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Biochar and industrial FeCl3 as additives to favor the consumption of volatile fatty acids during anaerobic digestion of food waste

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Biochar and industrial $FeCl_3$ as additives to favor the consumption of volatile fatty acids during anaerobic digestion of food waste

Short title: Biochar and industrial $FeCl_3$ to stabilize the anaerobic digestion of food waste

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Abstract

BACKGROUND

Although anaerobic digestion is a promising alternative for the valorization of complex substrates such as food waste, this process is yet to be optimized and options for its stabilization must be developed. The goal of this study was to assess for the first time if the addition of biochar, together with trace elements in the form of an industrial $FeCl_3$ solution, could serve as method for stabilizing anaerobic digestion of food waste.

RESULTS

Results from batch reactors demonstrated that the supplementation of both biochar and industrial $FeCl_3$ favored the digestion kinetics. Their addition improved the maximum methane production rates (from 886 up to 1498 ml·g VS^{-1}·d^{-1}) and the average daily methane production rates (from 280 up to 376 ml·d^{-1}), related to acetate and propionate consumption,

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respectively. Continuous reactors confirmed the batch results, with higher methane production rates (up to 1.75 $l \cdot l^{-1} \cdot d^{-1}$) and lower concentrations of both acetate and propionate when biochar and FeCl3 were added.

CONCLUSION

Addition of biochar and industrial FeCl3 favored the digestion kinetics, improving volatile fatty acid consumption and the methane production rates. Although more research is needed, these materials appear as an economically-feasible alternative for stabilizing the valorization of food waste at industrial scale.

Introduction

The increasing production of food waste (FW) and novel international regulations call for the development of novel sustainable technologies for its treatment and valorization (1). Among the existing options, anaerobic digestion (AD) is an environmental-friendly process that provides an efficient waste treatment, producing at the same time biogas and digestate. Nevertheless, FW AD is a complex biological process that often leads to inefficient results or reactor acidification. The first complication occurring during FW AD is related to the fast biodegradability of this substrate, which is mainly composed of easily degradable carbohydrates. Therefore, the reactors can be easily overloaded, especially in batch systems (2), causing an unbalance between the acidogenesis/acetogenesis and the methanogenesis steps, which results in an initial accumulation of volatile fatty acids (VFAs) and a consequent pH drop (1,3–6). The second issue to be dealt with is related to the high protein content of this substrate and its low water content (~20 % total solids; TS). During AD, organic nitrogen (usually in the form of proteins) is reduced into ammonia nitrogen (total ammonia nitrogen; TAN), which, in its free form (free ammonia nitrogen; FAN) is toxic to microorganisms, especially to methanogenic archaea, eventually resulting also in an accumulation of VFAs.

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Thus, different FW AD studies have reported inefficient performances due to high concentrations of FAN (7–9).

The main alternative that has been applied to favor the consumption of VFAs in FW AD is the supplementation of trace elements (TEs) (10–14). TEs have been found to favor VFA consumption in both mesophilic and thermophilic AD of FW, mainly due to a favored synthesis of critical enzymes in the process of syntrophic hydrogenotrophic methanogenesis (HM), which is known to be the predominant methane-producing pathway during FW AD (2,11,15–17). TEs addition has been successfully applied to avoid accumulation of VFAs at high organic loading rates (OLRs) (10,18,19) and to recover acidified reactors (20,21).

Another strategy to favor VFA consumption during AD that has gained attention in the last few years is the addition of conductive materials (22,23). This approach is based on the capability of these materials for improving microbial interactions, by allowing the formation of biofilm onto theirs surfaces and by facilitating the occurrence of direct interspecies electron transfer (DIET), a mechanism in which the transfer of electrons between species occurs through shared physical connections. DIET is a faster and more efficient route for electron transfer than mediated interspecies electron transfer (23) and, as molecular hydrogen is no longer formed, VFA oxidation is thermodynamically independent of its concentration (22,24). Moreover, recent studies have suggested that acetic acid (HAc), butyric acid (HBu) and propionic acid (HPr) can be metabolized through DIET (22,25–27). Materials such as granular activated carbon (GAC), carbon cloth or magnetite have been found to favor VFA consumption and methane production during AD (26,28,29). Among them, GAC appears as a particularly performant alternative that has been found to promote the consumption of HAc, HBu and HPr during AD of dog-food waste and FW (22,28,30).

However, both TEs and GAC are expensive and their application at an industrial scale is far from being feasible. Thus, cheaper alternatives must be found. A simple substitute for the TEs

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solution may be industrial $FeCl_3$, which consists on an acid solution, highly concentrated in different metals (mainly Fe) that is commonly applied in wastewater treatment plants and anaerobic digesters for pH control worldwide. Concerning GAC, an affordable substitute may be biochar, which is also a carbon-conductive material with the capacity of accepting and donating electrons (31). In addition, biochar may also improve the AD process by providing buffering capacity and by sorption of inhibitors (32). It also favors nutrient retention in the digestates, facilitating nutrient uptake if the digestate is spread on land for plant cultivation (32,33). Moreover, as biochar can be produced from digestate (34) or directly from FW (35), its addition for AD improvement clearly fits within the approach of environmental biorefinery.

