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## **Addition of biochar and trace elements in the form of industrial FeCl<sub>3</sub> to stabilize anaerobic digestion of food waste: dosage optimization and long-term study**

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**Biochar and industrial FeCl<sub>3</sub> as additives to favor the consumption of volatile fatty acids during anaerobic digestion of food waste**

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3 1 **Biochar and industrial FeCl<sub>3</sub> as additives to favor the consumption of volatile fatty acids**  
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5 2 **during anaerobic digestion of food waste**  
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8  
9 4 **Short title: Biochar and industrial FeCl<sub>3</sub> to stabilize the anaerobic digestion of food**  
10  
11 5 **waste**  
12  
13 6

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30  
31 14 **Abstract**

32  
33 15 **BACKGROUND**

34  
35 16 Although anaerobic digestion is a promising alternative for the valorization of complex  
36  
37 17 substrates such as food waste, this process is yet to be optimized and options for its  
38  
39 18 stabilization must be developed. The goal of this study was to assess for the first time if the  
40  
41 19 addition of biochar, together with trace elements in the form of an industrial FeCl<sub>3</sub> solution,  
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43 20 could serve as method for stabilizing anaerobic digestion of food waste.  
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46 21 **RESULTS**

47  
48 22 Results from batch reactors demonstrated that the supplementation of both biochar and  
49  
50 23 industrial FeCl<sub>3</sub> favored the digestion kinetics. Their addition improved the maximum  
51  
52 24 methane production rates (from 886 up to 1498 ml·g VS<sup>-1</sup>·d<sup>-1</sup>) and the average daily methane  
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54 25 production rates (from 280 up to 376 ml·d<sup>-1</sup>), related to acetate and propionate consumption,  
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3 26 respectively. Continuous reactors confirmed the batch results, with higher methane production  
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5 27 rates (up to  $1.75 \text{ l}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ ) and lower concentrations of both acetate and propionate when  
6  
7 28 biochar and  $\text{FeCl}_3$  were added.

## 8 9 29 **CONCLUSION**

10  
11 30 Addition of biochar and industrial  $\text{FeCl}_3$  favored the digestion kinetics, improving volatile  
12  
13 31 fatty acid consumption and the methane production rates. Although more research is needed,  
14  
15 32 these materials appear as an economically-feasible alternative for stabilizing the valorization  
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17 33 of food waste at industrial scale.

## 18 19 20 34 21 22 35 **Introduction**

23  
24 36 The increasing production of food waste (FW) and novel international regulations call for the  
25  
26 37 development of novel sustainable technologies for its treatment and valorization (1). Among  
27  
28 38 the existing options, anaerobic digestion (AD) is an environmental-friendly process that  
29  
30 39 provides an efficient waste treatment, producing at the same time biogas and digestate.  
31  
32 40 Nevertheless, FW AD is a complex biological process that often leads to inefficient results or  
33  
34 41 reactor acidification. The first complication occurring during FW AD is related to the fast  
35  
36 42 biodegradability of this substrate, which is mainly composed of easily degradable  
37  
38 43 carbohydrates. Therefore, the reactors can be easily overloaded, especially in batch systems  
39  
40 44 (2), causing an unbalance between the acidogenesis/acetogenesis and the methanogenesis  
41  
42 45 steps, which results in an initial accumulation of volatile fatty acids (VFAs) and a consequent  
43  
44 46 pH drop (1,3–6). The second issue to be dealt with is related to the high protein content of this  
45  
46 47 substrate and its low water content (~20 % total solids; TS). During AD, organic nitrogen  
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48 48 (usually in the form of proteins) is reduced into ammonia nitrogen (total ammonia nitrogen;  
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50 49 TAN), which, in its free form (free ammonia nitrogen; FAN) is toxic to microorganisms,  
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52 50 especially to methanogenic archaea, eventually resulting also in an accumulation of VFAs.  
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3 51 Thus, different FW AD studies have reported inefficient performances due to high  
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5 52 concentrations of FAN (7–9).  
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7 53 The main alternative that has been applied to favor the consumption of VFAs in FW AD is the  
8  
9 54 supplementation of trace elements (TEs) (10–14). TEs have been found to favor VFA  
10  
11 55 consumption in both mesophilic and thermophilic AD of FW, mainly due to a favored  
12  
13 56 synthesis of critical enzymes in the process of syntrophic hydrogenotrophic methanogenesis  
14  
15 57 (HM), which is known to be the predominant methane-producing pathway during FW AD  
16  
17 58 (2,11,15–17). TEs addition has been successfully applied to avoid accumulation of VFAs at  
18  
19 59 high organic loading rates (OLRs) (10,18,19) and to recover acidified reactors (20,21).  
20  
21 60 Another strategy to favor VFA consumption during AD that has gained attention in the last  
22  
23 61 few years is the addition of conductive materials (22,23). This approach is based on the  
24  
25 62 capability of these materials for improving microbial interactions, by allowing the formation  
26  
27 63 of biofilm onto their surfaces and by facilitating the occurrence of direct interspecies electron  
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29 64 transfer (DIET), a mechanism in which the transfer of electrons between species occurs  
30  
31 65 through shared physical connections. DIET is a faster and more efficient route for electron  
32  
33 66 transfer than mediated interspecies electron transfer (23) and, as molecular hydrogen is no  
34  
35 67 longer formed, VFA oxidation is thermodynamically independent of its concentration (22,24).  
36  
37 68 Moreover, recent studies have suggested that acetic acid (HAc), butyric acid (HBu) and  
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39 69 propionic acid (HPr) can be metabolized through DIET (22,25–27). Materials such as  
40  
41 70 granular activated carbon (GAC), carbon cloth or magnetite have been found to favor VFA  
42  
43 71 consumption and methane production during AD (26,28,29). Among them, GAC appears as a  
44  
45 72 particularly performant alternative that has been found to promote the consumption of HAc,  
46  
47 73 HBu and HPr during AD of dog-food waste and FW (22,28,30).  
48  
49 74 However, both TEs and GAC are expensive and their application at an industrial scale is far  
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51 75 from being feasible. Thus, cheaper alternatives must be found. A simple substitute for the TEs  
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3 76 solution may be industrial  $\text{FeCl}_3$ , which consists on an acid solution, highly concentrated in  
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5 77 different metals (mainly Fe) that is commonly applied in wastewater treatment plants and  
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7 78 anaerobic digesters for pH control worldwide. Concerning GAC, an affordable substitute may  
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9 79 be biochar, which is also a carbon-conductive material with the capacity of accepting and  
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11 80 donating electrons (31). In addition, biochar may also improve the AD process by providing  
12  
13 81 buffering capacity and by sorption of inhibitors (32). It also favors nutrient retention in the  
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15 82 digestates, facilitating nutrient uptake if the digestate is spread on land for plant cultivation  
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17 83 (32,33). Moreover, as biochar can be produced from digestate (34) or directly from FW (35),  
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19 84 its addition for AD improvement clearly fits within the approach of environmental  
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21 85 biorefinery.

