Thermophilic Biogas Upgrading via ex Situ Addition of H₂ and CO₂ Using Codigested Feedstocks of Cow Manure and the Organic Fraction of Solid Municipal Waste

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ABSTRACT: Bioconversion of renewable H₂ and waste CO₂ using methanogenic archaea is a promising technology for obtaining high-purity CH₄, which can serve as an alternative for natural gas. This process is known as ex situ biogas upgrading. This work highlights the pathway toward the bioconversion of renewable H₂ and CO₂ into high-purity biomethane by exploiting highly accessible agro-municipal residues: cow manure (CM) and the organic fraction of solid municipal waste (OFSMW), which used to be called “waste materials”. More specifically, an ex situ thermophilic (55 °C) biogas upgrading process was conducted by CM and OFSMW codigestion at different mass proportions: 100:0, 80:20, 70:30, 60:40, and 50:50. Maximum CH₄ concentrations of 92−97 vol % and biogas volumetric production rates of 4954−6605 NmL/L.d were obtained from a batch reactor of 3 L working volume. Feedstock characterization, pH monitoring, and the carbon-to-nitrogen ratio were critical parameters to evaluate during biogas upgrading experiments. In this work, the usefulness of agro-municipal substrates is highlighted by producing high-purity biomethane—an energetic chemical to facilitate renewable energy conversion, which supports various end-use applications. This process therefore provides a solution to renewable energy storage challenges and future sustainable and green energy supply.

1. INTRODUCTION

Recently, the advancement of renewable energies—such as biomass, wind, and solar—has received significant attention in efforts to reduce the environmental and social challenges associated with fossil fuels and their processing.¹ In first-world regions, such as the European Union, renewable energy targets of 20 and 27% have been set for 2020 and 2030.² Likewise, many developing countries have also initiated various strategies to fast-track renewable energy technology development. Global future renewable energy benefits include reduced electricity prices,³ the alleviation of environmental pollution, and new employment and local manufacturing opportunities.⁴ In addition, the export of renewable energies is made possible by the power-to-gas concept, which will bring future wealth to countries with vast renewable energy sources, which in the past had limited or zero fossil fuels for export purposes. Indeed, energy export to space-limited regions such as Japan and many European countries looks promising.

However, renewables such as wind and solar are susceptible to natural intermittency.⁵,⁶ Sometimes, up to 40% of the electricity is deemed to be in temporary surplus,⁷ whereas the electricity supply from wind energy could be near-zero during periods of low wind and solar energy has zero potential at night time. This has resulted in the development of alternative energy storage solutions in order to provide grid balancing, a technique to ease the fluctuations in power grids and provide more sustainable power supply from renewables.⁸

One attractive method of exploiting the surplus electricity from renewable sources is to split water into H₂ and O₂ (power-to-gas).⁹,¹⁰ Hydrogen is viewed as a clean energy carrier because of its carbon-free structure and the fact that it can be produced from any renewable energy resource.¹¹,¹² Nonetheless, its application has been hampered due to storage and transportation challenges.¹³,¹⁴ To circumvent these barriers, H₂ is used alongside CO₂ to produce CH₄ via the Sabatier reaction (eq 1).¹⁵ This process is known as the power-to-methane (PtM) concept.¹⁶ PtM has sparked great interest amongst industry and academia as a new pathway to accommodate the increased percentage of renewables in energy grids.¹⁷ When CH₄ produced from PtM conforms to natural gas standards, it is readily stored in existing gas...
CH4 production rates compared to mesophilic conditions and pressure. Generally, thermophilic fermentation provides faster hydrogenotrophic methanogens convert H2 and CO2 into CH4 and hydrogenotrophic methanogens. 32 The acetoclastic methanogenic species.34,35

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4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \ \ \ \Delta H^0 = -165 \text{ kJ/mol}
\] (1)

PtM is achieved through two processes: catalytic methanation and biological methanation. Catalytic methanation is a well-established process and is already being used for commercial purposes.21 However, this process employs catalysts such as nickel and ruthenium, which require high-temperature operation (300–500 °C) and above-atmospheric pressures (up to 25 bar).22,23 On the other hand, biological methanation can take place at mesophilic mesophilic (30–37 °C) or thermophilic (35–65°C) temperatures and at atmospheric pressure. Generally, thermophilic fermentation provides faster CH4 production rates compared to mesophilic conditions and is the preferred temperature range to improve digestion (e.g., breaking down fibrous feedstocks).24–28 Other benefits of biological methanation include a wide spectrum of feedstocks (including waste materials) and microorganisms that are found in diverse anoxic environments.16,29 The microorganisms that mediate CH4-forming biochemical reactions are known as methanogens.30,31 These archaeal species also form a syntrophic relationship with other organisms such as acidogenic and acetogenic bacteria.32 Methanogens can be classified into two distinct groups: acetoclastic methanogens and hydrogenotrophic methanogens.32 The acetoclastic methanogens convert acetate into CH4 and CO2.33 The hydrogenotrophic methanogens convert H2 and CO2 into CH4 (eq 1) and are reported to be the largest group of methanogenic species.34,35

