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1 Syngas biomethanation: in a transfer limited process, is CO inhibition 2 an issue?

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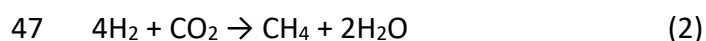
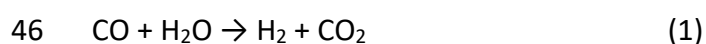
9 Abstract

10 Syngas biomethanation is a promising technology in the process chain converting wastes to
11 methane. However, gas-liquid mass transfer is a limiting factor of the biomethanation
12 process. To reach high methane productivity, increasing the pressure is an interesting
13 strategy to improve mass transfer. However, the CO content in the syngas raises concerns
14 about a potential inhibition of the microorganisms. Therefore, the aim of the research was
15 to assess the ability to work at high CO partial pressures. In this regard, a pressurized
16 continuous stirred column with a working volume of 10L was implemented and a consortium
17 adapted for syngas-biomethanation for 22 months was submitted to 100% CO and
18 increasing pressure. No inhibition phenomenon was observed for logarithmic P_{CO} as high as
19 1.8 bar (inlet pressure 5.0 bar), which was the first time that such a high CO partial pressure
20 was tested in continuous mode. Mass transfer limitations allowed for the carboxydrotrophic
21 microorganisms to consume CO faster than it was transferred, allowing for the dissolved CO
22 concentration to remain under inhibitory concentrations. These results question the habitual
23 consensus that CO inhibition is a limiting factor of syngas biomethanation.

24 **Key words:** CO inhibition – syngas fermentation – waste to energy – biomethanation – gas-
25 liquid mass transfer

26 1 Introduction

27 The growing world population leads concurrently to an increase in waste production and
28 therefore higher risk of environment pollution, and to increase energy demand. Waste
29 valorisation to energy is an interesting approach to address both these issues at the same
30 time. In this perspective, waste gasification allows to convert heterogeneous wastes to
31 energy in the form of syngas. Syngas is a mixt of H₂, CO, CO₂ and CH₄ and its composition
32 varies with the gasification technology employed, the type of waste input and the
33 operational parameters (Pradhan et al., 2015). Syngas can be upgraded to methane to take
34 advantage of the storage and gas grid infrastructures. In this regard, catalytic methanation is
35 a well-developed technology with existing commercial plant in the world (Ren et al., 2020).
36 However, it is sensitive to syngas pollutants such as tar, H₂S or HCl that can deactivate
37 catalysts (Ahn et al., 2021; Ducamp et al., 2018). Therefore, it requires extensive gas cleaning
38 which can increase costs. To face this challenge, biomethanation is a promising developing
39 technology that uses adapted microorganisms as the catalyst for the methanation reactions.
40 Indeed, biological processes present a higher resistance to poisoning compared to catalytic
41 processes (Klasson et al., 1992). Moreover, biomethanation doesn't require consistent gas
42 quality and specific H₂/CO ratio to be able to produce methane (Yasin et al., 2019).
43 Indeed, in thermophilic conditions and using a mixed adapted consortium, the complex
44 syntrophic interactions between the diverse microbial groups involved can be summarized
45 according to two independent global equations:



48 With CO and water being converted to H₂/CO₂ by carboxydrotrophic hydrogenogens (Eq.1)
49 and H₂/CO₂ to methane by hydrogenotrophic methanogens (Eq. 2) (Figueras et al., 2021;
50 Grimalt-Alemany et al., 2019; Guiot et al., 2011; Luo et al., 2013; Sipma et al., 2003).
51 Even though it is a promising technology, biomethanation has only been implemented at lab
52 scale yet (Figueras et al., 2021) and only once using a real syngas (Asimakopoulos et al.,
53 2021b). It still faces several scientific challenges, one of them being CO inhibition.
54 Indeed, carbon monoxide could at the same time act as a substrate for carboxydrotrophic
55 microorganisms or inhibit some microbial populations. Carboxydrotrophic methanogens
56 could be inhibited by CO partial pressure between 0.5 and 1 atm (Grimalt-Alemany et al.,
57 2018). However, it has been reported on a consortium adapted to syngas-biomethanation
58 that hydrogenotrophic methanogens could be more tolerant to high CO concentration
59 compared to carboxydrotrophic hydrogenogens (Figueras et al., 2021). The importance of the
60 inhibition depends on the quantity of CO experienced by the microorganisms. The maximum
61 dissolved CO concentration C_{CO}^* (mol/L) can be expressed according to Eq. 3:

$$62 \quad C_{CO}^* = H_{CO} \cdot y_{CO} \cdot P \quad (3)$$

63 With H_{CO} the Henry law constant (mol/L/bar), y_{CO} the molar fraction of CO in the gas and P
64 the total pressure (bar). Therefore, both the syngas composition and the operating pressure
65 of the process can impact the maximum dissolved CO concentration and therefore impact
66 the CO inhibition. Syngas composition and therefore the CO content depends on various
67 parameters, mainly the waste composition that influences the choice of reactor type (Couto
68 et al., 2013). The oxidizing agent used has also a strong impact on the CO proportion. In
69 particular, O₂ and steam allow for less N₂ in the syngas and higher CO percentages (up to
70 48% for a fixed bed downdraft gasifier with O₂ as an oxidizing agent (Couto et al., 2013)).

