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1 Evaluation of chemical-free microwave pretreatment on methane yield of two 2 grass biomass with contrasted parietal content

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8

9 Abstract

10 As a result of increasing demand for alternatives to fossil energy, the agricultural biogas
11 sector is in expansion and lignocellulosic biomass (LCB) represents an interesting renewable
12 feedstock. Nevertheless, due to the recalcitrance and complexity of its structure,
13 deconstructive pretreatments are necessary to render possible biochemical conversions and
14 efficient biomass exploitation. In this work, chemical-free, mild microwave pretreatment was
15 evaluated (through BMP tests) as a method to improve anaerobic biodegradability of two
16 grass biomass of industrial relevance and contrasted parietal content: corn stalks (low
17 parietal content, high soluble content) and miscanthus (high parietal content, low soluble
18 content). BMP tests carried out on raw biomass before pretreatment highlighted the negative
19 correlation of BMP value to lignin and cellulose contents and the positive correlation to
20 soluble and hemicellulose contents.

21 Efficiency of microwave pretreatment under two conditions, open vessel and high pressure (4
22 bars), with water as unique solvent was tested for tackling recalcitrance and results were
23 compared to conventional heating pretreatment and a control treatment. Solid and liquid
24 phases were separated after pretreatment with the aim to elucidate if microwave treatment
25 had an impact on organic matter solubilisation and/or on the residual solid phase, which
26 could improve the biodegradability of the pretreated solid fraction. To the authors'
27 knowledge, this is the first study to dissociate methane production of the solid phase from
28 that of the liquid phase after microwave pretreatment.

29 Observed BMP values in mesophilic conditions of raw biomass samples were 286
30 NLCH₄/kgVS for corn stalks and 228 NLCH₄/kgVS for miscanthus respectively (in agreement
31 with literature). No significant improvement in BMP value nor in CH₄ production kinetics
32 were observed following microwave pretreatment, while a harsh chemical pretreatment (10h
33 soaking in 10% w/w NaOH) allowed 30% increase in BMP value. These results highlight the
34 significant chemical effect -compared to thermal- on the biomass deconstruction and fibers
35 breakdown during chemical-free microwave pretreatment. A synergy microwave effect with
36 could allow to allow higher impact on biomass recalcitrance using lower NaOH amounts than
37 chemical treatment alone.

38

39 **Keywords**

40 microwave technology, anaerobic digestion, corn stalk, miscanthus, lignocellulosic biomass

41

42 **1. Introduction**

43 In September 2015, the 193 UN Member States adopted the Sustainable Development Agenda
44 2030, which encourages countries to “mobilize efforts to end all forms of poverty, fight
45 inequalities and tackle climate change”, with a vision of transforming our world by eradicating
46 poverty while ensuring transition to sustainable development (United Nations, 2020).
47 Following the objectives for 2020 focused on energy aspects, the European objectives for
48 2030 target GHG emissions reduction by 55% compared to 1990, 32% of renewable energy in
49 the overall energy mix, and at least 32.5% improvement in energy efficiency (European
50 Commission, 2020). Biogas is one of the renewable energy sources that can contribute to
51 attain these objectives (Bhatia et al., 2019) and LCB from agricultural residues and energy
52 crops represents a promising sourcing because of their energetic potential per hectare
53 (Rechberger et al., 2019). The ambitious objectives fixed by EU policies have greatly aroused
54 interest in LCB utilization for bioenergy and green chemistry applications, but solutions are
55 required in order to render biorefinery installations economically viable. Among other issues,
56 it is necessary to solve the problems of land-use conflict, ineffective biomass supply and
57 upstream transformation processes (ADEME, 2017).

58 World biogas production is still low with respect to the significant untapped potential that
59 represent the available sustainable feedstocks (EBA, 2019): according to the International
60 Energy Agency, biogas (plus biomethane) production in 2018 was around 35 Mtoe, while the
61 estimated overall sustainable potential is estimated to 570 Mtoe for biogas and 730 Mto for
62 biogas plus biomethane (IEA, 2020). In Europe for example, biogas contribution to bioenergy
63 was 7.8% in 2015 (Scarlat et al., 2018), but it is expected that this production increase in the
64 next years because of the implementation of national policies to develop the energy
65 production from renewable resources (García and Daboussi, 2016; IEA, 2020). Nevertheless,
66 this sector requires solutions to improve the yields of anaerobic digestion installations in
67 order to render them profitable. Among other issues, it is necessary to solve the problem of
68 resistance to degradation (or recalcitrance) of lignocellulosic by-products from agriculture
69 and food industries, considered as cheap substrates, but which pretreatment can require large
70 investment costs (Kampman et al., 2017).

71 Biomass cell wall, composed of cellulose, hemicelluloses and lignin, is organized as a physical
72 barrier limiting biological degradation thus pretreatments are necessary to deconstruct the
73 LCB network in order to allow biomass transformation processes (Bichot et al, 2018; Zhao et
74 al., 2012b). Indeed, development of efficient but sustainable pretreatments is one of the main
75 technico-economical challenges that limit the expansion of biogas installations, as
76 pretreatment has been considered the second most expensive step in the biomass-to-energy
77 transformation process (Den et al, 2018). LCB pre-treatments have thus been extensively
78 addressed and numerous works have been published in the last years. Multiple technologies
79 (thermal, biochemical, mechanical and enzymatic, or a combination of them) have been tested
80 in order to improve anaerobic biodegradability of a wide diversity of LCB substrates, as oil
81 palm empty fruit bunches pretreated by wet oxidation (Lee et al. (2020); combined
82 thermal-chemical treatment of rice straw (Kim et al., 2018); sonication of maize straw and
83 dairy manure (Zou et al., 2016) or municipal solid waste (Rasapoor et al., 2016),
84 hydrothermal treatment of grass (Phuttaro et al, 2019), among many others. A recent review
85 by Kumar and Sharma (2017) provide an update on different methods of pretreatment for
86 lignocellulosic biomass.

87 Mild microwave treatment was chosen in this study because of its potential as
88 low-environmental impact pretreatment: rapid heating in bulk biomass and the possibility of
89 using less water and less chemical reactants than other thermal treatments (Kostas et al.,
90 2017). Indeed, since the early 2000s, studies dealing with microwave pretreatment aiming at
91 deconstructing LCB have been carried out, but to a considerably lesser extent than other
92 physico-chemical pretreatments. According to literature review (Table S1), microwave
93 pretreatment studies concern mainly 3 applications: 1) Polysaccharides release (the greatest
94 proportion), for ethanol production mostly; applied to wheat straw (Saha et al., 2008; Xu et
95 al., 2011; Aguilar-Reynosa et al., 2017; Tsegaye et al., 2019), rice straw (Sakdaronnarong et al.,
96 2017), rapeseed straw (Lu et al., 2011), brewers' spent grain (Ravindran et al., 2018) and
97 sugar cane bagasse (Zhu et al, 2016; Moodley and Kana, 2017), among other substrates; 2)
98 Phenolic molecules release, applied to rice bran (Wataniyakul et al., 2012), bulrush (Oussaid
99 et al., 2018), spent grain (Moreira et al., 2012), among others; 3) Energy (H_2 or CH_4)
100 production, applied to wheat straw (Jackowiak et al., 2011a; Sapci, 2013; Nordmann et al.,
101 2014), rice straw (Kainthola et al., 2019), switchgrass and/or miscanthus (Jackowiak et al.,
102 2011b; Irmak et al., 2018), among others.

