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1 **Effect of total solids content on biohydrogen production and lactic acid accumulation**
2 **during dark fermentation of organic waste biomass**

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26

27 **Abstract**

28

29 Production of biohydrogen and related metabolic by-products was investigated in Solid
30 State Dark Fermentation (SSDF) of food waste (FW) and wheat straw (WS). The effect of
31 the total solids (TS) content and H₂ partial pressure (pp_{H₂}), two of the main operating
32 factors of SSDF, were investigated. Batch tests with FW at 10, 15, 20, 25 and 30 % TS
33 showed considerable effects of the TS on metabolites distribution. H₂ production was
34 strongly inhibited for TS contents higher than 15% with a concomitant accumulation of
35 lactic acid and a decrease in substrate conversion. Varying the pp_{H₂} had no significant
36 effect on the conversion products and overall degradation of FW and WS, suggesting that
37 pp_{H₂} was not the main limiting factor in SSDF. This study showed that the conversion of
38 complex substrates by SSDF depends on the substrate type and is limited by the TS
39 content.

40

41 **Keywords:** Biohydrogen; Lactic acid; Dark fermentation; Total solids; Food waste

42

43 **Highlights**

- 44 • H₂ and by-products production were investigated in solid state DF of food waste.
45 • Effects of TS content and H₂ partial pressure on substrate conversion were studied.
46 • 15% TS was found to be limiting for H₂ production during solid state dark
47 fermentation.

48

49

50 **1. Introduction**

51 Interest in Dark Fermentation (DF) bioprocesses has increased over the past decade because
52 it has shown the potential to produce ‘green’ hydrogen (H₂) from widely available and low-
53 cost organic biomass with a low environmental impact (Azwar et al., 2014). Renewable
54 waste biomass such as agricultural residues, organic fraction of municipal solid waste
55 (OFMSW) and agro-industrial waste might give an additional competitive economic
56 advantage to industrial units treating waste feedstocks by anaerobic digestion for local
57 supply of H₂ (De Gioannis et al., 2013; Guo et al., 2010; Urbaniec and Bakker, 2015).
58 OFMSW and lignocellulosic residues such as wheat and rice straw are potential substrates,
59 as they are abundant and do not compete with food crops as for the production of first
60 generation biofuels (Ghimire et al., 2015a; Guo et al., 2010).

61 Based on the total solids (TS) content of the digestion mixture, anaerobic fermentation
62 processes such as anaerobic digestion (AD) processes can be categorized as: i) wet AD,
63 which operates at low solids content (<15% TS) and ii) dry AD at high solids content
64 (>15% TS) (Motte et al., 2013). Alternatively, AD processes are categorized as: (i) wet
65 (<10% TS), (ii) semi-dry (10–20% TS) and (iii) dry (>20% TS) (Karthikeyan and
66 Visvanathan, 2012). Dry anaerobic fermentation processes can be operated at high substrate
67 concentrations with little water addition, which offers the advantages such as of: (i)
68 economic benefits by reducing reactor volumes and energy consumption for heating the
69 digester, (ii) more efficient handling of the digestate by reducing the drying post-treatment
70 operation and (iii) a higher simpler technicity (Karthikeyan and Visvanathan, 2012; Motte
71 et al., 2013). Dry AD processes have been successfully applied to many different
72 agricultural feedstocks (Liotta et al., 2015; Mussoline et al., 2012).

73 Dry ~~AD~~ anaerobic fermentation processes, in particularly dry DF, can also be attractive for
74 commercialization of bio-H₂ processes for the same reasons. Dry DF which operates at a
75 TS content higher than 15% (sometimes also defined as > 20% TS), is also known as Solid
76 State Dark Fermentation (SSDF). SSDF has recently been reported as suitable for
77 biohydrogen production from wheat straw (WS) (Motte et al., 2015, 2014). In their study,
78 Motte et al. (2015) proposed to combine SSDF with a mechanical pre-treatment process to
79 enhance the overall conversion of lignocellulosic biomass, which makes it more feasible for
80 upscaling in a biorefinery concept.

81 However, increasing the TS content during dry anaerobic fermentation can impact both the
82 total substrate degradation and the biogas production (Abbassi-Guendouz et al., 2012;
83 Motte et al., 2013). At high (19 – 25 %) TS content, mass and energy transfer are lowered
84 by the low water content (Motte et al., 2014; Valdez-Vazquez & Poggi-Varaldo, 2009).
85 These can lead to a local accumulation of by-products up to inhibitory concentrations or
86 result in decreased metabolic conversion rates. Fernández et al. (2008) reported 17%
87 decrease of the degradation rates in dry AD of OFMSW, when the TS content increased
88 from 20 to 30%. Cazier et al. (2015) showed that high hydrogen partial pressures (pp_{H₂})
89 inhibited ~~wheat straw~~ WS hydrolysis in dry AD, suggesting that local accumulation of
90 metabolic intermediates adversely impacted the overall conversion of the biological
91 process. More particularly, Motte et al. (2013) reported a significant decrease in H₂
92 production along with a sharp decrease of WS conversion when performing SSDF at 19 %
93 TS. Moreover, a reduction of the H₂ yields and shifts in the microbial community
94 composition towards lactic acid metabolism was observed at high TS content (Motte et al.,

95 2014). In contrast, only few studies have addressed the effect of increasing the TS content
96 on DF of food waste (Valdez-Vazquez and Poggi-Varaldo, 2009).
97 Therefore, this study aims to investigate the effect of the TS content on organic waste
98 conversion in SSDF using food waste (FW) and Wheat Straw as high and low
99 biodegradable substrate, respectively. In addition, the effect of the p_{H_2} on organic waste
100 hydrolysis and metabolic pathways was evaluated using SSDF batch tests.

