energies

MDPI

Communication

Lessons Learned from an Experimental Campaign on Promoting Energy Content of Renewable Biogas by Injecting H_2 during Anaerobic Digestion

Shiplu Sarker [1,*], Sander N. Wijnsma [2] and Kristian M. Lien [3]

[1] Department of Manufacturing and Civil Engineering, Norwegian University of Science and Technology (NTNU), 2815 Gjøvik, Norway
[2] Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway; sandernw@stud.ntnu.no
[3] Department of Energy and Process Engineering, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway; Kristian.m.lien@ntnu.no
* Correspondence: shiplu.sarker@ntnu.no; Tel.: +4791271077

Received: 13 June 2020; Accepted: 7 July 2020; Published: 9 July 2020

Abstract: Direct injection of H_2 to an anaerobic reactor enables biological fixation of CO_2 into CH_4 (biomethanation) and consequently boosts methane content in the produced biogas. However, there has been only a small amount of literature reporting results on this technique in a continuous reactor framework to date. To fill this gap, the present study devoted an experimental work to direct H_2 addition to a fed-batch semi-continuous reactor, where the injected H_2 concentration increased gradually (~3–30 mmol), spanning a moderate operational period of about 70 days. As the results revealed, the reactor continued anaerobic operation for each level of H_2 dosing and produced an average methane content in the biogas ranging between 65% and 72%. The exhibited biogas upgrading trend appeared to be under-developed, and thereby suggests the need for further research.

Keywords: biogas; biomethanation; H_2 injection; methane content; in situ; upgrading

1. Introduction

Fossil fuel-based energy is predicted to become obsolete in the future, given the diminishing resources and increasing population. This has led to accelerated generation of renewable energy from all available sources, e.g., wind, solar, biomass, and geothermal, among others. As a part of renewable sources, wind and solar energy are not available throughout the year and their production compared to demand does not coincide, resulting in generation of surplus or deficient energy at times [1]. Thus, the integration of a long-term storage as well as conversion of surplus electricity are indispensable requirements in incorporating these renewable resources into grid-based energy systems.

In a conventional approach, power-to-gas (P2G) technology enables conversion of electrical power to gas fuels [2]. Employing P2G, surplus electricity produced from seasonal renewable sources can be utilized to split water via electrolysis into H_2 and O_2, and subsequently H_2 synthesis into methane in the presence of CO_2 in a catalytic chemical reaction (the so-called "Sabatier process"). This approach, however, is energy intensive, less efficient ($\eta < 80\%$), and characterized by a high operating temperature (250–700 °C), high pressure, and the use of a catalyst (e.g., nickel) [2,3]. On the contrary, the bio-Sabatier process (i.e., biomethanation), mediated by archaea (i.e., a domain of single-cell microorganisms), occurs at a relatively lower temperature and normal pressure according to Equation (1) [4]:

$$CO_2 + 4H_2 = CH_4 + 2H_2O;\ \Delta G^{\circ} = -130.7\ \text{kJ/mol} \quad (1)$$

In an anaerobic digestion (AD) process (i.e., a set of complex biochemical reactions convert organic feedstocks into biogas, e.g., predominantly 60% CH_4 and 40% CO_2, under an anoxic environment and moderate temperature, e.g., 20–70 °C), bio-Sabatier proceeds through hydrogenothrophic methanogenesis (i.e., methane generation by hydrogenothrophes). Normally, less methane is produced via this route compared to acetoclastic methanogenesis (i.e., methane generation from acetic acid by acetoclasts) [5]. However, the syntrophy between methanogenesis archaea and fermentative bacteria (i.e., microbes present in the pre-methanogenesis steps), responsible for the degradation of various acids (e.g., proprionate, butarate), only becomes thermodynamically feasible if hydrogenotrophes remove H_2 quickly, which in turn depends on H_2 partial pressure [6,7]. With the objective of promoting hydrogenotrophic methanogenesis, direct injection of external hydrogen can interrupt optimum H_2 partial pressure [8], influencing syntrophy and consequently reducing pH level outside the microbes' operating limit [5], eventually causing formation of flocs, granules, and/or biofilms, or in the worst case process failure [9].

While keeping H_2 partial pressure within a safe limit is a pivotal requirement as far as process balance is concerned, mass transfer of H_2 from gas to liquid plays a vital role in effectively contributing to biomethanation [10,11], when H_2 is added directly. Among other factors, mass flow rate depends on volumetric mass flow rate as well as concentration gradient of H_2 between the different phases [12]. Previously, several efforts were made to improve mass flow rate, including varied mixing speeds [4,13], direct injection using different membranes [14–16] and alumina ceramic sponges [11], changing diffusion devices [17,18], as well as the use of modified reactor types [11,19]. It has been reported that direct injection allows bubble-free hydrogen input, greater biomass–substrate contact, less H_2 off-gasing, and even easier mass flow control [20]. Besides, direct injection eliminates the need for an additional reactor by allowing methane enrichment to proceed in the existing reactor, making the process financially compelling [4]. However, since pure H_2 is not a readily available gas and its production is associated with high cost and emissions, depending on the sources and technologies used (e.g., steam reforming, partial oxidation, biomass gasification, electrolysis, etc.), interest has been growing in the direct injection of alternative hydrogen-based compounds as well as microbial fuel cells (MFC) [12,21,22].