71 Other parameters such as the equivalence ratio can also influence the syngas composition
72 (Paniagua et al., 2022).

73 Gas-liquid mass transfer is a limiting step of the biomethanation process (Asimakopoulos et
74 al., 2018). According to Eq. 4, N_i (mol/L_{reactor}/h) the mass transfer rate of a component i is
75 proportional to the mass transfer coefficient $k_{L,i}$ (h⁻¹), and to the concentration gradient
76 between the bulk liquid $C_{i,L}$ (mol/L) and the saturation concentration, which corresponds to
77 the product of the Henry constant $H_{i, cp}$ by the partial pressure P_i .

$$78 \quad N_i = k_{L,i} * (H_{i, cp} * P_i - C_{i,L}) \quad (4)$$

79 Therefore, increasing the pressure allows for a better mass transfer rate. However, it can
80 also increase the dissolved CO up to inhibitory concentrations (Eq. 3). Yet, carboxydrotrophic
81 microorganisms consume CO and participate in lowering $C_{CO,L}$. Therefore, the CO
82 concentration experienced by the microorganisms is a question of equilibrium between the
83 rate at which CO is transferred and the rate at which it is consumed (Fig. 1). Even though
84 removing mass transfer limitations is a key aspect of the biomethanation process addressed
85 by many studies (Asimakopoulos et al., 2018; Jensen et al., 2021), one should keep in mind
86 the potential CO inhibition that could arise with a high-performance reactor in terms of
87 transfer.

88 On the other hand, the transfer rate is different for every study, notably because the reactor
89 geometry impacts the $k_{L,i}$ (Eq. 4). Hence, the CO partial pressure remains a good indicator to
90 compare the inhibition phenomena between various studies, as it indicates the highest C_{CO}^*
91 experienced by the microorganisms.

92 A few studies have explored the inhibition limit of consortia performing biomethanation of
93 100% CO. Navarro et al. (2016) observed in mesophilic conditions in batch bottles, for non-

94 acclimated sludge, that the maximum methane productivity was reached for a CO partial
95 pressure of 0.2 atm. Carboxydrotrophic activity increased with CO partial pressure. Yet,
96 methanogenic activity decreased with P_{CO} and totally stopped at 1 atm. However, after
97 adaptation to CO for 45 days, the consortium was able to convert 1 atm of CO with methane
98 production up to 90%.

99 Guiot et al. (2011) observed in mesophilic batch test on unacclimated sludge a stop of
100 methanogenesis for P_{CO} higher than 0.3 atm. However, using a gas lift reactor,
101 methanogenesis was able to occur up to 0.96 atm of P_{CO} in the feeding gas, after 97 days.
102 According to the authors, this was probably due to the dilution due to the gas recirculation.

103 Luo et al. (2013) observed in batch in thermophilic conditions on a non-adapted sludge that
104 for a P_{CO} higher than 0.25 atm, methanogenesis became limited, as well as carboxydrotrophic
105 activity. Methanogenesis was completely limited for a P_{CO} of 1 atm.

106 Alves et al. (2013), when performing an enrichment for syngas in thermophilic conditions
107 with gradual pressure increases, observed an interruption of methanogenesis after 4
108 transfers, at a P_{CO} of 0.18 bar. After 16 enrichment transfers, methanogenesis didn't start,
109 indicating that the consortium did not adapt to CO. It is unclear why it would not adapt after
110 a long period and perform methanogenesis, whereas it did for Navarro et al. (2016).
111 However, carboxydrotrophic activity was present.

112 To sum up, four studies have observed CO inhibition for P_{CO} higher than 0.2 atm, notably of
113 methanogenesis that appeared to be the most affected by increasing CO partial pressure.

114 However, a few studies have been able to convert CO at higher partial pressure. For
115 instance, a successful methane production was obtained at P_{CO} higher than 0.4 atm (Grimalt-
116 Alemany et al., 2019; Li et al., 2019; Westman et al., 2016; Youngsukkasem et al., 2015). Li et

117 al. (2020) obtained methane production with P_{CO} at 1 atm, with full CO conversion in 10 days
118 in batch tests. It could be argued that this is due to a higher biomass concentration: if the
119 microorganisms are inhibited by CO and only perform at a certain fraction of their maximum
120 rate, having a higher biomass concentration can compensate for the inhibition. Indeed, Luo
121 et al. (2013) have observed full methanogenesis inhibition at a P_{CO} of 1 atm with a biomass
122 concentration of 12.4 g/L, whereas Li et al. (2020) observed methane production at a
123 biomass concentration of 84.7 g/L (both in thermophilic conditions)