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23  
24 86 Biochar has been previously used as amendment for AD: (i) for biogas purification (removal  
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26 87 of  $\text{H}_2\text{S}$  or  $\text{CO}_2$ ) (36–38), (ii) as reactor packing for biofilm support (39), (iii) as AD substrate  
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28 88 (40), (iv) for nutrient supplementation (41), (v) as matrix for sorption of inhibitors (42,43) or  
29  
30 89 (vi) as means to increase the buffer capacity of the system (33). However, only three studies  
31  
32 90 have been carried out dealing with low-dosage of biochar for improving the AD kinetics. Luo  
33  
34 91 et al. (44) studied the addition of biochar for improving the kinetics of methane production  
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36 92 and VFA degradation (HAc and HBU) with glucose as substrate, concluding that a positive  
37  
38 93 effect existed. Zhao et al. (27) investigated the effect of biochar and ethanol supplementation  
39  
40 94 in reactors degrading HPr and HBU, observing that the degradation of both VFAs was  
41  
42 95 improved by adding biochar and concluding that bacteria known to participate in DIET (*i.e.*  
43  
44 96 *Geobacter* species) were attached onto the biochar surface. Finally, Sunyoto et al. (45)  
45  
46 97 assessed the influence of biochar addition in a two-phase AD reactor treating aqueous  
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48 98 carbohydrates FW, concluding that biochar supplementation increased the hydrogen and  
49  
50 99 methane production rates. They attributed this improvement to a promotion of the biofilm  
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52 100 formation.

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3 101 To the knowledge of the authors, no study has been carried out so far to assess if addition of  
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5 102 biochar can favor VFA consumption during AD of complex substrates such as FW. Moreover,  
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7 103 the stabilizing effect of jointly adding industrial  $\text{FeCl}_3$  (to provide TEs) and biochar on the  
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9 104 performance of FW AD has been investigated for the first time. Therefore, the goal of this  
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11 105 study was to: (i) optimize the concentrations of both biochar and industrial  $\text{FeCl}_3$  using a  
12  
13 106 batch experimental design to improve the kinetics of FW AD and (ii) evaluate the effect of  
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15 107 industrial  $\text{FeCl}_3$  and biochar supplementation on the AD performance of semi-continuous  
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17 108 pilot reactors treating commercial FW.  
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## 22 **Experimental**

### 23 *Inoculum, substrate and AD additives*

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26 112 The reactors were inoculated with digestate from an industrial plant treating different organic  
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28 113 streams at high TAN/FAN concentrations ( $5.04 \text{ g TAN}\cdot\text{l}^{-1}$ ;  $615 \text{ mg FAN}\cdot\text{l}^{-1}$ ). Thus, it was  
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30  
31 114 assumed that the microorganisms would be adapted to high the high TAN/FAN levels  
32  
33 115 associated with FW AD (9). The TS content of the inoculum was  $5.81 \pm 0.02 \%$ , with a  
34  
35 116 proportion of volatile solids (VS) of  $59.13 \pm 0.08 \%$ . The activity of the inoculum was  
36  
37 117 verified prior its utilization using ethanol as substrate. The commercial FW was collected  
38  
39 118 from five mayor producers from the region of the Grand Narbonne, in the south of France. A  
40  
41 119 proportional mixture (wet weight) was used as substrate. The main characteristics of the FWs,  
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43 120 as well as those of the mixture used as substrate and the inoculum are presented in Table 1.  
44  
45 121 The characteristics of the FW mixture were in agreement with typical values presented in the  
46  
47 122 literature (1), which indicated that it could be considered as a representative sample of a  
48  
49 123 general FW. It had a TS contents of 21 % (90.3 % VS), it was mainly composed of  
50  
51 124 carbohydrates ( $618 \text{ g}\cdot\text{kg TS}^{-1}$ ) and it had relatively low C/N ratios (16.1). No sulfur was  
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53  
54 125 detected. A more extensive characterization and a deeper discussion of the results can be  
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3 126 found in Capson-Tojo et al. (9).

4  
5 127 The industrial  $\text{FeCl}_3$  was provided by an industrial AD plant, where it is still used for pH  
6  
7 128 controlling purposes. The characteristics and composition of this industrial solution, rich in  
8  
9 129 TEs, are shown in Table 2.

10  
11 130 The biochar was natural slow-pyrolyzed wood charcoal, commonly found in the market for  
12  
13 131 several applications. Before utilization, the biochar was grinded and sieved at 600  $\mu\text{m}$ .

14  
15 132 *Batch experimental design for dosage optimization*

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17 133 A multilevel factorial design was used to optimize the dosage of biochar and industrial  $\text{FeCl}_3$   
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19 134 and to evaluate their individual effect on the methane production and the VFA production-  
20  
21 135 consumption kinetics. Digestate from continuous reactors digesting FW was used as inoculum  
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23 136 (after consumption of the remaining VFAs). This sludge had a TS content of 5.17 %, with  
24  
25 137 60.2 % corresponding to VS and had a TAN content of  $7.27 \text{ g}\cdot\text{l}^{-1}$ . Sixty g of FW were added  
26  
27 138 as substrate in all the reactors. A substrate to inoculum (S/X) ratio of  $1 \text{ g VS}\cdot\text{g VS}^{-1}$  was  
28  
29 139 applied, leading to initial FW concentrations of approximately  $27 \text{ g VS FW}\cdot\text{l}^{-1}$ . The reactors  
30  
31 140 were incubated at 37 °C. The working volumes ranged from 487 ml to 529 ml. Two different  
32  
33 141 concentrations of the  $\text{FeCl}_3$  solution ( $0.1$  and  $0.2 \text{ g Fe}\cdot\text{l}^{-1}$ ) and three of biochar (10, 55 and 100  
34  
35 142  $\text{g}\cdot\text{l}^{-1}$ ) were tested. As a result, an experimental design with 12 conditions was defined (6  
36  
37 143 conditions in duplicate; Table 3). Two consecutive batch feeding were carried out. The first  
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39 144 feeding served for inoculum adaptation (results not presented), and the results of the second  
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41 145 one were used for modelling purposes. It must be mentioned that, based on previous results, it  
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43 146 was decided not to include into the experimental design reactors containing only biochar and  
44  
45 147  $\text{FeCl}_3$ . As it was demonstrated in Capson-Tojo et al. (30), although the separate addition of  
46  
47 148 GAC and TEs also enhanced the VFAs degradation kinetics during FW AD, the best  
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49 149 performances were achieved when both of these reagents were added simultaneously. With  
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51 150 these results as starting point, the current experimental designed was defined. The same study  
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3 151 was also used to define the levels of industrial FeCl<sub>3</sub> and biochar to be tested (30).  
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5 152 This experimental design was chosen because it allows analyzing the effects of the selected  
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7 153 factors (biochar and FeCl<sub>3</sub> concentrations) on a chosen output through the entire experimental  
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9 154 region covered. This implied that it was possible to distinguish between the effect of biochar  
10  
11 155 and FeCl<sub>3</sub> without the need of single additive treatments. It allows so by predicting the  
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13 156 responses using a quadratic model (Eq. 1):  
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15 157

$$158 \quad y = a_0 + \sum_{i=1}^k a_i \cdot x_i + \sum_{i=1}^k a_{ii} \cdot x_i^2 + \sum_{i < j}^k a_{ij} \cdot x_i \cdot x_j \quad \text{Equation 1}$$

159

160 Where y is the response to be predicted, x<sub>i</sub> are the studied factors and a<sub>i</sub>, a<sub>ii</sub> and a<sub>ij</sub> are the  
161 parameters corresponding to each factor. These parameters represent the linear effects, the  
162 quadratic effects and the interactional effects, respectively. The first coefficient a<sub>0</sub> is required  
163 for fitting the mathematical model. The p-values from F-tests (95 % confidence) and the  
164 coefficient of determination R<sup>2</sup> were used to evaluate the fitness of the model. The experiment  
165 was designed and evaluated using the software STATGRAPHICS Centurion XVI Version  
166 16.1.03 (©StatPoint Technologies Inc.).

