



HAL
open science

Reversibility of hydrolysis inhibition at high hydrogen partial pressure in dry anaerobic digestion processes fed with wheat straw and inoculated with anaerobic granular sludge

Elisabeth A. Cazier, Eric Trably, Jean-Philippe Steyer, Renaud Escudié

► To cite this version:

Elisabeth A. Cazier, Eric Trably, Jean-Philippe Steyer, Renaud Escudié. Reversibility of hydrolysis inhibition at high hydrogen partial pressure in dry anaerobic digestion processes fed with wheat straw and inoculated with anaerobic granular sludge. *Waste Management*, 2019, 85, pp.498-505. 10.1016/j.wasman.2019.01.019 . hal-02625415

HAL Id: hal-02625415

<https://hal.inrae.fr/hal-02625415v1>

Submitted on 21 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Reversibility of hydrolysis inhibition at high hydrogen partial pressure in dry**
2 **anaerobic digestion processes fed with wheat straw and inoculated with anaerobic**
3 **granular sludge**

4 Elisabeth A. Cazier, Eric Trably*, Jean-Philippe Steyer, Renaud Escudie

5 LBE, Univ Montpellier, INRA, 102 avenue des Etangs, 11100, Narbonne, France

6 *corresponding author: eric.trably@inra.fr; Phone: (+33)(0) 468 425 172

7 **Abstract**

8 In dry anaerobic digestion (AD), methanogenic performances are lowered by high solid
9 contents. Low performances are often caused by a decrease of the gas-liquid transfer
10 kinetics leading to local accumulation of inhibitory by-products. Hydrogen was
11 previously identified as an inhibitor of hydrolytic and acetogenic microbial activities in
12 dry AD. CO₂ is also generated but its impact on the microbial activity remains
13 unknown. In this study, the reversibility of dry AD inhibition by high H₂ partial
14 pressure (P_{H_2} of 1 bar) was investigated by adding CO₂ (400 mbars) after 11 and 18
15 days of methanogenesis inhibition, in an AD process operated at 25% TS, using wheat
16 straw as substrate and inoculated with anaerobic granular sludge. As soon as CO₂ was
17 added, the methanogenic activity rapidly recovered within 3 days, from 0.41±0.1 to
18 3.77±0.8 and then 2.25±0.3, likely through the hydrogenotrophic pathway followed by
19 the acetoclastic pathway, respectively. This result was confirmed by the high abundance
20 of *Methanomicrobiales* (83%) and the emergence of *Methanosarcinales* sp (up to 17%)
21 within the methanogens. Furthermore, the recovery kinetics were impacted by the

22 duration of the inhibition period suggesting a different impact of the high P_{H_2} on
23 hydrogenotrophic and acetoclastic methanogens.

24

25 **Keywords**

26 Acidogenesis; Carbon dioxide; Gas transfer; Hydrogen; Solid-State Anaerobic
27 Digestion

28 **1 Introduction**

29 In anaerobic digestion, the organic matter is converted by microorganisms into (1) a
30 biogas composed of CH_4 and CO_2 , and (2) a residual digestate that may be further used
31 as fertilizer if sanitary and environmental requirements are met. Three types of
32 anaerobic digestion (AD) processes are distinguished according to the operational
33 conditions: (1) the wet AD operated at a total solid content (TS) lower than 10%, (2) the
34 semi-dry AD at a TS content between 10 and 20 % and (3) the dry AD, also called
35 solid-state AD, at a TS content above 20% (Abbassi-Guendouz et al., 2012). Since less
36 water is required in dry AD, the digester size as well as the energy demand are both
37 minimized. Dry AD has gained lot of interest for industrial purposes and is now being
38 widely implemented for the treatment of agricultural and ligno-cellulosic residues.

39 In counterpart, dry AD technologies present several disadvantages due to their high TS
40 content, such as a decrease of the AD performances with lower methane yields and
41 some handling difficulties due to the high heterogeneity and viscosity of the substrate
42 (Abbassi-Guendouz et al., 2012; Motte et al., 2013). When the TS content exceeds 30
43 %, the anaerobic digestion process can be rapidly blocked or even strongly inhibited

44 (Abbassi-Guendouz et al., 2012). Such inhibition phenomenon is characterized by a
45 decrease of the biogas production and an increase of the Volatile Fatty Acids (VFAs)
46 concentration (Abbassi-Guendouz et al., 2012; Motte et al., 2013). Indeed, the decrease
47 of the free available water at high TS content results in the reduction of mass transfer
48 kinetics of soluble molecules such as VFAs, or dissolved gases (Bollon et al., 2013).
49 Since dissolved gas diffusion and gas-liquid transfers become rapidly limiting, local
50 accumulation of these by-products can occur and lead to microbial local inhibition in
51 the bulk phase where microorganisms are active (Abbassi-Guendouz et al., 2012).

52 In particular, dissolved hydrogen accumulation rapidly makes the acetogenic reactions
53 thermodynamically unfavourable causing higher VFAs accumulation, a subsequent
54 decrease of the pH and finally methanogenesis inhibition (Guo et al., 2010). Under
55 anaerobic conditions, H_2 is produced by acidogenic bacteria and is immediately
56 consumed in combination with CO_2 by either homoacetogenic bacteria to produce
57 acetate, or hydrogenotrophs to produce methane (Amani et al., 2010). This latter
58 pathway represents about 30% of the CH_4 produced in anaerobic digestion (Amani et
59 al., 2010). All these reactions are reversible in the AD process where acetate oxidation
60 plays also a key role between methanogenic pathways (Karakashev et al., 2006). The
61 local H_2 partial pressure can transitorily increase but must remain low enough to avoid
62 inhibition of syntrophic acetogenic bacteria. When the hydrogen partial pressure (P_{H_2}) is
63 high, VFAs production increases, causing a decrease of the pH to lower value than 6
64 (Guo et al., 2010). Such variation in pH can impact biomass hydrolysis as well as the
65 following steps of acidogenesis and methanogenesis (Siegert and Banks, 2005; Veeken
66 et al., 2000). Indeed, the growth of methanogenic and acidogenic bacteria are strongly
67 affected by the pH (Luo and Angelidaki, 2013).

68 In addition, Cazier et al. (2015) reported that a high initial partial pressure of H₂ in the
69 headspace was the main inhibitory factor affecting wheat straw hydrolysis in dry AD. It
70 was suggested that CO₂ played a key role since H₂ inhibition occurred only in absence
71 of remaining CO₂. Indeed, hydrolysis inhibition did not occur when CO₂ was initially
72 present with H₂ in the reactor headspace. In that case, H₂ was rapidly consumed by
73 homoacetogenic bacteria and methanogens. When anaerobic digestion is efficiently
74 working, it can be assumed that CO₂ and H₂ are both biologically produced during
75 acidogenesis and acetogenesis and are continuously consumed by homoacetogenic
76 bacteria and methanogenic archaea. More CO₂ than H₂ is produced, the overall CO₂
77 content ranging from 30 to 50% of the biogas.

78 However, the exact role of CO₂ on the bacterial activity in AD remains unclear. On the
79 one hand, CO₂ has been reported as inhibitor of the production and degradation of
80 VFAs, as previously shown by Hansson and Molin (1979) and Arslan et al. (2012) who
81 worked on acetate and propionate accumulation at pH 7 and pH 4.5 and under 1 bar of
82 CO₂, respectively. Consistently, it was elsewhere reported that an inhibitory impact of
83 CO₂ on acetogenic and lactic acid bacteria at pH 5.3 (Kim et al., 2006) and on
84 acetoclastic methanogens at pH 7 (Hansson and Molin, 1981). On the other hand,
85 acidogenesis and more particularly H₂ production was shown to be improved by
86 sparging CO₂ before fermentation (at 30 to 300 ml_{CO₂}.min⁻¹) (Bru et al., 2012; Kim et
87 al., 2006). Nonetheless, an inhibitory effect was observed when CO₂ was sparged at
88 higher rate (500 ml_{CO₂}.min⁻¹) (Bru et al., 2012). In contrast, Park et al. (2005) reported
89 that fermentative H₂ production was improved by removing the CO₂. Since all
90 experiments were carried out under different operating conditions and different

91 microbial communities, concluding on the exact impact of CO₂ on the different AD
92 microbial activities remains unclear.