Biochar has been previously used as amendment for AD: (i) for biogas purification (removal of H_2S or CO_2) (36–38), (ii) as reactor packing for biofilm support (39), (iii) as AD substrate (40), (iv) for nutrient supplementation (41), (v) as matrix for sorption of inhibitors (42,43) or (vi) as means to increase the buffer capacity of the system (33). However, only three studies have been carried out dealing with low-dosage of biochar for improving the AD kinetics. Luo et al. (44) studied the addition of biochar for improving the kinetics of methane production and VFA degradation (HAc and HBu) with glucose as substrate, concluding that a positive effect existed. Zhao et al. (27) investigated the effect of biochar and ethanol supplementation in reactors degrading HPr and HBu, observing that the degradation of both VFAs was improved by adding biochar and concluding that bacteria known to participate in DIET (*i.e.* *Geobacter* species) were attached onto the biochar surface. Finally, Sunyoto et al. (45) assessed the influence of biochar addition in a two-phase AD reactor treating aqueous carbohydrates FW, concluding that biochar supplementation increased the hydrogen and methane production rates. They attributed this improvement to a promotion of the biofilm formation.

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To the knowledge of the authors, no study has been carried out so far to assess if addition of biochar can favor VFA consumption during AD of complex substrates such as FW. Moreover, the stabilizing effect of jointly adding industrial $FeCl_3$ (to provide TEs) and biochar on the performance of FW AD has been investigated for the first time. Therefore, the goal of this study was to: (i) optimize the concentrations of both biochar and industrial $FeCl_3$ using a batch experimental design to improve the kinetics of FW AD and (ii) evaluate the effect of industrial $FeCl_3$ and biochar supplementation on the AD performance of semi-continuous pilot reactors treating commercial FW.

Experimental

Inoculum, substrate and AD additives

The reactors were inoculated with digestate from an industrial plant treating different organic streams at high TAN/FAN concentrations (5.04 g TAN·l^{-1}; 615 mg FAN·l^{-1}). Thus, it was assumed that the microorganisms would be adapted to high the high TAN/FAN levels associated with FW AD (9). The TS content of the inoculum was 5.81 ± 0.02 %, with a proportion of volatile solids (VS) of 59.13 ± 0.08 %. The activity of the inoculum was verified prior its utilization using ethanol as substrate. The commercial FW was collected from five mayor producers from the region of the Grand Narbonne, in the south of France. A proportional mixture (wet weight) was used as substrate. The main characteristics of the FWs, as well as those of the mixture used as substrate and the inoculum are presented in Table 1. The characteristics of the FW mixture were in agreement with typical values presented in the literature (1), which indicated that it could be considered as a representative sample of a general FW. It had a TS contents of 21 % (90.3 % VS), it was mainly composed of carbohydrates (618 g·kg TS^{-1}) and it had relatively low C/N ratios (16.1). No sulfur was detected. A more extensive characterization and a deeper discussion of the results can be

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found in Capson-Tojo et al. (9).

The industrial $FeCl_3$ was provided by an industrial AD plant, where it is still used for pH controlling purposes. The characteristics and composition of this industrial solution, rich in TEs, are shown in Table 2.

The biochar was natural slow-pyrolyzed wood charcoal, commonly found in the market for several applications. Before utilization, the biochar was grinded and sieved at 600 µm.

Batch experimental design for dosage optimization

A multilevel factorial design was used to optimize the dosage of biochar and industrial $FeCl_3$ and to evaluate their individual effect on the methane production and the VFA production-consumption kinetics. Digestate from continuous reactors digesting FW was used as inoculum (after consumption of the remaining VFAs). This sludge had a TS content of 5.17 %, with 60.2 % corresponding to VS and had a TAN content of 7.27 $g \cdot l^{-1}$. Sixty g of FW were added as substrate in all the reactors. A substrate to inoculum (S/X) ratio of 1 g VS·g VS^{-1} was applied, leading to initial FW concentrations of approximately 27 g VS $FW \cdot l^{-1}$. The reactors were incubated at 37 °C. The working volumes ranged from 487 ml to 529 ml. Two different concentrations of the $FeCl_3$ solution (0.1 and 0.2 g $Fe \cdot l^{-1}$) and three of biochar (10, 55 and 100 $g \cdot l^{-1}$) were tested. As a result, an experimental design with 12 conditions was defined (6 conditions in duplicate; Table 3). Two consecutive batch feeding were carried out. The first feeding served for inoculum adaptation (results not presented), and the results of the second one were used for modelling purposes. It must be mentioned that, based on previous results, it was decided not to include into the experimental design reactors containing only biochar and $FeCl_3$. As it was demonstrated in Capson-Tojo et al. (30), although the separate addition of GAC and TEs also enhanced the VFAs degradation kinetics during FW AD, the best performances were achieved when both of these reagents were added simultaneously. With these results as starting point, the current experimental designed was defined. The same study

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was also used to define the levels of industrial $FeCl_3$ and biochar to be tested (30). This experimental design was chosen because it allows analyzing the effects of the selected factors (biochar and $FeCl_3$ concentrations) on a chosen output through the entire experimental region covered. This implied that it was possible to distinguish between the effect of biochar and $FeCl_3$ without the need of single additive treatments. It allows so by predicting the responses using a quadratic model (Eq. 1):

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

Where y is the response to be predicted, x_i are the studied factors and a_i, a_{ii} and a_{ij} are the parameters corresponding to each factor. These parameters represent the linear effects, the quadratic effects and the interactional effects, respectively. The first coefficient a_0 is required for fitting the mathematical model. The p-values from F-tests (95 % confidence) and the coefficient of determination R^2 were used to evaluate the fitness of the model. The experiment was designed and evaluated using the software STATGRAPHICS Centurion XVI Version 16.1.03 (©StatPoint Technologies Inc.).

The reactors were incubated in an Automated Methane Potential Testing System (AMPTSII) (Bioprocess Control, Sweden). Twelve reactors from the AMPTSII system, with a total volume of 600 ml, were used. According to the manufacturer instructions, they were connected to CO_2 traps (NaOH solutions) and to gas flow meters to determine continuously the methane flow rates. These reactors also allowed the follow-up of the dynamics of VFA production-consumption. A hole present in each vessel was used as sampling port, facilitating an easy sampling of the digestate. The reactors were agitated during one minute every 10 minutes at 40 rpm.

