

Article

Effect of N₂ on Biological Methanation in a Continuous Stirred-Tank Reactor with *Methanothermobacter marburgensis*

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Received: 20 May 2019; Accepted: 28 June 2019 ; Published: 2 July 2019



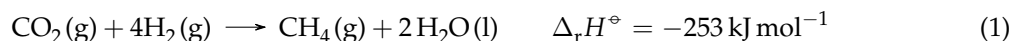
Abstract: In this contribution, the effect of the presence of a presumed inert gas like N₂ in the feed gas on the biological methanation of hydrogen and carbon dioxide with *Methanothermobacter marburgensis* was investigated. N₂ can be found as a component besides CO₂ in possible feed gases like mine gas, weak gas, or steel mill gas. To determine whether there is an effect on the biological methanation of CO₂ and H₂ from renewable sources or not, the process was investigated using feed gases containing CO₂, H₂, and N₂ in different ratios, depending on the CO₂ content. A possible effect can be a lowered conversion rate of CO₂ and H₂ to CH₄. Feed gases containing up to 47% N₂ were investigated. The conversion of hydrogen and carbon dioxide was possible with a conversion rate of up to 91% but was limited by the amount of H₂ when feeding a stoichiometric ratio of 4:1 and not by adding N₂ to the feed gas.

Keywords: biological methanation; CSTR; *Methanothermobacter marburgensis*; methane; carbon dioxide; dinitrogen; hydrogen; power-to-gas

1. Introduction

Today's demand for energy all over the world makes it necessary to reduce the use of fossil energy sources to a minimum. Therefore, renewable sources must be used to develop a sustainable energy management that does not end in overexploitation of natural resources and global warming. But the volatility of renewable energies is still a big problem when switching from conventional power plants to photovoltaics and wind power plants without having a system for distributing this energy to those places where it is needed. Since the Kyoto Protocol was adopted in 1997, which extends the United Nations Framework Convention on Climate Change (UNFCCC) from 1992, politics has officially cared about the emission of carbon dioxide (CO₂). To reduce the emissions of CO₂, it is necessary to recycle emissions that are not avoidable. This approach of recycling also increases the overall efficiency of a process where CO₂ is an unused product and reduces its carbon footprint. Biological methanation is a technology that combines these aspects and converts hydrogen from electrolysis (power-to-gas), operating with power from renewable energies and carbon dioxide, possibly from biogenic sources, and methanogenic microorganisms. It is a contribution to the solution of storing power that is not needed at the time of its generation, the smoothing of current peaks in the power grid, and reducing CO₂ emissions. Due to the lack of an adequate hydrogen (H₂) infrastructure in many countries like Germany, a further step of converting the hydrogen to methane is undertaken. Methanogenic archaea use substrates like acetate, methanol, carbon monoxide, or carbon dioxide as sources for carbon

assimilation [1]. In the case of carbon dioxide, some of those methanogenic organisms use hydrogen to convert adenosine diphosphate (ADP) to adenosine triphosphate (ATP) and reduce carbon dioxide to methane (CH₄) [2]. Those organisms are part of microbial consortia in, e.g., biogas plants, where they convert intermediates to methane in a last step—methanogenesis. *Methanothermobacter marburgensis* is such a hydrogenotrophic organism and was first isolated from an anaerobic sewage digester [3]. Equation (1) shows the chemical reaction of methanation.



Since the price of CO₂ emission certificates (European Union Allowance *EUA*) is increasing (see Figure 1), there will be a competition for purchasing, using, and recycling CO₂. This will lead to an increasing price for CO₂. Therefore, other sources of carbon-dioxide-containing gases have to be determined. These gases are of interest for biological methanation. While biogases consist mostly of methane and carbon dioxide (fractions $\geq 1\%$ vol.) [4,5], there are other gases, like weak gas from pressure swing adsorption (PSA) or water washing (WW), or mine gases with a high N₂ content, that are also possible carbon dioxide sources for the process of methanation (see Table 1) [6–9]. Steel mill gas, for example, consists of around 44% N₂, 23% CO, 21% CO₂, 10% H₂, and 2% CH₄ [10]. To use this gas as a source for methanation would mean to access a large potential, and the steel industry has a major interest in improving efficiency. Only for Germany, it could mean a potential of 20×10^6 t of carbon dioxide emissions [11]. This is about 10% of the CO₂ emissions of German industrial processes and the manufacturing industry.