93 The aim of this study was to evaluate the impact of adding CO₂ in mesophilic dry AD
94 when methanogenesis was artificially inhibited by high initial H₂ partial pressure in
95 headspace. Two inhibition durations (11 and 18 days) prior to CO₂ injection were
96 investigated to evaluate the persistence of the inhibitory effect on acidogenic and
97 methanogenic populations.

98 **2 Materials and methods**

99 *2.1 Substrate*

100 Wheat straw (*Triticum aestivum*) was used as substrate. After harvest and collection,
101 wheat straw was fractionated using a cutting miller through a 1 mm grid, and then
102 sieved to collect particles having a size between 400 µm and 1 mm. The TS content of
103 the wheat straw particles was 95%.

104 *2.2 Operating conditions of the batch tests*

105 Industrial UASB anaerobic granules were used to inoculate the batch reactors. The
106 granules were manually broken and mixed during 24 h at 35°C, and were then
107 centrifuged (7 841 g, 20 min, and 4°C) to obtain a homogeneous anaerobic inoculum.
108 The TS content of the inoculum ranged between 10 and 15 %. The substrate/inoculum
109 ratio was fixed at 3 (on basis of the volatile solid contents) (Liew et al., 2012). A buffer
110 solution of sodium bicarbonate (0.0026 g of NaHCO₃·g⁻¹ of substrate) was used to keep
111 the pH at 8 all along the experiment (data not shown). A solution of trace elements
112 (FeCl₂ 2 g·L⁻¹, CoCl₂ 0.5 g·L⁻¹, MnCl₂ 0.1 g·L⁻¹, NiCl₂ 0.1 g·L⁻¹, ZnCl₂ 0.05 g·L⁻¹,

113 H_3BO_3 0.05 $\text{g}\cdot\text{L}^{-1}$, Na_2SeO_3 0.05 $\text{g}\cdot\text{L}^{-1}$, CuCl_2 0.04 $\text{g}\cdot\text{L}^{-1}$, Na_2MoO_4 0.01 $\text{g}\cdot\text{L}^{-1}$) was
114 added (0.2 mL by flask) at start of the experiment, using the same protocol than Cazier
115 et al. (2015). Initial TS content of the mixture (inoculum, wheat straw, sodium
116 bicarbonate solution and trace elements) was fixed at 25% corresponding to the
117 maximal TS value where no inhibitory effect was observed in dry AD (Abbassi-
118 Guendouz et al., 2012; Motte et al., 2013) and to only investigate the reversibility of the
119 inhibition only caused by high hydrogen partial pressure (Cazier et al. (2015)).

120 First, the mixture was introduced into a reactor with a working volume of 3 L operated
121 during 10 days at 35°C under N_2 atmosphere to reach an active phase of
122 methanogenesis and homogenize the substrate/inoculum medium. Then, 20 g of this
123 pre-culture was put into the bottom of a 600 ml flask, which corresponded to a thin
124 layer of less than 1 cm of substrate to limit the influence of the gas diffusion in the
125 medium. The flasks were initially flushed with N_2 gas. Hydrogen was then added to
126 reach an initial H_2 partial pressure of 996 ± 27 mbars, under a total pressure of 1 500
127 mbars. A control, with only N_2 in headspace, was also carried out. All the flasks were
128 then incubated at 35°C for 32 days. In some of the flasks filled with hydrogen, 396 ± 44
129 mbars of CO_2 were added after 11 and 18 days of operation. Batch tests were carried out
130 in triplicates for each condition. Flasks were sampled at day 0, 11, 18, 25 and 32 for
131 further analysis of fermentative metabolite concentrations.

132 *2.3 Analytical methods*

133 Biogas production volume was periodically estimated by measuring the total pressure
134 and the biogas composition. The gas composition was determined using a gas
135 chromatograph Perkin Clarus 580 composed of an injector heated at 250°C and two
136 capillary columns heated at 60°C. The first column corresponded to an RtUbond for the

137 CO₂ and the second column an RtMolsieve used for the detection of the O₂, H₂, N₂ and
138 CH₄. The carrier gas was argon at 350 kPa and under a flowrate of 31.8 ml.min⁻¹. The
139 detection was ensured by a thermal conductivity detector kept at 150°C.

140 Metabolites were quantified by diluting 5 g of digestate in 20 g of deionized water for
141 30 minutes. The mixture was then centrifuged during 20 min at 39 121 g and 4°C and
142 filtrated at 0.2 µm with a nylon membrane using the same protocol than Cazier et al.
143 (2015). VFAs were measured with a gas chromatograph Perkin Clarus 580 equipped
144 with an Elite-FFAP crossbond® carbowax® 15 m column connected to a flame
145 ionization detector at 280°C. Nitrogen was used as carrier gas under a flow rate of 6
146 mL.min⁻¹ (Motte et al., 2013). Other metabolites than VFAs were quantified using high
147 performance liquid chromatograph, e.g. lactic acid and ethanol. The chromatograph was
148 composed of an automatic sampler (Water 717), a pre-column to filter residues (Micro
149 guard cation H refill cartridges, Bio-Rad) and an Aminex HPX-87H column (300 mm
150 on 7.8 mm, Bio-Rad). The carrier eluent was a sulfuric acid solution at 0.005 M under a
151 fixed flowrate of 0.4 ml.min⁻¹.

152 The microbial communities of *Archaea* and *Bacteria* were characterized after DNA
153 extraction and amplification of the V3 region of the 16S rRNA according to the
154 protocols of Braun et al. (2011) and Bru et al. (2012).

155 The PCR products were purified and sequenced, using the Illumina MiSeq System with
156 2x300 bp paired-end chemistry used at the GenoToul sequencing centre
157 (www.genotoul.fr). An average of 46 021 high quality sequences per sample for
158 *Archaea* and for *Bacteria* were retained after assembly, de-multiplexing and cleaning
159 with Mothur software version 1.33.2, as described by Schloss et al. (2009). SILVA

160 release 102 was used for alignment and taxonomic affiliation. Sequences are registered
161 on NCBI database under the accession numbers KY229870 to KY229893 for archaea,
162 and KY234504 – KY235143 for bacteria.

163 2.4 Data analysis

164 R software (version 2.15.2) coupled with the package Rcmdr (version 1.8-4) was used
165 for statistical analysis of the experimental data, using variance analysis (ANOVA).
166 Non-significant p-values were fixed > 0.05 and significant p-values were fixed when $<$
167 0.05 .

