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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 H<sub>2</sub> partial pressure (pp<sub>H2</sub>), 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. H<sub>2</sub> 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<sub>H2</sub> had no significant  
36 effect on the conversion products and overall degradation of FW and WS, suggesting that  
37 pp<sub>H2</sub> 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 • H<sub>2</sub> and by-products production were investigated in solid state DF of food waste.  
45 • Effects of TS content and H<sub>2</sub> partial pressure on substrate conversion were studied.  
46 • 15% TS was found to be limiting for H<sub>2</sub> 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 (H<sub>2</sub>) 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 H<sub>2</sub> (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-H<sub>2</sub> 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<sub>H<sub>2</sub></sub>)  
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 H<sub>2</sub>  
92 production along with a sharp decrease of WS conversion when performing SSDF at 19 %  
93 TS. Moreover, a reduction of the H<sub>2</sub> 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  $p_{\text{H}_2}$  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 H<sub>2</sub> (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 H<sub>2</sub> 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): FeCl<sub>2</sub> 2 , CoCl<sub>2</sub> 0.5 , MnCl<sub>2</sub> 0.1, NiCl<sub>2</sub>  
127 0.1 , ZnCl<sub>2</sub> 0.05 , H<sub>3</sub>BO<sub>3</sub> 0.05, ,Na<sub>2</sub>SeO<sub>3</sub> 0.05 , CuCl<sub>2</sub> 0.04 and Na<sub>2</sub>MoO<sub>4</sub> 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 H<sub>2</sub> (pp<sub>H2</sub>) on substrate conversion**

134 The study on the effect of the partial pressure of H<sub>2</sub> (pp<sub>H2</sub>) 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). H<sub>2</sub> gas was initially  
139 added in the headspace of 600 ml serum bottles according to the two following sets of tests:  
140 pp<sub>H2</sub> equivalent to 542 (± 32) mbars (33 ± 2 % H<sub>2</sub> in the headspace, named as “A”) and  
141 1087 (± 29) mbars (66 ± 1 % H<sub>2</sub> in the headspace, named as “B”). A control was carried

142 out with only N<sub>2</sub> in the headspace (named as “C”). In all tests, the initial total pressure was  
143 set at 1,500 mbars by adding N<sub>2</sub> 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). N<sub>2</sub> 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 CO<sub>2</sub> analysis, while the second column was a RtMolsieve used for the determination of the



166 O<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub> and CH<sub>4</sub> 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<sub>H2</sub>. 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<sub>H2</sub> 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. H<sub>2</sub> 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 H<sub>2</sub> 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 H<sub>2</sub> 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, H<sub>2</sub>  
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 H<sub>2</sub>, as reported and hypothesized by Cazier et al. (2015).

250

### 251 **3.2 Effect of pp<sub>H2</sub> on substrate conversion**

252 As potential inhibitory factor, the effect of the pp<sub>H2</sub> on FW and WS degradation at a high  
253 TS content (25 %TS) was investigated and different initial pp<sub>H2</sub> 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<sub>H2</sub>. The level of inhibition of pp<sub>H2</sub> on substrate hydrolysis was determined  
256 based on the difference in total substrate degradation (Fig. 3a). No significant effect of the  
257 initial pp<sub>H2</sub> 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<sub>H2</sub>) 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 T<sub>0</sub>  
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 ( $140.7 \pm 23$  g COD/kg VS<sub>added</sub> 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<sub>added</sub> at  
308 controlled pH 6.0, 35 °C) and Wang et al. (2014) (918 g COD/kg VS<sub>removed</sub> 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<sub>added</sub> at 23 % TS, 37  
314 °C) at an initial pH 8.5, as compared with this study (22 ± 2 g COD/kg TS<sub>added</sub> 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) H<sub>2</sub> production by photo-  
325 fermentation, v) feed for microbial electrolysis cells for production of H<sub>2</sub> 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 methane, i.e. both H<sub>2</sub> and CH<sub>4</sub> (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 H<sub>2</sub> production ceased at a  
334 TS content higher than 15%, accompanied by the accumulation of lactic acid. Moreover,  
335 the pp<sub>H2</sub> 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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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