Table 1. Composition of possible feed gases for methanation [5,9,12].

Source	CO ₂	CH ₄	N ₂	Other
weak gas (PSA)	91% to 99%	1% to 8%	0% to 3%	0% to 1%
weak gas (WW)	11% to 22%	0.1% to 0.3%	58% to 63%	17% to 20%
coal seam	24%	90% to 95%	1% to 8%	0%
inactive coalmine	8% to 15%	60% to 80%	5% to 32%	0%
blast furnace	23%	0%	49%	28%

This work investigated the effects of a N₂ content greater than 10% on the conversion rate and other process parameters. Therefore, N₂/CO₂ mixtures in different proportions were used as feed gases instead of pure carbon dioxide. For comparison, the experiments were also performed with pure CO₂. The following two observations might be possible. While in catalytic methanation NH₃ is formed from N₂ up to 600 ppm due to the process conditions [13], in biological methanation N₂ can be used enzymatically for nitrogen fixation (diazotrophy) by some archaea [14,15]. It was reported that *M. marburgensis* can grow diazotrophic [16]. This may cause a difference in the formation of methane due to a change in metabolism because the reactions for reducing N₂ to NH₃ are anabolic [14]. According to Equation (1), there is no reaction of N₂ in the catabolic methanogenesis of CO₂ [17]. Furthermore, when using a biological multiphase system, methane formation is limited by the molecular transport of CO₂ and H₂ from the gas phase into the liquid phase. Adding N₂ may result in a lower conversion rate.

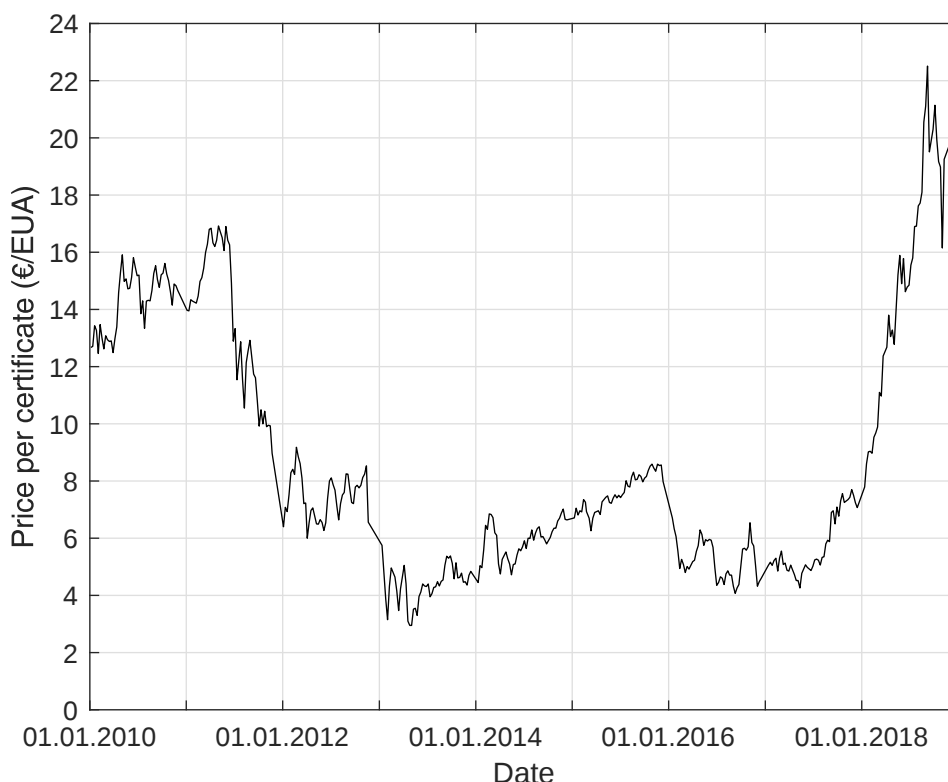


Figure 1. Price per certificate (European Union Allowance) for the allowance to emit the greenhouse gas equivalent of 1 t CO₂ from 1 January 2010 to 31 March 2019 [18].