Biological methanation is categorized as follows: (i) in situ biogas upgrading, where H2 (from a renewable origin) is injected into the reactor and is coupled with the endogenous CO2 to generate CH4 and (ii) ex situ biogas upgrading, which involves the parallel injection of renewable H2 and CO2 (acquired from an external source, e.g., brewery) into the liquid phase of the reactor.16 The latter process has some merits compared with the in situ process: in addition to the ex situ H2 fed to the reactor, acquiring the CO2 from an external source improves the rate of carbon conversion potential and thus the ultimate production rate of CH4. The in situ process is limited by the rate of CO2 produced by the microorganisms, and if H2 is fed in excess, the fermenter is more prone to acidification—CH4 production slows or ceases completely.

Anaerobic codigestion involves the mixing of two or more feedstocks to achieve complementary nutritional characteristics.36 As opposed to monodigestion, the codigestion of organic feedstocks offers several benefits, such as balance of nutrients, optimum carbon-to-nitrogen ratio, and increased buffering capacity, which subsequently leads to enhanced biogas production.37 Among the feedstocks, the organic fraction of solid municipal waste (OFMSW) is deemed a suitable feedstock for biogas upgrading processes because it is an abundant waste material, comprising of various food components (bread, apple, orange, carrot, cabbage, potato, etc.), some of which are high in carbohydrates such as starch (bread, potato), whereas others are high in fructose (apple, orange, etc.).38–41 Other components of OFMSW, such as paper, is cellulosic, and takes much longer to degrade into simple carbohydrates. Cow manure (CM) also supports numerous essential compounds (nitrogen, potassium, phosphorus, copper, zinc, etc.) that stimulate the activity of methanogenic species but is low in energetic value.42 The codigestion of CM and OFMSW is therefore promising to obtain enhanced biogas yields.

Many developing countries are experiencing increased amounts of organic waste residues because of industrialization and population growth. For instance, South Africa produces more than 60 million tons of organic waste per annum.43 These organic residues are usually dumped on landfills because of inadequate waste management systems, resulting in various environmental issues.43 These feedstocks could play a crucial role in the advancement of biological methanation technology by reducing associated substrate costs; substrates account for more than 60% of the overall bioprocessing costs.43

The anaerobic codigestion of CM and OFMSW is well known. For instance, it is common practice worldwide to co-digest these two common waste feedstocks for biogas production. This biogas, containing ~50–70 vol % CH4, is ultimately used for combined heat and power generation in MW-scale plants.24,44,45 An overview of the literature suggests that the effects of ex situ H2 and CO2 sparging has not been studied on the codigestion of CM and OFMSW substrates. The ex situ sparging process has indicated that CH4 concentrations >90 vol % can be obtained with various other inoculums and substrates summarized in Table 3. In this work, the performance of an ex situ sparged process using CM/OFMSW substrates will be investigated, while incorporating the advantages of thermophilic (55 °C) digestion. In particular, varying ratios of CM/OFMSW will be used to evaluate the digester’s operation and performance, that is, on biogas and CH4 yields, volatile fatty acids (VFAs) production and fermentation pH. In addition, this work provides information on the basic morphological properties of the prevalent CH4-forming microorganisms. Ultimately, this work motivates why upgraded biomethane (>90 vol % CH4) is much more than a source of fuel for combustion purposes. With minimal separation required, almost pure CH4 can be obtained, which acts as a building block for the chemical industry, a “green” automotive fuel, and energy storage medium that will facilitate an increased percentage of renewables in—and the sustainability of—associated energy systems.

2. MATERIALS AND METHODS

2.1. Feedstock Procurement. Fresh CM was collected from a local free-range cattle farm in Potchefstroom, North-West Province, South Africa. It is important to highlight that even though CM is considered as a feedstock that is widely used in codigestion, it supports the essential hydrogenotrophic methanogens that are required during the anaerobic codigestion process.46,47 Meanwhile, the OFMSW was obtained from a waste dumping site in Potchefstroom; by mass, this material consisted of paper (2%), bread (8%), orange (10%), apple (10%), cabbage (35%), and potato waste (35%).48 It is worth to mention that the composition of OFMSW can vary greatly at different locations. The OFMSW feedstock was dried at room temperature (~23 °C) and then reduced to a small particle size (2–3 mm) with a household blender in order to enhance its digestibility. The nutritional infrastructure (natural gas pipelines, >90 vol % CH4), as these pipelines offer energy storage capacity in the order of GWh to TWh. The combustion of CH4 in gas turbines during low electricity supply from renewables ultimately brings stabilization to the grid. In addition, CH4 can be used for various applications (e.g., chemical industry, mobility sector, and household heating).17–20
properties of CM and OFMSW were analyzed and are tabulated in Table 1.