168 Total Substrate Degradation (TSD) was estimated from a theoretical Chemical Oxygen
169 Demand (COD) mass balance between the start-up and the end of each experiment, as
170 described elsewhere (Cazier et al., 2015). All calculation was expressed according to the
171 initial TS content of wheat straw (TS_i expressed in grams of dry solids), as follows:

$$172 \text{ TSD} = \text{Final State} - \text{Initial State} = \frac{A_{\text{H}_2,\text{f}} + A_{\text{CH}_4,\text{f}} + A_{\text{met},\text{f}} + A_{\text{GC}}}{\text{TS}_i} - \frac{A_{\text{H}_2,\text{i}} + A_{\text{met},\text{i}}}{\text{TS}_i} \text{ (Eq. 1)}$$

173 where, $A_{\text{H}_2,\text{f}}$ is the amount of H_2 remaining at the end in the headspace, $A_{\text{CH}_4,\text{f}}$ the final
174 amount of accumulated CH_4 , $A_{\text{met},\text{f}}$ the final amount of metabolic products, A_{GC} the
175 total amount of gas (H_2 and CH_4) sampled for analyses, $A_{\text{H}_2,\text{i}}$ the initial amount of H_2
176 added and $A_{\text{met},\text{i}}$ the initial amount of metabolites in the medium. Since all these
177 parameters are expressed in grams of COD, TSD corresponded to gram of COD per
178 gram of initial TS of wheat straw.

179 3 Results and discussion

180 3.1 Recovery of the methanogenic activity after CO₂ addition

181 Figure 1.a shows the cumulated production of CH₄ along reactor operation time. For the
182 reactors carried out at high initial P_{H_2} and, thus, operated under inhibitory conditions,
183 CO₂ was added after 11 and 18 days of operation. The control corresponds to a reactor
184 without initial addition of H₂ in headspace.

185 In the control reactor, a maximal and constant CH₄ production rate was observed after a
186 lag phase of 5 days and reached a value of $2.7 \pm 0.32 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$, equivalent to
187 $2.98 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{VS}}^{-1} \cdot \text{day}^{-1}$. This result is significantly lower than previous reported values
188 of $12 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{VS}}^{-1} \cdot \text{day}^{-1}$ for wheat straw at 22% TS (Liew et al., 2012). Such difference
189 resulted either from different microbial inoculum origins or from a TS content slightly
190 higher in the present experiment (25% TS), considering that 28-30 % TS was previously
191 reported as a threshold value prior inhibition of the methanogenic and acidogenic
192 microbial activities (Abbassi-Guendouz et al. 2012; Motte et al. 2013).

193 In the reactors where H₂ was initially added, a small quantity of CH₄ accumulated the
194 first day of experiment at low production rates of 0.96 ± 0.52 and $0.96 \pm 0.42 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$
195 (Table 1): this production rates correspond to the mean values of the triplicates
196 used to evaluate the addition of CO₂ after 11 and 18 days of inhibition, respectively.
197 Thereafter, CH₄ production was strongly inhibited due to the presence of high partial
198 pressure of H₂ in the headspace, with average production rates of only 0.45 ± 0.1 and
199 $0.38 \pm 0.1 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$. In comparison, the control (only N₂) showed a methane
200 production rate ten times higher at $2.7 \pm 0.3 \text{ ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$ for the same experimental
201 time. Consequently, the amounts of cumulated methane after 11 and 18 days reached

202 only 4 ± 0.5 and 6 ± 1 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1}$ in the inhibited reactors against 20 ± 4 and 39 ± 4
203 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1}$ in the controls, respectively. It was concluded that methanogenesis was
204 clearly inhibited in presence of high initial H_2 partial pressure in headspace (996 ± 27
205 mbars). The corresponding concentration of dissolved H_2 in the medium at 35°C was
206 estimated at $0.58 \pm 5 \times 10^{-2}$ $\text{mg}_{\text{H}_2} \cdot \text{L}^{-1}$. Consistently, a similar value was reported as a
207 threshold H_2 concentration prior to wheat straw hydrolysis inhibition in AD by Cazier et
208 al. (2015).

209 When CO_2 was added in reactor headspace, the methane rapidly accumulated within the
210 first 3 days to reach values of 12 ± 1 and 10 ± 2 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1}$ in the reactors where CO_2
211 was added at day 11 and 18, respectively. This first phase of CH_4 production was called
212 “phase 1”, as shown in Figure 1.b. During phase 1, H_2 and CO_2 were both rapidly
213 consumed until total exhaustion of H_2 in headspace (data not shown). No significant
214 acetate accumulation was observed during the first 7 days after CO_2 addition (Figure 2).
215 It was therefore concluded that H_2 and CO_2 were most likely consumed by
216 hydrogenotrophic methanogens to produce CH_4 . Consistently, methane production rates
217 in phase 1 were higher than in the controls, with 4 ± 0.75 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$ and $3.55 \pm$
218 0.87 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$ for CO_2 added at day 11 and 18, respectively, versus an average
219 value of 2.7 ± 0.3 $\text{ml}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1} \cdot \text{day}^{-1}$ in the controls (Table 1). Such observation strongly
220 supports the fact that efficient hydrogenotrophic methanogenesis was the main
221 methanogenic pathway during phase 1. Moreover, the methanogenic activity recovered
222 immediately after CO_2 addition, suggesting that hydrogenotrophic methanogens were
223 not inhibited at high P_{H_2} , at least during the first 18 days. This is consistent with
224 previous observations where the production of CH_4 by hydrogenotrophic methanogens

225 was previously shown to be favoured at high P_{H_2} (> 5 mbars) in anaerobic digestion
226 systems (Demirel and Scherer, 2008; Schink, 1997).

227 After phase 1, a phase of 4 days, called 'plateau', was observed with only a small
228 amount of CH_4 that accumulated whatever the time of CO_2 addition (Figure 1.b). The
229 CH_4 production rate during the plateau phase was very low, *i.e.* 0.82 ± 0.26 and $0.41 \pm$
230 $0.27 \text{ ml}_{CH_4} \cdot \text{g}_{TS}^{-1} \cdot \text{day}^{-1}$ when the CO_2 was added at 11 and 18 days respectively (Table
231 1). Since no H_2 was present in headspace, this plateau phase corresponded probably to
232 the time for the microbial community to readapt to favourable conditions for substrate
233 degradation, as initially observed in the control, *i.e.* a lag phase of 4 days at the start of
234 the experiment.

235 Afterwards, methane production increased to reach a cumulated methane yield of 16 ± 1
236 $\text{ml}_{CH_4} \cdot \text{g}_{TS}^{-1}$ and $11 \pm 3 \text{ ml}_{CH_4} \cdot \text{g}_{TS}^{-1}$ in 7 days, when CO_2 was added at day 11 and 18,
237 respectively. This second production phase was denominated 'phase 2' (Figure 1.b). In
238 phase 2, the methane production rates decreased by half when compared to phase 1
239 (Table 1), with 2.74 ± 0.45 and $1.61 \pm 0.23 \text{ ml}_{CH_4} \cdot \text{g}_{TS}^{-1} \cdot \text{day}^{-1}$ when the CO_2 was added
240 at 11 and 18 days respectively. Since the CH_4 production rates were substantially
241 different during for the first and second phase, two different methanogenic pathways
242 were likely involved. Indeed, it is well established that hydrogenotrophic
243 methanogenesis is faster than the acetoclastic methane producing pathway (Pan et al.,
244 2016). While CH_4 production in phase 1 seemed to be mainly due to hydrogenotrophic
245 methanogens, methanogenesis was most probably resulting from the degradation of
246 acetate by acetoclastic methanogens in phase 2 (Demirel and Scherer, 2008).

247 Interestingly, CH₄ production rates were slightly higher when CO₂ was added after 11
248 days of inhibition (4 ± 0.75 and 2.74 ± 0.45 ml_{CH₄}·g_{TS}⁻¹·day⁻¹ for phases 1 and 2,
249 respectively) than 18 days (3.55 ± 0.87 and 1.77 ± 0.23 ml_{CH₄}·g_{TS}⁻¹·day⁻¹ for phases 1
250 and 2, respectively). Such a difference suggests a cumulative inhibitory effect of the
251 time of exposure to H₂ on both hydrogenotrophic and acetoclastic methanogens.