2. Materials and Methods

2.1. Instrumentation

For methanation, a fermenter with a total volume of $V_R = 42$ L was used. A stirrer with three Rushton turbines ($d = 105$ mm) from Präzisions-Rührer Gesellschaft mbH (Warburg, Germany) was able to perform an agitation at $n = 1000$ min⁻¹. Gases were fed with thermal mass flow controllers EL-FLOW select (Bronkhorst Deutschland Nord GmbH, Kamen, Germany) from compressed gas bottles into the fermenter via two ring gas spargers on the bottom. Temperature and pressure were both set constant at $\vartheta = 65$ °C and $p = 1$ bar(g) with a pressure control valve EL-PRESS (Bronkhorst Deutschland Nord GmbH, Kamen, Germany). CO₂, H₂, and CH₄ were measured in the off-gas with BCP gas sensors via infrared (IR) (carbon dioxide and methane) and thermal conductivity (hydrogen) (BlueSens gas sensor GmbH, Herten, Germany). The pH value was measured with a CPS11D (Endress+Hauser Messtechnik GmbH & Co. KG, Weil am Rhein, Germany) and regulated with H₂SO₄ ($c_{\text{H}_2\text{SO}_4} = 1$ mol L⁻¹) and (NH₄)₂CO₃ ($c_{(\text{NH}_4)_2\text{CO}_3} = 1$ mol L⁻¹). Redox potential (ORP) was measured with an EasyFerm Plus ORP Arc 120 (Hamilton Bonaduz AG, Bonaduz, Switzerland).

2.2. Nutrition Media, Microorganism, and Gases

At first, the reactor was filled with 25 L nutrition solution consisting of 0.45 g L⁻¹ NaCl, 6.00 g L⁻¹ NaHCO₃, 0.10 g L⁻¹ MgSO₄, 0.225 g L⁻¹ KH₂PO₄, 0.225 g L⁻¹ K₂HPO₄, 0.225 g L⁻¹ (NH₄)₂SO₄, 0.06 g L⁻¹ CaCl₂, 0.002 g L⁻¹ (NH₄)₂FeSO₄·6 H₂O, 0.002 g L⁻¹ (NH₄)₂Ni(SO₄)₂·6 H₂O, 10 mL L⁻¹ mineral solution [1.5 g L⁻¹ nitrilotriacetic acid, 3 g L⁻¹ MgSO₄·7 H₂O, 0.5 g L⁻¹ MnSO₄·H₂O, 1 g L⁻¹ NaCl, 0.1 g L⁻¹ FeSO₄·7 H₂O, 0.18 g L⁻¹ CoSO₄·7 H₂O, 0.1 g L⁻¹ CaCl₂·2 H₂O, 0.18 g L⁻¹ ZnSO₄·7 H₂O, 0.01 g L⁻¹ CuSO₄·5 H₂O, 0.02 g L⁻¹ KAl(SO₄)₂·12 H₂O, 0.01 g L⁻¹ H₃BO₃, 0.01 g L⁻¹ Na₂MoO₄·2 H₂O, 0.03 g L⁻¹ NiCl₂·6 H₂O, 0.3 mg L⁻¹ Na₂SeO₃·5 H₂O, 0.4 mg L⁻¹ Na₂WO₄·

2 H₂O, ad. 1000 mL distilled water], 10 mL L^{−1} vitamin solution [2 mg L^{−1} biotin, 2 mg L^{−1} folic acid, 10 mg L^{−1} pyridoxine-HCl, 5 mg L^{−1} thiamine-HCl, 5 mg L^{−1} riboflavin, 5 mg L^{−1} nicotinic acid, 5 mg L^{−1} D-Ca-pantothenate, 0.1 mg L^{−1} vitamin B₁₂, 5 mg L^{−1} aminobenzoic acid, 5 mg L^{−1} α-lipoic acid, ad. 1000 mL distilled water] and filled up with distilled water. The reactor was flushed with CO₂ for 180 min to obtain an anaerobic atmosphere. A total of 20 mL of Na₂S · 9 H₂O (*c* = 1 mol L^{−1}) was added to reduce the remaining O₂, and 20 mL cell suspension of *Methanothermobacter marburgensis* (DSM-2133, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) were added to the reactor. Then H₂ was added until a ratio of H₂:CO₂ 4:1 was reached. H₂ had a nominal purity of ≥ 99.9% (Linde AG, München, Germany), and CO₂ (later referred to as C100) had a nominal purity of 99.8% (Westfalen AG, Münster, Germany). To get a sufficient cell concentration of *M. marburgensis*, the organisms were cultured for *t* = 5 d. The doubling time of *M. marburgensis*, according to the literature, is *t_d* = 2 h [19,20]. In this work, a doubling time of about *t_d* = 2.4 h was observed. Experiments were performed with different CO₂-containing premixed feed gases. CO₂/N₂ mix gases BIOGON® C20 (CO₂ (20 ± 2)%, rest N₂), C30 (CO₂ (30 ± 3)%, rest N₂), and C40 (CO₂ (40 ± 4)%, rest N₂) were obtained in pressure gas bottles from Linde AG. The resulting composition of the feed gases containing the CO₂/N₂ mix and H₂ is shown in Table 2. In addition, an anaerobe solution of Na₂S · 9 H₂O (*c* = 0.5 mol L^{−1}) was added periodically to the reactor to maintain the supply of sulfur for the growth of *M. marburgensis* [21].

