Simultaneous CO_2 and CO methanation using microbes

Kohlmayer Matthias[a,b,c]#, Huber Robert[c,d], Brotsack Raimund[b,c] Mayer Wolfgang[e]

[a]Technical University of Munich, Munich, Germany

[b]Deggendorf Institute of Technology, Deggendorf, Germany

[c]MicroPyros GmbH, Straubing, Germany

[d]Municipal vocational school for biological-technical assistants Straubing, Straubing, Germany

[e]University of Applied Sciences Kempten, Kempten, Germany

#Address correspondence to Kohlmayer Matthias, matthias.kohlmayer@tum.de.

ABSTRACT

In this study, we developed a method for simultaneous bio-methanation of CO_2 and CO with H_2 in a single bioreactor using a combination of carboxydotrophic bacteria and methanogenic archaea for industrial applications. Methanogenic archaea generally use H_2 and CO_2 to produce methane, whereas very few methanogenic archaea methanize CO, and these grow slowly and consequently produce low reactant gas turnover rates. Thus, to achieve fast and simultaneous transformation of CO and CO_2, we identified a combination of carboxydotrophic and hydrogenogenic bacteria and methanogenic archaea that can produce H_2 and CO_2 from CO, and then methanize CO_2 and H_2. The present screening experiments identified carboxydotrophic bacteria and methanogenic archaea that can cohabitate at the same thermophilic temperature and pH ranges and in the same growth medium. In these experiments, combinations of *Carboxydocella thermautotrophica* (DSM 12326), *Carboxydocella sporoproducens* (DSM 16521), and three thermophilic rod-shaped methanogenic archaeal cultures from MicroPyros GmbH formed unique microbial co-cultures that transformed CO_2, H_2, and CO to methane. The successful combination of these microbes could be used to gasify biowastes, such as sewage sludge, as alternative sources of hydrogen for microbial power-to-gas processes. Accordingly, gasification under these conditions produced H_2-rich gas containing CO_2 and CO, theoretically allowing various types of biowastes to be converted to biomethane, which is CO_2-neutral, storable, and widely applicable as an energy source.

IMPORTANCE:

In this study, we hypothesized that the simultaneous bio-methanation of CO_2 and CO with H_2 in a single bioreactor can support the Power-to-Gas technology, a storage technology for renewable

energies. We formed a novel co-culture tool that efficiently achieved the fast and simultaneous transformation of CO and CO_2. That novel co-culture consists of *Caroxydocella thermautotrophica* (DSM 12326), *C. sporoproducens* (DSM 16521), and three thermophilic rod-shaped methanogenic archaeal cultures from MicroPyros GmbH.

Introduction

We hypothesized that the efficient catalysis of the methanation of CO_2 and CO in a single bioreactor can aid in promoting the expansion of renewable energy sources to slow climate change. For the efficient catalysis, we have developed a combination of carboxydotrophic bacteria and methanogenic archaea.

Power-to-Gas (PtG) storage technologies have been developed to support the use of renewable energies, and these use electrical power from renewable sources to split water and generate hydrogen and oxygen. Hydrogen from such hydrolysis reactions can be stored as methane following reactions with CO_2 as follows: $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$. Hence, catalysis of such reactions by methanogenic archaea will constitute a microbial PtG technology.

The workload and profitability of PtG technologies can be increased using alternative sources of hydrogen when electrical power for hydrogen production is expensive. To this end, gasification of sewerage sludge may provide an adjustable, ecological, and economically rational alternative H_2 source for microbial PtG processes (1), (2). The ensuing gasification produces H_2-rich gas that also contains CO_2 and CO, and could be used directly as a gas mixture for the PtG processes, pending on the methanation of CO.

In contrast with chemical-catalyzed synthesis of methane from H_2 and CO_2 using the so-called Sabatier process, microorganism biocatalysts can adapt to gas pollution, are robust to fluctuations of gas contents, and can work at lower temperatures and pressures (3). Multiple methanogenic archaea have been shown to methanize CO_2 and H_2, although very few reportedly methanize CO (4), (5), (6), (7), and these grow slowly and produce low rates of reactant gas turnover (8). However, efficient CO-oxidizing, H_2-producing bacteria have been identified, and these use CO as their only source of carbon and energy (9).

Klasson *et al.* (10) previously reported the use of a single co-culture comprising *Rhodospirillum rubrum* to convert CO and H_2 to methane and *Methanobacterium formicicum* and *Methanosarcina barkeri* to convert H_2 and CO_2 to methane at 34°C. Herein, we identified microbes that could simultaneously methanize CO_2, H_2, and CO at thermophilic growth temperatures. We then devised a novel combination of CO-utilizing and H_2-producing bacteria ($CO + H_2O \rightarrow CO_2 + H_2$) with methanogenic archaea ($CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$) and showed optimal growth at around 63°C.

Our data demonstrate a highly efficient microbial co-culture that transforms CO_2, H_2, and CO to methane. This co-culture comprised a combination of *Carboxydocella thermautotrophica* (DSM 12326), *Carboxydocella sporoproducens* (DSM 16521), and three methanogenic archaea cultures from MicroPyros GmbH. The present innovation may provide the basis for gasification of sewage sludge as an alternative source of hydrogen for industrial scale microbial PtG processes.