101

102 **2. Material and Methods**

103 **2.1 Substrate feedstock**

104 Batch SSDF experiments were carried out with synthetic FW (20.6% TS and 19.7% VS),
105 freshly prepared with a well-defined composition as described elsewhere (Ghimire et al.,
106 2015b) and similar to the composition of FW in European countries reported by
107 VALORGAS (2010). WS with a TS and VS content of 95% and 97%, respectively, was
108 used as a model lignocellulosic biomass substrate.

109

110 **2.2 Source of inoculum**

111 Waste activated sludge was used as inoculum after heat-shock treatment (90 °C, 15 min) to
112 enrich spore forming *Clostridium* sp. and inhibit methanogenic and homoacetogenic
113 activities that consume H₂ (Ghimire et al., 2015a). It was sampled from the secondary
114 clarification unit of a municipal wastewater treatment plant located in the center of France
115 with a capacity of 285,000 population equivalents. The waste activated sludge was
116 centrifuged at 6,500 rpm for 20 min (4 °C) to reach 13.9% TS and 9.7% VS content for
117 achieving a higher TS content during all experiments.

118

119 **2.3 Experimental set-up**

120 **2.3.1 Effect of TS content on H₂ production and substrate conversion**

121 Batch SSDF tests were carried out in triplicates at 10%, 15%, 20%, 25% and 30% TS
122 content of FW. In 600 ml flasks, 53.4 g of an inoculated biomass mixture composed of FW
123 (20.3 g wet weight with 19.7% VS) and inoculum (4.1 g wet weight with 9.7% VS) with a
124 substrate (S) to inoculum (X) ratio (S/X) of 10 g VS substrate/g VS inoculum were mixed
125 with 16.0 g of 2-(N-morpholino) ethanesulfonic acid (MES) buffer, 12 ml of 3.2 % NaOH
126 and 1 ml of trace metal solution containing (g/L): FeCl₂ 2 , CoCl₂ 0.5 , MnCl₂ 0.1, NiCl₂
127 0.1 , ZnCl₂ 0.05 , H₃BO₃ 0.05, ,Na₂SeO₃ 0.05 , CuCl₂ 0.04 and Na₂MoO₄ 0.01 . This
128 mixture had an initial pH of 5.5. The amount of distilled water to be added was calculated
129 with a mass balance on the TS content, including substrate, inoculum, buffer and the other
130 solutions added to obtain final TS contents of 10.0 (± 0.01), 14.98 (± 0.03), 19.89 (± 0.04),
131 24.92 (± 0.02) and 30.0 (± 0.07) %TS. Batch tests were then incubated at 37 (± 1) °C for 14
132 days.

133 **2.3.2 Effect of partial pressure of H₂ (pp_{H2}) on substrate conversion**

134 The study on the effect of the partial pressure of H₂ (pp_{H2}) was carried out in four replicated
135 batch tests operated with FW or WS at a S/X ratio of 10 and a final TS content of 25 (± 1)
136 %. A final TS of 25% was selected to ensure the effect of a high TS content on substrate
137 conversion. Tests were carried out with a thin layer of digestate (< 1 cm), approximately 22
138 (± 2) g, to minimize the effect of gas diffusion (Cazier et al., 2015). H₂ gas was initially
139 added in the headspace of 600 ml serum bottles according to the two following sets of tests:
140 pp_{H2} equivalent to 542 (± 32) mbars (33 ± 2 % H₂ in the headspace, named as “A”) and
141 1087 (± 29) mbars (66 ± 1 % H₂ in the headspace, named as “B”). A control was carried

142 out with only N₂ in the headspace (named as “C”). In all tests, the initial total pressure was
143 set at 1,500 mbars by adding N₂ at the start of the experiments for all conditions. The initial
144 pH was adjusted at 5.5 using the MES buffer. Batch tests were incubated at mesophilic
145 temperature (37 ± 1 °C) for two fermentation periods of 14 and 21 days each.

146

147 **2.4 Analytical methods**

148 Prior to liquid metabolite measurements, 5 g of digestate was diluted in 5 g of deionized
149 water, mixed during 30 minutes, centrifuged at 39,121g during 20 min at 4°C and then
150 filtrated at 0.2 µm with a nylon membrane. The remaining liquid phase was then used to
151 quantify organic acids, other metabolic end-products and soluble sugars. Dark fermentation
152 metabolites were measured at the beginning and the end of each experiment.

153 Organic acids were quantified with a gas chromatograph (Perkin Clarus 580) using an Elite
154 - FFAP crossbond® carbowax® 15 m column connected to a flame ionization detector at
155 280°C, as described elsewhere (Cazier et al., 2015). N₂ was used as carrier gas at a flow
156 rate of 6 mL/min. High performance liquid chromatography (HPLC) was used to quantify
157 other metabolites, e.g. lactic acid, alcohols, and soluble sugars. The HPLC chain was
158 composed of an Aminex HPX-87H column (300 mm on 7.8 mm, Bio-rad), a pre-column to
159 filter residues (Micro guard cation H refill cartridges, Bio-rad) and an automatic sampler
160 (Water 717). A diluted sulfuric acid solution of 0.005 M in milliQ water was used as eluent
161 at a flow rate of 0.4 ml/min.