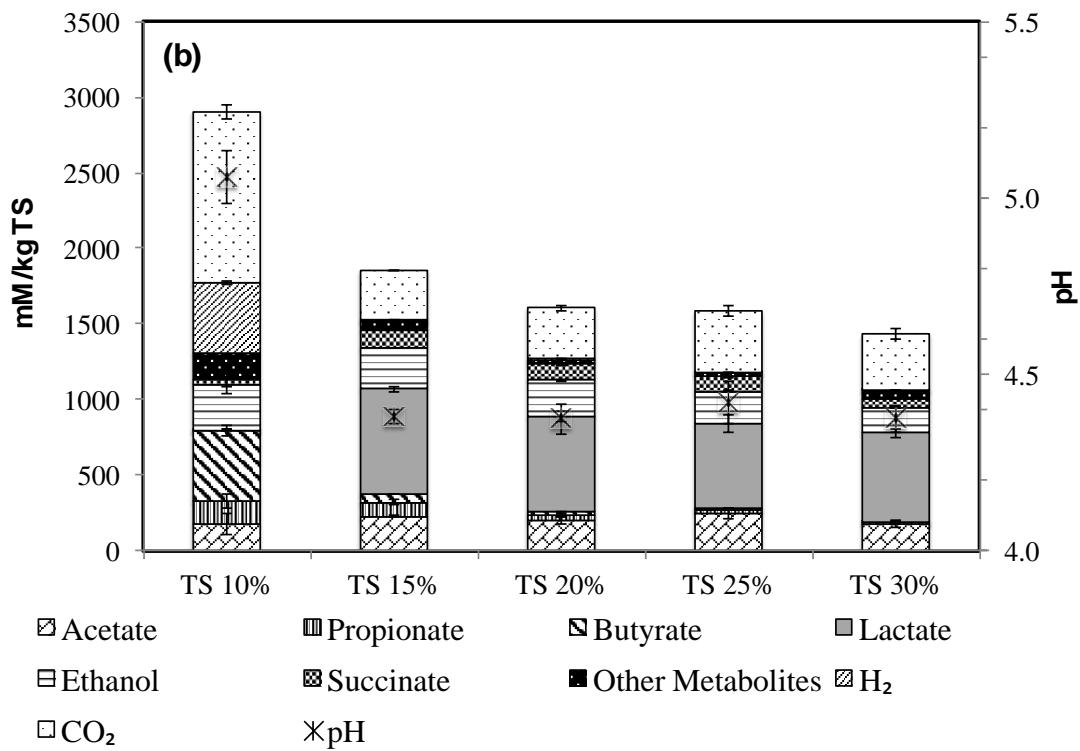
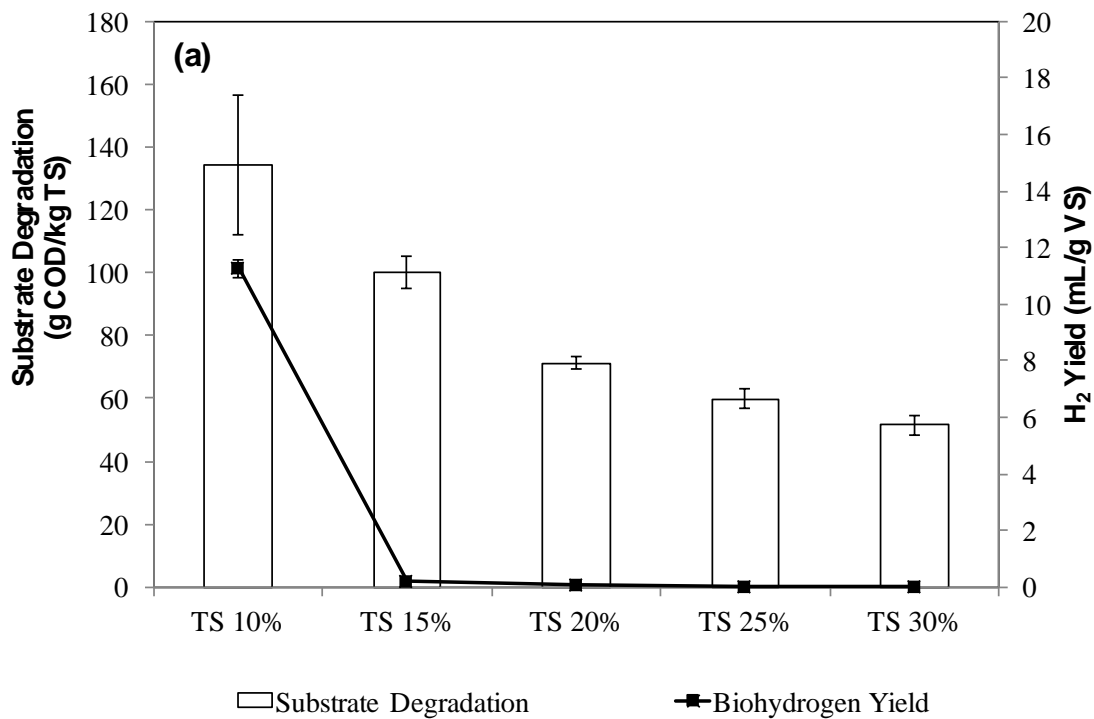
485 **Fig. 3.** Substrate degradation (in g COD per kg of initial TS) at 25% TS using FW and WS  
486 (a) as well as end metabolites accumulation (mM per kg of initial TS) in SSDF of FW (b)  
487 and WS (c) at different  $pp_{H_2}$  and at the end of two fermentation times (14 d and 21 d). A =  
488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

