

Biogas production through bio-methanation of syngas

Pegah Parichehreh Dizaji

Thesis Submitted to the University of Ottawa in Partial Fulfillment of the Requirements for
the Degree of Master of Applied Science in Civil Engineering
Master of Applied Science in Civil Engineering

Department of Civil Engineering
Faculty of Engineering
University of Ottawa

Abstract

Sustainable and environmentally friendly waste-to-energy conversion technologies, such as anaerobic digestion (AD) and gasification, have received significant attention in recent energy research. These technologies have proven their ability to reduce reliance on fossil fuels and greenhouse gas emissions by converting organic waste into products and fuels with market value, such as biomass, biogas, and synthetic gas.

Since the syngas produced by biomass gasification contains highly toxic CO and flammable H₂, converting syngas into renewable natural gas has recently gained a lot of interest. By coupling AD with syngas, microbial consortium in the AD reactor converts the syngas into methane through a process known as biomethanation. Feeding syngas into the AD reactor is a method that not only can enhance methane production by conversion of CO₂ to CH₄ during the AD process but also converts syngas into methane as pure energy.

This study aims to assess and compare the effect of different syngas compositions on methane production and optimize the SB process by identifying the best syngas composition and gas-biomass ratio under mesophilic temperature conditions. The study was conducted using batch and semi-continuous reactors in a lab-scale setting. The results of this study can contribute to the development of more efficient and sustainable methods for SB.

In phase I of this study, syngas biomethanation under different syngas compositions was conducted under three different gas-biomass ratios (0.5, 1 and 1.5) in bench-scale experiments to study the impact on CO and H₂ partial pressure and CO toxicity on operation parameters (e.g., pH and VFA) and syngas conversion efficiency. The results showed that the optimum syngas composition with the highest amount of CH₄ is H₂-rich syngas (CO₂:CO; H₂ 1:1:7) and syngas with stoichiometric ratios between H₂ and CO/CO₂ (CO:H₂ 1:3; CO₂:H₂ 1:4) because of the sufficient available amount of hydrogen in the headspace. Methane content in the produced biogas reached 80.0%, 63.6% and 57.7%, respectively, compared to the control sample with 30.2% methane in the headspace.

In phase II, the optimum syngas compositions were selected for experimenting with semi-continuous mode to 1) investigate the effect of injecting syngas in several stages in increasing syngas conversion efficiency, 2) adapt microorganisms to hydrogen and enhance biohydrogen production, and 3) test higher stoichiometric ratio between H₂ and CO/CO₂ to enhance syngas biomethanation efficiency. The data indicated higher methane content and syngas conversion in a semi-continuous mode. The biogas had methane concentration of 82.3, 76.9, 73.8, 84.9 and 81.7% in samples CO₂:CO: H₂ (1:1:7), CO:H₂ (1:3), CO₂:H₂ (1:4), CO: H₂ (1:4) and CO₂:H₂ (1:5).

By injecting gas into the biomass in several stages, methane levels in the produced biogas in each stage increased, demonstrating the adaptation of microorganisms to the injected hydrogen and carbon-sourced gases. A higher stoichiometric ratio of H₂ to CO/CO₂ promoted the growth and activity of methanogens, leading to increased methane production.

List of Abbreviations

AD	Anaerobic Digestion
RNG	Renewable natural gas
VFAs	Volatile fatty acids
GHG	Greenhouse gases
SB	Syngas bio methanation
BMP	Biochemical Methane Potential
CSTR	Continuous stirred-tank reactor

Acknowledgement

I would like to express my sincere gratitude to my supervisors, Professor Majid Sartaj and Dr. Niloofar Abdehagh, for their unwavering support, trust, encouragement, and guidance during my graduate studies. I am grateful to Dr. Mohammad Adghim for his precious time and valuable suggestions on developing this thesis. The completion of this work would have been impossible without their help.

Finally, I would like to especially thank my beloved parents and my husband for their endless love, support, patience, and care throughout my life.

Contents

Abstract	ii
List of Abbreviations	iv
Acknowledgement	v
Contents	vi
List of Figures	x
List of Tables	xiv
Chapter 1	1
1. Introduction	1
1.1 Background and Context.....	1
1.2 Problem Statement	2
1.3 Research Objectives and Scope	5
1.4 Contribution	5
1.5 Thesis Organization	6

Chapter 2.....	8
2. Literature Review.....	8
2.1 Anaerobic Digestion	8
2.1.1 Stages of AD	8
2.1.2 Operational Parameters	10
2.1.3 Biogas Upgrading	14
2.2 Gasification and Syngas Production	15
2.3 Coupling Gasification and Anaerobic Digestion	16
2.3.1 Gasification of Anaerobic Digestion Residues	17
2.3.2 Biomethanation of Syngas in AD Reactors	17
2.4 Syngas Biomethanation Pathways	18
2.4.1. Bioconversion of CO and H ₂ /CO ₂	18
2.4.2 Bioconversion of H ₂	21
2.4.3 Bioconversion of CO ₂	22
2.5 Syngas Biomethanation Operational Conditions	24
2.6 Recent Advancements in Syngas Biomethanation	28
2.7 Summary and Knowledge Gaps.....	32
Chapter 3.....	34
3. Methodology	34
3.1 Overview	34
3.2 Biomass and Syngas	34
3.3 Experimental Procedures	35
3.3.1 Phase I.....	37
3.3.2 Phase II.....	40
3.4 Analytical methods and Calculation	42
3.4.1 Biomass Characteristics	42

3.4.2 Biogas Composition.....	42
3.4.3 Biogas Volume.....	43
3.4.4 Conversion Efficiency and Methane Yield.....	45
Chapter 4.....	46
4. Results and Discussion	46
4.1 Biomass Characteristics	46
4.2. Phase 1 Results: Syngas single injection in batch mode.....	46
4.2.1 Biogas Production in the Control Sample.....	46
4.2.2 CO ₂ Biomethanation (Sample A).....	47
4.2.3 CO Biomethanation (Sample B)	50
4.2.4 H ₂ Biomethanation (H ₂ a and H ₂ b)	53
4.2.5 CO and H ₂ (CO:H ₂ 1:3) biomethanation (Sample C)	55
4.2.6 CO ₂ and H ₂ (CO ₂ :H ₂ 1:4) biomethanation (Sample D).....	59
4.2.7 Syngas (CO ₂ :CO:H ₂ 1:2:3) biomethanation (Sample E).....	62
4.2.8 Syngas (CO ₂ :CO:H ₂ 1:1:7) biomethanation (Sample F).....	65
4.3 Comparing results of Phase I:	68
4.4 Phase II Results: Biomethanation of Syngas in semi-continuous mode.....	72
4.4.1 Biogas production in the control sample	72
4.4.2 H ₂ c Bio-methanation in semi-continuous mode	72
4.4.3 CO and H ₂ biomethanation in semi-continous mode with stoichiometric ratio CO: H ₂ 1:3 (Sample G).....	75
4.4.4 CO and H ₂ biomethanation in semi-continous mode with stoichiometric ratio CO: H ₂ 1:4 (Sample H).....	77
4.4.5 CO ₂ and H ₂ biomethanation in semi-continous mode with stoichiometric ratio CO ₂ : H ₂ 1:4 (Sample I).	79
4.4.6 CO ₂ and H ₂ biomethanation in semi-continous mode with stoichiometric ratio CO ₂ : H ₂ 1:5 (Sample J).	81
4.4.7 Syngas bio methanation in semi-continuous mode with stoichiometric ratio CO ₂ :CO:H ₂ 1:2:3 (Sample K).....	83

4.5 Comparing results of Phase II:.....	87
Chapter 5.....	89
5. Conclusions and Future Work	89
Chapter 6.....	93
6. References.....	93
Appendix.....	101

List of Figures

Figure 2.1. Anaerobic digestion stages and processes [21].	10
Figure 2.2. Anaerobic digestion and Gasification coupling scheme [18].	16
Figure 2.3. Syngas bio-methanation by coupling gasification and AD [17].	18
Figure 2.4. Microbial metabolic pathways convert syngas to CH ₄ . Methanogenic reactions are represented by red-dotted arrows, acetogenic reactions by blue-small-dashed hands, and hydrogenogenic reactions by orange-big-dashed arrows [36].	19
Figure 2.5. Anaerobic respiratory process coupled to CO oxidation [31].	20
Figure 2.6. Wood-Ljungdahl pathway [47].	24
Figure 2.7. Conversion pathway of CO to CH ₄ in AD. A: low CO pressure (<0.5atm), B: high CO pressure (≥1 atm) and C: 100% CO concentrations after acclimation. The thickness of the arrow represents the possibility of pathways. (thick: 60–80%, intermediate: 20–40%, thin:5-20% and dotted lines: blocked pathway) [54].	27
Figure 3.1. Incubator.	36
Figure 3.2. BMP bottles with inoculum and substrate inside the incubator.	36

Figure 3.3. Spectrophotometer for measuring VFAs.....	42
Figure 3.4. GOW-MAC 350 and 400 Instruments.	43
Figure 3.5. U-tube water manometer	45
Figure 4.1. Accumulative biogas production in the control sample.	47
Figure 4.2. Net accumulative biogas under CO ₂ (Sample A) biomethanation under the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).....	48
Figure 4.3. CH ₄ and CO ₂ contents in the produced gas in sample A.....	49
Figure 4.4. Net accumulative biogas under CO (sample B) biomethanation with the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).....	51
Figure 4.5. CH ₄ and CO ₂ contents in the produced gas in sample B.....	52
Figure 4.6. Accumulative biogas in samples a) H ₂ a and b) H ₂ b.....	54
Figure 4.7. Net accumulative biogas under CO: H ₂ 1:3 (sample C) biomethanation under the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).	56
Figure 4.8. CH ₄ and CO ₂ contents in the produced gas in sample C (CO: H ₂ 1:3 biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).	58
Figure 4.9. Net accumulative biogas under CO ₂ and H ₂ (sample D) biomethanation under the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).	60
Figure 4.10. Methane and CO ₂ contents in the produced gas in sample D (CO ₂ :H ₂ 1:4 biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).	61
Figure 4.11. Net accumulative biogas under syngas biomethanation in sample E under the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).	63
Figure 4.12. CH ₄ and CO ₂ contents in the produced gas in sample E (syngas (CO ₂ :CO: H ₂ 1:2:3) biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).....	64
Figure 4.13. Net accumulative biogas under syngas biomethanation in sample F under the biomass-gas ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).	66

Figure 4.14. CH ₄ and CO ₄ contents in the produced gas in sample F (syngas (CO ₂ :CO:H ₂ 1:1:7) biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).	67
Figure 4.15. Accumulative biogas in the control sample.....	72
Figure 4.16. Net accumulated biogas under H ₂ c Bio methanation.	73
Figure 4.17. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection (Sample H ₂ c).	73
Figure 4.18. Net accumulated biogas under CO and H ₂ biomethanation Sample G (CO:H ₂ 1:3).	76
Figure 4.19. CH ₄ and CO ₄ contents in the produced gas on the last day of each injection Sample G (CO:H ₂ 1:3).	76
Figure 4.20. CO Conversion efficiency on the last day of each injection for Sample G (CO: H ₂ 1:3) and Sample H (CO: H ₂ 1:4).	77
Figure 4.21. Net accumulated biogas under CO and H ₂ bio methanation Sample H (CO:H ₂ 1:4).	78
Figure 4.22. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection Sample H (CO:H ₂ 1:4).	78
Figure 4.23. Net accumulated biogas under CO ₂ and H ₂ bio methanation Sample I (CO ₂ :H ₂ 1:4).	80
Figure 4.24. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection Sample I (CO ₂ :H ₂ 1:4).	80
Figure 4.25. CO ₂ Conversion efficiency on the last day of each injection for Sample I (CO ₂ : H ₂ 1:4) and Sample J (CO ₂ : H ₂ 1:5).	81
Figure 4.26. Net accumulated biogas under CO ₂ and H ₂ bio methanation Sample J (CO ₂ : H ₂ 1:5).	82

Figure 4.27. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection Sample J (CO ₂ : H ₂ 1:5).	82
Figure 4.28. Net accumulated biogas under Syngas bio methanation Sample K (CO ₂ :CO:H ₂ 1:2:3).	84
Figure 4.29. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection Sample K (CO ₂ :CO:H ₂ 1:2:3).	84
Figure 4.30. CO and CO ₂ Conversion efficiency on the last day of each injection for Sample K (CO ₂ :CO:H ₂ 1:2:3) and Sample L (CO ₂ :CO: H ₂ 1:1:7).	85
Figure 4.31. Net accumulated biogas under Syngas bio methanation Sample L (CO ₂ :CO: H ₂ 1:1:7).	86
Figure 4.32. CH ₄ and CO ₂ contents in the produced gas on the last day of each injection Sample L (CO ₂ :CO: H ₂ 1:1:7).	86

List of Tables

Table 2.1. Summary of Recent Advancements in Syngas Bio methanation	32
Table 3.1. Setup of syngas bio-methanation experiments at the gas-to-biomass ratio of 0.5 (mL gas/mL biomass) (Phase I).....	38
Table 3.2. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1 (mL gas/mL biomass) (Phase I).....	38
Table 3.3. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1.5 (mL gas/mL biomass) (Phase I).....	39
Table 3.4. Set up of H ₂ bio-methanation experiments (Phase I).....	39
Table 3.5 Setup of syngas bio-methanation experiments with multiple injections (Phase II).	41
Table 3.6. Set up of H ₂ bio-methanation experiments with multiple injections (Phase II).	41
Table 4.1. VFAs and pH in Sample A on the last day of the experiment.....	50
Table 4.2. VFAs and pH in Sample B on the last day of the experiment.	53
Table 4.3. VFA and pH in Samples H ₂ a and H ₂ b on the last day of the experiment.....	54
Table 4.4. VFA and pH in Sample C on the last day of the experiment.....	59

Table 4.5. VFAs and pH in Sample D on the last day of the experiment.....	62
Table 4.6. VFA and pH in Sample E on the last day of the experiment.....	65
Table 4.7. VFA and pH in Sample F on the last day of the experiment.....	67
Table 4.8. Biogas content and CO/CO ₂ Conversion efficiency in the samples of Phase I.....	71
Table 4.9. CO/CO ₂ Conversion efficiency in the samples of Phase II.	87
Table 0.1. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 0.5 (mL gas/mL biomass) (Phase I).....	101
Table 0.2. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1 (mL gas/mL biomass) (Phase I).....	102
Table 0.3. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1.5 (mL gas/mL biomass) (Phase I).....	102
Table 0.4. Set up of H ₂ bio-methanation experiment (Phase I).	103
Table 0.5. Setup of syngas bio-methanation experiments with multiple injections (Phase II).	104
Table 0.6. Setup of H ₂ bio-methanation experiments with multiple injections (Phase II). ...	104

Chapter 1

1. Introduction

1.1 Background and Context

In today's world, the need to develop and improve sustainable energy sources has become very important because of the limited resources of fossil fuels, the unsustainable nature of fossil fuels due to the emission of harmful greenhouse gases (GHG) into the atmosphere, and the rising energy demand [1]. Thus, alternative forms of energy from sustainable and environmentally compatible sources are urgently needed [2]. Many countries are implementing policies and regulations, investing in renewable energy, promoting sustainable transportation and circular economy, supporting research and innovation, and engaging with international organizations to move towards net-zero emissions and a more sustainable future [3]. In the last couple of decades, many green technologies have been developed; some are available for commercial use, including anaerobic digestion, solar power, wind power, hydropower, biofuels, electric vehicles, and sustainable aviation fuels [4].

Biomass is an abundant renewable resource that, if not managed, can contribute to greenhouse gas emissions. At the same time, organic solid wastes from municipal and agricultural sectors are overgrowing [4]. Anaerobic digestion (AD) is a viable green technique that converts organic waste into energy by producing biogas, which can be converted to heat and electrical

energy while reducing greenhouse emissions and odours [5]. AD is a biochemical process in which complex organic pollutants are reduced by microbial activity through extracellular (hydrolysis and disintegration) and intracellular reactions (acidogenesis, acetogenesis, and methanogenesis) for biogas production [6]. Biogas is a combustible gas composed of primarily methane (CH_4) (55–75%) as the only source of energy, carbon dioxide (CO_2) (25–45%), and trace amounts of other gases, such as hydrogen sulphide (H_2S), ammonia (NH_3), nitrogen (N_2), and water vapour [7].

Biogas is converted to electricity and heat, with 27 % of biogas being upgraded to renewable natural gas (RNG) (biomethane) in Canada [8]. Numerous biogas facilities have been installed in Canada due to feed-in tariff rules promoting RNG [4]. Based on the existing records, there were 44 on-farm digesters in Canada in 2020, with 23 working in the food and beverage sector [9]. Biogas generation in Canada is a small but developing business compared to Europe. Canada is committed to reducing greenhouse gas (GHG) emissions as outlined in the Paris Agreement. Under this international agreement, Canada pledged to reduce GHG emissions by 30% below 2005 levels by 2030. In addition, Canada has set a goal to reach net-zero emissions by 2050, which involves balancing any remaining emissions with removing carbon from the atmosphere [10]. Therefore, Canada needs to revamp its policy on renewable energy and financial incentives to achieve these targets [9]. A few factors could contribute to the rapidly expanding Canadian RNG business today. Firstly, Canada already has more than 480,000 km of natural gas pipelines. Additionally, the production capacity of RNG from organic waste is potentially vast in Canada [12].

1.2 Problem Statement

Produced biogas typically contains impurities (25–45% CO_2 and trace amounts of H_2S and NH_3), which can limit its potential use as a fuel. Upgrading biogas involves removing these

impurities and increasing the methane content of the gas to improve its quality and make it a more valuable fuel. Upgrading biogas can be brought up to the standards required for injection into natural gas pipelines [13]. Several physicochemical techniques for upgrading biogas, such as polyglycolic adsorption, chemical treatment, pressure swing adsorption, and water washing, already exist for removing CO₂ from biogas. However, since high pressure or the addition of chemicals is needed, these methods can raise the overall costs of generating biomethane [6], [11], [14]. To avoid these drawbacks and utilize moderate treatments that need less chemical material and energy, the biological conversion of CO₂ to CH₄ for upgrading biogas can address the disadvantages mentioned above of physicochemical biogas upgrading techniques [14]. Biological conversion reduces CO₂ emissions and results in higher biomethane production per unit weight of organic waste. This biological process, called biomethanation, involves using microorganisms to convert CO₂ into CH₄ using H₂ and CO as inorganic electron donors, which increases the purity and energy content of the biogas. In the presence of inorganic electron donors, methanogens can use them to reduce CO₂ through the hydrogenotrophic methanogenesis pathway or produce CH₄ through the acetolactic methanogenesis [15].

Another inevitable product of AD is digestate. It mainly consists of solids, liquids, nutrients, microorganisms, and trace elements. Digestate is used as a soil amendment or fertilizer due to its high nutrient content and potential for improving soil health. However, there are rising concerns about the land application of digestate since heavy metals, nitrogen surplus, and pathogens may spread to the ground in addition to contributing to GHG emissions [14], [17]. Some thermo-chemical processes have been developed to further treat the digestate and recover energy and nutrients, including pyrolysis and gasification [16], [17]. Gasification converts biomass into a mixture of gases called syngas (synthetic gas), mainly consisting of N₂, CO₂, CO, and H₂. The process involves heating the biomass at high temperatures in the absence of oxygen, which results in the breakdown of the complex organic compounds in the biomass into

simpler molecules [6], [11]. Since CO is a toxic gas and H₂ is flammable, handling and utilizing syngas requires specialized equipment and safety measures; therefore, converting syngas to natural gas has gained much attention in recent years [16]. By coupling AD with syngas, syngas biomethanation (SB) can happen inside the AD reactor and increase the energy efficiency of syngas utilization by converting CO and H₂ into methane [18]. SB by anaerobic microorganisms has been demonstrated as an economical and practical method that takes place under ambient pressure, with few by-products and is insensitive to impurities [16], [19]. However, different environmental and operational factors, such as pH, temperature, pressure, accumulation of volatile fatty acids (VFAs), syngas composition, and gas-to-liquid transfer, could affect the process and its efficiency that should be considered during the operation [20]. Researchers have tested different approaches for optimizing SB by changing process conditions, improving microbial consortia, using different reactors designs, and changing syngas loading rate. However, to the author's knowledge, biomethanation of CO, CO₂ and H₂ alone, different combinations, and with higher stoichiometric ratios than the general composition of syngas that is produced in gasifying plants has not been tested thoroughly in batch and semi-continuous mode under mesophilic temperature. Since the composition of syngas can vary widely depending on the feedstock and the process used, it is necessary to investigate the effect of each gas in syngas on biomethanation in AD reactors before scaling up from laboratory-scale to pilot-scale and industrial-scale. Additionally, semi-continuous gas injection with time intervals between each injection has been employed to enhance microorganisms' adaptability to the gases.

1.3 Research Objectives and Scope

The main objective of this study is to test syngas biomethanation (SB) under different CO, CO₂ and H₂ compositions in batch and semi-continuous phases under mesophilic temperature.

The following points summarize the specific objectives of this study:

Phase I batch mode.

- Investigate and compare the effect of different syngas compositions on biogas upgrading.
- Test three different gas-biomass ratio systems to investigate the effect of CO and H₂ partial pressure on operation parameters (e.g., pH and VFAs) and syngas conversion efficiency.

Phase II semi-continues mode.

- Investigate the effect of injecting syngas in several stages in enhancing syngas conversion efficiency.
- Assess the adaptability of microorganisms to hydrogen and enhance biohydrogen production.
- Test higher stoichiometric ratio between H₂ and CO/CO₂ to improve syngas bio methanation efficiency.

1.4 Contribution

Investigating the effect of each gas in syngas on biogas upgrading in an AD reactor can improve the literature and current knowledge about SB in several ways.

- This study can help identify which gases in the syngas mixture are most beneficial or inhibitory to the bio methanation process. This information can be used to optimize syngas composition to maximize biogas yields and improve the efficiency of the AD process. Results can help develop new catalysts and reactor designs tailored for specific gas mixtures. This can

lead to more efficient and cost-effective biomethanation, benefiting the energy sector and other industries producing waste gases.

- Improved knowledge about SB can inform policy and decision-making regarding waste management, renewable energy, and climate change mitigation. This can help promote adopting sustainable practices that benefit the environment, society, and the economy.

Overall, this research will contribute to further identifying potential challenges and evaluating countermeasures of this technology.

1.5 Thesis Organization

This thesis will be presented in a monograph format, and the organization of the dissertation is as follows:

- Chapter 1: Introduction

This chapter introduces the importance of the topic and its impacts on the biogas upgrading technology and contribution to the industry and field advancements.

- Chapter 2: Literature Review

This chapter covers the fundamentals of AD, CO, CO₂ and H₂ biomethanation, environmental and operational factors for biogas production and SB optimization and limiting factors. In addition, this chapter summarizes previous work and research results and the knowledge gap.

- Chapter 3: Research Methodology

This chapter will discuss the experimental plan and the general materials and methods used in the study.

- Chapter 4: Results and Discussion

In this chapter, the results of the study will present in tables and graphs, and the findings will be explained to address the research goals.

- Chapter 5: Conclusion

This chapter summarizes the conclusions from this research and recommendations for future work.

- Chapter 6: References

This chapter presents the full citation of the references used in the thesis.

Chapter 2

2. Literature Review

This chapter covers the fundamentals of anaerobic digestion, gasification, biogas upgrading, syngas biomethanation (SB) and its challenges in anaerobic digester reactors, and a summary of previous work and research results.

2.1 Anaerobic Digestion

Anaerobic Digestion (AD) is a biological process for treating organic wastes that breaks down organic matter, such as plant material, food waste, animal manure, and wastewater sludge, in the absence of oxygen. This process is carried out by a group of microorganisms called anaerobes, which convert the organic matter into biogas (a mixture of methane, carbon dioxide, and trace amounts of other gases) and digestate (a nutrient-rich liquid or solid residue) [21].

2.1.1 Stages of AD

The AD process involves a series of biological activities that break down complex organic structures such as proteins, carbohydrates, and fats into the final AD products, i.e., biogas and

digestate [6]. AD's four consecutive microbiological stages are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Figure 2.1 shows the inputs and outputs of each AD stage.

Hydrolysis: The hydrolysis phase is the first stage in the anaerobic digestion process, where the complex organic matter in the substrate is broken down into simpler molecules by hydrolytic enzymes produced by bacteria. This phase is crucial as it transforms organic matter into soluble monomers, which are then available for uptake by other microorganisms to convert into biogas. The hydrolysis phase occurs within the first few days of the process and is influenced by microbial community composition. Overall, the hydrolysis phase is essential to the success of anaerobic digestion as it sets the stage for the subsequent stages of the process [1], [6], [21].

Acidogenesis: The acidogenesis phase is the second stage of the AD process, where acidogenic bacteria further degrade hydrolysis products into organic acids such as acetic, propionic, and butyric acid. Organic acids produced during this phase are essential intermediates which methanogenic bacteria can use for methane production. Optimal conditions during this phase can lead to higher organic acid production and a more efficient AD process [1], [6], [21].

Acetogenesis: In the third phase, anaerobic oxidation reactions occur, and organic acids are broken down into acetic acid (the primary precursor for methane production), hydrogen, and carbon dioxide. Hydrogen production increases the hydrogen partial pressure that may cause inhibition to the AD process. Acetogenesis is critical to the success of the AD process as it generates a significant proportion of the acetic acid needed for the methanogenesis phase [1], [6], [21].

Methanogenesis: In the last step, acetate is converted by acetoclastic methanogens to methane and carbon dioxide, which form under strictly anaerobic conditions.

Hydrogenotrophic methanogens also produce methane by consuming carbon dioxide and hydrogen [6], [21].

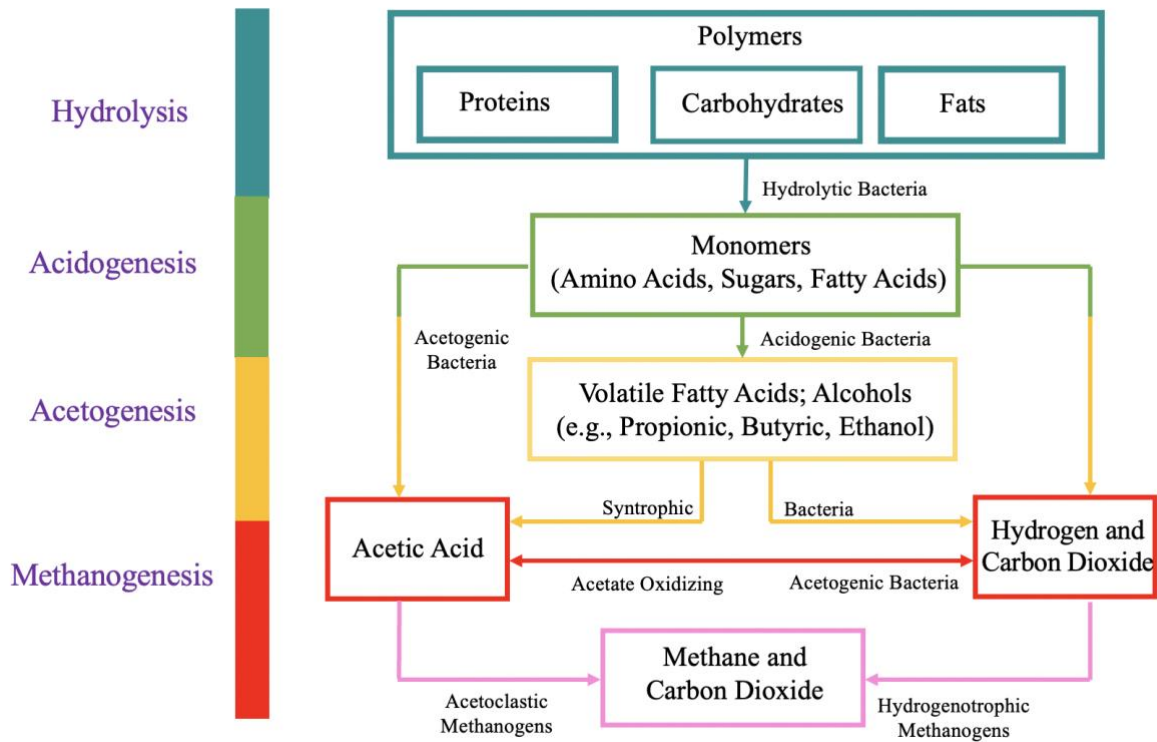


Figure 2.1. Anaerobic digestion stages and processes [21].

2.1.2 Operational Parameters

Since AD is a biological process, factors such as pH, temperature, carbon-to-nitrogen ratio (C:N), hydraulic retention time (HRT) and the total amount of organic matter must be taken into consideration to optimize the process [6].

pH: Anaerobic digestion is a complex process involving the activity of a wide range of microorganisms, including acidogens, acetogens, and methanogens. Each group of microorganisms plays a crucial role in the overall process, and their activity is affected by changes in pH. Acidogens have an optimal pH range of 7 to 9, are relatively insensitive to changes in pH, and can tolerate a wider range of pH levels than other microorganisms. However, if the pH drops below 5.0, the activity of acidogens can be inhibited, leading to a decrease in the production of organic acids and, ultimately, a reduction in methane production.

Acetogens have an optimal pH range of 6.5 to 7.5. If the pH drops below the optimal range, the activity of acetogens can be inhibited. Methanogens have a narrow pH range for optimal activity. Generally, methanogens prefer a pH range of 6.5 to 7.5 [22]. pH levels are regularly monitored during the AD process using a pH meter.