Materials and Methods

Based on the methods reported by Hofbauer *et al.* (11), we used the synthetic steam gasifier and synthetic air gasifier gas compositions shown in Table 1. Gases were purchased from Tyczka Industrie-Gase of Mannheim, Germany.

The present co-cultures were established as combinations of *Carboxydocella thermautotrophica* (DSM 12326; 12), *Carboxydocella sporoproducens* (DSM 16521; 13; so-called Carbos) from Deutsche Sammlung für Mikroorganismen und Zellkulturen of Brunswick, Germany, and a cell mixture of three thermophilic rod-shaped methanogenic archaeal cultures (TRMC) that were kindly supplied by MicroPyros GmbH of Straubing, Germany. TRMC are autotrophic isolates from the biogas plant of Zweckverband Abfallwirtschaft of Straubing, Germany and were selected in screens for reproducible growth in newly designed medium (see below) with synthetic steam gasifier gas and synthetic air gasifier gas. The present isolates showed the best and most reproducible growth among all strains tested and were selected from the MicroPyros culture collection. *Carboxydocella sporoproducens* cells grow more slowly than *Carboxydocella thermautotrophica* cells but can form stable spores that survive periods of CO deprivation (12), (13). To ensure sustained supply, cultures were transferred into fresh media weekly (5% inoculation) and were incubated at 63°C for 24 h. All experiments were performed in triplicate for at least five transfers.

Growth media were prepared as described by Zhao *et al.* (14), except that the expensive vitamin and yeast extract supplements were omitted for future industrial scale-up. The final media contained (g/L) KCl (0.33), $MgCl_2 \times 6H_2O$ (0.102), $CaCl_2 \times 2H_2O$ (0.015), NH_4Cl (0.33), K_2HPO_4 (0.14), and $NaHCO_3$ (0.42) in demineralized water. A trace element solution (10 mL/L) and a

selenite-tungstate solution (0.1 mL/L) were added as described for DSMZ medium 14 and DSMZ medium 385.

Oxygen was expelled from media solutions using the argon method described by Hungate (15) with minor modifications. Subsequently, 0.7 g/L of $Na_2S \times 9H_2O$ was added, and media were introduced to anaerobic chambers and were dispensed into 28 mL serum tubes at 5 mL per tube. Tubes were then sealed with rubber stoppers and were pressurized to 3 bar absolute using the desired gas phase. Gas phases in glass tubes were then exchanged by degassing and gassing three times. Pressurized tubes were then autoclaved at 121°C for 15 min (15), (16) and the pH of the autoclaved medium was about 6.5. Culture tubes were incubated almost horizontally to maximize contact areas of gas and liquid phases, and gases entered liquid phases containing microorganisms solely by diffusion. Samples of cultures were taken using syringes (Omnifix-F®), and numbers of cells were counted using fluorescence microscopy (Olympus BX53F) with a Thoma counting chamber. Numbers of cells in TRMC were determined by counting fluorescent cells, and pH was determined using pH sticks (pH-Fix; 4.5–10.0, Roth). Pressure levels in culture tubes were determined using a portable WAL 0–4 bar absolute membrane pressure unit (Wal Mess- und Regelsysteme GmbH of Oldenburg, Germany).

H_2, CO, CO_2, CH_4, and N_2 contents were quantified using a Thermo Fisher Scientific Trace 1310 gas chromatograph with a thermal conductivity detector. In these determinations, 400 µL aliquots of gas were taken from the headspaces of culture tubes and were added to a packed Supelco Carboxen-1000 column (Gas Syringe A-2, Macherey-Nagel of Düren, Germany). The column was then heated to 108°C and maintained at this temperature for 6.25 min, followed by heating to 177°C at 120°C/min and maintenance at this temperature for 3.2 min. Finally, the column was heated at 120°C/min to 222°C and was maintained at this temperature for 4.6 min. Argon was

119 used as the carrier gas at a flow rate of 11 mL/min. The injector and detector were adjusted to
120 125°C and 235°C, respectively. Measured GC values were standardized to 100%. Finally,
121 methane production rates (MPR) were calculated as ml of CH_4 produced/(mL medium *h).

122 **Results**

123 **Co-culture**

124 *Carboxydocella thermautotrophica, Carboxydocella sporoprducens* (Carbos), and cells from the
125 three thermophilic rod-shaped methanogenic archaeal isolates (TRMC) proliferated well together
126 in the newly designed medium without vitamin solution or yeast extract and under the growth
127 conditions are presented in Table 2 (11), (12).

128

129

130 **Metabolic properties of TRMC isolates**

131 Mixtures of the three methanogenic archaeal isolates methanated CO_2 and consumed H_2 from the
132 gas phase in the volumes shown in Fig. 1. However, CO conversion was not observed with either
133 steam gasifier or air gasifier gas (Fig. 1).