### Table 1. Characteristics of Feedstocks Used in Biogas Upgrading Experiments

| parameter | CM (wt %) | OFMSW (wt %) |
|-----------|-----------|--------------|
| TS        | 21.40     | 36.45        |
| VS        | 18.65     | 26.75        |
| VS/TS ratio\(^a\) | 0.87 | 0.73         |
| C         | 38.75     | 40.29        |
| H         | 7.35      | 7.38         |
| N         | 1.74      | 1.53         |
| S         | 0.29      | 0.22         |
| O         | 51.87     | 50.58        |
| C/N ratio\(^a\) | 22.27 | 26.33        |
| protein   | 10.38     | 10.13        |
| fat       | 1.14      | 3.91         |
| carbohydrate | 58.16 | 75.14        |

\(^a\)Unitless parameter.

### 2.2. Reactor Configuration and Experimental Setup.

The ex situ thermophilic biogas upgrading experiments were conducted in a batch reactor. The reactor was fabricated using 2 mm thick stainless steel (SS304), supporting a total volume of 4 L (height 275 mm, ID 140 mm) and working volume of 3 L. The lid was made from polyvinyl chloride material (thickness 15 mm). It consisted of four ports: a gas inlet, stirring shaft, sampling port, and gas outlet. The lid was clamped to the reactor flange and sealed with a silicone rubber O-ring to ensure airtightness. The stirring shaft was connected to a Heidolph RZR 2020 mixer (Heidolph Instruments GmbH, Germany), supported with a retort stand and clamp. The stirring speed was in the range of 40−100 rpm. The temperature of the reactor was maintained using a temperature-regulated water bath (Labotec Pty Ltd., South Africa). A schematic representation of the ex situ thermophilic biogas upgrading process is shown in Figure 1.

Before use, the reactor was sterilized with 70 vol % ethanol. The experimental design was adopted from the work of Khayum and co-workers.\(^{46}\) Brieﬂy, five separate codigestion processes (CM100OF0, CM80OF20, CM70OF30, CM60OF40, and CM50OF50), consisting of 100:0, 80:20, 70:30, 60:40, and 50:50, respectively, on a mass basis of CM/OFMSW, were carried out. The experiments were performed at a final working volume of 3 L. The biomethanation processes were enriched with a nutrient medium which consisted of the following (in g/L): glucose (10.0), KH₂PO₄ (0.5), K₂HPO₄ (0.5), KCl (0.05), NH₄Cl (0.5), FeSO₄·H₂O (0.005), NiCl₂·6H₂O (0.0015), Na₂HPO₄·2H₂O (0.25), MgCl₂·6H₂O (0.05), NaHCO₃ (0.05), and MnCl₂ (0.015). These nutrients were added at the start of each biomethanation experiment to stimulate bacterial growth. The initial pH and temperature were set at 7.6 and 55 °C, respectively. These parameters were set in accordance with a previous study that recorded high CH₄ content of up to 90 vol %.\(^{35}\) Furthermore, biomethanation experiments were carried out at thermophilic conditions because it poses several benefits, such as a high hydrolysis rate, inhibition of toxins, less foam occurrence, and high biogas production.\(^{35}\)

Prior to each biomethanation process, the reactor was sparged with N₂ (99.999 vol %) for 5 min to remove oxygen in the headspace, to facilitate the growth of methanogenic species. Batch experiments (100 rpm, 20 days) were conducted in duplicate at a constant temperature of 55 °C. After 24 h of fermentation, the processes were continuously injected with the gaseous mixture (H₂/CO₂=4:1 by volume, according to the Sabatier reaction). The total flow rate was 2400 NmL/L d (1920 NmL/L d H₂ and 480 NmL/L d CO₂). This flow rate was then reduced to 960 NmL/L d (768 NmL/L d H₂ and 192 NmL/L d CO₂) after peak CH₄ production to improve the utilization of gas by the methanogenic species.\(^{49}\) A Bronkhorst mass flow controller (Bronkhorst High-Tech BV, Netherlands) was used to regulate the gaseous feed. Furthermore, control experiments that represent the nonsparged processes (CM100OF0-C to CM50OF50-C) were conducted in parallel with the main experiments, under similar operating conditions.

### 2.3. Analytical Methods.

The composition of biogas (CH₄, CO₂, and H₂) was analyzed using a SRI 8610C gas chromatograph (SRI Instruments, USA), equipped with a molecular sieve 13X column (6 ft) and a thermal conductivity detector. Argon was used as carrier gas at a flow rate of 26 mL/min. The oven temperature was programmed as follows: 50 °C for 3 min, then ramped up to 200 °C at a rate of 30 °C/min and held for 2 min. In addition, the biogas flow rate was measured daily using a bubble flow meter, at ambient temperature and pressure. The biogas and CH₄ flow rates were then converted to normal conditions (20 °C, 1 bar) for reporting purposes in this paper.