Buffering capacity and alkalinity are important operational parameters in the AD process, as they affect the system's ability to resist changes in pH. Buffering capacity refers to the system's ability to resist changes in pH when an acid or a base is added. The buffering capacity of the AD system is mainly determined by the concentration of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, which act as natural buffers. Alkalinity, however, refers to concentrations of chemicals that can neutralize acids. It measures the system's capacity to absorb hydrogen ions (H^+) and maintain a stable pH range [23]. The relationship between buffering capacity, alkalinity, and pH during AD is complex. Generally, when the pH of the AD system decreases, i.e., it becomes more acidic, the buffering capacity and alkalinity also decrease. This means that the system becomes more sensitive to further pH changes, which can lead to a cascade of adverse effects, including reduced microbial activity, decreased biogas production, and increased risk of system failure [23]. Adding alkalinity sources, such as calcium carbonate (CaCO_3) or sodium bicarbonate (NaHCO_3), can help buffer the system against pH fluctuations and maintain a stable pH range. Additionally, controlling the feedstock composition, adjusting the hydraulic retention time (HRT) and temperature, and monitoring the pH regularly can help regulate the pH and maintain optimal conditions for microbial activity and the biogas production [24].

Temperature: The metabolism and growth rate of the microbial consortia during AD performance are strongly influenced by temperature. Mesophilic (35 °C) and thermophilic (55 °C) environments are often suitable for AD. The choice of temperature depends on several factors, including the type of feedstock, the microbial community, and the desired biogas

production. In general, mesophilic AD is more common than thermophilic AD, but both have advantages and disadvantages.

Mesophilic AD is more tolerant of variations in feedstock composition and requires less energy to maintain the optimal temperature range. Additionally, mesophilic microorganisms typically have higher growth rates and produce more biogas per unit of feedstock than thermophilic microorganisms. Mesophilic AD is also less sensitive to process upsets, such as pH changes, which can lead to system failure. On the other hand, thermophilic AD can provide several benefits over mesophilic AD. The higher operating temperature can improve the pathogen reduction, resulting in a safer digestate product. Additionally, thermophilic microorganisms can break down complex organic compounds more efficiently, resulting in a higher rate of biogas production and potentially higher biogas quality. However, thermophilic AD requires more energy to maintain the higher temperature range and is more sensitive to variations in feedstock composition. Also, higher temperatures encourage the increase of free ammonia, which inhibits microbial activity [20], [24].

C:N: For the AD plants to run effectively, the C:N ratio in the feedstock must be kept in a particular range, typically 20:1 to 30:1. This is because microorganisms consume carbon 20 to 30 times more quickly than nitrogen [25]. If the C:N ratio is too low (meaning there is too much nitrogen compared to carbon), the digestion process can become unstable, resulting in high ammonia levels, reduced biogas yields, and potential inhibition of the microbial communities involved in the process [26]. On the other hand, if the C:N ratio is too high (meaning there is too much carbon compared to nitrogen), microbial activity may be limited, leading to slow or incomplete digestion. To fix a C:N ratio problem in an AD process, there are a few options that can be considered depending on the specific situation. Co-digestion is an approach for balancing the C:N ratio. For instance, adding animal waste to a feedstock mix that is high in carbon but low in nitrogen can help to improve the C:N ratio. If the C:N ratio is too

low adding carbon-rich materials such as paper, cardboard, straw, or sawdust can help to balance the ratio. Alternatively, reducing the amount of nitrogen-rich materials (such as food waste or manure) in the feedstock mix may also be effective. Alternatively, if the C:N ratio is too low, increasing the retention time in the AD system can allow more time for the microbial communities to adjust to the feedstock and break down the organic matter. This can help to reduce the ammonia levels and stabilize the digestion process [25].

Hydraulic retention time (HRT): HRT represents the average retention time of the biomass in the reactor before discharging. Since the growth of methanogens is slow, a short retention time increases the risk of loss of active microorganisms. However, an extended retention period necessitates a larger digester volume, which raises the final cost. The optimum HRT has been reported to be around 16 to 20 days [25], [27].

Volatile fatty acids (VFAs): VFAs are important transitional compounds produced by microorganisms in the AD process. VFAs are typically composed of acetic, propionic, butyric, and valeric acids, among others. The accumulation of VFAs can have both positive and negative effects on the AD process. In low concentrations, VFAs are important substrates for methanogens in the methanogenesis stage of AD, and their presence can increase biogas production. However, when VFAs accumulate to high concentrations, they can cause a range of negative effects, including pH reduction, microbial stress, inhibition of methanogenesis, and even system failure [6], [21].

Ammonia: Although ammonia (NH_3) is essential for bacteria growth, high amounts of free ammonia are toxic for methanogenesis. Generally, ammonium ions (NH_4^+) and NH_3 are combined and presented as the total ammonia nitrogen (TAN) in AD [20]. pH and temperature have a proportionate impact on the quantity of NH_3 . NH_3/TAN increases from 1.1 to 11.3% when the pH rises from 7.0 to 8.0 at mesophilic temperature. But the NH_3 content of the TAN increases from 5.0 to 27% when the pH is raised from 7.0 to 8.0 at the thermophilic temperature

[28]. Methanogenic archaea are particularly sensitive to NH_3 as they can readily enter the cell membrane of methanogenic archaea and alter their K^+ efflux, resulting in a lower rate of CH_4 production. The range of TAN and NH_3 concentrations that can inhibit anaerobic digestion depends on various factors, including the type of feedstock, operating conditions, and microorganisms involved. Thus, the reported range of TAN concentrations to inhibit the methanogenesis process and cause the failure of the reactor varies and is 1500–5000 mg $\text{NH}_3\text{-N/L}$ [20], [28], [29].

2.1.3 Biogas Upgrading

Biogas upgrading consists of removing carbon dioxide and thus increasing the heating value of the biogas by increasing its CH_4 content. In general, there are physical, chemical, biological, or a combination of different strategies for improving the methane content of biogas. The conventional physical and chemical technologies are membrane separation, cryogenic separation, absorption, pressure swing adsorption, water washing or organic solvent [26]. Although these technologies offer certain benefits, they also have some drawbacks, such as high energy and investment costs and the need to utilize toxic solvents in some cases. In addition, while removing CO_2 , a small quantity of CH_4 is also released, contributing to rising levels of greenhouse gases [30], [14]. Recently, biological technologies such as syngas biomethanation (SB) have been at the centre of research and practice. Biological upgrading is advantageous because CO_2 can be captured and recycled into new products [31]. This process has several advantages over conventional methods, including lower energy requirements, cost-effectiveness, flexibility, and environmental benefits. This method can treat a wide range of biogas feedstocks and remove impurities [32]. Syngas production and biomethanation which are steps toward biological biogas upgrading will be discussed in the following paragraphs.

2.2 Gasification and Syngas Production

Gasification is a thermochemical conversion technology that converts biomass into a fuel gas called syngas. Gasification produces three additional by-products: char, ash, and tar/organic compounds. The gasification process involves the partial oxidation of the biomass in a high-temperature (500-1500 °C) and pressures (1-80 psi) and low-oxygen environment [31]. The range of oxygen during gasification is typically very low, usually between 0.1% and 10% by volume. The oxygen is added to the gasifier in the form of air or pure oxygen to initiate the gasification reactions. Still, the amount of oxygen is carefully controlled to prevent combustion and ensure optimal syngas production. Maintaining a low-oxygen environment is crucial to the success of the gasification process. Too much oxygen can lead to combustion, which reduces the efficiency of the gasification process and generates unwanted emissions. On the other hand, too little oxygen can limit the gasification reactions and reduce the syngas yield [33].

The syngas mixture consists mainly of CO, CO₂, H₂ and CH₄, and N₂ as by-products [18]. Typically, CO and H₂ make up 85% of the total volume of syngas, with CO₂ and CH₄ making up most of the remaining 15% [20]. The syngas produced by gasification can be used as a fuel for power generation or as a feedstock to produce chemicals and fuels [33], [31]. Gasification has several advantages over traditional combustion technologies, including higher energy efficiency, lower emissions of pollutants, and the ability to utilize a wide range of feedstocks, including biomass, municipal solid waste, and coal. Biomass is the most plentiful energy source. It is seen to be a viable option to develop sustainable, renewable, and environmentally friendly energy sources, which currently account for 14% of global energy consumption [34]. The gasification process typically involves four main steps: drying and pyrolysis, gasification, combustion, and gas cleaning. During the drying and pyrolysis step, the biomass is heated to a high temperature in the presence of a limited amount of oxygen, which drives off the moisture and volatile components of the biomass. The resulting char is then converted into syngas

through the gasification step. After removing impurities such as sulphur and particulates, the resulting syngas is combusted to produce heat, which is used to drive a turbine and generate electricity [33], [31]. For biological biogas upgrading and reducing waste after AD, AD and gasification can be combined to create a more efficient and effective waste management system. Methods, challenges, and advantages of this combination are discussed in the following sections.

2.3 Coupling Gasification and Anaerobic Digestion

Gasification and anaerobic digestion are two processes for converting organic materials into fuel or energy. However, although AD and thermochemical methods such as gasification are key processes for developing a circular economy and essential pillars of resource efficiency, further technological leaps are needed. In this respect, a conceptual coupling system of AD and gasification, i.e., syngas bio methanation, has been proposed in the literature [35].

Coupling AD and gasification processes can have several benefits, including upgrading produced biogas during AD, converting residual biomass after AD into fuel, increasing overall energy conversion efficiency of gasification, increasing flexibility in feedstock selection, and reducing environmental impact, which will be discussed further in the following paragraphs. As indicated in Figure 2.2, AD and gasification can be coupled by using biomass after AD in gasifier reactors or utilizing produced syngas after gasification during AD [36].

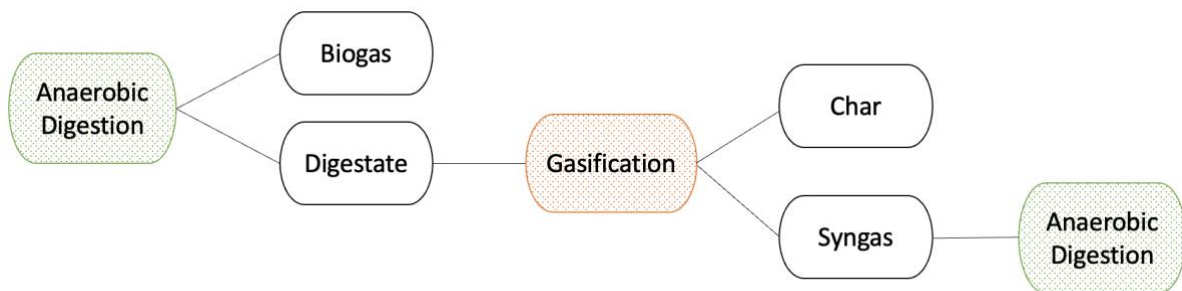


Figure 2.2. Anaerobic digestion and Gasification coupling scheme [18].

2.3.1 Gasification of Anaerobic Digestion Residues

The quality of syngas produced through gasification highly depends on the biomass type. Poor-quality syngas (low heating value (4 MJ/m^3)) can create issues for the downstream energy systems. Therefore, compared to single-stage gasification, a two-stage approach, an AD system followed by gasification, has demonstrated its benefits in improving overall waste-to-energy efficiency. This coupling method converts non-readily biodegradable substrates like hemicellulose, lignin, and cellulose to higher heating value gas (24 MJ/m^3) [35]. Combining AD and gasification by gasifying the dried biomass after AD can also resolve the disposal of the biomass simultaneously [18]. Another factor in generating high-quality syngas with the gasification process is keeping the initial moisture level of the feedstock less than 30%. Pre-drying uses a lot of energy; however, the heat generated during the gasification process can be saved to dry the wet biomass after AD, improving the effectiveness of the coupling system [18], [36].

2.3.2 Biomethanation of Syngas in AD Reactors

Syngas biomethanation (SB) is a biological biogas upgrading process and relies on acetogenic and hydrogenotrophic methanogen's ability to produce CH_4 from injected electron donor gases, i.e., H_2 and CO (Figure 2.3). In the AD reactor, acetogens and methanogens use syngas and act as biocatalysts that consume the syngas and generate CH_4 and CO_2 [37]. Therefore, methane production from syngas involves two steps: thermochemical processes (converting biomass to syngas) and SB (converting syngas to methane) [6], [16]. SB is applicable in both in-situ and ex-situ systems. In the in-situ approach, the inorganic electron donor gases are injected into an AD reactor to be consumed with the available CO_2 and converted into CH_4 by hydrogenotrophic methanogens. The ex-situ alternative involves the injection of syngas into

an external reactor containing enhanced hydrogenotrophic cultures, where the cultures transform syngas into CH_4 [30], [38].

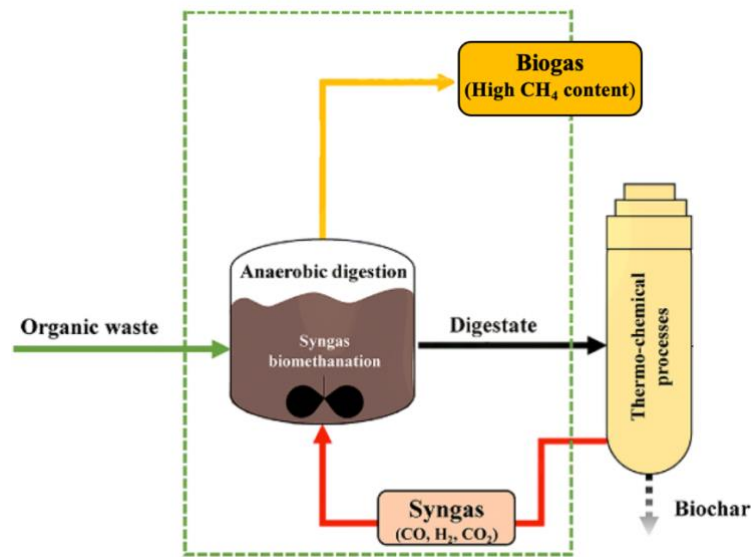


Figure 2.3. Syngas bio-methanation by coupling gasification and AD [17].

2.4 Syngas Biomethanation Pathways

SB could proceed through different pathways; some are direct, while others are indirect [39]. This is because there is a wide range of interconnected biochemical reactions and syntrophic relationships between microbial groups that can utilize syngas and use products of other microorganisms (Figure 2.4) [40].

2.4.1. Bioconversion of CO and H₂/CO₂

The direct pathway of CH_4 production comprises hydrogenotrophic methanogenesis (Eq. 1) and carboxydrotrophic methanogenesis (Eq. 4). Based on the equations and Figure 2.4, hydrogenotrophic methanogens use H_2 and CO_2 to produce CH_4 , while carboxydrotrophic methanogens convert CO directly to CH_4 . Therefore, hydrogenotrophic methanogens activity increases the stability of the whole process by metabolizing hydrogen and CO_2 into Methane [39].

In addition, CO and H₂/CO₂ can be converted indirectly to CH₄ with acetate and H₂/CO₂, which can produce methanogenic precursors. The indirect conversion can take place in homoacetogenesis (Eq. 5), carboxydrotrophic acetogenesis (Eq. 3) or syntrophic acetate oxidation (Eq. 7) with the reductive acetyl-coenzyme A (Acetyl-CoA) pathway. The methanogenic precursors are subsequently transformed to CH₄ with hydrogenotrophic methanogenesis (Eq.1) and acetoclastic methanogenesis (Eq. 6). Also, the production of CH₄ can take place through carboxydrotrophic hydrogenogenesis (Eq. 8) [14], [21], [31], [32],

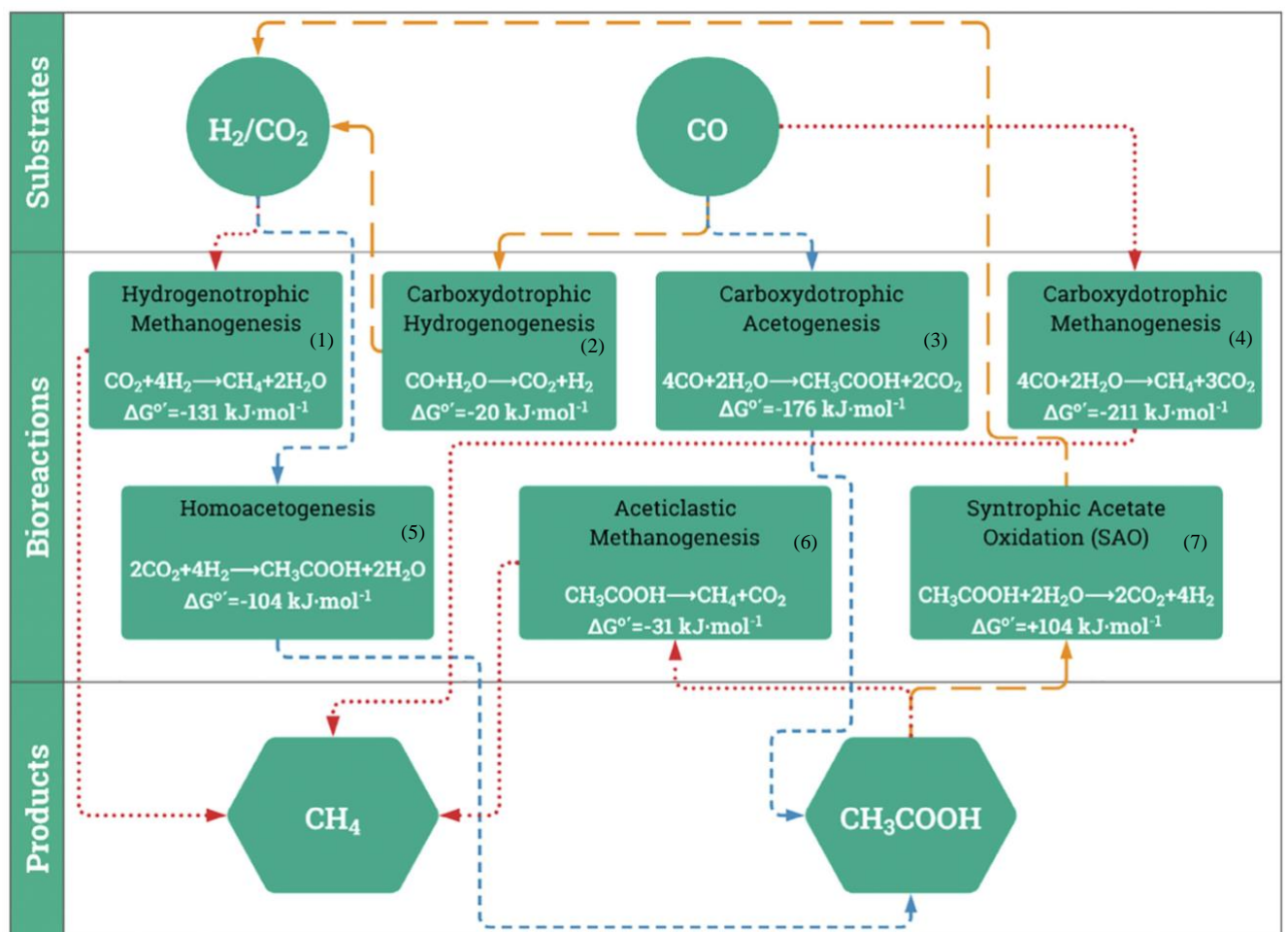
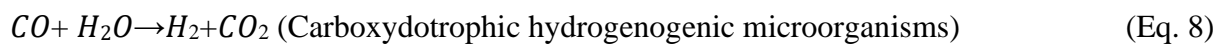
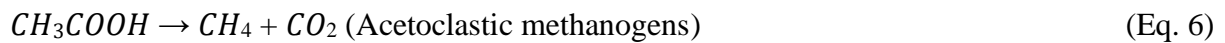
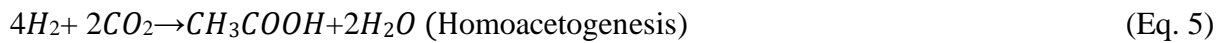
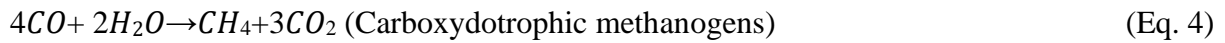
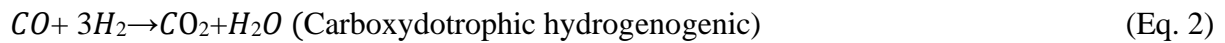
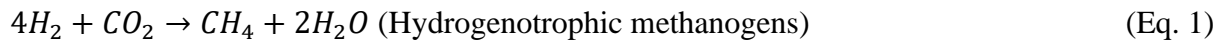


Figure 2.4. Microbial metabolic pathways convert syngas to CH₄. Methanogenic reactions are represented by red-dotted arrows, acetogenic reactions by blue-small-dashed hands, and hydrogenogenic reactions by orange-big-dashed arrows [36].

[39], [43].



As shown in Figure 2.5, CO-dehydrogenase complex (CODH) is an essential enzyme in the biological conversion of CO. CO oxidation is catalyzed by the CODH enzyme complex, as shown in (Eq. 9) [6], [31]. The CODH function can vary: CODH can be monofunctional and catalyze the CO oxidation to form CO₂, or it can be bifunctional and produce H₂ and acetyl coenzyme A (acetyl-CoA). Sulphate and sulphur metabolism may occur with the oxidation of CO, producing hydrogen sulphide (H₂S) [6], [31].

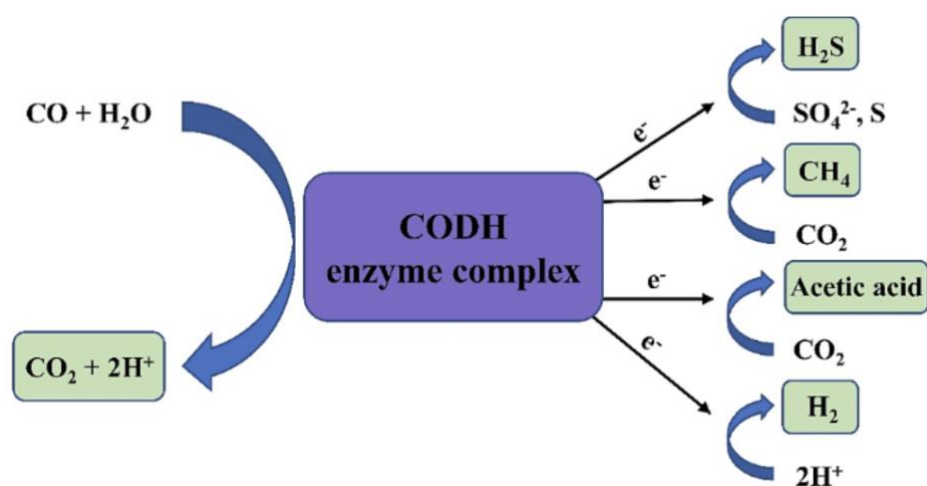


Figure 2.5. Anaerobic respiratory process coupled to CO oxidation [31].

2.4.2 Bioconversion of H₂

As mentioned before, the addition of H₂ into the AD reactor enhances the methanogenesis process as the hydrogenotrophic bacteria consume the H₂ with CO₂ and produce methane as a by-product (Eq. 1). More than 90% of the injected hydrogen converts to methane which increases the potential energy function of biogas, and the combustion properties can be improved by unconverted hydrogen. Also, based on Eq. 1, the consumption of CO₂ increases CH₄ content and decreases the upgrading cost [14]. Based on the methanogenesis process, hydrogenotrophic methanogens exist in an AD reactor. Therefore, injecting H₂ into AD can increase the hydrogenotrophic methanogenesis and upgrade methane production in the reactors by having more hydrogen in the reactor [6].

Based on Eq. (1), hydrogenotrophic methanogenic archaea consume four moles of H₂ and one mole of CO₂ to produce one mole of CH₄. Also, carboxydophilic hydrogenogenic microorganisms (Eq. 2) require 3 moles of H₂ and one mole of CO to generate a high-quality biomethane gas [44]. However, produced syngas by gasifying biomass does not have this gas mixture. Therefore, additional sources of H₂ might be required. H₂ for the biogas improvement must also come from renewable sources for the whole process to be considered green energy. For renewable sources to provide the H₂ balance of the upgrading process, the concept of power to gas has been suggested by [Ahern et al. 2015]. Excess electricity from windmills can be converted to hydrogen with an electrolysis process [45]. Also, there are other primary renewable sources of hydrogen, such as petroleum refineries, biomass gasification, petrochemical plants, and soda manufacturing [14].

A central technical challenge with hydrogen addition is the pH increase to values over 8.5, which inhibits methanogens' activities [38]. Typically, an injection of H₂ exceeding a stoichiometric ratio of 4:1 (H₂ to CO₂) would abruptly reduce CO₂ in the reactor, increasing pH because of the removal of bicarbonate (Eq. 10). Additionally, it will prevent autotrophic

hydrogenotrophic methanogenesis since this process needs CO₂ as a carbon source to convert H₂ to CH₄ [46]. Using pH control to maintain pH throughout the process is a strategy for overcoming this challenge; however, it requires the addition of chemicals which is not favourable. [38].



Besides, high H₂ partial pressure can inhibit the activity of methanogenic microorganisms and lead to the accumulation of VFAs. Therefore, if a sudden and high H₂ concentration is injected into an AD reactor, it can promote the accumulation of electron sinks like ethanol, lactate, butyrate, and propionate. Accordingly, because of excessive acidification by VFAs accumulation, the methanogenic activity of archaea would no longer be possible, and the system can become imbalanced or even completely shut down [38]. Therefore, the H₂ to biomass ratio and the effect of other components in syngas on the AD process should be evaluated to overcome the syngas limitations [6].

A recent study observed that acetate accumulated in batch reactors when H₂ was injected at a higher stoichiometric ratio for hydrogenotrophic methanogenesis. However, during long exposures to H₂, the hydrogenotrophic population increases the ability to utilize H₂ and reverts the inhibition [38].

2.4.3 Bioconversion of CO₂

Limited and conflicting information about the utilization and bioconversion of injected additional CO₂ is reported in the literature. Additionally, the potential application of CO₂ and possible effects of CO₂ injection on the microorganisms and final produced biogas are not reported rigorously. Since the reactions involved in AD are complicated and variable, finding the pathways in which the injected CO₂ is used and converted to CH₄ is challenging. Previously, no study reported a mass balance of ex-situ injection of CO₂ without adding H₂ in the AD [47].

An improvement in the hydrogenotrophic pathway by using acetic acid (VFAs) (Eq. 7) as an alternative supply of H_2 is reported by Alimahmoodi et al. (2008) [48]. However, this contradicts the studies of Salomoni et al. (2011) [45], where a two-stage AD system was tested with CO_2 injection at the first stage, where no hydrogenotrophic methanogenesis is active. In these studies, increased carbon assimilation (the process by which living organisms convert inorganic carbon like carbon dioxide to organic compounds) by the Wood-Ljungdahl pathway was proposed. This formation causes the production of acetate that will be utilized by acetoclastic methanogenesis at the final stages (Eq. 6) [49].

The Wood-Ljungdahl route uses CO_2 by reducing it to methyl and carbonyl branches for the precursor of CO_2 fixation. In the methyl branch, one molecule of CO_2 is converted to Formate ($CHOOH$) and then, by reduction with four more electrons, is converted to CH_3-H_4 -folate to form a methyl group. In the carbonyl branch, one molecule of CO_2 is converted to carbon monoxide by reducing two electrons (Figure 2.6) [50]. The produced methyl and carbonyl groups then form acetyl-CoA, which converts to carbon or acetyl-phosphate, which can lead to acetic acid formation. Acetoclastic methanogenesis (Eq.6) would then be an indirect pathway to produce CH_4 because of its high availability in the substrate [47].

The Wood-Ljungdahl route is dependent on the availability of an electron donor, such as H_2 , CO , or Formate. In the absence of sufficient electron donors, the microorganisms may switch to alternative metabolic pathways to generate energy and reduce CO_2 . In addition, if the injected CO_2 is not fully used during the AD process, the dissolved CO_2 in the final biomass may increase and contribute towards uncontrolled GHG emissions in later stages. Also, carbonation because of excess dissolved CO_2 in the liquid can lead to a pH drop in the reactor and inhibit methanogens activity. Therefore, before a full-scale application, it is necessary to estimate the amount of external CO_2 added to the reactors to optimize bio methanation and minimize GHG emissions [47].

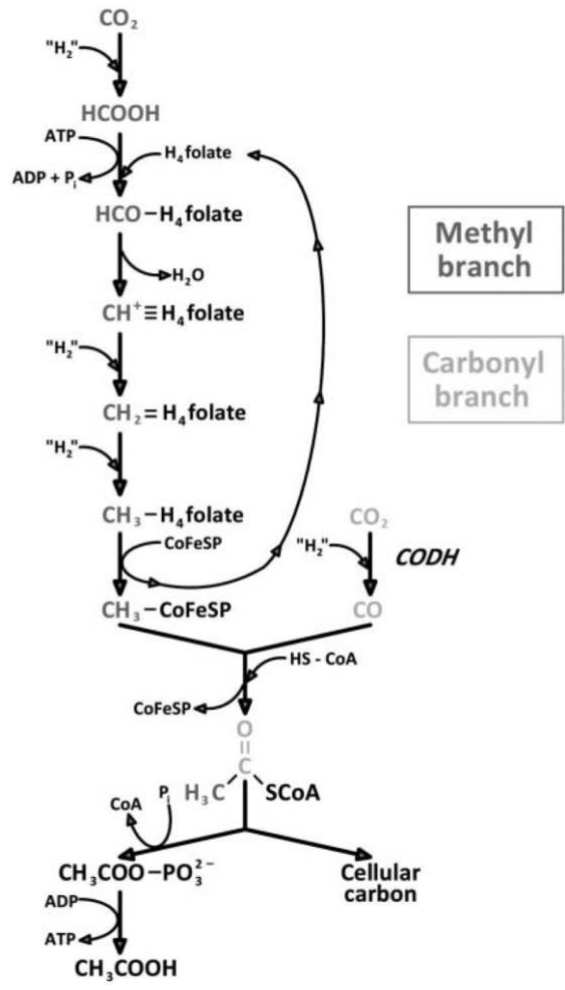


Figure 2.6. Wood-Ljungdahl pathway [47].

