Organic Matter Characterization and Phytotoxic Potential Assessment of a Solid Anaerobic Digestate Following Chemical Stabilization by an Iron-Based Fenton Reaction

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Abstract

Digestates, the by-product of the anaerobic bioconversion of organic wastes for the production of biogas, are highly variable in chemical and biological properties thus limiting their potential use in agriculture as soil amendment. Using a lab-scale glass reactor we aimed to assess the feasibility to chemically stabilize the solid fraction of an anaerobic digestate applying a Fenton reaction under constant pH (3.0), temperature (70 °C), reaction time (8 h) and various combinations of H_2O_2 and Fe^{2+}. In Fenton-treated samples the phytotoxic potential (determined on a test plant), total phenols and the bad smell odor index markedly declined, whereas total C and N remained unaltered. Thermogravimetric (TG) analysis and Fourier transform infrared (FT-IR) spectroscopy revealed contrasting changes in extracted humic and fulvic fractions being increased or depleted, respectively, in aromatic substances. Process feasibility and optimum conditions for an effective biomass stabilization were achieved with a H_2O_2/Fe^{2+} ratio between 0.02 and 0.03.

Keywords: biomass valorization; elemental analysis; FT-IR spectroscopy; humic-like compounds; odor impact; post-digestion treatment; TG analysis; phenols.

Introduction

In recent years, sustainable processes of energy production have received an increased attention among several governments across the European countries. In particular, a greater attention is being devoted to the anaerobic digestion (AD) technology, which was originally developed for treating biodegradable wastes and sewage sludge.[1,2] The AD process relies upon the biological conversion of organic by-products under low oxygen content for the production of a biogas, rich in methane (50-80% v/v), together with carbon dioxide and other contaminant gases (i.e. H_2S). Biogas can be used directly as a fuel, in combined heat and power gas engines, or upgraded to natural gas-quality biomethane.[3] Besides biogas, the AD process leads to the production of a partially degraded, nutrient-

rich by-product currently named digestate.[4] Owing to its chemical composition and content of essential plant nutrient elements – especially N-reduced forms and soluble potassium - anaerobic digestate has the potential as soil conditioner to improve physical properties, and release soluble plant nutrient elements thus reducing the supply of synthetic fertilizers.[5,6] Moreover, it can increase soil C storage[7] and contribute to soil pest control.[8] Nevertheless, a complete exhaustion of the AD process is rarely achieved and, unless digestates undergo a proper post-processing treatment including composting,[9–11] soil incorporation of yet unstable or immature by-product can negatively affect the soil-plant system.[10] Most known undesirable features are: high levels of heavy metals,[12] phytotoxic compounds,[13] pathogenic bacteria,[14] bad odor emission, unbalanced nutrient content and excess inorganic N forms. As well as this, recent European[15] and National[16] regulations together with local guidelines have established the maximum annual amounts of anaerobic digestate to be incorporated into arable fields to protect groundwater from nitrate contamination. Therefore, anaerobic digestates need to be fully stabilized and characterized for their residual phytotoxicity and chemical composition before their safe use in agricultural systems.

Although known for more than one century, the Fenton reaction (first described by H.J.H. Fenton[17] and based on Fe^{2+}-catalyzed H_2O_2 decomposition in strongly oxidizing hydroxyl radicals) was not applied in environmental protection technologies until the late 1960s. Nowadays the classical Fenton reaction is considered as one of a set of advanced oxidation processes (AOPs)[18,19] and is widely used for the chemical treatment of wastewater, industrial sludge, landfill leachate, soils and sediments which are contaminated with biorefractory organic compounds such as phenols, dyes, pesticides, organic solvents, pharmaceuticals, domestic chemicals, etc.[20–24] Popularity and widespread applications of the Fenton oxidation process are due to the following features: 1) it works at near-ambient temperature and pressure conditions, 2) it requires cheap, relatively reactive and easy-to handle reagents, 3) it is rapid and effective, and 4) it can be easily integrated in more sophisticated chemical technologies for the treatment of a broad range of hazardous wastes, including solid agricultural matrices such as digestates from AD plants. Although few studies have recently