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

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

Continuous pilot scale reactors

In parallel to the batch reactors, two different pilot scale reactors were run: a control system simply digesting FW and a doped reactor supplemented with biochar and the $FeCl_3$ solution. Both reactors were incubated at 37 °C and had a working volume of 12 l. The reactors were fed once per day, initially with an OLR of 1.4 g $VS \cdot l^{-1} \cdot d^{-1}$, corresponding to a hydraulic retention time (HRT) of 110 days. This value was increased to 2.8 g $VS \cdot l^{-1} \cdot d^{-1}$ (HRT of 55 days) after 77 days of operation. An equivalent amount of digestate was withdrawn to keep the volume of the reactors constant. The $FeCl_3$ solution was diluted with water (x20 vol:vol) and dosed into the supplemented reactor to keep a constant concentration of 100 mg $Fe \cdot l^{-1}$ (value calculated from optimal results reported in the literature (10–12)). This corresponded to concentrations of 0.38 mg $Mn \cdot l^{-1}$, 0.19 mg $Zn \cdot l^{-1}$, 0.03 mg $Ni \cdot l^{-1}$, 0.01 mg $Co \cdot l^{-1}$ and 0.01 mg $Mg \cdot l^{-1}$ in the reactor. The initial concentration of biochar in the supplemented reactor was 10 $g \cdot l^{-1}$. As it will be further explained, this concentration was increased up to 50 $g \cdot l^{-1}$ to favor the consumption of the accumulated VFAs, according to the results obtained from the batch experimental design.

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

Analytical methods

Physico-chemical characterization of the FW

The TS and VS contents were measured as described in the standard methods of the American Public Health Association (46). The concentrations of carbohydrates and lipids were determined using the Dubois method (47) and by a gravimetric method based on accelerated

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solvent extraction (46), respectively. The protein content was calculated using the total Kjeldahl nitrogen (TKN) contents (6.25 g protein·g N^{-1} (48)). The TKN and NH_4^+ concentrations were determined using an AutoKjehdahl Unit K-370, BUCHI. The contents of organic (TOC) and inorganic carbon (IC) were measured with a Shimadzu (Kyoto, Japan) TOC-V_{CSN} Total Organic Carbon Analyzer coupled to a Shimadzu ASI-V tube rack. The C/N ratio was calculated as TOC/TKN. A WTW (London, United Kingdom) pHmeter series inoLab pH720 was used for pH measurement. Finally, the biochemical methane potentials (BMPs) of the substrates were measured according to Motte et al. (49).

Gas quantification and analysis

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

Analysis of metabolites and final products of the digestion

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

Data analysis

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

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

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initial mass of VS of substrates. They were corrected to take into account the digestate removed when sampling. The concentrations of FAN in the reactors were determined according to Rajagopal et al. (8), as a function of temperature, pH, ionic strength and TAN concentration. The concentrations of the main ions present in the reactors (Cl^-, Na^+, NH_4^+, K^+, Mg^{2+}, H^+, VFAs and Ca^{2+}) were taken into account in this calculation.

Results and discussion

Optimization of biochar and $FeCl_3$ dosing in batch reactors

As aforementioned, the first objective of this study was to evaluate the effect of different concentrations of both biochar and industrial $FeCl_3$, aiming at optimizing their dosage. The results of the experimental design (using the second consecutive batch) are shown in Table 4. All the reactors produced methane efficiently, with yields ranging from 456 to 505 ml $CH_4 \cdot g\ VS^{-1}$ and final pH values between 8.15 and 8.42. These high pH values were caused by the high TAN concentrations, up to 9.75 $g \cdot l^{-1}$. As it can be observed, the main differences were related to the AD kinetics. The maximum methane production rates (consequence of the initial consumption of HAc) varied widely, from 907 to 1498 $ml \cdot g\ VS^{-1} \cdot d^{-1}$. The total time for consumption of HPr (most recalcitrant VFA to be degraded) ranged from 18.9 to 24.4 days. The average daily methane production was calculated by dividing the total volume of methane produced by the batch duration (*i.e.* time to completely degrade HPr), according to Capson-Tojo et al. (30). It varied from 302 to 376 ml $CH_4 \cdot d^{-1}$. The values of the methane yields, the maximum methane rates and the average daily methane production rates were used as inputs for the quadratic model (Equation 1), obtaining the results presented in Table 5. The raw kinetic curves of the methane yields and production rates, the pH values and the concentrations of the different VFAs are presented in Figure S1 and Figure S2 (supplementary material).

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Starting with the methane yields, the modeled results indicated that both linear coefficients from the biochar and the $FeCl_3$ (*i.e.* $a_{biochar}$ and a_{FeCl3}) had a significant effect on this variable (p-values linear coefficients < 0.05). However, the quadratic parameter $a_{biochar\text{-}biochar}$ was statistically different than zero with a 99 % of certainty (p-value < 0.01) and the high value of this parameter indicated that the experimental design was not properly centered for predicting the methane yield. The relatively low R^2 value (86.7 %) also suggests the lack of fit of the model. In addition, the Durbin-Wilson test (with a p-value < 0.05) could not exclude that correlations existed due to the order in which the data were used as input. All these results suggest that the model was not able to predict any direct effect of the biochar or the $FeCl_3$ on the final methane yields obtained, indicating that no significant differences existed between the obtained results. Considering that the biodegradable matter content of these two additives should be negligible, this is a logical outcome.