2.5 Syngas Biomethanation Operational Conditions

SB performance depends on environmental and operational factors such as pH, temperature, pressure, and syngas composition. This dependency is because of the activity of microorganisms engaged in SB.

pH: The hydrogenogenic microbial group generally has optimal growth at neutral pH. Additionally, most methanogens thrive at a pH of 6.8 to 8.5, close to natural and alkaline ranges. Consequently, SB is preferably conducted at neutral pH (7.0 and 7.6), at which both bacteria and archaea can grow and function [19], [32]. One of the primary factors that can impact pH during SB is the production of acids by microorganisms. The acetogens convert CO

and H₂ to acetic acid, which can release protons (H⁺) and acetate ions (CH₃COO⁻) into the solution and decrease pH.

The conversion of acetic acid by methanogens during AD can also result in changes in pH in the reactor. Methanogens use acetic acid as a substrate to produce methane and carbon dioxide, and the reaction involves the consumption of H⁺, which can increase the pH in the reactor. The acetoclastic methanogenesis pathway, which consists of the conversion of acetic acid directly to CH₄ and CO₂, can increase pH due to the consumption of H⁺. This pathway is typically favoured in AD systems operating at neutral to slightly acidic pH values. The hydrogenotrophic methanogenesis pathway, which involves the conversion of H₂ and CO₂ to CH₄ in the presence of acetate, can also result in a pH increase due to the consumption of H⁺. This pathway is typically favoured in AD systems operating at more alkaline pH values. Overall, the net effect of acetic acid conversion on pH during AD depends on the balance of the acidogenic and methanogenic reactions in the reactor. The pH can decrease if the acidogenic reactions produce more hydrogen ions than the methanogenic reactions consume. On the other hand, if the methanogenic reactions consume more hydrogen ions than the acidogenic reactions produce, then the pH can increase [51].

In addition, the pH can also be influenced by other factors such as the buffering capacity of the substrate, the microbial populations present in the reactor, the substrate loading rate and retention time in the AD reactor. A high loading rate can result in the rapid production of acids, leading to a decrease in pH, while a longer retention time can produce more CH₄, which consumes protons and can help buffer the pH [32].

Temperature: Microbial activity during SB can proceed under both thermophilic and mesophilic temperatures. However, temperature increments can influence the interactions between microbes and determine prevailing metabolic pathways used by consortiums [32]. Although gas solubility under thermophilic conditions is low, the microbial growth rate

approximately doubles with every 10 °C rise in temperature. Thus, the temperature can significantly shift microbial communities and growth rates and improve chemical reactions in the liquid phase [16], [28].

CO Partial Pressure: The partial pressure of CO can significantly impact the performance of SB. In general, higher partial pressures of CO (≥ 1 atm) can inhibit the activity of the microorganisms responsible for the bio-methanation process, thereby reducing the overall conversion efficiency of the process [52]. In general, CO is toxic to many organisms involved in the methanation process, such as methanogens and acetogens, and can inhibit their activity even at relatively low concentrations. The toxicity range of CO on microorganisms depends on several factors, including the concentration and exposure time of CO and the type of microorganisms involved. CO can bind to the same active sites on microorganisms as H₂, reducing the availability of active sites for H₂ oxidation. This competition for active sites can limit the rate of methanogenesis, reducing methane production. Additionally, CO can inhibit enzymes involved in H₂ oxidation, further reducing the rate of methanogenesis. Furthermore, the presence of CO can also lead to the formation of intermediates such as CO₂ and formate, which can further inhibit the methanation process [53]. The degree of inhibition caused by CO can vary depending on the concentration of CO in the syngas feedstock and the duration of exposure. The inhibition may be reversible, and the microorganisms can recover once the concentration of CO decreases [54], [55].

Achieving higher methanogenic potential under a 100% CO atmosphere after acclimation of the sludge to CO is possible due to the ability of some microorganisms to adapt to the presence of CO and utilize it as a substrate for methanogenesis. The process of acclimation involves exposing the organisms in the sludge to gradually increasing concentrations of CO over time. During this process, the microorganisms that can use CO as a substrate will adapt and become more efficient at converting CO to methane. This adaptation can involve changes in gene

expression, enzyme production, and metabolic pathways. However, it is essential to note that achieving higher methanogenic potential under a 100% CO atmosphere may not always be desirable, as it can also result in the production of intermediates [54], [55] (Figure 2.7).

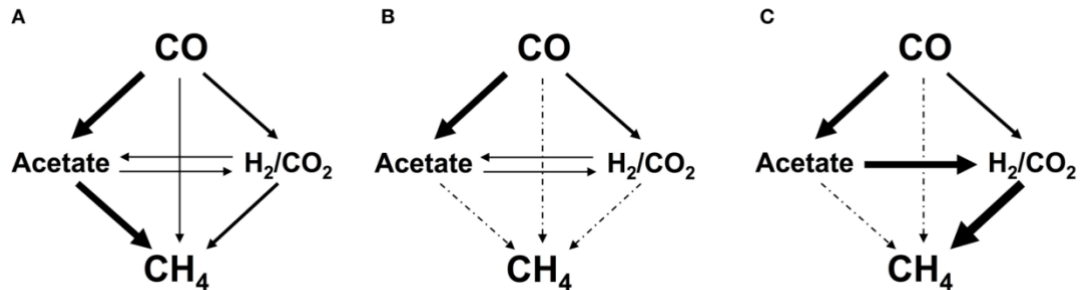


Figure 2.7. Conversion pathway of CO to CH₄ in AD. A: low CO pressure (<0.5atm), B: high CO pressure (≥1 atm) and C: 100% CO concentrations after acclimation. The thickness of the arrow represents the possibility of pathways. (thick: 60–80%, intermediate: 20–40%, thin:5-20% and dotted lines: blocked pathway) [54].

Figure. 2.7 is in line with the findings of Guiot et al. (2011) [26], where different CO partial pressures (from 0.42 to 0.96 atm) on SB were investigated under mesophilic temperatures. In conclusion, the highest CO conversion efficiency was 75% at a CO partial pressure of 0.6 atm [33].

Gas-liquid transfer: One of the limiting factors in SB is the insufficient gas-liquid transfer of synthetic gas into the liquid phase [56]. Based on Henry's law, mass transfer depends on many variables, including the gas flow rate, gas partial pressure, gas solubility in the liquid phase, temperature, liquid characteristics, reactor type, and mixing speed. Unlike CO₂, CO and H₂ have low solubility in the liquid, leading to lower conversion rates, reduced methane production, and restricting the system's ultimate productivity. At standard temperature and pressure (STP), the solubility of CO₂, CO and H₂ in water is approximately 0.034, 2.8×10^{-3} and 1.6×10^{-4} mol/L [57], [58].

The gas solubility can be increased by lowering the temperature of the reactor and/or increasing pressure. However, because of the sensitivity of microbial growth under low temperatures and high pressure, the most common approach for improving gas conversion is operating the

system using reactors with different configurations and designs [56]. Examples of reactor types used for overcoming the low gas solubility issue are continuously-stirred tank reactors (CSTR), stripping columns, CSTR with hollow fibre membrane modules (HFMBR), gas-lift reactors, trickle bed reactors, U-loop bioreactors, bulk-gas-to-atomized-liquid reactor and multi-orifice baffled bioreactor [56].

Another approach for increasing gas-liquid transfer is diffusing gas to an AD reactor using bubble generation devices. The diffusers create a high interfacial area between the gas and the liquid, allowing for efficient mass transfer and gas dissolution in the liquid. Bubble gas diffusers are typically made up of a porous material, such as ceramics, plastics, or metals, that contain small pores or orifices through which the gas is released. The size and geometry of the pores can be adjusted to control the size of the bubbles and the rate of gas flow. As the bubbles rise through the liquid, they create turbulence and mixing, which enhances the contact between the gas and the liquid, promoting efficient mass transfer. The performance of bubble gas diffusers can be influenced by several factors, including the pore size and distribution, gas flow rate, liquid properties (such as temperature, pressure, and viscosity), and the presence of other materials in the liquid that can affect the gas-liquid mass transfer. Optimizing these factors can help to maximize the efficiency of bubble gas diffusers and ensure effective gas dissolution in a variety of applications [59], [60].

2.6 Recent Advancements in Syngas Bio methanation

Kozak et al. (2022) reported the effect of different CO₂:H₂ ratios on the hydrogenotrophic methanogenesis of an anaerobic digester of a municipal wastewater treatment plant in batch-mode experiments in both mesophilic and thermophilic temperatures. CH₄ content in the produced biogas resulting from syngas injection with CO₂:H₂ molar ratios of 1:1, 1:2, 1:3 and 1:4 was 24, 45, 63 and 73%, respectively, at 55°C and 23, 42, 58 and 69%, respectively, at 37

°C. The maximum CH₄ content was for the sample with the stoichiometric ratio of 1:4 at 55°C. This study demonstrated the influence of the stoichiometric relationship between CO₂ and H₂ and thermophilic temperature, which led to hydrogenotrophic methanogens increment responsible for increasing methane content in the biogas [61].

The above findings align with Corbellini et al. (2019), where hydrogenotrophic methanogen counts were improved in semi-continuous mode. Four CO₂ and H₂ molar ratios from 1:1 to 1:4 was injected into the AD reactor. The produced biogas in the case with the ratio of 1:4 showed the highest methane content (about 80%). Using ratios higher than the stoichiometric value and a longer acclimation time while achieving or exceeding the stoichiometric H₂/CO₂ ratio was suggested to further enhance methane and biogas production [62].

Li et al. (2022) investigated the effect of reactor mixing speed (low speed (300 rpm), medium speed (500 rpm), and high speed (800 rpm)) on SB by injecting syngas into biomass with a CO₂:CO: H₂ molar ratio of 1:4:5. Increasing the mixing speed improved the gas-liquid mass transfer, which was noted by increasing CO conversion efficiency from 82.6% to 99.8%. Also, the results showed that it was feasible to carry out SB at high CO concentrations (40% CO). Increasing the rotation speed significantly improved CH₄ concentration and altered the process toward the CO-to-H₂ pathway [53].

Yang et al. (2020) investigated injecting syngas at CO:H₂ molar ratio of 4:5 with different syngas loading rates (starting from 560 mL/d to 5300 mL/d). Two continuous stirred-tank reactors (CSTR) with a working volume of 4 L, mixing speed of 300 r/min and uncontrolled pH were operated on both mesophilic and thermophilic temperatures. Food waste as feedstock and sewage sludge from a sewage treatment plant as biomass was tested for SB. An aeration basket was utilized to diffuse gas to reactors to increase the gas-liquid mass transfer rate. Results indicated that methane production in the thermophilic condition was almost stable in the experiment's lifetime and was 5 to 30% higher than in mesophilic temperature. Also, the

CO conversion rate increased with temperatures increasing from 37 °C to 55 °C. Under the mesophilic condition, an abrupt VFAs increment was observed that caused inhibition in biogas production. This result indicated that CO was more toxic to microorganisms in the methanogenesis step. However, the methanogens were more tolerant to CO addition in the reactor operating at thermophilic temperature. For both reactors, the conversion efficiency of syngas was over 97%, regardless of the syngas loading rate. Results indicated the need for a higher stoichiometric ratio of H₂ to convert all the injected CO in the reactor [16].

On the other hand, Andreides et al. (2022) observed that increasing the syngas loading rate from 1 to 1.5 L/(L_R·d) negatively impacted SB performance. CO and H₂ conversion efficiency were reduced, and CH₄ production was one-third of the stage with the syngas loading rate from 1 L/(L_R·d). The limiting parameters were low gas-liquid mass transfer rate and poor gas diffuser to the reactors performance [43].

The effects of temperature and mixing speed in mesophilic CSTRs with syngas composition of CO₂:CO: H₂ with molar ratio of 1:4:5 on SB were tested by Yeqing Li et al. (2019). Increasing the mixing speed of the reactor from 400 rpm to 500 rpm increased methane content from 43% to 48%. This again indicated the positive effect of high, mixing speeds on facilitating CO and H₂ conversion to CH₄. But syngas flow rate increments reduced methane content from 48% to 44%. Some of the injected gas was not consumed because of low gas-liquid transfer. Compared to the study of Yang et al. (2020) [16], there was little difference in the final methane content of reactors at 37 °C and 55 °C, and even the mesophilic system was more stable during the experiment [63].

Achinas et al. (2020) investigated the effect of different liquid-to-gas ratios on the metabolic preference of microorganisms in batch reactors flashed with syngas (H₂ 55%, CO 25%, CO₂ 10% and CH₄ 10%). In all ratios, a substantial increase in CH₄ content was observed. With a liquid-to-gas ratio 1:1, methane concentration reached 60% after four days of injection; higher

gas-to-liquid ratios (1:3 and 1:5) took eight days to get the methane content of 60%. Higher ratios decreased the production rate of CH₄ and reduced the speed at which the CO was consumed. On day four of the experiment, CO in the 1:1 ratio was completely consumed, whereas 20% unconsumed CO was observed in the bottles with higher gas-to-liquid proportions. This also agrees with theoretical calculations because lower methanogens were present for degrading substrates in ratios where more syngas was injected [64].

Fernández et al. (2014) investigated CO₂ bio methanation in AD for biogas upgrading. CO₂ bio methanation was tested by injecting CO₂ at 0, 0.3, 0.6 and 0.9 molar fractions in batch reactors treating food waste or sludge. The higher increase in CH₄ concentration is reported in reactors with sludge than in food waste. The limiting factor in the food waste reactor was the toxic concentrations of NH₃ that inhibited acetoclastic methanogens. Daily methane production increased by 96–138% for the reactor treating sludge on the first day of the experiment, and carbon dioxide in the produced biogas was reduced by 8–34% [65].

Several articles used mathematical modelling to investigate how to optimize and better understand an AD reactor's performance. Shah et al. (2017) developed a version of the ADM-1 to incorporate SB into the model. In the modified ADM-1, the gas-liquid transfer of CO and H₂ is adjusted by considering the concentrations of dissolved CO and H₂ and the mass transfer coefficient (kLa (kg/m³/h)). kLa included operational factors like mixing, gas pressure, substrate properties and temperature. Syngas with high H₂ concentration (85%) showed a methane production increment of 47% with a methane content of 81% in the produced biogas. The respective methane production increment and content in low-hydrogen syngas (45%) were 42 and 67%. Also, pure H₂ addition resulted in a methane content of 92 %; however, biogas production was increased only by 33%. This is because pH increased to inhibitory levels due high CO₂ consumption rate. According to the simulated models, the optimal syngas

composition to inject into AD for improving methane generation should have a 70–80% hydrogen percentage [11], [66].

2.7 Summary and Knowledge Gaps

As indicated in Table 2.1 researchers have been exploring various techniques to improve the process of SB inside AD reactors. These approaches included optimizing process conditions, improving gas-liquid transfer, and adjusting the rate and composition at which syngas is injected into the system. The importance of stoichiometric ratios between gases, the effect of liquid mixer speed and reactor temperature on the conversion efficiency of gases to methane, the method of gas diffusing to the liquid phase, and mathematical modelling for increasing SB have been repeated in previous experiments. However, it is worth noting that the current information is still limited regarding the biomethanation of different combinations of CO, CO₂, and H₂ gases, both individually, in varying stoichiometric ratios between gases, and in different gas-biomass ratios. No comprehensive research is reported on this topic, particularly in batch and semi-continuous mode and under mesophilic temperature conditions.

Table 2.1. Summary of Recent Advancements in Syngas Bio methanation

Gas type and Ratio	Enhancement Method	Reactor Type	Temperature	Results
CO ₂ :H ₂ 1:1 to 1:4	Increasing Hydrogen ratio.	Batch	Mesophilic and Thermophilic	Max CH ₄ content (73%) at 1:4 ratio and 55°C.
CO ₂ :H ₂ 1:1 to 1:4	Increasing Hydrogen ratio.	Semi-continues	Mesophilic	Max CH ₄ content (80%) at 1:4 ratio.
CO ₂ :CO: H ₂ 1:4:5	Increasing mixing speed.	CSTR	Thermophilic	CO conversion increased to 99.8%.
CO:H ₂ 4:5	Increasing syngas loading rate.	CSTR	Mesophilic and Thermophilic	Reduced CO and H ₂ conversion efficiency. Thermophilic system was more stable.

CO ₂ :CO: H ₂ 1:4:5	Increasing mixing speed and syngas loading rate.	CSTR	Mesophilic and Thermophilic	CO and H ₂ conversion increased by increasing mixing speed. Syngas flow rate increments reduced methane content. Mesophilic system was more stable.
CO ₂ at 0, 0.3, 0.6 and 0.9 molar fractions.	Increasing ratio.	CO ₂ Batch	Mesophilic	Daily methane production increased by 96–138%, and carbon dioxide in the produced biogas reduced by 8–34%.
Syngas with pure H ₂ , high H ₂ concentration (85%) and low-H ₂ syngas (45%)	Increasing Hydrogen ratio.	CSTR	Mesophilic	Optimal syngas composition for improving methane generation is syngas with 70–80% H ₂ .

Due to the wide variability in the composition of syngas, which is influenced by the feedstock and processing method, it is essential to explore the impact of different gases in syngas on biogas upgrading during the AD process. This investigation should occur before scaling up from laboratory-scale to pilot-scale and industrial-scale operations. By understanding the effects of each gas component, researchers can optimize the process and improve its efficiency at larger scales.

Chapter 3

3. Methodology

3.1 Overview

This chapter covers the materials and methods used throughout the experimental laboratory work. The experiments consisted of two phases using the same type of materials, i.e., syngas and biomass, but differed in the composition and frequency of injection. Phase I represents batch mode testing. In phase I, there was only one gas injection at the start. In addition to testing different syngas compositions, three gas-to-liquid ratios (0.5, 1 and 1.5 mL gas/mL biomass) were tested. Phase II represents a semi-continuous mode where multiple gas injections with time intervals were tested. The best syngas compositions from Phase I were selected for testing in a semi-continuous mode during Phase II. Additionally, syngas with higher stoichiometric ratios between gases was investigated in Phase II.

3.2 Biomass and Syngas

The anaerobic digestion (AD) biomass was obtained from Fepro farm, a biogas plant in Ottawa, treating cattle manure and corn silage at 35°C. The biomass was used for the experiment on the day it was collected from the plant. The CO, CO₂ and H₂ were used as the gaseous substrates

for all the syngas bio-methanation (SB) experiments. Each gas was transferred from a gas cylinder (purchased from Messer Canada Inc.) into a Tedlar bag which was then used to draw the amount needed for each sample.

3.3 Experimental Procedures

Serum bottles with a working volume of 250 mL (total volume of 300 mL) were used for all batch and semi-continuous experiments, and all the experiments were conducted in triplicates. The experiment procedure was as follows: 20 mL biomass was added to the serum bottles. N₂ was flushed for 5 min to remove the air in the headspace of the serum bottle, and then bottles were sealed with rubber stoppers and plastic caps immediately. The appropriate volume of gases was drawn from the corresponding Tedlar bags using a syringe and injected into the biomass using a long needle that allowed injection directly into the liquid phase. In batch mode, all the gases were injected into the bottles immediately after sealing the bottles. Then, the bottles were placed in a temperature-controlled shaking incubator at 35°C and 450 rpm (Figures 3.1 and 3.2). The shaking speed was set at the incubator's maximum capacity to increase gas-to-liquid conversion efficiency. Biogas production and composition were monitored using a manometer and gas chromatography (GC) machines every two days for the first ten days and then reduced to every four days.

The same procedure was repeated for the semi-continued method; however, gases were injected in multiple stages with time intervals. After the first injection, bottles were placed in the incubator. Biogas production and gas composition was monitored every day. When H₂ was depleted (not detected in the headspace of the bottles), the next injection took place. Injections were repeated four times.

Control samples consisted of 20 mL biomass with no gas injection to estimate the contribution of the biomass organic matter to biogas production and allow the calculations for net biogas production of samples with the substrate prepared for both phases.

The experiments continued until the methane production levelled out, and no biogas production was observed in the samples. The primary objective of the experiments is to observe the effect of different syngas components and gas-biomass ratio on the AD process. To achieve this goal, the biogas production and conversion rate of varying syngas composition to methane was monitored and compared with the control reactor.



Figure 3.1. Incubator.



Figure 3.2. BMP bottles with biomass and substrate inside the incubator.

3.3.1 Phase I

In the first phase of the experiment, different individuals and combination of H₂, CO, and CO₂ in three different gas-to-liquid ratios (0.5, 1, 1.5 mL gas/mL biomass) were tested (Tables 3.1, 3.2 and 3.3).

The single substrate was CO₂ for condition A and CO for condition B. Dual substrate combinations were tested in conditions C and D, and H₂ was added with stoichiometric ratios of 1:3 for CO:H₂ and 1:4 for CO₂:H₂. In condition E, the stoichiometric ratio of 1:2:3 for CO₂:CO:H₂ was selected to examine a combination of syngas with the composition produced after the industrial gasifier, gasifies biomass. More H₂ than stoichiometric was added to reach a ratio of 1:1:7 for CO₂:CO:H₂ for converting all the CO and CO₂ into methane in condition F. As the sole substrate, pure H₂ was added based on Table 3.4 under two conditions (H₂a and H₂b). Control bottles in which biomass without gas injection was tested for a period of 45 days were considered as a source for calculating the total injection volume of H₂. Since there is a 1:4 molar ratio between CO₂ and H₂, the total volume of H₂ under condition H₂a was calculated to be four times of the total CO₂ the control sample produced without any gas substrate. The total volume was calculated as 60 mL of H₂. Under condition H₂b, the total injected H₂ was reduced to 40 mL to test the effect of lower H₂ partial pressure on gas conversion efficiency to methane.

The total biogas (CH₄ and CO₂) produced over 45 days in the control sample was subtracted from total biogas production in conditions A through F to estimate the net effect of injected gases in upgrading biogas production. For the samples where only H₂ as substrate was fed to the bottles, because of operational inhibitions, the total produced methane was lower than the control system. Therefore, the graphs will show the results of total produced biogas without the reduction of biogas produced under the control system to avoid negative results, which be discussed further in the following chapter.

Table 3.1. Setup of syngas bio-methanation experiments at the gas-to-biomass ratio of 0.5 (mL gas/mL biomass) (Phase I).

Substrate	Control	A		B		C		D		E		F	
CO ₂	0	0.45 mmol	10 ml	0		0		0.45 mmol	10 ml	0.137 mmol	3.0 ml	0.206 mmol	4.6 ml
CO	0	0		0.41 mmol	10 ml	0.41 mmol	10 ml	0		0.274 mmol	6.7 ml	0.206 mmol	5.00 ml
H ₂	0	0		0		1.23 mmol	27 ml	1.8 mmol	40 ml	0.411 mmol	9.3 ml	1.44 mmol	32.3 ml
CO ₂ : CO: H ₂	na	1:0:0		0:1:0		0:1:3		1:0:4		1:2:3		1:1:7	
Biomass (ml)	20	20		20		20		20		20		20	

Table 3.2. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1 (mL gas/mL biomass) (Phase I).

Substrate	Control	A		B		C		D		E		F	
CO ₂	0	0.9 mmol	20 ml	0		0		0.9 mmol	20 ml	0.274 mmol	6.10 ml	0.411 mmol	9.1 ml
CO	0	0		0.82 mmol	20 ml	0.82 mmol	20 ml	0		0.548 mmol	13.3 ml	0.411 mmol	10 ml
H ₂	0	0		0		2.45 mmol	54.6 ml	3.6 mmol	80.9 ml	0.822 mmol	18.5 ml	2.88 mmol	64.6 ml
CO ₂ : CO: H ₂	na	1:0:0		0:1:0		0:1:3		1:0:4		1:2:3		1:1:7	
Biomass (ml)	20	20		20		20		20 ml		20 ml		20 ml	

Table 3.3. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1.5 (mL gas/mL biomass) (Phase I).

Substrate	Control	A		B		C		D		E		F	
CO ₂	0	1.35 mmol	30 ml	0		0		1.35 mmol	30 ml	0.411 mmol	9.1 ml	0.617 mmol	13.7 ml
CO	0	0		1.23 mmol	30 ml	1.23 mmol	30 ml	0		0.822 mmol	20 ml	0.617 mmol	15 ml
H ₂	0	0		0		3.68 mmol	81.9 ml	5.4 mmol	121.3 ml	1.233 mmol	27.7 ml	4.32 mmol	97 ml
CO ₂ : CO: H ₂	na	1:0:0		0:1:0		0:3:1		1:0:4		1:2:3		1:1:7	
Biomass (ml)	20	20		20		20		20		20		20	

Table 3.4. Set up of H₂ bio-methanation experiments (Phase I).

Substrate	H ₂ a		H ₂ b	
CO ₂	0		0	
CO	0		0	
H ₂	2.67 mmol	60 ml	1.76 mmol	40 ml
CO ₂ : CO: H ₂	na		na	
Biomass (ml)	20		20	

*The volume of the gases in the tables is the amount injected into the bottle sample at the lab temperature (24 °C); for STP volumes, see the appendix.

3.3.2 Phase II

In the second phase (Tables 3.5 and 3.6), the mixture of gases with higher gas conversion rate to methane (CO:H₂ 1:3, CO₂:H₂ 1:4 and CO:CO₂:H₂ 1:1:7) and syngas with the industrial mixture (CO:CO₂:H₂ 1:2:3) for optimizing coupling SB and AD with the real syngas composition and pure hydrogen (H_{2a}) was selected from phase I. The total volume of gas injected into the bottles was divided into four equal portions according to (Tables 3.6 and 3.5) with time intervals to experiment with the semi-continued mode and investigate the effect of adaptability of biomass to the injected gases. Moreover, in phase II higher stoichiometric ratios (CO:H₂ 1:4 and CO₂:H₂ 1:5) were tested to investigate their effect on increasing methane content in the produced biogas.

In phase II, the total biogas (CH₄ and CO₂) produced in 30 days in the control sample was subtracted from all the samples to obtain the net effect of injected gases in upgrading biogas production.

Table 3.5 Setup of syngas bio-methanation experiments with multiple injections (Phase II).

Substrate	Control	G		H		I		J		K		L	
CO ₂	0	0		0		1.2 mmol	27.0 ml	1.2 mmol	27.0 ml	0.36 mmol	8.10 ml	0.55 mmol	12.0 ml
CO	0	1.10 mmol	27.0 ml	1.10 mmol	27.0 ml	0		0		0.73 mmol	18.0 ml	0.55 mmol	13.5 ml
H ₂	0	3.3 mmol	73.5 ml	4.4 mmol	98.0 ml	4.8 mmol	108.0 ml	6.0 mmol	135.0 ml	1.10 mmol	24.5 ml	3.84 mmol	86.5 ml
CO ₂ : CO: H ₂	na	0:1:3		0:1:4		1:0:4		1:0:5		1:2:3		1:1:7	
Biomass (ml)	20	20		20		20		20		20		20	

Table 3.6. Set up of H₂ bio-methanation experiments with multiple injections (Phase II).

Substrate	H ₂ c	
CO ₂	0	
CO	0	
H ₂	3.5 mmol	80 ml
CO ₂ : CO: H ₂	na	
Biomass (ml)	20 ml	

*The volume of the gases in the tables is the amount injected into the bottle sample at the lab temperature (24 °C); for STP volumes, see the appendix

3.4 Analytical methods and Calculation

3.4.1 Biomass Characteristics

pH was measured using a pH meter (HACH). Volatile fatty acids (VFAs) were measured using the Esterification method (HACH TNT 872 50-2500 mg CH₃COOH/L) and spectrophotometer at the beginning and end of the incubation (Figure 3.3). The total solids concentration in the biomass sample was calculated using the gravimetric technique. For the TS, 10 g of homogenized sample was poured into pre-weighed aluminum dishes. Samples were put in an oven at 105°C for 6 hours and then weighed [67]. After weighing the previous dishes dried at 105 °C, residues produced from Total Solids were ignited in a furnace at 550 °C for 3 hours and then weighed to measure the volatile solids [68].



Figure 3.3. Spectrophotometer for measuring VFAs.

3.4.2 Biogas Composition

The percentage of each gas in the produced biogas was analyzed using GOW-MAC 350 and GOW-MAC 400 (Figure 3.4). The H₂ and CO in the headspace of bottles were measured using GOW-MAC 350 equipped with a thermal conductivity detector (TCD) and Molsieve 13x

80/100 (8'x48") column with Argon as carrier gas. Setup parameters were as follows; the Carrier flow rate: 30 mL/min on each side, the TCD temperature: 100°C, the column temperature: 80°C, the bridge current: 80 ma and the detector temperature: 120°C.

N₂, CO₂ and CH₄ were measured by GOW-MAC 400 equipped with a TCD and Molsieve T 100/120 (5'x125") column with Helium as carrier gas. Setup parameters are as follows: 35 mL/min on each side, TCD temperature: 100°C, column temperature: 80°C, bridge current: 50 ma, and detector temperature: 120.

Since two GCs were used for gas analysis, calibration equations for each detectable gas were created before gas sampling and analysis were started.



Figure 3.4. GOW-MAC 350 and 400 Instruments.

3.4.3 Biogas Volume

The volume of produced biogas production from each bottle was measured using a U-tube water manometer and water displacement method (Figure 3.5). U-tube water is connected to the bottles with a needle. The biogas in the headspace flows into the cylindrical flask and displaces the liquid. The volume of replaced water is taken as the volume of gas (Eq.13) [69] [70]. The quantity of methane and carbon dioxide was calculated using the percentage of each gas (gas composition), the volume of produced biogas and bottle headspace.

$$V_{\text{CH}_4,n} \text{ (mL)} = ((V_{\text{biogas},n} + V_{\text{headspace}}) \times \frac{\%_{\text{CH}_4,n}}{100}) - (V_{\text{headspace}} \times \frac{\%_{\text{CH}_4,n-1}}{100}) \quad (\text{Eq.13})$$

$V_{\text{CH}_4,n}$: the methane generation volume (mL).