134

135 Fig. 1: Gas compositions at 0 and 71.3-h incubation of TMRC in air gasifier gas

136

Growth experiments with Carbos and TRMC

In initial experiments with combinations of Carbos and TRMC, microscope analyses showed the presence of fluorescent methanogenic archaea and the morphologically shorter non-fluorescent carboxydotrophic, hydrogenogenic bacteria growing in co-culture (Fig. 2), with an estimated TRMC to Carbos ratio of about 2:1 (Fig. 2).

Fig. 2: Co-culture of methanogenic archaea (TRMC; blue-colored rods) with carboxydotrophic bacteria (Carbos; black-colored rods) in steam gasifier gas after 96 h incubation at 63°C

Consumption of steam gasifier gas by co-cultures

Figure 3 shows simultaneous decreases in H_2 and CO concentrations and concomitant increases in CH_4 contents of the gas phase. In these experiments, calculated maximum methane concentrations in the headspace were achieved within 96 h, based on reaction equations $CO + H_2O \rightarrow H_2 + CO_2$ [1] and $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$ [2]. Moreover, CO_2 concentrations were increased immediately and then remained approximately constant, and pressure (p) profiles (p actual / p start) dropped during methanation to about 44% of the initial pressure.

Fig. 3: Changes in gas compositions and pressure during incubation in steam gasifier gas; error bars indicate standard deviations of three measurements

Initial hydrogen concentrations were 50% and dropped to 0.5% within 72 h, and CO concentrations decreased from 20% to 3% over the same time. Concomitantly, methane concentrations increased from 10% to 53%, and after 96 h incubation, H_2 concentrations were 0%, CO concentrations were 2%, and methane concentrations were 55% of the entire volume. Moreover, increases in methane concentrations increased by about 1.0 vol%/h between 24 and 48 h, corresponding with a methane production rate of 0.03 mL/(mL*h).

The maximum possible methane concentration in the steam gasifier gas phase following complete H_2 and CO methanation according to equations [1] and [2] was 55 vol%, and the total methane production rate from 10 vol% to 55 vol% methane over the entire 96 h incubation time was 0.02 mL/(mL*h).

During growth experiments in steam gasifier gas, numbers of cells in Carbos and TRMC increased during the first period of methanation, and then decreased and remained stable at about 1×10^8 cells/mL (Fig. 4).

Fig. 4: Changes in cell numbers over the incubation period in steam gasifier gas at 63°C; standard deviations of three measurements are indicated by error bars

Consumption of air gasifier gas by co-cultures

H_2 and CO contents decreased with increases in CH_4 concentrations in the gas phase (Figure 5), and the calculated maximum methane concentration in the headspace was achieved within 72 h. Simultaneously, pressure profiles in the gas phase of the glass tubes (p actual / p start) dropped to about 69% of the initial pressure during methanation. In accordance, hydrogen concentrations in air gasifier gas dropped from 15% to 1.5% over 48 h. Concomitantly, CO concentrations

179 decreased from 20% to 3%, and methane concentrations increased from 10% to 21%. After 96 h,
180 the hydrogen concentration was 0%, the CO concentration was 1%, and the methane
181 concentration was 22% of the total volume, and this concentration was maximal in air gasifier
182 gas following complete H_2 and CO methanation, according to equations [1] and [2]. Moreover, in
183 calculations of MPR, methane concentrations increased by 0.22 vol%/h between 24 and 48 h
184 incubation, corresponding with a production rate of 0.01 mL/(mL*h). Similarly, the total methane
185 production rate from 10 vol% methane at 0 h to 22 vol% at 72 h was 0.01 mL/(mL*h).

186

187 Fig. 5: Changes in gas composition and pressure over the incubation period in air gasifier gas;
188 standard deviations of three measurements are indicated by error bars

189

190 Growth experiments in air gasifier gas showed initial increases in cell numbers in Carbos and
191 TRMC, followed by decreases to 0.5×10^8/mL and 0.8×10^8/mL, respectively (Fig. 6).

192

193 Fig. 6: Changes cell numbers over the incubation period in air gasifier gas; standard deviations of
194 three measurements are indicated by error bars

195

196 **Discussion**

197 In the present novel combination cultures of thermophilic methanogenic archaea and
198 thermophilic carboxydotrophic and hydrogenogenic bacteria, growth of all three organisms in

199 synthetic steam gasifier gas and in synthetic air gasifier gas was observed, and CO, CO_2, and H_2
200 were converted into methane. In addition, the ensuing increases in methane concentrations
201 corresponded with the stoichiometric ratios indicated by the equations $CO + H_2O \rightarrow H_2 + CO_2$
202 and $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$.