Nevertheless, the model was able to reproduce precisely the maximum methane production rates (R^2 of 94.2 % and p-value Durbin-Wilson 0.20) and the average daily methane production rates (R^2 of 100 %), indicating that highly significant differences existed between the reactors. The model responses for these two variables are presented in Figure 1.

The maximum methane production rate was mainly affected by the biochar concentration, with a negligible influence of the industrial $FeCl_3$ concentration. This can be verified by the p-values of the parameters in Table 5, where it can be observed that the only parameter with a p-value < 0.5 was $a_{biochar}$. This indicates that addition of biochar clearly improved the degradation of the HAc that accumulated at the beginning of the AD process, when the maximum methane production rates were registered (Figure S1 and Figure S2). This is in agreement with previous results, where GAC addition enhanced the HAc consumption during batch FW AD (30). Regarding the average daily methane production rates (estimated by dividing the total volume of methane produced by the time to completely degrade the HPr),

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the value of this variable also increased greatly at higher biochar concentrations. However, in this case the effect of the $FeCl_3$ was significant, with both $a_{biochar}$ and a_{FeCl3} showing p-values lower than 0.5. This variable was mainly affected by the batch duration, which was determined by the time required for HPr consumption. Thus, it can be concluded that both biochar and industrial $FeCl_3$ addition favored the degradation of the HPr accumulated during the first days of the batch process. This can be related to favored syntrophic interactions and to the occurrence of DIET in the case of biochar and to the synthesis of enzymes for the industrial $FeCl_3$ (*i.e.*, TEs contained in this solution). Again, this is in agreement with previous results presented in the literature. Adding GAC and TEs into batch reactors, a reduction of the time required to completely degraded the accumulated HPr of around 40 % was observed during batch FW AD (30).

Although optimal values could be extrapolated (162 $g \cdot l^{-1}$ of biochar at 0.1 g $Fe \cdot l^{-1}$ for maximizing the methane production rate and 111 $g \cdot l^{-1}$ of biochar at 0.2 g $Fe \cdot l^{-1}$ for maximizing the average methane production rates), it is clear that the experimental design was no properly centered and therefore further experiments must be performed to precisely optimize the dosing of these reagents.

Performance of the continuous pilot reactors

Two continuous pilot reactors were run in parallel to the batch reactors during 196 days: a control reactor fed only with FW and a reactor supplemented with biochar and industrial $FeCl_3$. The main operational parameters and the obtained results are presented in Figure 2. After starting the reactors at an OLR of 1.4 g $VS \cdot l^{-1} \cdot d^{-1}$, it was required to wait around 30-40 days to achieve a stable methane production of 10 $l \cdot d^{-1}$ in both systems. Interestingly, differences between both reactors could already be appreciated in this start-up period. After 15 days, the reactor supplemented with biochar and industrial $FeCl_3$ showed higher methane production rates than the control (Figure 2B), which were associated with higher initial

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methane yields, lower concentrations of HAc and HPr (*i.e.* peaks of HAc of 13 and 16 $g \cdot l^{-1}$, respectively) and higher pH values. This suggests that the added AD enhancers favored the consumption of the VFAs accumulated during the start-up period, even at a biochar concentration of 10 $g \cdot l^{-1}$. This is in agreement with the batch results, confirming the positive effect of adding these reagents on the initial HAc consumption (leading to a lower extent of HPr accumulation) and on the HPr degradation.

Moving forwards, after 60 days both reactors achieved efficient methane productions at the first OLR applied, with yields around 400 ml $CH_4 \cdot g\ VS^{-1}$ (95 % of the BMP), with relatively low concentrations of VFAs in the reactors (2 $g \cdot l^{-1}$ of HAc and 0.3 $g \cdot l^{-1}$ for HPr) and with high pH values (around 8.1). It must be mentioned that high TAN concentrations were already present in the reactors at this point, with values around 8 $g \cdot l^{-1}$ in both reactors at day 68. It must also be commented that the average TAN concentrations in the reactors were 8513 ± 362 $mg \cdot l^{-1}$ in control reactor and 8432 ± 777 $mg \cdot l^{-1}$ in the biochar-supplemented reactor (corresponding to 398 and 550 mg $FAN \cdot l^{-1}$, respectively). This indicates that no significant differences existed and that the observed improvement of the AD performance was no caused by a reduction in the TAN/FAN concentrations related to its adsorption on the biochar particles.

However, due to the low OLR applied, relatively low methane production rates (around 10 l $CH_4 \cdot d^{-1}$) were obtained. Therefore, the OLR was doubled in day 77 to reach 2.8 g $VS \cdot l^{-1} \cdot d^{-1}$. This caused a sudden drop in the methane yields to 160 ml $CH_4 \cdot g\ VS^{-1}$, which was associated with an increase in the HAc and HPr concentrations in the reactors. In agreement with the previous results, the levels of both VFAs were always lower in the supplemented reactor.

In an attempt to reduce the intensity of VFA accumulation in the reactor containing biochar and $FeCl_3$, the biochar concentrations were increased to 20 $g \cdot l^{-1}$ on day 105 and to 50 $g \cdot l^{-1}$ on day 146 (based on the results from the batch optimization experiment described above). While

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the first increase did not have significant effects, the second one (to 50 $g \cdot l^{-1}$) caused a drop in the HAc concentration from 13 to 7 $g \cdot l^{-1}$, raising the methane yields up to 350 ml $CH_4 \cdot g\ VS^{-1}$. A consequent decrease in the HPr concentration was observed (from 3.1 to 1.8 $g \cdot l^{-1}$). Sadly, on day 167 a problem occurred with the heating of the biochar-supplemented reactor. This caused a temperature drop, which led to a sudden decrease of the methane yields obtained and, again, an accumulation of HAc at the end of the operational period (days 167 to 196). Besides the aforementioned complication, Figure 2 clearly shows that addition of biochar and industrial $FeCl_3$ decreased the HPr concentrations in reactors, with considerable differences between the supplemented reactor and the control. This discrepancy was particularly important after increasing the biochar concentrations, with HPr levels of 7.2 $g \cdot l^{-1}$ in the control reactor and of 1.8 $g \cdot l^{-1}$ in the reactor containing biochar and industrial $FeCl_3$ at the end of the operational period.