Biogas enrichment through bio-Sabatier by means of H_2 injection to AD is considered an easily retrofittable technology for commercial application, where the infrastructure for gas storage and connections for gas grids are easily accessible and well established. However, the commercial implementation of bio-Sabatier so far is rare or next to none [23,24]. In fact, the focus of the existing literature on bio-Sabatier is mostly based on lab or small scales, emphasizing batch mode feeding [25]. As a result, there is a lack of knowledge, experience, and R&D efforts on the viability of this approach in continuous reactor plants. Considering this, an experimental trial using a fed-batch semi-continuous reactor operated with cattle manure was carried out where externally produced H_2 with variable concentration was introduced directly to the reactor's headspace. The purpose of the work was to determine the feasible H_2 injection regime by identifying the threshold of reactor operation and using this experience to develop future experiments, focusing more on enhancing technological robustness and addressing the current challenges so that further improvement of the energetic content of the upgraded biogas can be achieved.

2. Materials and Methods

Anaerobic digestion used for the present study was carried out by means of a 5 L working volume 6.6 L bioreactor (Biostat® A, Sartorius, UK) integrated with built-in sensors for real-time automatic measurement of temperature (provided by a surrounding heating element), pH, and mixer rpm, and interfaced with a programmable logic controller (PLC) to enable user-defined customization and data acquisition. The experimental procedure involved in different processing steps is shown in Figure 1a.

At start-up, ~2000 g of inoculum and ~11 g of cattle manure (CM), collected from a food waste biogas plant (63.75° N, 11.92° E, Ecopro AS, Trondheim, Norway) and a cattle farm (63.67° N, 9.49° E, Trondheim, Norway), respectively, were added to the reactor. The feeding was given to an inlet port located at the top of the reactor (Figure 1b), where three additional ports served as options for gas release (normally open and connected with a 5 L Tedlar bag, Sigma-Aldrich, Darmstadt, Germany), liquid sampling, and gas sampling. After feeding, an anaerobic environment was established by purging the reactor with N_2 (99.99% purity, Linde-gas AS, Oslo, Norway) for 20–30 min at 4–5 bar. At this point, incubation began, and the reactor was run at constant 39 ± 1 °C (mesophilic temperature) throughout, with routine feeding of CM 3 to 4 times a week. The feeding scheme undertaken for the selected period of the experiment is listed in Table 1.