162 The gas composition was measured with a gas chromatograph (Perkin Clarus 580)
163 equipped with a thermal conductivity detector at 150°C and an injector heated at 250°C and
164 two capillary columns heated at 60°C. The first column corresponded to a RtU bond for
165 CO₂ analysis, while the second column was a RtMolsieve used for the determination of the

166 O₂, H₂, N₂ and CH₄ concentration as described elsewhere (Cazier et al., 2015). Argon at a
167 pressure of 350 kPa and a flow rate of 31.8 ml/min was used as carrier gas. Gas production
168 was monitored through the increase in gas pressure, which was periodically measured with
169 a digital manometer (2000, Leo2 Keller, Winterthur, Switzerland).

170

171 **2.5 Microbial diversity analysis**

172 For microbial analysis, 0.2 – 0.3 g of solid samples were collected at the end of the
173 fermentation period of 14 days from the SSDF batch tests operated at different pp_{H2}. PCR
174 based pyrosequencing, described elsewhere by Carrillo-Reyes et al. (2016), was carried out
175 in order to elucidate the classification of the microorganisms present in the SSDF. The
176 samples for microbial analysis were taken at different pp_{H2} during SSDF of FW and WS.

177

178 **2.6 Data analysis**

179 Substrate degradation was estimated by calculating a theoretical chemical oxygen demand
180 (COD) mass balance. For this, the difference in metabolic end-products (accumulated in
181 both the gaseous and liquid phase) at the initial and final state were compared. Direct
182 measurement of COD was not considered in this study, as suggested by Cazier et al.
183 (2015), since the COD measurements of the complex organic residues, such as
184 lignocellulosic biomass and FW, may vary more than 10 %, which may be higher than the
185 overall substrate degradation during the SSDF process.

186 Total substrate degradation was estimated from the amount of COD produced during DF of
187 the substrate estimated per kg of TS added initially, and corresponded to:

188
$$\text{Total Substrate Degradation} = \text{COD of Final State} - \text{COD of Initial State}$$

189
$$\text{Total Substrate Degradation} = \frac{A_{H_2,f} + A_{met,f} + A_{GC}}{kg\ TS} - \frac{A_{H_2,i} + A_{met,i}}{kg\ TS}$$

190 Where: $A_{H_2,f}$ is the amount of H_2 remaining at the end in the headspace, $A_{met,f}$ the final
191 amount of metabolites accumulated, A_{GC} the total amount of gas (H_2) sampled for analyses,
192 $A_{H_2,i}$ the initial amount of H_2 added and $A_{met,i}$ the initial amount of metabolites in the
193 medium.

194 R (OSX version 3.1.3) with the package Rcmdr (OSX version 2.1.7) was used for statistical
195 analysis of the experimental data. The P value was set at 0.05 and the significance of the
196 results was tested with P values of: * < 0.05; ** < 0.01; *** < 0.001; while results were not
197 significantly different when $P > 0.05$. A multivariate analysis by Principal Components was
198 carried out using FactoMineR (version 1.24, more on <http://factominer.free.fr/>), an
199 extension of the R software, to correlate the H_2 production with the substrate degradation
200 and formation of other metabolic by-products (Lê et al., 2008).

201

202 **3. Results and discussion**

203 **3.1 Effect of TS content on H_2 production and substrate conversion**

204 SSDF tests were carried out with FW at different TS contents (Fig. 1). Overall, the H_2
205 production was strongly impacted by increasing the initial TS content, which is in
206 accordance with earlier studies (Motte et al., 2014; Valdez-Vazquez & Poggi-Varaldo,
207 2009). Fig. 1a shows the FW degradation after 14 days of incubation as a function of the
208 TS content. Fig. 1b presents the molar yield (mmol/kg TS) of the major metabolic end
209 products. Substrate conversion decreased and biohydrogen production was significantly
210 lowered when the initial TS content increased (Fig.1b). A simultaneous shift of the
211 metabolic pathways was observed at a high TS content. The maximum and minimum

212 substrate degradation of 134 (\pm 22) and 51 (\pm 3) g COD/kg TS was achieved at 10 and 30
213 % TS, respectively. H₂ accumulated in the headspace only at a TS content of 10%.
214 Hydrogen production drastically decreased and lactic acid production was favored at TS
215 contents higher than 10%. Indeed, from 15% TS onwards, the metabolic pathways shifted
216 towards lactic acid conversion, thus explaining the decrease in H₂ production (Fig. 1b).
217 The highest substrate conversion of 2,901 (\pm 143) mM metabolites/kg TS was observed at
218 wet conditions of (10% TS) (Fig. 1b), while it was the lowest (1,435 \pm 13 mM
219 metabolites/kg TS) at 30% TS (Fig. 1b). As shown in Fig. 1a and b, such adverse effect of
220 the TS content started between 10 and 15%. Motte et al. (2014) showed similar decrease of
221 the hydrogen yields, related to a metabolic shift with an accumulation of lactic acid, but at
222 higher TS content (19%) for wheat straw only. Further narrowing the study of the TS
223 content within the range 10 – 15 % TS or considering the soluble carbohydrates available to
224 microbial degradation could further give new insights on the mechanisms behind this
225 limiting effect of the TS content in SSDF.