Although the obtained results further suggest that addition of biochar and industrial $FeCl_3$ can improve the AD kinetics and favor VFA consumption during FW AD, it is also clear that further continuous experiments must be carried out, allowing longer operational periods. These experiments should aim to reach an operational steady-state, with results that can be extrapolated to a potential industrial-scale facility.

Biochar as feasible option for enhancing FW AD

The results obtained both in batch and continuous reactors are in agreement with different studies carried out to study the effect of biochar on AD. The kinetics of consumption of HAc and HBu were reported to be faster when adding biochar using glucose as substrate (44). Also the direct degradation of HPr and HBu has been improved by supplementing biochar and ethanol (27). Sunyoto et al. (45) observed that biochar supplementation increased the methane production rates and enhanced the consumption of HAc and HBu in the second stage of a 2-phase AD reactor treating aqueous FW.

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Using GAC as carbon-based AD enhancer, different studies have suggested that HAc, HBu and HPr can be directly metabolized through DIET, improving the kinetics of consumption of these VFAs (22,25–27). In addition, biochar has also been found to promote the growth of bacteria known to participate in DIET (*i.e. Geobacter* species) onto its surface (27). Therefore, it can be hypothesized that the improved VFA degradation kinetics could be related to an enhancement of the syntrophic interactions between microorganisms via biofilm formation and to the occurrence of DIET. The degradation of HAc through DIET has already been proposed in the literature and, although being more limited thermodynamically (9), DIET may have also played an important role in the oxidation of HPr. Besides, even if direct DIET of HPr might not have occurred extensively, its degradation would be favored anyway due to lower HAc and hydrogen/formate concentrations. Further studies analyzing the microbial communities attached on the biochar, as well as the properties of the biochar used, should be performed to verify this hypothesis. Concerning the industrial $FeCl_3$ addition, this additive favored the HPr degradation due to the supplementation of TEs, critical for enzyme synthesis (11).

These experiments proved that a regular biochar (natural slow-pyrolyzed wood charcoal) could also improve greatly the AD performance using complex FW as substrate. It must also be considered that, other than the concentration applied, many parameters and variables which have not been considered in this study have a huge potential for optimization when considering biochar as AD enhancer. It is clear that the textural characteristics of the biochar (*e.g.* specific surface, pore volume, pore size or pore distribution) as well as its surface chemistry (*e.g.* hydrophobicity) or its particle size play a major role on biofilm formation. In addition, also its resistivity (conductivity) might have a huge impact on its capability for favoring DIET. All these characteristics are dependent on different variables that clearly deserve further study, such as the raw material used for biochar production (41), the

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temperature and pressure applied during pyrolysis (*i.e.* slow or fast pyrolysis) or the pretreatment applied to the biochar before its addition into the AD reactor (*i.e.* mechanical grinding) (32).

Although deep techno-economic analyses must be carried out before considering its application at industrial scale (particularly at the high concentrations of biochar applied, which would require recirculation of the solid fraction of the digestate for biochar reutilization), the obtained results suggest for the first time that biochar and industrial $FeCl_3$ can be a feasible alternative for stabilizing AD of FW, favoring the consumption of VFAs and improving the methane productivities. When compared to substrate dilution (1:1 vol:vol), which is the most commonly applied stabilization method in industrial facilities (increasing greatly the HRTs and the volumes of digestate produced and requiring an input of clean water), the proposed technology is clearly a more environmental-friendly option that can be coupled with other waste treatment processes (*i.e.* green waste pyrolysis).