124 The difference in temperature conditions could possibly explain the discrepancy between
125 the results from the literature. Indeed, the temperature conditions impact the predominant
126 microbial species (Asimakopoulos et al., 2020), which could present various tolerance to CO
127 inhibition. However, amongst the four studies reporting CO inhibition for CO partial pressure
128 higher than 0.2 atm, two operated in mesophilic conditions (Guiot et al., 2011; Navarro et
129 al., 2016) and two in thermophilic conditions (Alves et al., 2013; Luo et al., 2013). Moreover,
130 other studies were able to obtain methane production with CO partial pressure higher than
131 0.4 atm in both thermophilic and mesophilic conditions (Asimakopoulos et al., 2020; Li et al.,
132 2019; Youngsukkasem et al., 2015). Therefore, temperature conditions are not the sole
133 factor explaining the different CO tolerances observed by various studies. To our knowledge,
134 the highest CO partial pressure tested is 1.8 bar by Sipma et al. (2003) in batch mode.

135 Various sludges were tested, and some were able to produce methane. It appeared in this
136 study that this was dependent on the structure of the sludge, and that granular sludges were
137 more resistant to CO inhibition. Indeed, crushing the sludge led to a stop of methane
138 production. The granule structure appears to protect sensible microorganisms that are
139 located in the center (Fang, 2000). Therefore, the structure of the sludge is also to consider
140 when comparing studies.

141 The CO inhibition threshold is still unclear in terms of partial pressure. Literature data are
142 scattered, and no clear conclusion can be drawn. The time of adaptation, the biomass
143 concentration and the structure of the sludge need to be considered when comparing
144 studies. When considering working at high pressure to enhance mass transfer and reach high
145 methane productivity, one should consider the impact that CO could have at high dissolved
146 concentrations. However, CO inhibition has not been evaluated for P_{CO} higher than 1.8 bar.
147 Hence, the aim of this study is to explore for the first time CO inhibition at higher pressure in
148 continuous mode.

149 2 Material and Methods

150 The reactor set-up was similar to the one described in a previous paper (Figueras et al.,
151 2021) and is described in Fig. 2 . The reactor was a gas-tight stainless-steel tank (height:
152 588mm, inner diameter: 162mm), continuously supplied with gas injection. The tank was
153 pressurized with a pressure controller. Inlet and outlet mass flow rates were monitored, as
154 well as outlet gas composition. Thermophilic conditions were chosen as they allow higher
155 methane productivity (Asimakopoulos et al., 2020; Grimalt-Alemany et al., 2019;
156 Youngsukkasem et al., 2015) and because hydrogenotrophic methanogens present higher
157 growth rates in thermophilic conditions (Rafrafi et al., 2020). Therefore, the temperature
158 was regulated with a thermostat at 55°C by circulating hot water in the water jacket of the
159 tank. Mass transfer was enhanced by mechanical agitation, with three Rushton turbines, at
160 1000 rpm. The working volume was 10L. CO (>99%) was supplied with a gas bottle.

161 The reactor was initially incubated with a mesophilic anaerobic sludge sampled from the
162 sludge digester of the municipal wastewater treatment plant of La Feysine, Lyon, France.

163 The sludge was suspended and didn't form floc or granules in the biomethanation reactor

164 due to the high agitation rate. It was adapted for syngas biomethanation at 40% CO, 40% H₂,
165 and 20% CO₂ at 4 bar for 22 months, corresponding to a P_{CO}ⁱⁿ of 1.6 bar. To choose this
166 syngas composition, we considered syngas compositions from gasification plant using either
167 steam or O₂ as oxidizing agents. Indeed, these agents help reduce the N₂ content in the
168 syngas, which is more favorable to biomethanation. Before the beginning of the experiments
169 described in this work, 100% CO at 1 atm at 7.5 L_{STP}/h was supplied to the consortium for
170 one day, which led to methane production.

171 2.1 Gradual pressure increase

172 The first experiment was done to test the impact of increasing CO partial pressure on
173 carboxydrotrophic and methanogenic activity. For this purpose, the total pressure was
174 increased by 0.1 bar steps, every hour. The pressure was not modified during the weekends,
175 leading to longer exposure to a single pressure. The pressure was first increased up to 2.4
176 bars (run 1), but the experiment was interrupted due to technical issues. The experiment
177 was restarted one month later (run 2). Since no inhibition had been observed during run 1, it
178 was decided to make the first steps quicker starting at 1 bar, then 1.5, 2, 3 and 3.5 bar with a
179 change every hour. Then, the total pressure was increased by 0.1 bar steps every hour, up to
180 5 bar which is the limit of the capacity of the reactor. The experiments are described in Table
181 1.

182

183 Before the beginning of run 1, the total solid (TS) concentration was 9.0 g/L, and the volatile
184 suspended solids (VSS) concentrations 6.9 g/L. The compositions of the liquid media for run
185 1 and 2 are described in Table 2. The analytical methods are detailed in a previous article
186 (Figueras et al., 2021). Since no deficiency was observed at these concentrations, no nutrient

187 solution was added during the experiment. In run 1, 5 mL of a solution of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (6.77
188 g_s/L) was supplied daily. This supply was interrupted for the rest of the experiments because
189 the sulfur content appeared to be enough to allow methane production.