approached the chemical stabilization of the anaerobic digestate,[25–27] they primarily focused on dewaterability and changes in the chemical characteristics of treated organic wastes. Whereas no attempt has been made to assess the phytotoxic potential and investigate specific changes in spectroscopic features of digestate-derived humic-like compounds, which are key to restoring the fertility level once incorporated into arable soils. Aim of the present work was to assess the effectiveness of an iron-based Fenton reaction as a fast and low-cost, post-digestion, chemical technology to stabilize the solid fraction of an anaerobic digestate, reduce its phytotoxic potential and promote the formation of humic-like compounds, thus providing suitable features for its safe use in agriculture for managing the fertility of soil. To this aim, we employed a lab-scale glass reactor to investigate various combinations of reduced H_2O_2 and Fe^{2+} dosages on physical (bad odor emission), chemical (total phenols, organic matter, electrical conductivity) and phytotoxic properties of a solid anaerobic digestate. Fenton-treated digestates and their extracted humic and fulvic fractions were also characterized by elemental analysis, FT-IR spectroscopy and TG analysis. Findings from the research represent the first step towards Fenton process optimization in real plants for AD by-products stabilization.

Materials and Methods

Anaerobic digestate

The anaerobic digestate was provided by a local full-scale biogas producing plant (Fattoria della Piana soc. Agricola, RC, Italy) fed with an ingestate constituted by a mix of dairy cattle slurry, as the major component, together with milk serum, maize silage and, in minor amount, olive waste and citrus pulp. The rated power of the plant was 999 kWh with a hydraulic retention time (HRT) of 60 days in two continuously stirred tank reactors (CSTR) of a total capacity of 7500 m^3 (2500 m^3 tank reactor 1 + 5000 m^3 tank reactor 2), operating under mesophilic conditions (40 °C). The total volume loaded per day was 120 m^3, the hydraulic retention time (HRT) 60 days, and the minimum guaranteed retention time (MGRT) 16 h at 40 °C. The digestate used in the present research was subjected to mechanical

119 solid/liquid separation (by press screw) to separate the aqueous fraction (named liquor), which was
120 discarded, from the solid fraction which was collected and then characterized (Table 1) as previously
121 described[4,12] before use. Briefly, the electrical conductivity (EC) and pH were measured in a
122 biomass/water (1:10) and (3:50) (w/v) mixture, respectively. The ash content was determined after
123 combustion at 550 °C for 8 h, whereas total organic C and total N were measured by an automatic
124 elemental analyzer CN628 LECO (LECO Corporation, USA). NH_4^+-N and NO_3^--N contents in 2 M
125 KCl extracts (1:10 v/v) were determined colorimetrically by using a Flow Injection Analysis System
126 (FIAS 400 PerkinElmer, Inc., CT, USA) equipped with an AS90 Autosampler (PerkinElmer) and
127 linked to an UV/Vis spectrophotometer Lambda 25 (PerkinElmer). Total K, S, Ca, Mg, Cl, F, Mn, B,
128 Cd, Cr, Pb, Ni, Hg, Cu and Zn were determined by inductively coupled plasma mass spectrometry
129 (ICP-MS ICAP-Q Thermo Scientific, CA) after microwave (Ethos up, Milestones srl, I) acid
130 digestion with HNO_3/H_2O_2 (7:1 v/v). Enzymatic (alkaline phosphatase, dehydrogenase and
131 hydrolysis of fluorescein diacetate) activities were determined colorimetrically.[28] All analyses were
132 carried out in triplicate.
133
134 **Iron-based Fenton reaction**
135 The iron-based Fenton oxidation of the solid anaerobic digestate was carried out using a laboratory-
136 scale glass reactor apparatus (Figure S1). Freshly sampled solid anaerobic digestate (equivalent to
137 100 g dry weight) was finely ground (particle size < 3 mm) and then placed into the glass reactor
138 together with the Fenton reagents: Fe^{2+} (as the catalyst) provided in form of high purity epta-hydrate
139 ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 99.5% purity) (VEBI Istituto Biochimico s.r.l., Italy) and analytical
140 grade hydrogen peroxide solution (H_2O_2, 30% w/w) (Panreac Applichem, Spain) (as the oxidant),
141 which was properly diluted to the desired final concentration. Reagent dosage and H_2O_2/Fe^{2+} ratio of
142 the differing treatments as well as Fenton reaction operating conditions were as detailed in Table 2.
143 A stirring devise was properly adapted to fit the glass reactor and maintain the mixture under
144 continuous stirring at 50 rpm. The air-tight glass reactor was placed into a water bath set at 70 °C. An

internal pressure gradient of -100 mbar, respect to ambient pressure, was maintained throughout the entire process so as to allow the sample to become completely dry. The pH mixture was allowed declining to pH 3.0 and maintained until the reaction had completed. The reaction took as long as 8 hours to complete, when any repelling odor was no longer appreciable and the mixture reached a complete dryness. To sum up, ten chemically and thermally treated (A1-A5, B1-B5) and one thermally treated samples with no reagent addition (Ctrl) were produced during the Fenton stabilization process, and then immediately stored in air-tight bags before any further analysis. Untreated solid anaerobic digestate was oven-dried at 105 °C until constant weight and taken as a reference treatment (Dig).