167 The reactors were incubated in an Automated Methane Potential Testing System (AMPTSII)  
168 (Bioprocess Control, Sweden). Twelve reactors from the AMPTSII system, with a total  
169 volume of 600 ml, were used. According to the manufacturer instructions, they were  
170 connected to CO<sub>2</sub> traps (NaOH solutions) and to gas flow meters to determine continuously  
171 the methane flow rates. These reactors also allowed the follow-up of the dynamics of VFA  
172 production-consumption. A hole present in each vessel was used as sampling port, facilitating  
173 an easy sampling of the digestate. The reactors were agitated during one minute every 10  
174 minutes at 40 rpm.

175 The criterion followed to decide when the batch had finished was the total consumption of the

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3 176 HPr in the reactors (26 days). Blank reactors (without substrate addition) were carried out to  
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5 177 account for the endogenous respiration of the inoculum.

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7 178 Continuous pilot scale reactors

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9 179 In parallel to the batch reactors, two different pilot scale reactors were run: a control system  
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11 180 simply digesting FW and a doped reactor supplemented with biochar and the FeCl<sub>3</sub> solution.  
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13 181 Both reactors were incubated at 37 °C and had a working volume of 12 l. The reactors were  
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15 182 fed once per day, initially with an OLR of 1.4 g VS·l<sup>-1</sup>·d<sup>-1</sup>, corresponding to a hydraulic  
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17 183 retention time (HRT) of 110 days. This value was increased to 2.8 g VS·l<sup>-1</sup>·d<sup>-1</sup> (HRT of 55  
18  
19 184 days) after 77 days of operation. An equivalent amount of digestate was withdrawn to keep  
20  
21 185 the volume of the reactors constant. The FeCl<sub>3</sub> solution was diluted with water (x20 vol:vol)  
22  
23 186 and dosed into the supplemented reactor to keep a constant concentration of 100 mg Fe·l<sup>-1</sup>  
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25 187 (value calculated from optimal results reported in the literature (10–12)). This corresponded to  
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27 188 concentrations of 0.38 mg Mn·l<sup>-1</sup>, 0.19 mg Zn·l<sup>-1</sup>, 0.03 mg Ni·l<sup>-1</sup>, 0.01 mg Co·l<sup>-1</sup> and 0.01 mg  
28  
29 189 Mg·l<sup>-1</sup> in the reactor. The initial concentration of biochar in the supplemented reactor was 10  
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31 190 g·l<sup>-1</sup>. As it will be further explained, this concentration was increased up to 50 g·l<sup>-1</sup> to favor the  
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33 191 consumption of the accumulated VFAs, according to the results obtained from the batch  
34  
35 192 experimental design.

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37 193 The pilot reactors consisted of jacketed cylindrical vessels made of stainless steel that had  
38  
39 194 inner stirring blades to provide continuous agitation. A more detailed description of the  
40  
41 195 reactors can be found elsewhere (9). The experiments lasted for 196 days.

42  
43 196 Analytical methods

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45 197 *Physico-chemical characterization of the FW*

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47 198 The TS and VS contents were measured as described in the standard methods of the American  
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49 199 Public Health Association (46). The concentrations of carbohydrates and lipids were  
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51 200 determined using the Dubois method (47) and by a gravimetric method based on accelerated  
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3 201 solvent extraction (46), respectively. The protein content was calculated using the total  
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5 202 Kjeldahl nitrogen (TKN) contents (6.25 g protein·g N<sup>-1</sup> (48)). The TKN and NH<sub>4</sub><sup>+</sup>  
6  
7 203 concentrations were determined using an AutoKjeldahl Unit K-370, BUCHI. The contents of  
8  
9 204 organic (TOC) and inorganic carbon (IC) were measured with a Shimadzu (Kyoto, Japan)  
10  
11 205 TOC-V<sub>CSN</sub> Total Organic Carbon Analyzer coupled to a Shimadzu ASI-V tube rack. The C/N  
12  
13 206 ratio was calculated as TOC/TKN. A WTW (London, United Kingdom) pHmeter series  
14  
15 207 inoLab pH720 was used for pH measurement. Finally, the biochemical methane potentials  
16  
17 208 (BMPs) of the substrates were measured according to Motte et al. (49).

#### 209 *Gas quantification and analysis*

210 The amount of methane produced was automatically measured in the AMPTSII system and  
211 the volume of biogas produced in the pilot reactors was continuously measured using Ritter  
212 MilliGascounters MGC-1 V3.0. The composition of the biogas was determined by gas  
213 chromatography as described in Cazier et al. (50).

#### 214 *Analysis of metabolites and final products of the digestion*

215 A sample of digestate from the pilot reactors was taken once per week for measurement of the  
216 concentrations of VFAs and ionic species in the reactors. Concerning the batch study, a  
217 plastic tube submerged in the reaction media served for digestate sampling when required.  
218 Before sampling, a clip was used for blocking the gas output and the equivalent volume of  
219 digestate to be removed was injected as nitrogen gas, avoiding an overestimation of the  
220 produced gas. The concentrations of VFAs and ionic species in the digestates were measured  
221 as described in Motte et al. (51), by gas and ion chromatography, respectively.

#### 222 *Data analysis*

223 The concentration of FAN was calculated according to Chen et al. (52) as a function of  
224 temperature, pH, and TAN concentration.

225 The methane yields were calculated by dividing the total volume of methane produced by the

1  
2 226 initial mass of VS of substrates. They were corrected to take into account the digestate  
3  
4 227 removed when sampling. The concentrations of FAN in the reactors were determined  
5  
6 228 according to Rajagopal et al. (8), as a function of temperature, pH, ionic strength and TAN  
7  
8 229 concentration. The concentrations of the main ions present in the reactors ( $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  
9  
10 230  $\text{Mg}^{2+}$ ,  $\text{H}^+$ , VFAs and  $\text{Ca}^{2+}$ ) were taken into account in this calculation.  
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13 231