226 In addition, acetate and butyrate were the two of the major as metabolic by-products in a
227 TS content of 10%, while lactate dominated at higher TS contents. The PCA correlation
228 plot (Fig. 2a) shows that H₂ production correlated only with butyrate production,
229 suggesting that acetate resulted from DF and homoacetogenesis pathways. Theoretically,
230 the presence of acetate and butyrate as metabolic by-products is correlated with hydrogen
231 production pathways (Ghimire et al., 2015b). However, a correlation with butyrate
232 production as shown in this study (Fig. 2a), is consistent with previous observations made
233 by Guo et al. (2014) for solid waste where homoacetogenesis can occur. Interestingly, H₂
234 production was also linked to the total substrate degradation, while lactate production did
235 not correlate well with the substrate conversion but more with the initial TS content

236 suggesting an adverse effect of high TS content likely due to the local microbial
237 environment (Fig. 2b). Indeed, the decrease of substrate conversion at increasing TS might
238 be attributed to VFA accumulation, especially lactic acid, decreasing locally the culture pH
239 and inhibiting the hydrolysis process, even though a high amount of MES buffer was added
240 to the medium. Such reaction is very similar to the ensiling process where pH lower than
241 4.5 are favored to stop the microbial process (Kafle & Kim, 2013). Consistently, Lin et al.
242 (2006) reported that xylose removal decreased from 85% to 37% when the culture pH
243 decreased from 8 to 5. Similarly, Fang and Liu (2002) reported a 10% decrease in glucose
244 degradation when the pH decreased from 5.5 to 4. The low culture pH could also have had
245 an adverse effect on hydrolytic enzymes. Indeed, Veeken et al. (2000) reported a decrease
246 in hydrolysis of complex substrates with a decrease in pH, since hydrolytic enzymes are
247 optimally functional at neutral pH (Parawira et al., 2005).
248 Another explanation for the decrease in substrate degradation at higher TS content could be
249 the local accumulation of H₂, as reported and hypothesized by Cazier et al. (2015).

250

251 **3.2 Effect of pp_{H2} on substrate conversion**

252 As potential inhibitory factor, the effect of the pp_{H2} on FW and WS degradation at a high
253 TS content (25 %TS) was investigated and different initial pp_{H2} were tested. Fig. 3a shows
254 the substrate degradation (expressed as g COD/kg TS) from FW after 14 and 21 days of DF
255 at different pp_{H2}. The level of inhibition of pp_{H2} on substrate hydrolysis was determined
256 based on the difference in total substrate degradation (Fig. 3a). No significant effect of the
257 initial pp_{H2} on biomass hydrolysis (ANOVA test, P-values > 0.05) was observed at 25%
258 TS. This was further evident from the metabolic products that accumulated at the end of the

259 experimental period, which showed no significant shift when changing pp_{H_2} (Fig 3b).
260 Substrate degradation slightly increased with the fermentation time, and lactic acid and
261 ethanol accumulated as major metabolites in all tested pp_{H_2} with FW.
262 Further tests were carried out with WS under similar experimental conditions with the
263 objective to confirm the results obtained with FW. Although the pH did not significantly
264 decrease at the end of the experimental period, Fig. 3 and the ANOVA analysis (P-values >
265 0.05) show that the pp_{H_2} did not affect the DF of the substrate WS. However, the difference
266 in metabolites accumulated between 14 and 21 d suggests that hydrolysis occurred.
267 Therefore, it seems that some dark fermentative communities present in the SSDF tests
268 investigated with WS were not strongly affected by the presence of H_2 . These results
269 contrast with the study of Cazier et al. (2015) who reported an inhibition by high pp_{H_2} of
270 WS hydrolysis in dry AD, at pH 7. In their work, inhibition was observed at a pp_{H_2} > 742
271 mbars and degradation of wheat straw decreased from 90 (\pm 10) to 20 (\pm 10) g COD/kg TS
272 in the controls and at a pp_{H_2} of 1,555 mbars, respectively. In contrast, in this study, no
273 evidence of the effect of pp_{H_2} was observed, suggesting the inhibition of the substrate
274 conversion is mainly due to organic acids (especially lactate) accumulation. Although this
275 difference might be attributed to the lower operational pH in the present study (pH 3.7 -
276 5.5) compared to the dry AD (pH 7 - 8) process of Cazier et al. (2015), the WS degradation
277 in the control tests, i.e. 22.4 (\pm 2) g COD/kg TS (at pH 5.4), was interestingly very similar
278 to the one reported by Cazier et al. (2015), i.e. 20 (\pm 10) g COD/kg TS, when maximum
279 hydrolysis inhibition occurred at a pp_{H_2} of 1,555 mbars at pH 7 - 8. Therefore, this
280 difference could be attributed to the fact that substrate degradation in this study was
281 strongly related to the high content in soluble carbohydrates present in the FW substrate, as
282 reported by Guo et al. (2013), but not to the substrate to be hydrolyzed such as WS.