252 3.2 Impact of the P_{H₂} on other metabolic by-products dynamics

253 Figure 2 presents the accumulation of metabolic by-products (VFAs and methane), the
254 remaining hydrogen in the controls and in the reactors carried out with high initial P_{H₂}.
255 In the controls, microbial metabolites, *i.e.* all VFAs, formate, succinate, and ethanol,
256 transitorily accumulated after 11 and 18 days of operation (35 ± 2 and 39 ± 5
257 mg_{COD}·g_{TS}⁻¹ at day 11 and 18, respectively) likely because of the high TS content, and
258 then decreased to 7 ± 2 mg_{COD}·g_{TS}⁻¹ at day 32, confirming the efficient methanogenic
259 activity even at 25% TS.

260 In comparison, the amount of metabolites was higher in the reactors where H₂ was
261 initially added. The concentrations of metabolites reached 58 ± 5 mg_{COD}·g_{TS}⁻¹ at day 11
262 and 82 ± 17 mg_{COD}·g_{TS}⁻¹ at day 18 prior to CO₂ addition (Figure 2). These values
263 corresponded to a total concentration in metabolites of about 20 ± 2 g·L⁻¹ at day 11, and
264 28 ± 6 g·L⁻¹ at day 18, respectively. Such value is above the inhibitory limit of 20 g·L⁻¹
265 as previously reported in wet AD processes (Siegert and Banks, 2005). Interestingly,
266 most of the hydrogen consumed at day 11 and at day 18 (69 ± 2 mg_{COD}·g_{TS}⁻¹ and 72 ± 2
267 mg_{COD}·g_{TS}⁻¹) corresponds to the concentration of produced metabolites (51 ± 2
268 mg_{COD}·g_{TS}⁻¹ and 75 ± 17 mg_{COD}·g_{TS}⁻¹ at day 11 and 18 prior to CO₂ addition,
269 respectively). The small difference at day 11 between the hydrogen recoveries into
270 metabolites (18 mg_{COD}·g_{TS}⁻¹) could correspond to the methane produced during this time

271 $(17.5 \pm 4 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1})$. Such high concentration of metabolites might have been the
272 cause of the strong inhibition of methanogenic activity at high P_{H_2} , prior to CO_2
273 addition. Since VFAs did not accumulate during this period, it can also be concluded
274 that hydrolysis and/or acidogenesis may also have been inhibited under these
275 conditions, prior to CO_2 addition, as previously reported by Cazier et al. (2015).

276 Furthermore, the increase in the total amount of metabolites at high initial P_{H_2} was
277 mostly due to an increase of acetate, and, at a lower extent, butyrate and isobutyrate.
278 The acetate concentration increased from $2 \pm 0 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ to $35 \pm 4 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ at
279 day 11 and $43 \pm 11 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ at day 18, prior to CO_2 addition. Meanwhile, the
280 butyrate and isobutyrate concentration increased from $1.7 \pm 0 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ at start to $9 \pm$
281 $0.2 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ and $18 \pm 4 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ at day 11 and 18, respectively. Such increase of
282 acetate and butyrate concentrations under an atmosphere rich in H_2 was previously
283 observed during the anaerobic conversion of carbohydrates-rich wastes (Arslan et al.,
284 2012). These authors reported an increase of 31% and 51% of acetate and butyrate
285 production, respectively, under a P_{H_2} of 2 bars in comparison to only N_2 .

286 When CO_2 was added after 11 days of inhibition, no metabolite degradation was
287 observed during the first 7 days after CO_2 addition (phase 1), confirming the assumption
288 of a dominant hydrogenotrophic pathway producing methane (Figure 2). The decrease
289 of the total COD concentration between the day of addition of CO_2 (day 11) and 7 days
290 after, was probably due to the fact that the analysis of metabolites and acetate was only
291 done in one sacrificed replicate and not all replicates. Therefore, ANOVA was used to
292 statistically compare the results for each.

293 7 days after CO₂ addition, a decrease of the total metabolites concentration was
294 observed, and was mostly due to acetate consumption. This observation is consistent
295 with the recovery of the methanogenic activity. The acetate content decreased from 33 ±
296 2.4 mg_{COD}·g_{TS}⁻¹ to 8 ± 6 mg_{COD}·g_{TS}⁻¹ between day 7 and 14, respectively. Such
297 difference between the acetate concentration at day 0 and 14 after CO₂ addition was
298 statistically significant (ANOVA, p-value <0.05). The decrease of acetate concentration
299 was likely due to the conversion of acetate into CH₄ by acetoclastic methanogens
300 (Pavlostathis and Giraldo-Gomez, 1991) or to the oxidation of acetate by acetate-
301 oxidizing bacteria into H₂ and CO₂ that are then converted to CH₄ by hydrogenotrophic
302 methanogens (Karakashev et al., 2006). A similar trend was observed when CO₂ was
303 added after 18 days with an acetate content that decreased from 30 ± 10 mg_{COD}·g_{TS}⁻¹ at
304 day 7 to 18 ± 3 mg_{COD}·g_{TS}⁻¹ at day 14 (Figure 2). Interestingly, acetate degradation from
305 day 7 to 14 (phase 2) was slower when CO₂ was added after 18 days of inhibition. This
306 result was likely due to the time of exposure of acetate-degrading methanogens at high
307 H₂ partial pressure. In other studies, a specific inhibitory effect was observed on the
308 growth of *Methanosarcina* sp. when H₂ partial pressure was increased from 2.5 to 20
309 mbars and a specific effect on acetate degradation was observed (Ahring et al., 1991).
310 Such observation is also supporting the fact that hydrogenotrophic methanogens were
311 most probably the most efficient CH₄ producers in phase 1 since no significant
312 difference between the two times of exposure.

313 3.3 Impact of the P_{H_2} on the overall substrate degradation

314 To estimate the impact of the P_{H_2} on the global microbial activity, the overall substrate
315 degradation was calculated in mg_{COD}·g_{TS}⁻¹ using Equation 1, which takes in
316 consideration the amount of H₂ initially added (Figure 3).

317 In the reactors where CO₂ was added at day 11 or day 18, the substrate degradation was
318 very similar 14 days after CO₂ addition, with $55 \pm 9 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ and 54 ± 17
319 $\text{mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$, respectively. Therefore, the impact of the time exposure on the methane
320 production rate in phase 2, was probably not due to a persistent effect on the global
321 microbial activity since the overall substrate degradation was the same after 14 days, but
322 more likely to a transitory accumulation of metabolites due to a slower methanogenic
323 activity, as shown in Figure 2. Nevertheless, when comparing these values to the
324 control, the global substrate degradation was lower in the reactors operated at high
325 initial P_{H_2} for a close duration of operation. About $80 \pm 5 \text{ mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$ were reached at
326 day 11 in the control that is substantially higher than in inhibited reactors. All these
327 observations suggest that the high initial P_{H_2} had very likely a persistent inhibitory
328 effect on the hydrolytic activity of the consortium.