VFAs were determined using a gas chromatograph (6890N, Agilent Technologies, USA) equipped with a polar ZB FFAP capillary column (length 60 m, ID 0.32 mm, film thickness 50 µm).
μm). Helium was used as a carrier gas at a flow rate of 2.9 mL/min. The injector temperature was maintained at 240 °C in splitless mode. The oven temperature was programmed as follows: 70 °C for 2 min, then ramped up to 240 °C at a rate of 12 °C/min, and held for 5 min.

The total solids (TS) and volatile solids (VS) were determined according to standard methods.30 The elemental compositions (C, H, N, and S) of these feedstocks were obtained using an elemental analyzer (Vario EL Cube, Germany). The O content was calculated from the balance of C, H, N, and S. The fat, protein, and carbohydrate contents were calculated using the method reported by Shen and colleagues.30 Meanwhile, the pH was measured daily using an Orion Star A214 benchtop pH meter (Thermo Scientific, South Africa). The morphology of the prevalent CH4-producing microorganisms was observed using a light microscope (Leica Camera AG, Germany).

### 3. RESULTS AND DISCUSSION

#### 3.1. Characterization of Feedstocks

The characteristics of the feedstocks used (CM and OFSMW) are given in Table 1. The VS/TS ratio is an indicator of organic matter in these feedstocks.37 In this study, high VS/TS ratios for CM and OFSMW of 0.87 and 0.73, respectively, imply that these substrates are easily biodegradable. This was confirmed by the high carbohydrate content (58.16 and 75.14 wt %) and low contents of protein (10.38 and 10.13 wt %) and fats (1.14 and 3.91 wt %). Studies have shown that carbohydrate-rich feedstocks such as OFSMW biodegrade easily by fermentable bacteria to form VFAs and H2 during acidogenesis and acetogenesis.11,36 The resulting metabolites (H2 and VFAs) are then used by the methanogenic species in the final stage of anaerobic digestion.37 Hence, the formation of these intermediates is important because they affect the overall production of CH4. Furthermore, the C/N ratio for CM and OFSMW was 22.27 and 26.33, respectively. A C/N ratio of 20–30 is considered to be optimum for maintaining nutrient balance and stimulating CH4-producing organisms during anaerobic codigestion processes.37,46

#### 3.2. Methane Concentration and Biogas Flow Rate during Biogas Upgrading Experiments

The exogenous addition of gases (H2 and CO2) in the biomethanation processes led to a significant increase in the CH4 concentration (Figure 2) in comparison with the nonsparged control experiments (CM100OF0-C to CM50OF50-C) (Figure 3). For the sparged processes, the maximum CH4 concentrations were in the range of 92–97 vol %, occurring between days 6 to 12, respectively. The volumetric CH4 production rates were recorded as 4557–5886 NmL/L d for the five processes (each value reported on the day of maximum CH4 content). The maximum attained values are summarized in Table 2. The total biogas flow rates for the five experiments are illustrated in Figure 4, with maximum values of 4954–6605 NmL/L d. At maximum CH4 production there was also a simultaneous decrease in the concentration of H2 within the reactors because of its consumption (1640–1920 NmL/L d) by the hydrogenotrophic methanogens.49

The increase in the concentration of CH4 was attributable to the favorable characteristics of the thermophilic conditions, such as improved digestibility of feedstocks, enhanced metabolic rates, and resistance against inhibitors, as has been reported in similar studies.28,51 In addition, the injection of H2 and CO2 was advantageous in these processes because this prevented a sharp decline in pH, unlike in the conventional anaerobic digestion processes. In conventional processes, the acidogenic–solventogenic transition phase causes an increase in the accumulation of metabolites such as VFAs and alcohols, resulting in a sharp decline in the fermentation pH, which ultimately leads to the inhibition of CH4-producing pathways (as discussed in Section 3.3).52,53 In this work, the pH was in the range of 5.62–6.58 in all of the biogas upgrading experiments at end of the fermentations. It has been reported that the optimization of pH is crucial in anaerobic digestion because it enhances the buffering capacity/alkalinity of the biomethanation process.45,54,55