$V_{\text{biogas},n}$: the biogas generation volume (mL).

V_h : the headspace volume (mL) of each bottle.

$\%_{\text{CH}_4,n}$: the methane percentage of the generated biogas calculated by GC.

$\%_{\text{CH}_4,n-1}$: the methane percentage of generated biogas in the previous sample.

The same equation was applied for calculating the volume of CO₂ in the headspace.

To focus solely on the production of biogas and consumption of injected gases, the total volume of gas in the headspace was adjusted by eliminating the remaining volume of N₂.

After each reading and calculation, the daily biogas production volume was converted to STP temperature and pressure (STP 273 K, 1.01325 Pa) condition [71].



Figure 3.5. U-tube water manometer

3.4.4 Conversion Efficiency and Methane Yield

Syngas bio methanation performance was evaluated by calculating the following parameters: methane yield (y_{CH_4} , mol CH_4 /mol H_2) and the biological conversion efficiency of CO and CO_2 . Measured gas volume fractions were converted to molar fractions using the ideal gas law under STP conditions.

Methane yield (y_{CH_4} , mol CH_4 /mol H_2), was calculated as the total mol of produced CH_4 divided by mol of injected H_2 according to (Eq.14). The biological conversion efficiency of H_2 , CO and CO_2 (η_i) were calculated according to (Eq.15). S_i is the mol of injected H_2 , CO, CO_2 or CO plus CO_2 [61], [72], [73].

$$\text{Methane Yield} = \frac{(CH_4)_{out}}{(H_2)_{in}} \quad (\text{Eq.14})$$

$$\eta_i (\%) = \frac{(CH_4)_{out}}{(S_i)_{in}} \times 100 \quad (\text{Eq.15})$$

Chapter 4

4. Results and Discussion

This chapter presents and discusses the results obtained from Phases I and II. The primary objective of the experiments was to observe the effect of different syngas compositions and gas-biomass ratios on the conversion efficiency of injected gas to methane. The biogas production and conversion rate of different syngas compositions to methane were monitored and compared with the control sample.

4.1 Biomass Characteristics

The biomass was characterized and it contained a total solid (TS) of 42.07 ± 0.18 g/L, volatile solid (VS) of 31.14 ± 0.03 g/L, pH of 7.75 ± 0.06 , and VFAs of $9,366 \pm 1.14$ mg/L for Phase I and TS of 45.33 ± 0.1 g/L, VS of 36.03 ± 0.05 g/L, pH of 7.83 ± 0.08 , and VFAs of $9,042 \pm 1.71$ mg/L for Phase II.

4.2. Phase 1 Results: Syngas single injection in batch mode

4.2.1 Biogas Production in the Control Sample

A control sample was used while testing Biochemical Methane Potential (BMP) to determine the amount of methane produced by the biomass itself in order to calculate the net effect of

bio-methanation of injected gases (Figure 4.1). VFAs and pH on the last day of 45 days experiment were measured. VFA was reduced to $8,670\pm 1.71$ mg/L and pH increased to 8.05 ± 0.18 in the control sample without any injected gases.

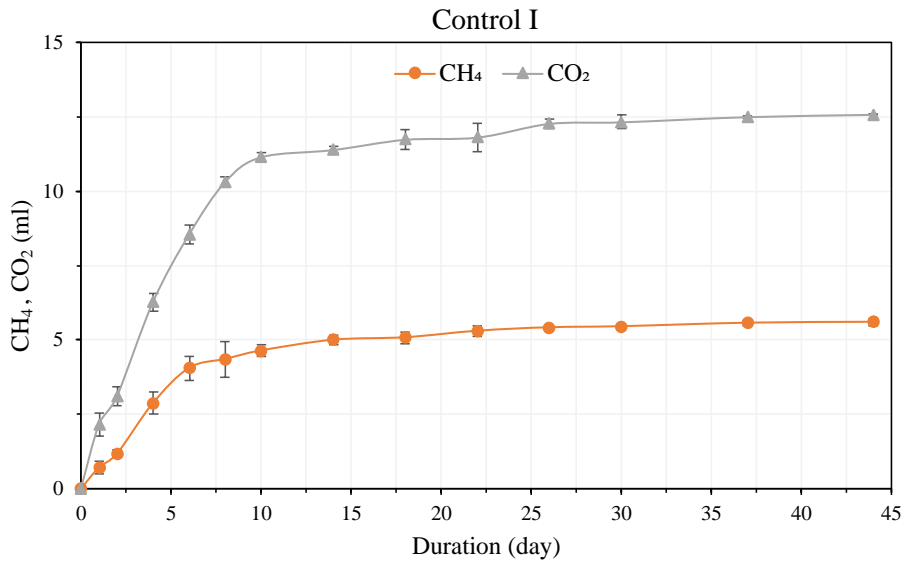


Figure 4.1. Accumulative biogas production in the control sample.

4.2.2 CO₂ Biomethanation (Sample A)

The average net accumulative CO₂ consumption and CH₄ production in three gas-liquid ratios are presented in Figure 4.2. In all three conditions, methane production increased with the consumption of CO₂, indicating the utilization of CO₂ substrate by different bacterial genera. The conversion efficiency of 33.1%, 27.8% and 25.2% was observed respectively for ratios 0.5, 1 and 1.5 (mL gas/mL biomass) during 45 days with a methane content of 40.9%, 36.8% and 32.3% (Figure 4.3). As expected, methane content and conversion rate were lower in higher gas-to-liquid ratios. This was mainly because of a lower portion of gas and hence more biomass and bacterial genera available in a ratio of 0.5 (mL gas/mL biomass). Also, gas-to-liquid conversion can be another inhibitory factor, which causes CO₂ in higher ratios not to be able to convert totally to methane inside the biomass and remain untouched in the headspace of the samples.

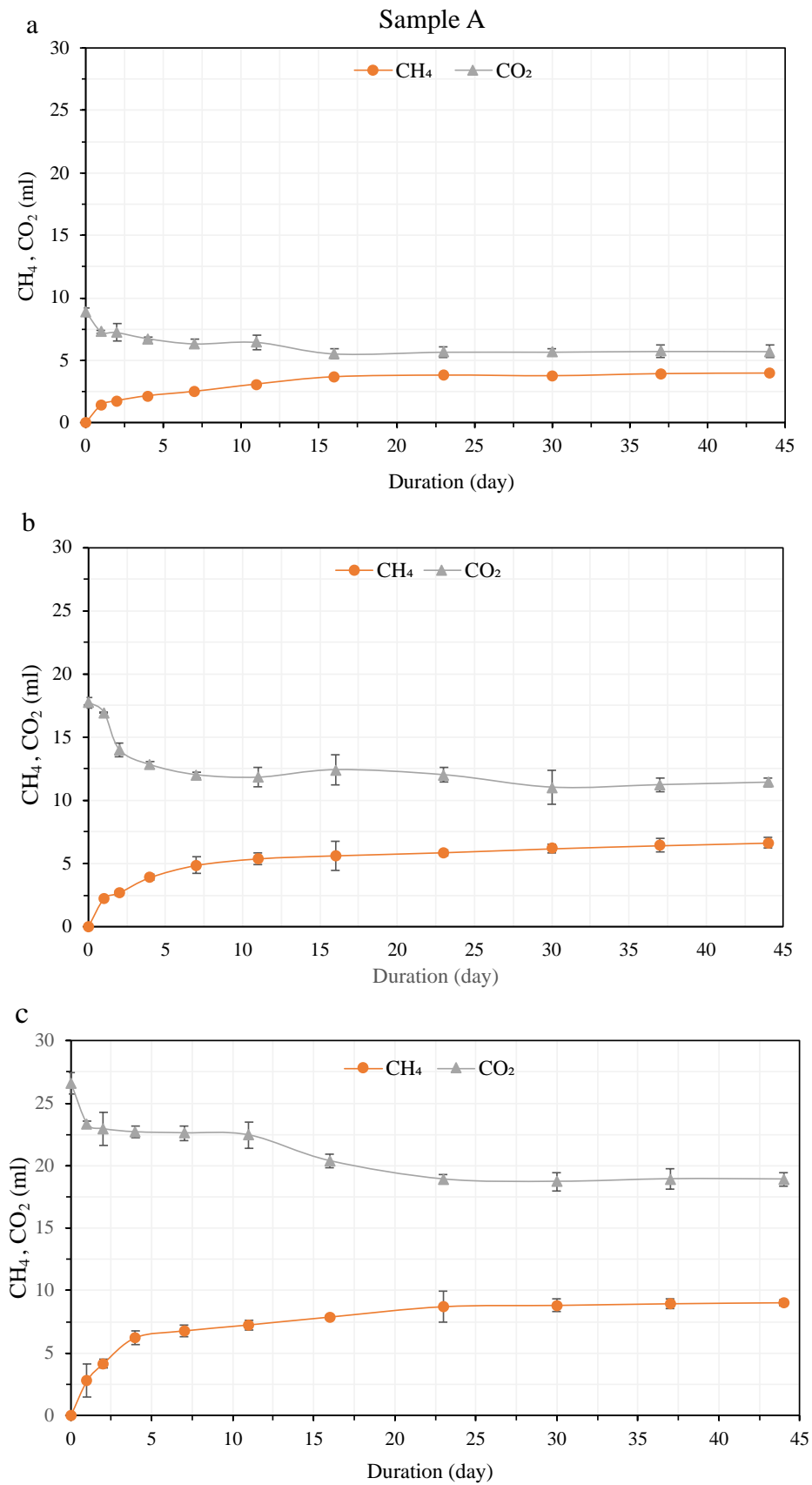


Figure 4.2. Net accumulative biogas under CO₂ (Sample A) biomethanation under the gas-biomass ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

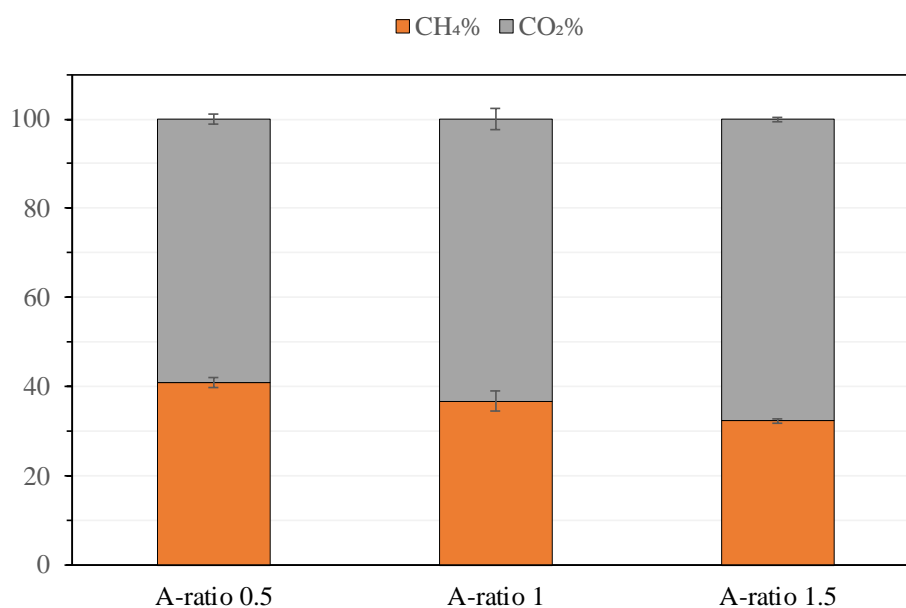


Figure 4.3. CH₄ and CO₂ contents in the produced gas in sample A (CO₂ biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).

Because the reactions in ADs are complicated, there is a lot of uncertainty about how CO₂ can be used, and bio-converted to CH₄. Also, part of the injected CO₂ could have been changed into other type of materials instead of CH₄, which makes it harder to indicate which pathway for CO₂ conversion is used [65]. Based on previous experiments, if the conversion route of CO₂ to CH₄ takes place in the Wood-Ljungdahl pathway, CO₂ conversion should increase the production of volatile fatty acids (VFAs) through the acetoclastic path of CH₄ production, where CO₂ is converted, and acetate is made (Eq.5 and Figure 2.6) [65]. However, low conversion efficiency and lack of H₂ in the system, indicate a small portion of CO₂ was converted to acetic acids and then to CH₄. This theory is consistent with the experiment's results, where VFAs in samples reached $9,396 \pm 1.6$, $9,576 \pm 1.65$ and $9,516 \pm 1.48$ mg/L, respectively, on the last date of the investigation (Table 4.1) which is very close to the VFA before CO₂ injection.

Compared to the control system, an enhancement of CH₄ production was observed in all ratios, demonstrating the potential of ADs to utilize and convert injected CO₂. However, considering the increase in CH₄, it is important to note that the concurrent rise in CO₂ poses a limitation to the effectiveness of this process in upgrading biogas.

Table 4.1. VFAs and pH in Sample A on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass *	0.5**	1	1.5
VFAs mg/L	9,366±1.14	9,396 ± 1.6	9,576 ± 1.65	9,516 ± 1.48
pH	7.83±0.08	7.76 ± 0.02	7.35 ± 0.05	7.52 ± 0.07

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios

4.2.3 CO Biomethanation (Sample B)

Net accumulative CO consumption and methane generation performances in three gas-liquid ratios are presented in Figure 4.4. As indicated in Figure 2.4 and based on Eq.1, 2, 3 and 4 demonstrating CO conversion pathways, the theoretical conversion is 25.0% of the volume of the injected CO. At condition B, the CO conversion rate to methane reached 19.7%, 15.8% and 15.3% of total injected gas respectively in ratios 0.5, 1 and 1.5 (mL gas/mL biomass). This indicates a slight methane production increase in all ratios and utilization of CO substrate with different bacterial genera. However, in all three proportions with CH₄ increment, CO₂ was also increased. During the possible CO conversion pathways (methanogenic, acetogenic and hydrogenogenic reactions (Eq. 2, 3, 4, 6 and 7)), along with CH₄ production, CO₂ is produced. Because of the lack of hydrogen in the system, a high portion of produced CO₂ remains in the headspace, which is undesirable.

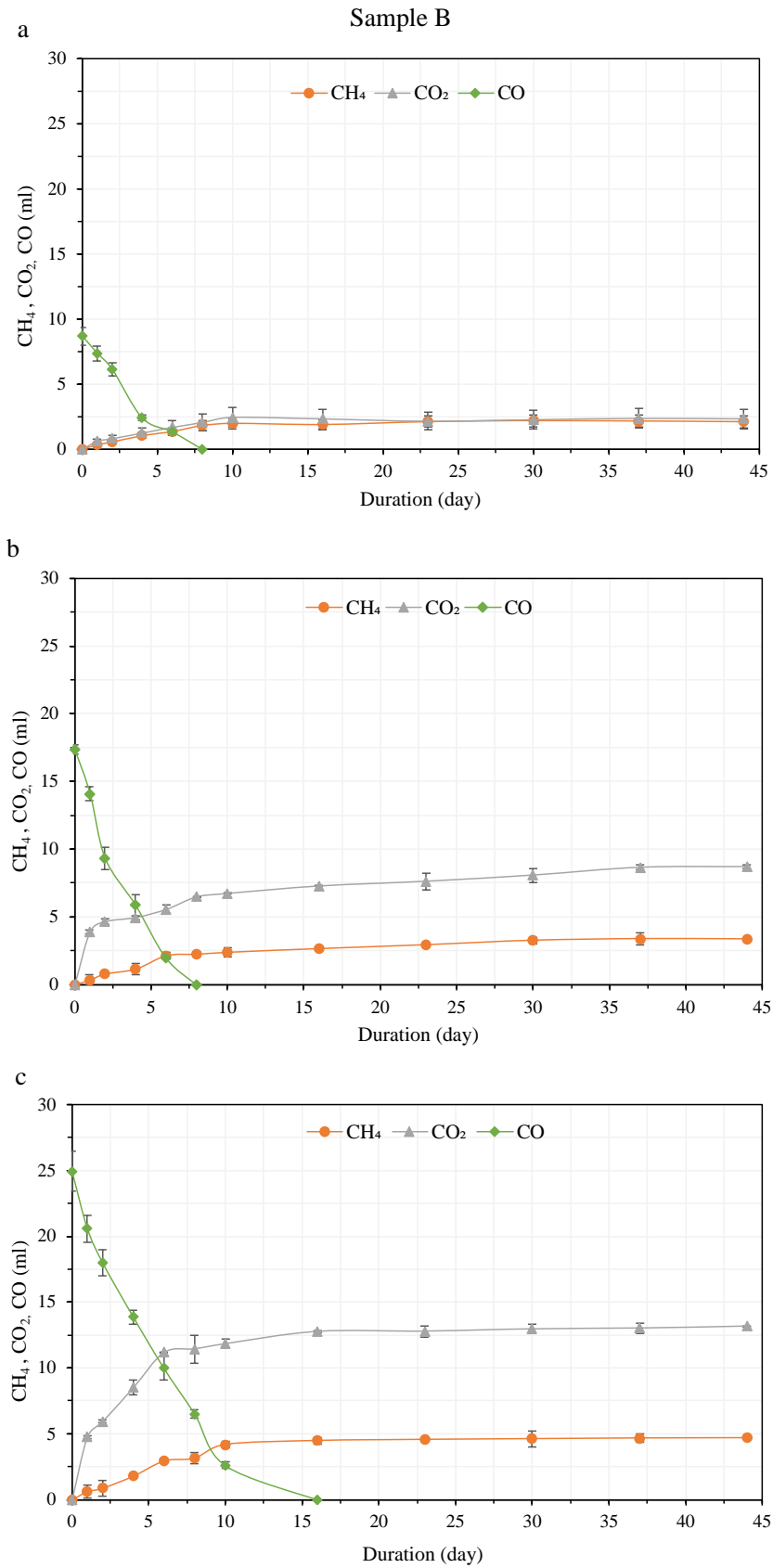


Figure 4.4. Net accumulative biogas under CO (sample B) biomethanation with the ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

Also, the increased production of CO₂, accumulation of VFAs and pH drop (Table 4.2) at the end of the experiment might be because of the utilization of CO through the carboxydrotrophic acetogenesis pathway, where CO gets converted to acetic acid and then to H₂/CO₂ or CH₄/CO₂. As expected, methane content and conversion rate were lower in higher gas-to-liquid ratios. The highest methane content (47.4%) (Figure 4.5) and the conversion rate (19.7%) were observed in gas to liquid at a ratio of 0.5 (mL gas/mL biomass). Methane content was 27.8% and 26.2%, for ratios 1 and 1.5 (mL gas/mL biomass). In ratios 1 and 1.5 (mL gas/mL biomass), a higher volume of CO₂, more VFA and, therefore, a lower volume of CH₄ was observed. Possible explanations for these results are the availability of more bacterial genera in the lower gas-liquid ratio because of the lower volume of gases.

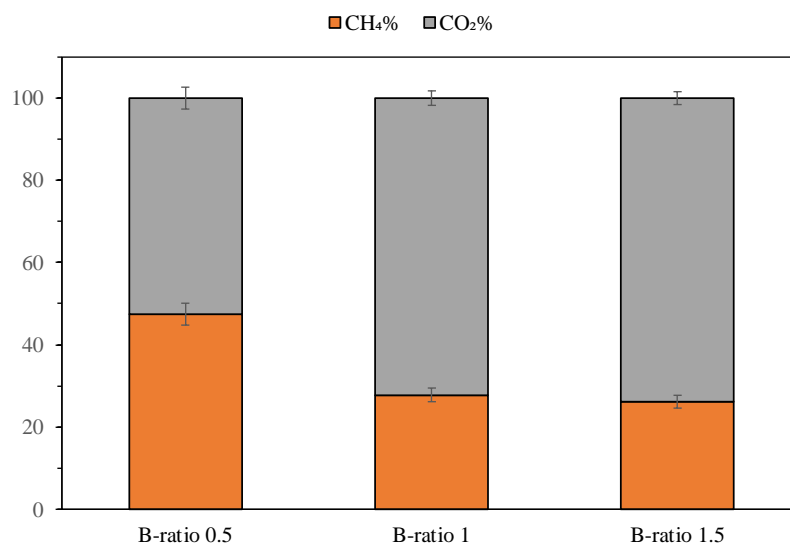


Figure 4.5. CH₄ and CO₂ contents in the produced gas in sample B (CO biomethanation) under gas-biomass ratio of 0.5, 1 and 1.5 (mL gas/mL biomass).

Compared to the control sample, in addition to CH₄ total volume of CO₂ was increased in all the ratios. Therefore, injecting CO alone into the AD reactor can reduce the digestion process's overall efficiency and is not a recommended method for optimizing methane production. CO is toxic to many of the microorganisms involved in AD and can interfere with their ability to break down organic matter to produce methane and increase CO₂ in the produced biogas.

Table 4.2. VFAs and pH in Sample B on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass*	0.5**	1	1.5
VFAs mg/L	9,366±1.14	9,906 ± 2.05	10,063 ± 0.62	10,282 ± 1.93
pH	7.83±0.08	7.21 ± 0.03	7.01 ± 0.05	6.88 ± 0.07

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios

4.2.4 H₂ Biomethanation (H₂a and H₂b)

Figure 4.6 indicates the accumulative hydrogen consumption and biogas production after hydrogen addition. In sample H₂a, although CO₂ concentration was zero until day 4 and lower than the control sample in the whole experiment period, the batch experiment ultimately failed as the CH₄ production was also lower than the control system. Several technical barriers limited the efficiency of in-situ biogas upgrading in the samples with hydrogen. In H₂a sample, by feeding H₂ into the bottles, during the hydrogenotrophic pathway, methanogens use H₂ and CO₂ in the biomass and/or CO₂ produced in the acetoclastic path. With the consumption of CO₂, pH increased to 9.2 and caused inhibition. The added H₂ elevated H₂ partial pressure and inhibited oxidation of VFAs which resulted in an increase to 10,359 mg/L in H₂a samples (Table 4.3). Therefore, for this sample where only H₂ as substrate was fed to the bottles, the graphs show results of total produced methane and CO₂ without any reduction to avoid negative values in the graph. Methane yield reached 0.042 mol H₂/mol CH₄ which compares with the theoretical values of 0.25 mol H₂/mol CH₄.

In H₂b sample, although CO₂ was lower than in the control system and methane production was higher than in the control system, methane yield did not reach the theoretical value and was 0.16 mol H₂/mol CH₄. The addition of in-situ H₂ into the bioreactor caused an increase in pH level to 8.8 in sample H₂b. This occurred due to the removal of naturally produced CO₂ in the liquid phase, inhibiting the hydrogenotrophic methanogenesis process and other functions.

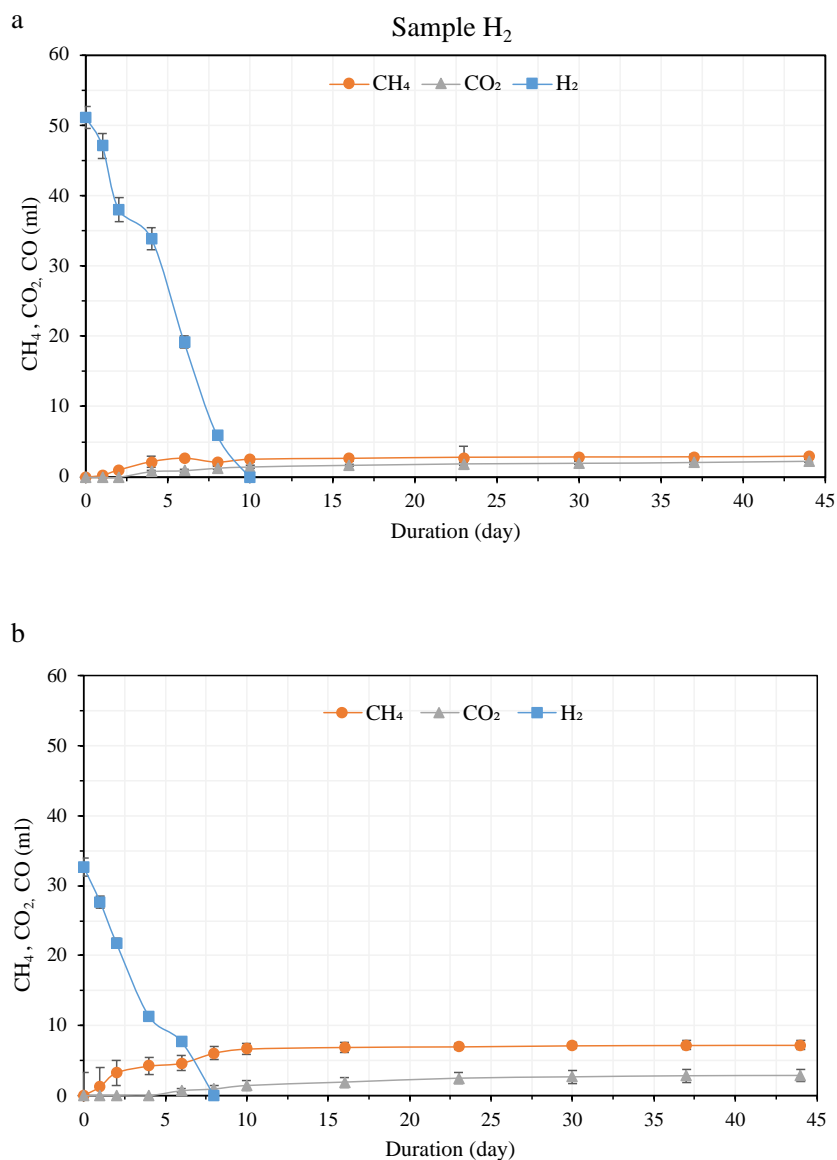


Figure 4.6. Accumulative biogas in samples a) H₂ a and b) H₂ b.

Table 4.3. VFA and pH in Samples H₂ a and H₂ b on the last day of the experiment.

	Biomass*	H ₂ a**	H ₂ b
VFAs mg/L	9,366±1.14	10,722 ± 0.87	9,823 ± 1.89
pH	7.83±0.08	9.2 ± 0.08	8.84 ± 0.1

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios.

Therefore, semi-continuous injection of gases is repeated for the sample with H₂ as a sole substrate to test if lower exposures of microorganisms to H₂ through multiple injections can

increase hydrogenotrophic ability and observe the effect of reducing the high and sudden partial pressure of H₂ into microorganisms on yielding higher conversion rate.

4.2.5 CO and H₂ (CO:H₂ 1:3) biomethanation (Sample C)

As an essential reductant and energy carrier, H₂ is critical in SB. Methanogenic microorganisms use H₂ and CO in AD to produce methane, the primary component of biogas. Adding H₂ and CO gives the microorganisms access to additional substrates, increasing biogas production. To convert CO to methane, stoichiometric H₂ (CO:H₂ 1:3) was added in condition C. As indicated in Figure 4.7, in ratios 0.5 and 1 (mL gas/ mL biomass), CO and H₂ were consumed synchronically and faster because of the availability of more microorganisms. However, in the ratio of 1.5, CO consumption was reduced by adding higher amounts of H₂. The reason is that injected H₂ preferred to react with bicarbonate in the biomass liquid. This can be observed in the pH increase to 8.95 from 7.83 in sample C in the ratio 1.5 system. This was not followed in ratios 0.5 and 1 because of the lower volume of the injected gas and the strong buffer capacity provided by the biomass as lower gas was in the system under conditions 0.5 and 1 (mL gas/ mL biomass). As a result, the pH level raised when more H₂ was introduced into the SB process, which is a significant concern. One suggested approach for addressing this issue involves using a phosphate buffer in a continuous syngas bio-methanation process.

Mass balance through all three ratios indicates no serious inhibitions occurred during the experiment because of partial pressure of CO and H₂ and methanogens were able to convert all the injected CO and H₂ to biogas.