190 2.2 Starting at high pressure

191 To identify the pressure at which the inhibition starts to lift, the consortium was submitted
192 to high initial P_{CO} followed by incremental decreases. Considering the scientific literature
193 presented earlier, it was considered that the microorganisms would be completely inhibited
194 at 4 bars. The idea was thus to start at high CO partial pressure and to make sure that the
195 saturation concentration was reached in the liquid phase. Therefore, the pressure was set at
196 maximal pressure of 4 bar to reach inhibition and prevent CO conversion. Then, it was
197 planned to lower the pressure step by step, every 24h. It was assumed that once the
198 inhibition limit would be lifted, CO conversion would start, and a precise value of inhibitory
199 CO concentration would be deduced.

200 To guarantee a P_{CO} of 4 bars, the gaseous atmosphere of the reactor had to be composed of
201 100% CO. Therefore, the CO conversion by the microorganisms had to be interrupted. For
202 this purpose, the stirring was stopped, and the pressure was initially set at 1 atm to limit CO
203 mass transfer flux to the liquid. The CO inlet gas flow was set to its maximal value (24 L_{STP}/h)
204 to purge the reactor from other gases. When the outlet gas composition reached 100% CO,
205 the pressure was set at 4 bars and the input gas flow rate at 7.5 L_{STP}/h. Then the stirring was
206 launched.

207 This experiment was performed 3.5 months after run 2. Due to cellular lysis, the microbial
208 consortium was probably different than the one used in run 1 and 2.

209

210 Regarding CO inhibition in continuous mode, as CO is converted, a gradient of gas
211 composition establishes between the inlet and the outlet of the reactor. To estimate the
212 maximal CO concentration experienced by the microorganisms according to Eq. 3, the
213 logarithmic partial pressure (P_{CO}^{log}) is a good compromise (Doran, 2013; Villadsen et al.,
214 2011):

$$215 \quad P_{CO}^{log} = \frac{P_{CO}^{in} - P_{CO}^{out}}{\ln(P_{CO}^{in}) - \ln(P_{CO}^{out})} \quad (5)$$

216 With P_{CO}^{in} the CO partial pressure at the inlet of the reactor, and P_{CO}^{out} at the outlet of the
217 reactor (bar).

218

219 3 Results

220 3.1 Gradual pressure increase

221 During the first experiment the inlet pressure was gradually increased up to 5 bar (run 1 and
222 2), which is the limit of the capacity of the reactor. With the gradual increase of CO partial
223 pressure, we observed an increase in CO conversion efficiency from around 24 mmol/L/h at
224 0.6 bar of P_{CO}^{log} to around 31 mmol/L/h at 1.8 bar (Fig. 3). Similarly, CO conversion
225 efficiencies also increased with CO logarithmic partial pressure, around 70% at 0.6 bar of
226 P_{CO}^{log} to around 92% at 1.8 bar. The increase in conversion efficiencies can be explained by
227 the enhanced mass transfer rate that increased with pressure (Eq. 4). Therefore, it appears
228 that there is no CO inhibition on carboxydrotrophic activity.

229 In this study, we did not test an inlet CO partial pressure inferior to 1 bar. However, during
230 the adaptation time on syngas (40% CO, 40% H₂, 20% CO₂) mentioned in Material and
231 Methods, a test has been made at atmospheric pressure for 7.5 L_{STP}/h of syngas. This

232 corresponds to an inlet P_{CO} of 0.4 bar and a CO flow rate of 3 L_{STP}/h . In these conditions, CO
233 conversion rate was 11 mmol/L/h ($P_{CO}^{log}=0.21$ bar). This shows that the CO conversion rate
234 follows the same trend from low to high CO partial pressure.

235 Moreover, one can observe in Fig. 4 that the methane productivity increased with the
236 logarithmic CO partial pressure, from around 5.9 mmol/L/h at 0.6 bar of P_{CO}^{log} to around 7.8
237 mmol/L/h at 1.8 bar. Methane yield observed the same trend, from around 70% at 0.6 bar to
238 93% at 1.8 bar. Therefore, no drop in methane productivity was observed as the pressure
239 increased. On the contrary, methane production increased, probably due to the increase in
240 mass transfer with the increase of partial pressure (Eq. 4).

241 Therefore, no inhibition of methanogenesis was observed for a logarithmic CO partial
242 pressure as high as 1.81 bar. Sipma et al. (2003) reported that anaerobic granules were able
243 to convert CO to methane at a similar operating pressure. They also observed in the same
244 batch experiment that crushing the granules led to CO conversion with no methane
245 production. This is likely due to diffusional mass transfer limitation in the granule that
246 protects the inner microorganism. However, in our case, the microorganisms do not form
247 flocs or granules. Yet, they were still able to perform CO biomethanation at 1.8 bar. The
248 main difference between the two studies could be the adaptation time: their experiments
249 were performed on unacclimated sludge for 35 days, whereas our consortium had been
250 adapted for syngas-biomethanation for 22 months.