103 Always according to this literature review, the effect of microwaves on recovery of
104 polysaccharides or phenolic molecules is improved, while the effect on anaerobic
105 biodegradability, assessed by BMP tests (box 1) is less clear: methane production kinetics
106 increase by 68% using microwave pretreated switchgrass was observed by Jackowiak et al.
107 (2011b), while BMP value was not modified using microwave pretreated wheat straw
108 (Nordmann et al., 2014) or increased by 28% (Jackowiak et al., 2011a). Moreover, Sapci
109 (2013) did not observe any improvement in BMP value of different straws (wheat, oat or
110 barley straws) even using harsh microwave conditions (between 200°C and 300°C for
111 15min); while Kan et al (2018) reported 52% increase of BMP value of brewer's spent grain
112 after microwave pretreatment. So far, it can be said that the effect of microwave on biogas
113 production is not clear-cut: it can be positive or neutral.

114 The objective of the present study was thus to determine the effect of microwave
115 pretreatment on anaerobic biodegradability of two LCB of industrial interest: corn stalks (CS)

116 and miscanthus (MSC). Both have good energetic potential per hectare (189 GJ/ha for
117 miscanthus and 170 GJ/ha for corn stalks, according to Somer et al. (2014)), but whose
118 energetic yield could be improved with an adapted pretreatment. Two microwave conditions
119 (open vessel and pressurized vessel) were tested and results were compared to conventional
120 heating pretreatment and a control. Pretreatment effect on biomass was evaluated in terms of
121 methane production (BMP and kinetics) by gDM separately on solid and liquid phases with
122 the aim to elucidate if microwave treatment had an impact on organic matter solubilisation
123 and on the lignocellulosic network of the residual solid phase which could improve the
124 biodegradability of the pretreated solid fraction. To the authors' knowledge, this is the first
125 study to dissociate methane production of the solid phase from that of the liquid phase after
126 pretreatment.

127

128 **Box 1** BMP (Biochemical Methane Potential) is the “maximum amount of methane that can be
129 recovered from a substrate per mass of substrate organic matter as volatile solids (VS) or
130 chemical oxygen demand (COD)” according to Koch et al, 2020. BMP test is a protocol widely
131 used to assess the methane potential/anaerobic biodegradability of a substrate (Filer et al,
132 2019). It is performed by monitoring the biogas produced per specific amount of substrate
133 (S_0) by a specific amount of inoculum (X_0), i.e. anaerobic sludge, in a closed reactor.

134

135 2. Materials and Methods

136 The following section describes the different materials and methods involved in this study and
137 particularly develops pretreatments setting up and BMP tests performing.

138

139 2.1. Raw biomass and inoculum

140 Two corn stalks (CS) genotypes were involved in this study: F 98902 noted CS1 and F 7025
141 noted CS2. Both were harvested in September 2016 by INRAE IJPB (Versailles-Grignon unit,
142 Versailles Cedex, 78026, France). Three miscanthus clones (MSC) were studied and noted
143 MSCB for *M. x giganteus* Britannique, MSCF for *M. x giganteus* Floridulus and MSCR for *M.*
144 *sinensis* Rotsilber. They were harvested in February 2017 by INRAE Agrolmpact (Estrées

145 Mons experimental unit, Péronne, 80203, France). Samples were grounded to 1mm using two
146 successive crushers (Viking, model GE 220, STIHL, Stuttgart, Germany and Fritsch Pulverisette
147 19), sieved to retain only particles between 0.2mm and 1mm and kept in closed boxes at
148 ambient temperature before usage. Biomass composition were compared between
149 2016/2017 and 2018 (date of the study) in order to determine whether storage had an
150 impact on the biomass. No significant differences in biomass composition were detected
151 before and after storage (results not shown) and biomass were considered to be of identical
152 composition between 2018 and the harvest date.

153 The inoculum used in the study was provided by EMIN LEYDIER paper mill (573 Route des
154 Ortis, 26240 Laveyron, FRANCE). It consisted of anaerobic sludge, stored at 35°C before
155 usage.

156

157 *2.2. Chemicals and biomass composition analysis*

158 All treatments and analysis were performed using chemicals from Merck and High purity
159 water (Merck Millipore Quantum TEX).

160 Before any treatment, dry matter rate (DM) and volatile solid rate (VS) were determined. DM
161 corresponds to a sample dry residue after total evaporation of water at 105°C (NREL, 2008).
162 Volatile solid (VS) is the mass of organic matter contained in a dry residue, obtained after, at
163 least 2 hours, carbonization at 550°C (NREL, 2008).

164 Biomass composition was determined using Van Soest protocol (Van Soest and Goering,
165 1970) which is based on mass sequential partitioning of cell walls, from most extractible to
166 less extractible, with successive extractions using different solvents (water, neutral detergent
167 solution, acid detergent solution and sulfuric acid 72%). Van Soest protocol permitted to
168 determine alterations in the amount of parietal polymers, consisting of hemicelluloses,
169 cellulose and lignin, between the raw biomass and the pretreated biomass.

170 COD (Chemical Oxygen Demand) measurement was carried out on solid biomass and liquid
171 phase after pretreatment using kits (AQUALYTIC 420721 Küvettentest CSB Vario MR-COD
172 Vario). COD of liquid phase was expressed in mgO₂/L by diluting 0.2mL of the liquid sample in
173 1.8mL of high purity water in the kit. COD of solid sample was expressed in mgO₂/gDM. 1g of

174 the sample was first soaked in 5mL H₂SO₄ for 12 hours, with stirring. After this time, the
175 sample was considered fully diluted in the acid and high purity water was added to reach a
176 volume 250mL. 0.5mL was collected and mixed in the kit with 1.5mL of high purity water. In
177 both case COD was determined by reading absorbance at 610nm.

178

179 *2.3. Pretreatments*

180 Various pretreatments have been tested in this study including microwave pretreatment,
181 compared to conventional heating pretreatment and a control pretreatment with no heating.
182 The operating conditions are described below.

183

184 *2.3.1. Microwave pretreatment*

185 Microwave pretreatments were performed using a Minilabotron 2000 (SAIREM, FRANCE) lab
186 pilot, operating at 2.45GHz with a maximum power of 2kW. This equipment was used to
187 perform two pretreatments types: microwave pretreatment heating at atmospheric pressure
188 (open vessel) named MWH and pressurized microwave pretreatment heating, named PMWH.
189 These two pretreatments were chosen to evaluate the impact of two very different microwave
190 conditions on the BMP. At atmospheric pressure, the microwave conditions have been
191 optimized in a previous paper (Bichot et al., 2019a) to release phenolic acids and it would be
192 interesting to see if these conditions also increased the BMP. Under pressure, the operating
193 conditions demonstrated a more important impact on the biomass structure than at
194 atmospheric pressure, especially concerning hemicelluloses solubilisation, which could allow
195 to increase the BMP (Bichot et al., 2020). In both cases, the operating conditions were thought
196 out upstream and adapted to the microwave pilot used in order to be as adequate as possible.
197 All treatments were performed in duplicate and at constant incident power. As the objective
198 of this study was to develop green physico-chemical pretreatment, water without chemical
199 reactants was used as solvent.

200 The microwave pretreatment at atmospheric pressure, named MWH for microwave heating,
201 was performed using a glass reactor in the following conditions: 14g of raw material were
202 mixed with 285g of water, corresponding to 4.7%DM (dry matter). These conditions have

203 been determined as optimal ones with the microwave pilot and the biomass used and were
204 determined in a previous study (Bichot et al., 2019a). After one hour of soaking in water at
205 ambient temperature, the reactor was closed with a glass cover connected to a refrigerant for
206 avoiding water evaporation during treatment. The treatment lasted 800s at 710W
207 corresponding to an incident power density of 2.4W/g. The development of this pretreatment
208 has been described by Bichot et al. (2019a).