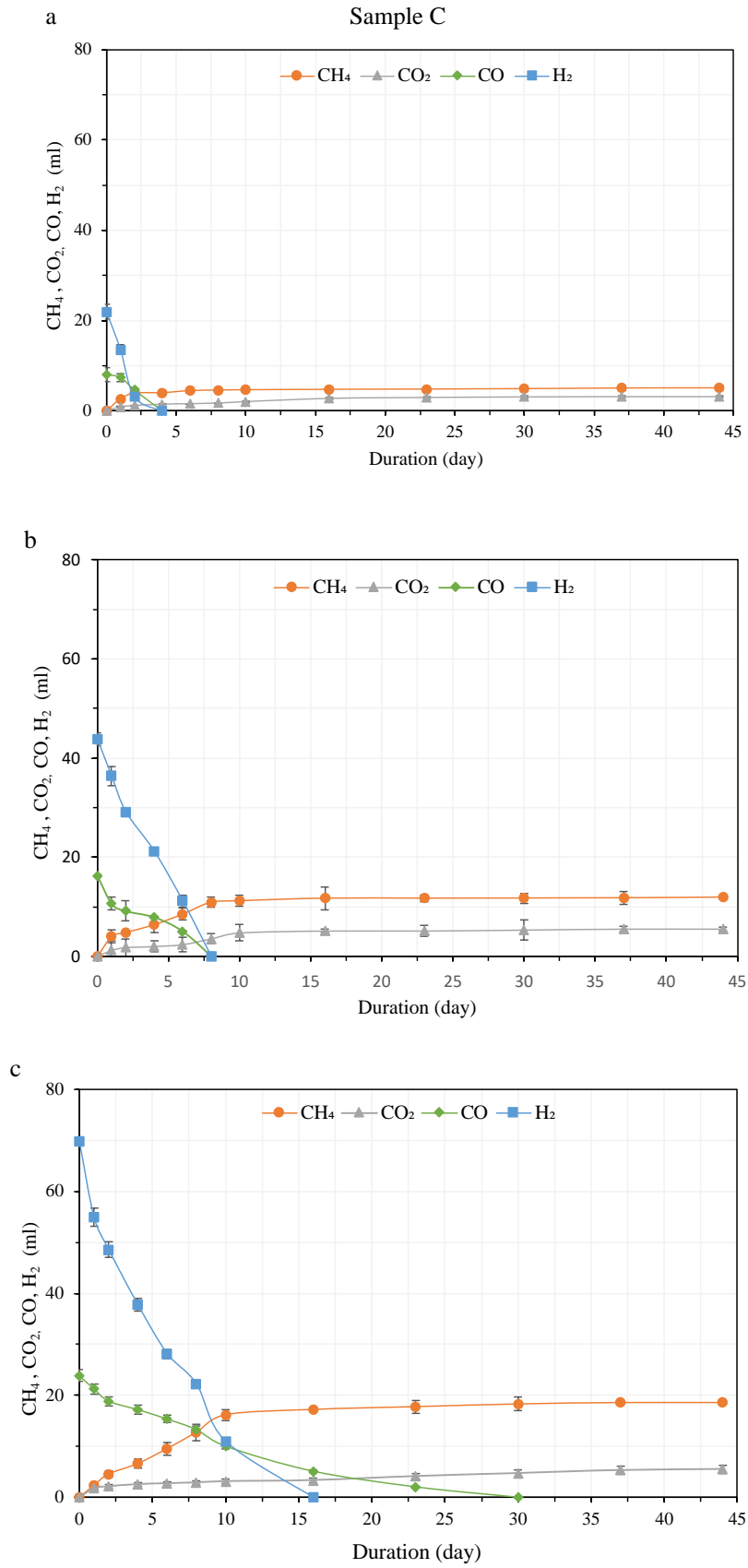


Figure 4.7. Net accumulative biogas under CO:H₂ 1:3 (sample C) biomethanation under the gas-biomass ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

The results showed 30.4%, 145.6% and 194.4% higher methane percentage in sample C than in sample B (CO alone), respectively, in different ratios. However, the conversion rate of injected CO to CH₄ did not reach 100% of the theoretical in any conditions, it was 51.4%, 59.7% and 63.6%, respectively, in 0.5, 1 and 1.5 (mL gas/ mL biomass) ratios. The presence of CO₂ in the produced biogas and VFAs accumulation in all the ratios are the reasons for not reaching desirable conversion efficiency. This shows that the conversion of CO inside of reactor occurred through multiple pathways (Figure 2.4). Compared to the control system, VFAs in sample C was increased in three ratios demonstrating the conversion of CO to methane through indirect pathways (Table 4.4). The presence of CO₂ and VFAs shows the conversion of CO mostly through Carboxydrotrophic acetogenesis (Eq.3) and Carboxydrotrophic hydrogenogenesis (Eq.2) which converts CO to acetate and CO₂. Produced acetate is then converted to methane and CO₂ through Acetoclastic methanogenesis (Eq.6). In both pathways CO₂ is again generated and a portion of it was converted to methane through Hydrogenotrophic methanogenesis (Eq.1) since enough H₂ was not available to convert all the produced CO₂ through the mentioned pathways. In addition, the presence of H₂ may alter the microbial community, which can have an additional impact on their ability to convert CO and affect overall conversion performance. Some microorganisms, such as homoacetogenic bacteria, can use hydrogen to produce acetic acid instead of methane. This can reduce the overall methane yield of the AD process and increase the concentration of VFAs, inhibiting methanogenic archaea (Eq. 5). In this experiment methane yield reach 0.2 mmol CH₄/mmol H₂ in the ratio 1.5 (mL gas/ mL biomass) with highest methane content which compares with the theoretical value of 0.33.

Conversion of CO through different pathways can be a possible reason for higher methane content and syngas conversion efficiency in higher gas-to-liquid ratios (Figure 2.7 and 4.8).

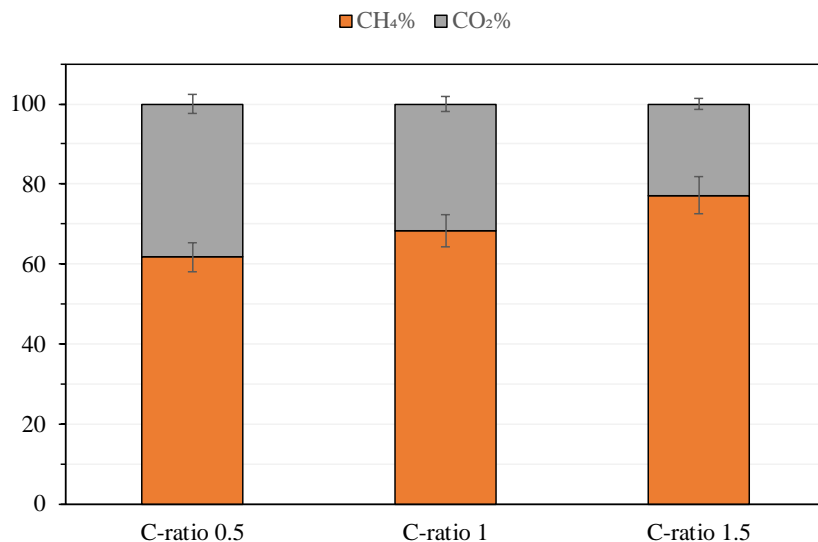


Figure 4.8. CH₄ and CO₂ contents in the produced gas in sample C (CO: H₂ 1:3 biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).

CO is a crucial substrate for both carboxydrotrophic methanogens and carboxydrotrophic acetogens and its concentration in the feed gas can play a significant role. Since the partial pressure of CO in three ratios was lower than 0.5 atm; according to Figure 2.4, there is the possibility of CO conversion to CH₂ through all the pathways [54]. Based on the concentration of CO₂ and CO conversion efficiency to methane, this experiment indicated that higher CO concentrations have favoured direct CO conversion to methane through carboxydrotrophic methanogenesis where methanogens directly utilize CO to produce methane. On the other hand, lower CO concentrations promoted the carboxydrotrophic acetogenesis pathway, where acetogens convert CO to acetate and CO₂. Also, the hydrogen partial pressure in the system influenced the metabolic pathways. Higher hydrogen partial pressure favoured the carboxydrotrophic methanogenesis pathway, while lower hydrogen partial pressure promoted the carboxydrotrophic acetogenesis pathway. It can be seen in this experiment where Methane yield reaches 0.2 mmol CH₄/mmol H₂ in the ratio of 1.5 mL gas/ mL biomass from 0.15 mmol CH₄/mmol H₂ in the ratio of 0.5 (mL gas/ mL biomass). Moreover, similar to other metabolic pathways, the pH of the system affected the prevalence of carboxydrotrophic methanogenesis

or carboxydrotrophic acetogenesis. As demonstrated in (Table 4.4), the environment of the experiment was more acidic in a higher ratio than the two other ratios.

Table 4.4. VFA and pH in Sample C on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass*	0.5**	1	1.5
VFA mg/L	9,366±1.14	9,518 ± 1.21	9,621 ± 1.05	9,441 ± 1.22
pH	7.83±0.08	7.03 ± 0.06	7.31 ± 0.12	8.95 ± 0.07

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios.

4.2.6 CO₂ and H₂ (CO₂:H₂ 1:4) biomethanation (Sample D)

To convert CO₂ to methane, stoichiometric H₂ (CO₂:H₂ 1:4) was added in condition D. As indicated in Figure 4.9 in all the ratios, the net accumulative CH₄ production reached the maximum on the same day hydrogen reduced to zero. This proves the critical role of H₂ in the bio-methanation of CO₂. In three ratios, compared to sample A (CO₂ alone), methane content was increased by 37.5%, 76.6 % and 105.6%, respectively. Methane content in different ratios increased to 56.3, 64.9 and 66.5% and was enhanced compared to the control system which was 30.8%. The conversion efficiency of the CO₂ in syngas mixture to Methane reached 46.6%, 55.8% and 57.7%, respectively, in 0.5, 1 and 1.5 gas-biomass ratios. The increase of CO₂ in the produced biogas indicates that H₂ and CO₂ were not completely converted to CH₄ through Hydrogenotrophic methanogenesis. Also, the increase of CO₂ might be because of the reaction of injected H₂ with dissolved bicarbonate in the biomass which was not measured before injection and therefore lack of H₂ in the system for conversion of injected CO₂ and/or conversion of CO₂ through the acetoclastic pathway (Eq.6) to methane which can increase production of CO₂.

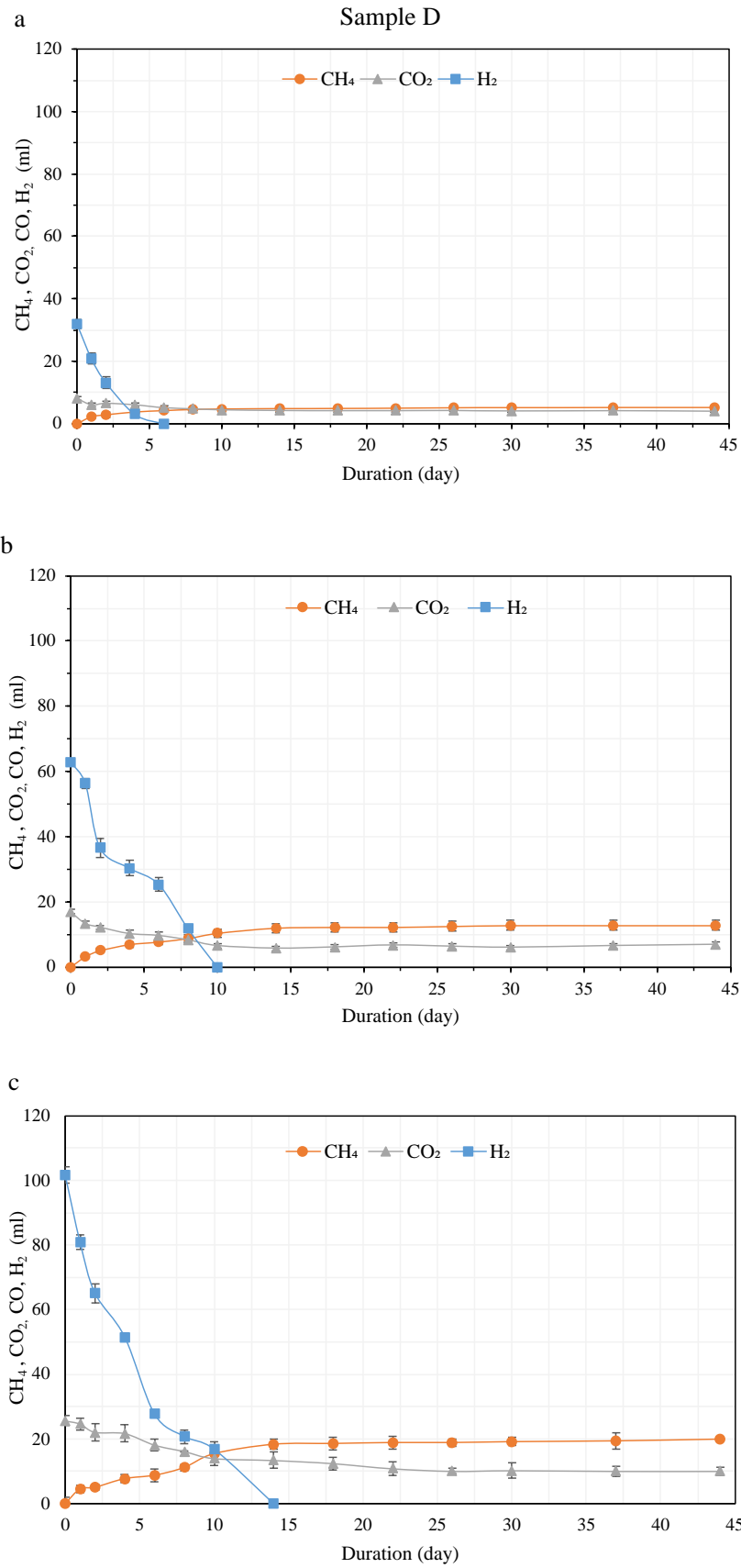


Figure 4.9. Net accumulative biogas under CO₂ and H₂ (sample D) biomethanation under the gas-biomass ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

Additionally, the H₂ partial pressure will thermodynamically affect whether the conversion would be endergonic or exergonic. A previous study also reported that H₂ could act as an electron donor and resulted in changes in the microbial community. Also, as shown in Figure 4.9, H₂ in the headspace of the bottles were not observed on day 5, 10 and 15, respectively, in ratio 0.5, 1 and 1.5 (mL gas/mL biomass) but was not employed for CH₄ production entirely. H₂ might be transferred to the liquid phase and consumed due to biomass adaptation to the substrate [38].

In this sample, methane yield was 0.15 mmol CH₄/mmol H₂ in the ratio of 1.5 (mL gas/ mL biomass) with the highest methane content which was expected to be 0.25. Therefore, semi-continuous injection of gases is repeated Phase II to exercise longer exposures of microorganisms to H₂ and increase the hydrogenotrophic ability to reach a higher conversion rate.

According to Table 4.5 pH and VFA were stable through the experiment in three ratios and close methane content (Figure 10) and conversion efficiency especially in ratios 1 and 1.5 (mL gas/mL) biomass demonstrating that partial pressure of gases in higher ratio and low solubility of H₂ did not affect the operation.

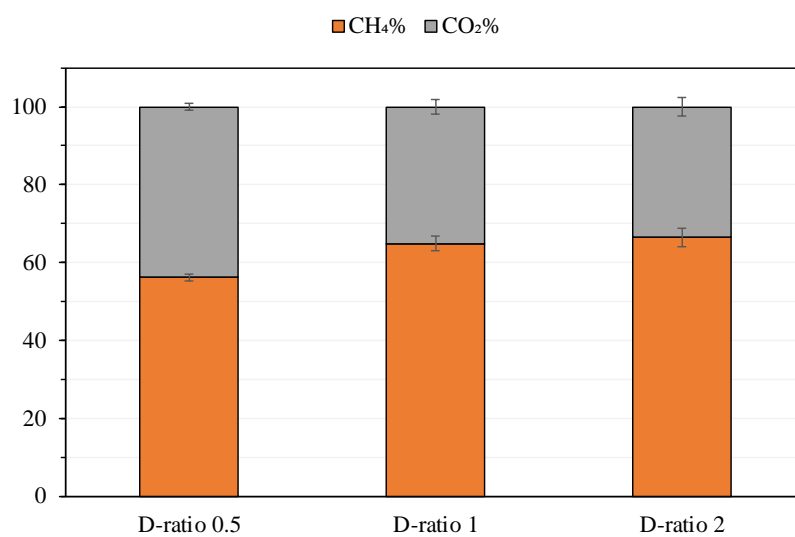


Figure 4.10. Methane and CO₂ contents in the produced gas in sample D (CO₄:H₂ 1:4 biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).

Conversion of CO₂ through two different pathways can be a possible reason for higher methane content and syngas conversion efficiency in higher gas-to-liquid ratios. Higher hydrogen partial pressure favours hydrogenotrophic methanogenesis, where H₂ is directly utilized by methanogenic microorganisms to produce methane CH₄. The presence of high levels of H₂ can suppress homoacetogenic bacteria and promote the methanogenic pathway. It is confirmed by the fact that Methane yield reached 0.15 mmol CH₄/mmol H₂ in the ratio of 1.5 (mL gas/ mL biomass) from 0.12 mmol CH₄/mmol H₂ in the ratio of 0.5 (mL gas/ mL biomass).

The pH of the system can affect the metabolic pathways. Hydrogenotrophic methanogenesis is favoured at slightly acidic to neutral pH, while homoacetogenesis is more favourable at lower pH values (Table 4.5).

Table 4.5. VFAs and pH in Sample D on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass*	0.5**	1	1.5
VFAs mg/L	9,366±1.14	9,521 ± 1.35	9,546 ± 1.81	9,486 ± 2.3
pH	7.83±0.08	6.63 ± 0.08	6.78 ± 0.09	8.62 ± 0.11

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios

4.2.7 Syngas (CO₂:CO:H₂ 1:2:3) biomethanation (Sample E)

Sample E consists of syngas with the composition of CO₂:CO: H₂ 1:2:3 that is produced in an actual gasifier reactor that converts biomass to syngas (The National Research Council Canada). CH₄ concentration in sample E in all ratios was higher than in control and samples A and B (CO₂ and CO alone). Methane production was increased during the experiment compared to the control system. However, since CO and CO₂ in syngas were not completely converted to CH₄, CO₂ in the produced biogas was also increased (Figure 4.11).

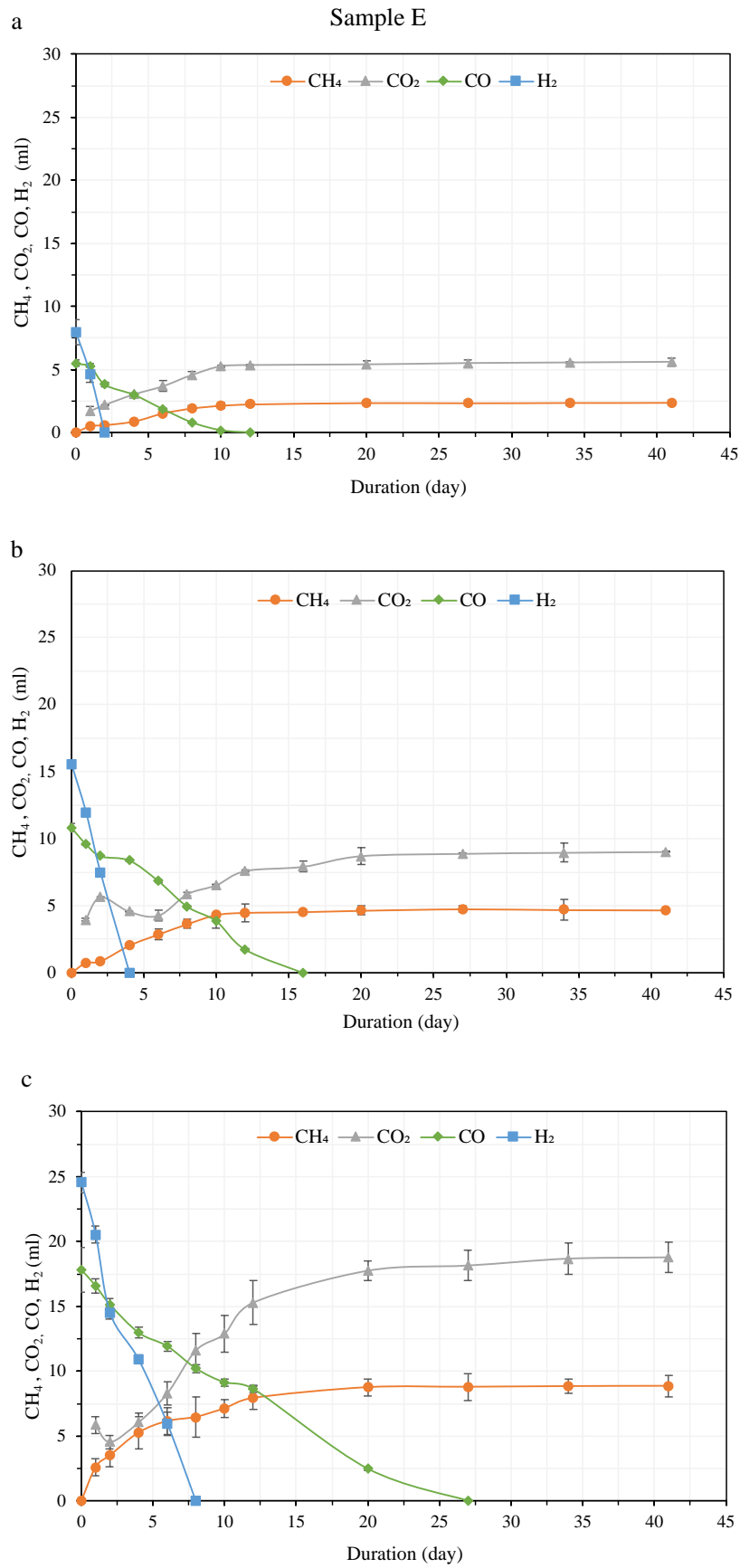


Figure 4.11. Net accumulative biogas under syngas bimethanation in sample E under the gas-biomass ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

Similar results were achieved in the study of (Chunxing Li et al.2021) [19] and (S.Shah et al.2016) [56], experimenting with low H₂ syngas in which the produced biogas content reached 38% and 42% of methane content (Figure 4.12).

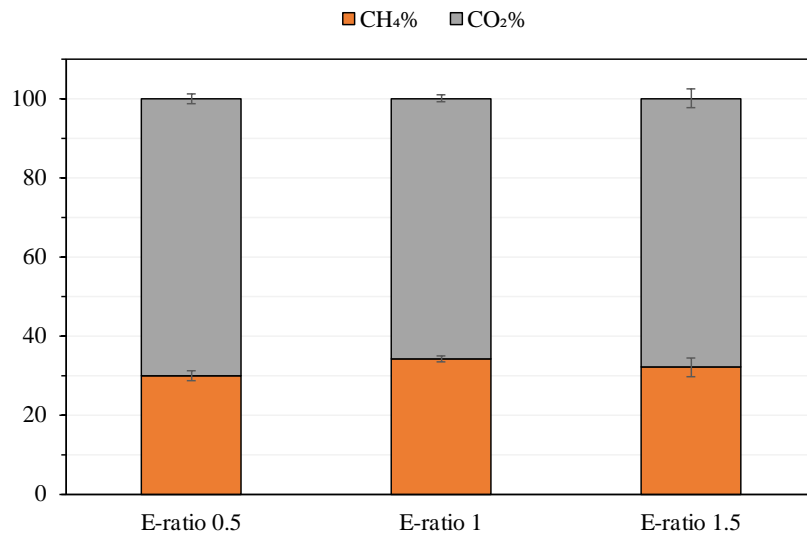


Figure 4.12. CH₄ and CO₂ contents in the produced gas in sample E (syngas (CO₂:CO: H₂ 1:2:3) biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).

However, CH₄ concentration was lower in all the ratios than in samples C and D (CO and CO₂ with stoichiometric H₂). This is due to a lack of sufficient quantity of hydrogen in the system for converting all the injected CO and CO₂. As indicated in Figure 4.11, injected H₂ was consumed in the first days of the experiment, but CO was still observed in the headspace. Therefore, the remaining CO might be consumed by acetogenic microorganisms to acetate and H₂/CO₂ and then to methane and carbon dioxide which caused the conversion efficiency of total injected CO and CO₂ to Methane in the H₂ deficient syngas reached to 23.6, 38.4 and 34.9%, respectively, in three ratios.

Compare to samples A and B (CO₂ and CO alone) slight VFAs accumulation was observed proving the effect of even a low concentration of H₂ in the system on biological methanation. pH was almost constant in all the ratios of sample E in the last of the experiment. The reason

behind pH consistency is the injection of CO and CO₂ into the biomass that buffered the pH during the experiment.

Table 4.6. VFA and pH in Sample E on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass*	0.5**	1	1.5
VFA mg/L	9,366±1.14	9,384 ± 1.83	9,675 ± 2.29	9,224 ± 1.95
pH	7.83±0.08	7.98 ± 0.03	7.88 ± 0.03	7.63 ± 0.07

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios.

4.2.8 Syngas (CO₂:CO:H₂ 1:1:7) biomethanation (Sample F)

The highest methane production and conversion of injected gases to methane were observed in sample F (syngas with the composition of CO₂:CO:H₂ 1:1:7), where the highest amount of hydrogen was added to the system and therefore make it the most efficient syngas composition for biogas upgrading. As indicated in Figure 4.13 In all the samples, CO was consumed before hydrogen reached zero which shows a high consumption rate of CO in the presence of a sufficient amount of H₂.

As indicated in Figure 4.14, methane concentration reached 62.8%, 75.2% and 79.8% in the produced biogas compared to the control system, which was 30.80%. This result was also observed in the investigation of (Chunxing Li et al.2021) testing SB in batch reactors under thermophilic temperature with sufficient hydrogen content where all the injected syngas bio converted to methane, and the concentration of methane in the headspace reached 98% [19].

Also, adding CO and CO₂ together buffered pH and caused pH stability during the experiment. Accumulation of VFAs was not marked on the last day of the experiment (Table 4.7) proving a favourable condition for syngas bio methanation.

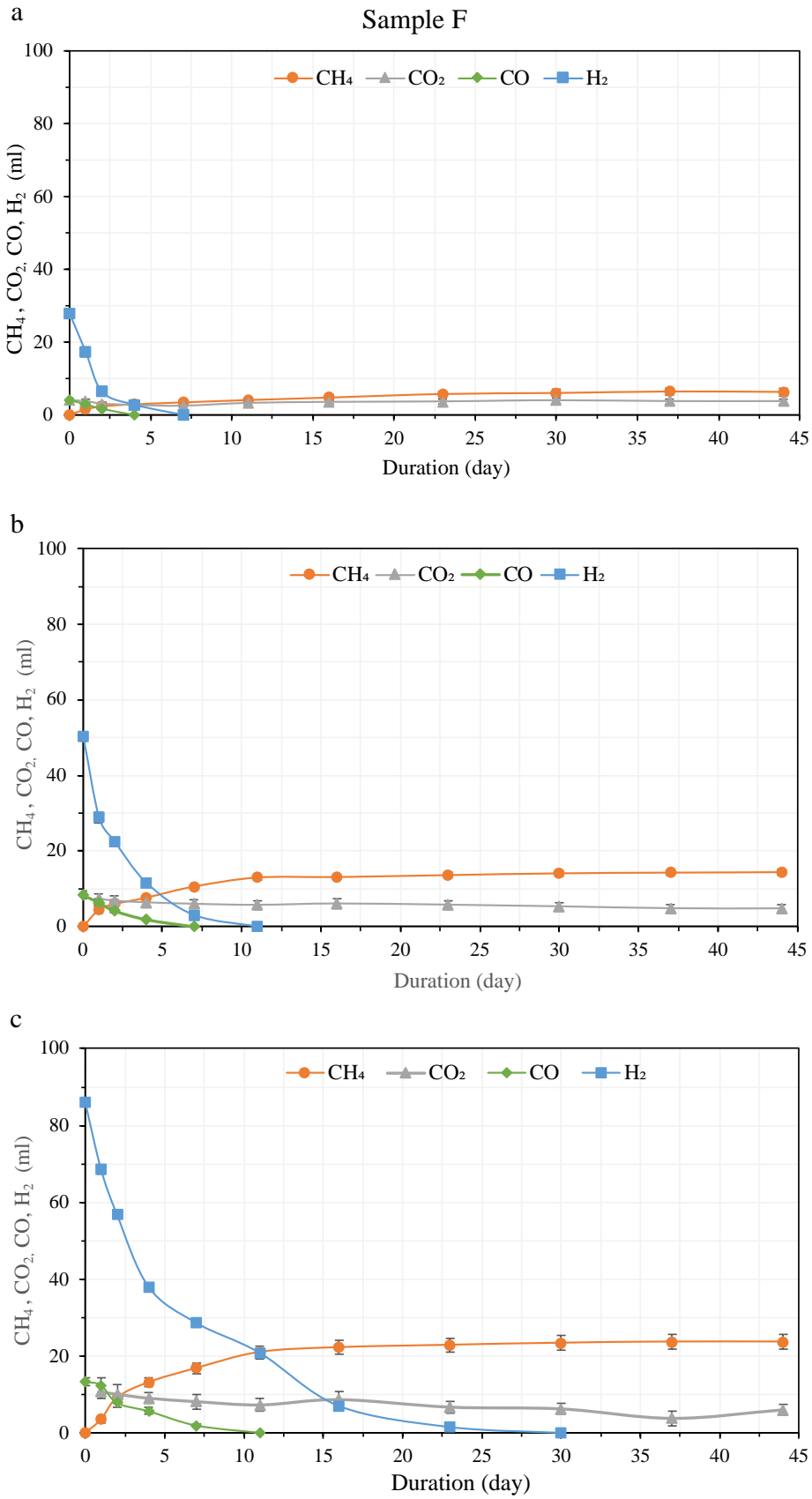


Figure 4.13. Net accumulative biogas under syngas bimethanation in sample F under the gas-biomass ratio of a) 0.5, b) 1 and c) 1.5 (mL gas/mL biomass).

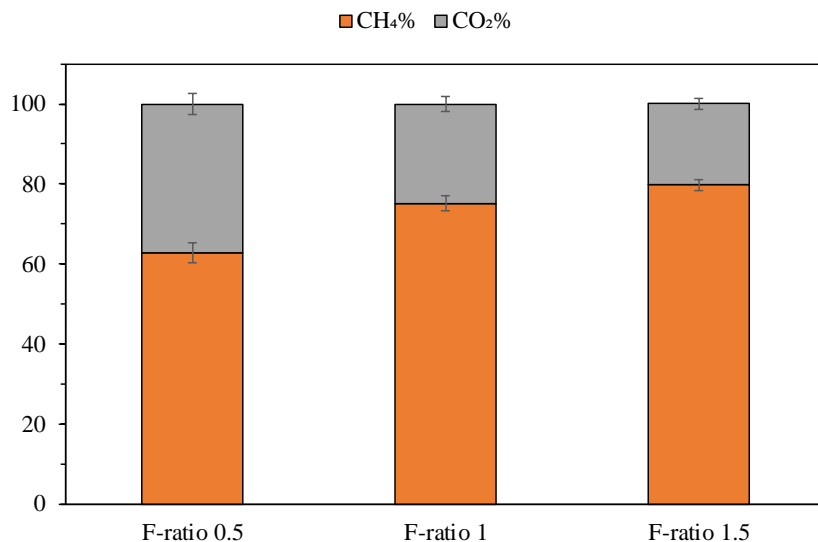


Figure 4.14. CH₄ and CO₂ contents in the produced gas in sample F (syngas (CO₂:CO:H₂ 1:1:7) biomethanation) under ratios 0.5, 1 and 1.5 (mL gas/mL biomass).