251 It appears that gradually increasing the CO partial pressure allowed for the microorganisms
252 to convert CO and maintain a low CO concentration in the liquid bulk. Therefore, we
253 designed the next experiment to start at a high dissolved CO concentration to try to reach an
254 inhibitory concentration.

255 3.2 Starting at high pressure

256 The experiment starting at high CO partial pressure is described in Fig. 5. The agitation was
257 stopped, and the reactor was purged at high CO flow rate and atmospheric pressure until
258 the CO composition in the outlet gas reached a high percentage of 90% (1.). Then the
259 pressure was increased from 1 to 4 bar (2.). Finally, the agitation was restarted (3.). We can
260 observe that as soon as the agitation was restarted, the CO conversion began, and CO
261 conversion efficiency reached 94% in 1.6 hour with methane production.

262 Indeed, before the agitation was restarted (step 2.), mass transfer rate was only controlled
263 by the concentration gradient and the CO pressure (which was the same in the headspace
264 and at the inlet: 4 bar). Therefore, the mass transfer rate was slow. When starting the
265 agitation, the mass transfer rate was accelerated, and CO was transferred faster to the liquid
266 bulk. However, as soon as it began to be transferred, the carboxydrotrophic microorganisms
267 began to consume it.

268 Much probably, under these operating conditions, the CO uptake rate was fast enough to
269 keep the dissolved concentration of CO under the inhibition limit. This means that, for a
270 reactor that is transfer limited, and for a well-adapted consortium, CO inhibition is difficult
271 to observe even at CO inlet partial pressure as high as 4 or 5 bars.

272 However, this is at the conditions that the carboxydrotrophic microorganisms are fully active
273 and not limited by other factors. Indeed, in a previous study (Figueras et al., 2021), we have
274 suspected CO inhibition of the carboxydrotrophic population in one occasion. It happened
275 after a maintenance period. CO conversion was inhibited for P_{CO}^{log} up to 2 bar and the
276 activity was recovered as soon as the pressure was lowered. However, methane production
277 was still occurring. In this study, we were not able to reach a P_{CO}^{log} higher than 1.81 bar due

278 to equipment limitations and safety issues. It is therefore possible that at higher CO inlet
279 pressure, P_{CO}^{log} would reach an inhibitory limit that could be around $P_{CO}^{log} = 2$ bar. Therefore,
280 if the carboxydophilic microorganisms are not active and efficient, for example at a restart
281 of the process after a maintenance as stated earlier, the limiting factor could become the
282 biological CO conversion rate instead of the CO transfer rate.

283 It can be observed in Fig. 5 that the conversion of a gas containing 100% CO led to the
284 production of only 23% of CH_4 in the outlet gas and 69% of CO_2 . Considering mass transfer
285 limitations, this is consistent with the stoichiometry of the reactions involved in
286 biomethanation (Eq. 1 and 2). Indeed, according to the stoichiometry, 100% CO would
287 theoretically be converted to 25% CH_4 and 75% CO_2 . Regarding syngas-biomethanation,
288 theoretically, the higher the hydrogen content in the syngas is, the higher the methane
289 content in the outlet gas will be, which has been demonstrated experimentally by several
290 studies (Asimakopoulos et al., 2021a; Li et al., 2019, 2017). Usually, syngas compositions are
291 richer in H_2 (Couto et al., 2013), which would allow for a higher methane content in the gas
292 produced by biomethanation.

293 Finally, the experimental set-up was not appropriate to reach an inhibitory concentration,
294 and an experimental set-up able to function at higher pressure would be more adapted.

295 Another option would be to saturate in CO a cell-free medium using high CO partial
296 pressure, then to inject the microorganisms at high pressure. Another perspective for this
297 research could be to study the evolution of the microbial population when it is submitted to
298 an increase of the CO partial pressure to observe if it varies and adapts. This could also help
299 to identify predominant microorganisms and their tolerance to CO.

300 Considering the use of a real syngas to produce methane through biomethanation, in
301 addition to the determination of CO inhibition thresholds, other parameters should be
302 considered to optimize the process. The methane yield could be improved either by using a
303 syngas rich in H₂ or by supplying exogenous hydrogen that could be produced from excess
304 electricity from renewable wind or solar energy (Asimakopoulos et al., 2021a). Furthermore,
305 the potential inhibitory effect of the impurities contained in the syngas (tar, H₂S, NH₃) on
306 the microorganisms (Xu et al., 2011) should be assessed.

307 4 Conclusion

308 To conclude, this study demonstrated the high tolerance to CO of a consortium adapted to
309 syngas-biomethanation. An inlet CO partial pressure up to 5 bar was reached with no
310 apparent CO inhibition on either carboxydrotrophic or methanogenic activity. When setting
311 the initial pressure up to 5 bar, the fact that CO was consumed faster than it was transferred
312 allowed for the dissolved CO concentration to remain below an inhibitory limit. Therefore,
313 with an efficient and adapted consortium, because of the mass transfer limitations, the CO
314 inhibition is not necessary a limiting factor of the syngas biomethanation process.