209 After the treatment, the reactor was air-cooled for 15min before opening. Reaction mixture
210 was filtered through a 200 μ m sieve. Solid was washed with 300mL of deionized water to
211 remove by-products. The solid fraction was placed in an oven for 7 days at 40°C to dry in
212 order to be stored without deterioration. Moreover, drying permitted to measure dry matter
213 content to determine the amount of solubilised matter during processing. The amount of
214 recovered solid ($\text{g}_{\text{pretreated solid biomass}}/\text{g}_{\text{dry raw matter}}$) was an indicator of the effectiveness of the
215 treatment. The supernatant was filtered through cellulose filter (2.7 μ m) and stored at -20°C
216 until BMP tests. The final volume was considered as the initial volume subtracted from the
217 volume absorbed by the material, called swelling volume and equal to 1mL/gDM. No
218 evaporation occurred in open vessel trials due to the refrigerant.

219 The pressurized microwave pretreatment, named PMWH for pressurized microwave heating,
220 was performed using a PTFE hydrolyzing digestion vessel (PTFE/TFM.BOLA (T18) with
221 membranes (Cat. No. A250-08) resisting pressure up to 20bar. Following preliminary tests
222 (Bichot et al., 2020), 2g biomass were added to 40mL water in the reactor corresponding to
223 4.7%DM. No magnetic nor mechanical stirring was implemented as the reaction mixture
224 mixed itself during boiling. Samples underwent one-hour pre-soaking in water at ambient
225 temperature before the microwave treatment, which lasted 180 seconds at 300W,
226 corresponding to an incident power density of 7.03W/g. After treatments, samples were
227 processed as described before. Energy consumption was not discussed here, but the energy
228 balance was done and was presented elsewhere (Bichot et al., 2019b).

229

230 2.3.2. Conventional pretreatment

231 Conventional heating (CH) treatment was used to compare thermal effects on methane
232 production from microwave heating and from conventional heating. 14g of raw biomass were
233 mixed to 285g water in the glass reactor. After one-hour soaking, the glass cover connected to
234 the refrigerant was immersed in an oil bath at 110°C for 800s. After treatments, samples were
235 processed as described before.

236

237 2.3.3. Control treatment

238 A control treatment, with no heating (NoH), was also carried out: 14g of biomass were added
239 to 285g water in a beaker. Liquid and solid phases were separated after one hour of soaking at
240 room temperature, without any heating. After treatments, samples were processed as
241 described before.

242

243 2.4. Biochemical methane potential tests

244 The BMP (Biochemical Methane Potential) tests were carried out according to the standard
245 protocol of the laboratory in 569mL serum bottles covered with rubber stoppers. Each bottle
246 contained 2gVS of inoculum and 1gVS of raw or pretreated solid or 1g of COD (liquid phase) in
247 order to attain a S_0/X_0 ratio of 0.5. The bottles were N_2 flushed before being closed and
248 incubated at 35°C with constant agitation for at least 60 days. For each pretreatment
249 condition studied, four bottles were prepared: two for the solid phase and two for the liquid
250 phase.

251 Two positive controls in which substrate was replaced by ethanol, easily biodegradable, were
252 carried out to verify the good activity of the inoculum, which was always the case during this
253 study. Moreover, two negative controls without substrate were also prepared to determine
254 the residual methane potential of the inoculum. This endogenous production was then
255 removed to each test production to calculate the net methane potential.

256 Biogas production was measured every two days for the two first weeks and subsequently
257 every three or four days. Produced biogas was analysed with a gas chromatograph (Varian
258 Micro GC CP 4900). Vector gas was nitrogen, injection volume was 200nL for an injection time
259 of 40ms. The two columns used were: Molsieve 5Å for the separation and analysis of N_2/O_2 ,

260 CH₄, CO and NO, operating at 40°C and 21psi; and OPoraPLOT Q for the separation and
261 analysis of CO₂, SO₂, operating at 40°C and 21psi.

262 BMP results were expressed in NLCH₄/kgVS for solid phases or LCH₄/kgCOD for liquid phases.
263 In order to evaluate the impact of pretreatment on the kinetics of methane production, the
264 first-order kinetic constants were calculated using the least-squares fit of methane production
265 vs. time (t) with the following equation:

$$V = V_{\max} (1 - e^{-kt}),$$

266 with V the volume of methane in NLCH₄/kgVS, V_{max} the maximum producible methane volume
267 in NLCH₄/kgVS, k the first-order kinetics constant in days⁻¹ and t the digestion time in days.
268 The Microsoft Excel Solver function was used to determined V_{max} and k. The model had
269 already been applied under the same operating conditions and with the same devices by
270 Thomas et al. (2018), that demonstrated the relevance of the model to miscanthus raw
271 biomass with R²>0.95. This unique model was chosen as the objective of the study was not to
272 determine the model that best matched the data but to highlight the impact of pretreatments
273 on BMPs value.

274

275 3. Results and discussion

276 Results of the study are presented in the following: first the raw biomass compositions were
277 analysed and BMP values determine. Then BMP tests were carried on two biomass of interest
278 in order to produce more biogas.

279

280 *3.1. Raw biomass composition and BMP results*

281 As a first step, raw materials composition were analysed and an effort was made to
282 understand the impact of biomass composition on BMP values. Then biomass were pretreated
283 by various pretreatments (microwave heating MWH, pressurized microwave heating PMWH,
284 conventional heating CH and no heating considered as control NoH) and BMP tests were
285 carried out in order to determine the effect of the pretreatment on BMP values.

286

287 3.1.1. Raw biomass composition

288 In order to determine the biomass composition, biomass samples were analysed in triplicate
 289 by Van Soest method and results are summarized in Table 1.

290

291

Table 1: Composition of raw biomass used for this study

		CS1	CS2	MSCB	MSCF	MSCR
Dry matter	%	92	92	92	92	92
Van Soest soluble	%DM	37.30 ± 1.9	35.70 ± 1.3	8.29 ± 0.2	5.77 ± 2.4	6.63 ± 4.5
Cell wall	%DM	62.70 ± 1.9	64.30 ± 1.3	91.71 ± 0.2	94.23 ± 2.4	93.37 ± 4.5
Distribution:						
Hemicelluloses	%DM	26.00 ± 0.9	30.09 ± 1.5	22.91 ± 3.6	25.86 ± 0.4	35.68 ± 1.2
Cellulose	%DM	28.51 ± 0.9	27.36 ± 1.5	52.78 ± 3.5	51.78 ± 1.6	47.33 ± 2.4
Lignin	%DM	6.85 ± 1.5	5.30 ± 1.0	15.46 ± 0.4	16.21 ± 0.8	10.14 ± 2.1
Ash	%DM	1.18 ± 0.4	1.55 ± 0.6	0.56 ± 0.4	0.38 ± 0.38	0.22 ± 1.9
BMP	NLCH ₄ /kgVS	287 ± 23	285 ± 7	228 ± 8	250 ± 2	278 ± 5