489

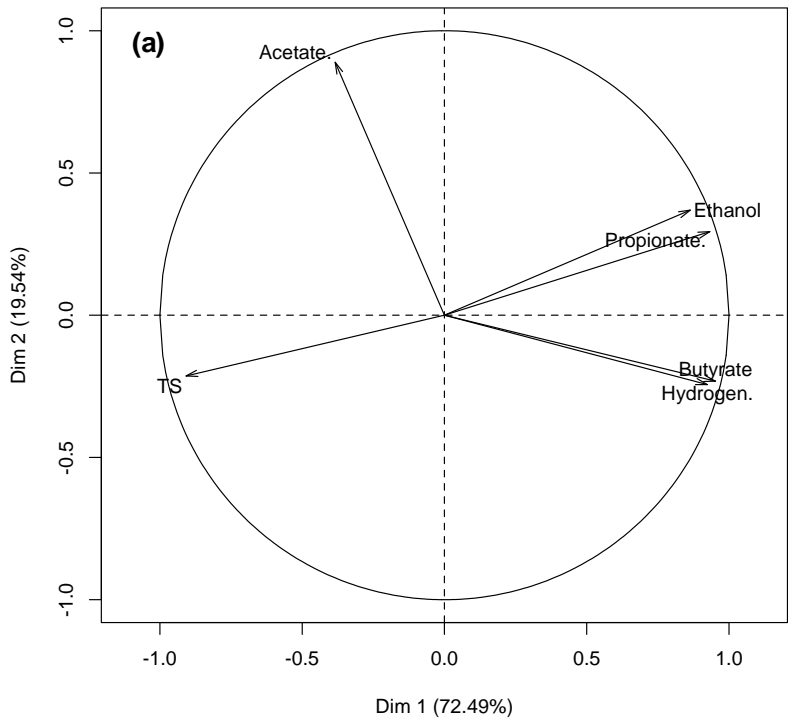
490 **Fig. 4.** Microbial community distribution at family level of the sequenced samples during  
491 the SSDF using FW (a) and WS (b) at the beginning ( $T_0$ ) and end of the 14 d incubation at  
492 different  $pp_{H_2}$  (0, 542 ( $\pm$  32) mbars, and 1087 ( $\pm$  30) mbars) considering most abundant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