These results are comparable to other studies reported in the literature. Siddique et al.51 observed a 50–65% increase in CH4 production and a 98% reduction in chemical oxygen demand during a thermophilic codigestion process of pretreated petrochemical wastewater and beef and dairy CM, that was conducted at an operational pH of 6.5. Guneratnam and co-workers56 reported a high CH4 concentration of 92 vol % from an ex situ thermophilic biomethanation process using mixed cultures that was conducted at a pH of 7.7–8.2. Furthermore, Alitalo et al.56 obtained high-purity CH4 (>90 vol %) in a H2/CO2-sparged biomethanation process using methanogenic cultures at an operational pH of 6.9. These results were also substantiated by microbial physiology studies which indicated that several species of methanogens such as Methanosarcina thermophila, Methanobacterium formicicum, Methanosarcina barkeri, and Methanosarcina mazei thrive in the pH range of 6.0–7.0.52,57,58

The chosen substrates also contributed toward the enhancement of CH4 concentration, as highlighted in Section 3.1. OFSMW consists of various organic materials that are utilized by microorganisms during methanogenesis, as earlier reported by Han and Shin.59 This substrate consisted primarily of

![Figure 2. Methane concentration during ex situ thermophilic biogas upgrading experiments of CM100OF0 to CM50OF50.](https://dx.doi.org/10.1021/acsomega.0c01725)

![Figure 3. Methane concentration in nonsparged biomethanation control experiments CM100OF0-C to CM50OF50-C.](https://dx.doi.org/10.1021/acsomega.0c01725)
Alongside CH4 during methanogenesis. Its accumulation during the declining phase. Microorganisms that compete with CH4 producers thrive during this stage and also possible during the declining phase. Microorganisms that participate during the later stages (days 12−20) of the biogas upgrading experiments (CM100OF0 to CM50OF50). A similar trend in CH4 concentrations for all the experiments (CM100OF0 to CM50OF50). This is attributed to the depletion of nutrients as opposed to in continuous systems where a steady-state is established.9 A transition in microbial communities is established.9 A transition in microbial communities is observed to that of the four stages of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis, and methanogenesis).64 The CH4 concentration (Figure 2) was significantly lower in the later stages (days 12−20) of the biogas upgrading processes (CM100OF0 to CM50OF50). A similar trend in the biogas flow rates (Figure 4) was observed to that of the CH4 concentrations for all the experiments (CM100OF0 to CM50OF50). This is attributed to the depletion of nutrients— as this phenomenon is often observed in batch fermentation systems—as opposed to in continuous systems where a steady-state is established.9 A transition in microbial communities is also possible during the declining phase. Microorganisms that compete with CH4 producers thrive during this stage and utilize the CH4 as their energy source.15 Likewise, the CH4 content was also low in the later stages of the nonsparged control experiments (Figure 3). This can also be attributed to the rapid accumulation of VFAs, which disrupt the pH of the fermentation medium (further discussed in Section 3.3).

It was observed that CO2 was partially converted during biogas upgrading experiments, as shown by the low consumption rate of 59−298 NmL/L d (Table 2). This low conversion may be because CO2 is endogenously produced alongside CH4 during methanogenesis. Its accumulation resulted in additional CO2 content within the biogas, as opposed to the accumulation of H2, which is used as a precursor during the syntrophic interaction between certain bacteria and methanogenic archaea, leading to CH4 formation.15,59 Despite the incomplete conversion of CO2, the quality of the biogas in CM100OF0 to CM50OF50 fulfills the objective of achieving a high-purity CH4 (>90 vol % CH4) that could potentially be injected into natural gas pipelines with minimal purification.

The production of CH4 was also monitored in the nonsparged control experiments (CM100OF0-C to CM50OF50-C) (Figure 3). A maximum CH4 concentration of up to 78.45 vol % was achieved in CM80OF20-C when CM was codigested with OFSMW at a ratio of 80:20 wt %. This increase in the CH4 composition is probably due to the availability of the essential nutrients which were supplied in the correct proportions.46 These results are comparable with the results of other studies reported in the literature. Nielfa et al.55 obtained a high CH4 yield of 221 mL CH4/g VS and a CH4 fraction of >60 vol % in the codigestion process of CM with OFSMW at a ratio of 80:20 wt %. Khayum and co-workers46 reported a high CH4 concentration of 71 vol % in an anaerobic digestion process where CM was codigested with spent tea waste at a ratio of 70:30 wt %. These results also suggest that there were sufficient microorganisms at a ratio of 80:20 wt %, which improved CH4 production. Moreover, the abundance of CH4-producing organisms might have promoted the interspecies electron transfer during the biodegradation of OFSMW.66

Several reactor designs have been evaluated, as it has been shown that the biocatalytic conversion of H2 and CO2 into CH4 is highly dependent on reactor type. For example, Kougias et al.9 conducted ex situ biogas upgrading experiments in three different reactor systems and observed that a high CH4 concentration of up to 98 vol % is achievable in a bubble column reactor. Burkhardt et al.67 reported an optimum CH4 concentration of 98 vol % in a trickle bed reactor that was immobilized with hydrogenotrophic cultures. Other reactor types such as continuously stirred tank reactors, fixed bed reactors, and upflow reactors have been successfully used in the biocatalytic conversion of H2 and CO2 into CH4.66,68 Moreover, operational parameters, such as gas recirculation,69 the gas injection device design,70 and stirring intensity71