292

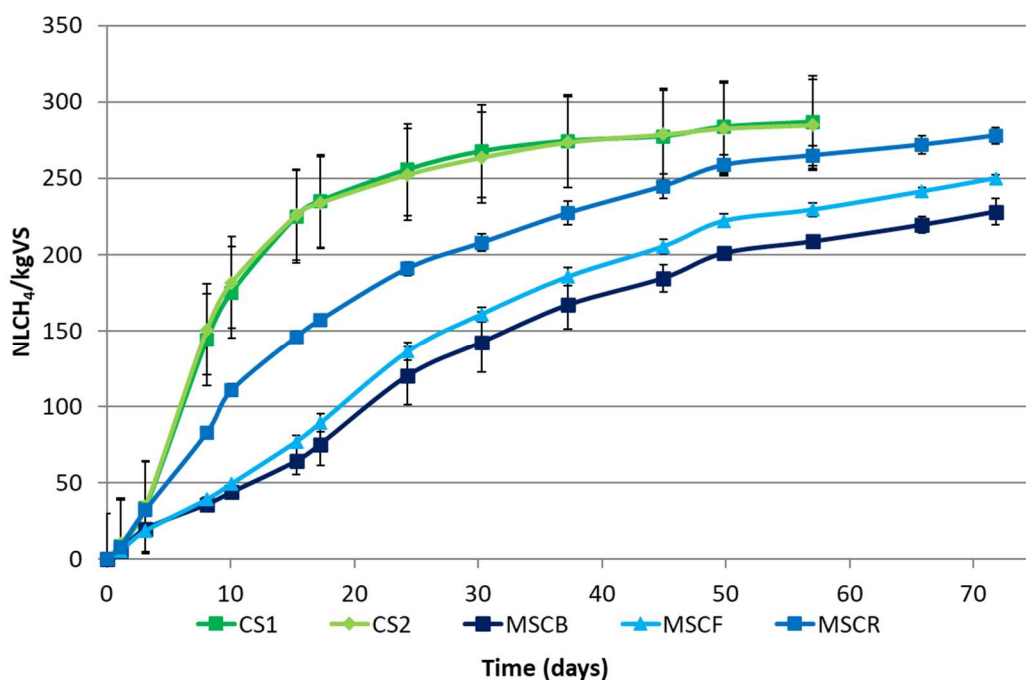
293 According to Table 1, CS and MSC were very different in terms of composition. The proportion
 294 of cell wall (equivalent to parietal polymers) vs Van Soest soluble content in MSC is more than
 295 10 times higher than in CS, respectively 13.5:1 and 1.7:1 ratio in average of the DM%. More
 296 precisely, lignin contents in MSC were on average twice as much as corn stalks content in
 297 lignin. Within MSC types, the highest BMP value observed was for MSCR, which had the lowest
 298 lignin content and the highest hemicelluloses content. The lowest BMP observed was related
 299 to the MSCB, which had the highest cellulose content and a high lignin content. These results
 300 concerning raw CS and MSC composition were consistent with those in literature. Van der
 301 Weijde et al. (2013) outlined proportions of 27-40% cellulose, 25-34% hemicelluloses and
 302 9-15% lignin in corn stover and 28-49% cellulose, 24-32% hemicelluloses and 15-28% lignin
 303 in miscanthus.

304

305 3.1.2. BMP of raw biomass

306 The Biochemical Methane Potential (BMP) of the different raw biomass samples were first
 307 determined, with the aim of choosing the genotype for CS - or the clone for MSC- with the

308 lowest methane potential (having the more room for improvement). Figure 1 presents
309 methane production curves with respect to time for the raw biomass studied.



310
311

Figure 1: Methane production curve vs. time for raw biomass (CS1, CS2, MSCB, MSCF and MSCR)

312 From Figure 1, it can be observed that anaerobic biodegradation pattern is not the same for CS
313 and MSC. For both CS1 and CS2, methane production led to a BMP value of 286 NLCH₄/kgVS
314 on day 57, with significant error bars between the duplicates, certainly due to samples
315 heterogeneity. Sun et al. (2015) and Sawatdeenarunat et al. (2015) respectively registered
316 256 NLCH₄/kgVS and 291 NLCH₄/kgVS for corn stalks methane potential and thus it can be
317 considered that BMP values observed in the present study were consistent with literature
318 analysis.

319 Concerning MSC, differences were observed in BMP final values for the different clones, with
320 lower error bars than those observed with corn samples, certainly due to lower samples
321 heterogeneity. Indeed, BMP value of MSCB and MSCF were significantly lower than MSCR
322 (respectively -17% and -10%) (p value = 0.00463). This result was in agreement with
323 Thomas et al. (2019) who observed that miscanthus BMP was largely dependent on the clone
324 considered. Thus, in the present study, the initial biomass had a significant impact on BMP (p
325 = 0.01688). For MSC, biodegradation was two times slower for MSCR, 3 times slower for
326 MSCF and 4 times slower for MSCB than for CS samples; and led to a BMP value respectively

327 2.8%, 12.5% and 19.5% lower than CS BMP value, but biodegradation was not completed
328 when the experience was stopped on day 75; actually, the stationary phase had not yet been
329 reached (Figure 1).

330 No lag phase was observed, neither for CS nor for MSC and the methane production increased
331 in an exponential way since the start-up of the tests, especially in the case of CS (Figure 1).
332 The fast methane production at the early stages of the reaction corresponded to the
333 biodegradation of molecules easily degradable by microorganisms, such as soluble sugars. It
334 could also be explained by the very small particle size, as Filer et al. (2019) recommended to
335 crush the particles at less than 10mm, which was largely the case in this study. Then, the
336 methane production slowed down, this phase corresponding to the hydrolysis of less
337 accessible molecules such as parietal sugars (Phuttaro et al., 2019). As soluble content,
338 predominantly sugars, in CS was higher than in MSC, methane production rate was higher. On
339 the contrary, MSC had a higher parietal content, that had to be first hydrolysed by
340 microorganisms explaining the slower kinetics in the case of MSC than CS. (Phuttaro et al.,
341 2019).

342 Using the first order kinetic model, V_{\max} and k could be determined for each raw biomass with
343 a reliable approximation ($R^2 > 0.98$) and values are summarized in Table 2. For a good fit of a
344 model, Joglekar and May (1987) suggested that the R^2 should be superior to 0.8, which was
345 the case in the present study Table 2. The kinetic constant k was equal to 0.09 for CS and was
346 more than 4 times inferior (0.02) for MSC, confirming the previous predictions that MSC
347 kinetic was slower than CS. Moreover, according to Table 2, the maximum theoretical volumes
348 were 289 NLCH₄/kgVS and 286 NLCH₄/kgVS for CS1 and CS2 respectively. These values were
349 close to the actual volumes produced (Figure 1), meaning that the BMP tests were finished
350 and permitted to reach the maximum volumes. On the contrary, the maximum theoretical
351 volumes were 350 NLCH₄/kgVS, 341 NLCH₄/kgVS and 286 NLCH₄/kgVS for MSCB, MSCF and
352 MSCR respectively. Except for MSCR, these values were more than 100 NLCH₄/kgVS higher
353 than those actually measured, implying that BMP tests were not finished and by running the
354 tests longer, a higher biogas volume would be produced. As they had already been in progress

355 for two and a half months and the production increase was minimal, it was decided to stop the
356 MSC tests.

357

358

359 *Table 2: V_{max} and k determined on raw biomasses (CS1, CS2, MSCB, MSCF and MSCR) using the first order*
360 *model*

	V_{max} mod (NLCH₄/kgVS)	k (day⁻¹)	R²
CS1	289 ± 29	0.0872 ± 0.005	0.99
CS2	286 ± 8	0.0905 ± 0.000	0.99
MSCB	350 ± 93	0.0177 ± 0.008	0.98
MSCF	341 ± 9	0.0197 ± 0.002	0.99
MSCR	286 ± 6	0.0445 ± 0.001	0.99

361

362 [3.1.3. Correlation between raw biomass composition and BMP values](#)

363 In order to understand the link between raw biomass composition and BMP values, a
364 correlation matrix was constructed (Figure 2) using as variables BMP (NLCH₄/kgVS), soluble
365 content from Van Soest analysis (%DM), hemicellulose content (%DM), cellulose content
366 (%DM), lignin (%DM) and ash content (%DM). In the matrix (Figure 2), the larger the
367 number (from -1 to +1) the more positive the correlation between two variables. This
368 correlation was also represented by the colour of the box at the intersection of the variables:
369 blue corresponded to a positive correlation, red to a negative correlation and white to no
370 correlation.