329

330 The exact mechanisms behind microbial hydrolysis are still uncertain and probably
331 highly diverse when considering complex substrates. Two main mechanisms have been
332 proposed in the AD model (ADM1): (1) the enzymes are directly secreted into the
333 liquid phase by hydrolytic microorganisms with a direct effect on substrate hydrolysis
334 that releases free sugars on the bulk phase or (2) the microorganisms attach on the
335 substrate surface with the formation of a biofilm and produce enzymatic complexes to
336 disrupt the organic material (Batstone et al., 2002). Recently, Cazier et al. (2015)
337 reported an initial a strong inhibitory effect of high P_{H_2} on the hydrolytic activity in dry
338 AD. High P_{H_2} could have either influenced the production or secretion of extracellular
339 enzymes by retro-inhibition, or reduced the physiological activity of the
340 microorganisms. Similarly to the present study, these experiments were carried out with

341 thin layer of substrate to reduce gas transfer limitation and investigate the local effect of
342 H₂ partial pressure. In dry AD reactors, the effect of gas transfer limitation must also to
343 be considered. Since diffusion coefficients decrease when TS contents increase (Bollon
344 et al., 2013), dissolved gas diffusion and gas-liquid transfer may become a limiting
345 factor (Abbassi-Guendouz et al., 2012), with a local accumulation of H₂ and CO₂ in the
346 medium. With a substrate rich in carbohydrates and at high TS content, hydrolysis and
347 acidogenesis is highly favoured with a rapid production of VFAs, CO₂ and H₂. That
348 could lead to a local accumulation of H₂ since CO₂ could be dissolved in carbonates at
349 high pH. Therefore, if the local P_{H_2} is high enough, hydrolysis may therefore be
350 inhibited, especially if the local P_{H_2} is low. The results of the present study suggest that
351 the addition of CO₂ in dry AD digester may improve the methanogenic performances
352 not only by increasing the gas-transfer kinetics, but also by reducing the local P_{H_2}
353 through H₂ consumption. Adding CO₂ may also present inhibitory effects on AD
354 performances if the medium is not properly buffered since CO₂ could decrease the pH
355 down to 6 that has a strong inhibitory effect on methanogens (Ward et al., 2008).
356 However, this is unlikely to occur since tests operated under similar conditions with
357 only CO₂ added in headspace (no H₂) were carried out and no impact on dry AD
358 performances was observed (data not shown).

359 3.4 Impact of the P_{H_2} on microbial community dynamics

360 The compositions in *Archaea* and *Bacteria* of the microbial communities were
361 determined in the control reactor, and in the reactors containing a high P_{H_2} before CO₂
362 addition, and 7 days (end of 'plateau' phase) and 14 days (end of phase 2) after CO₂
363 addition (Table 2).

364 First, the composition in *Archaea* in the inoculum (day 0) was mainly dominated by
365 hydrogenotrophic methanogens (*Methanobacteriales*: 91.6%) followed by mostly
366 acetoclastic methanogens (*Methanosarcinales*: 5.8%), as already described by Amani et
367 al. (2010). Interestingly, in the control, the relative proportion of acetoclastic
368 methanogens increased over the experimental time to reach 17 % after 18 days. Such
369 variation in the type of methanogens was already reported in dry AD (31% TS) during
370 the start-up period followed by a stabilization period, for a semi-continuous
371 thermophilic reactor treating the organic fraction of municipal solid waste (Montero et
372 al. 2008). A higher proportion in *Methanosarcinales* sp. might indicate that an efficient
373 microbial process occurred in the controls, as previously suggested in dry AD by
374 Abbassi-Guendouz (2013).

375 In the reactor where CO₂ was added at day 11, the overall composition of the archaeal
376 community did not significantly change. Indeed, the microbial community was
377 composed of 90-91% *Methanobacteriales* and only 6-8% *Methanosarcinales* all along
378 the experiment. Since hydrogenotrophic methanogens (*Methanobacteria* sp. and several
379 *Methanosarcina* sp.) were present in much higher concentration than acetoclastic
380 methanogens (*Methanosarcina* sp. only), hydrogenotrophic CH₄ production from H₂
381 and CO₂ was likely more efficient than from acetate. Such microbial community
382 structure is in accordance with an absence of acetate accumulation during the first 7
383 days after CO₂ addition. After a time between 7 and 14 days necessary to reactivate
384 acetotrophic pathways by *Methanosarcinales*, a subsequent decrease of acetate
385 concentration was observed.

386 In comparison, the composition of the archaeal community was significantly different
387 when CO₂ was added at day 18 (Table 2). In that case, the percentage of

388 *Methanosarcinales* amongst *Archaea* was not only initially higher but also increased
389 from 12% to 17% at the end of the experiment. This result suggests that CH₄ production
390 from acetate was most probably higher when CO₂ was added after 18 days than after 11
391 days. As reported elsewhere, acetate metabolism in *Methanosarcinales* starts to be
392 inhibited with only 2.5 mbars of H₂ (Ahring et al., 1991). Therefore, a higher
393 composition in *Methanosarcinales* supports a higher persistence of the inhibitory effect
394 of the initial high P_{H_2} . Nonetheless the final increase in *Methanosarcinales* relative
395 abundance might indicate a recovery of efficient methanogenesis in these conditions.

396 Characterization of the bacterial community showed clear differences between control
397 reactors and inhibited reactors (either 11 or 18 days). Although all other clusters of
398 bacteria remained in similar proportion, the relative abundance of *Clostridiales*
399 increased in inhibited reactors at a similar extent from 30-31% to 40-42% during the
400 first 7 days after CO₂ addition. Meanwhile, the proportion of *Bacteroidales* decreased
401 from 24-32% to 19-21% in inhibited reactors even though their relative abundance
402 reached up to 47% after 18 days in the control. Since many members of *Clostridiales*
403 are involved in hydrolytic and acidogenic activities in AD, a reactivation of the
404 hydrolytic activity after H₂ inhibition seemed have to be carried out by members of the
405 *Clostridiales* order. The imbalance between *Bacteroidales* and *Clostridiales* orders
406 might have resulted from a differential sensitivity to inhibitor exposure. Consistently,
407 Abbassi-Guendouz et al. (2013) reported a *Clostridium* sp. enrichment when dry AD of
408 cardboard was inhibited by metabolite accumulation suggesting a higher resistance of
409 these microorganisms to detrimental conditions of growth (low pH, high P_{H_2}).

410 **4 Conclusion**

411 In this study, inhibition of dry AD at high initial H₂ partial pressure was found to be
412 reversible by adding CO₂ whatever the time of exposition to H₂. The reversibility
413 occurred in two steps, with a very probable first consumption of H₂ and CO₂ by
414 hydrogenotrophic methanogens followed by acetoclastic methanogen. Methanogenic
415 performances depended then on the time of exposure to high P_{H_2} with a persistent
416 impact on AD kinetics. These results suggest that injecting CO₂ may represent a
417 solution to improve solid-state AD at high TS content by avoiding local inhibition of
418 H₂.

419

420 **Acknowledgements**

421 We thank Gaëlle Gevaudan for the technical assistance with the microbial analysis of
422 the experiments.

423 This work was supported by the French Environment and Energy Management Agency
424 (ADEME) and the French Institute for Agricultural and Food Research (INRA), by
425 funding EA Cazier's PhD.

426 **5 References**

427 Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgènes, J.-P., Steyer,
428 J.-P., Escudié, R., 2012. Total solids content drives high solid anaerobic digestion
429 via mass transfer limitation. *Bioresour. Technol.* 111, 55–61.