Table 2. Composition of mixed gases and volumetric amounts of H₂, CO₂, and N₂ in the feed gas.

CO ₂ -Containing Feed Gas	Measured Mole Fraction		Resulting Composition of the Whole Feed Gas		
	<i>x</i> _{N₂}	<i>x</i> _{CO₂}	H ₂	CO ₂	N ₂
C20	0.816	0.184	42.4%	10.6%	47.0%
C30	0.738	0.262	51.2%	12.8%	36.0%
C40	0.640	0.360	59.0%	14.8%	26.2%
C100	0	1	80.0%	20.0%	0%

2.3. Calculations

For the following calculations, it was assumed that $\dot{V}_{N_2,feed} = \dot{V}_{N_2,off}$. For the calculation of the feed gas compositions shown in Table 2, Equations (2)–(4) were used.

$$c_{H_2,feed} = \frac{100 \cdot 4}{4 + 1 + \frac{x_{N_2}}{x_{CO_2}}} \quad (2)$$

The carbon dioxide/hydrogen ratio was chosen to be 1 to 4 according to Equation (1), resulting in Equation (3).

$$c_{CO_2,feed} = \frac{1}{4} \cdot c_{H_2,feed} \quad (3)$$

The dinitrogen content depended on the composition of the mixed gas. Thus, it follows Equation (4).

$$c_{N_2,feed} = \frac{x_{N_2}}{x_{CO_2}} \cdot c_{CO_2,feed} \quad (4)$$

To compare the experiments, it is possible to consider the ratio of outgoing methane flow and carbon dioxide feed flow. This relationship is called the conversion rate *r_c* in the following descriptions. The conversion rate *r_c* of CO₂ to CH₄ was calculated via Equation (5) and can also be applied to the conversion rate in a H₂/CO₂/CH₄ gas system without N₂. It describes the degree of conversion from CO₂ to CH₄.

$$r_c = \frac{\dot{V}_{CH_4,off}}{\dot{V}_{CO_2,feed}} \quad (5)$$

Therefore, the gas flows of CO₂ and CH₄ had to be determined. $\dot{V}_{CO_2,feed}$ can be calculated from the CO₂ content (x_{CO_2}) and the volume flow of the CO₂/N₂ mix ($\dot{V}_{mix,feed}$), as presented in Equation (6). $\dot{V}_{CH_4,off}$ is defined in Equation (7).

$$\dot{V}_{CO_2,feed} = \dot{V}_{mix,feed} \cdot x_{CO_2} \quad (6)$$

$$\dot{V}_{CH_4,off} = c_{CH_4,off} \cdot \frac{\dot{V}_{CO_2,feed} + \dot{V}_{H_2,feed}}{1 + \frac{\dot{V}_{H_2}}{\dot{V}_{CO_2,feed}} \cdot c_{CH_4,feed}} \quad (7)$$

3. Results

The obtained parameters, pH, oxidation reduction potential (ORP), temperature, and pressure, which were observed and are not presented in this section, did not differ from a normal methanation process using just carbon dioxide and hydrogen as feed gases. All experiments were performed 4 to 6 times with the same results. For each experiment, a typical result was considered. Figure 2 shows the off-gas concentrations of CO₂, H₂, CH₄, and N₂ in such a typical experiment for each gas mixture. The axes are scaled identically in all subfigures. There was no change of values after $t = 8$ h in any of them, so here just a duration of 8 h is presented. Sensor data for each parameter were recorded every 10 s. The mean value of each parameter was calculated. These mean values are presented in Tables 3–6.