Extraction of humic-like fractions

Fulvic (FA) and humic (HA) acids were extracted and fractionated as described previously.[29] Briefly, an amount of the organic matrix equivalent to 2 g dry weight was transferred into a 250-mL Teflon centrifuge tube containing 100 mL of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ solution. The headspace of each tube was flushed with N_2 to create an anaerobic environment, and then the tubes were shaken (80 rpm) at 65 °C for 24 h. The suspension was centrifuged at 6000 rpm for 20 min and filtered through a 0.80 µm membrane filter. An aliquot of 25 mL of the filtered extract was acidified to pH 2 with diluted H_2SO_4 (1:1 in water) to separate HA from FA. Following centrifugation (6000 rpm, 20 min), pelleted HA were collected, while the supernatant containing FA was further purified by passing it through a 10 cm^3 of an insoluble polyvinylpyrrolidone resin (Fluka Analytical) equilibrated with 0.005 M H_2SO_4. The eluate containing the non-humified fraction was discarded, while the retained fraction was eluted with 0.5 M NaOH and collected. HA and FA fractions were freeze-dried and stored at -20 °C before any further characterization.

Odor emission

Odor emission from either dry (immediately after the oxidation treatment) or rewetted (few weeks after the treatment) Fenton-treated and control samples was estimated by a panel test made up of five people using the following class rating system of bad odor emission: class 1 (no perception), class 2 (light perception), 3 (very strong perception). The mean value of the scores given to each treatment has been reported as BSI (Bad Smell Index).

Chemical analyses

The electrical conductivity (EC) of Fenton-treated and control samples was measured in a biomass/water (1:10, w/v) mixture; whereas the total phenolic compounds (TPC) content was determined as described before.[30] Briefly, a suitable amount (0.500 g) of the organic matrix was extracted with ethyl acetate under shaking at room temperature for 16 h. The extract was concentrated by evaporation under vacuum (LABOROTA 4000, Heidolph, D) and then dissolved in 5 mL of methanol. Content of TPC in the extracts was determined by using the Folin-Ciocalteu reagent (Sigma, Italy). Briefly, 20 µL of the digestate extract was added with distilled water (830 µL), the Folin-Ciocalteu reagent (50 µL) and, after 3 minutes 100 µL of 6% (w/v) NaOH. Following 1 h incubation, the content of TPC was determined spectrophotometrically at 725 nm. Analytical readings were expressed as mg of catechol equivalent per kg dry weight. The determination of organic matter (OM) content of Fenton digestates was done by loss on ignition according to the method 5A described in the Kellogg Soil Survey Laboratory Methods Manual.[31] Elemental C and N contents in Fenton digestates and their extracted humic-like fractions were determined by an automatic elemental analyzer CN628 LECO (LECO Corporation, USA). All the chemical analyses were carried out in triplicate.

FT-IR analysis

Fourier-transform infrared (FT-IR) spectroscopy analysis of Fenton-treated and control samples and their extracted HA and FA fractions was performed in the wavenumber range of 400-4000 cm^{-1} by