- 430 Ahring, B.K., Westermann, P., Mah, R.A., 1991. Hydrogen inhibition of acetate
431 metabolism and kinetics of hydrogen consumption by *Methanosarcina*
432 *thermophila* TM-1. *Arch. Microbiol.* 157, 38–42.
- 433 Amani, T., Nosrati, M., Sreekrishnan, T., 2010. Anaerobic digestion from the viewpoint
434 of microbiological, chemical, and operational aspects — a review. *Environ. Rev.*
435 18, 255–278.
- 436 Arslan, D., Steinbusch, K.J.J., Diels, L., De Wever, H., Buisman, C.J.N., Hamelers,
437 H.V.M., 2012. Effect of hydrogen and carbon dioxide on carboxylic acids patterns
438 in mixed culture fermentation. *Bioresour. Technol.* 118, 227–34.
- 439 Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V, Pavlostathis, S.G., Rozzi, A.,
440 Sanders, W.T.M., Siegrist, H., Vavilin, V. a, 2002. The IWA Anaerobic Digestion
441 Model No 1 (ADM1). *Water Sci. Technol.* 45, 65–73.
- 442 Bollon, J., Benbelkacem, H., Gourdon, R., Buffière, P., 2013. Measurement of diffusion
443 coefficients in dry anaerobic digestion media. *Chem. Eng. Sci.* 89, 115–119.
- 444 Braun, F., Hamelin, J., Gévaudan, G., Patureau, D., 2011. Development and application
445 of an enzymatic and cell flotation treatment for the recovery of viable microbial
446 cells from environmental matrices such as anaerobic sludge. *Appl. Environ.*
447 *Microbiol.* 77, 8487–93.
- 448 Bru, K., Blazy, V., Joulain, C., Trably, E., Latrille, E., Quéméneur, M., Dictor, M.-C.,
449 2012. Innovative CO₂ pretreatment for enhancing biohydrogen production from

450 the organic fraction of municipal solid waste (OFMSW). *Int. J. Hydrogen Energy*
451 37, 14062–14071.

452 Cazier, E.A., Trably, E., Steyer, J.P., Escudié, R., 2015. Biomass hydrolysis inhibition
453 at high hydrogen partial pressure in solid-state anaerobic digestion. *Bioresour.*
454 *Technol.* 190, 106–113.

455 Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic
456 methanogens during anaerobic conversion of biomass to methane: A review. *Rev.*
457 *Environ. Sci. Biotechnol.* 7, 173–190.

458 Guo, X.M., Trably, E., Latrille, E., Carrère, H., Steyer, J.-P., 2010. Hydrogen
459 production from agricultural waste by dark fermentation: A review. *Int. J.*
460 *Hydrogen Energy* 35, 10660–10673.

461 Hansson, G., Molin, N., 1981. End product inhibition in methane fermentations: effects
462 of carbon dioxide on fermentative and acetogenic bacteria. *Eur. J. Appl. Microbiol.*
463 *Biotechnol* 13, 242–247.

464 Karakashev, D., Batstone, D.J., Trably, E., Angelidaki, I., 2006. Acetate oxidation is the
465 dominant methanogenic pathway from acetate in the absence of
466 *Methanosaetaceae*. *Appl. Environ. Microbiol.* 72, 5138–41.

467 Kim, D., Han, S., Kim, S., Shin, H., 2006. Effect of gas sparging on continuous
468 fermentative hydrogen production. *Int. J. Hydrogen Energy* 31, 2158–2169.

469 Liew, L.N., Shi, J., Li, Y., 2012. Methane production from solid-state anaerobic
470 digestion of lignocellulosic biomass. *Biomass and Bioenergy* 46, 125-132.

471 Luo, G., Angelidaki, I., 2013. Co-digestion of manure and whey for in situ biogas
472 upgrading by the addition of H₂: process performance and microbial insights.
473 *Appl. Microbiol. Biotechnol.* 97, 1373–81.

474 Motte, J.-C., Escudié, R., Bernet, N., Delgènes, J.-P., Steyer, J.-P.P., Dumas, C.,
475 Delgenes, J.-P.P., Steyer, J.-P.P., Dumas, C., 2013. Dynamic effect of total solid
476 content, low substrate/inoculum ratio and particle size on solid-state anaerobic
477 digestion. *Bioresour. Technol.* 144, 141–148.

478 Pan, X., Angelidaki, I., Alvarado-Morales, M., Liu, H., Liu, Y., Huang, X., Zhu, G.,
479 2016. Methane production from formate, acetate and H₂/CO₂; focusing on kinetics
480 and microbial characterization. *Bioresour. Technol.* 218, 796–806.

481 Park, W., Hyun, S.H., Oh, S.-E., Logan, B.E., Kim, I.S., 2005. Removal of Headspace
482 CO₂ Increases Biological Hydrogen Production. *Environ. Sci. Technol.* 39, 4416–
483 4420.

484 Pavlostathis, S.G., Giraldo-Gomez, G., 1991. Kinetics of anaerobic treatment. *Water*
485 *Sci. Technol.* 24, 35–59.

486 Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation.
487 *Microbiol. Mol. Biol. Rev.* 61, 262–280.

488 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,
489 Lesniewski, R. a., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,
490 Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-

491 source, platform-independent, community-supported software for describing and
492 comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.

493 Siegert, I., Banks, C.J., 2005. The effect of volatile fatty acid additions on the anaerobic
494 digestion of cellulose and glucose in batch reactors. *Process Biochem.* 40, 3412–
495 3418.

496 Veeken, A., Kalyuzhnyi, S., Scharff, H., Hamelers, B., Kalyuzhnyi, S., 2000. Effect of
497 pH and VFA on hydrolysis of organic solid waste. *J. Environ. Eng.* 6, 1076–1081.

498 Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the
499 anaerobic digestion of agricultural resources. *Bioresour. Technol.* 99, 7928–40.

500

501 **6 Tables captions**

502 **Table 1 :** Methanogenic activity performances (cumulated CH₄ production, CH₄
503 production rate) for the different phases, in the control (with no H₂ initially added) and
504 for reactors with initial H₂ in headspace at a partial pressure of 996 ± 27 mbars and
505 where CO₂ was added at day 11 and 18.

506 **Table 2 :** Phylum and class of *Archaea* and *Bacteria* presents in the control (without
507 gas added) and when the CO₂ was added at 11 and 18 days in % (results of the
508 sequencing) at 0, 11, 18, 25 and 32 days after the beginning of the experiment.