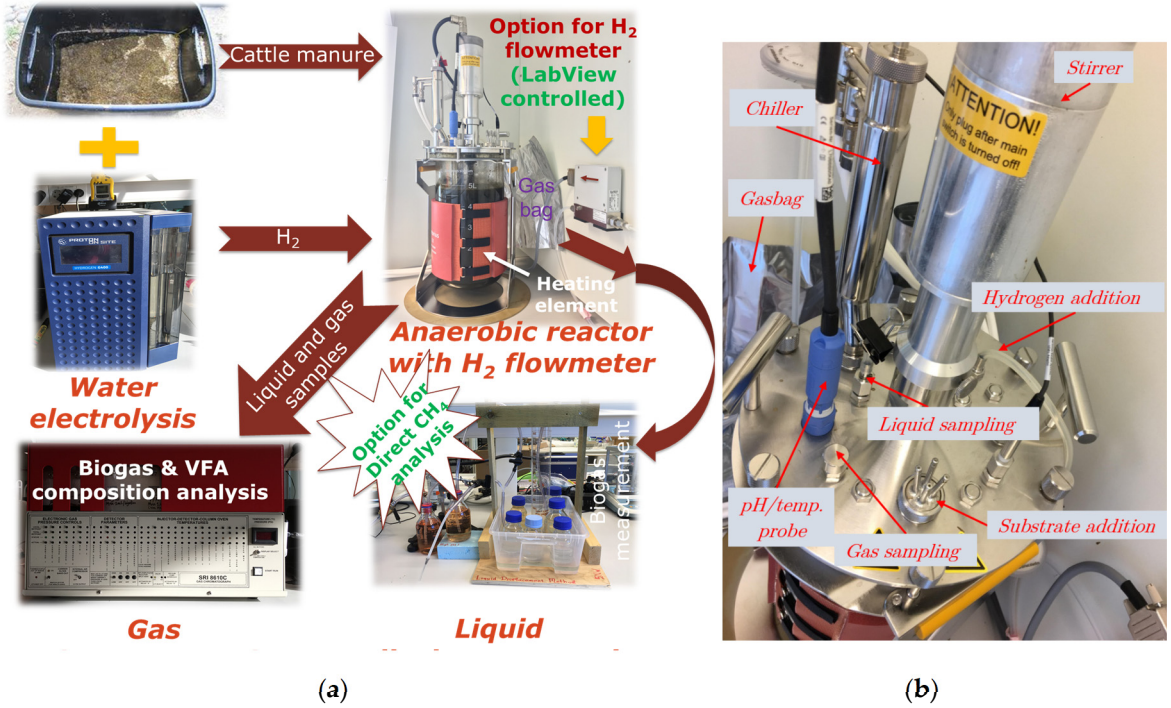

Figure 1. Experimental set-up: (**a**) different processing steps; (**b**) top view of the reactor Biostat® A showing location of different ports and sensors.

Table 1. Cattle manure (CM) feeding scheme with corresponding digester substrate to inoculum (S:I) ratios.

Day	39	40	47	49	51	53	57	61	71
CM input, g/d	64.1	71	69.3	70.5	61	71.9	69.5	64.3	72.5
S:I	2.32	2.61	3.19	3.48	3.73	4.03	4.6	5.08	6.29

As the reactor had been supplemented with CM, and there was no withdrawal of digestate, the substrate to inoculum ratio (S:I) inside the reactor accumulated and varied from 0.05 at day 1 to 6.29 at day 88 (at the end), making the mode of the reactor operation fed-batch. The S:I was calculated based on the volatile solids of substrate and inoculum, while the characterization of substrate, inoculum, and digester liquids was done using in situ facilities and an external laboratory (Eurofins AS, Trondheim, Norway), when needed (Table 2). Among the parameters analyzed, total solids (TS) and volatile solids (VS) were measured according to the protocol American Public Health Association (APHA) 2005 [26], as described by Sarker [27]; total ammonium nitrogen (TAN) using spectrophotometry (Specroquant Pharo 300 with Spectroquant® kit:1.14559.0001, Merck, Kenilworth, NJ, USA) according to the standard APHA 1995 [28]; and volatile fatty acids (VFA) using gas chromatography (Hewlett

Plackard 6890), as described by Bergland et al. [29]. pH values were obtained directly from the pH sensor measurement through PLC, and carbohydrates, lipids, and proteins were evaluated by Eurofins AS. The biogas, as produced, filled the connected gas bag (5 L Tedlar PLV bag, Sigma-Aldrich, Darmstadt, Germany), which was disconnected every time for measurement and analyzed separately for biogas quantity (calibrated at standard temperature and pressure (STP)) using liquid displacement equipment (Figure 1a), and then connected back to the reactor to enable filling until the next measurement. In parallel to biogas quantity, the quality of biogas in terms of CH_4 and CO_2 was also measured using gas chromatography (SRI 8610C, SRI Instruments, Torrance, CA, USA) (Figure 1a), analyzing samples collected regularly in glass vials of 10 mL (Apodan A/S, Hørsholm, Denmark) [27].

Table 2. Measured properties of inoculum and substrate.

Properties	Unit	Inoculum before Exp.	Substrate before Exp.
TS	wt%	0.83 ± 0.03	12.11 ± 0.07
VS	wt%	0.39 ± 0.04	9.54 ± 0.04
Carbohydrates	wt%	0.0	5.3
Proteins	wt%	<0.30	1.56
Lipids	wt%	2.18	0.93
pH	pH	7.38	7.41
TAN	mg/L	584	1590
Total VFA	mg/L	52.95 ± 1.3	5518.23 ± 19.1
Acetic acid	mg/L	n.d.	4263.85 ± 17.2
Propionic acid	mg/L	n.d.	694.18 ± 0.6
Iso-butyric acid	mg/L	n.d.	118.00 ± 0.1
n-butyric acid	mg/L	n.d.	240.38 ± 0.4
Iso-valeric acid	mg/L	n.d.	201.82 ± 1.2
n-valeric acid	mg/L	n.d.	n.d.

n.d.: not detected.

After the reactor had reached a steady state in terms of stable biogas production [30], biogas upgrading through biological conversion of CO_2 to CH_4 (bio-Sabatier process) was attempted. Accordingly, H_2 produced from an external water electrolyzer (PROTON G400, Wallingford, USA) (Figure 1a) was introduced manually into the reactor headspace through the biogas exit port using a needle and a gas-tight glass syringe (Hamilton 101 RN, Sigma-Aldrich, Darmstadt, Germany). However, an option to inject hydrogen automatically via a mass flowmeter (8711 MFC, Burkert Norway AS, Skjetten, Norway) (Figure 1a) interfaced with LabView for flow control has been under development for future campaigns.