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Figure and Table captions

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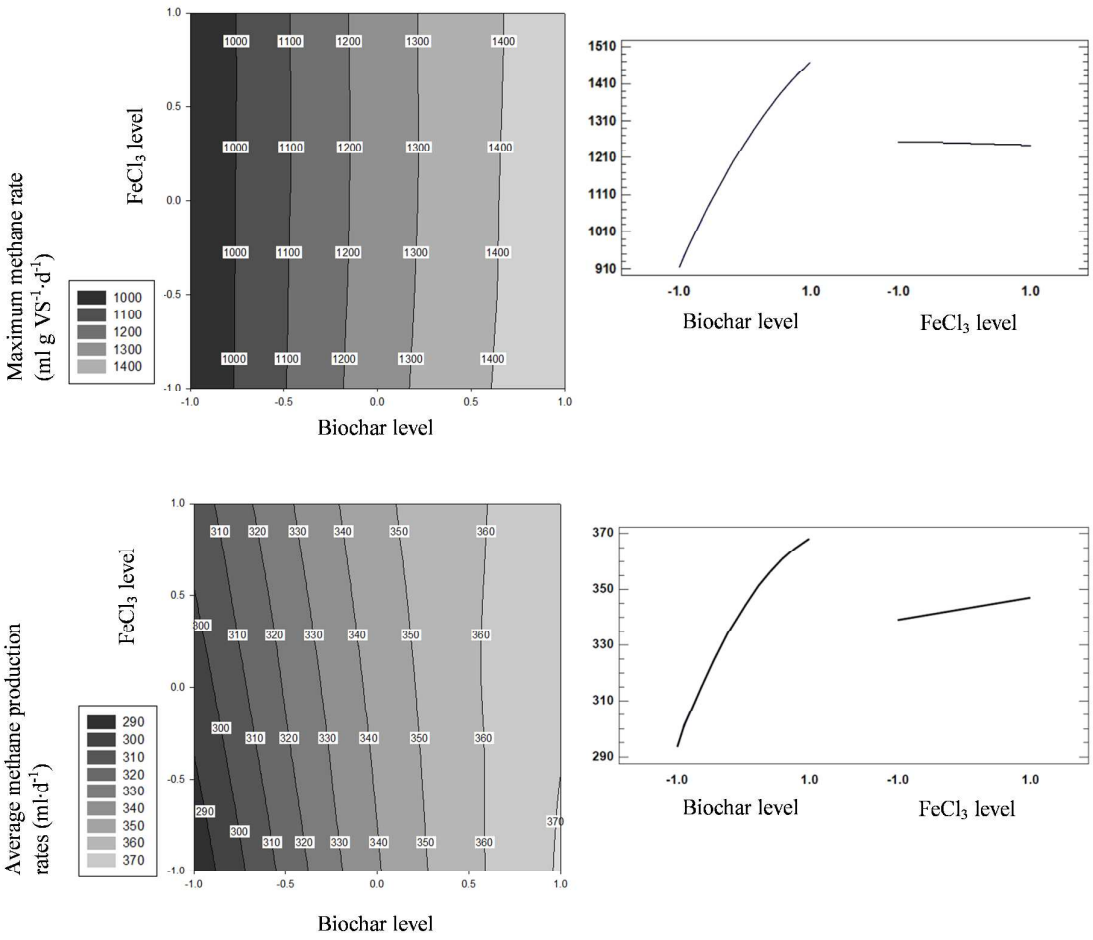

Figure 1. Surface responses (left) and average individual effects (right) of (up) the maximum methane rates and (down) the average daily methane production rates

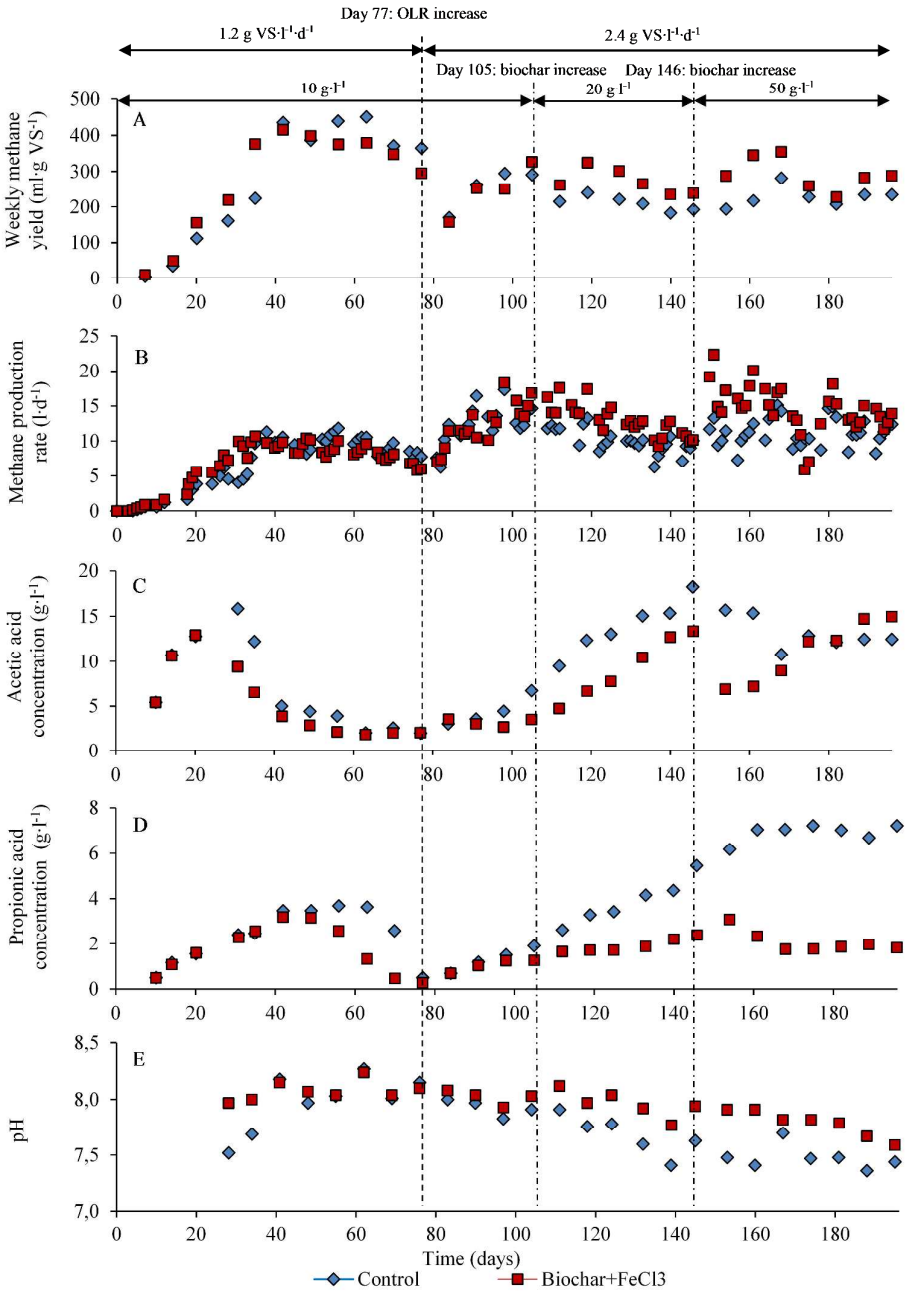

Figure 2. Evolution of the (A) average weekly methane yield, (B) methane production rate, (C) acetic acid concentration, (D) propionic acid concentration and (E) pH in the pilot reactors. The days in which an operational parameter (*i.e.* OLR or biochar concentration) was modified are also indicated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