Table 4.7. VFA and pH in Sample F on the last day of the experiment.

	Gas-liquid ratios (mL gas/mL biomass)			
	Biomass*	0.5**	1	1.5
VFA mg/l	9,366±1.14	9,272 ± 1.3	9,352 ± 2.17	9,263 ± 2.09
pH	7.83±0.08	7.55 ± 0.07	7.42 ± 0.08	7.12 ± 0.09

* Biomass characteristics before gas injection.

** Biomass characteristics after gas injection in three ratios.

Higher syngas ratios in the headspace positively affected conversion efficiency and methane production. Methane yield reached 0.21 mmol CH₄/mmol H₂ in the ratio of 1.5 (mL gas/ mL biomass) from 0.17 mmol CH₄/mmol H₂ in the ratio of 0.5 mL gas/ mL biomass. Also, conversion efficiencies in all three ratios were higher than samples C and D (CO and CO₂ align with stoichiometric H₂). This result again indicates that in this experiment higher concentration and partial pressure of gases created a favourable condition for the conversion of CO and CO₂ through the methanogenesis pathway rather than acetogenesis. However, 60.6%, 69.7% and 79.9% of injected syngas were converted to methane in three ratios of sample F and efficiency did not reach 100% of the theoretical value, and CO₂ is still in the produced biogas. Previous

studies have proved that a portion of hydrogen is used for biomass adaptation of substrate and can be not utilized in hydrogenotrophic pathways [32].

4.3 Comparing results of Phase I:

By analyzing the CH₄ content and gas conversion efficacy of various gas mixtures, the biomethanation of two and three gas compositions seem more promising for biogas upgrading. For the purpose of upgrading, it is desirable to have lower CO₂ levels and higher CH₄ content. Additionally, when taking conversion rates into account, a higher H₂ content is preferred and in terms of carbon source, CO is favored over CO₂.

As indicated in Table 4.9 methane content and conversion efficiency of syngas with the composition of CO:H₂ reached a maximum of 77.1% and 63.6% for a ratio of 1.5 (mL gas/mL biomass), respectively. For the syngas with the composition of CO₂:H₂ methane content and conversion efficiency of syngas were 66.5 and 57.7 for a ratio of 1.5 (mL gas/mL biomass), respectively. And for syngas H₂ rich they were 79.8% and 79.9% for a ratio of 1.5 (mL gas/mL biomass). Therefore, as indicated in Tables 4.5 and 4.6, the biomethanation of CO and CO₂ alone which showed both methane content and conversion efficiency below 50% was not practised for the next phase.

Except for samples A and B (CO and CO₂ alone), a significant increase in CH₄ concentration can be seen in all the ratios of samples C, D, E and F. However, the production yield of CH₄ did not approach 100% of the theoretical yield and CO₂ concentration did not go below 20% in any samples or ratios.

There are several explanations for these results. The microbial community in the biomass is diverse and consists of various species with different metabolic capabilities. Some microorganisms are specialized in converting CO/CO₂ and H₂ to CH₄, while others are better at metabolizing CO₂ to produce more CO₂. Therefore, the presence of certain microorganisms

and their activity can influence the conversion of syngas components. Also, some microorganisms, such as homoacetogenic bacteria, can use hydrogen to produce acetic acid instead of methane. This can reduce the overall methane yield of the AD process and increase the concentrations of VFAs as a by-product. Except for sample H₂ alone, a high accumulation of VFAs was not observed, thus it can be concluded that the produced VFAs was converted to biogas through acetoclastic methanogenic pathways which can increase the concentration of CO₂.

To achieve a higher conversion efficiency of injected gases to methane, the next phase of the experiment involved testing injection of mixture of gases with higher ratio of hydrogen. This adjustment aims to provide a greater quantity of hydrogen for the conversion of CO₂, which is generated through acetogenesis pathways. Phase II of the experiment was conducted in a semi-continuous mode due to certain limitations associated with increasing hydrogen partial pressure to higher levels. One limitation is the potential toxicity of elevated H₂ levels on microorganisms, which can have a reverse effect on the activity and overall performance of SB. Additionally, higher H₂ partial pressure can lead to an increased accumulation of VFAs and a wider pH range, both of which can negatively impact the stability and efficiency of the process [74]. This limitation was also observed in samples H₂ a and H₂ b, where a high and sudden injection of hydrogen resulted in an increase in both VFAs and pH levels, ultimately inhibiting the bio-methanation process.

This approach was also adopted to prevent gas leakage and the risk of explosion resulting from high-pressure buildup within the bottles. Additionally, in Phase II (semi-continuous mode), syngas compositions consisting of double and triple combinations with the same stoichiometric ratios will be tested again. This is done first to compare their outcomes with samples containing H₂ concentrations exceeding the stoichiometric ratio. Secondly to assess the impact of multiple

injection of H₂ at specific time intervals on the adaptability of microorganisms to the injected gas.

Table 4.8. Biogas content and CO/CO₂ Conversion efficiency in the samples of Phase I

Sample	A (CO ₂)			B (CO)			C (CO:H ₂ 1:3)		
Gas-to-liquid ratio (ml gas/ml biomass)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Conversion efficiency to Methane %	33.1	27.8	25.2	19.7	15.8	15.3	51.4	59.7	63.6
CH ₄ content %	41.0	36.8	32.3	47.4	27.8	26.2	61.8	68.3	77.1
CO ₂ content %	59.1	63.2	67.7	52.6	72.2	73.8	38.2	31.7	22.9
Sample	D (CO ₂ :H ₂ 1:4)			E (CO ₂ :CO:H ₂ 1:2:3)			F (CO ₂ :CO:H ₂ 1:1:7)		
Gas-to-liquid ratio (ml gas/ml biomass)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Conversion efficiency to Methane %	46.6	55.8	57.7	23.6	38.4	34.9	60.6	69.7	79.9
CH ₄ content %	56.3	64.9	66.5	29.9	34.2	32.0	62.8	75.2	79.8
CO ₂ content %	43.7	35.1	33.5	70.1	65.9	68.0	37.2	24.8	20.3

4.4 Phase II Results: Biomethanation of Syngas in semi-continuous mode

4.4.1 Biogas production in the control sample

A control sample is used while testing Biochemical Methane Potential (BMP) to ensure that the results obtained from the test are accurate and can be compared to other samples (Figure 4.15). Also, it is used to determine the amount of methane that is produced by the biomass itself. VFAs and pH on the last day of 30 days experiment were measured at $8,854 \pm 0.99$ mg/L and 7.88 ± 0.08 in the control sample without any injected gases.

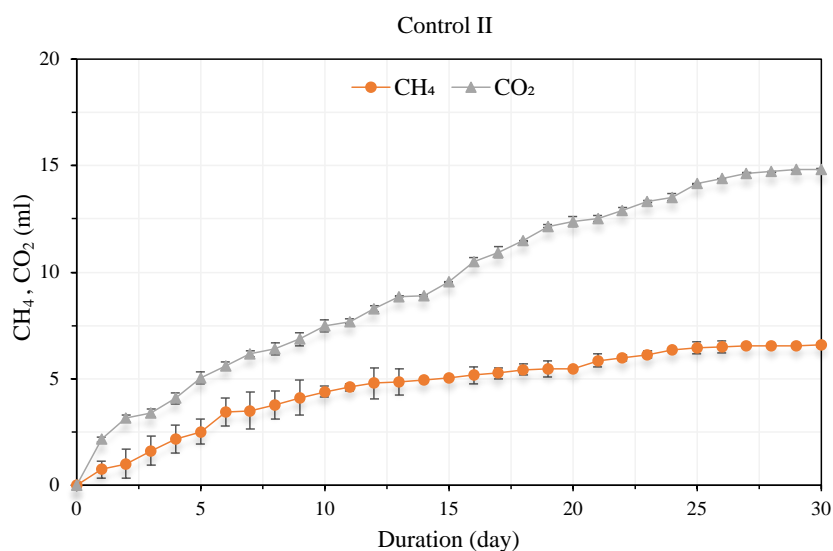


Figure 4.15. Accumulative biogas in the control sample.

4.4.2 H₂c Bio-methanation in semi-continuous mode

The addition of hydrogen in a semi-continued mode showed great potential to improve biogas upgrading injection of H₂ alone. As indicated in Figure 4.16, by addition of hydrogen in each stage, methane production was increased. Results showed the influence of increasing hydrogenotrophic methanogenesis activity by higher utilization of CO₂ in the biomass than in the control sample. Figure 4.17 shows depletion in the percentage of CO₂ from day five till the end of the experiment.

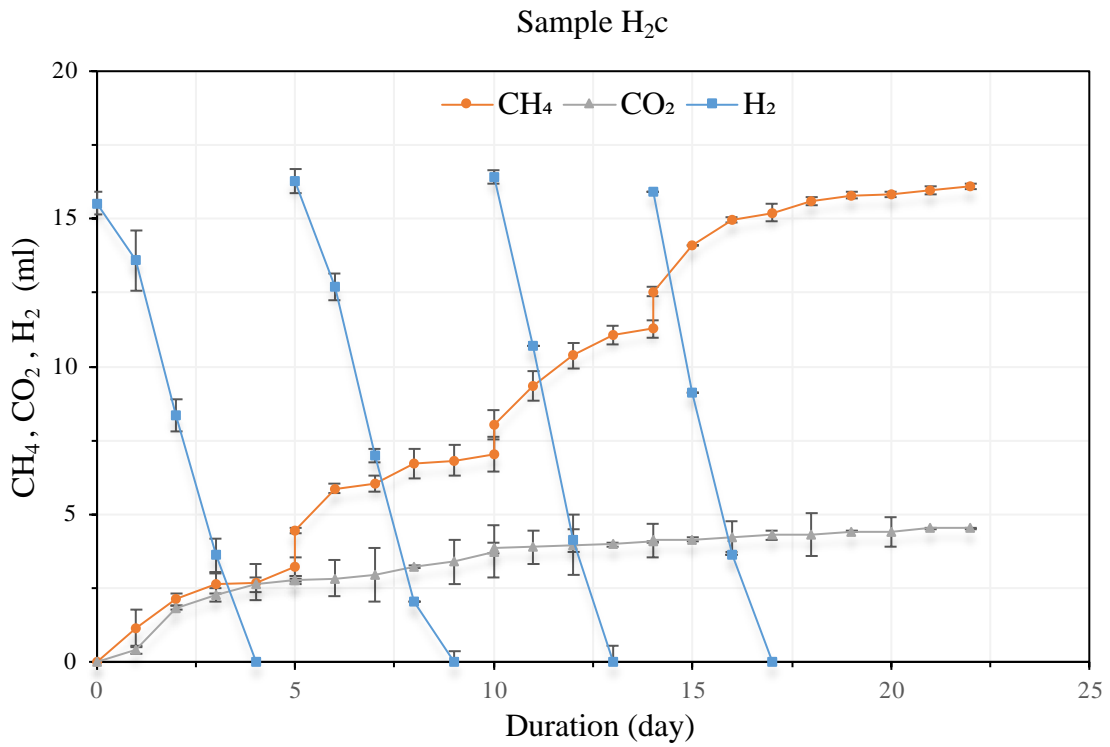


Figure 4.16. Net accumulated biogas under H₂c Bio methanation.

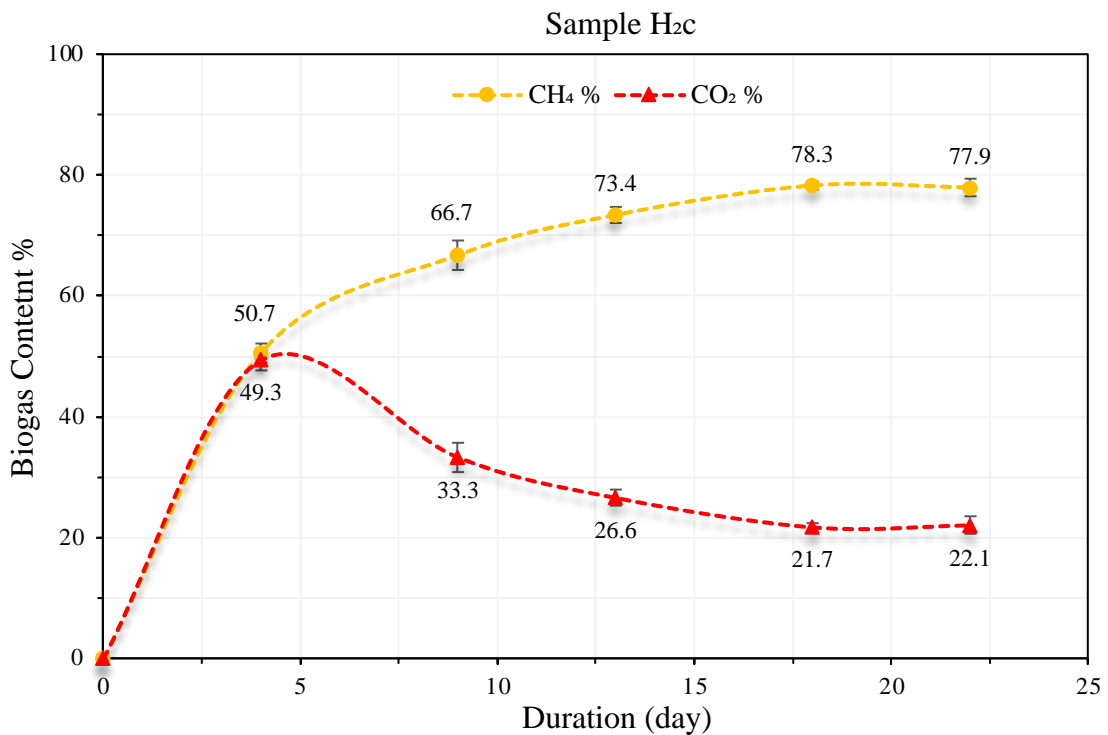


Figure 4.17. CH₄ and CO₂ contents in the produced gas on the last day of each injection (Sample H₂c).

Injecting hydrogen in several steps into an AD reactor can potentially increase the hydrogenotrophic ability of the microbial community in the reactor. By injecting H₂ in several stages, the microbial community was exposed to a pulsatile H₂ supply that promoted the growth and activity of hydrogenotrophic bacteria. The pulsatile H₂ supply prevented the inhibition of the methanogenic archaea, which can occur when the H₂ concentration is too high and cause a buildup of H₂ in the reactor.

Methane yield reached 0.2 mmol CH₄/ mmol H₂ compared with 0.16 in phase I and resulted in the CH₄ concentration of 77.9%, compared with 56% in phase I and 30.80% in the control sample. As indicated in Figure 4.17, although the total volume of H₂ injected was measured to be four times the expected CO₂ production, 22.1% of the headspace composition consists of CO₂. The reason might be microorganisms' utilization of H₂ in the first days for the adaption or reaction of dissolved CO₂ in the biomass with the injected H₂. Therefore, it is expected that the biogas can be upgraded by increasing the concentration of hydrogen gas injected or an additional injection stage since a reduction in CO₂ concentration is observed after each stage.

Adding hydrogen in 4 stages alleviated the inhibitory factors that caused the reactor to fail in phase I. Compared to phase I, VFAs accumulation was not observed, and it was reduced to 8,020±3.08 mg/L from 9,042±1.04 mg/L as the hydrogen pressure was lower and more favourable for VFAs oxidation. Also, multiple injection of hydrogen prevented the pH drop during the experiment and was measured at 8.2±0.17, increasing reactor performance.

4.4.3 CO and H₂ biomethanation in semi-continuous mode with stoichiometric ratio CO: H₂ 1:3 (Sample G)

Figure 4.18 shows the net accumulative biogas produced from the simulated syngas (CO and H₂ stoichiometric ratio of CO: H₂ 1:3). Increment in the percentages of CH₄ and CO₂ in the produced biogas on the last day of each feeding stage can be seen. As expected, methane concentration is higher on the last day of 4th injection than in the first stage (Figure 4.19). Figure 4.20 indicates CO conversion efficiency enhancement after each gas injection stage. Semi-continuous injection provided a controlled and steady supply of CO and H₂ to the reactor. This allowed the microorganisms to adapt and adjust to the changing conditions in the reactor, resulting in a more stable and efficient process. This effect can be observed in the methane yield increment from 0.15 in the first injection to 0.22 mmol CH₄/mmol H₂ on the last day of the last injection. While injecting large amounts of CO and H₂ all at once created a shock loading effect that stressed the microorganisms and reduced their activity, leading to lower conversion efficiency, which was proven in phase I, where CO conversion efficiency reached 63.6% and methane yield to 0.20 mmol CH₄/mmol H₂ on the last day under ratio 1.5 (mL gas/mL biomass) with highest methane content. No VFAs accumulation was observed, and it was reduced to 9,018±3.8 from 9,042 mg/L, indicating a favourable condition for VFAs conversion to methane in the semi-continuous mode. pH was also in the optimal pH range of 7.43±0.04.

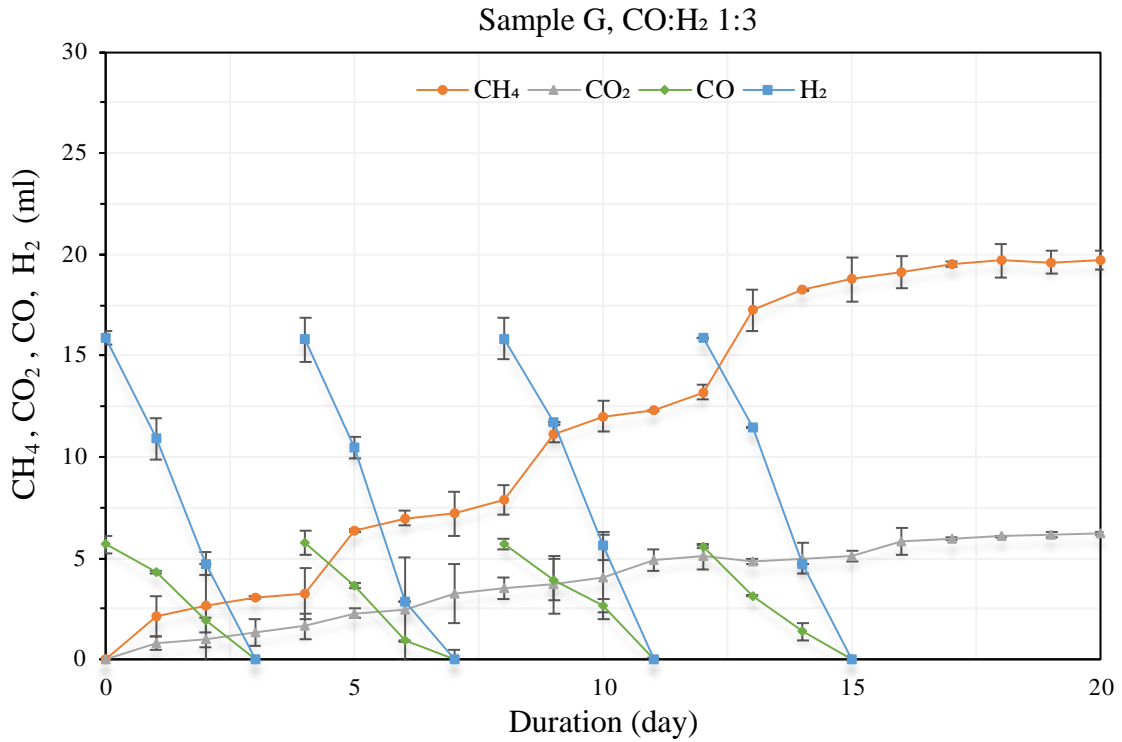


Figure 4.18. Net accumulated biogas under CO and H₂ biomethanation Sample G (CO:H₂ 1:3).

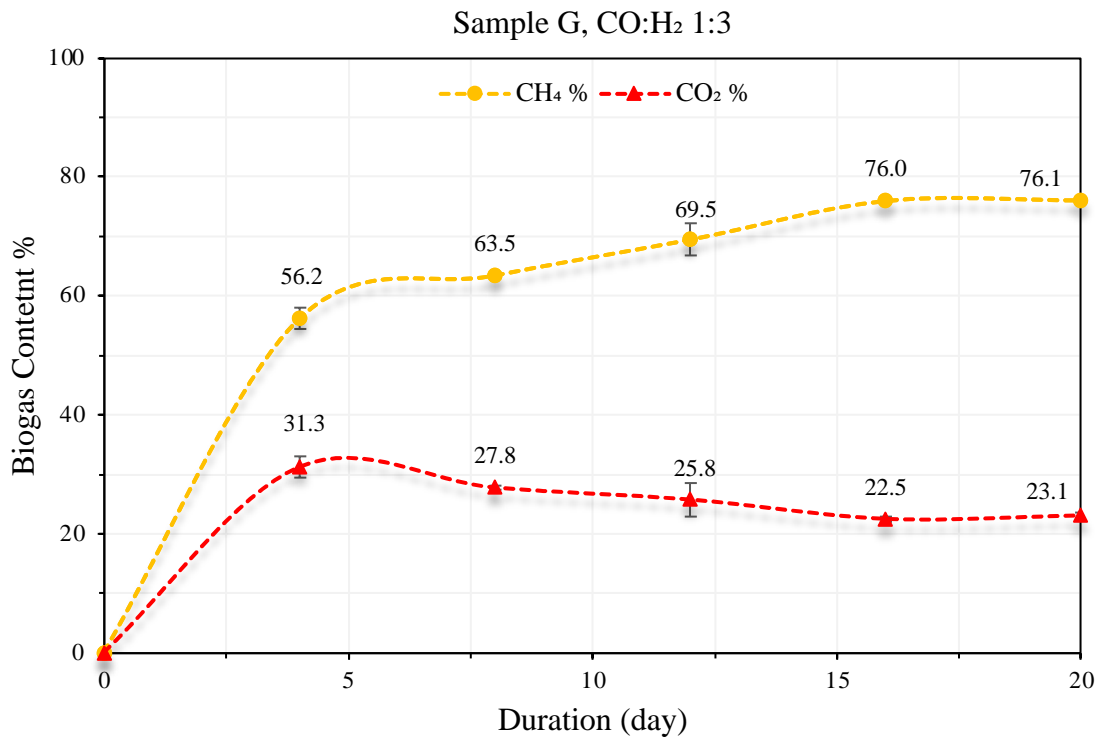


Figure 4.19. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample G (CO:H₂ 1:3).

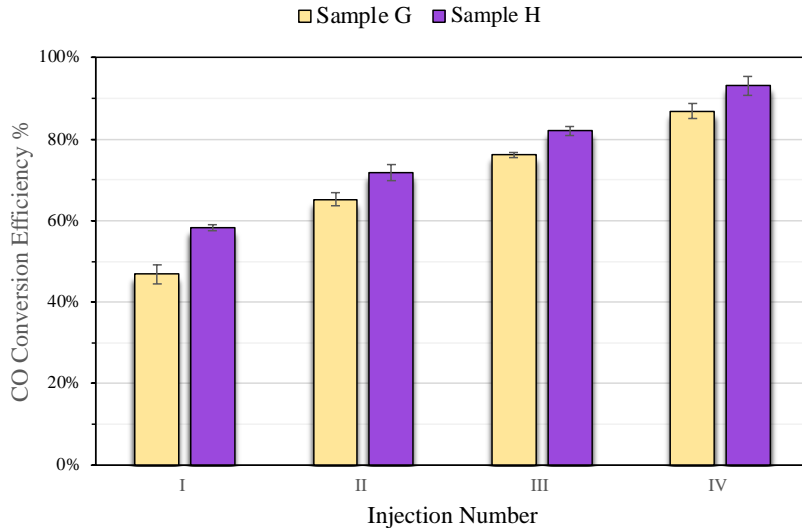


Figure 4.20. CO Conversion efficiency on the last day of each injection for Sample G (CO: H₂ 1:3) and Sample H (CO: H₂ 1:4).

4.4.4 CO and H₂ biomethanation in semi-continuous mode with stoichiometric ratio CO: H₂ 1:4 (Sample H)

Figure 4.21 illustrates the net accumulative biogas generated from the simulated syngas, consisting of CO and H₂ with a higher stoichiometric ratio (CO: H₂ 1:4). The percentages of CH₄ and CO₂ in the produced biogas increased noticeably on the final day of each feeding stage. It is observed that the methane concentration is higher on the last day of the fourth injection compared to the initial stage (Figure 4.22). Furthermore, Figure 4.20 demonstrates the enhancement in CO conversion efficiency following each gas injection stage.

Comparing the results of samples G and H, in which CH₄ content increased by 13.1% and CO₂ decreased by 21.2%, it is concluded that elevating the H₂/CO ratio can enhance the methane yield generated from SB and enhance the quality of biogas produced. Methane yield in sample H was also higher reaching 0.25 mmol CH₄/mmol H₂ on the last day of the last injection from 0.14 mmol CH₄/mmol H₂ after the first injection. Syngas can be converted to methane through different pathways, potentially leading to increased production of CO₂ and VFAs as by-products. Therefore, an appropriate increase in the H₂/CO ratio can promote CO conversion and simultaneously increase the methane content.

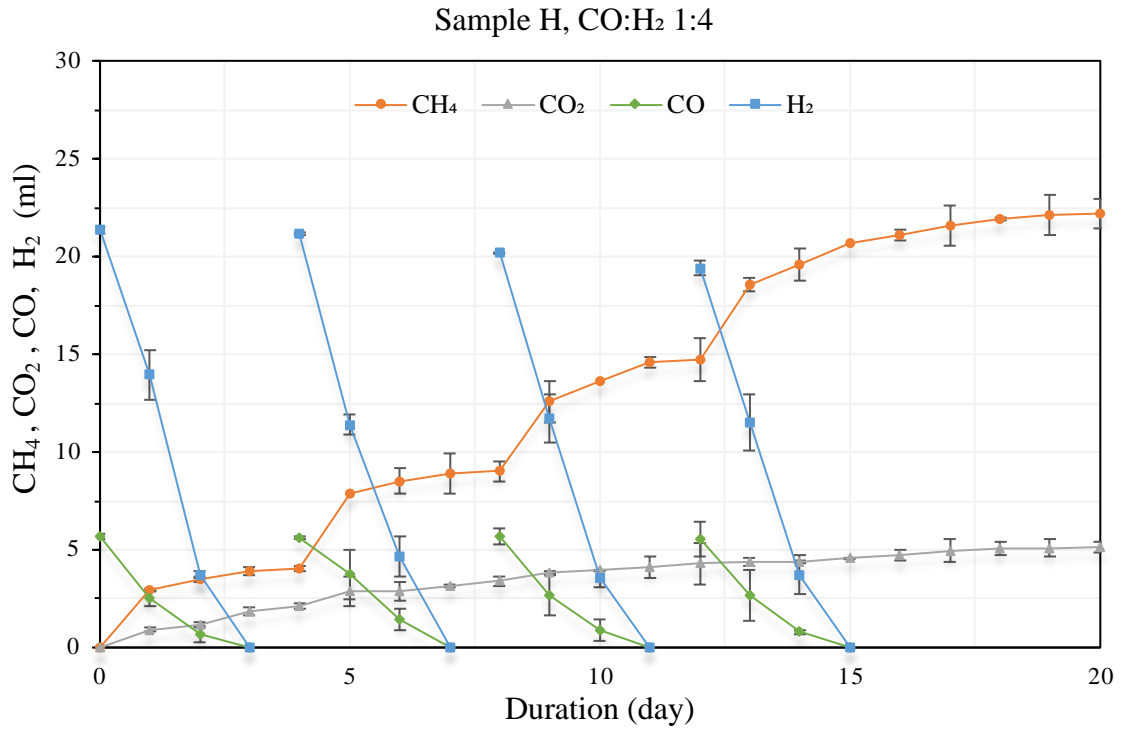


Figure 4.21. Net accumulated biogas under CO and H₂ bio methanation Sample H (CO:H₂ 1:4).

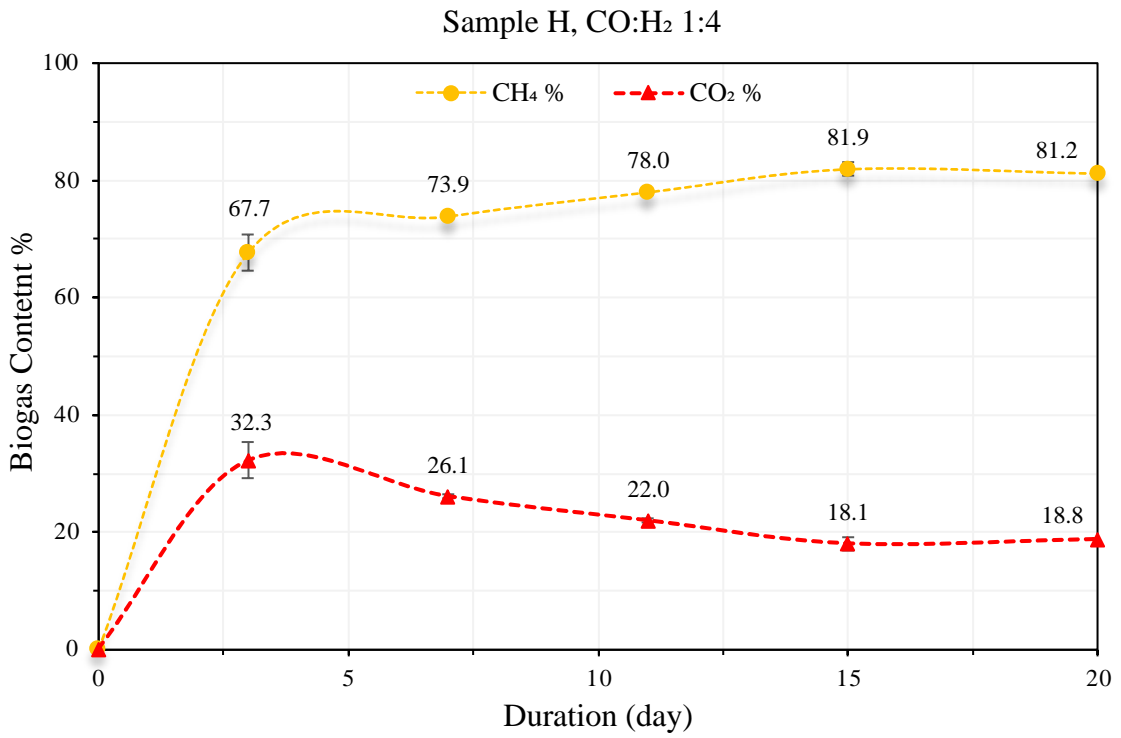


Figure 4.22. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample H (CO:H₂ 1:4).