283 Moreover, the pH might have affected microbial selection and the related metabolic
284 pathways. Lactic acid or ethanol were not present as major intermediates in the SSDF of
285 WS (Fig. 3c), in contrast to tests with FW (Fig. 3b). This suggests that the microorganisms
286 producing these metabolites are dominant in moisture limiting and nutrient rich
287 environments such as FW, but not in WS (Sikora et al., 2013).

288 This is in good agreement with the characterization of the microbial communities. The
289 microbial community characterization (Fig. 4) shows that mainly 3 families represented the
290 most abundant OTUs, showing the community present in the SSDF residue after 14 d
291 incubation had a low diversity, although the number of distinct operational taxonomic units
292 (OTUs) was 1127 and 1877 for the SSDF samples of FW and WS, respectively.. The
293 hydrogen (pp_{H2}) favored the development of a *Lactobacillales* population (Fig. 4a). The
294 high abundance of *Lactobacillales* (Fig. 4) explains the high lactic acid concentration
295 during SSDF of FW (Fig. 3c). Moreover, the high abundance of *Lactobacillales* at T₀
296 during SSDF of FW (Fig. 4a) compared to WS (Fig. 4b) implies FW as their major source
297 of inoculum. On the other hand, during SSDF of WS, ~~the~~ *Clostridiales* were present
298 abundantly, which agrees with the production of several other metabolites such as acetate,
299 propionate and butyrate. This observation is also in good agreement with Motte et al.(2014)
300 who previously reported that high TS contents impacted the microbial composition through
301 the selection of lactic-acid bacteria from the *Bacilli* class.

302

303 The production of organic acids by fermentative conversion processes is commonly
304 significantly affected by the operating parameters such as culture pH, temperature and
305 substrate concentration (Wang et al., 2014, 2015). However, the highest conversion of FW
306 (140.7 ± 23 g COD/kg VS_{added} at initial pH 5.5, 25 % TS, 14 days SRT and 37 °C) in this

307 study is lower compared to the study by Wang et al. (2015) (799 g COD/kg VS_{added} at
308 controlled pH 6.0, 35 °C) and Wang et al. (2014) (918 g COD/kg VS_{removed} at controlled pH
309 6.0, 30 °C). This could explain the higher conversion of the waste biomass into
310 fermentative products reported in these two studies, compared to this study where the pH
311 was not controlled and the low pH conditions (3.7 - 5.1) finally inhibited the microbial
312 activity. Similarly, a comparison can be made between the study of Motte et al. (2015) who
313 obtained 6 fold higher organic acids production (140 ± 6 g COD/kg TS_{added} at 23 % TS, 37
314 °C) at an initial pH 8.5, as compared with this study (22 ± 2 g COD/kg TS_{added} at 25 % TS,
315 37 °C) at initial pH 5.5. ~~Therefore, it can be concluded that under SSDF, the substrate~~
316 ~~conversion depends on the culture pH, TS content, substrate type and microbial~~
317 ~~community.~~

318 The total substrate conversion under SSDF also depends on the substrate type. FW has a
319 high VS content (21 to 27% VS) and is particularly suitable for a concomitant production
320 of bio-hydrogen and platform molecules such as short-chain volatile fatty acids, lactic acid
321 and / or alcohols (Uçkun Kiran et al., 2015; VALORGAS, 2010). Soluble by-products of
322 DF can be further applied in biological processes for (i) wastewater treatment (Elefsiniotis
323 et al., 2004), (ii) production of platform molecules such as biopolymers (Ntaikou et al.,
324 2009), (iii) micro-algal lipids production (Turon et al., 2015), iv) H₂ production by photo-
325 fermentation, v) feed for microbial electrolysis cells for production of H₂ and other value
326 added chemicals (El Mekawy et al., 2014) or vi) anaerobic digestion for a higher energy
327 recovery, in the form of hythane, i.e. both H₂ and CH₄ (Ghimire et al., 2015a).

328

329

330 **5. Conclusion**

331 This study highlighted the effects of limiting parameters on substrate conversion during
332 SSDF of FW and WS. The initial TS content significantly affected the substrate
333 degradation and metabolic by-products during SSDF of FW. The H₂ production ceased at a
334 TS content higher than 15%, accompanied by the accumulation of lactic acid. Moreover,
335 the pp_{H2} did not affect the SSDF of food waste due to lactic acid production lowering the
336 culture pH, which played a major role in limiting the substrate conversion, as confirmed
337 during SSDF of WS.

338

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343

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477 **Figure Captions**

478 **Fig. 1.** Effect of TS content on substrate degradation (in g COD per kg of initial TS) (a);
479 and accumulation of end metabolites ~~accumulation~~ (mM per kg of initial TS) (b); ~~at~~
480 ~~different TS content~~

481

482 **Fig. 2.** Principal component analysis correlation circle plots: (a) Hydrogen and major
483 metabolic by-products production and (b) substrate degradation and metabolic products