With gradual increases in quantity (between 70 and 670 mL, or 3 and 30 mmol; Table 3), H_2 was successfully injected at 9 instances (between days 40 and 71), resulting in available stoichiometric H_2:CO_2 varying between 0.40 and 2.86. Attempts to further increase H_2:CO_2 constrained the reactor, forcing the discontinuation of H_2 injection after day 71 followed by the end of the experiment at day 88.

3. Results and Discussions

The H_2 injection strategy together with the corresponding development of H_2:CO_2, biogas amount, and methane content in the biogas are displayed in Table 3, while Figures 2 and 3 illustrate the effect of H_2 injection on biogas composition and VFA development, respectively.

After start-up, at around day 39, the biogas production stabilized, i.e., 5% variation of biogas yield between the two/three successive days [30], with average biogas yield of 525 mL/d, methane content of ~69%, pH of 7.2, and total VFA of 500 mg/L corresponding to a cumulative S:I of 2.5. At this point, the "Batch 1" (Table 3) of H_2 was introduced through the biogas exit port, which was then closed so that no H_2 could potentially leak through the system unreacted. About 24 h after the H_2 augmentation, the biogas port was opened, immediately causing the produced biogas to fill the connected gas bag. At the same time, the digester liquid was sampled and the real-time temperature, pH, and rpm values

from the PLC display were recorded. As measured, the biogas yield, methane content, pH, and VFA after 24 h of H_2 addition amounted to the following: 591 mL/d, 72%, and ~450 mg/L, respectively. The biogas yield and methane content at this point improved compared to the steady state without H_2 supplement. This indicates that the small doses of H_2 did not inhibit the digestion process, and since the target was to gradually achieve a headspace H_2:CO_2 molar ratio of 4:1, an increased dose of H_2 was supplemented at the second instance at day 41 and onward using the same protocol of injection and samplings as "Batch 1".

Table 3. H_2 injection strategy with corresponding methane content in biogas (methane content values after 24 h of H_2 injections are highlighted with underlined numbers).

H_2 Injection Batch	Day	H_2 Injection Amount, mL	H_2 Injection Amount, mmol	H_2:CO_2	CH_4 Content in Biogas	Biogas Amount, mL/d
Batch 1	39	70.0	3.12	0.40	69%	525
Batch 2	40	95.8	4.27	0.76	72%	591
	41	-	-	0.76	72%	590
Batch 3	47	100.0	4.46	0.27	62%	
	48	-	-	0.27	65%	666
Batch 4	49	200.0	8.92	0.62	68%	
	50	-	-	0.62	65%	778
Batch 5	51	300.0	13.38	0.71	63%	
	52	-	-	0.71	72%	686
Batch 6	53	400.0	17.85	0.97	65%	
	54	-	-	0.97	67%	690
Batch 7	57	300.0	13.38	1.11	67%	
	58	-	-	1.11	65%	493
Batch 8	61	400.0	17.85	1.78	65%	
	62	-	-	1.78	65%	391
Batch 9	71	670.0	29.89	2.86	69%	
	72	-	-	2.86	64%	325