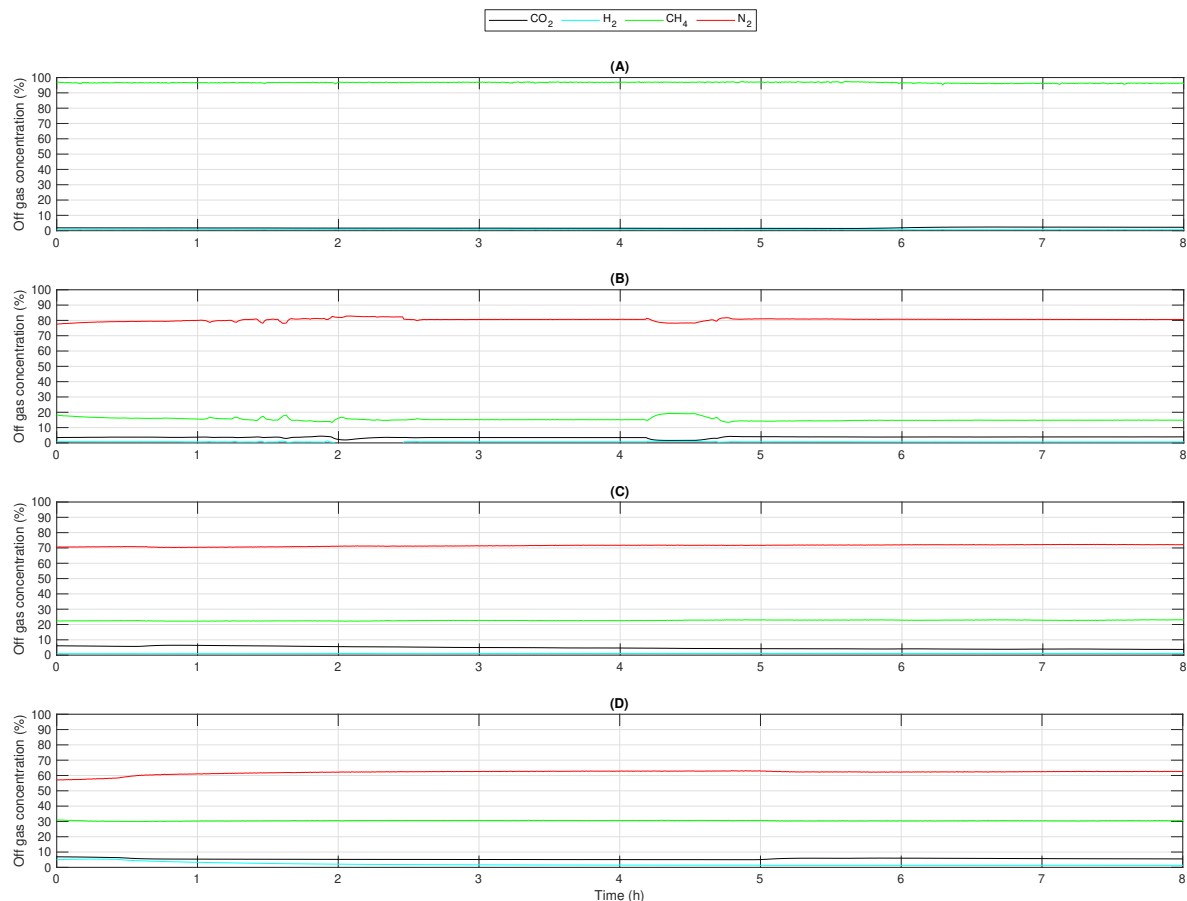


Figure 2. Concentrations of H₂, CO₂, CH₄ (measured), and N₂ (calculated) in the off-gas when using feed gases with different CO₂ mole fractions: (A) C100 ($x_{CO_2} = 1$), (B) C20 ($x_{CO_2} = 0.186$), (C) C30 ($x_{CO_2} = 0.262$), (D) C40 ($x_{CO_2} = 0.360$).