509

510 **7 Figures captions**

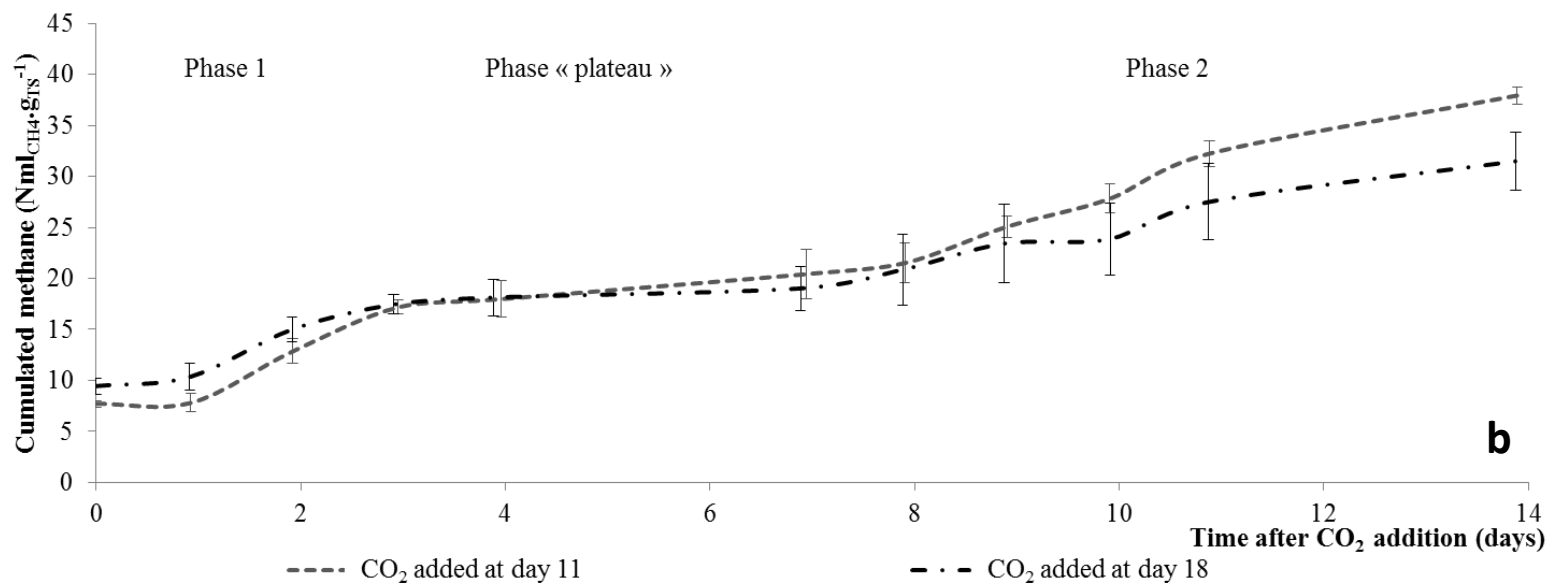
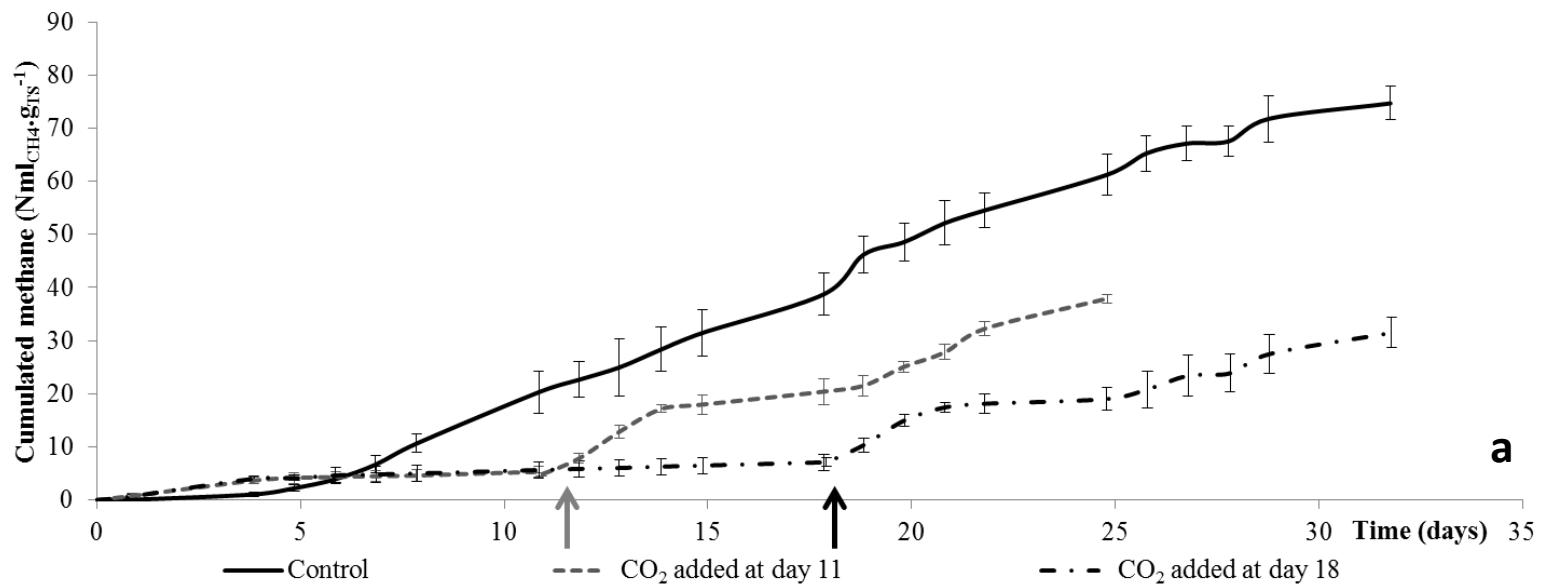
511

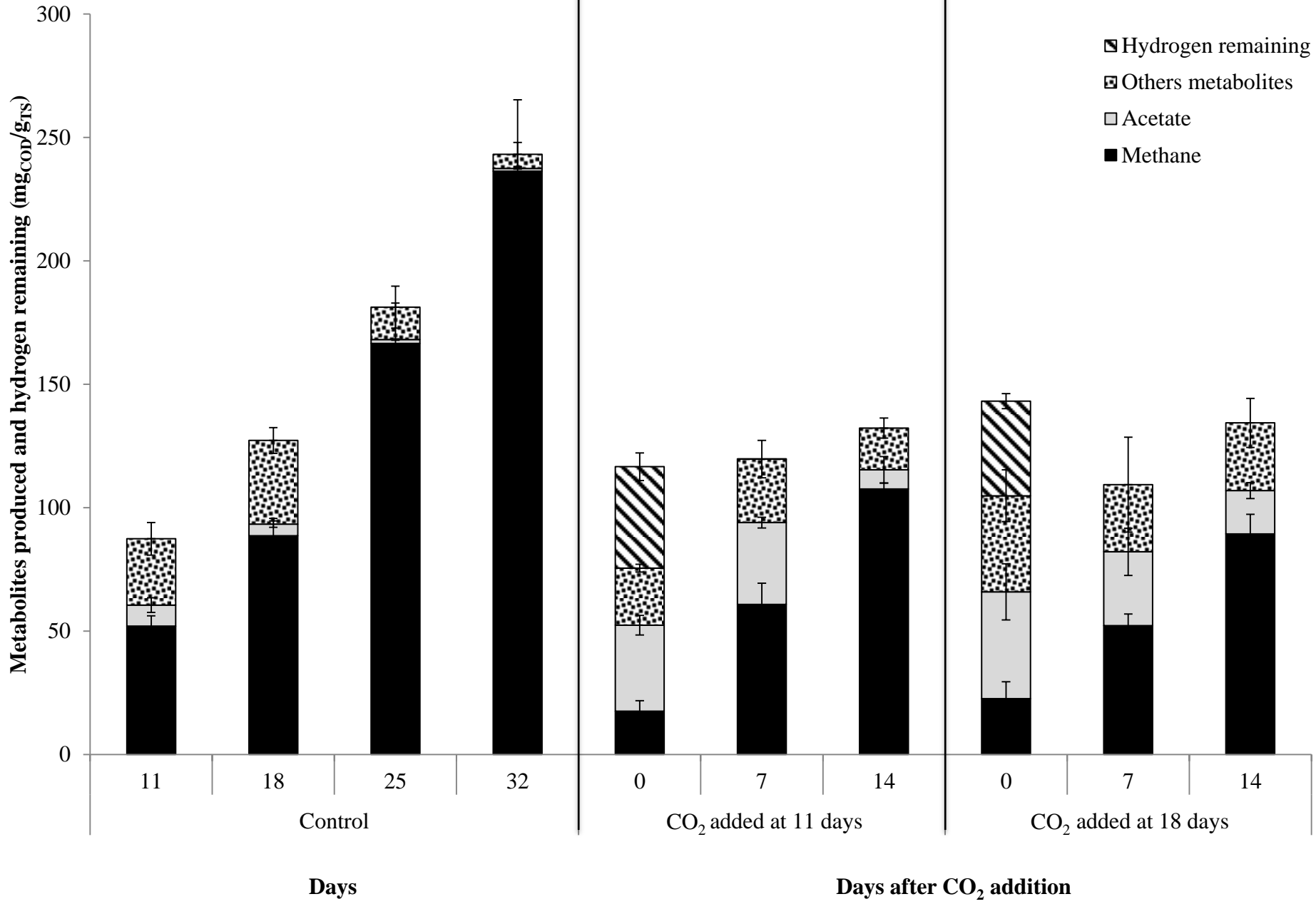
512 **Figure 1:** Cumulative methane production (in $\text{mL}_{\text{CH}_4} \cdot \text{g}_{\text{TS}}^{-1}$), for reactors with H_2
513 initially present in headspace and where CO_2 was added after 11 and 18 days of
514 operation; according to (A) the time of reactor operation or (B) the normalized time
515 after CO_2 addition . Tests were operated at pH 8, 25% TS and at 35°C . The grey and
516 black arrows show the time when CO_2 was added at 11 and 18 days, respectively.