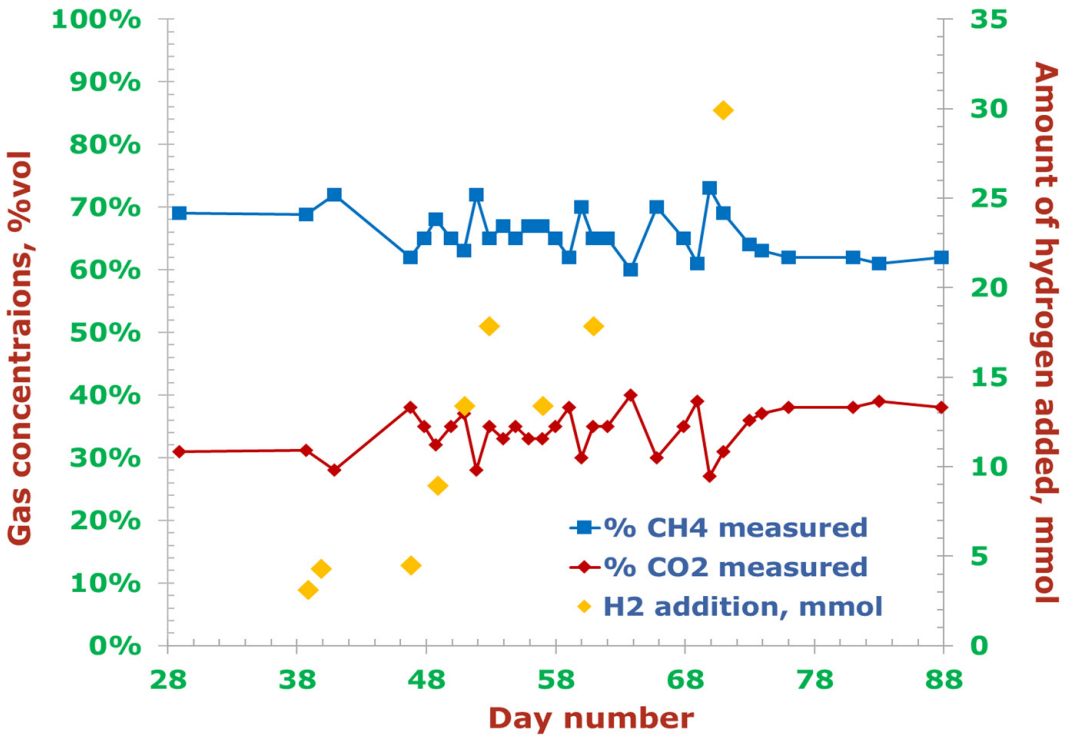

Figure 2. Effect of H_2 injection on biogas composition (*v*/*v*) in terms of standard temperature and pressure (STP) normalized CH_4 and CO_2 contents.

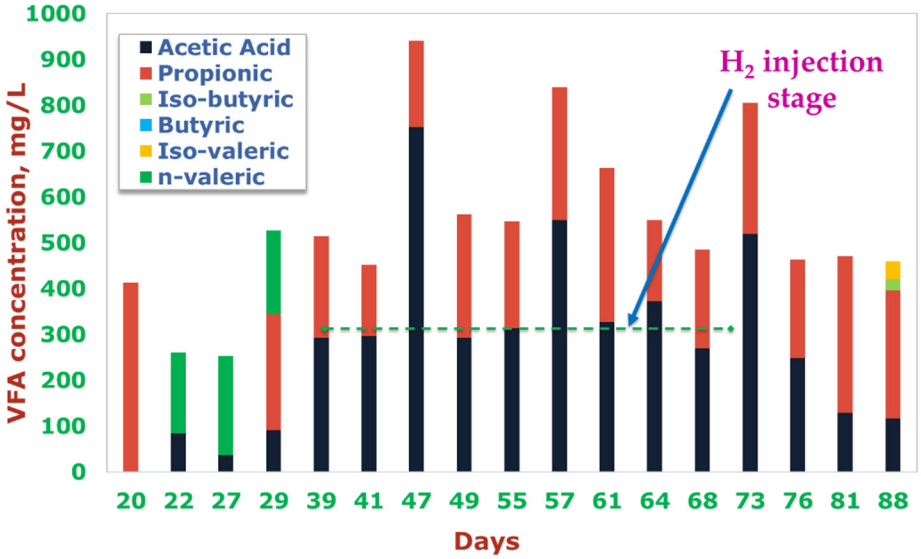

Figure 3. Effect of H_2 injection on the digester's volatile fatty acids (VFA) development. The VFA data presented in this graph correspond to the values at sampling days on which analyses were conducted, and hence should not be interpreted as the actual VFA effect on those days. The day-specific actual VFA effect corresponding to the experimental progress might remain undetected, as the interval between the measurements was high and the analysis results obtained were dependent on an external lab. Regardless, the VFA formation trend is clearly demonstrated.

Measured biogas yield and methane content after "Batch 2" (Table 3) injection remained almost constant since the "Batch 1" with 590 mL/d and 72%, respectively, indicating that the increased H_2 feeding did not contribute to improving the spontaneity of hydrogenotrophic methanogenesis, and hence the methane content. Meanwhile, the VFA and pH at this stage settled to an average of 480 mg/L and 7.1, respectively. The stable pH implies that the process presumably was not affected by the hydrogen partial pressure. However, since the H_2 was input directly to the reactor headspace, liquid mass transfer might not be effective, resulting in poor hydrogen utilization efficiency, and in turn no effect on methane content improvement. Jensen et al. [16] reported that mass transfer of hydrogen is more dependent on injected bubble size and distribution than on the amount of H_2 injected. Their computational fluid dynamics (CFD) modeling results demonstrated that smaller H_2 bubble sizes dispersed through the reactor more evenly, resulting in greater contact area and consequently enhanced gas–liquid mass transfer. The injected H_2 bubble sizes and distribution however were not investigated and therefore could not be confirmed in the present context.

While the biogas yield, methane content, and pH after H_2 addition during "Batch 2" remained almost constant, surprisingly, total VFA formation almost doubled near to the H_2 feed during "Batch 3" (Table 3). This perhaps was due to the slight substrate overloading, as S:I input kept increasing (Table 1) [7,31], which ultimately resulted in imbalance between acedogenesis and methanogenesis and/or acetogenesis and methanogenesis. However, since the level of developed VFA was still below 1 g/L and the pH remained stable at around 7.2, the increased VFA neither compromised the reactor stability nor was it directly correlated to the elevated level of H_2 feed.