Using pure CO₂ resulted in $r_c = 99.3\%$ and an off-gas concentration of methane $c_{CH_4,off} = 96.6\%$, which is shown in Table 3. The off-gas composition is presented in Figure 2A. While the experiment was performed, c_{CH_4} was greater than 95%. H₂ varied from 0.6% to 0.8% and CO₂ from 1.4% to 2.4%. According to [22], this composition is sufficient for an injection into the natural gas grid. These values fit other observations [23,24] and support the results made for this contribution. It is a typical off-gas composition for the biological methanation performed with CO₂ and H₂, even though the flow rates were very low.

Feeding C20 resulted in a N₂ off-gas concentration of 80% and a CH₄ concentration of 15% (see Figure 2B). Moreover, about 4% CO₂ was left in the off-gas. While using C30 as a feed gas (Figure 2C), the off-gas consisted of 72% N₂, 23% CH₄, and 5% CO₂. Experiments with C40 led to an off-gas composition of about 63% N₂, 30% CH₄, and 5% CO₂, which is presented in Figure 2D. Hydrogen concentrations in all experiments were at about 1%. The resulting conversion rates were 85% to 91%. All results are also shown in Tables 4–6, Figure 3.

Table 3. Overview of the mean feed and off-gas concentrations and flows using pure CO₂ (C100). Concentration of CO₂, H₂, and CH₄ were measured values, N₂ was calculated.

	H ₂	CO ₂	N ₂	CH ₄	r_c
c_{feed}	80%	20%	0%	0%	0
$\dot{V}_{feed}$	300 mL min ^{−1}	75 mL min ^{−1}	0 mL min ^{−1}	0 mL min ^{−1}	
c_{off}	0.7%	2.7%	0%	96.6%	99.3%
$\dot{V}_{off}$	0.5 mL min ^{−1}	2.1 mL min ^{−1}	0 mL min ^{−1}	74.5 mL min ^{−1}	

Table 4. Overview of the mean feed and off-gas concentrations and flows using C20. Concentration of CO₂, H₂, and CH₄ were measured values, N₂ was calculated.

	H ₂	CO ₂	N ₂	CH ₄	r_c
c_{feed}	42.4%	10.6%	47.0%	0%	0
$\dot{V}_{feed}$	220 mL min ^{−1}	55 mL min ^{−1}	243.9 mL min ^{−1}	0 mL min ^{−1}	
c_{off}	0.6%	3.9%	80.5%	15.0%	84.9%
$\dot{V}_{off}$	2.0 mL min ^{−1}	12.1 mL min ^{−1}	243.9 mL min ^{−1}	46.7 mL min ^{−1}	

Table 5. Overview of the mean feed and off-gas concentrations and flows using C30. Concentration of CO₂, H₂, and CH₄ were measured values, N₂ was calculated.

	H ₂	CO ₂	N ₂	CH ₄	r_c
c_{feed}	51.2%	12.8%	36.0%	0%	0
$\dot{V}_{feed}$	300 mL min ^{−1}	75 mL min ^{−1}	211.3 mL min ^{−1}	0 mL min ^{−1}	
c_{off}	1.0%	4.7%	71.6%	22.7%	90.9%
$\dot{V}_{off}$	3.1 mL min ^{−1}	14.3 mL min ^{−1}	211.3 mL min ^{−1}	68.2 mL min ^{−1}	

Table 6. Overview of the mean feed and off-gas concentrations and flows using C40. Concentration of CO₂, H₂, and CH₄ were measured values, N₂ was calculated.

	H ₂	CO ₂	N ₂	CH ₄	r_c
c_{feed}	59.0%	14.8%	26.2%	0%	0
$\dot{V}_{feed}$	300 mL min ^{−1}	75 mL min ^{−1}	133.3 mL min ^{−1}	0 mL min ^{−1}	
c_{off}	1.5%	5.4%	62.7%	30.5%	89.9%
$\dot{V}_{off}$	3.2 mL min ^{−1}	12.0 mL min ^{−1}	133.3 mL min ^{−1}	67.4 mL min ^{−1}	

When reaching a full conversion, which means $r_c = 1$ and no H_2 and CO_2 left, the off-gas only consisted of N_2 for C20 and C30, CH_4 for C40, and just CH_4 when using C100. This means that the off-gas must show the same composition as the carbon-dioxide-containing feed gas, but CO_2 is fully replaced by CH_4 . In every experiment, N_2 was close to its mole fraction in the mixed gas (without H_2). It can also be seen that the concentration of H_2 in the off-gas was always close to 1% and therefore, was almost fully converted to methane. The carbon dioxide concentration was 3.9% to 5.4% when feeding CO_2/N_2 , so there was carbon dioxide left in the off-gas that could be converted. There was also a difference between $\dot{V}_{CO_2,feed}$ and $\dot{V}_{CO_2,off} + \dot{V}_{CH_4,off}$. Volumetric flows were calculated and not measured.