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^{-1})	618	n.m.[1]
Proteins (g·kg TS^{-1})	187	n.m.[1]
Lipids (g·kg TS^{-1})	121	n.m.[1]
BMPs (ml CH_4·g VS^{-1})	420	n.m.[1]
pH	5.02	8.10
TAN (g·kg TS^{-1})	0.90	5.04
TKN (g·kg TS^{-1})	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 $FeCl_3$ solution

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Parameter	Unit	Value
Density at 20 °C	($g \cdot cm^{-3}$)	1.45
$FeCl_3$	(%)	41.1
Cl total	($g \cdot l^{-1}$)	397
Fe total	($g \cdot l^{-1}$)	206
HCl	($g \cdot l^{-1}$)	2.2
Mn	($mg \cdot l^{-1}$)	780
Zn	($mg \cdot l^{-1}$)	390
Pb	($mg \cdot l^{-1}$)	220
Ni	($mg \cdot l^{-1}$)	67
Co	($mg \cdot l^{-1}$)	28
Cu	($mg \cdot l^{-1}$)	65
Cr	($mg \cdot l^{-1}$)	45
Ca	($mg \cdot l^{-1}$)	540
Na	($mg \cdot l^{-1}$)	110
Al	($mg \cdot l^{-1}$)	100
Mg	($mg \cdot l^{-1}$)	15

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

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FW at an S/X ratio of 1 g VS·g VS^{-1} and incubated at 37 °C

Reactor	Normalized level		Real concentrations ($g \cdot l^{-1}$)	
	Biochar	$FeCl_3$	Biochar	$FeCl_3$
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

Table 4. Results of the batch experimental design

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Reactor	Methane yield (ml·g VS^{-1})	Maximum methane rate (ml·g VS^{-1}·d^{-1})	Time for HPr consumption (d)	Average daily methane production rates (ml·d^{-1})[1]	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

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

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

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design

Parameter/coefficient	Methane yield ($ml \cdot g\ VS^{-1}$)	Maximum methane rate ($ml \cdot g\ VS^{-1} \cdot d^{-1}$)	Average daily methane production rates ($ml \cdot d^{-1}$)[1]
a_0	465	1250	343
$a_{biochar}$	-9.54*	278.2**	37.3***
a_{FeCl3}	-9.04*	-4.71	4.02***
$a_{biochar\text{-}biochar}$	28.3**	-57.9	-12.2***
$a_{biochar\text{-}FeCl3}$	-8.03	3.25	-6.94***
R^2	86.7 %	94.2 %	100 %
p-value Durbin-Wilson	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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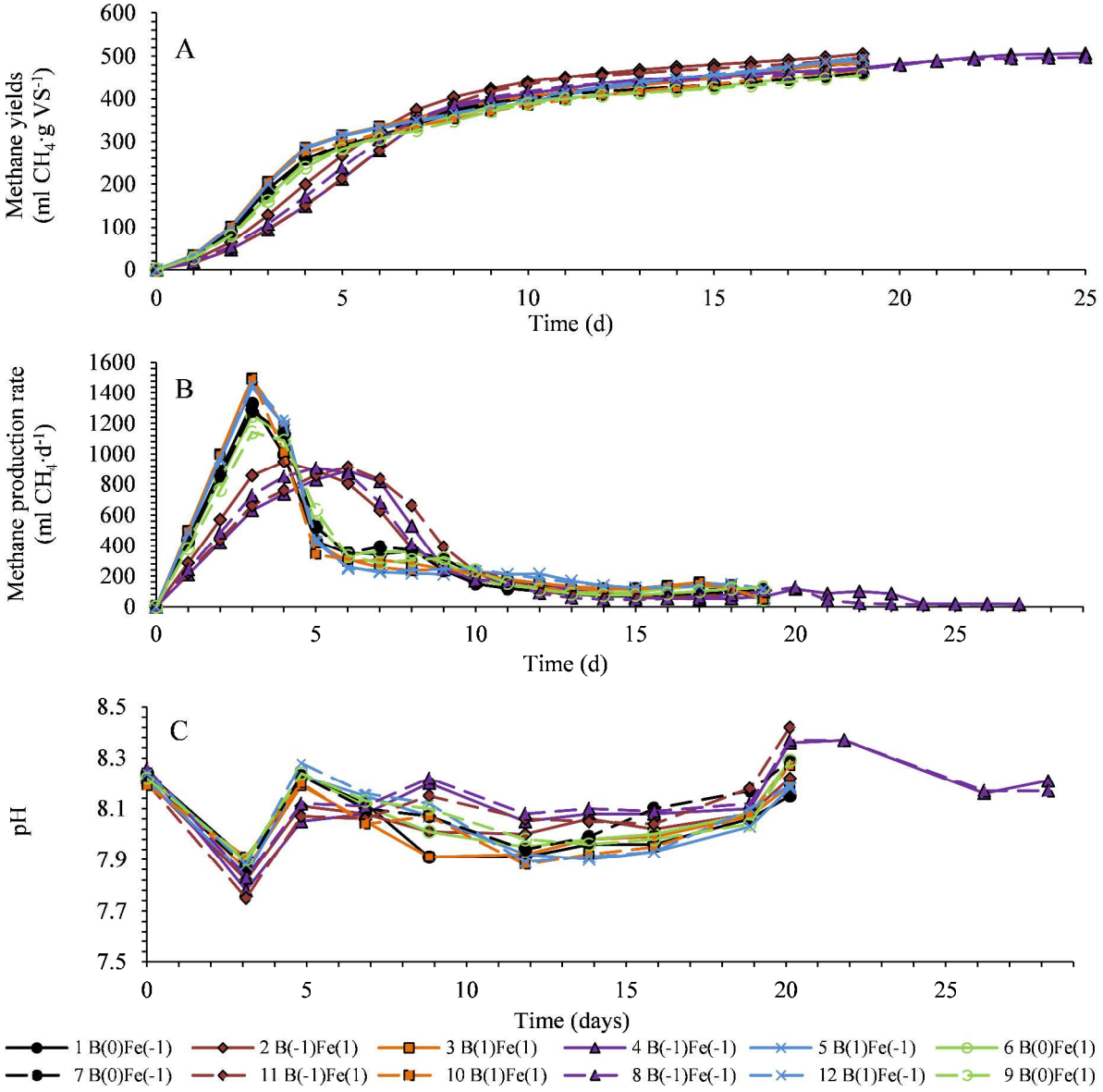