### Table 2. Performance Summary of the Biogas Upgrading Experiments

| feedstock ratio (CM/OFSMW)  | anaerobic digestion process |
|-----------------------------|-----------------------------|
|                             | day of maximum CH4 content in biogas | biogas content (vol %) | H2 | CO2 | CH4 | H2 feed rate (NmL/L/d) | CO2 feed rate (NmL/L/d) | total H2 consumed of feed (NmL/L/d) | total CO2 consumed of feed (NmL/L/d) | CH4 production rate (NmL/L/d) | C/N ratio | final pH |
|-----------------------------|-----------------------------|
| CM100OF0                    | 9                           | 0.0 ± 0.0                  | 0.5 ± 5.3                  | 0.3 ± 1.2                  | 6.0 ± 2.7                  | 4.0 ± 0.1                  | 1920                         | 1892 ± 167                     | 1903 ± 58                  | 1903 ± 58              | 1920       | 1920     |
| CM80OF20                    | 7                           | 3.0 ± 4.9                  | 7.5 ± 5.3                  | 3.7 ± 1.6                  | 0.0 ± 2.3                  | 4.0 ± 0.1                  | 480                          | 480                          | 480                          | 480                          | 480         | 480      |
| CM70OF30                    | 12                          | 97.0 ± 4.9                 | 92.0 ± 0.0                 | 96.0 ± 2.8                 | 94.0 ± 4.9                 | 92.0 ± 0.0                 | 1920                         | 1920                         | 1920                         | 1920                         | 1920       | 1920     |
| CM60OF40                    | 6                           | 298 ± 0.0                  | 59 ± 298                   | 266 ± 59                   | 480 ± 100                  | 282 ± 4                   | 298                          | 59 ± 298                     | 266 ± 59                   | 480 ± 100                  | 282 ± 4    | 282 ± 4 |
| CM50OF50                    | 8                           | 5886 ± 1387                | 5165 ± 1387                | 5548 ± 700                 | 4656 ± 575                 | 4557 ± 27                  | 5886                         | 5165 ± 1387                  | 5548 ± 700                 | 4656 ± 575                  | 4557 ± 27  | 4557 ± 27 |

*Note: Table 2 provides a summary of the biogas upgrading experiments with different feedstock ratios (CM/OFSMW). The table includes the day of maximum CH4 content in biogas, biogas content (vol %), and other relevant parameters such as H2, CO2, CH4 feed rates, total H2 and CO2 consumed of feed, CH4 production rate, C/N ratio, and final pH. The results are presented for different scenarios (CM100OF0 to CM50OF50). The experimental error is based on a standard deviation calculated from the duplicate digestion process for each scenario. A transition in microbial communities is also possible during the declining phase. Microorganisms that compete with CH4 producers thrive during this stage and utilize the CH4 as their energy source.15 Likewise, the CH4 content was also low in the later stages of the nonsparged control experiments (Figure 3). This can also be attributed to the rapid accumulation of VFAs, which disrupt the pH of the fermentation medium (further discussed in Section 3.3).

**Figure 4.** Biogas flow rate during ex situ thermophilic biogas upgrading experiments of CM100OF0 to CM50OF50.
strongly influence the performance of the biogas upgrading process.

3.3. pH Profile during Biogas Upgrading Experiments. The pH plays a crucial role during biomethanation processes because it influences the diversity of microbial communities, biodegradability of feedstocks, and the accumulation of metabolites. In our biogas upgrading experiments (CM100OF0 to CM50OF50), the final pH varied from 5.62 to 6.58 (Figure 5). A similar observation has been reported by Omar et al., with an optimum pH range of 6.0−7.0. The near-neutral pH range in the biogas upgrading processes is caused by the enhanced buffering capacity and the microbial consumption of H₂ and CO₂. With the addition of ex situ CO₂, the pH is mediated by the equilibrium formation of carbonic acid and its dissolution toward bicarbonate ions (eq 2). The equilibrium dissolution led to a concomitant stabilization in the fermentation pH. In contrast, there was a steady decrease in the fermentation pH of the nonsparged control experiments (CM100OF0-C to CM50OF50-C) over the 20 day fermentation period (Figure 6). The final pH in these bioprocesses varied from 4.1 to 4.53. This low pH range was caused by the rapid accumulation of VFAs and alcohols. The systems depended entirely on endogenous CO₂ formation, which limited the buffering capacity (bicarbonate content). VFAs (and protons) accumulated, causing a systematic drop in pH. Besides, the drop in pH also causes a shift in metabolic pathways because of the production of CH₄-scavenging organisms.