315 Declaration of Competing Interest

316 The authors declare that they have no known competing financial interests or personal
317 relationships that could have appeared to influence the work reported in this paper.

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329

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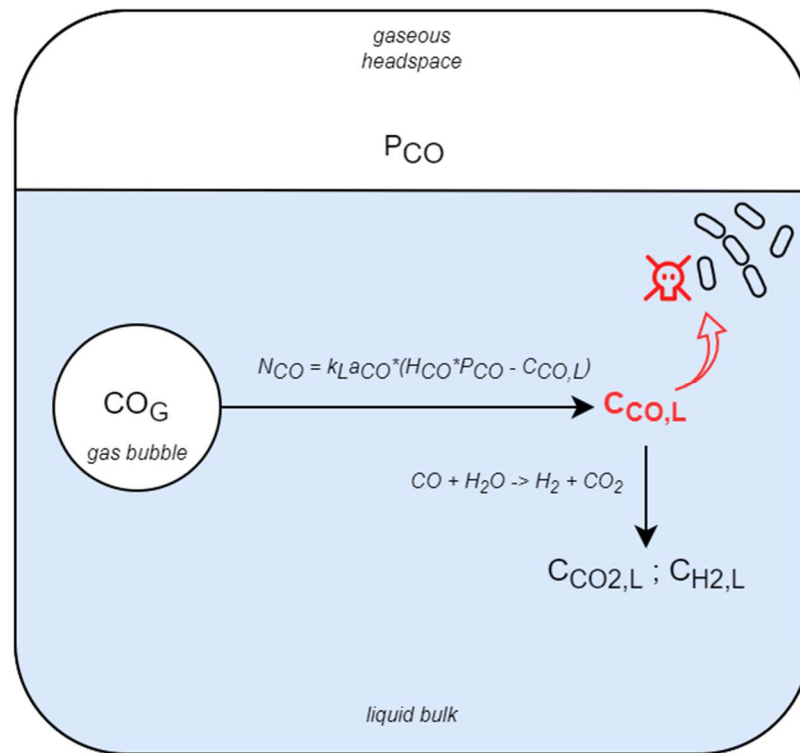
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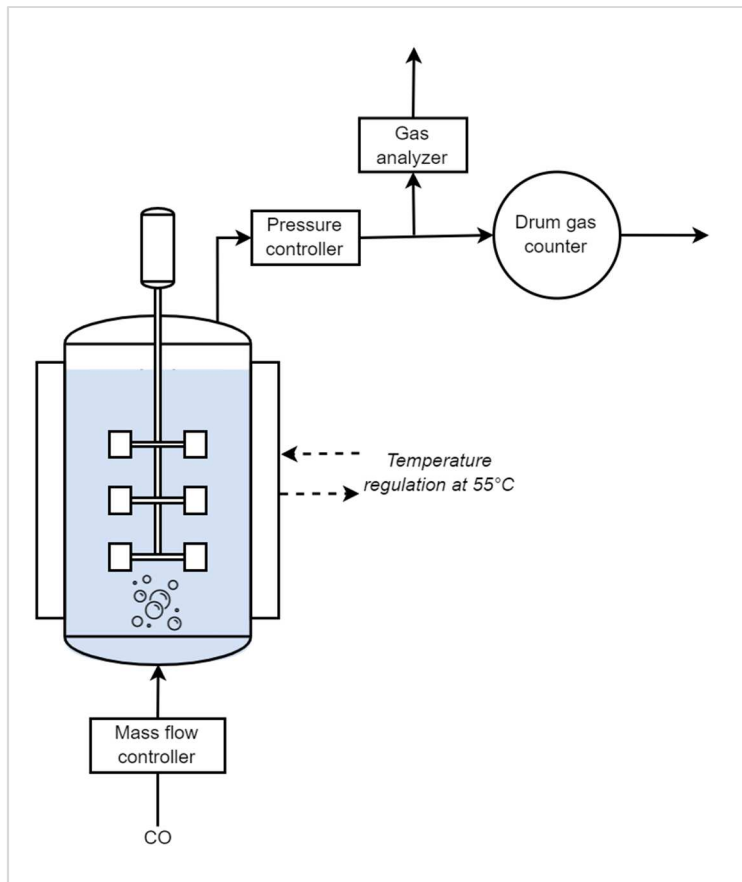
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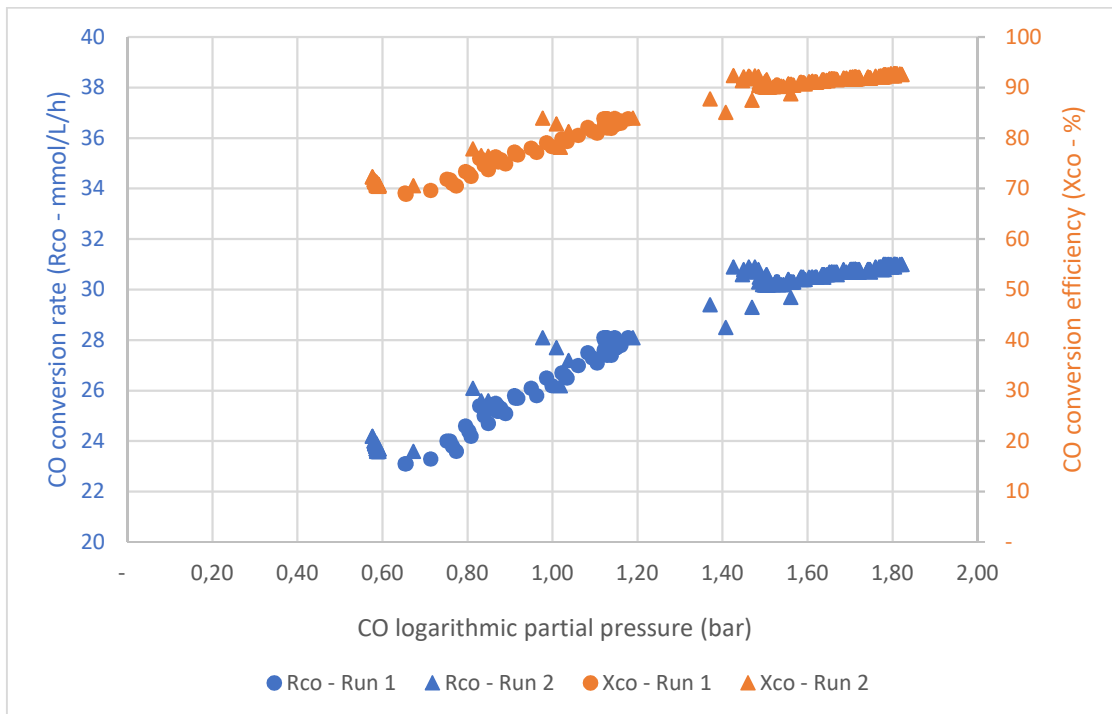
Fig. 1: Simplified representation of the mechanisms impacting the dissolved CO concentration ($C_{CO,L}$). The CO_G from the gas phase is transferred to the liquid according to equation 4, at a rate that depends on the $k_L a$ and the CO partial pressure. Moreover, the dissolved CO is consumed by carboxydophilic microorganisms and converted to H_2 and CO_2 (equation 1). Therefore, $C_{CO,L}$ is a question of equilibrium between the rate at which CO is transferred and the rate at which it is consumed. It should be noted that $C_{CO,L}$ is the concentration of CO experienced by the microorganisms that can induce an inhibition.