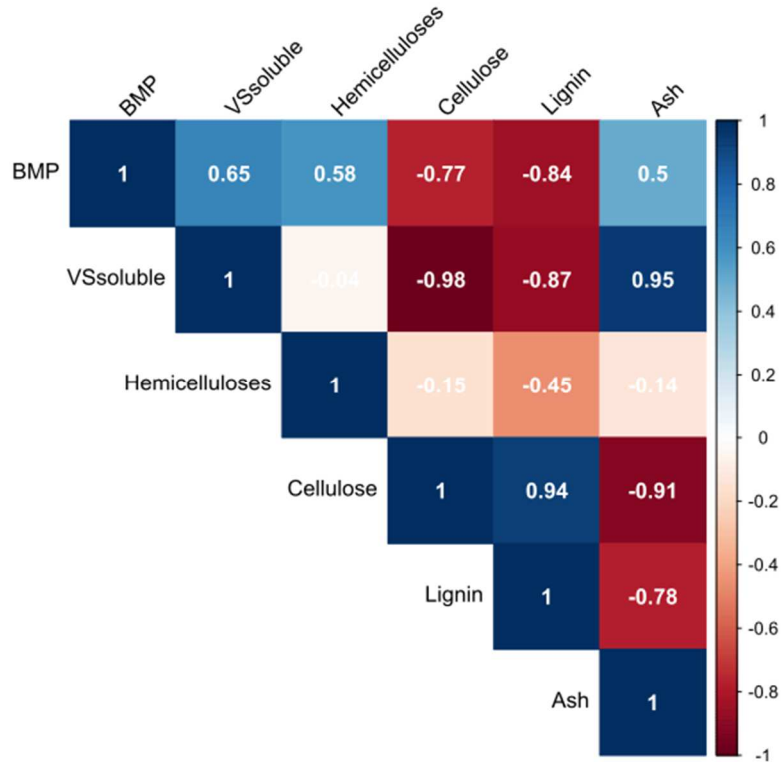


Figure 2: Correlation matrix of BMP and raw biomass composition

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374 In this study and for both tested biomass, BMP value was negatively correlated to lignin and
 375 cellulose contents (-0.84 and -0.77 respectively) and positively correlated to soluble and
 376 hemicellulose contents (0.65 and 0.58 respectively). The higher the lignin and cellulose
 377 contents in the biomass, the lower the BMP. This result was consistent with Monlau et al.
 378 (2012) study that showed the negative correlation between the BMP value and lignin content
 379 and between the BMP value and crystalline cellulose content on 20 lignocellulosic materials
 380 including rice straw, sorghum, maize stalks and sunflower stalks. Various studies highlight the
 381 critical role of lignin and cellulose in parietal protection against external attacks (Miedes et al.,
 382 2014) and their negative correlation within BMP value (Triolo et al., 2011; Kobayashi et al.,
 383 2004). Indeed, crystalline cellulose reduces biodegradability because of the highly resistant
 384 hydrogen bonds network forming a recalcitrant wall to enzymes and microbial attacks,
 385 compared to amorphous cellulose (Zhao et al., 2012a). Concerning lignin, it acts as a physical
 386 barrier, limiting the access of enzymes to cellulose and adsorbing enzymes during enzymatic
 387 hydrolysis due to its hydrophobic structural features (Zoghalmi and Paës, 2019). The lower
 388 BMP value observed for miscanthus compared to corn stalks can thus be understood, as

389 miscanthus is richer in lignin and cellulose than corn stalks (Table 2). Moreover, the slower
 390 methane production rate observed during the first period of the BMP test for miscanthus
 391 compared to corn stalks can also be explained by the smaller amount of soluble content
 392 (sugars, proteins...), corresponding to the more easily degradable material content, in average
 393 6.9%DM and 36.5%DM respectively for MSC and CS (Phuttaro et al., 2019).

394

395 *3.2. BMP of pretreated biomass*

396 From the results obtained in the previous section, MSCB and CS1 were selected for the
 397 microwave pretreatment study; the first one because its BMP value was the lowest observed
 398 between the three MSC (more room for biodegradability improvement) and the second
 399 because there was not substantial difference between CS BMP.

400 MSCB and CS1 were pretreated by conventional heating (CH), classic microwave heating
 401 (MWH, 710W, 800s), pressurized microwave heating (PMWH, 300W, 180s) and control
 402 treatment (NoH). The different pretreatments were performed with the aim to compare
 403 microwave heating pretreatment with conventional heating pretreatment. After treatment
 404 and phase separation, COD and Volatile Solid analysis were performed on solid and liquid
 405 phases in order to determine the dry matter solubilisation obtained after the pretreatments
 406 tested. Results are summarized in Table 3.

407 *Table 3: COD and VS analysis on solid and liquid phases after treatments. MWH classic microwave heating,*
 408 *CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating*
 409 *treatment*

	Solid phase		Liquid phase	
	COD (gO ₂ /gDM)	VS (%) (g/gDM)	COD _{sol} (gO ₂ /L)	DM (%) (g/g)
CS1 Raw	1.25	88.87%		
CS1 MWH	1.25	91.74%	12.4	1.12%
CS1 CH	1.26	91.57%	12.0	1.08%
CS1 NoH	1.25	92.05%	10.1	0.90%
CS1 PMWH	1.26	91.51%	14.2	1.26%
MSCB Raw	1.30	92.20%		
MSCB MWH	1.32	96.09%	2.31	0.18%
MSCB CH	1.30	95.64%	1.68	0.13%
MSCB NoH	1.29	95.52%	1.12	0.09%
MSCB PMWH	1.49	94.74%	2.04	0.16%

410

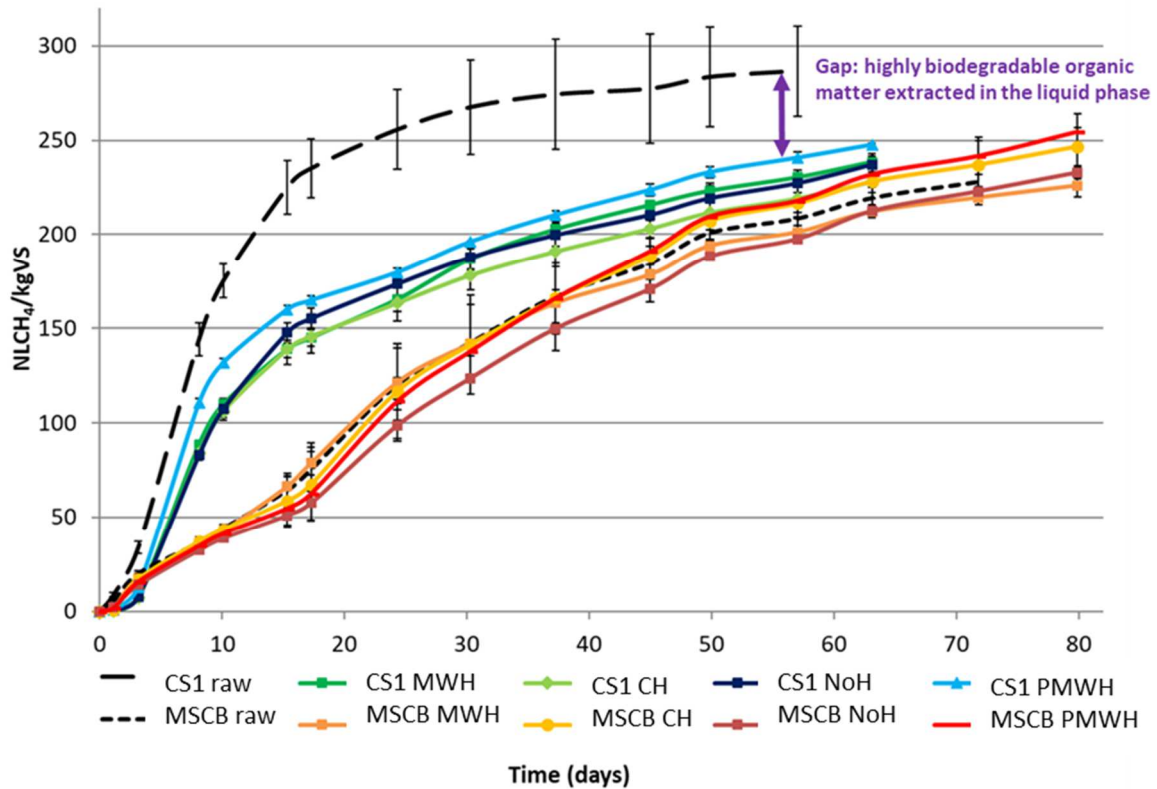
411 From Table 3, it can be seen that no significant effect on organic matter solubilisation was
412 observed after microwave pretreatment (MWH or PMWH); as measured soluble organic
413 matter content in the liquid phase of pretreated (MWH, PMWH and CH) and no pretreated
414 samples (NoH) were similar. DM and COD content in the liquid phases of CS samples,
415 whatever the treatment, was higher than in MSC liquid phase, which could be explained by the
416 higher parietal content of miscanthus, as discussed previously (Table 1), with no link to the
417 pretreatment performed.