In sample H, no VFA accumulation was observed, and it was reduced to $9,874 \pm 1.11$ from $9,042$ mg/L, indicating a favourable condition for VFA conversion to methane in the semi-continuous mode. pH was also in the optimal pH range of 7.92 ± 0.03 at the end of the experiment.

4.4.5 CO₂ and H₂ biomethanation in semi-continuous mode with stoichiometric ratio CO₂: H₂ 1:4 (Sample I).

Figure 4.23 depicts the net accumulative biogas generated from the simulated syngas, characterized by a stoichiometric ratio of (CO₂: H₂ 1:4). Noticeable increases in the percentages of CH₄ and CO₂ can be observed in the biogas produced on the final day of each feeding stage. As anticipated, the concentration of methane is higher on the last day of the fourth injection compared to the initial stage (Figure 4.24). Figure 4.25 indicates that during all four enrichment stages in sample I, an increment in the CO₂ conversion efficiency is observed. Lower CO₂ conversion efficiency at the beginning of the experiment demonstrates that an essential fraction of H₂ was utilized for biomass adaptation to the substrate. After biomass had fully acclimated to the gas substrates, a significant amount of H₂ was used for methane production [75]. This can be proved in the enhancement of Methane yield from 0.12 in the first injection to 0.22 mmol CH₄/mmol H₂ on the last day of the last injection. VFA accumulation was not observed in sample I, and it reached $9,159 \pm 1.11$ and pH was in the optimal pH range of 7.45 ± 0.04 which proves the effectiveness of microorganisms' adaption by injecting H₂ in several steps.

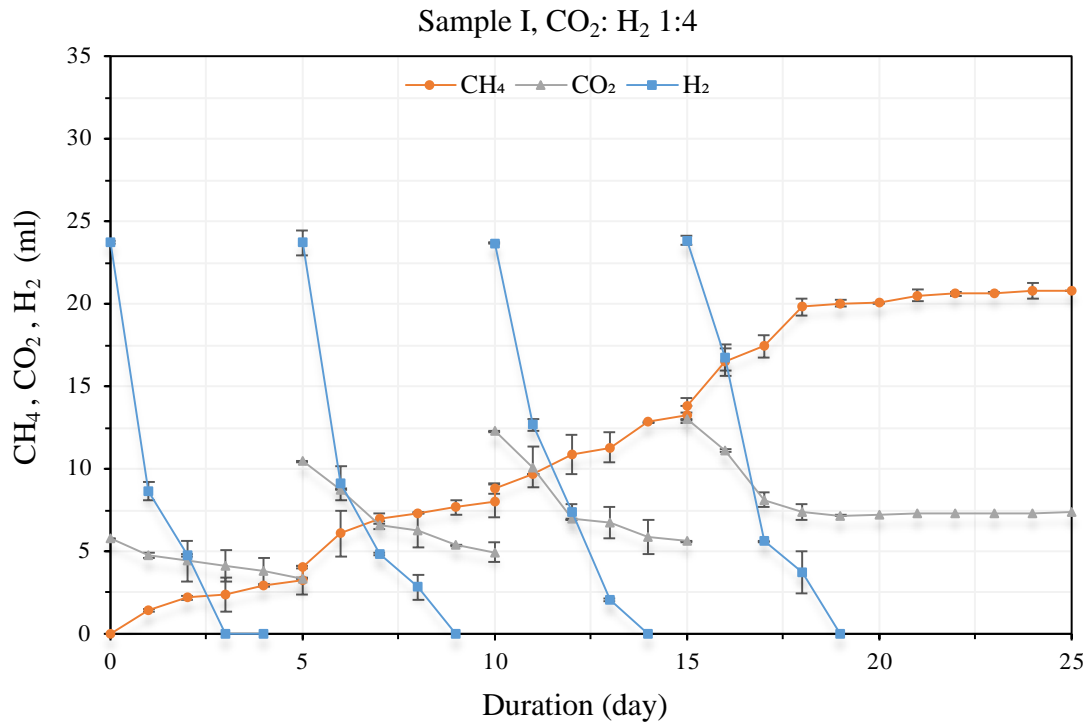


Figure 4.23. Net accumulated biogas under CO₂ and H₂ bio methanation Sample I (CO₂:H₂ 1:4).

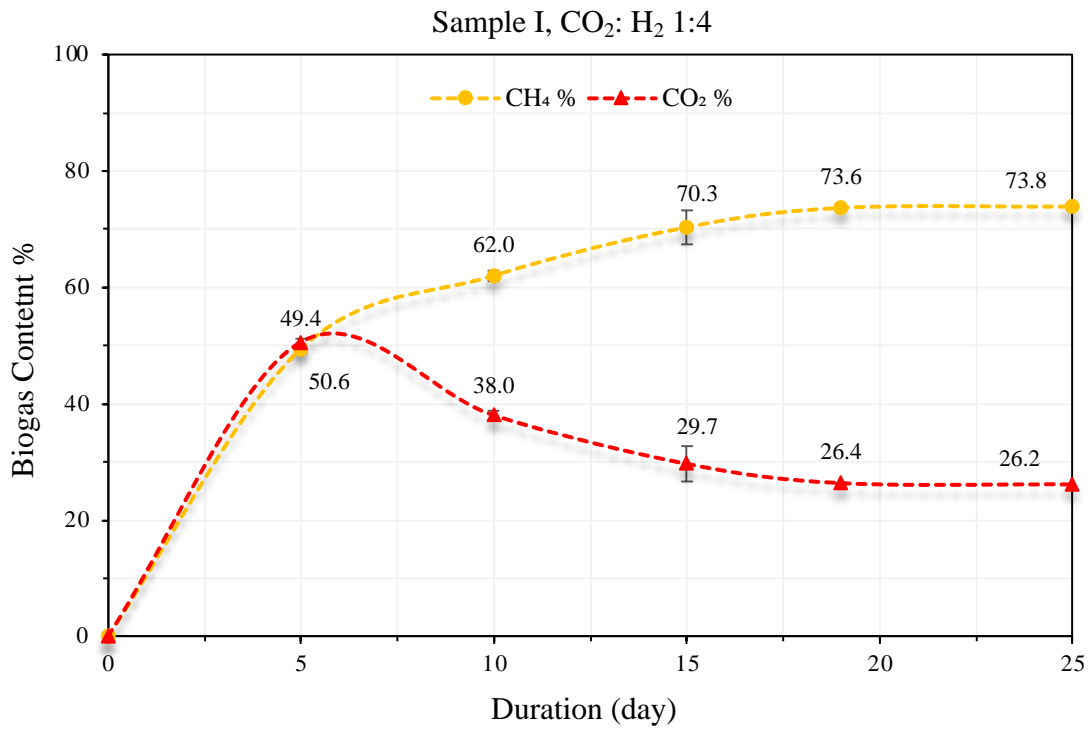


Figure 4.24. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample I (CO₂:H₂ 1:4).

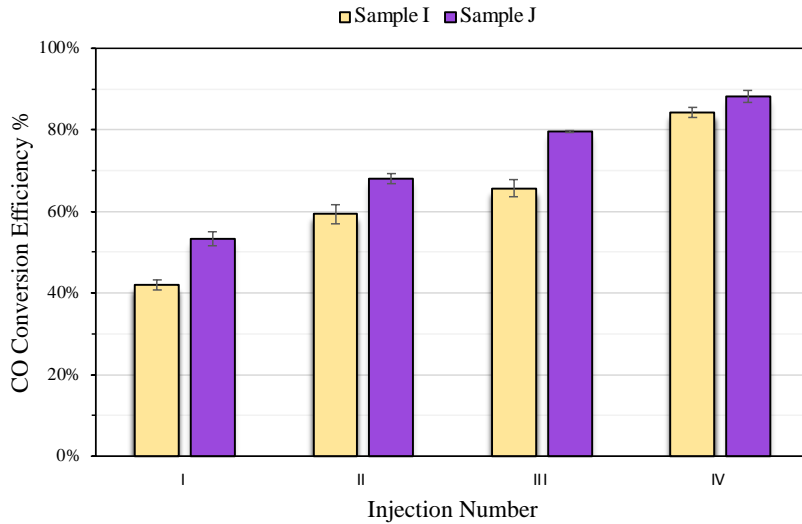


Figure 4.25. CO₂ Conversion efficiency on the last day of each injection for Sample I (CO₂: H₂ 1:4) and Sample J (CO₂: H₂ 1:5).

4.4.6 CO₂ and H₂ biomethanation in semi-continuous mode with stoichiometric ratio CO₂: H₂ 1:5 (Sample J).

Figure 4.26 shows the net accumulative biogas produced from the simulated syngas (CO₂ and H₂ with a higher stoichiometric ratio (CO: H₂ 1:4). Increment in the percentages of CH₄ and CO₂ in the produced biogas on the last day of each feeding stage can be seen. As expected, methane concentration is higher on the last day of 4th injection than in the first stage (Figure 4.27).

Figure 4.25 indicates that during all four enrichment phases in sample J, an increment in the CO₂ conversion efficiency is observed. Lower CO₂ conversion efficiency at the beginning of the experiment demonstrates that an essential fraction of H₂ was utilized for biomass adaptation to the substrate. As shown Figure 4.26 after biomass had fully acclimated to the gas substrates, a significant amount of H₂ was used for methane production (Methane yield increment from 0.12 in the first injection to 0.23 mmol CH₄/mmol H₂ on the last day of the last injection) [75]. At the final stage of sample J, the methane content of 81.7 % was achieved, which is 10.7% higher than sample I, thus making attractive, in a biological biogas upgrading process operation, the use of an H₂/CO₂ ratio above the stoichiometric value to boost CO₂ conversion,

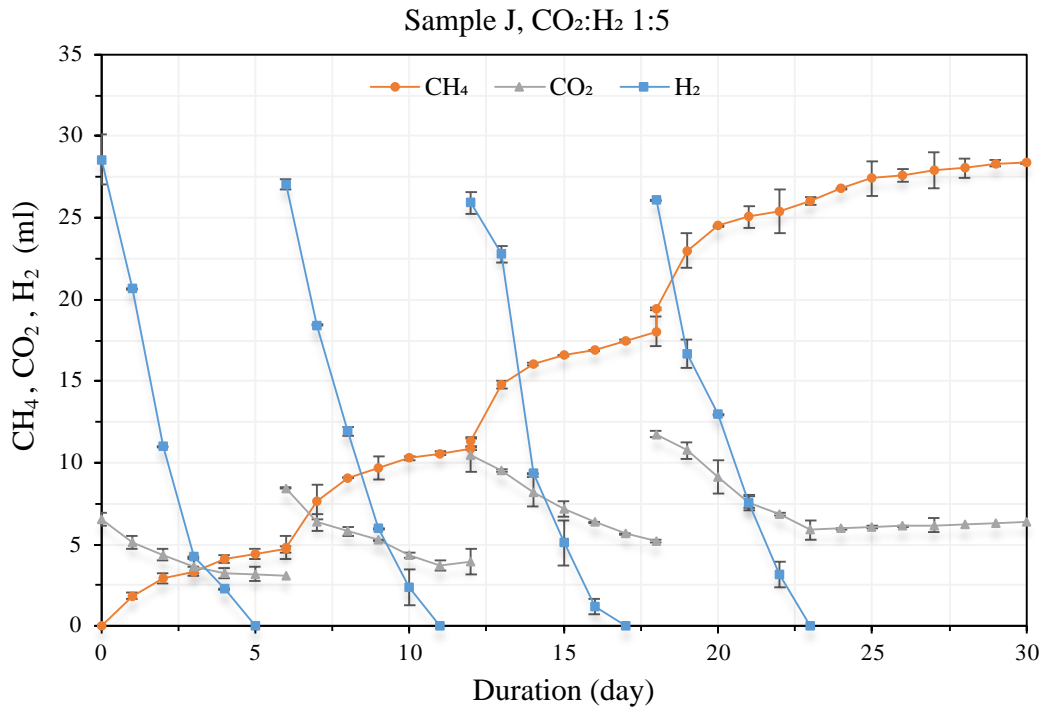


Figure 4.26. Net accumulated biogas under CO₂ and H₂ bio methanation Sample J (CO₂: H₂ 1:5).

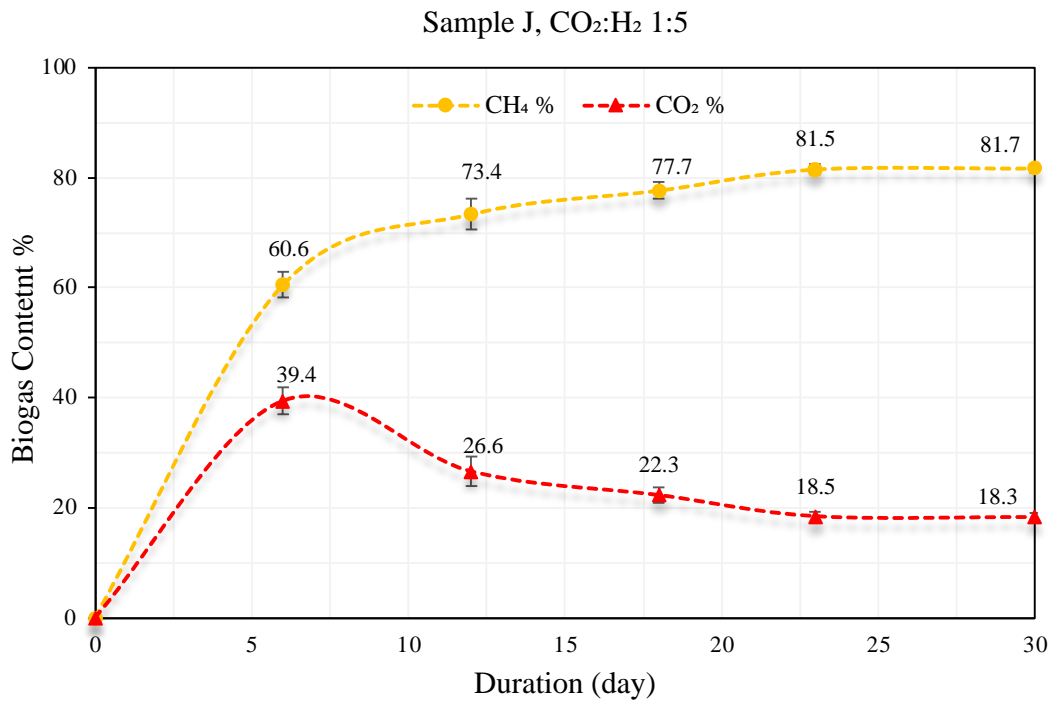


Figure 4.27. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample J (CO₂: H₂ 1:5).

resulting in increasing methane yield and methane content in the biogas produced. No accumulation of VFA was detected, and it reached a level of $8,922 \pm 2.46$ mg/L. The pH level remained within the optimal range of 7.72 ± 0.08 .

4.4.7 Syngas bio methanation in semi-continuous mode with stoichiometric ratio CO₂:CO:H₂ 1:2:3 (Sample K).

Although the results of sample E in phase I (Syngas CO₂:CO:H₂ 1:2:3) showed the lowest biogas upgrading in reducing CO₂ content in the produced biogas compared to other syngas compositions, it was tested again in a semi-continuous mode in phase II. The main reason was to optimize the coupling of AD with the syngas composition produced in industrial gasifying reactors. As in phase I, because of deficient H₂ in the design of syngas, in each stage, CO content was not consumed entirely by consumption of H₂ (Figure 4.28). Methane content on the last day of the experiment, when CO was not detected in the sample's headspace, reached 58.6% (Figure 4.29). In sample K, VFA was increased to 9692 ± 1.25 mg/L, and pH was reduced to 7.12 ± 0.05 demonstrating the conversion of gases mostly to acetic acids and CO₂. However, the same as other samples with injecting gases continuedly with time intervals increment in the total gas conversion to methane were indicated (Figure 4.30).

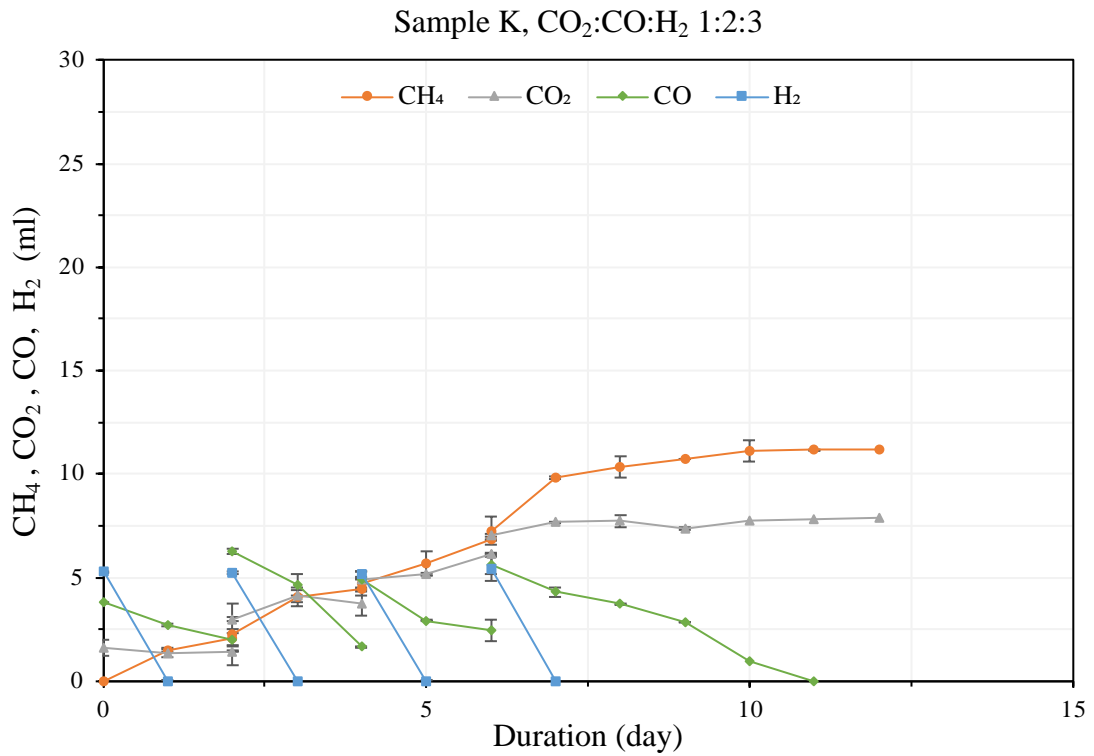


Figure 4.28. Net accumulated biogas under Syngas bio methanation Sample K (CO₂:CO:H₂ 1:2:3).

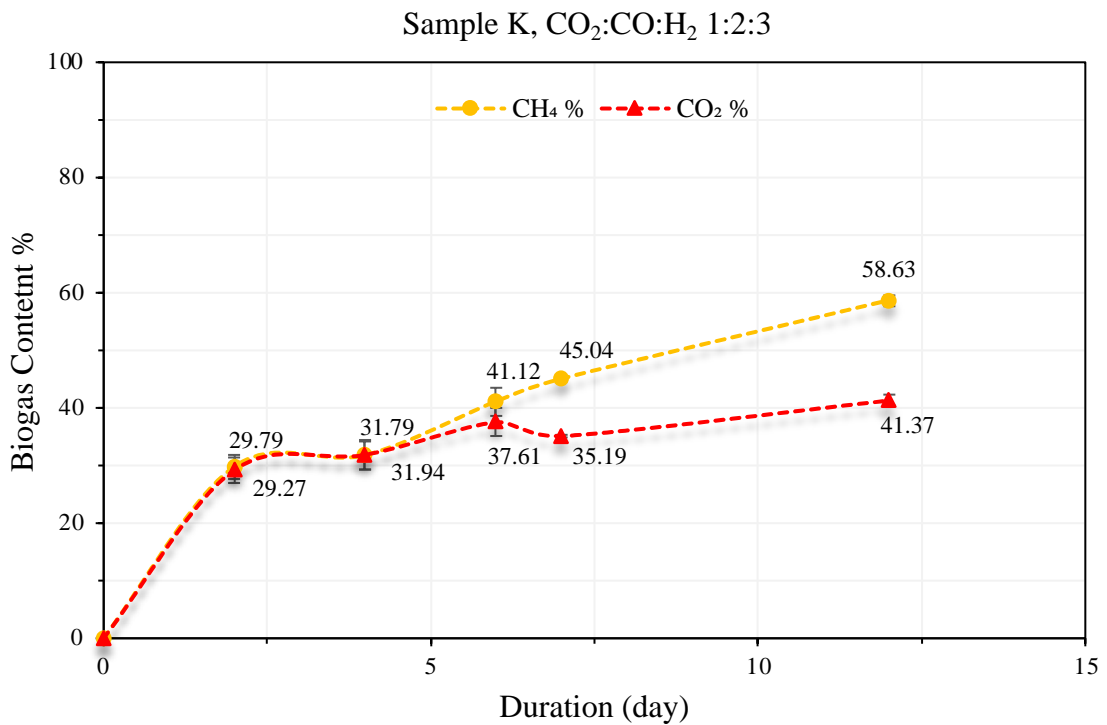


Figure 4.29. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample K (CO₂:CO:H₂ 1:2:3).

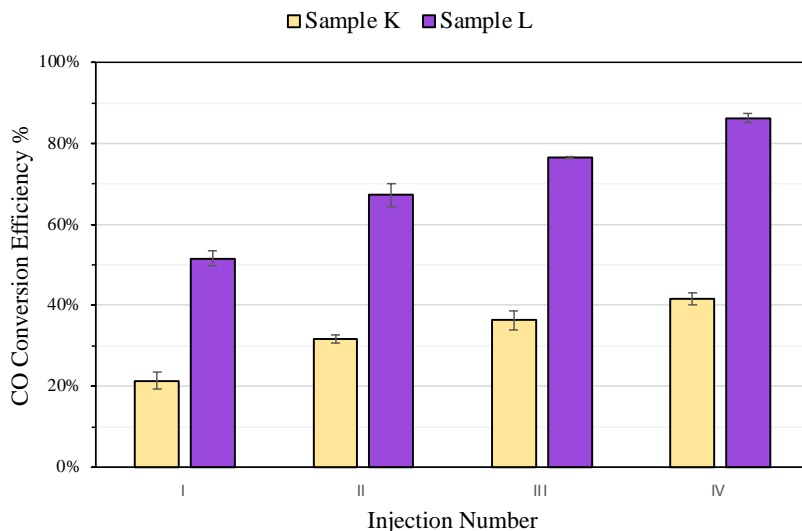


Figure 4.30. CO and CO₂ Conversion efficiency on the last day of each injection for Sample K (CO₂:CO:H₂ 1:2:3) and Sample L (CO₂:CO: H₂ 1:1:7).

4.4.8 Syngas biomethanation in semi-continuous mode with stoichiometric ratio CO₂:CO: H₂ 1:1:7 (Sample L).

As in sample F in phase I (syngas CO₂:CO: H₂ 1:1:7), where a sufficient volume of hydrogen was in the headspace, methane content was boosted more than all other syngas compositions. During each stage, CO was consumed with the consumption of H₂ (Figure 4.31) and CO conversion to Methane was increased after each injection (Figure 4.30). Methane yield was also increased to 0.24 mmol CH₄/mmol H₂ on the last of the 4th injection from 0.14 after first injection which is higher than any other samples. However, as with different samples, produced biogas still constituted a considerable amount of CO₂ (Figure 32), demonstrating not all the CO, CO₂ and H₂ were converted to methane via hydrogenotrophic methanogenesis, and CO, CO₂ conversion efficiency reached 81.2% on day 20th of the experiment. In sample L, VFA reduction was observed to 8,813±1.25 mg/L, and pH was 7.52±0.05 demonstrating the stability of the reactors during the experiment.

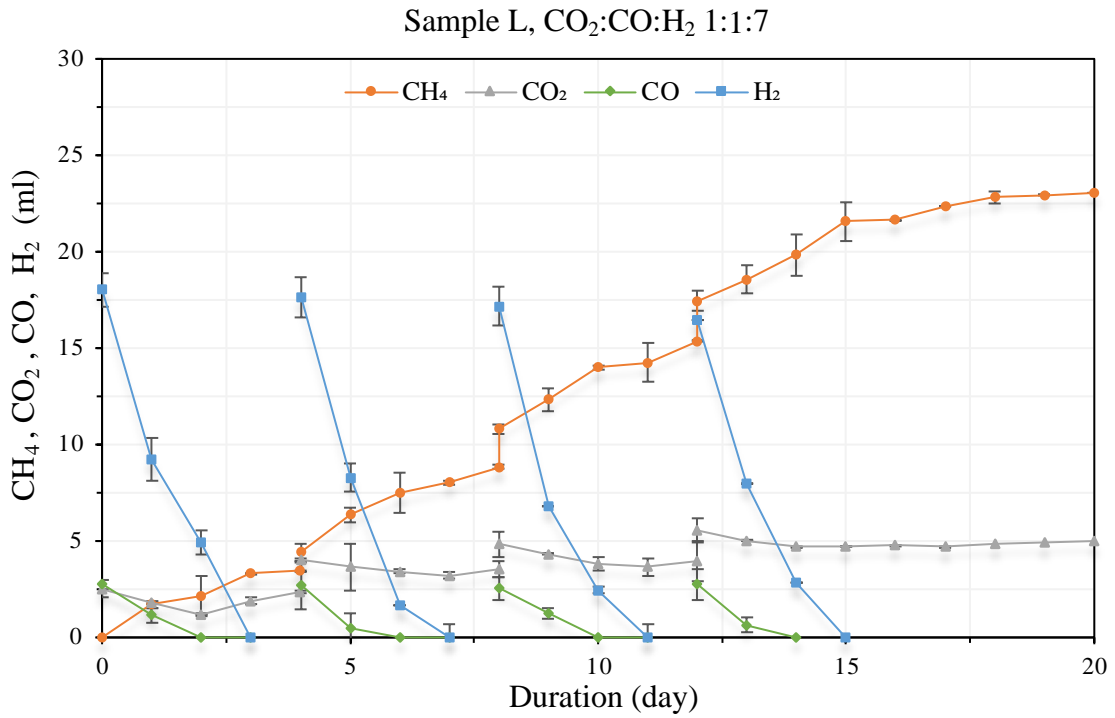


Figure 4.31. Net accumulated biogas under Syngas bio methanation Sample L (CO₂:CO: H₂ 1:1:7).

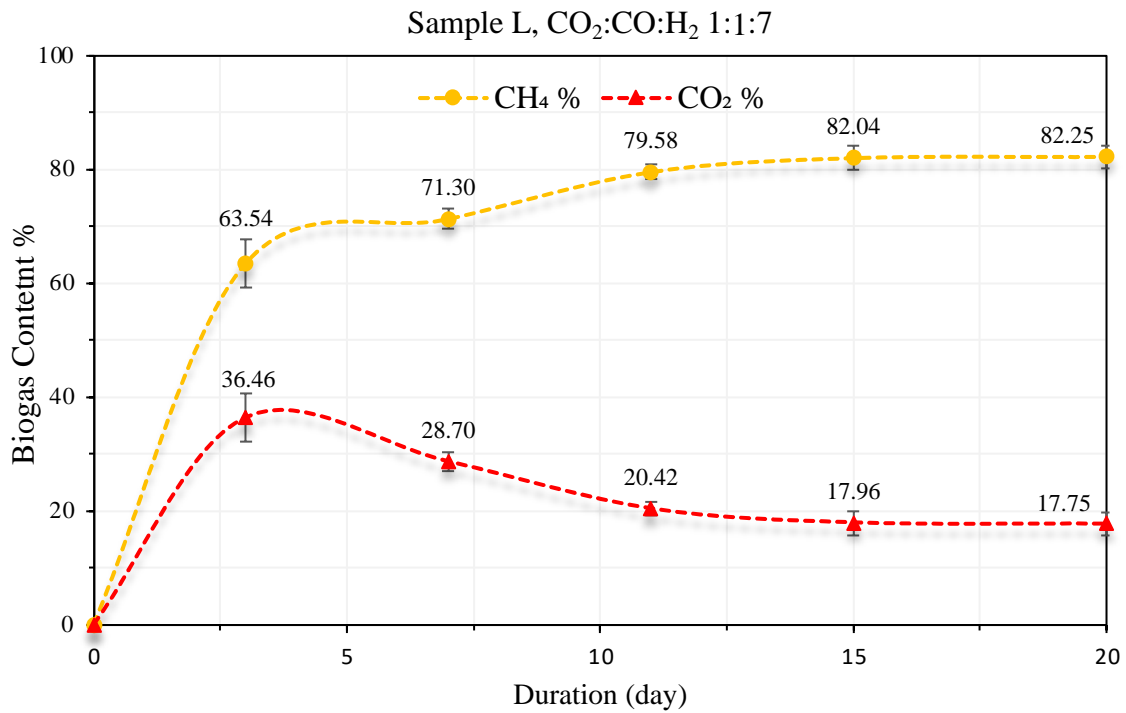


Figure 4.32. CH₄ and CO₂ contents in the produced gas on the last day of each injection Sample L (CO₂:CO: H₂ 1:1:7).