484

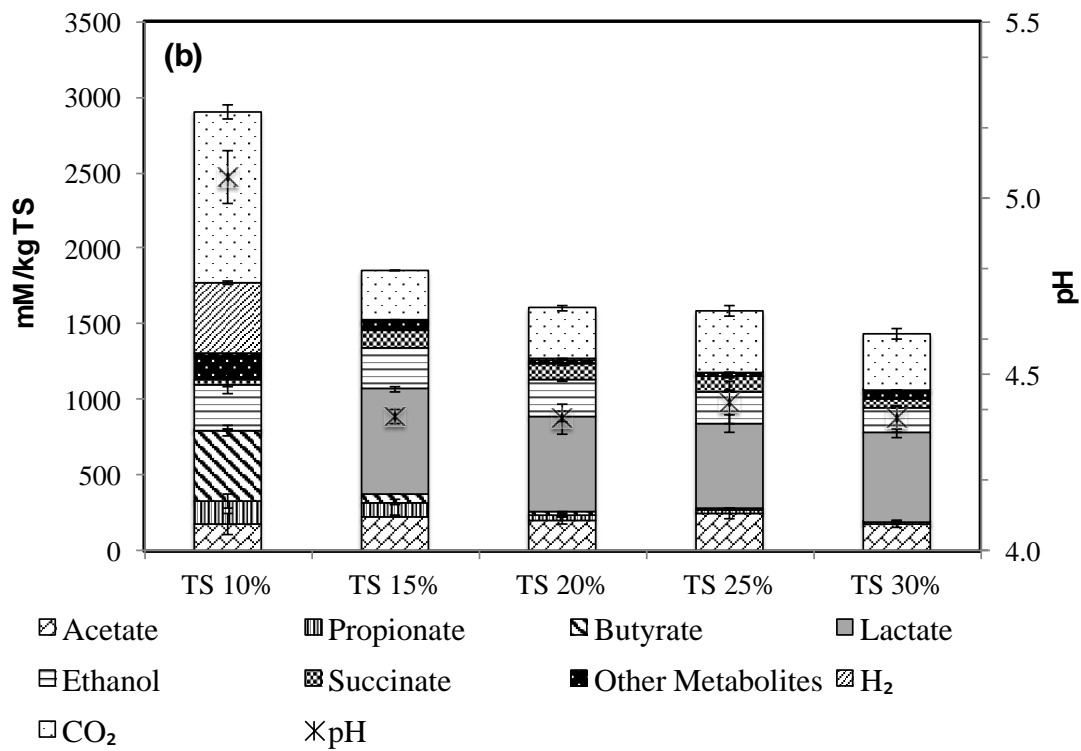
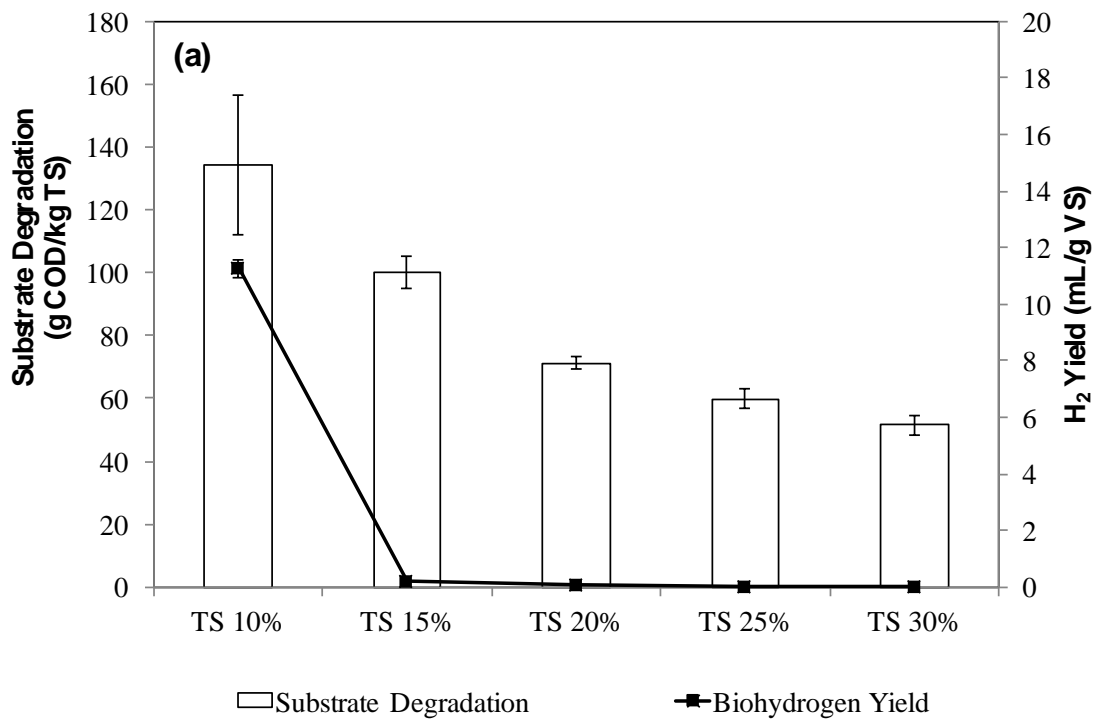
485 **Fig. 3.** Substrate degradation (in g COD per kg of initial TS) at 25% TS using FW and WS
486 (a) as well as end metabolites accumulation (mM per kg of initial TS) in SSDF of FW (b)
487 and WS (c) at different pp_{H_2} and at the end of two fermentation times (14 d and 21 d). A =
488 542 (\pm 32) mbars, B= 1087 (\pm 30) mbars, C = 0 mbars, 14 = 14 d and 21 = 21 d