418 Consequently from previous results, it was physically impossible to add 1gVS of the liquid
419 samples into the BMP flasks in order to keep the 0.5 S_0/X_0 ratio (Initial substrate VS/Initial
420 Inoculum VS) in agreement with standard laboratory protocol. As a consequence of the low
421 S_0/X_0 ratio applied (0.045), it was expected that methane production due to the organic load
422 would be difficult to dissociate from the methane production due to the endogenous
423 production. BMP tests were performed in duplicate and results were expressed in
424 $NLCH_4/kgVS$ for solid phase and in $NLCH_4/kgCOD$ for liquid phase.

425

426 3.2.1. BMP of solid phase of pretreated samples

427 The methane production curves of the solid phases of pretreated samples (CS1 and MSCB) are
428 presented in Figure 3.



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Figure 3: Methane production vs. time from solid phase after various treatments for CS1 and MSCB. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

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According to Figure 3, whatever the treatment, the methane production rate was similar for all CS1 and MSCB pretreated samples. Nevertheless, production rate was slower for MSCB samples than for CS1, which can certainly be explained by the more lignified structure of the miscanthus. As discussed in the previous section with Figure 2, lignin content being negatively correlated to BMP (-0.84), this could also affect the methane production rate. In Figure 3, the observed BMP value of pretreated CS1 solid phase, 250 NLCH₄/kgVS, was lower than BMP of the raw CS1, which was 286 NLCH₄/kgVS. This was due to the easy solubilisation of soluble organic matter observed during the pretreatment, as soluble content represented 37.3 % of the CS1 DM, whereas MSCB soluble content was only 8.29 %DM. The gap in BMP final value between raw and pretreated CS1 equals to the biodegradable soluble COD fraction that was removed following solubilisation and Liquid:Solid phase separation of pretreated CS1 samples. Biodegradability of released soluble compounds is discussed later.

In the case of MSCB, the BMP of raw and solid phase pretreated biomass were most probably similar (at 5% risk, according to student test, *p* value was largely superior to 0.05), meaning

447 that a non-significant amount of organic matter was solubilised during the heating treatment
448 or during soaking. These hypotheses are discussed in the following section. At 5% risk,
449 methane production rate and BMP values obtained for pretreated MSC were not significantly
450 lower than raw MSC BMP values (with p values equal to 0.6367). Pretreatments did not allow
451 organic matter solubilisation neither weakening of the lignocellulosic network, which could
452 improve the samples biodegradability of the pretreated solid fraction, in terms of methane
453 production kinetics and BMP.

454 Most studies on the effects of LCB pretreatment - not necessarily thermal pretreatments - on
455 anaerobic biodegradability show an increase in the BMP after pretreatment. For example, in
456 the study of Thomas et al. (2019), BMP value of NaOH treated miscanthus at atmospheric
457 pressure and ambient temperature, increased by 55% and in the case of Siddhu et al. (2016)
458 BMP of steam-exploded corn stover, increased by 56%, demonstrating the positive
459 pretreatment effect on methane production. Nevertheless, in the case of microwave
460 pretreatment, results are more unclear. In this way, Jackowiak et al. (2011b) demonstrated
461 that the BMP produced from switchgrass pretreated by high-pressure microwave (260°C and
462 33bars) was not improved when compared to raw switchgrass but the reaction rate was
463 improved: a reduction of 4.5 days to reach 80% of the methane volume was observed. In
464 comparison, the maximum methane volume increased by 28% using wheat straw pretreated
465 in the same microwave conditions (Jackowiak et al., 2011a). Similarly, Kainthola et al. (2019)
466 demonstrated an increase of more than 100 NmLCH₄/gVS after treating rice straw with
467 microwave for 4min at 190°C, reaching 325 NmL/gVS. On the contrary, Sapci (2013)
468 pretreated by microwave, under temperatures between 200° and 300°C, different LCB
469 (barley, oat, spring and winter wheat) and demonstrated that the microwave treatment did
470 not improve the anaerobic digestion and that the increase in temperature led to a lower
471 methane production. The microwave conditions described above were more severe than
472 those used in the present study, but prove that the microwave pretreatment is not always
473 effective in increasing the BMP value of a lignocellulosic biomass. Studies tried to understand
474 microwave effects on biomass organization using both Field Emission Scanning Electron
475 Microscopy and Fourier Transform Infrared. When BMP increased after microwave

476 pretreatment, this could be explained by a breakdown in polysaccharides parietal polymers
 477 (Kainthola et al., 2019).

478 In this study, the first order kinetic model was implemented on methane production data for
 479 CS1 and MSCB to better understand the link between biomass pretreatment and anaerobic
 480 digestion kinetic. Results are summarized in Table 4.

481

482 *Table 4: Methane production during BMP tests, V_{max} and k determined on pretreated biomasses solid phases*
 483 *using the first order model. MWH classic microwave heating, CH conventional heating treatment, NoH*
 484 *control treatment, PMWH pressurized microwave heating treatment*

		V_{max} mod (NLCH ₄ /kgVS)	k (day ⁻¹)	R ²
CS1	NoH	236.4 ± 4.5	0.0552 ± 0.002	0.98
	CH	227.7 ± 5.7	0.0544 ± 0.002	0.98
	MWH	243.0 ± 3.5	0.0509 ± 0.004	0.98
	PMWH	242.0 ± 4.4	0.0639 ± 0.004	0.97
MSCB	NoH	398.6 ± 56	0.0120 ± 0.003	0.99
	CH	386.1 ± 73	0.0130 ± 0.009	0.98
	MWH	290.8 ± 23	0.0206 ± 0.003	0.99
	PMWH	415.4 ± 40	0.0128 ± 0.001	0.98

485

486 For both pretreated biomass with the fourth treatments, the model fitted well with the
 487 experimental kinetic with R² superior to 0.97 (Table 4). Concerning CS1, the predicted volume
 488 production was the same as the experimental volume production, whatever the treatment,
 489 demonstrating that CS1 digestion was complete at the end of the 60 days of digestion and this
 490 was also reflected in the methane production curve (Figure 3) which tends to a plateau from
 491 day 50. Concerning MSCB, the predicted maximal volume was higher than the experimental
 492 maximal volume, up to 165 NLCH₄/kgVS in the case of PMWH MSCB. Moreover, the standard
 493 deviations were high, between 23 NLCH₄/kgVS and 73 NLCH₄/kgVS. The difference between
 494 the two values can be explained by the uncomplete biodegradation of the samples at the time
 495 the BMP tests were stopped: the model predicted that production could continue and thus no
 496 plateau was observed on the MSCB methane production curves (Figure 3). There was no
 497 difference for MSCB methane production kinetics between raw and pretreated solid phase,
 498 with a value of 0.02 day⁻¹ because of the low organic matter solubilised in the liquid phase.