After "Batch 3" and "Batch 4" (Table 3) injections, which made H_2:CO_2 increase from 0.27 to 0.61, the biogas yield improved from 666 to 778 mL/d, while methane content kept constant at 65%. This again implies that external H_2 did not have any influence in enriching biogas with enhanced methane. However, the increased biogas yield at this stage may indicate that the reactor experienced improved biodegradability [32] as a result of the available S:I feed, i.e., between 3.19 and 3.48 (see Table 1), which could also be correlated with the reduced VFA level (~550 mg/L; Figure 3).

"Batch 5" to "Batch 7" (Table 3) of H_2 addition resulted in a steady decline of biogas yield from around 686 to 493 mL/d with corresponding downfall of methane content from 72% to 65%, respectively.

In contrast, the VFA concentration increased (~820 mg/L; Figure 3), which however was less likely to contribute to any process imbalance [7]. Despite the increased H_2 loading, the decreased biogas yield at this stage maybe attributed to overdosed S:I, while the methane content might be correlated with weaker methanogenesis spontaneity, either caused by inefficient H_2 mass transfer or by thermodynamic instability [33]. In fact, during this period of operation, foam/scum formation on the top of the reactor fluid was observed to have overgrown regardless of a constant mixing at 100 rpm. Although this occurs frequently with cattle manure AD [34], the enlarged foam layer reduces the possibility of efficient H_2 mass transfer by reducing close contact between feed and microorganisms and also with H_2. With the H_2 injection strategy undertaken in the current work, this phenomenon was likely to be aggravated at the back end of the experiment, resulting in compromised biogas yield as well as the formation of methane.

Further declines in methane content (i.e., from 65% to 64%) and biogas yield (i.e., from to 325 mL/d) were evidenced after "Batch 9" (Table 3) injection, although VFA content tended to stabilize (Figure 3) and pH kept constant at ~7.2, as normal. It can thus be concluded that step-wise increase of H_2 concentration coupled with elevated S:I might lead to decreased process performance as a result of multiple phenomena associated with a poor hydrogen utilization efficiency [35], elevated residual substrate accumulation [36], and decreased activity of acetogens or methanogens [37]. Nevertheless, the reactor did not show any sign of collapse during and after the H_2 injection stint and kept producing biogas with reasonable methane content (Figure 2) for the whole duration of the experiment. However, as there was not a great deal of methane content improvement during the H_2 input phase, reaching a H_2:CO_2 level of 4:1 as per the original target was found to be irrelevant, causing H_2 injection to stop at day 71 with a H_2:CO_2 of 2.86. Approaching a H_2:CO_2 of 4:1 and more thus remains the scope of future experiments to be developed based on the present campaign. It is worthwhile to note that, as per the literature [37], greater methane content improvement can be achieved by utilizing a H_2:CO_2 of more than 4:1 and up to 10:1. However, several process difficulties including elevated VFA development were evidenced when H_2:CO_2 extended beyond 4:1 [37].

4. Conclusions

The feasibility of a H_2 injection window in a realistic anaerobic digestion context was examined, and based on the obtained results, the following concluding points are revealed: (a) the methane and VFA compositions, although developed erratically, were not found to be directly correlated with the H_2 partial pressure, as the reactor pH stayed stable (~7.2) throughout; (b) because H_2 was manually injected directly to the reactor headspace, the H_2 gas–liquid mass transfer was not effective, and thereby the desired level of methane content in the produced biogas could not be obtained; (c) poor H_2 mass transfer appeared to be aggravated by the elevated foam formation on digester liquid at the back end of the process, resulting in compromised methane content and biogas yield; (d) due to the unsatisfactory methane content improvement, the H_2 injection at stoichiometric 4:1 H_2:CO_2 or more could not be justified and hence was not exploited.

As for future experimental campaigns, the focus should be placed more on circumventing the challenges encountered here (i.e., inefficient liquid mass transfer, poor methane upgrading performance) and on developing avenues for further improvement taking into consideration wider operational H_2:CO_2, continuous long-term production of high-quality biomethane (e.g., volumetric CH_4 content at or over 90%), and assessment of techno-economic and environmental soundness (e.g., life cycle assessment).

Author Contributions: Conceptualization, K.M.L. and S.S.; methodology, S.S. and S.N.W.; software, S.N.W.; validation, S.S. and S.N.W.; formal analysis, S.S. and S.N.W.; investigation, S.N.W. and S.S.; resources, K.M.L. and S.S.; data curation, S.S. and S.N.W.; writing—original draft preparation, S.S.; writing—review and editing, S.S.; visualization, S.S. and S.N.W.; supervision, S.S. and K.M.L.; project administration, K.M.L.; funding acquisition, K.M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors gratefully acknowledge the lab facility provided by the Department of Energy and Process Technology at NTNU (Norwegian University of Science and Technology) throughout the course of this work. Special thanks go to ENERSENSE (Energy and Sensor Systems, NTNU) for support with the finance. In addition, Rune Bakke, Carlos Dinamarca, and Eshetu Janka Wakjera from the Department of Energy, Process and Environmental Engineering at the University of South Eastern Norway are acknowledged for their assistance with some sample analyses in their lab.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