203 Consistent with kinetic expectations, methane production slowed with decreasing reactant gas
204 concentrations and lower pressures in culture tubes, reflecting decreased substrate gas
205 availability. However, in air gasifier gas, methanation proceeded more slowly due to lower
206 hydrogen partial pressures compared with those in steam gasifier gas. Klasson *et al.* previously
207 showed simultaneous conversion of the gases CO and H_2 by *Rhodospirillum rubrum* and H_2 and
208 CO_2 by *Methanobacterium formicicum* and *Methanosarcina barkeri* in growing co-cultures at
209 34°C. In their study, *R. rubrum* growth was dependent on the presence of tungsten, light, and a
210 carbon source other than CO, such as sugars, acetate, or yeast extract, and vitamin supplements
211 were also provided (10), (17). In contrast, the present unique microbial combination grew
212 optimally at 63°C and remained viable in the absence of light and expensive vitamin supplements
213 and carbon sources, thus increasing the convenience and cost-effectiveness of large-scale
214 cultures. Moreover, in the study by Klasson *et al.*, methane yields from H_2 were 83% of the
215 theoretical maximal yield (10), whereas we achieved 100% of theoretical maximal methane
216 concentrations in the gas phase using synthetic steam and air gasifier gases. As a final advantage,
217 the present co-culture system with spore-forming *Carboxydocella sporoproducens* was robust to
218 periods of CO deficiency.

219 In summary, we demonstrated the use of a novel co-culture tool that efficiently allows the use of
220 biowastes as sources of carbon and energy using gasification followed by biological methanation.
221 This novel technology could also be applied to other CO_2, H_2, and CO-containing gases, such as

exhaust gases from steel production, allowing use as raw materials for the production of CO_2-neutral methane in industrial processes (18).

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Figure Legends

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284 standard deviations of three measurements are indicated by error bars

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286 three measurements are indicated by error bars