499 In addition, the observed methane production kinetics of the solid phase of the pretreated CS
500 samples were slower than the raw sample, a difference of 0.04 day^{-1} , as a consequence of the
501 L:S phase separation (soluble, easily biodegradable compounds were removed) (Figure 3).

502 These observations suggested that microwave heating did not favour organic matter
503 solubilisation neither weakening of the lignocellulosic network, which could improve the
504 samples biodegradability of the pretreated solid fraction, in terms of methane production
505 kinetics and BMP.

506 To compare results obtained with microwave pretreatment, a chemical NaOH pretreatment
507 allowing to obtain an efficient breakdown of the lignocellulosic network (Monlau et al, 2012)
508 was implemented. At ambient temperature and with the same operating conditions as NoH
509 treatment, 10g CS1 were pretreated for 10 hours with 10%w/w NaOH before performing
510 BMP tests on the mixture solid+ liquid phase, in duplicate. In these conditions, BMP reached
511 $405.5 \text{ NLCH}_4/\text{kgVS}$ representing an increase of more than $100 \text{ NLCH}_4/\text{kgVS}$ compared to raw
512 CS1 (equivalent to +30%). During alkaline pretreatment ester bonds between lignin and
513 hemicelluloses were saponified resulting in biomass delignification (Zhao et al., 2012b) and
514 allowing better action of microorganisms producing biogas. This was in agreement with
515 Thomas et al. (2019) demonstrating an increase of 55% in miscanthus BMP results after 6
516 days of treatment with 10% NaOH. Chemical pretreatment, by subjecting biomass to difficult
517 conditions, dislocated the cell wall structure and thus facilitated the production of biogas by
518 microorganisms.

519

520 3.2.2. BMP of liquid phase of pretreated samples

521 BMP tests were implemented in duplicate with the liquid phases after the different
522 pretreatments (MWH, PMWH, CH and NoH), in order to determine the biodegradability of the
523 COD fraction solubilised by microwave pretreatments (MWH and PMWH) and to compare it
524 to the COD released by the control treatment without heating (NoH). The methane production
525 curves of CS1 and MSCB liquid phases are presented in Figure 4.

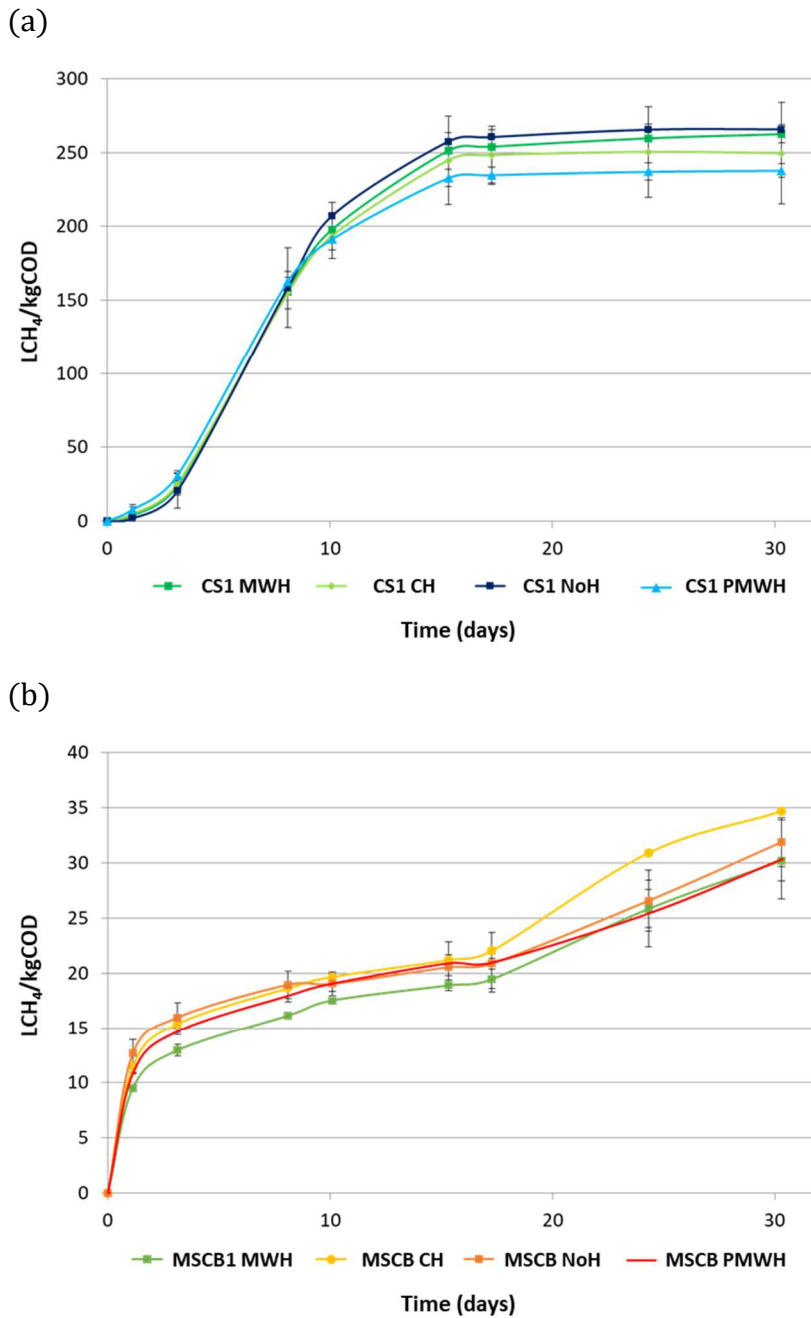


Figure 4: Methane production vs. time from liquid phases after various treatments for CS1 (a) and MSCB (b). MWH classic microwave heating, CH conventional heating treatment, NoH control treatment, PMWH pressurized microwave heating treatment

526 According to Figure 4a, biodegradability of the released COD in liquid phase for CS1 was not
 527 influenced by pretreatments. Whatever the pretreatment, final BMP values reached 265
 528 LCH₄/kgCOD, which was close to the raw BMP value and meant that the solubilised COD was
 529 71% biodegradable. Moreover, the soluble COD was fast biodegradable as in 10 days, the BMP
 530 value was already 200 LCH₄/kgCOD whereas it took 35 days for the solid fraction (Figure 3).
 531 This result was consistent with Table 1 demonstrating that raw CS1 was rich in soluble

532 content easily biodegradable and with Figure 2, illustrating that soluble compounds were
 533 positively correlated with biogas production (0.65).

534 Concerning MSCB, the trend in Figure 4b was different than for CS1: the total volume
 535 produced was low, without exceeding 35 LCH₄/kgCOD, corresponding to a biodegradability of
 536 10%. These results could partially be explained by the low soluble content in miscanthus
 537 (Table 1) and the hardly biodegradable nature of solubilised molecules. Nevertheless, these
 538 results must be taken with caution, given the COD values out of range in the liquid phase that
 539 could lead to misinterpretations.

540 To the authors' knowledge, this is the first study to dissociate methane production of the solid
 541 phase from that of the liquid phase after microwave pretreatment.

542

543 3.2.3. Total methane production from solid and liquid phase

544 A balance of the methane production from the pretreated solid and liquid phases was carried
 545 out for each pretreatment in order to determine whether or not pretreatment had an effect on
 546 anaerobic biodegradability and methane production. Results are reported in Table 5.