AD	Anaerobic Digestion
APHA	American Public Health Association
CM	Cattle Manure
CFD	Computational Fluid Dynamics
MFC	Microbial Fuel Cells
P2G	Power to Gas
PLC	Programmable Logic Control
R&D	Research and Development
S:I	Substrate to Inoculum ratio
STP	Standard Temperature and Pressure
TAN	Total Ammonium Nitrogen
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids

References

1. Weschenfelder, F.; Leite, G.N.P.; da Costa, A.C.A.; de Castro Vilela, O.; Ribeiro, C.M.; Ochoa, A.A.V.; Araújo, A.M. A review on the complementarity between grid-connected solar and wind power systems. *J. Clean. Prod.* **2020**, *257*, 120617. [CrossRef]
2. Götz, M.; Lefebvre, J.; Mörs, F.; Koch, A.M.; Graf, F.; Bajohr, S.; Reimert, R.; Kolb, T. Renewable Power-to-Gas: A technological and economic review. *Renew. Energy* **2016**, *85*, 1371–1390. [CrossRef]
3. Liu, W.; Wen, F.; Xue, Y. Power-to-gas technology in energy systems: Current status and prospects of potential operation strategies. *J. Mod. Power Syst. Clean Energy* **2017**, *5*, 439–450. [CrossRef]
4. Luo, G.; Angelidaki, I. Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture. *Biotechnol. Bioeng.* **2012**, *109*, 2729–2736. [CrossRef]
5. Bassani, I.; Kougias, P.G.; Treu, L.; Angelidaki, I. Biogas upgrading via hydrogenotrophic methanogenesis in two-stage continuous stirred tank reactors at mesophilic and thermophilic conditions. *Environ. Sci. Technol.* **2015**, *49*, 12585–12593. [CrossRef]
6. McCarty, P.L.; Smith, D.P. Anaerobic wastewater treatment. *Environ. Sci. Technol.* **1986**, *20*, 1200–1206. [CrossRef]
7. Sarker, S.; Lamb, J.J.; Hjelme, D.R.; Lien, K.M. A review of the role of critical parameters in the design and operation of biogas production plants. *Appl. Sci.* **2019**, *9*, 1915. [CrossRef]
8. Hattori, S. Syntrophic acetate-oxidizing microbes in methanogenic environments. *Microbes Environ.* **2008**, *23*, 118–127. [CrossRef]
9. Mulat, D.G.; Mosbæk, F.; Ward, A.J.; Polag, D.; Greule, M.; Keppler, F.; Nielsen, J.L.; Feilberg, A. Exogenous addition of H 2 for an in situ biogas upgrading through biological reduction of carbon dioxide into methane. *Waste Manag.* **2017**, *68*, 146–156. [CrossRef]
10. Pauss, A.; Andre, G.; Perrier, M.; Guiot, S.R. Liquid-to-gas mass transfer in anaerobic processes: Inevitable transfer limitations of methane and hydrogen in the biomethanation process. *Appl. Environ. Microbiol.* **1990**, *56*, 1636–1644. [CrossRef]