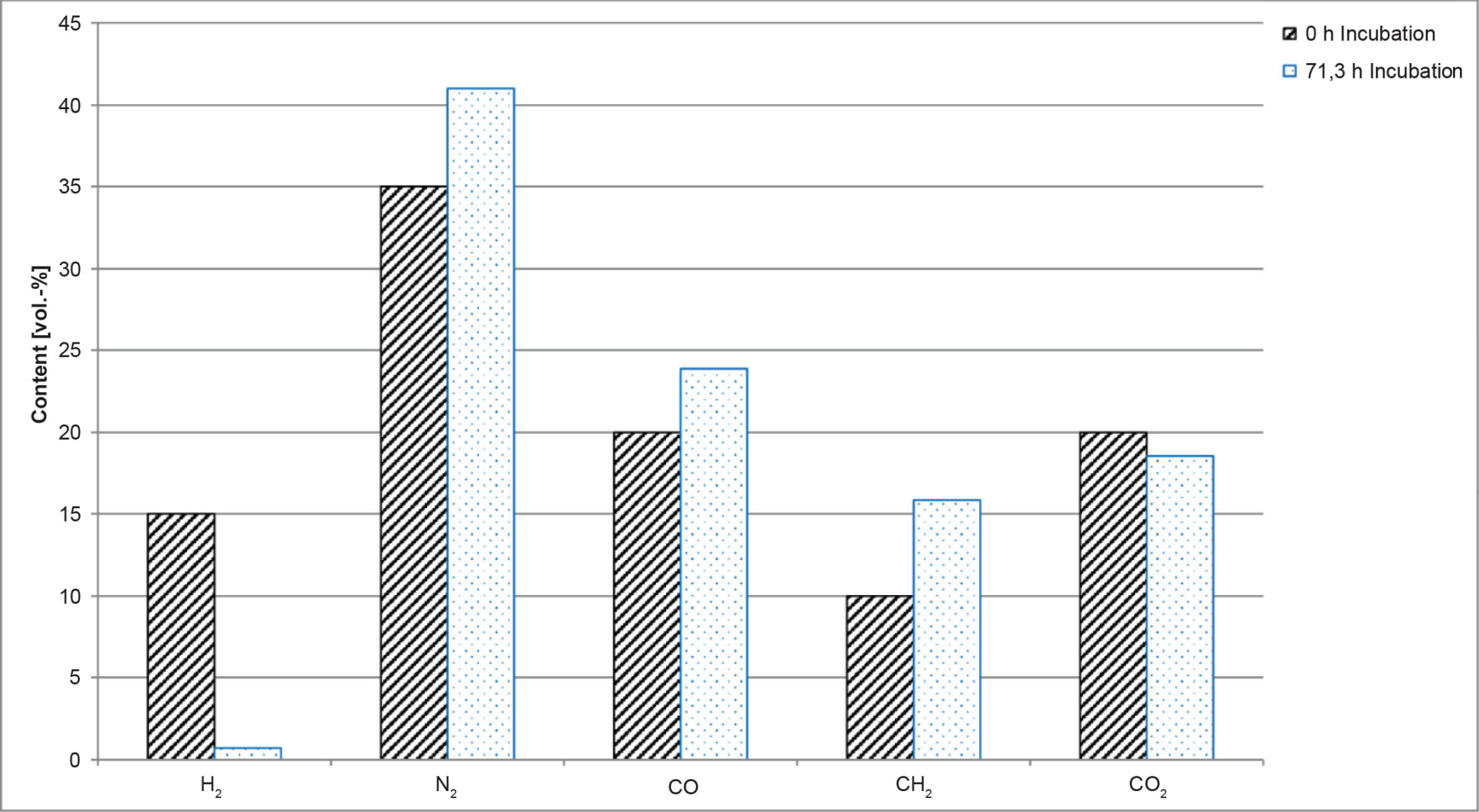
287

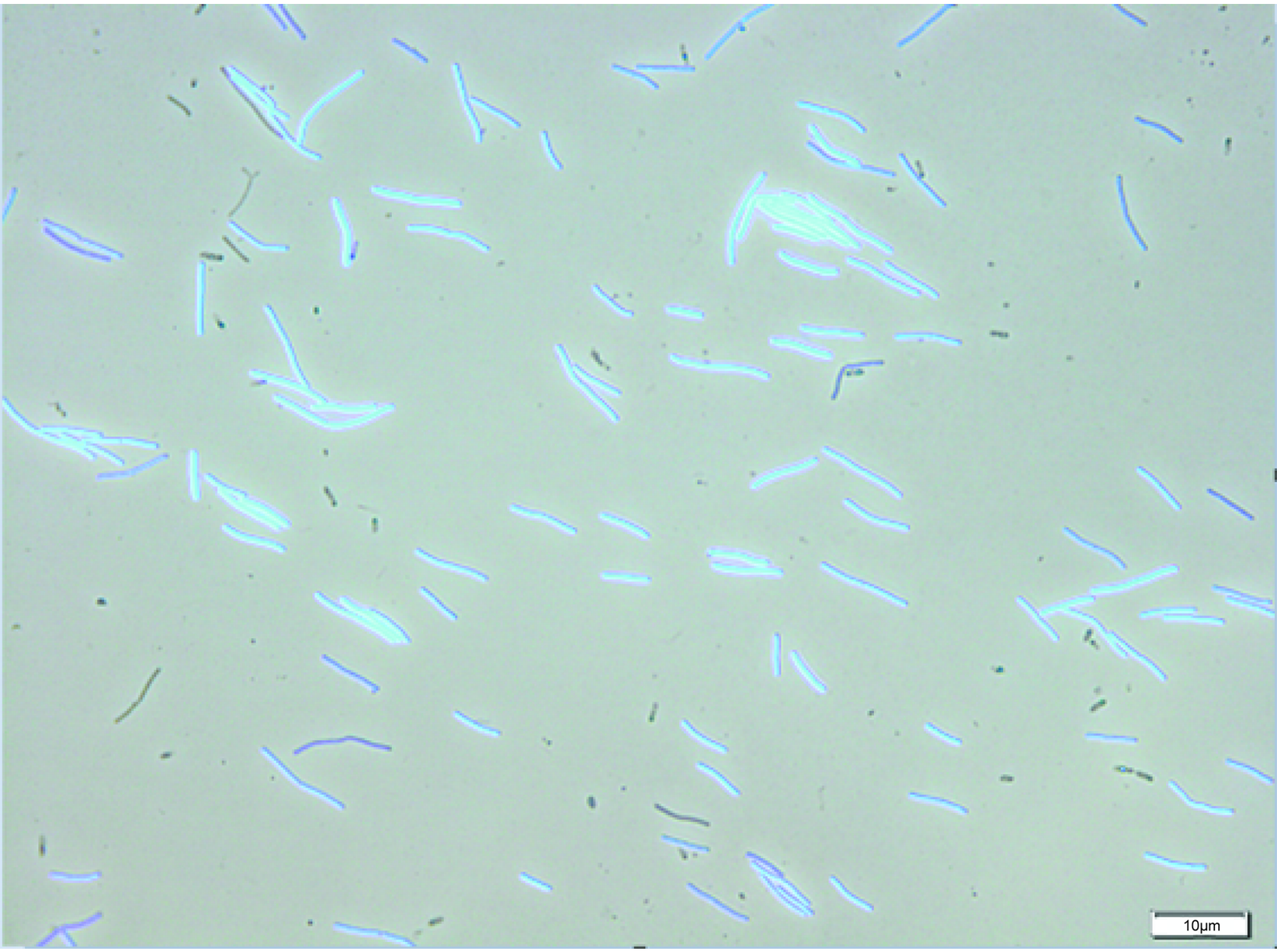
Table 1 Gas concentrations of steam gasifier and air gasifier gases