## 15 232 **Results and discussion**

### 17 233 Optimization of biochar and $\text{FeCl}_3$ dosing in batch reactors

19  
20 234 As aforementioned, the first objective of this study was to evaluate the effect of different  
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22 235 concentrations of both biochar and industrial  $\text{FeCl}_3$ , aiming at optimizing their dosage. The  
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24 236 results of the experimental design (using the second consecutive batch) are shown in Table 4.  
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26 237 All the reactors produced methane efficiently, with yields ranging from 456 to 505 ml  $\text{CH}_4\cdot\text{g}$   
27  
28 238  $\text{VS}^{-1}$  and final pH values between 8.15 and 8.42. These high pH values were caused by the  
29  
30 239 high TAN concentrations, up to 9.75  $\text{g}\cdot\text{l}^{-1}$ . As it can be observed, the main differences were  
31  
32 240 related to the AD kinetics. The maximum methane production rates (consequence of the  
33  
34 241 initial consumption of HAc) varied widely, from 907 to 1498  $\text{ml}\cdot\text{g}\text{VS}^{-1}\cdot\text{d}^{-1}$ . The total time for  
35  
36 242 consumption of HPr (most recalcitrant VFA to be degraded) ranged from 18.9 to 24.4 days.  
37  
38 243 The average daily methane production was calculated by dividing the total volume of methane  
39  
40 244 produced by the batch duration (*i.e.* time to completely degrade HPr), according to Capson-  
41  
42 245 Tojo et al. (30). It varied from 302 to 376  $\text{ml}\text{CH}_4\cdot\text{d}^{-1}$ . The values of the methane yields, the  
43  
44 246 maximum methane rates and the average daily methane production rates were used as inputs  
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46 247 for the quadratic model (Equation 1), obtaining the results presented in Table 5. The raw  
47  
48 248 kinetic curves of the methane yields and production rates, the pH values and the  
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50 249 concentrations of the different VFAs are presented in Figure S1 and Figure S2 (supplementary  
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52 250 material).  
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3 251 Starting with the methane yields, the modeled results indicated that both linear coefficients  
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5 252 from the biochar and the  $\text{FeCl}_3$  (*i.e.*  $a_{\text{biochar}}$  and  $a_{\text{FeCl}_3}$ ) had a significant effect on this variable  
6  
7 253 (p-values linear coefficients  $< 0.05$ ). However, the quadratic parameter  $a_{\text{biochar-biochar}}$  was  
8  
9 254 statistically different than zero with a 99 % of certainty (p-value  $< 0.01$ ) and the high value of  
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11 255 this parameter indicated that the experimental design was not properly centered for predicting  
12  
13 256 the methane yield. The relatively low  $R^2$  value (86.7 %) also suggests the lack of fit of the  
14  
15 257 model. In addition, the Durbin-Wilson test (with a p-value  $< 0.05$ ) could not exclude that  
16  
17 258 correlations existed due to the order in which the data were used as input. All these results  
18  
19 259 suggest that the model was not able to predict any direct effect of the biochar or the  $\text{FeCl}_3$  on  
20  
21 260 the final methane yields obtained, indicating that no significant differences existed between  
22  
23 261 the obtained results. Considering that the biodegradable matter content of these two additives  
24  
25 262 should be negligible, this is a logical outcome.  
26  
27 263 Nevertheless, the model was able to reproduce precisely the maximum methane production  
28  
29 264 rates ( $R^2$  of 94.2 % and p-value Durbin-Wilson 0.20) and the average daily methane  
30  
31 265 production rates ( $R^2$  of 100 %), indicating that highly significant differences existed between  
32  
33 266 the reactors. The model responses for these two variables are presented in Figure 1.  
34  
35 267 The maximum methane production rate was mainly affected by the biochar concentration,  
36  
37 268 with a negligible influence of the industrial  $\text{FeCl}_3$  concentration. This can be verified by the p-  
38  
39 269 values of the parameters in Table 5, where it can be observed that the only parameter with a p-  
40  
41 270 value  $< 0.5$  was  $a_{\text{biochar}}$ . This indicates that addition of biochar clearly improved the  
42  
43 271 degradation of the HAc that accumulated at the beginning of the AD process, when the  
44  
45 272 maximum methane production rates were registered (Figure S1 and Figure S2). This is in  
46  
47 273 agreement with previous results, where GAC addition enhanced the HAc consumption during  
48  
49 274 batch FW AD (30). Regarding the average daily methane production rates (estimated by  
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51 275 dividing the total volume of methane produced by the time to completely degrade the HPr),  
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3 276 the value of this variable also increased greatly at higher biochar concentrations. However, in  
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5 277 this case the effect of the  $\text{FeCl}_3$  was significant, with both  $a_{\text{biochar}}$  and  $a_{\text{FeCl}_3}$  showing p-values  
6  
7 278 lower than 0.5. This variable was mainly affected by the batch duration, which was  
8  
9 279 determined by the time required for HPr consumption. Thus, it can be concluded that both  
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11 280 biochar and industrial  $\text{FeCl}_3$  addition favored the degradation of the HPr accumulated during  
12  
13 281 the first days of the batch process. This can be related to favored syntrophic interactions and  
14  
15 282 to the occurrence of DIET in the case of biochar and to the synthesis of enzymes for the  
16  
17 283 industrial  $\text{FeCl}_3$  (*i.e.*, TEs contained in this solution). Again, this is in agreement with  
18  
19 284 previous results presented in the literature. Adding GAC and TEs into batch reactors, a  
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21 285 reduction of the time required to completely degraded the accumulated HPr of around 40 %  
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23 286 was observed during batch FW AD (30).

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25  
26 287 Although optimal values could be extrapolated ( $162 \text{ g}\cdot\text{l}^{-1}$  of biochar at  $0.1 \text{ g Fe}\cdot\text{l}^{-1}$  for  
27  
28 288 maximizing the methane production rate and  $111 \text{ g}\cdot\text{l}^{-1}$  of biochar at  $0.2 \text{ g Fe}\cdot\text{l}^{-1}$  for  
29  
30 289 maximizing the average methane production rates), it is clear that the experimental design  
31  
32 290 was no properly centered and therefore further experiments must be performed to precisely  
33  
34 291 optimize the dosing of these reagents.

### 35 36 37 292 Performance of the continuous pilot reactors

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39 293 Two continuous pilot reactors were run in parallel to the batch reactors during 196 days: a  
40  
41 294 control reactor fed only with FW and a reactor supplemented with biochar and industrial  
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43 295  $\text{FeCl}_3$ . The main operational parameters and the obtained results are presented in Figure 2.  
44  
45 296 After starting the reactors at an OLR of  $1.4 \text{ g VS}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ , it was required to wait around 30-40  
46  
47 297 days to achieve a stable methane production of  $10 \text{ l}\cdot\text{d}^{-1}$  in both systems. Interestingly,  
48  
49 298 differences between both reactors could already be appreciated in this start-up period. After  
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51 299 15 days, the reactor supplemented with biochar and industrial  $\text{FeCl}_3$  showed higher methane  
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53 300 production rates than the control (Figure 2B), which were associated with higher initial  
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3 301 methane yields, lower concentrations of HAc and HPr (*i.e.* peaks of HAc of 13 and 16 g·l<sup>-1</sup>,  
4  
5 302 respectively) and higher pH values. This suggests that the added AD enhancers favored the  
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7 303 consumption of the VFAs accumulated during the start-up period, even at a biochar  
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9 304 concentration of 10 g·l<sup>-1</sup>. This is in agreement with the batch results, confirming the positive  
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11 305 effect of adding these reagents on the initial HAc consumption (leading to a lower extent of  
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13 306 HPr accumulation) and on the HPr degradation.