494

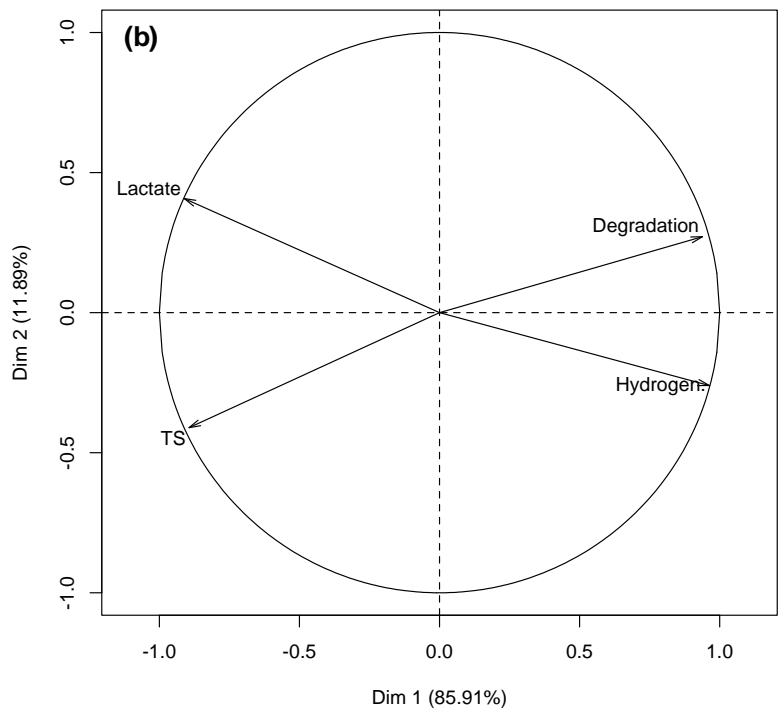
495 **Fig. 1.**



497 **Fig. 2.**  
498



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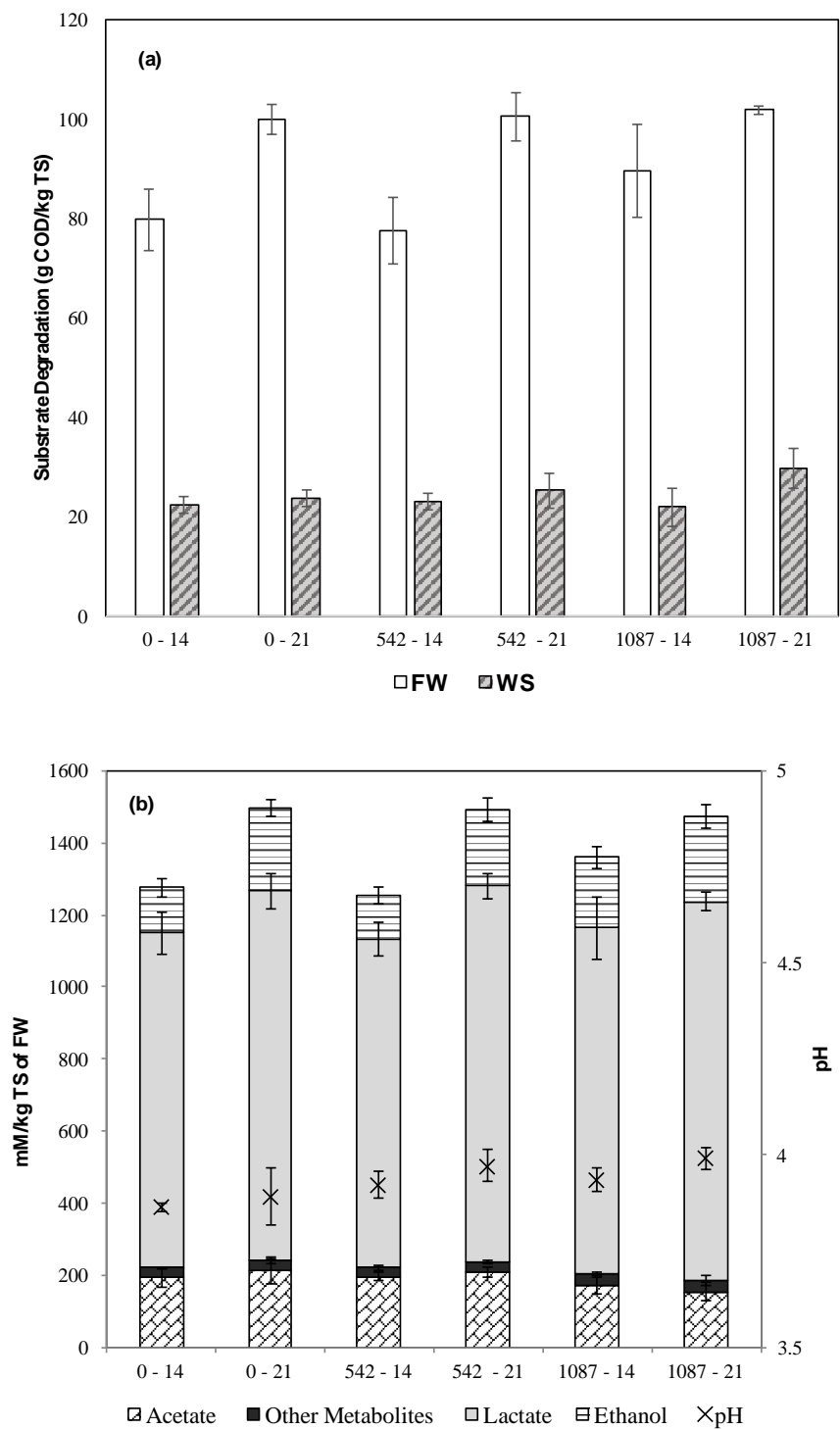