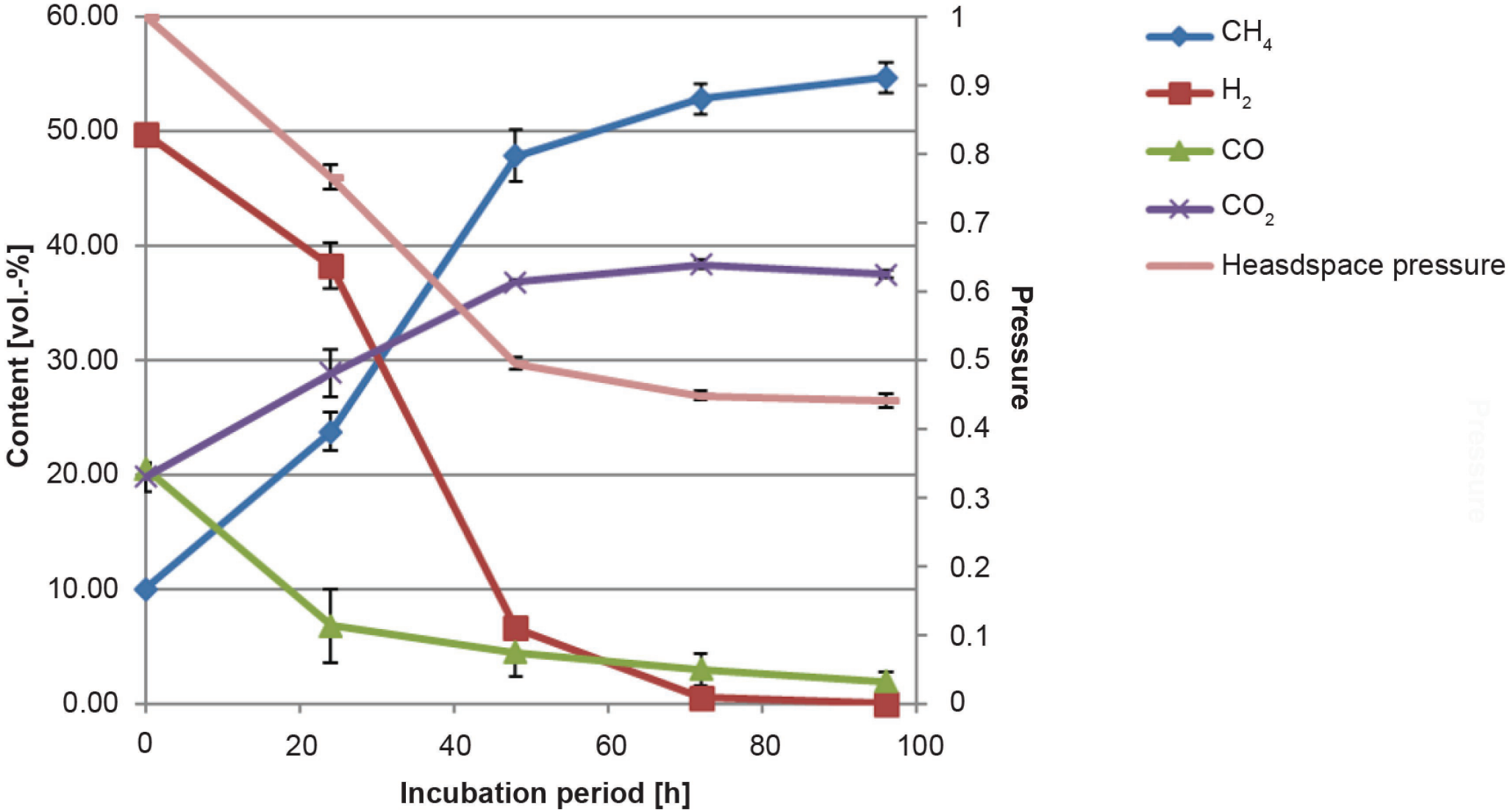
	Steam gasifier gas [vol%]	Air gasifier gas [vol%]
H_2	50	15
CO	20	20
CO_2	20	20
CH_4	10	10
N_2	0	35

Table 2 Growth conditions

Growth conditions	*Carboxydocella thermautotrophica* (Range; Optimum)	*Carboxydocella sporoproducens* (Range; Optimum)	TRMC (Culture Conditions: MicroPyros)	Conditions used for the co-culture (This study)
T [°C]	40–68; 58	50–70; 60	63	63
pH	6.5–7.6; 7	6.2–8.0; 6.8	6.5	6.5
Metabolic reaction	$CO+H_2O->CO_2+H_2$	$CO+H_2O->CO_2+H_2$	CO_2+4H_2 $->CH_4+2H_2O$	This study


45
40
35
30
25
20
15
10
5
0
Content [vol.-%]
0 h Incubation
71,3 h Incubation
H2
N2
CO
CH2
CO2