196 using a Spectrum One FT-IR spectrometer (PerkinElmer, CT, USA). One milligram of each organic
197 sample was ground up with 400 mg KBr (FT-IR grade) and homogenized in an agate mortar. KBr
198 pellets were compressed under vacuum, under a pressure of 10000 kg cm^{-2} for 10 min. Thirty-two
199 scans were collected and corrected against a control pellet containing only KBr.
200
201 **TG analysis**
202 Thermogravimetric (TG) analysis of dried and milled Fenton-treated and control samples and their
203 extracted HA and FA fractions (5 mg dry weight) was performed by using a Simultaneous Thermal
204 Analyser (STA) 6000 PerkinElmer (PerkinElmer, CT, USA) operating within the range 30 - 900 °C
205 at a heating rate of 15 °C min^{-1}, under synthetic air atmosphere (21 ± 1% O_2 and 79 ± 1% N_2 at a 100
206 mL min^{-1} flow rate). TG profiles and differential thermogravimetry (DTG) curves were obtained in
207 terms of the percentage of weight loss of the sample and from the first derivative of TG profiles
208 representing the rate of weight loss, respectively. In order to obtain the real organic matter content
209 (C_{corr}), the weight loss attributed to the residual water was subtracted from DTG peaks (DTG2 and
210 DTG3) as follows:
211 $$C_{corr} = 100 \cdot \left(\frac{C_i}{100 - C_{water}}\right) \quad (1)$$
212 where C_i is the weight loss of peak DTG2 or DGT3 registered in the DTG profiles (as an example,
213 the DTG profile of Dig is reported in Figure S2), and C_{water} is the first DTG peak (DTG1) occurring
214 to 60 °C and corresponds to the dehydration of the residual water content.[32]
215
216 **Phytotoxicity assay**
217 The phytotoxic assay of either the native or Fenton-treated solid anaerobic digestate was carried out
218 in accordance to previously described[33] adopting a completely randomized design with five
219 replications. Briefly, Fenton-treated digestates were extracted with water (1:20, w/v, on dry weight
220 basis) under shaking (120 rpm) at room temperature for 24 h. Then, the suspension was filtered

(Whatman® no. 42) and the extract was diluted with sterile distilled water so as to obtain the following concentrations: 0, 0.1, 1, 5, 10, 20, 80, and 100%. Germination and root growth of lettuce (*Lactuca sativa* L.) seeds exposed to the digestate aqueous extracts were evaluated. Briefly, lettuce seeds were surface-sterilized by soaking in 15% (v/v) NaClO solution for 15 minutes and then rinsed with distilled water. Ten seeds were evenly distributed into a Petri dish (Ø 6 cm), on a double layer of filter paper previously moistened with 2 mL of each dilution of the aqueous extract. Petri dishes were then placed in the dark into a growth chamber at 24 ± 1 °C and 70% relative humidity. After 48 hours, germinated seeds were counted and their root length measured by using a WinRhizo pro STD 1600 software (Instruments Régent Inc, Canada). The Germination Index [GI (%)] was calculated by multiplying the germination percentage by the root length percentage, divided by 100 as reported before.[34]

Statistical analysis

Chemical data shown in Tables 3 and 4 are reported as mean values ($n = 3$), and a Tukey's honest significant difference (HSD) post hoc test was run to compare means at a $P < 0.05$ level of significance. Data from the phytotoxic assay (Figures 4 and 5) were first tested for deviation from normality (Kolmogorov-Smirnov test) and homogeneity of within-group variances (Levene's test). After running a one-way ANOVA to check any significant effect of the treatment (extract dose) on the variability of the data (the block effect in the experimental design was found to be not significant at $P < 0.05$), multiple pairwise comparison of means was done by Tukey's HSD test at $P < 0.05$ level of significance. In order to compare the effects of aqueous extracts from differently treated digestates, a non-linear regression model based on a log-logistic function[35] was used to estimate ED_{50} values, which represents the percent extract dose lowering the maximum GI (%) by 50%. The model parameters were estimated by the least square method (TableCurve 2D v.4.0 software, Jandel Scientific Ekrath, D) using the Levenburg-Marquardt algorithm for fitting non-linear equations. Non-linear regressions were repeated several times in order to minimize the sum of the square of deviation

247 between the predicted and experimental values to less than 0.01% between two consecutive fits. The
248 quality of curve fitting was assessed by *F* test for non-fit based on analysis of variance at $P < 0.05$.
249 Statistical analyses were performed using the Systat 13.0 version 13.1 software package (SYSTAT
250 Software Inc.).
251
252 **Results**
253 **Solid anaerobic digestate characterization**
254 Chemical characterization of the digestate (Table 1) showed a slightly high dry mass content (> 30%),
255 but still consistent with what generally found in final products from AD of agroindustrial wastes.[5,10]
256 The final biomass showed an alkaline reaction, as expected, because volatile fatty acids degradation
257 and ammonia production occurring during the AD process leads to an increase of the pH.[4] Low values
258 of EC and ash content were also found, thus indicating that the soluble salts did not represent a
259 limiting factor for agricultural use of the by-product. The digestate showed also an interesting content
260 of macronutrients (namely K and S) and a low concentration of inorganic contaminants (particularly
261 Cd, Pb, Ni, Hg, Cu and Zn), which were in compliance with mandatory limits. However, the Cr(VI)
262 content was almost five-fold the limit value according to the Italian legislation,[16] whereas it was only
263 slightly above the current limit of 2 mg kg^{-1} reported in the recently approved Regulation EU n.
264 1009/2019.[15] Noticeably, most N occurred as organic form (ammonium-N was approximately ~12%
265 of total N), whereas the nitrate-N content was negligible. Total C was in accordance to literature,[36,37]
266 while a considerably high (17.86%) content of humified fractions (C_{HA} + C_{FA}) was found.
267 Nonetheless, this latter finding was consistent with the observed BOD_5 value lower than 2.5 g L^{-1}
268 (Table 1) thus designating an anaerobic digestate suitable for use as fertilizer also in accordance with
269 Alburquerque et al.[5] Even though the C/N ratio was higher than 25 (Table 1), once incorporated into
270 the soil the mineralization of the more labile organic fractions could lead to the release of soluble-N
271 forms. Finally, the residual biomass showed noticeable enzymatic activities; whereas levels of