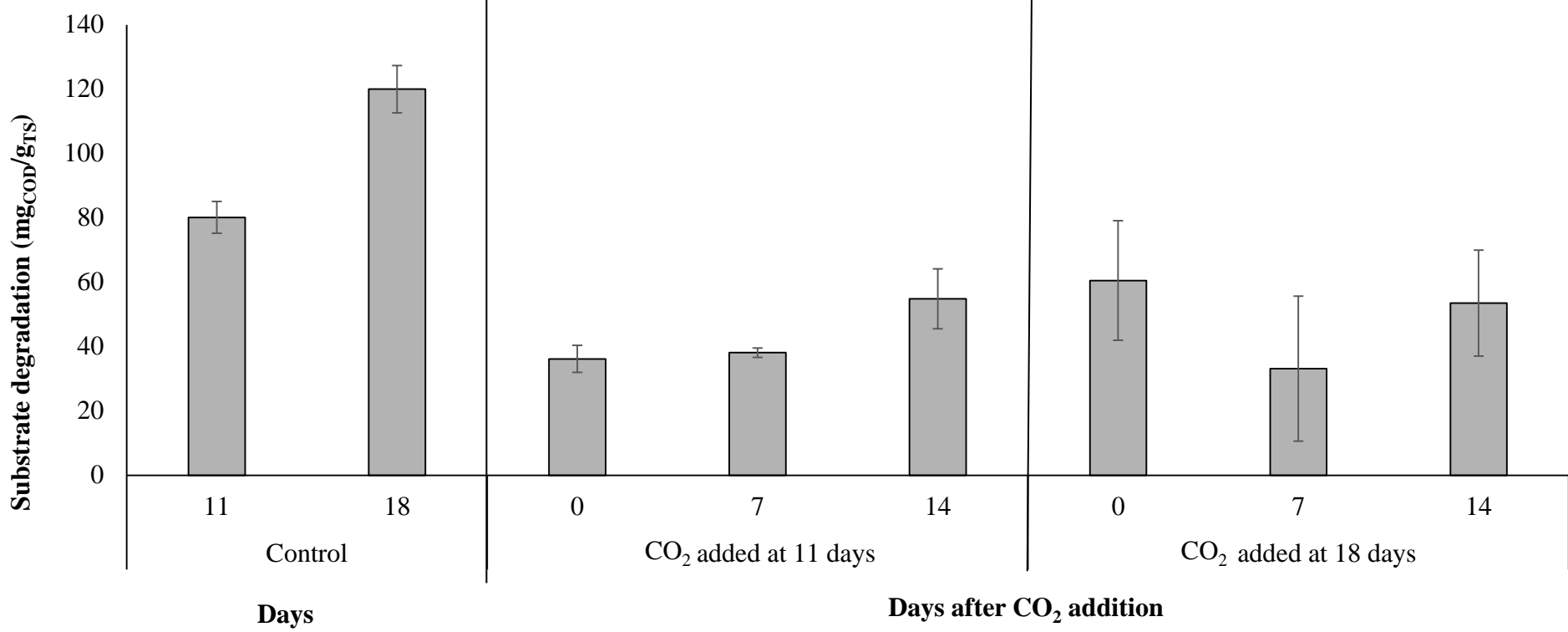
517 **Figure 2:** Metabolites production (in $\text{mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$) according to the time of operation in
518 the control (only N_2 initially in headspace), and according to the time after CO_2 addition
519 after 11 and 18 days of operation of the reactors running at high P_{H_2} . All tests were
520 carried out at pH 8, 25% TS and 35°C .

521 **Figure 3:** Substrate degradation in $\text{mg}_{\text{COD}} \cdot \text{g}_{\text{TS}}^{-1}$, according to the time after adding CO_2
522 (CO_2 added at 11 and 18 days) and since the beginning (control)

523







		Phases	Time (days of operation)	Time since CO ₂ addition (days)	Cumulated CH ₄ produced per phase (Nml.g _{TS} ⁻¹)	Average CH ₄ production rate (Nml.g _{TS} ⁻¹ .day ⁻¹)
Control		Lag phase	0 to 5		2 ± 0.6	0.45 ± 0.13
		Exponential phase	5 to 32		72 ± 3	2.7 ± 0.32
CO₂ added at 11 days	Before CO₂ addition	Start phase	0 to 1		0.8 ± 0.4	0.96± 0.52
		Inhibition phase	1 to 11		4 ± 0.5	0.45 ± 0.14
	After CO₂ addition	CH ₄ production phase 1	11 to 14	0 – 3	12 ± 1	4 ± 0.75
		Lag phase	14 to 18	3 – 7	4 ± 2.4	0.82 ± 0.27
		CH ₄ production phase 2	18 to 25	7 - 14	16 ± 1	2.74 ± 0.45
CO₂ added at 18 days	Before CO₂ addition	Start phase	0 to 1		0.8 ± 3	0.96 ± 0.42
		Inhibition phase	1 to 18		6 ± 1	0.38 ± 0.1
	After CO₂ addition	CH ₄ production phase 1	18 to 21	0 – 3	10 ± 2	3.55 ± 0.87
		Lag phase	21 to 25	3 – 7	3 ± 2.1	0.41± 0.27
		CH ₄ production phase 2	25 to 32	7 – 14	11 ± 3	1.77 ± 0.23

Total operation time (days)		Control			CO ₂ added at day 11			CO ₂ added at day 18		
		0	11	18	11	18	25	18	25	32
Time after CO ₂ addition (days)		--	--	--	0	7	14	0	7	14
Class	Order									
<i>Archaea</i>										
<i>Methanobacteria</i>	<i>Methanobacteriales</i>	91.6%	81.7%	78.1%	90.8%	89.6%	89.8%	83.4%	83.0%	79.8%
<i>Methanomicrobia</i>		5.8%	15.6%	17.0%	6.2%	6.9%	7.8%	13.4%	13.0%	17.3%
	<i>Methanomicrobiales</i>	0%	1%	1%	0.2%	0.3%	0.2%	0%	1%	1%
	<i>Methanosarcinales</i>	6%	15%	16%	6%	7%	8%	13%	12%	17%
<i>Thermoplasmata</i>		0.5%	0.1%	0.4%	0.5%	0.7%	0.6%	0.3%	0.5%	0.5%
<i>Bacteria</i>										
<i>Clostridia</i>	<i>Clostridiales</i>	26.4%	29.5%	19.7%	31.0%	39.8%	46.8%	30.3%	41.9%	39.9%
<i>Bacteroidia</i>	<i>Bacteroidales</i>	29.2%	41.5%	46.9%	24.4%	20.5%	18.5%	31.9%	25.2%	20.9%
<i>Spirochaetes</i>	<i>Spirochaetales</i>	10.3%	15.0%	17.6%	10.5%	7.7%	15.5%	10.6%	6.1%	20.5%
<i>Synergistia</i>	<i>Synergistales</i>	5.6%	1.7%	2.1%	4.1%	3.9%	2.6%	5.3%	4.5%	3.0%
<i>Anaerolineae</i>	<i>Anaerolineales</i>	5.1%	1.3%	1.4%	7.7%	7.4%	3.3%	5.3%	5.2%	2.2%
<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	3.8%	1.4%	1.3%	2.4%	2.7%	1.2%	2.8%	4.3%	1.8%