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40.00
30.00
20.00
10.00
0.00
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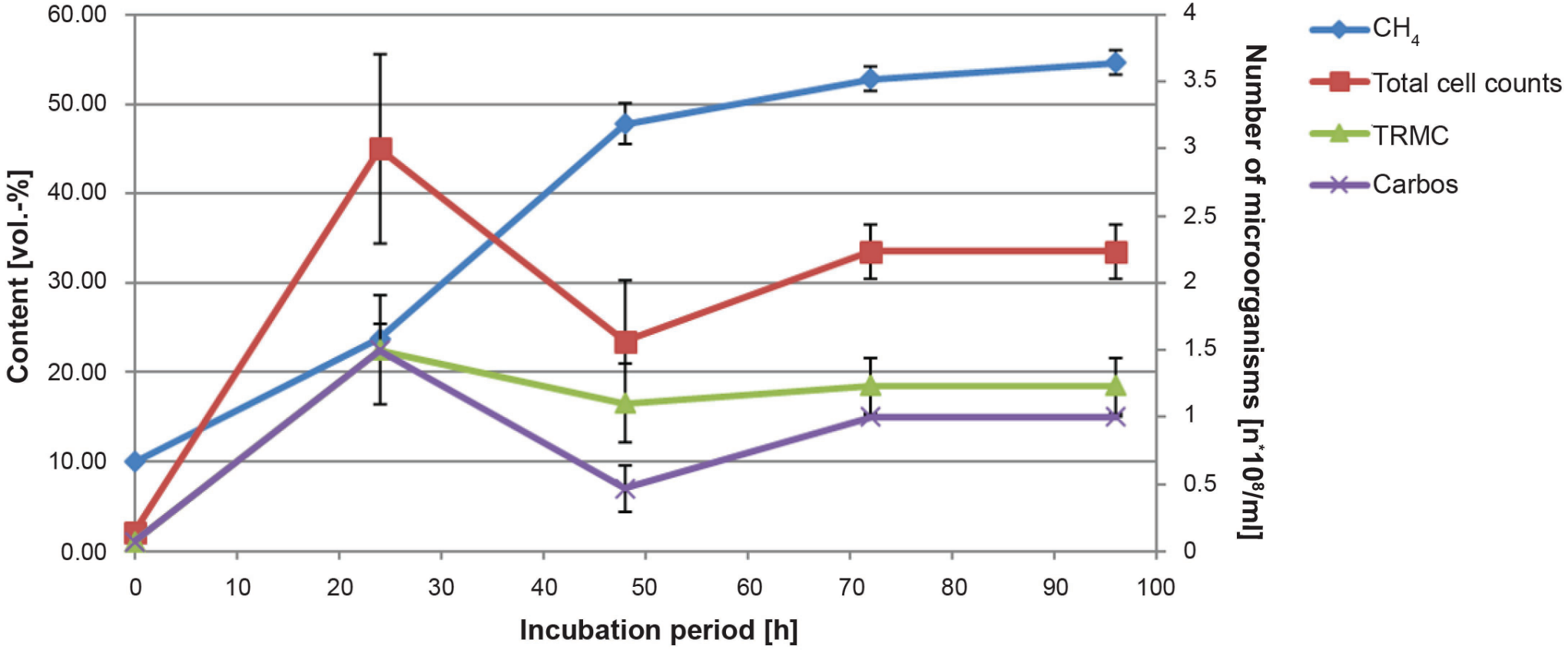
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Pressure
0
20
40
60
80
100
Incubation period [h]
CH4
H2
CO
CO2
Heasdspace pressure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30.00
20.00
10.00
0.00
Content [vol.-%]
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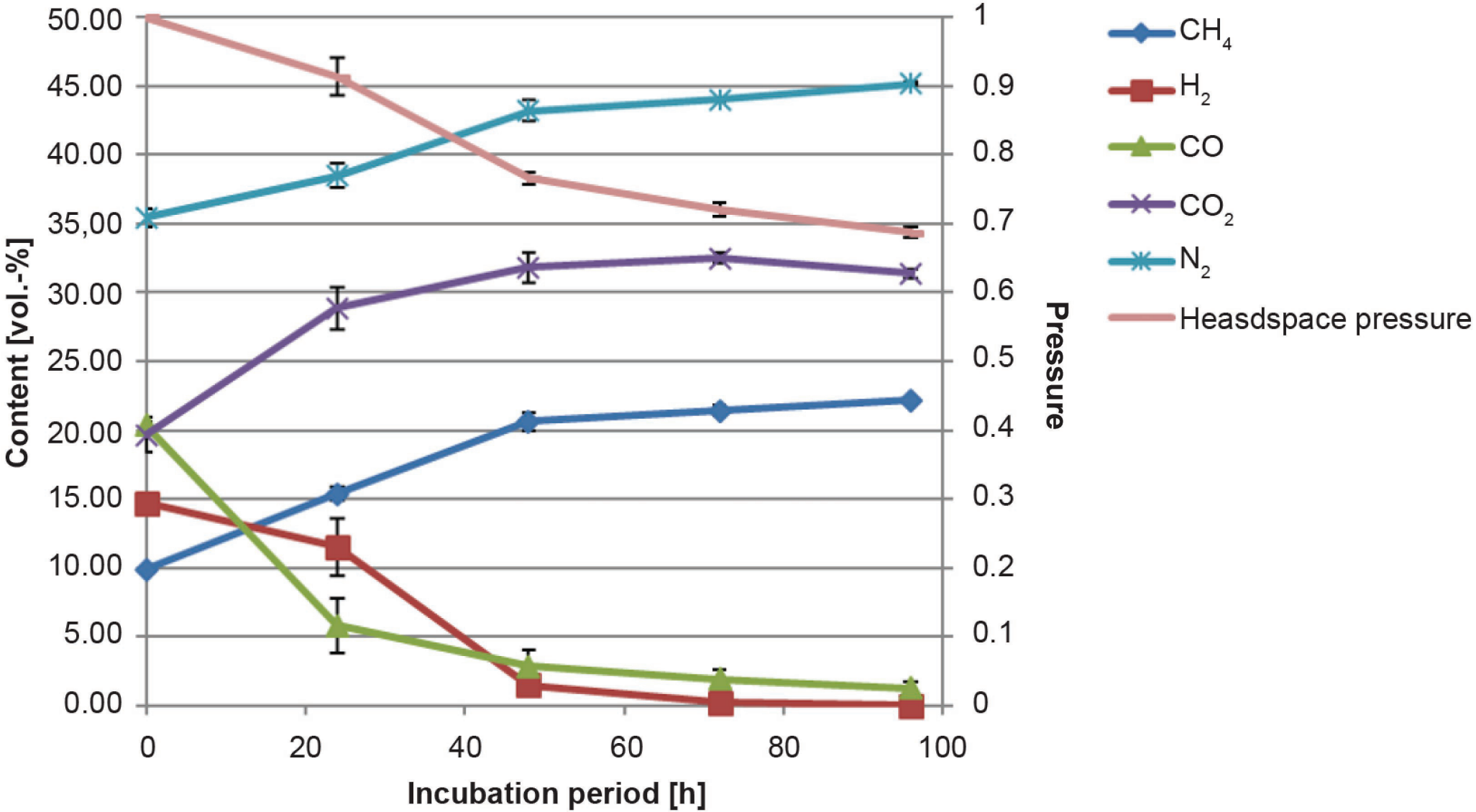
10
20
30
40
50
60
70
80
90
100
Incubation period [h]
4
3.5
3
2.5
2
1.5
1
0.5
0
Number of microorganisms [n*10⁸/ml]
CH4
Total cell counts
TRMC