11. Bassani, I.; Kougias, P.G.; Angelidaki, I. In-situ biogas upgrading in thermophilic granular UASB reactor: Key factors affecting the hydrogen mass transfer rate. *Bioresour. Technol.* **2016**, *221*, 485–491. [CrossRef] [PubMed]
12. Sarker, S.; Lamb, J.J.; Hjelme, D.R.; Lien, K.M. Overview of recent progress towards in-situ biogas upgradation techniques. *Fuel* **2018**, *226*, 686–697. [CrossRef]
13. Bhattacharyya, D.; Singh, K.S. Understanding the mixing pattern in an anaerobic expanded granular sludge bed reactor: Effect of liquid recirculation. *J. Environ. Eng.* **2009**, *136*, 576–584. [CrossRef]
14. Oyama, S.; Lee, D.; Hacarlioglu, P.; Saraf, R. Theory of hydrogen permeability in nonporous silica membranes. *J. Membr. Sci.* **2004**, *244*, 45–53. [CrossRef]
15. Luo, G.; Angelidaki, I. Hollow fiber membrane based H2 diffusion for efficient in situ biogas upgrading in an anaerobic reactor. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 3739–3744. [CrossRef]
16. Jensen, M.B.; Kofoed, M.V.W.; Fischer, K.; Voigt, N.V.; Agneessens, L.M.; Batstone, D.J.; Ottosen, L.D.M. Venturi-type injection system as a potential H_2 mass transfer technology for full-scale in situ biomethanation. *Appl. Energy* **2018**, *222*, 840–846. [CrossRef]
17. Díaz, I.; Pérez, C.; Alfaro, N.; Fdz-Polanco, F. A feasibility study on the bioconversion of CO_2 and H_2 to biomethane by gas sparging through polymeric membranes. *Bioresour. Technol.* **2015**, *185*, 246–253. [CrossRef]
18. Luo, G.; Angelidaki, I. Co-digestion of manure and whey for in situ biogas upgrading by the addition of H2: Process performance and microbial insights. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 1373–1381. [CrossRef]
19. Rachbauer, L.; Voitl, G.; Bochmann, G.; Fuchs, W. Biological biogas upgrading capacity of a hydrogenotrophic community in a trickle-bed reactor. *Appl. Energy* **2016**, *180*, 483–490. [CrossRef]
20. Kim, H.W.; Marcus, A.K.; Shin, J.H.; Rittmann, B.E. Advanced control for photoautotrophic growth and CO_2-utilization efficiency using a membrane carbonation photobioreactor (MCPBR). *Environ. Sci. Technol.* **2011**, *45*, 5032–5038. [CrossRef]
21. Linville, J.L.; Shen, Y.; Ignacio-de Leon, P.A.; Schoene, R.P.; Urgun-Demirtas, M. In-situ biogas upgrading during anaerobic digestion of food waste amended with walnut shell biochar at bench scale. *Waste Manag. Res.* **2017**, *35*, 669–679. [CrossRef]
22. Shen, Y.; Linville, J.L.; Urgun-Demirtas, M.; Schoene, R.P.; Snyder, S.W. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO_2 removal. *Appl. Energy* **2015**, *158*, 300–309. [CrossRef]
23. Zhang, L.; Kuroki, A.; Tong, Y.W. A Mini-Review on In situ Biogas Upgrading Technologies via Enhanced Hydrogenotrophic Methanogenesis to Improve the Quality of Biogas from Anaerobic Digesters. *Front. Energy Res.* **2020**. [CrossRef]
24. Satyanarayana, T.; Johri, B.N.; Das, S.K. *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 1. Microbial Diversity in Normal & Extreme Environments*; Springer: Berlin/Heidelberg, Germany, 2019.
25. Hupfauf, S.; Winkler, A.; Wagner, A.O.; Podmirseg, S.M.; Insam, H. Biomethanation at 45 °C offers high process efficiency and supports hygienisation. *Bioresour. Technol.* **2020**, *300*, 122671. [CrossRef]
26. APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
27. Sarker, S. By-products of fish-oil refinery as potential substrates for biogas production in Norway: A preliminary study. *Results Eng.* **2020**, *6*, 100137. [CrossRef]
28. APHA; WPCF. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association/American Water Works Association/Water Environment Federation: Washington, DC, USA, 1995.
29. Bergland, W.H.; Dinamarca, C.; Toradzadegan, M.; Nordgård, A.S.R.; Bakke, I.; Bakke, R. High rate manure supernatant digestion. *Water Res.* **2015**, *76*, 1–9. [CrossRef] [PubMed]
30. Varel, V.; Isaacson, H.; Bryant, M. Thermophilic methane production from cattle waste. *Appl. Environ. Microbiol.* **1977**, *33*, 298–307. [CrossRef]
31. Sarker, S.; Møller, H.B. Regulating feeding and increasing methane yield from co-digestion of C 5 molasses and cattle manure. *Energy Convers. Manag.* **2014**, *84*, 7–12. [CrossRef]
32. Carlsson, M.; Lagerkvist, A.; Morgan-Sagastume, F. The effects of substrate pre-treatment on anaerobic digestion systems: A review. *Waste Manag.* **2012**, *32*, 1634–1650. [CrossRef]

33. De Bok, F.; Plugge, C.; Stams, A. Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Res.* **2004**, *38*, 1368–1375. [CrossRef]
34. Ong, H.; Greenfield, P.; Pullammanappallil, P. Effect of mixing on biomethanation of cattle-manure slurry. *Environ. Technol.* **2002**, *23*, 1081–1090. [CrossRef] [PubMed]
35. Angelidaki, I.; Karakashev, D.; Batstone, D.J.; Plugge, C.M.; Stams, A.J. Biomethanation and its potential. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 2011; Volume 494.
36. Velmurugan, B. Anaerobic digestion of vegetable wastes for biogas production in a fed-batch reactor. *Int. J. Emerg. Sci.* **2011**, *1*, 478.
37. Agneessens, L.M.; Ottosen, L.D.M.; Voigt, N.V.; Nielsen, J.L.; de Jonge, N.; Fischer, C.H.; Kofoed, M.V.W. In-situ biogas upgrading with pulse H 2 additions: The relevance of methanogen adaption and inorganic carbon level. *Bioresour. Technol.* **2017**, *233*, 256–263. [CrossRef] [PubMed]