$$\text{H}_2\text{O} + \text{CO}_2 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \quad (2)$$

3.4. VFA Production during Biogas Upgrading Experiments. During the biomethanation process, metabolites such as VFAs are produced alongside CH₄ because of the syntrophic interactions amongst different microorganisms, such as acidogens, acetogens, and methanogens. The composition of VFAs is highly dependent on the type of substrate and inoculum used during the biomethanation process. In this study, acetic acid was the main VFA compound in all of the biogas upgrading reactors (Figure 7), followed by other acids such as propionic acid, valeric acid, and isovaleric acid, present in small quantities. The concentration of these VFAs (particularly acetic acid at 2665−1409 mg/L) was high during the lag phase (days 5−10), as a result of the acidogenesis and acetogenesis anaerobic pathways. However, their concentrations were significantly reduced (<300 mg/L) in the later stages of fermentation (days 15−20) because of their utilization in CH₄ production. A similar trend in VFA production was observed by Guneratnam and co-workers in an ex situ thermophilic biogas upgrading process. They also reported acetic acid to be the main VFA during the course of the biomethanation process.

The presence of VFAs during biogas upgrading may also be attributed to the formation of non-CH₄-forming reactions, such as the homoacetogenic pathways, in which the gaseous substrates (H₂ and CO₂) are metabolized to form acetic acid instead of CH₄. Nevertheless, it has been reported that the methanogenic species can use these VFAs during biogas upgrading when the concentration of H₂ is low within the reactor, as demonstrated in this study.

3.5. C/N Ratio during Biogas Upgrading Experiments. The carbon-to-nitrogen (C/N) ratio is another crucial parameter that is used to investigate the nutrient balance in organic substrates. For enhanced biogas production, the C/N ratio should be in the range of 20−30. Hence, the nutritional impact of CM and OFSMW during biogas upgrading experiments was examined by calculating the C/N ratios. A favorable C/N ratio of 20.01−26.33 was observed at the end of the ex situ thermophilic biogas upgrading experiments of CM100OF0 to CM50OF50 (Table 2). The C/N ratio attained herein is attributed to the synergistic effects of CM and OFSMW because of the carbon and nitrogen nutrients that were supplied in appropriate proportions. This in turn contributed to the enhanced activity of the CH₄ producers. A similar C/N ratio (20−25) has been reported by Pavi et al. in an anaerobic codigestion process of OFSMW and fruit and vegetable waste (FWV). Furthermore, they recorded

**Figure 5.** Variation in pH during biogas upgrading processes of CM100OF0 to CM50OF50.

**Figure 6.** Variation in pH during nonsparged biomethanation processes of CM100OF0-C to CM50OF50-C.

**Figure 7.** Production of VFAs during ex situ biogas upgrading experiments AD1 to AD5 (CM100OF0 to CM50OF50, respectively).
increases in the CH₄ yield of 141 and 43.8%, compared with the monodigestion processes of OFSMW and FVW, respectively. These results underscore the importance of utilizing nutritionally rich substrates in biogas upgrading approaches; these biowastes are highly beneficial to the growth and activity of CH₄-producing organisms.⁴⁶,⁴⁷

3.6. Morphological Characteristics of Methane Producers. The morphology of the prevalent CH₄-producing microorganisms during biogas upgrading experiments (CM100OF0 to CM50OF50) was examined during peak CH₄ production, using a light microscope (Figure 8). The broths consisted mainly of thin rod-shaped cells, which could indicate the presence of methanogenic species within the reactors. However, it is highly possible that other non-CH₄-forming species such as *Clostridium* were present during the biogas upgrading processes due to the fact that these hydrolytic organisms are highly active during anaerobic digestion and they are also rod-shaped.⁷⁹−⁸¹ Moreover, they contribute to the formation of the key intermediates such as VFAs and H₂, which are later consumed by the methanogenic species.⁸² These results are in agreement with literature, as some members of hydrogenotrophic and acetotrophic methanogens have previously been shown to have rod-shaped morphologies.⁸³⁻⁸⁴ However, in another study, the presence of cocci-shaped cells during the biomethanation process has been reported.⁸²

These conflicting reports clearly demonstrate the diversity in microbial communities during the biogas upgrading process. Consequently, research studies are now shifting from the use of conventional methods to advanced microbial characterization techniques in efforts to acquire deeper insights into the microbial population dynamics during biogas enrichment. It should be noted that the morphology presented here is not a full representation of the microbial population structure in the biogas upgrading processes. Future work will therefore focus on high-throughput screening techniques to acquire further knowledge on the microbial species prevalent during the biogas upgrading process.