Carbos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15.00
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1
0.9
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Pressure
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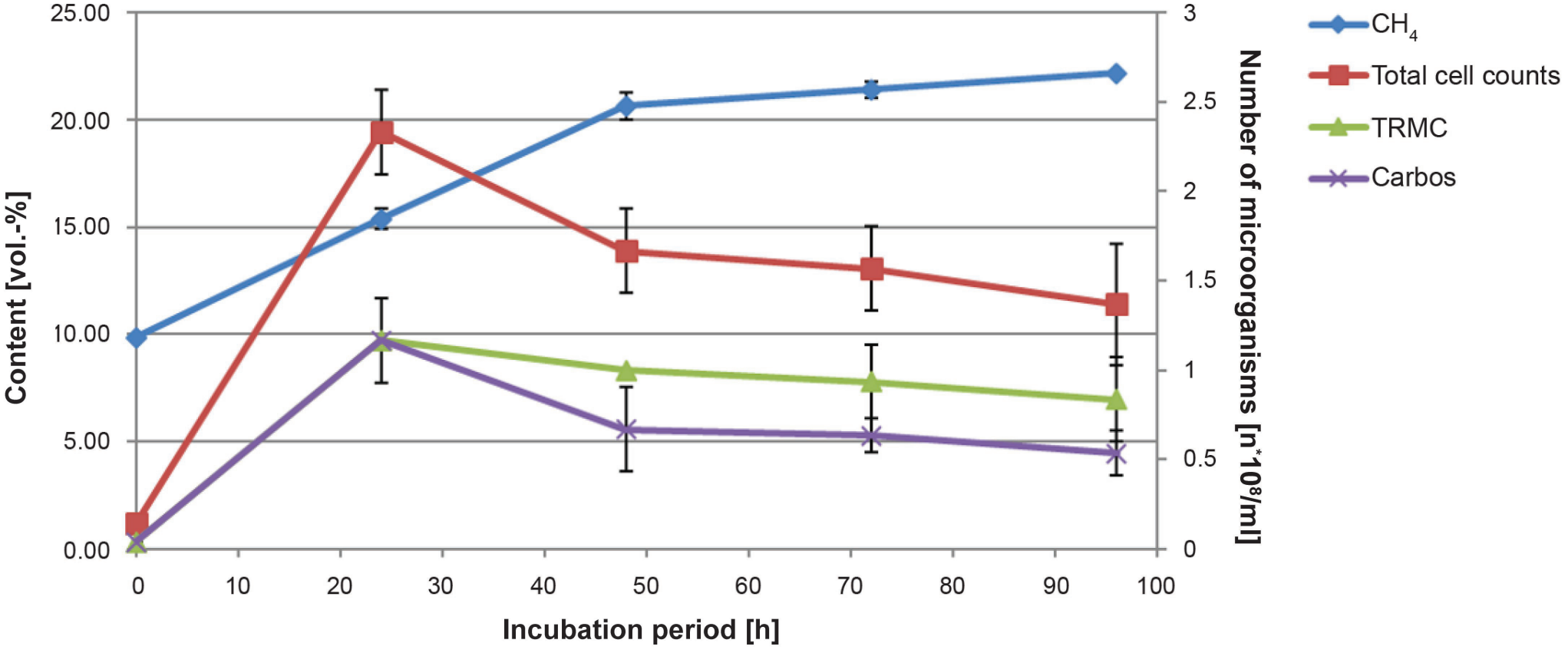
40
60
80
100
Incubation period [h]
CH4
H2
CO
CO2
N2
Heasdspace pressure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10.00
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Content [vol.-%]
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2
1.5
1
0.5
0
Number of microorganisms [n*10⁸/ml]
0
10
20
30
40
50
60
70
80
90
100
Incubation period [h]
CH4
Total cell counts
TRMC
Carbos