95	This study
Ex situ	CSTR	Batch	35	0.69	82.7	0.065	[36]
Ex situ	BCR	Continuous	37	2	87	4.42	[37]
Ex situ	CSTR	Semi-continuous	37	5	94.8	0.36	[38]
Ex situ	CSTR	Batch	37	0.1	79	NA	[39]
Ex situ	PFR	Batch	55	17	94	2.5	[40]

VR = Liquid Reactor Volume; FB-STR = fed-batch stirred-tank reactor; CSTR = Continuously Stirred Tank Reactor; BCR = Bubble Column Reactor; PFR = Plug flow reactor; NA = MER was not reported in the original study and could not be calculated from the available data.

Previous studies have shown that intermittent H₂ supply can support flexible methane production, while standby phases allow microbial and process recovery, promote the degradation of accumulated VFAs, and help reactor conditions return toward baseline levels [34]. In the present reactor, intermittent cyclic H₂ feeding was associated with low residual H₂ concentrations and stable performance during Stage III. Comparisons with continuous and semi-continuous systems should nevertheless be interpreted cautiously because reactor configuration, gas loading, working volume, mixing conditions, and analytical procedures differed among the studies.

As summarized in Table 4, although some batch-operated systems reported slightly higher CH₄ concentrations under comparable conditions (e.g., temperature and gas feeding strategy), their MER values remained lower than those achieved in the present study.

This comparison highlights the effectiveness of the implemented operational strategy in balancing methane purity and volumetric productivity.

Notwithstanding the promising performance observed under laboratory-scale conditions, several aspects require further investigation before plant-scale implementation. Long-term operation at low dissolved CO₂ setpoints should be assessed carefully. However, a low concentration of molecular dissolved CO₂ does not necessarily correspond to low total inorganic carbon availability in the liquid phase. Total inorganic carbon depends on carbonate-system speciation, which is mainly governed by pH and alkalinity through the equilibria among CO₂ (aq), HCO₃[−], and CO₃^{2−}. Therefore, the possible occurrence of inorganic-carbon limitation for methanogenic archaea cannot be inferred from the dissolved CO₂ setpoint alone and should be assessed through the combined monitoring of total inorganic carbon, alkalinity, pH, and biological performance.

An additional limitation of the present setup was the interruption of liquid feeding and digestate withdrawal during weekends while gas supply and recirculation continued. Consequently, the transient VFA increase observed during these periods reflected both biological transformations and the absence of liquid-phase dilution and removal. Future studies should compare continuous and interrupted liquid-feeding conditions under otherwise equivalent operating conditions. Before progressing towards plant-scale implementation, a mechanistic model integrating gas–liquid mass transfer, carbonate-system speciation, pressure dynamics, microbial kinetics, and competition among metabolic pathways should be developed and validated. Such a model would help define appropriate operating ranges, assess the risk of inorganic-carbon limitation, and optimize the control strategy.

An integrated control strategy based on reactor pressure and dissolved CO₂ concentration, with pH used as a monitored and indirectly regulated process variable, does not in itself represent a substantial barrier to scale-up. Sensors, automated valves, and automation systems for monitoring and controlling these variables are mature technologies that can be implemented in high-efficiency reactors. Nevertheless, reactor configuration, pressure-cycle management, the integration of pressure, dissolved CO₂, and pH control, the energy demand associated with gas compression and recirculation, and the robustness of the control logic should be evaluated through dedicated techno-economic feasibility studies and pilot-scale experimental validation.

4. Conclusions

This study evaluated an ex situ biological H₂ methanation process in a single fed-batch mesophilic reactor operated under intermittent gas-feeding conditions without external additives. Under the tested conditions, the combined implementation of pressure-controlled H₂ injection and dissolved CO₂ regulation was associated with stable methane production during Stage III. The results support the hypothesis that the combined regulation of dissolved CO₂ concentration and pressure-controlled H₂ feeding can maintain substrate availability while limiting sustained VFA accumulation and the associated acidification of the system under the operating conditions investigated in this study.

Although temporary VFA accumulation and pH reduction were observed during Stage II, the subsequent adjustment of the dissolved CO₂ control condition was followed by the recovery of pH, VFA stability, methane concentration, and H₂ utilization. Under stabilized conditions, methane concentrations reached 93.3%, with a hydrogen utilization efficiency of approximately 99.2% and an average MER of 3.95 NLCH₄ L_{VR}^{−1} d^{−1}. The recovery of methane production, pH, and H₂ utilization after the Stage II disturbance is consistent with the restoration of methane-forming activity. However, the individual contributions of hydrogenotrophic methanogenesis, acetoclastic methanogenesis, homoacetogenesis, and other biological processes could not be experimentally separated.

Overall, the results support the laboratory-scale feasibility of the proposed operating strategy for producing methane from H_2 and CO_2 under the tested conditions.

Given that the present study was conducted at 38 °C, further investigations under thermophilic conditions (50–60 °C) are warranted to evaluate potential improvements in reaction kinetics and methane productivity. In addition, the development and evaluation of high-pressure reactor configurations (>2000 mbar) should be explored, as elevated pressure may enhance gas–liquid mass transfer and improve overall conversion efficiency. A comprehensive assessment of heat integration and energy recovery will also be essential for optimizing system-level efficiency and supporting large-scale implementation.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app16157623/s1>. The Supplementary Materials associated with this article include the MATLAB control script used for operation of the ex situ biomethanation reactor (Supplementary Method S1) and the analytical procedures used for inoculum and feedstock characterization (Supplementary Method S2).

Author Contributions: A.S.: Writing—original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. G.C.: Review and editing, Validation, and formal analysis, conceptualization, methodology. A.C.: Methodology, review and editing. B.R.: Review and editing, Methodology, formal analysis. M.Z.: Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

MER	Methane Evolution Rate
VFAs	Volatile Fatty Acids
CSTR	Continuous Stirred Tank Reactor
TS	Total Solids
VS	Volatile Solids
tCOD	Total Chemical Oxygen Demand
sCOD	Soluble Chemical Oxygen Demand
HRT	Hydraulic Retention Time
P2G	Power-to-Gas

PtM	Power-to-Methane
HM	Hydrogenotrophic Methanogens
HAC	Homoacetogenic bacteria
FOS	Volatile Organic Acids
TAC	Total Alkalinity
PFR	Plug Flow Reactor
IBBR	Immobilized Bed Bioreactor
BCR	Bubble Column Reactor

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