3.7. Comparison of the CH₄ Concentration Here with That Reported in the Literature. Interestingly, the CH₄ concentration achieved in this work was 1.1 and 1.4 times higher than the CH₄ concentration that was achieved in biogas upgrading studies of Seifert et al.⁵ and Luo et al.,⁷ respectively (Table 3). It should be noted that these studies were carried out under similar operational parameters: thermophilic conditions (55−65 °C) and neutral pH range (6.85−7.2). The results obtained in this work highlight the effectiveness of codigesting CM with OFSMW during thermophilic ex situ biogas upgrading processes, as mentioned earlier. Nonetheless, research is still ongoing to determine the effects of other important parameters (i.e., reactor design, substrate type, and inoculum sources) on the performance of the biogas upgrading processes. Although the obtained CH₄ concentration (>90 vol %) during the day of maximum production fulfills the requirements for injection into natural gas pipelines, a comprehensive techno-economic assessment is also essential to determine the economic feasibility of large-scale implementation.

![Figure 8. Morphology of CH₄-producing microorganisms during biogas upgrading processes of CM100OF0 to CM50OF50. The rod-shaped cells are indicated with red arrows.](image-url)

| reactor type | inoculum | substrate | gas feed | temp (°C) | pH | working volume (L) | CH₄ content (vol %) | references |
|--------------|----------|-----------|----------|-----------|----|--------------------|-------------------|------------|
| CSTR         | pure culture | growth medium | ex situ | 65 | 6.85 | 10 | 85 | Seifert et al.⁵ |
| CSTR         | manure   | cattle manure | in situ | 55 | 7.2 | 3.5 | 65 | Luo et al.⁷ |
| CSTR         | anaerobic digestate | straw | in situ | 38 | 8.43 | 2 | 76.8−100 | Agneessens et al.¹⁵ |
| trickle bed | mixed cultures | trace elements | ex situ | 37 | 7.4−7.7 | 5.78 | 96 | Rachbauer et al.¹⁶ |
| trickle bed | mixed cultures | trace elements | ex situ | 37 | 7.2−7.4 | 88 | 98 | Burkhardt et al.¹⁷ |
| CSTR         | anaerobic sludge | trace elements | ex situ | 35 | 5.5 | 100 | 92 | Kim et al.¹⁸ |
| batch        | anaerobic sludge | food waste | in situ | 55 | 8.6 | 0.55 | 77.5−98.1 | Linville et al.¹⁹ |
| batch        | anaerobic sludge | corn stover | in situ | 55 | 7.5−9.0 | 0.6 | 90 | Shen et al.²⁰ |
| batch        | mixed cultures | CM/OFMSW | ex situ | 55 | 7.6 | 3 | 92−97 | this work |

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4. CONCLUSIONS AND RECOMMENDATIONS

This study highlights an alternative pathway toward the efficient and economic bioconversion of renewable H₂ and waste CO₂ into a clean and energetic fuel (high-purity biomethane), by exploiting highly accessible agro-municipal waste residues such as CM and OFSMW. The exogenous addition of H₂ and CO₂ in the biomethanation experiments (CM100OF0 to CM50OF50) led to significant increases in CH₄ production, compared with the control experiments (CM100OF0-C to CM50OF50-C). CH₄ concentrations of 92–97 vol % and biogas production rates of 4954–6605 Nm³/L d were obtained during maximum production (days 6–12). The combination of CM and OFSMW supported a nutritional balance for hydrogenotrophic methanogens to metabolize the ex situ feed of H₂ and CO₂, while the reduction of VFAs during the course of the experiments (days 10–20) indicated the presence of acetotrophic methanogens to support CH₄ formation. Furthermore, the ex situ addition of H₂ and CO₂ stabilized the pH in a near-neutral range (5.62–5.65) at the conclusion of the experiments. Of particular interest was that satisfactory biogas upgrading, even at high codigestion ratios of OFSMW (CM70OF30 to CM50OF50), leads to believe that this conversion pathway is a promising technology to convert substantial amounts of municipal organic waste.

Renewable energy-derived biomethane is therefore an important process that supports carbon recycling, which will facilitate an increased number of renewables in—and the sustainability of—associated energy systems. Despite these promising results, biogas upgrading technology is still in its infancy.

Subsequent work to be explored includes:

- Characterization of the methanogenic archaeal species that actively participate during the ex situ thermophilic biogas upgrading process, via microbial profiling methods.
- Evaluation of a continuous biogas upgrading process with extended fermentation times beyond 100 days. In addition, undertaking advanced CFD modeling of different reactor configurations to assess parameters such as stirring speed and mixing intensity, gas/liquid interactions during the sparging process, and the effects of recirculative flow—all of which could improve fermenter efficiency.
- A techno-economic assessment to determine the CAPEX and OPEX of CH₄ production in biogas upgrading processes. Typically, continuous flow anaerobic digesters are preferred industrially, which could be used to believe that pilot-scale digesters also based on continuous systems can provide essential information on the economic feasibility of the biogas upgrading process.

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Notes

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