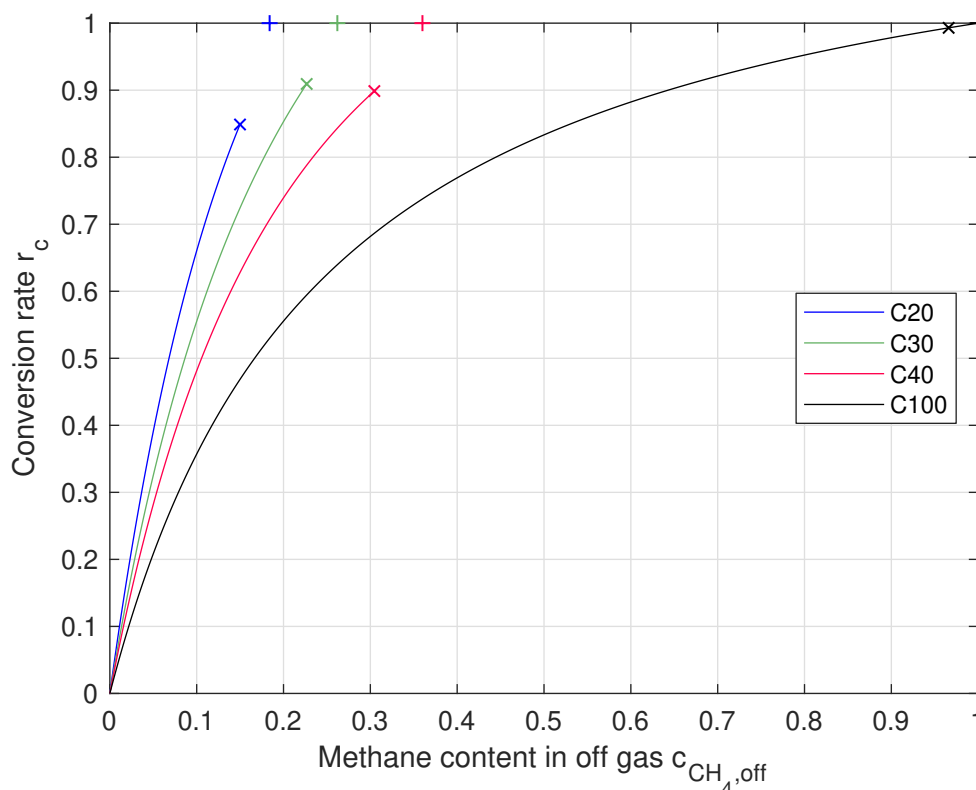


Figure 3. Conversion rate r_c with respect to CH_4 content in off-gas $c_{CH_4,off}$ for each feed gas composition. Markers: the “x” show mean values the processes were running at, the “+” show theoretical maximum.

4. Discussion

The aim of this contribution was to determine the effect of N_2 in the feed gas of a biological methanation performed in a continuous stirred-tank reactor (CSTR) with *M. marburgensis*. Methanation was performed in the common way, feeding H_2 and CO_2 with a ratio of 4:1 into the fermenter. It was shown that the target of 95% methane in the off-gas is possible to reach, which is a requirement for natural gas grid injection in Germany and other European countries [22]. To show whether there is an effect of an additional presumed inert gas in the feed gas, CO_2 was replaced with a CO_2/N_2 premixed gas. Mixtures with different compositions from 20% to 40% CO_2 were used and the rest was N_2 . These compositions were chosen to simulate possible CO_2 sources as they are presented in Section 1.