microorganisms harmful to human health (*Salmonella* spp., *Escherichia coli*) were below the detectable threshold.

Characterization of Fenton-treated digestate

Data from the BSI panel test clearly show that a H_2O_2 dosage equal or greater than 18.9 mg L^{-1} provided conditions suitable for stabilization as observed for treatments A4, A5, B4, and B5, that reached a score as low as 1 (Table 3). This condition is achieved at both Fe^{2+} catalyst concentration levels: 846 and 1692 mg L^{-1}. However, the higher the Fe^{2+} catalyst, the lower the stability in terms of odor emission as in the case of Fenton-treated digestates B2 and B3 *vs* A2 and A3 (Table 3). This finding suggests that beside a proper single catalyst dosage, also a H_2O_2/Fe^{2+} ratio larger that 0.01 is equally decisive for ensuring optimum conditions for the Fenton oxidation process. Expedectly, no reduction in the BSI was found when one or both catalysts were omitted (Table 3). It is interesting to note that this finding is consistent with the larger amount of HA and FA recovered in samples A4, A5, B4, and B5, which appeared also to be chemically stabilized.

The EC of the Fenton-treated samples significantly increased with increasing the catalytic Fe^{2+} dosage used during the oxidative process (i.e. control samples < treatments A < treatments B). Moreover, within the same iron dosage an increasing trend was found along with the H_2O_2 concentration increased (Table 3).

The TPC content in Fenton-treated digestates was strongly influenced by the oxidative treatments. In particular, it declined significantly by 31% in the thermally-treated control (Ctrl) compared to the untreated solid anaerobic digestate (Dig, 363.2 mg catechol kg^{-1}) (Table 3). A further significant decrease of TPC was observed after the Fenton reaction, and this decline was especially linked to the increasing dosage of H_2O_2 (from 0 to 24.12 mg L^{-1}) rather than that of catalytic Fe^{2+} (from 846 to 1692 mg L^{-1}), which indeed did not bring about any TPC reduction (Table 3).

The amount of organic matter in the biomass was not influenced by the oxidation process, and it ranged from 88.8% to 91.7% with no significant differences among treatments.

As for the elemental composition, the iron-based Fenton treatment did not affect the total C and N content despite the reagents dosage or their H_2O_2/Fe^2 ratio (Table 3), with the only exception represented by the Ctrl treatment that showed the larger elemental N content. The C/N ratio varied accordingly: the samples B1, B4, and B5 were characterized by a C/N value greater than that of Dig and particularly of the other samples (Table 3).

Humic and fulvic fractions of digestates

The iron-based Fenton process influenced the extraction yield and altered considerably the elemental C and N content of extracted HA and FA (Table 4). In brief, the thermal treatment at 70 °C induced a slight, but negligible, increase of the HA extraction yield. The same did not occur for FA where yield raised from 0.9% of control native digestate (Dig) to 2.7% of thermally treated sample Ctrl (Table 4). Moreover, increasing amounts of H_2O_2 enhanced the HA yield, but this trend was more evident at the lowest Fe^{2+} dosage (846 mg L^{-1}) and at a H_2O_2/Fe^{2+} ratio larger that 0.01: HA yields in samples B3, B4, and B5 were always lower than those found in samples A3, A4, and A5 (Table 4). A similar trend was also evidenced in the FA extraction yield that reached the highest values in A4 and B4 treatments (Table 4).