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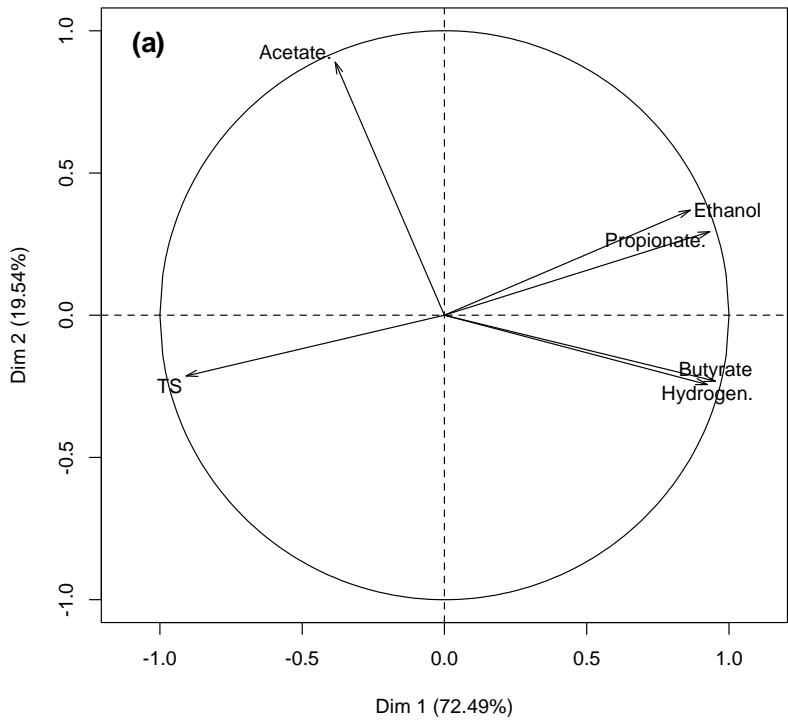
490 **Fig. 4.** Microbial community distribution at family level of the sequenced samples during
491 the SSDF using FW (a) and WS (b) at the beginning (T_0) and end of the 14 d incubation at
492 different pp_{H_2} (0, 542 (\pm 32) mbars, and 1087 (\pm 30) mbars) considering most abundant
493 OTUs (only OTUs with a presence of $>1.0\%$ were considered)

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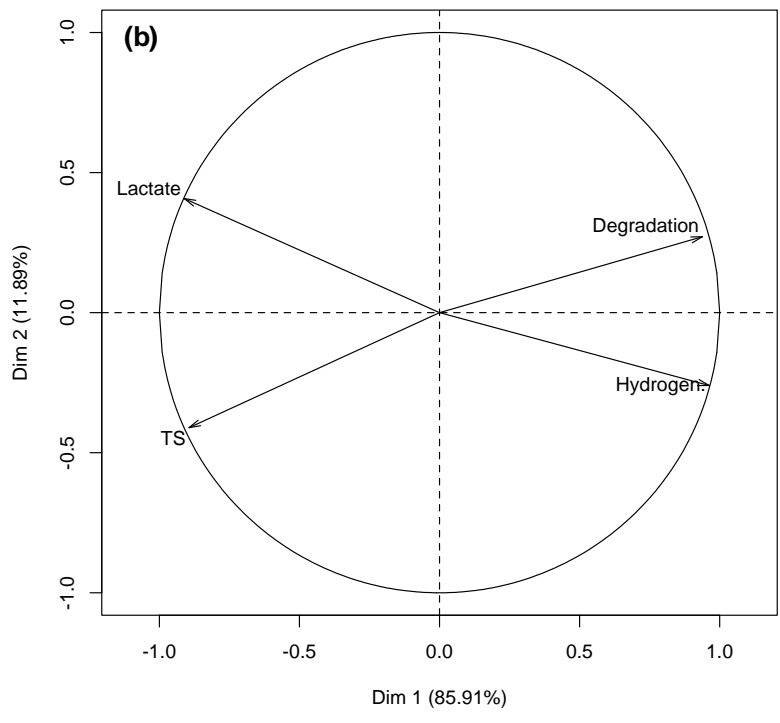
495 **Fig. 1.**