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Fig. 2. Simplified scheme of the reactor system.

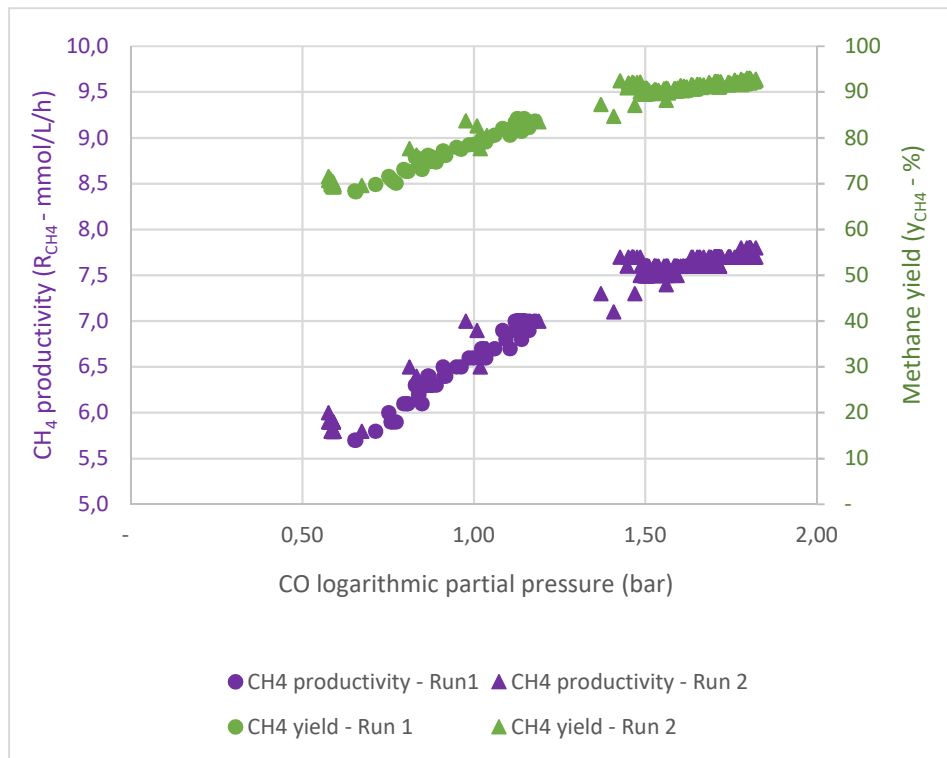


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Fig. 3: Evolution of the CO conversion rate and CO conversion efficiencies with increasing logarithmic CO partial pressure, for run 1 (circles) and run 2 (triangles).