15 307 Moving forwards, after 60 days both reactors achieved efficient methane productions at the  
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17 308 first OLR applied, with yields around 400 ml CH<sub>4</sub>·g VS<sup>-1</sup> (95 % of the BMP), with relatively  
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19 309 low concentrations of VFAs in the reactors (2 g·l<sup>-1</sup> of HAc and 0.3 g·l<sup>-1</sup> for HPr) and with high  
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21 310 pH values (around 8.1). It must be mentioned that high TAN concentrations were already  
22  
23 311 present in the reactors at this point, with values around 8 g·l<sup>-1</sup> in both reactors at day 68. It  
24  
25 312 must also be commented that the average TAN concentrations in the reactors were 8513 ± 362  
26  
27 313 mg·l<sup>-1</sup> in control reactor and 8432 ± 777 mg·l<sup>-1</sup> in the biochar-supplemented reactor  
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29 314 (corresponding to 398 and 550 mg FAN·l<sup>-1</sup>, respectively). This indicates that no significant  
30  
31 315 differences existed and that the observed improvement of the AD performance was no caused  
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33 316 by a reduction in the TAN/FAN concentrations related to its adsorption on the biochar  
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35 317 particles.

39 318 However, due to the low OLR applied, relatively low methane production rates (around 10 l  
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41 319 CH<sub>4</sub>·d<sup>-1</sup>) were obtained. Therefore, the OLR was doubled in day 77 to reach 2.8 g VS·l<sup>-1</sup>·d<sup>-1</sup>.  
42  
43 320 This caused a sudden drop in the methane yields to 160 ml CH<sub>4</sub>·g VS<sup>-1</sup>, which was associated  
44  
45 321 with an increase in the HAc and HPr concentrations in the reactors. In agreement with the  
46  
47 322 previous results, the levels of both VFAs were always lower in the supplemented reactor.

50 323 In an attempt to reduce the intensity of VFA accumulation in the reactor containing biochar  
51  
52 324 and FeCl<sub>3</sub>, the biochar concentrations were increased to 20 g·l<sup>-1</sup> on day 105 and to 50 g·l<sup>-1</sup> on  
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54 325 day 146 (based on the results from the batch optimization experiment described above). While

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3 326 the first increase did not have significant effects, the second one (to  $50 \text{ g}\cdot\text{l}^{-1}$ ) caused a drop in  
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5 327 the HAc concentration from  $13$  to  $7 \text{ g}\cdot\text{l}^{-1}$ , raising the methane yields up to  $350 \text{ ml CH}_4\cdot\text{g VS}^{-1}$ .  
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7 328 A consequent decrease in the HPr concentration was observed (from  $3.1$  to  $1.8 \text{ g}\cdot\text{l}^{-1}$ ). Sadly,  
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9 329 on day 167 a problem occurred with the heating of the biochar-supplemented reactor. This  
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11 330 caused a temperature drop, which led to a sudden decrease of the methane yields obtained  
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13 331 and, again, an accumulation of HAc at the end of the operational period (days 167 to 196).  
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15 332 Besides the aforementioned complication, Figure 2 clearly shows that addition of biochar and  
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17 333 industrial  $\text{FeCl}_3$  decreased the HPr concentrations in reactors, with considerable differences  
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19 334 between the supplemented reactor and the control. This discrepancy was particularly  
20  
21 335 important after increasing the biochar concentrations, with HPr levels of  $7.2 \text{ g}\cdot\text{l}^{-1}$  in the  
22  
23 336 control reactor and of  $1.8 \text{ g}\cdot\text{l}^{-1}$  in the reactor containing biochar and industrial  $\text{FeCl}_3$  at the end  
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25 337 of the operational period.  
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28 338 Although the obtained results further suggest that addition of biochar and industrial  $\text{FeCl}_3$  can  
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30 339 improve the AD kinetics and favor VFA consumption during FW AD, it is also clear that  
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32 340 further continuous experiments must be carried out, allowing longer operational periods.  
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34 341 These experiments should aim to reach an operational steady-state, with results that can be  
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36 342 extrapolated to a potential industrial-scale facility.