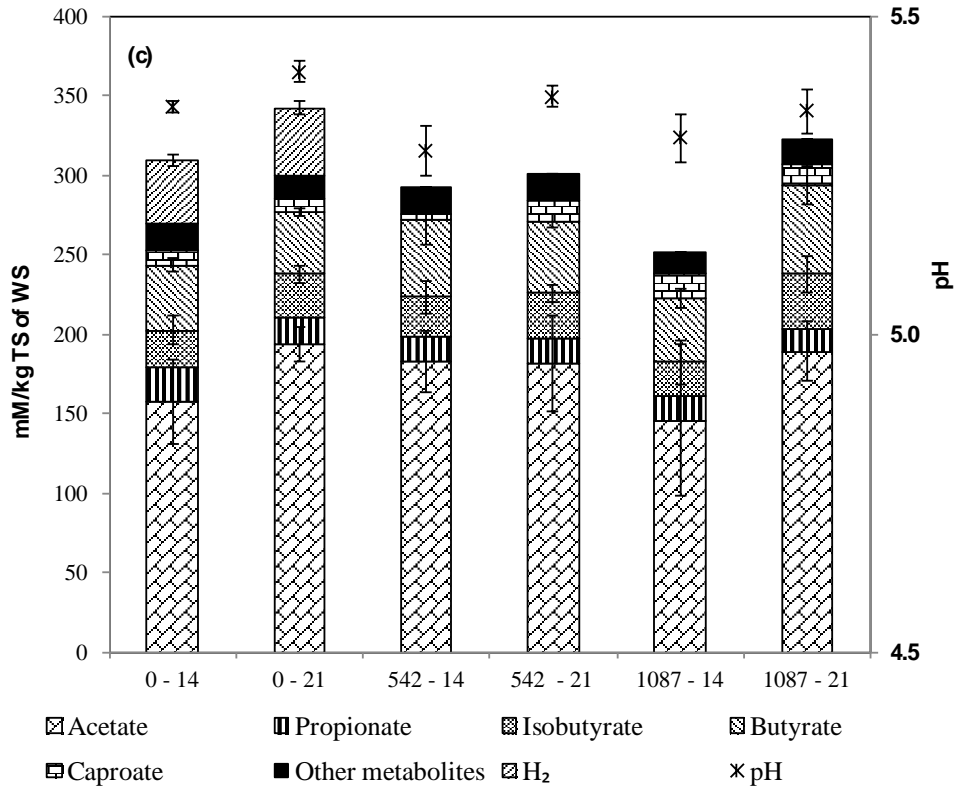
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503 Fig. 3.





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