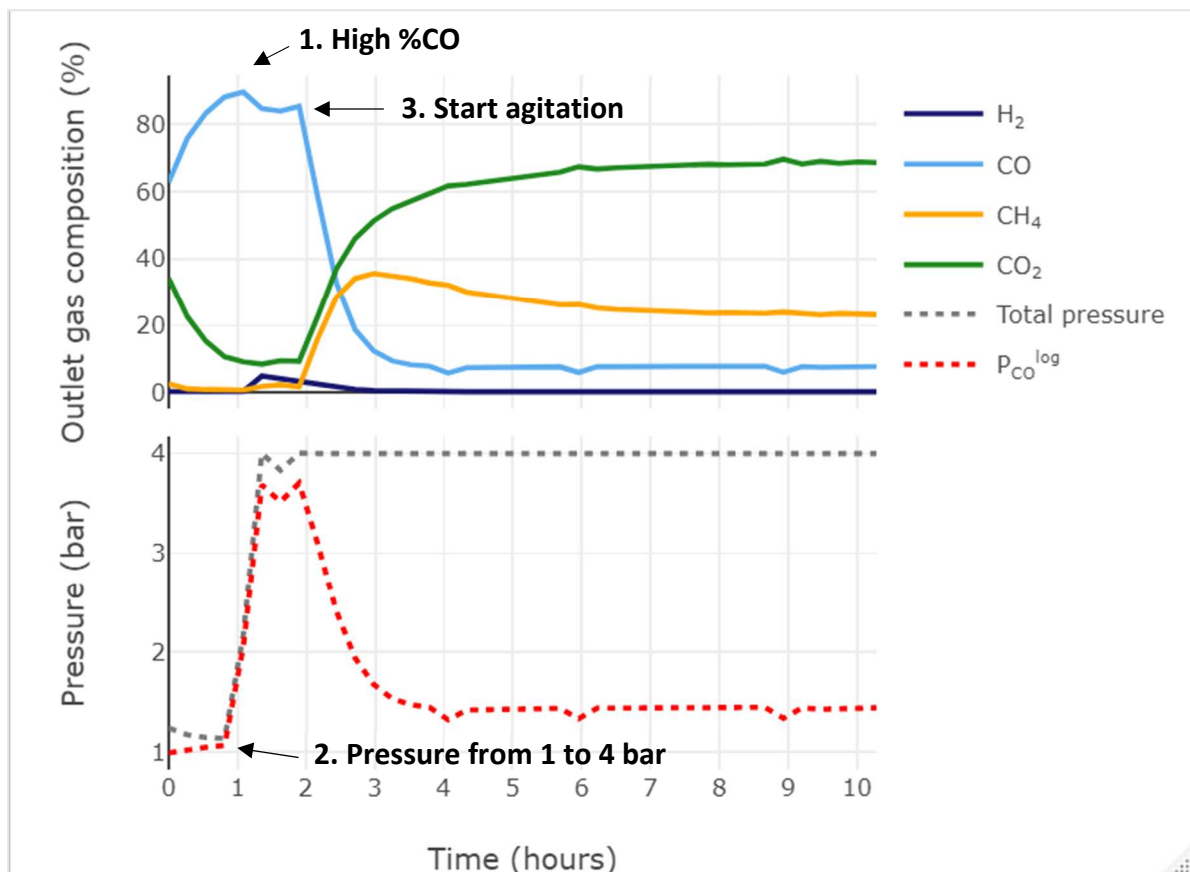
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Fig. 4: Evolution of the methane productivity and methane yield with increasing logarithmic CO partial pressure. Runs 1 and 2 were performed one month apart.

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443 *Fig. 5: Evolution of the outlet gas composition and pressure with time, during the experiment testing an initial high CO*
 444 *partial pressure. 1. Without agitation in order to stop the gas-liquid mass transfer, the reactor was purged with CO until a*
 445 *high outlet CO composition was reached (>90%). 2. The pressure was then increased from 1 to 4 bar. 3. The agitation was*
 446 *restarted, and CO conversion can be observed straight away without apparent CO inhibition.*

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448 *Table 1: Description of the conducted experiments.*

First experiment: gradual pressure increase

1 → 2.4 bar ; 0.1 bar/h Run 1

1 → 3.5 bar ; 0.5 bar/h Run 2

3.5 → 5 bar ; 0.1 bar/h

Second experiment: starting at high pressure

4 bar

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Table 2: Initial pH and concentrations of the liquid medium.

Initial concentrations and pH	Run 1	Run 2
pH	6.5	6.2
Acetic acid (g/L)	0.59	0.60
Propionic acid (g/L)	1.0	1.2
Ammonium (mg/L)	211	291
B (mg/L)	0.367	0.346
Co (mg/L)	0.047	0.089
Cu (mg/L)	0.270	0.173
Fe (mg/L)	1.18	2.02
K (mg/L)	28.1	26.9
Mg (mg/L)	5.24	5.61
Ni (mg/L)	0.622	0.611
S (mg/L)	173	68.0
Zn (mg/L)	0.99	0.952

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