#### 39 343 Biochar as feasible option for enhancing FW AD

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41 344 The results obtained both in batch and continuous reactors are in agreement with different  
42  
43 345 studies carried out to study the effect of biochar on AD. The kinetics of consumption of HAc  
44  
45 346 and HBU were reported to be faster when adding biochar using glucose as substrate (44). Also  
46  
47 347 the direct degradation of HPr and HBU has been improved by supplementing biochar and  
48  
49 348 ethanol (27). Sunyoto et al. (45) observed that biochar supplementation increased the methane  
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51 349 production rates and enhanced the consumption of HAc and HBU in the second stage of a 2-  
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53 350 phase AD reactor treating aqueous FW.  
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3 351 Using GAC as carbon-based AD enhancer, different studies have suggested that HAc, HBu  
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5 352 and HPr can be directly metabolized through DIET, improving the kinetics of consumption of  
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7 353 these VFAs (22,25–27). In addition, biochar has also been found to promote the growth of  
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9 354 bacteria known to participate in DIET (*i.e. Geobacter* species) onto its surface (27).  
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11 355 Therefore, it can be hypothesized that the improved VFA degradation kinetics could be  
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13 356 related to an enhancement of the syntrophic interactions between microorganisms via biofilm  
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15 357 formation and to the occurrence of DIET. The degradation of HAc through DIET has already  
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17 358 been proposed in the literature and, although being more limited thermodynamically (9),  
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19 359 DIET may have also played an important role in the oxidation of HPr. Besides, even if direct  
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21 360 DIET of HPr might not have occurred extensively, its degradation would be favored anyway  
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23 361 due to lower HAc and hydrogen/formate concentrations. Further studies analyzing the  
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25 362 microbial communities attached on the biochar, as well as the properties of the biochar used,  
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27 363 should be performed to verify this hypothesis. Concerning the industrial FeCl<sub>3</sub> addition, this  
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29 364 additive favored the HPr degradation due to the supplementation of TEs, critical for enzyme  
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31 365 synthesis (11).  
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33 366 These experiments proved that a regular biochar (natural slow-pyrolyzed wood charcoal)  
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35 367 could also improve greatly the AD performance using complex FW as substrate. It must also  
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37 368 be considered that, other than the concentration applied, many parameters and variables which  
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39 369 have not been considered in this study have a huge potential for optimization when  
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41 370 considering biochar as AD enhancer. It is clear that the textural characteristics of the biochar  
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43 371 (*e.g.* specific surface, pore volume, pore size or pore distribution) as well as its surface  
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45 372 chemistry (*e.g.* hydrophobicity) or its particle size play a major role on biofilm formation. In  
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47 373 addition, also its resistivity (conductivity) might have a huge impact on its capability for  
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49 374 favoring DIET. All these characteristics are dependent on different variables that clearly  
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51 375 deserve further study, such as the raw material used for biochar production (41), the  
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2 376 temperature and pressure applied during pyrolysis (*i.e.* slow or fast pyrolysis) or the  
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4 377 pretreatment applied to the biochar before its addition into the AD reactor (*i.e.* mechanical  
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6 378 grinding) (32).  
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9 379 Although deep techno-economic analyses must be carried out before considering its  
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11 380 application at industrial scale (particularly at the high concentrations of biochar applied,  
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13 381 which would require recirculation of the solid fraction of the digestate for biochar  
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15 382 reutilization), the obtained results suggest for the first time that biochar and industrial FeCl<sub>3</sub>  
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17 383 can be a feasible alternative for stabilizing AD of FW, favoring the consumption of VFAs and  
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19 384 improving the methane productivities. When compared to substrate dilution (1:1 vol:vol),  
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21 385 which is the most commonly applied stabilization method in industrial facilities (increasing  
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23 386 greatly the HRTs and the volumes of digestate produced and requiring an input of clean  
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25 387 water), the proposed technology is clearly a more environmental-friendly option that can be  
26  
27 388 coupled with other waste treatment processes (*i.e.* green waste pyrolysis).  
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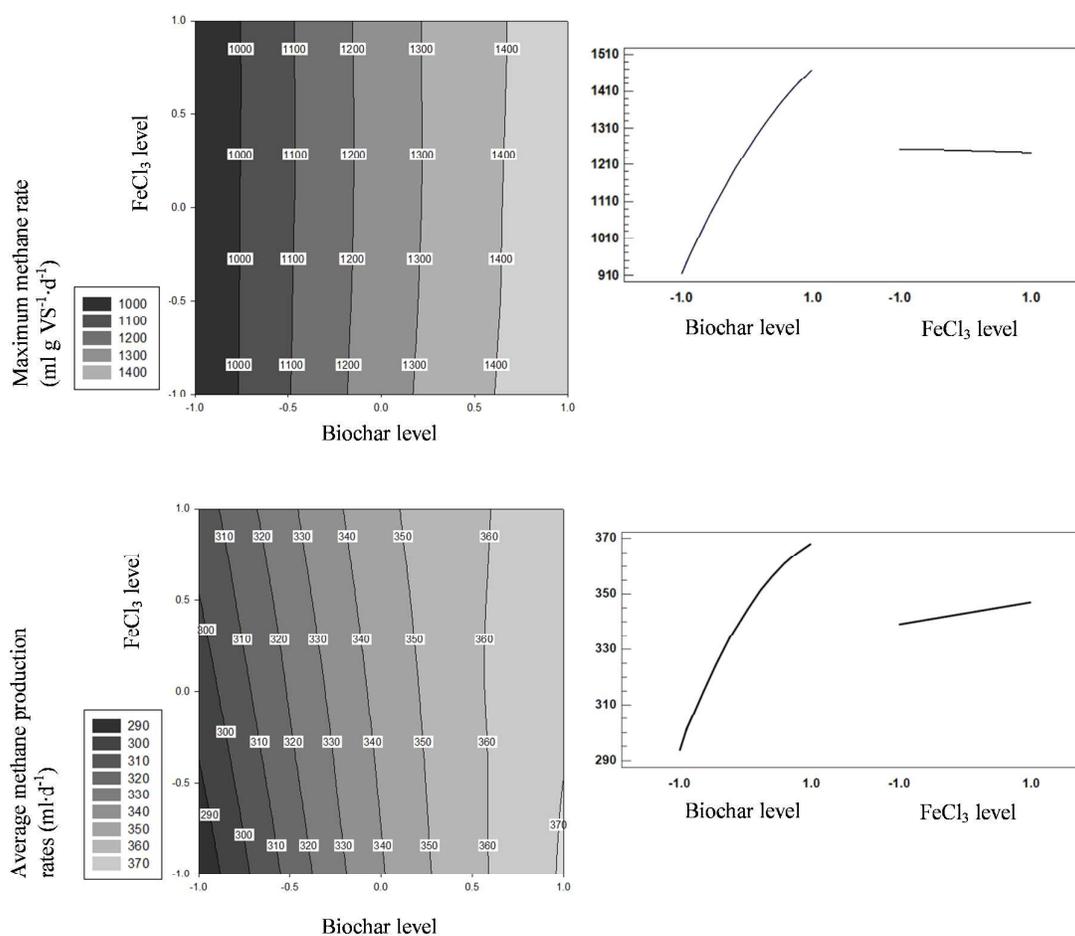
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565 **Figure 1.** Surface responses (left) and average individual effects (right) of (up) the maximum

566 methane rates and (down) the average daily methane production rates

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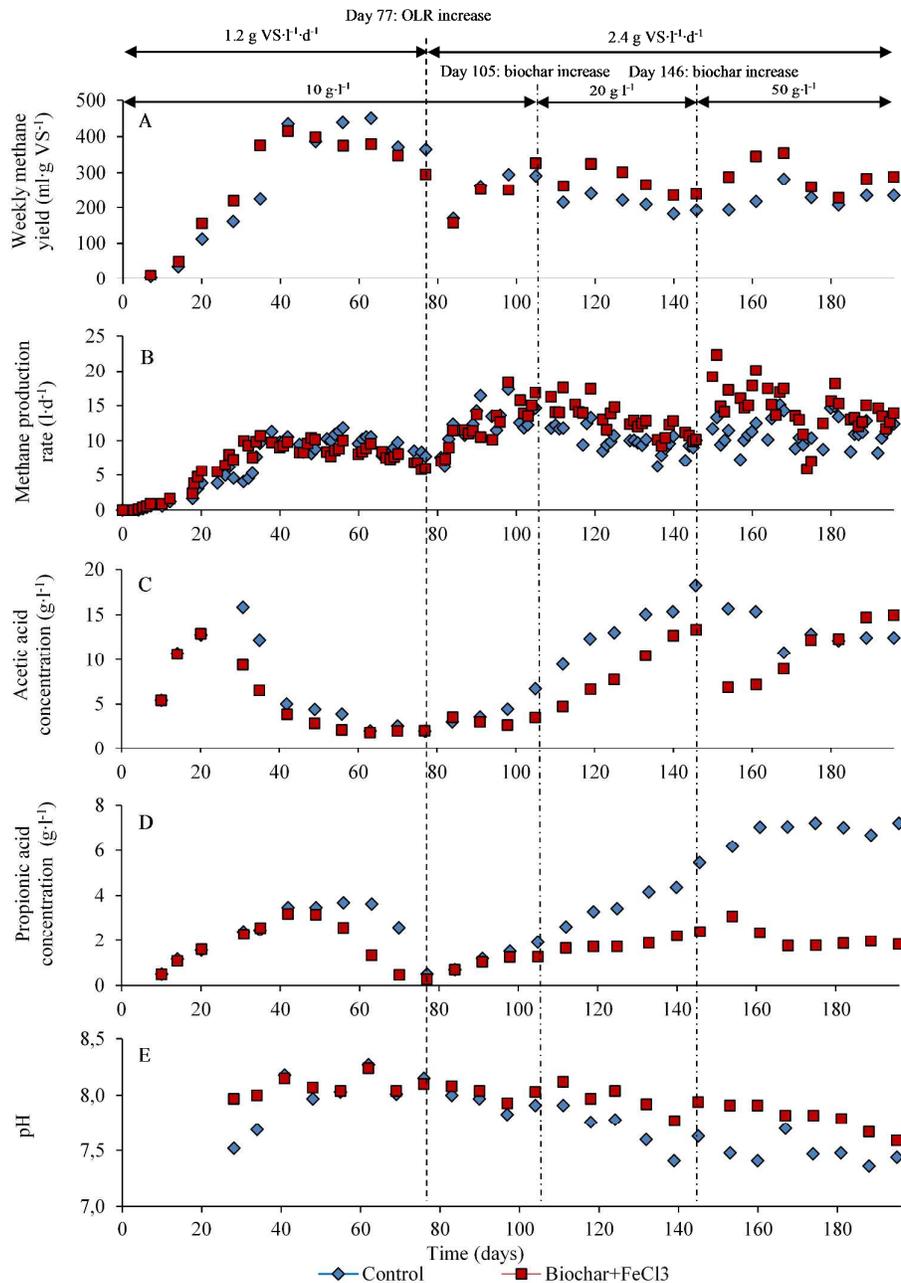
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580 **Figure 2.** Evolution of the (A) average weekly methane yield, (B) methane production rate,  
 581 (C) acetic acid concentration, (D) propionic acid concentration and (E) pH in the pilot  
 582 reactors. The days in which an operational parameter (*i.e.* OLR or biochar concentration) was  
 583 modified are also indicated (vertical lines)