497 **Fig. 2.**
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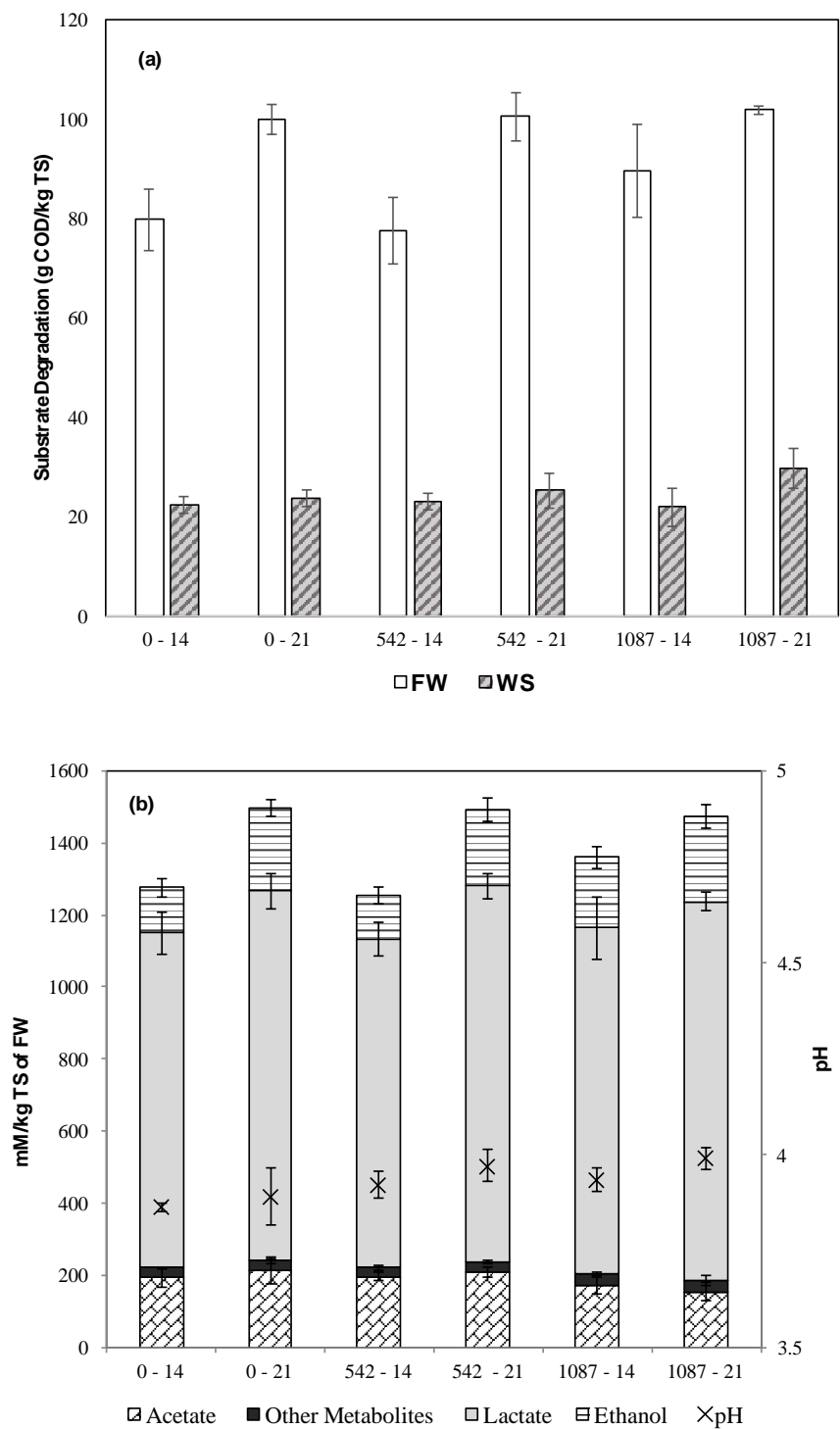


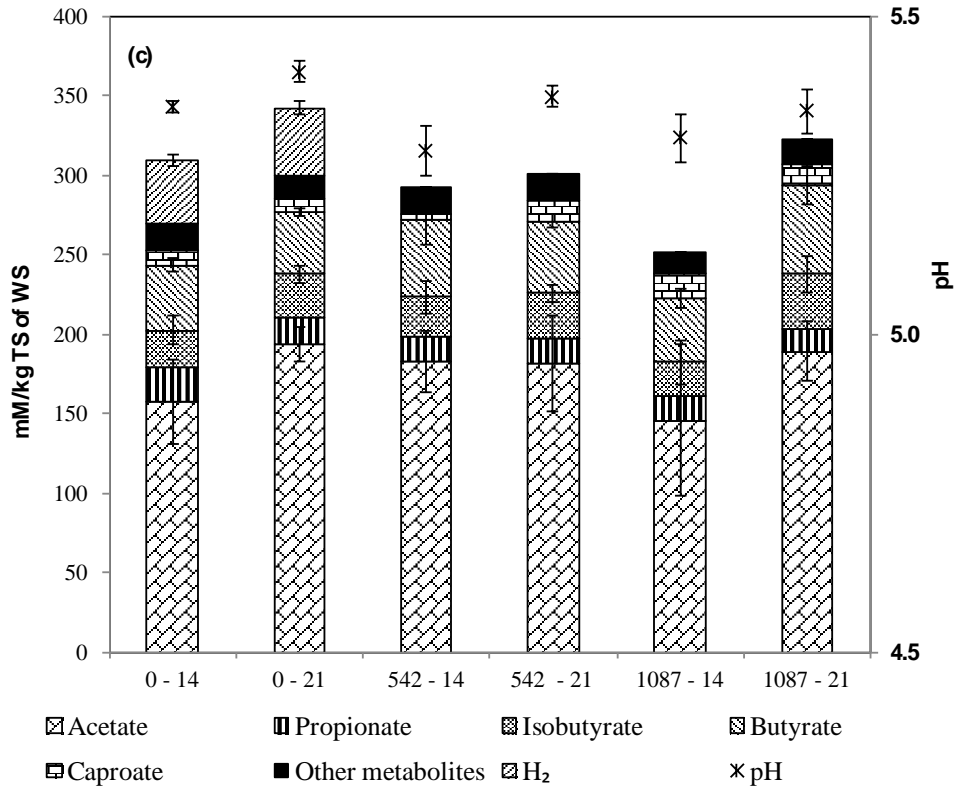
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503 Fig. 3.





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