547

548 *Table 5: Detailed methane production for 1g of biomass CS1 or MSCB (solid and liquid phase), equivalent to*
 549 *0.92gDM. MWH classic microwave heating, CH conventional heating treatment, NoH control treatment,*
 550 *PMWH pressurized microwave heating treatment*

		CS1				MSCB				
		NoH	CH	MWH	PMWH	NoH	CH	MWH	PMWH	
Raw COD	gO ₂ /gDM	1.253				1.304				
Raw BMP	mLCH ₄ /gCOD	193.12				160.86				
	mLCH ₄ /gDM	222.62				192.98				
Solid phase	COD	gO ₂ /gDM	1.245	1.259	1.253	1.258	1.286	1.301	1.322	1.489
	BMP	mLCH ₄ /gCOD	174.4	166.8	174.6	180.2	172.9	144.0	164.3	161.9
	Mass recovered	gDM/gDM	0.7	0.66	0.7	0.68	0.93	0.93	0.91	0.84
	Methane produced	mLCH ₄ /gDM	152.0	138.6	153.2	154.1	206.8	174.3	197.6	202.5
Liquid phase	COD	gO ₂ /L	10.12	11.97	12.36	14.15	1.12	1.68	2.322	2.04
	BMP	mLCH ₄ /gCOD	265.7	249.5	262.4	237.8	31.9	34.7	30.2	30.3
	Volume	mL/gDM	20	20	20	19	20	20	20	19
	Methane produced	mL/gDM	53.8	59.7	64.9	63.9	0.7	1.2	1.4	1.2
Total methane produced	mLCH ₄ /gDM	205.7	198.3	218.0	218.1	207.6	175.4	199.0	203.7	

551

552 From Table 5, it can be seen that the sum of methane production from solid and liquid phases
553 for each pretreatment was not significantly different from the raw biomass methane
554 production. In the case of PMWH treated CS1, the maximum volume produced was 218
555 mLCH₄/g raw biomass, not significantly different from raw CS1 BMP, 222 mLCH₄/gDM. In the
556 case of PMWH treated MSCB, the maximum volume produced was 204 mLCH₄/g raw biomass,
557 close to raw MSCB BMP, 192 mLCH₄/gDM. In the case of MSCB, the methane production from
558 the liquid phase was insignificant compared to that of the solid phase. In conformity with part
559 3.2.2., this result confirmed that no organic matter was solubilised in the liquid phase during
560 the treatment and thus methane production was not observed in this phase. Results from
561 Table 5 could be compared to those obtained in the same conditions without separating solid
562 and liquid phase during BMP tests: another set of experiments were conducted on raw CS1,
563 NoH CS1 and MWH CS1. Obtained results were 275 mLCH₄/gDM, 286 mLCH₄/gDM and 308
564 mLCH₄/gDM for raw, NoH and MWH respectively. The different inoculum used during these
565 experiments could explain the higher values obtained compared to those from Table 5.
566 Moreover, for the three conditions tested, high standard deviations (about 20 mLCH₄/gDM)
567 were calculated and made it impossible to compare results with one another: NoH and MWH
568 pretreatments seemed having no effects on BMP values, which was similar to the result
569 obtained by separating the solid phase from the liquid phase.

570

571 This study demonstrates that the tested microwave pretreatments had no significant effect on
572 methane production, certainly due to the very mild microwave conditions: even in the case of
573 pretreatment under pressure, the temperature did not exceed 140°C and the pressure 4 bar.

574 For example, Thomas et al. (2019) demonstrated an improvement in methane production up
575 to 55% when miscanthus was pretreated with NaOH 10g/100 g_{TS}⁻¹NaOH (without microwave
576 pretreatment), demonstrating the importance of chemical pretreatment and specially the
577 significant effect of chemicals as NaOH in improving biodegradability. In another study, Kan et
578 al. (2018) optimized brewers' spent grain microwave-assisted alkali pretreatment and
579 demonstrated an increase in BMP value up to 52% under optimized conditions: microwave
580 power 70.7W, treatment time 3.31min and alkali/biomass 0.25. Nevertheless, the most

581 impacting term in the second-order polynomial model fitting to the BMP results remained the
582 alkali loading, with a 2.8728 positive coefficient, meaning that under any microwave
583 conditions, microwave are currently unable to compete with chemicals. Indeed, by doubling
584 the pressure (8 bar), Phuttaro et al. (2019) increased the napier grass BMP by 35% by
585 carrying out a hydrothermal pretreatment for 90min at 175°C. However, higher temperatures
586 (200°C) were not recommended as they can cause the formation of anaerobic digestion
587 inhibitors, such as 5-hydroxymethyl furfural resulting from the hemicelluloses degradation.
588 As an example, Wang et al. (2018) observed a rice straw BMP value of only 200 NmLCH₄/gTS
589 following a thermal treatment at 210°C, whereas it reached 300 NmLCH₄/gTS at 180°C.
590 Chemical pretreatments have an important effect on the biomass structure and fibers
591 breakdown. Thus, on olive pomace, alkaline pretreatment combined with microwave for a few
592 minutes permitted to obtain similar BMP (an increase by 13%) to alkaline pretreatment alone
593 during 2 days: pretreatment time was largely reduced using microwave (Elalami et al., 2019).
594 In another study (Kumar Singh et al., 2019), it was the alkaline concentration that can be
595 reduce from 6% to 4% when microwave were combined to chemical treatment for 30min to
596 pretreat kitchen residues. But it is worth mentioning that these results were obtained on very
597 different raw materials than grass biomass: olive pomace were still very rich in fatty acids and
598 kitchen residues in proteins.
599 In this study, we focused on physical pretreatment with the objective to limit the use of
600 chemicals as much as possible. Chemical-free microwave pretreatment having appeared to be
601 ineffective to increase methane yield, the next step is to study combined microwave/chemical
602 pretreatment at low chemical concentration (synergy effect). Our aim is to develop greener
603 pretreatment technologies, with low chemical consumption.

604

605 4. Conclusions

606 Chemical-free microwave pretreatments (in open vessel and under pressure) were performed
607 on two LCB of industrial interest (corn stalks and miscanthus) with the aim of evaluating
608 microwave chemical-free pretreatment as a method of improving anaerobic biodegradability
609 of biomass, by reducing its recalcitrance. BMP tests carried out on raw biomass before

610 pretreatment highlighted the negative correlation of BMP value to lignin and cellulose
611 contents and the positive correlation to soluble and hemicellulose contents, and made it
612 possible to select the least “efficient” genotype and clone (with the more room for
613 biodegradability improvement), on which pretreatments could be tested: corn stalk genotype
614 F 98902 noted CS1 and miscanthus clone *M. x giganteus* Britannique, noted MSCB,
615 respectively.

616 From biomass analysis, it appeared that depending on raw biomass, liquid phase could
617 account for a significant percentage of total BMP, up to 25% in the case of corn stalks (cell
618 wall rich in soluble content). On the contrary in the case of miscanthus, the liquid phase
619 represented only 0.5% of the total BMP (cell wall rich in parietal elements for miscanthus).
620 According to our experimental results, chemical-free microwave pretreatment (open vessel or
621 under pression) did not allow to increase BMP value of miscanthus nor corn stalks samples,
622 because these conditions were not harsh enough to affect the lignocellulosic network, as it
623 was observed following 10 hours 10%w/w NaOH pretreatment (+30% increase of BMP
624 value). To conclude, with the tested operating conditions, no improvements in BMP could be
625 reached, but this work constitutes a basis for further microwave pretreatment investigations.
626 An interesting perspective would be combining microwave heating to low NaOH (or other
627 chemicals) proved to be efficient for biomass deconstruction. A synergy microwave effect
628 could allow to obtain higher impact on recalcitrance using lower NaOH amounts than
629 chemical treatment alone. Finally, it is important to emphasize that the energy recovery from
630 biomass must remain only the last step in a cascade process.

631

632 **Credit statements**

633 Conceptualization, J.P.D., V.M. and D.G.B.; methodology, A.B. and M.L.; formal analysis, A.B.;
634 investigation, A.B. and D.G.B.; writing—original draft preparation, A.B.; writing—review and
635 editing, J.P.D., D.G.B., V.M., and N.B. All authors have read and agreed to the published version of
636 the manuscript.

637

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644

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