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585 **Table 1.** Characteristics of the food waste mixture and the inoculum (9)

Parameter	Food waste mixture	Inoculum
TS (%)	21.0	5.81
VS/TS (%)	90.3	59.1
Carbohydrates (g·kg TS <sup>-1</sup> )	618	n.m. <sup>1</sup>
Proteins (g·kg TS <sup>-1</sup> )	187	n.m. <sup>1</sup>
Lipids (g·kg TS <sup>-1</sup> )	121	n.m. <sup>1</sup>
BMPs (ml CH <sub>4</sub> ·g VS <sup>-1</sup> )	420	n.m. <sup>1</sup>
pH	5.02	8.10
TAN (g·kg TS <sup>-1</sup> )	0.90	5.04
TKN (g·kg TS <sup>-1</sup> )	30.0	93.0
C/N	16.1	3.04

1. n.m. stands for "not measured"

**Table 2.** Characteristics and composition of the industrial FeCl<sub>3</sub> solution

Parameter	Unit	Value
Density at 20 °C	(g·cm <sup>-3</sup> )	1.45
FeCl <sub>3</sub>	(%)	41.1
Cl total	(g·l <sup>-1</sup> )	397
Fe total	(g·l <sup>-1</sup> )	206
HCl	(g·l <sup>-1</sup> )	2.2
Mn	(mg·l <sup>-1</sup> )	780
Zn	(mg·l <sup>-1</sup> )	390
Pb	(mg·l <sup>-1</sup> )	220
Ni	(mg·l <sup>-1</sup> )	67
Co	(mg·l <sup>-1</sup> )	28
Cu	(mg·l <sup>-1</sup> )	65
Cr	(mg·l <sup>-1</sup> )	45
Ca	(mg·l <sup>-1</sup> )	540
Na	(mg·l <sup>-1</sup> )	110
Al	(mg·l <sup>-1</sup> )	100
Mg	(mg·l <sup>-1</sup> )	15

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**Table 3.** Experimental design of the batch experiment. All the reactors were fed with 60 g of

625 FW at an S/X ratio of 1 g VS·g VS<sup>-1</sup> and incubated at 37 °C

Reactor	Normalized level		Real concentrations (g·l <sup>-1</sup> )	
	Biochar	FeCl <sub>3</sub>	Biochar	FeCl <sub>3</sub>
1	0	-1	55	0.1
2	-1	1	10	0.2
3	1	1	100	0.2
4	-1	-1	10	0.1
5	1	-1	100	0.1
6	0	1	55	0.2
7	0	-1	55	0.1
8	-1	-1	10	0.1
9	0	1	55	0.2
10	1	1	100	0.2
11	-1	1	10	0.2
12	1	-1	100	0.1

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640 **Table 4.** Results of the batch experimental design

Reactor	Methane yield (ml·g VS <sup>-1</sup> )	Maximum methane rate (ml·g VS <sup>-1</sup> ·d <sup>-1</sup> )	Time for HPr consumption (d)	Average daily methane production rates (ml·d <sup>-1</sup> ) <sup>1</sup>	Final pH
1	483	1327	18.9	363	8.15
2	505	948	23.0	302	8.22
3	484	1498	18.9	376	8.19
4	509	886	24.4	280	8.21
5	489	1436	18.9	374	8.18
6	459	1249	18.9	356	8.29
7	461	1281	20.1	329	8.29
8	501	907	21.5	316	8.17
9	456	1142	20.1	323	8.29
10	466	1489	18.9	361	8.27
11	496	913	21.5	326	8.42
12	496	1457	20.1	360	8.19

641 1. Calculated as final methane yield divided by the time required for HPr consumption (batch duration)

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657 **Table 5.** Coefficients of the quadratic model for the main responses of the experimental

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658 design

Parameter/coefficient	Methane yield (ml·g VS <sup>-1</sup> )	Maximum methane rate (ml·g VS <sup>-1</sup> ·d <sup>-1</sup> )	Average daily methane production rates (ml·d <sup>-1</sup> ) <sup>1</sup>
<b>a<sub>0</sub></b>	465	1250	343
<b>a<sub>biochar</sub></b>	-9.54*	278.2**	37.3***
<b>a<sub>FeCl3</sub></b>	-9.04*	-4.71	4.02***
<b>a<sub>biochar-biochar</sub></b>	28.3**	-57.9	-12.2***
<b>a<sub>biochar-FeCl3</sub></b>	-8.03	3.25	-6.94***
<b>R<sup>2</sup></b>	86.7 %	94.2 %	100 %
<b>p-value Durbin-Wilson</b>	0.025	0.20	-