To compare both scenarios, namely feeding H_2 with pure CO_2 and feeding H_2 with a CO_2/N_2 mixture, the ratio of $\dot{V}_{CH_4,off}$ and $\dot{V}_{CO_2,feed}$ was defined as the conversion rate r_c . N_2 concentration did not affect this value. Using pure CO_2 led to $r_c = 99.3\%$ with an off-gas concentration of 96.6% methane. According to former assumptions, this rate must be (nearly) the same when adding N_2 to the feed gas. Regarding Tables 4–6, the r_c in all experiments was about 84.9% to 90.9%. Comparing

r_c in all experiments, there was a slight difference. Regarding the accuracy of the used equipment, the authors cannot determine if this deviation comes from inaccuracies in measurement or from a possible consumption of N_2 in the process. In particular, the cross-sensitivity of the H_2 sensor towards CO_2 and CH_4 and the low CO_2 concentration resulted in a possibly high error of the gas sensors. We tried to compensate for this cross-sensitivity by doing a compensation calculation according to the manufacturer. These observations led to the assumption that in the process of biological methanation, N_2 did not influence the process of converting carbon dioxide into methane. N_2 behaves like an inert gas, and an overall concentration in feed gas from 0% to 47% can be used for biological methanation. Yet, the stoichiometric ratio of 4:1 hydrogen to carbon dioxide is not optimal. The limiting factor in this conversion was hydrogen. It was almost completely converted, with around 5% CO_2 left in the off-gas. To convert the remaining CO_2 , an additional experiment feeding more H_2 into the reactor, but deviating from the $H_2:CO_2$ ratio 4:1, resulted in $r_c = 99.3\%$. The ratio in this experiment was 4.3:1. This experiment is not presented in this contribution.

Regarding the first hypothesis made in the introduction, it is not possible to determine whether there is a formation of ammonia or not, considering the results obtained for this contribution. Due to Equation (1), N_2 does not participate in the catabolic reaction. On the other hand, the catalytic methanation process has been investigated while using N_2 in addition to CO and H_2 [13]. N_2 was added there to reduce the temperature in the reactor, and NH_3 was formed. Even if the target—to use dinitrogen for dilution of the synthesis gas and to cool down the catalytic process—is different from using new N_2 -containing gases as a carbon source, a CO_2/N_2 mix should be considered for biological methanation processes. Further experiments can be performed to investigate the formation of NH_3 and its presence in both the liquid and the gas phase. If there is a formation of ammonia, a substitution of NH_4^+ from the nutrition solution can be considered.

In addition, the second hypothesis states that there should be an effect of any N_2 content on the mass transfer into the liquid phase. The presence of dinitrogen in the feed gas led to lower concentrations of hydrogen and carbon dioxide than in a system without N_2 . According to Fick's law of diffusion, lower concentrations of CO_2 and H_2 result in lower mass flows, which also increases the limitation of the process. This might have had an effect on the conversion rate, but this could not have been determined during this work. By using a higher $\dot{V}_{feed}$, such a possible limitation could be determined.

Of course, a downstream processing of the off-gas would be necessary to separate N_2 and remaining CO_2 from CH_4 [5,25] to fit the requirements for a natural gas grid injection, according to [22]. The following step of upgrading the N_2 -containing off-gas to a gas to fit the grid injection requirements is not part of this contribution and can be found in the literature [5]. Alternatively, it can be directly used as a fuel in internal plant applications without downstreaming.

Author Contributions: Conceptualization, M.P.H., T.B., and J.S.; methodology, M.P.H.; investigation, M.P.H.; resources, J.S.; data curation, M.P.H., T.B., and J.S.; writing—original draft preparation, M.P.H.; writing—review and editing, M.P.H.; visualization, M.P.H.; supervision, J.S.; project administration, J.S.; funding acquisition, M.P.H., T.B., and J.S.

Funding: This research was funded by the German Federal Ministry of Education and Research (BMBF) grant number 13FH240PX6.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
C20	Gas mix with approx. $x_{\text{CO}_2} = 0.2$ and $x_{\text{N}_2} = 0.8$
C30	Gas mix with approx. $x_{\text{CO}_2} = 0.3$ and $x_{\text{N}_2} = 0.7$
C40	Gas mix with approx. $x_{\text{CO}_2} = 0.4$ and $x_{\text{N}_2} = 0.6$
C100	Gas with approx. $x_{\text{CO}_2} = 1$
CSTR	Continuous stirred-tank reactor
DSMZ	Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
IR	Infrared
ORP	Oxidation reduction potential
PSA	Pressure swing adsorption
UNFCCC	United Nations Framework Convention on Climate Change
WW	Water washing

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