4.5 Comparing results of Phase II:

Results of Phase II have proven that injecting additional H₂ into an AD reactor can be an effective method for increasing syngas conversion efficiency and methane content. As presented in Table 4.10 and comparing results of samples G and H; I and J; K and L extra H₂ in the reactor resulted in a noticeable enhancement in syngas conversion efficiency. Moreover, Table 4.10 illustrates that the biogas composition produced from the simulated syngas, with a higher stoichiometric ratio, exhibited increased percentages of CH₄ on the last day of each feeding stage.

Table 4.9. CO/CO₂ Conversion efficiency in the samples of Phase II.

Sample	G (CO:H ₂ 1:3)		H (CO:H ₂ 1:4)		I (CO ₂ :H ₂ 1:4)	
Injection Stages	Last Stage	Total	Last Stage	Total	Last Stage	Total
CO/CO ₂ conversion efficiency (%)	86.9	70.9	93.1	80.2	84.2	65.5

Sample	J (CO ₂ :H ₂ 1:5)		K (CO: CO ₂ :H ₂ 1:2:3)		L (CO: CO ₂ :H ₂ 1:1:7)	
Injection Stages	Last Stage	Total	Last Stage	Total	Last Stage	Total
CO/CO ₂ conversion efficiency (%)	88.2	79.0	41.7	47.5	87.6%	81.3%

It can be observed from the result that by using hydrogen as an electron donor, hydrogenotrophic methanogenesis offers a promising approach to biologically converting CO₂ and CO to CH₄, which could result in improved environmental and economic advantages of biogas technologies. The results verified that H₂ bio methanation could allow the biological conversion to occur satisfactorily. This outcome also emphasizes another route for the

economical and practical bioconversion of renewable H₂ and waste CO₂ into a clean and energy-rich fuel (pure biomethane) using hydrogenotrophic methanogens.

Results showed that distributing the hydrogen loading into an AD reactor over time could increase the conversion rate of H₂ to CH₄. By gradually introducing hydrogen into the reactor, the microorganisms responsible for the conversion process can adapt and optimize their metabolic activities. Initially, microorganisms in the AD reactor may have limited capability to utilize hydrogen efficiently. If a large amount of hydrogen is added all at once, it can result in inhibitory effects and reduce the conversion efficiency. However, by slowly increasing the hydrogen loading rate over time, the microorganisms have an opportunity to acclimate and develop a more robust population capable of utilizing hydrogen effectively.

Chapter 5

5. Conclusions and Future Work

The investigation involved examining various syngas compositions to assess the effectiveness of the coupling process in SB in both batch (Phase I) and semi-continues (Phase II) under mesophilic temperature.

5.1 Phase I:

- Regarding gas-liquid ratios, except for sample A (CO alone) and sample B (CO₂ alone), ratio 1.5 showed higher methane content and injected gas conversion efficiency. It is assumed that CO and H₂ partial pressure in this ratio was more in favour of the direct pathway CO and CO₂ to methane.

- In the case of biomethanation of individual syngas components, biomethanation of CO₂ was more promising than CO as it showed higher methane production and conversion efficiency (33.1, 34.8 and 27.8% and 19.7, 15.8 and 15.3% respectively for CO₂ and CO in ratios 0.5, 1 and 1.5 (mL gas/mL biomass)). H₂ biomethanation failed because of the high and

sudden injection of H₂, which increased the pH and resulted in lower biogas production than the control system.

- In the case of biomethanation of syngas with different components, the best syngas composition that gives the highest amount of CH₄ was syngas hydrogen-rich (78% H₂, 11% CO₂, 11% CO) and syngas with stoichiometric relation between hydrogen and carbon dioxide or carbon oxide (75% H₂, 25% CO) and (80% H₂, 20% CO₂). According to the findings, there is a correlation between the H₂ concentration in syngas and the CH₄ content in biogas, meaning that lower H₂ concentrations lead to lower CH₄ contents in biogas.

- Gas conversion efficiency to methane did not reach 100% in any of the samples because the production of CO₂ aligns with CH₄ as in all the samples acetoclastic pathways were utilized by microorganisms for the conversion of injected gases.

- The carbon mass balance in all samples indicated the conversion of injected gases to biogas or VFAs, with comparable amounts of VFAs observed in both the gas-injected samples and control samples. This demonstrates the utilization of produced VFAs during the experiment by microorganisms for biogas production through indirect pathways.

5.2 Phase II:

- Based on the results, injecting syngas to an AD reactor in semi-continuous mode led to higher CH₄ content than a sudden syngas injection. The microorganisms gradually adjusted and utilized the syngas more efficiently. In a semi-continuous mode, the organisms slowly adapted to the increased H₂, CO and CO₂ over time, increasing CH₄ production. Injected gas conversion efficiency to methane was increased while preceding the following stages and reached higher than 80% in all the samples with enough H₂ in the system.

- The CH₄ content in the produced biogas achieved 82.2 % with H₂-rich syngas composition, 73.8 for syngas with stoichiometric relation between H₂ and CO₂, 76.9% for

syngas with stoichiometric relation between H₂ and CO and 58.6% for the industrial composition of syngas.

- The study found that increasing the H₂/CO and H₂/CO₂ stoichiometric ratio can raise the methane content in SB bio methanation and enhance biogas quality. The CH₄ content in the produced biogas reached to 81.7 for syngas with higher stoichiometric relation between H₂ and CO₂, and 84.9 % for syngas with higher stoichiometric relation between H₂ and CO.

5.3 Future work:

- As results of phase I and II indicated, still, none of the samples reached 100% of the theoretical value. Any of the operational parameters like temperature variation, low gas-liquid transfer, pH variation, VFAs accumulation, gas partial pressure and gas leaking can change microbial construction and reduce the efficiency. Therefore, checking all these parameters during the experiment and the microbial analysis of the systems should be studied in future to investigate further the impact of injecting syngas on the microbial population.

- Gas diffusers and membranes can provide promising approaches for optimizing SB, addressing distinct aspects of the process to achieve enhanced outcomes by improving mass transfer and increasing the efficiency of bio methanation.

- The development of novel catalysts or catalyst formulations might be solution for enhancing the conversion of syngas components (CO, CO₂, and H₂) into methane. Catalysts with high activity, selectivity, and stability can significantly improve the bio-methanation process.

- Investigation of the microbial communities involved in syngas bio-methanation to better understand their roles and interactions. This knowledge can be utilized to optimize and engineer microbial consortia for improved syngas conversion efficiency and stability.

- The co-production of value-added products along with methane production, such as biofuels, platform chemicals, or other bio-based products. This can enhance the overall process economics and contribute to a more circular and sustainable bioenergy system.

Chapter 6

6. References

- [1] A. Wellinger, J. Murphy, D. Baxter, “The Biogas Handbook: Science, Production and Applications,” *Elsevier Inc*, pp. 1–126, 2008.
- [2] K. F. Adekunle and J. A. Okolie, “A review of biochemical process of anaerobic digestion,” *Advances in Bioscience and Biotechnology*, vol. 6, no. 03, p. 205–212, 2015.
- [3] IEA, “Net zero by 2050: A roadmap for the global energy sector,” *International Energy Agency 224*, March. 2021.
- [4] S. Kaza, L. Yao, P. Bhada-Tata, and F. Van Woerden, “What a waste 2.0: a global review of solid waste management,” *World Bank*, pp. 1–100, 2018.
- [5] E. Ara, M. Sartaj, and K. Kennedy, “Enhanced biogas production by anaerobic co-digestion from a trinary mix substrate over a binary mix substrate,” *Waste Management and Research.*, vol. 33, no. 6, pp. 578–587, 2015.
- [6] S. Shah, W. H. Bergland, and R. Bakke, “Methane from Syngas by Anaerobic Digestion,” in *Proceedings of the 58th Conference on Simulation and Modelling (SIMS 58) Reykjavik, Iceland, September 25th – 27th, 2017*, vol. 138, pp. 114–120, 2017.
- [7] V. N. Gunaseelan, “Anaerobic digestion of biomass for methane production: a review,” *Biomass and bioenergy*, vol. 13, no. 1–2, pp. 83–114, 1997.

- [8] Canadian Biogas Association, “Hitting Canada ’ s Climate Targets with Biogas & RNG Executive Summary,” March, 2022, *Available at:* https://biogasassociation.ca/resources/page/hitting_canadas_climate_targets_with_biogas_rng.
- [9] B McCabe, R Kroebel ,M Pezzaglia,C Lukehurst,C Lalonde,M Wellisch,J Murphy, “Integration of Anaerobic Digestion into Farming Systems in Australia, Canada, Italy, and the UK,” *IEA Bioenergy*, vol. 37, p.1–58, August. 2020.
- [10] B. Parr, M. Paterson, and D. Henry, “Opportunities and challenges for Canada to exceed its Paris Agreement target,” 2017, *Available at: www.sustainable.unimelb.edu.au*.
- [11] H. Sun , Z. Yang , Q. Zhao, M. Kurbonova ,R. Zhang ,G. Liu , W. Wang , “Modification and extension of anaerobic digestion model No. 1 (ADM1) for syngas biomethanation simulation: From lab-scale to pilot-scale,” *Chemical Engineering Journal*, vol. 403, p.1261–77, 2021.
- [12] O. Norouzi and A. Dutta, “The Current Status and Future Potential of Biogas Production from Canada’s Organic Fraction Municipal Solid Waste,” *Energies*, vol. 15, no. 2, p. 475–92, 2022.
- [13] Q. Sun, H. Li, J. Yan, L. Liu, Z. Yu, and X. Yu, “Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation,” *renewable and Sustainable Energy Reviews*, vol. 51, pp. 521–532, 2015.
- [14] G. Luo and I. Angelidaki, “Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture,” *Biotechnology and bioengineering*, vol. 109, no. 11, pp. 2729–2736, 2012.
- [15] A. I. Adnan, M. Y. Ong, S. Nomanbhay, K. W. Chew, and P. L. Show, “Technologies for biogas upgrading to biomethane: A review,” *Bioengineering*, vol. 6, no. 04, p. 92–115, 2019.
- [16] Z. Yang *et al.*, “Improvement of biofuel recovery from food waste by integration of anaerobic digestion, digestate pyrolysis and syngas biomethanation under mesophilic and thermophilic conditions,” *Journal of Cleaner Production*, vol. 256, p. 120594–604, 2020.
- [17] D. Andreides, J. I. B. Quispe, J. Bartackova, D. Pokorna, and J. Zabranska, “A novel

- two-stage process for biological conversion of syngas to biomethane,” *Bioresource Technology*, vol. 327, p. 124811–819, 2021.
- [18] M. Pecchi and M. Baratieri, “Coupling anaerobic digestion with gasification, pyrolysis or hydrothermal carbonization: a review,” *Renewable and Sustainable Energy Reviews*, vol. 105, pp. 462–475, 2019.
- [19] C. Li, X. Zhu, and I. Angelidaki, “Syngas biomethanation: effect of biomass-gas ratio, syngas composition and pH buffer,” *Bioresource Technology*, vol. 342, p. 125997–6008, 2021.
- [20] J. I. Bautista Quispe, “Biological conversion of energetic gases to biomethane,” M.S Thesis, IHE Delft Institute for Water Education, Delft, Netherlands, 2020, Available at: <https://cdm21063.contentdm.oclc.org/digital/collection/masters2/id/111969>.
- [21] S. Aslanzadeh, “Pretreatment of cellulosic waste and high-rate biogas production.” PhD thesis, University of Borås, School of Engineering, 2014, Available at: <http://hdl.handle.net/2320/12853>.
- [22] P. Elefsiniotis and W. K. Oldham, “Influence of pH on the acid-phase anaerobic digestion of primary sludge,” *Chemical Technology & Biotechnology*, vol. 60, pp. 89–96, 1994.
- [23] X. Zhou, Y. Lu, L. Huang, Q. Zhang, X. Wang, and J. Zhu, “Effect of pH on volatile fatty acid production and the microbial community during anaerobic digestion of Chinese cabbage waste,” *Bioresource Technology*, vol. 336, p. 125338, 2021.
- [24] I. Angelidaki and W. Sanders, “Assessment of the anaerobic biodegradability of macropollutants,” *Reviews in Environmental Science & Bio Technology*, vol. 3, pp. 117–129, 2004.
- [25] T. R. Sreekrishnan, S. Kohli, and V. Rana, “Enhancement of biogas production from solid substrates using different techniques—a review,” *Bioresource technology*, vol. 95, no. 1, pp. 1–10, 2004.
- [26] A. A. Akindele and M. Sartaj, “The toxicity effects of ammonia on anaerobic digestion of organic fraction of municipal solid waste,” *Waste Management*, vol. 71, pp. 757–766, 2018.
- [27] N. Pisutpaisal and U. Sirisukpoca, “Development of rapid chemical oxygen demand

- analysis using ozone as oxidizing agent,” *Energy Procedia*, vol. 50, pp. 711–718, 2014.
- [28] O. Yenigün and B. Demirel, “Ammonia inhibition in anaerobic digestion: a review,” *Process Biochemistry*, vol. 48, no. 5–6, pp. 901–911, 2013.
- [29] S. Dong and M. Sartaj, “Statistical analysis and optimization of ammonia removal from landfill leachate by sequential microwave/aeration process using factorial design and response surface methodology,” *Journal of environmental chemical engineering*, vol. 4, no. 1, pp. 100–108, 2016.
- [30] S. Fu, I. Angelidaki, and Y. Zhang, “In situ biogas upgrading by CO₂-to-CH₄ bioconversion,” *Trends Biotechnology*, vol. 39, no. 4, pp. 336–347, 2021.
- [31] D. Andreides, K. O. Fliegerova, D. Pokorna, and J. Zabranska, “Biological conversion of carbon monoxide and hydrogen by anaerobic culture: Prospect of anaerobic digestion and thermochemical processes combination,” *Biotechnology Advancement*, vol. 58, p. 107886, 2022.
- [32] A. G.-A. I. V Skiadas and H. N. Gavala, “Syngas biomethanation: state-of-the-art review and perspectives,” *Biofuels Bioprod Biorefining*, vol. 12, pp. 139–158, 2018.
- [33] S. R. Guiot, R. Cimpoaia, and G. Carayon, “Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas,” *Environmental science & technology*, vol. 45, no. 5, pp. 2006–2012, 2011.
- [34] N. A. Samiran, M. N. M. Jaafar, J.-H. Ng, S. S. Lam, and C. T. Chong, “Progress in biomass gasification technique—with focus on Malaysian palm biomass for syngas production,” *Renewable and Sustainable Energy Reviews*, vol. 62, pp. 1047–1062, 2016.
- [35] N. Antoniou, F. Monlau, C. Sambusiti, E. Ficara, A. Barakat, and A. Zabaniotou, “Contribution to Circular Economy options of mixed agricultural wastes management: Coupling anaerobic digestion with gasification for enhanced energy and material recovery,” *Journal of cleaner production*, vol. 209, pp. 505–514, 2019.
- [36] K. Xiang, Y. Zhiyi, Z. Jingxin, T. Yen Wah, Y. Wenming, D. Yanjun, W. Chi-Hwa, “Energy performance of an integrated bio-and-thermal hybrid system for lignocellulosic biomass waste treatment,” *Bioresource technology*, vol. 228, pp. 77–88, 2017.
- [37] B. Gunes, “A critical review on biofilm-based reactor systems for enhanced syngas fermentation processes,” *Renewable and Sustainable Energy Reviews*, vol. 143, p.

- 110950, 2021.
- [38] A. Irini, T.Laura, T.Panagiotis, L.Gang, C.Stefano, W.Henrik, K.Panagiotis., “Biogas upgrading and utilization: Current status and perspectives,” *Biotechnology Advances*, vol. 36, no. 2, pp. 452–466, Mar. 2018.
- [39] J. Zabranska and D. Pokorna, “Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens,” *Biotechnology Advancment.*, vol. 36, no. 3, pp. 707–720, 2018.
- [40] A. Konstantinos, G.Antonio, M.Antonios, W.Zhiyou, G.Hariklia N, S.Ioannis V, “Temperature effects on syngas biomethanation performed in a trickle bed reactor,” *Chemical Engineering*, vol. 393, p. 124739, 2020.
- [41] J. Daniell, M. Köpke, and S. D. Simpson, “Commercial biomass syngas fermentation,” *Energies*, vol. 5, no. 12, pp. 5372–5417, 2012.
- [42] J. Sipma, P. N. L. Lens, A. J. M. Stams, and G. Lettinga, “Carbon monoxide conversion by anaerobic bioreactor sludges,” *FEMS Microbiology Ecology.*, vol. 44, no. 2, pp. 271–277, 2003.
- [43] D. Andreides, D. Pokorna, and J. Zabranska, “Assessing the syngas biomethanation in anaerobic sludge digestion under different syngas loading rates and homogenisation,” *Fuel*, vol. 320, p. 123929, 2022.
- [44] M. A. Voelklein, D. Rusmanis, and J. D. Murphy, “Biological methanation: Strategies for in-situ and ex-situ upgrading in anaerobic digestion,” *Applied Energy*, vol. 235, pp. 1061–1071, 2019.
- [45] E. P. Ahern, P. Deane, T. Persson, B. Ó. Gallachóir, and J. D. Murphy, “A perspective on the potential role of renewable gas in a smart energy island system,” *Renewable Energy*, vol. 78, pp. 648–656, 2015.
- [46] S. Sarker, A. S. R. Nordgård, J. J. Lamb, and K. M. Lien, “Biogas and hydrogen,” in *Hydrogen, Biomass and Bioenergy*, Elsevier, 2020, pp. 73–87.
- [47] Y. Bajón Fernández, A. Soares, K. Koch, P. Vale, and E. Cartmell, “Bioconversion of carbon dioxide in anaerobic digesters for on-site carbon capture and biogas enhancement—A review,” *Critical Reviews in Environmental Science and Technology*, vol. 47, no. 17, pp. 1555–1580, 2017.

- [48] M. Alimahmoodi and C. N. Mulligan, “Anaerobic bioconversion of carbon dioxide to biogas in an upflow anaerobic sludge blanket reactor,” *Journal of the Air & Waste Management Association*, vol. 58, no. 1, pp. 95–103, 2008.
- [49] C. Salomoni, A. Caputo, M. Bonoli, O. Francioso, M. T. Rodriguez-Estrada, and D. Palenzona, “Enhanced methane production in a two-phase anaerobic digestion plant, after CO₂ capture and addition to organic wastes,” *Journal of the Air & Waste Management Association*, vol. 102, no. 11, pp. 6443–6448, 2011.
- [50] Y. Bajón Fernández, K. Green, K. Schuler, A. Soares, P. Vale, P. L. Alibardi, E. Cartmell, “Biological carbon dioxide utilisation in food waste anaerobic digesters,” *Water research*, vol. 87, pp. 467–475, 2015.
- [51] S. Paniagua, R. Lebrero, and R. Muñoz, “Syngas biomethanation: Current state and future perspectives,” *Bioresource Technology*, p. 127436, 2022.
- [52] S. Sancho Navarro, “Biomethanation of syngas: identification of metabolic pathways from CO in a natural anaerobic consortium,” Master Thesis, Faculty of Medicine, University of Montreal, Available at: <https://doi.org/1866/8636>, 2012.
- [53] L. Yeqing, L. Yinjun, W. Ximeng, L. Sen, S. Dongfang, J. Hao, Z. Hongjun, P. Junting, F. Lu, “Biomethanation of syngas at high CO concentration in a continuous mode,” *Bioresource Technology*, vol. 346, p. 126407, 2022.
- [54] S. Sancho Navarro, R. Cimpioia, G. Bruant, and S. R. Guiot, “Biomethanation of syngas using anaerobic sludge: shift in the catabolic routes with the CO partial pressure increase,” *Frontiers in microbiology*, vol. 7, p. 1188, 2016.
- [55] M. F. Serge R. Guiot, Ruxandra Cimpioia, Silvia Sancho-Navarro, “Bio-upgrading of syngas into renewable natural gas (methane),” in *proceedings of the 13th world congress on anaerobic digestion*, 2013.
- [56] H. N. Gavala, A. Grimalt-Alemany, K. Asimakopoulos, and I. V. Skiadas, “Gas biological conversions: the potential of syngas and carbon dioxide as production platforms,” *Waste and Biomass Valorization*, pp. 1–26, 2021.
- [57] A. M. Henstra, J. Sipma, A. Rinzema, and A. J. M. Stams, “Microbiology of synthesis gas fermentation for biofuel production,” *Current Opinion in Biotechnology*, vol. 18, no. 03, pp. 200–206, 2007.

- [58] I. K. Stoll, N. Boukis, and J. Sauer, “Syngas fermentation at elevated pressure—experimental results,” in *European Biomass Conference and Exhibition Proceedings*, 2019, pp. 1255–1261.
- [59] Y. Bao, J. Jia, S. Tong, Z. Gao, and Z. Cai, “A review on single bubble gas–liquid mass transfer,” *Current Opinion in Biotechnology*, vol. 28, no. 11, pp. 2707–2722, 2020.
- [60] L. Han and M. H. Al-Dahhan, “Gas–liquid mass transfer in a high pressure bubble column reactor with different sparger designs,” *Chemical Engineering Science*, vol. 62, no. 1–2, pp. 131–139, 2007.
- [61] M. Kozak, E. O. Köroğlu, K. Cirik, and Z. Zaimoğlu, “Evaluation of ex-situ hydrogen biomethanation at mesophilic and thermophilic temperatures,” *International Journal of Hydrogen Energy*, vol. 47, no. 34, pp. 15434–15441, 2022.
- [62] V. Corbellini, A. Catenacci, and F. Malpei, “Hydrogenotrophic biogas upgrading integrated into WWTPs: enrichment strategy,” *Water Science. Technology*, vol. 79, no. 4, pp. 759–770, 2019.
- [63] L.Yeqing, W.Zhenxin, H.Ziying, L.Sen, S.Dongfang, J.Hao, Z.Hongjun, X.Quan, “Effects of temperature, hydrogen/carbon monoxide ratio and trace element addition on methane production performance from syngas biomethanation,” *Bioresource Technology*, vol. 295, p. 122296, 2020.
- [64] S. Achinas, S. J. Mulder, and G. J. W. Euverink, “Influence of Liquid-to-Gas Ratio on the Syngas Fermentation Efficiency: An Experimental Approach,” *Bioengineering*, vol. 7, no. 4, p. 138, 2020.
- [65] Y. B. Fernández, A. Soares, R. Villa, P. Vale, and E. Cartmell, “Carbon capture and biogas enhancement by carbon dioxide enrichment of anaerobic digesters treating sewage sludge or food waste,” *Bioresource Technology*, vol. 159, pp. 1–7, 2014.
- [66] S. Shah, W. H. Bergland, and R. Bakke, “Methane from Syngas by Anaerobic Digestion,” *Proc. 58th Conf. Simul. Model. (SIMS 58) Reykjavik, Iceland, Sept. 25th – 27th, 2017*, vol. 138, pp. 114–120, 2017.
- [67] A.Sluite, B.Hames, D.Hyman, C.Payne, R.Ruiz, C.Scarlata, J.Sluite, D.Templeton, J.Wolfe, “Determination of total solids in biomass and total dissolved solids in liquid process samples,” *National Renewable Energy Laboratory.*, vol. 9, pp. 1–6, 2008.

- [68] US Environmental Protection Agency Engineering and Analysis Division, Office of Water-Office of Science and Technology, “Total, fixed, and volatile solids in water, solids, and biosolids,” 2001.
- [69] H. Yen, DE. Brune, “Anaerobic co-digestion of algal sludge and waste paper to produce methane,” *Bioresource Technology*, vol. 98, no. 1, pp. 130–134, 2007.
- [70] J. Filer, H. H. Ding, S. Chang, “Biochemical methane potential (BMP) assay method for anaerobic digestion research,” *Water*, vol.11, no. 5, pp. 921, 2019.
- [71] N. Alemahdi, H. C. Man, N. Nasirian, and Y. Yang, “Enhanced mesophilic biohydrogen production of raw rice straw and activated sewage sludge by co-digestion,” *International Journal of Hydrogen Energy*, vol. 40, no. 46, pp. 16033–16044, 2015.
- [72] N. A. Borjabad, “From biogas to biomethane. Biological conversion of H₂ and CO₂ to CH₄,” Ph.D Thesis Universidad de Valladolid, Spain, 2018.
- [73] D. Andreides, D. Stransky, J. Bartackova, D. Pokorna, and J. Zabranska, “Syngas biomethanation in countercurrent flow trickle-bed reactor operated under different temperature conditions,” *Renewable Energy*, vol. 199, pp. 1329–1335, 2022.
- [74] V. Sivalingam, T. Haugen, A. Wentzel, and C. Dinamarca, “Effect of Elevated Hydrogen Partial Pressure on Mixed Culture Homoacetogenesis,” *Chemical Engineering Science*. vol. 12, p. 100118, 2021.
- [75] N. Alfaro, M. Fdz-Polanco, F. Fdz-Polanco, and I. Díaz, “Evaluation of process performance, energy consumption and microbiota characterization in a ceramic membrane bioreactor for ex-situ biomethanation of H₂ and CO₂,” *Bioresource Technology*, vol. 258, pp. 142–150, 2018.

Appendix

Phase I:

Table 0.1. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 0.5 (mL gas/mL biomass) (Phase I).

Substrate	Blank	A	B	C	D	E	F
CO ₂	0	8.86 ml	0	0	8.86 ml	2.7 ml	4.05 ml
CO	0	0	8.86 ml	8.86 ml	0	5.9 ml	4.43 ml
H ₂	0	0	0	24.2 ml	35.85 ml	8.19 ml	28.65 ml
CO ₂ : CO: H ₂	na	1:0:0	0:1:0	0:1:3	4:0:1	1:2:3	1:1:7
Biomass (ml)	20	20	20	20	20	20	20

Table 0.2. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1 (mL gas/mL biomass) (Phase I).

Substrate	Blank	A	B	C	D	E	F
CO ₂	0	17.22 ml	0	0	17.72 ml	5.4 ml	8.09 ml
CO	0	0	17.22 ml	17.22 ml	0	11.81ml	8.86 ml
H ₂	0	0	0	48.4 ml	71.71 ml	16.38 ml	57.31ml
CO ₂ : CO: H ₂	na	1:0:0	0:1:0	0:1:3	1:0:4	1:2:3	1:1:7
Biomass (ml)	20	20	20	20	20 ml	20 ml	20 ml

Table 0.3. Setup of syngas bio-methanation experiments at the gas-biomass ratio of 1.5 (mL gas/mL biomass) (Phase I).

Substrate	Blank	A	B	C	D	E	F
CO ₂	0	26.59 ml	0	0	26.59 ml	8.09 ml	12.14 ml
CO	0	0	26.59 ml	26.59 ml	0	17.72 ml	13.3 ml
H ₂	0	0	0	72.6 ml	107.56 ml	24.56 ml	85.97 ml
CO ₂ : CO: H ₂	na	1:0:0	0:1:0	0:3:1	1:0:4	1:2:3	1:1:7
Biomass (ml)	20	20	20	20	20	20	20

Table 0.4. Set up of H₂ bio-methanation experiment (Phase I).

Substrate	H ₂ a	H ₂ b
CO ₂	0	0
CO	0	0
H ₂	53.18 ml	35.45 ml
CO ₂ : CO: H ₂	na	na
Biomass (ml)	20	20

Phase II:

Table 0.5. Setup of syngas bio-methanation experiments with multiple injections (Phase II).

Substrate	Blank	G	H	I	J	K	L
CO ₂	0	0	0	24.0 ml	24.0 ml	7.2 ml	10.6 ml
CO	0	24.0 ml	24.0 ml	0	0	16.0 ml	12.0 ml
H ₂	0	64.8 ml	86.8 ml	95.7 ml	119.7 ml	21.7 ml	76.7 ml
CO ₂ : CO: H ₂	na	0:1:3	0:1:4	1:0:4	1:0:5	1:2:3	1:1:7
Biomass (ml)	20	20	20	20	20	20	20

Table 0.6. Setup of H₂ bio-methanation experiments with multiple injections (Phase II).

Substrate	H ₂ c
CO ₂	0
CO	0
H ₂	53.18 ml
CO ₂ : CO: H ₂	na
Biomass (ml)	20 ml

The concentration of H₂, CH₄, CO₂, and CO N₂ in 50 μL of gas samples was calculated using the following Equations [71].

H₂ area /525.09 = qH₂ quantity in 50 μL gas sample.

CO area /52.519 = qCO quantity in 50 μL gas sample.

N₂ area /20.571 = qN₂ quantity in 50 μL gas sample.

CH₄ area /102.05 = qCH₄ quantity in 50 μL gas sample.

CO₂ area /242.16 = qCO₂ quantity in 50 μL gas sample.

The percentage of each gas in the headspace of bottles was calculated using the following equations.

$$100 \text{ qH}_2 / \text{qH}_2 + \text{qCH}_4 + \text{qCO}_2 + \text{qCO} + \text{qN}_2 = \text{H}_2 \%$$

$$100 \text{ qCH}_4 / \text{qH}_2 + \text{qCH}_4 + \text{qCO}_2 + \text{qCO} + \text{qN}_2 = \text{CH}_4 \%$$

$$100 \text{ qCO}_2 / \text{qH}_2 + \text{qCH}_4 + \text{qCO}_2 + \text{qCO} + \text{qN}_2 = \text{CO}_2 \%$$

$$100 \text{ qCO} / \text{qH}_2 + \text{qCH}_4 + \text{qCO}_2 + \text{qCO} + \text{qN}_2 = \text{CO} \%$$

$$100 \text{ qN}_2 / \text{qH}_2 + \text{qCH}_4 + \text{qCO}_2 + \text{qCO} + \text{qN}_2 = \text{N}_2 \%$$