1. Calculated as final methane yield divided by the time required for HPr consumption (batch duration)

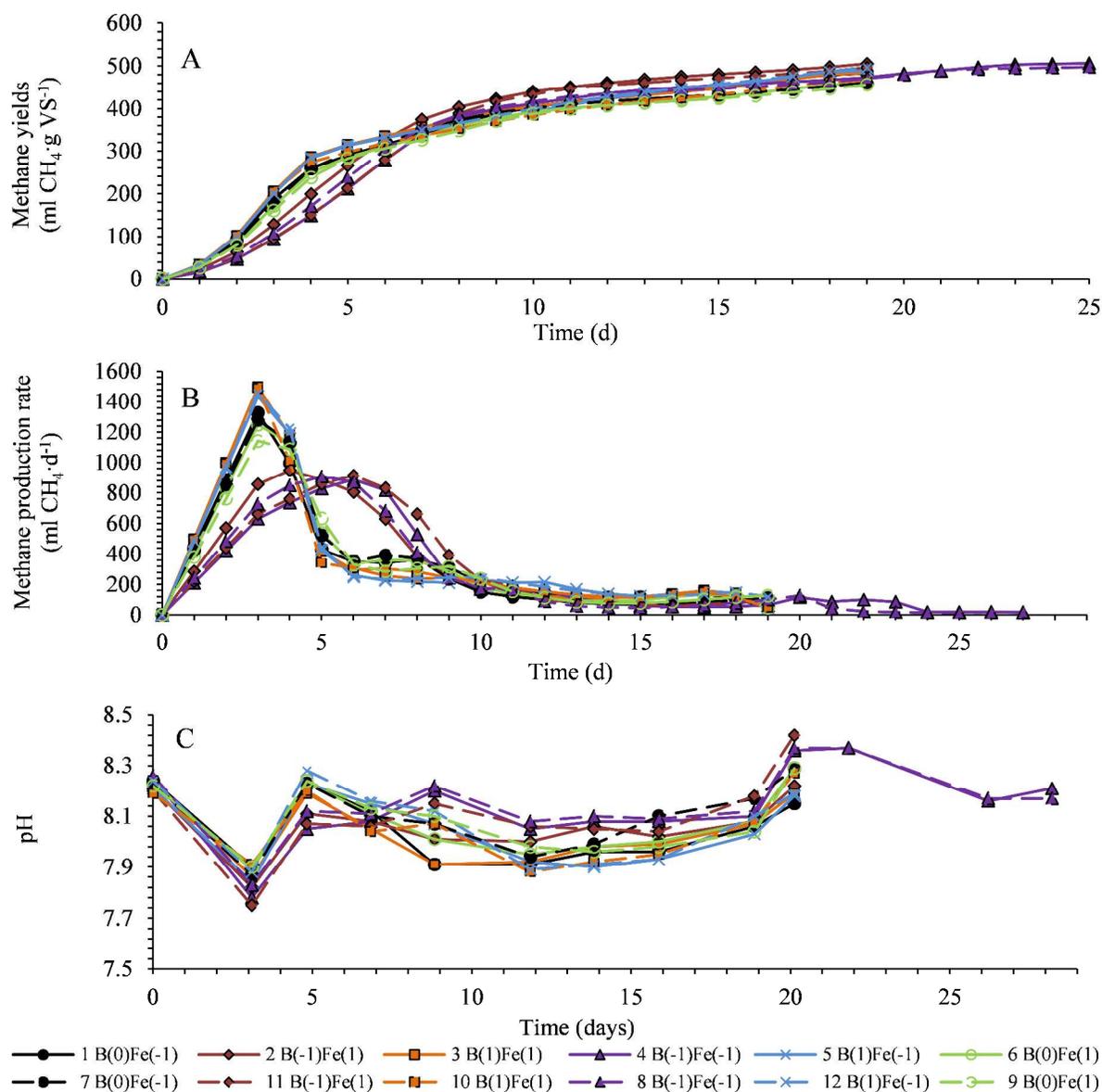
\* p-value < 0.05

\*\* p-value < 0.001

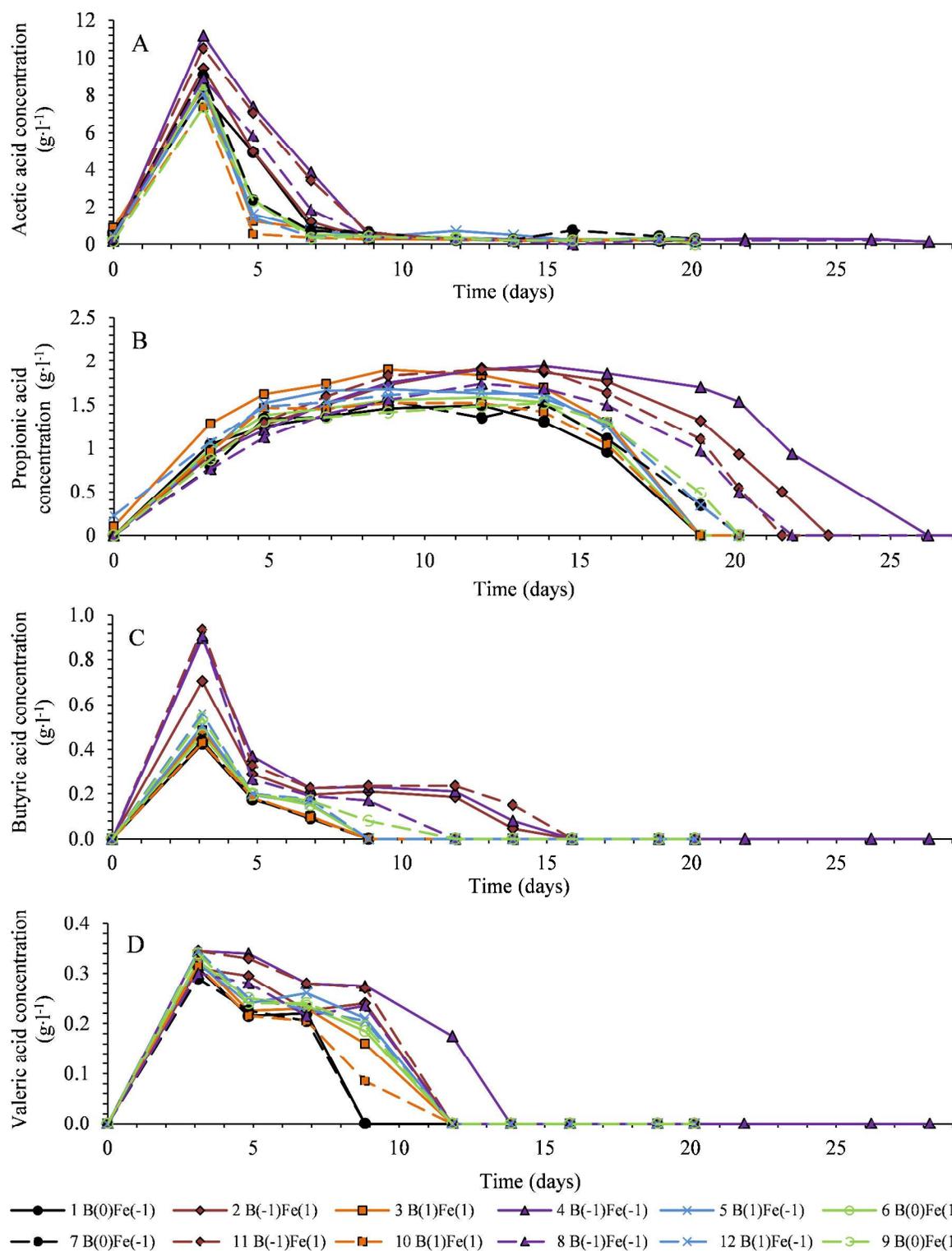
\*\*\* p-value < 0.0001

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**Figure S1.** Evolution of (A) the methane yields, (B) the methane production rates and (C) the pH in the reactors. The legend indicates the reactor number and the normalized levels of each factor (B stands for biochar and Fe for FeCl<sub>3</sub> solution)



**Figure S2.** Evolution of the concentrations of (A) acetic acid, (B) propionic acid, (C) butyric acid and (D) valeric acid in the reactors. The legend indicates the reactor number and the normalized levels of each factor (B stands for biochar and Fe for  $\text{FeCl}_3$  solution)