Figure S1. Evolution of (A) the methane yields, (B) the methane production rates and (C) the pH in the reactors. The legend indicates the reactor number and the normalized levels of each factor (B stands for biochar and Fe for $FeCl_3$ solution)

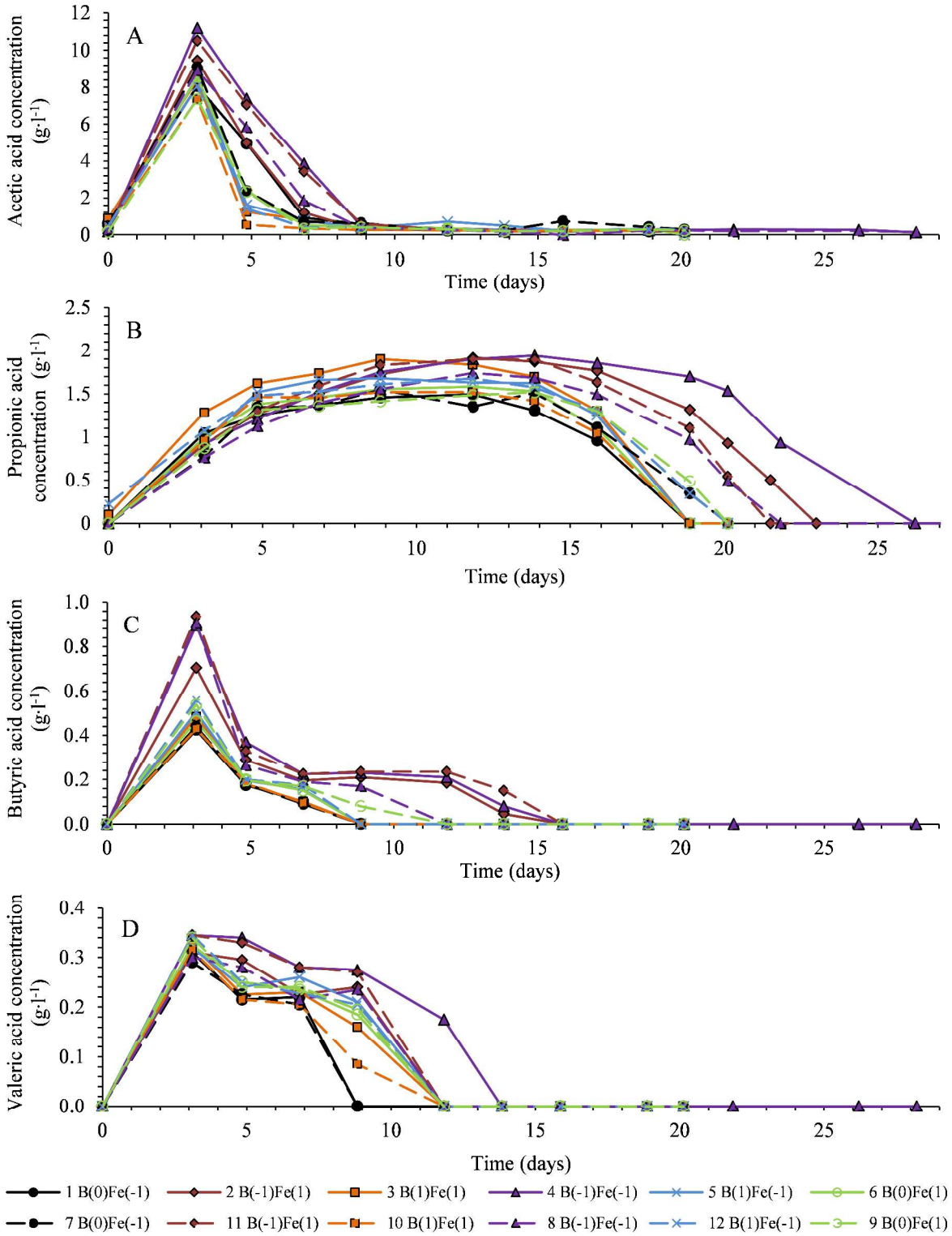

Figure S2. Evolution of the concentrations of (A) acetic acid, (B) propionic acid, (C) butyric acid and (D) valeric acid in the reactors. The legend indicates the reactor number and the normalized levels of each factor (B stands for biochar and Fe for $FeCl_3$ solution)