Elemental C and N contents in extracted HA remained practically unchanged in Dig and Ctrl treatments, while they decreased significantly in all Fenton-treated digestates. In particular, the smaller the H_2O_2/Fe^{2+} ratio of the catalysts used during the process, the lesser the C content found in the HA fractions (Table 4). Moreover, treatments with similar H_2O_2/Fe^{2+} ratios (i.e. A2 and B3, A3, and B5) showed the smaller C content was observed when the larger Fe^{2+} catalyst dosage was applied (Table 4). On the other hand, a declining trend of elemental N content of extracted HA was observed in all Fenton-treated digestates, particularly in those from samples treated with the greater catalytic Fe^{2+} dosage (Table 4).

Conversely, elemental C and N contents in extracted FA followed a slightly different trend, and they declined considerably in all samples, including Ctrl, when compared to Dig (Table 4). Precisely, a

decreasing C content was observed in FA extracted from samples treated with a smaller H_2O_2/Fe^{2+} ratio of catalysts used during the process. Noticeably, the N trend distanced itself from that of the C content: N sensitively decreased in FA extracted from Fenton digestates treated at greater H_2O_2/Fe^{2+} ratios (Tables 2 and 4).

FT-IR spectroscopy

The FT-IR spectra of Fenton-treated digestates and control samples are reported in Figure 1. Relevant peaks assigned to special functional groups and compounds are summarized in Table 5. The FT-IR analysis from native and Fenton-treated digestates evidenced peaks of OH of phenols, alcohols and carboxylic groups (3300-3400 cm^{-1}) and C–H stretching of alkyl structures (2847-2955 cm^{-1}). The absorbance bands at 1710-1772 cm^{-1} and 1590-1686 cm^{-1} were assigned to C=O stretching in carboxyl groups, carboxylic acids and ketones and aromatic C=C, C=O in amides I, ketone and quinone groups, respectively. In particular, the peak at 1738 cm^{-1} raised by increasing both Fe^{2+} and H_2O_2 rate. The absorbance bands at 1508-1560 cm^{-1} and 1444-1460 cm^{-1} were assigned to N–H stretching of amide II and C–H stretching in aliphatic structures, respectively. At 1370-1381 cm^{-1} the peak related to COO^- antisymmetric stretching, C–H and bending of CH_2 and CH_3 groups of amide III or aromatic ethers was observed (Figure 1). The absorbance band at 1100-1270 cm^{-1} was assigned to C–O stretching of aryl ethers and phenols, and to C–O stretching of secondary alcohols. The peak at 1040 cm^{-1} was attributed to C–O stretching of polysaccharides. In all spectra the absorption bands at 874-896 cm^{-1} were assigned to C–O bonds in the carbonate ion.[32,38–40]

The FT-IR spectra of HA and FA extracted from Fenton-treated digestates and control samples are shown in Figure 2. Precisely, FT-IR analysis of HA evidenced two peaks at 2931 cm^{-1} and at 2954 cm^{-1}, which were attributed to C–H stretching of alkyl structures (Figure 2a). These peaks were more pronounced in HA from Dig than in those from Fenton-treated digestates. The shoulder at 1720 cm^{-1} assigned to C=O stretching in carboxyl groups, carboxylic acids and ketones was observed only in HA from Dig control sample. Moreover, Dig spectra showed a greater relative intensity of peaks at

1510 and 1460 cm^{-1} assigned to amide II of N–H stretching and C–H stretching of aliphatic structures, respectively (Figure 2a). The peak at 1600 cm^{-1} assigned to aromatic C=C, C=O in amides I, ketone, and quinone groups was more intensive in HA extracted from Fenton treated samples (Figure 2a). In the spectra the adsorption at 1198 cm^{-1} assigned to C–O stretching and OH deformation of COOH[38,40] appeared in the HA extracted from treatments B2 and B4. In addition, the peak at 1125 cm^{-1}, corresponding to C–O stretching of aryl ethers and phenols, C-O stretching of secondary alcohols, was more intensive in treatment A1, B1, B2 and B3. In particular, in sample B3 the signal drew two bands (1120 cm^{-1} and 1140 cm^{-1})[32,38–40] (Figure 2a).

As regards FT-IR analysis of FA, only FT-IR spectra of samples A1 and B1, where 846 and 1492 mg L^{-1} of Fe^{2+} was applied in the Fenton oxidation, respectively, showed the peak at 1381 cm^{-1} assigned to COO^- antisymmetric stretching, C–H and bending of CH_2 and CH_3 groups (Figure 2b). The C–O stretching and OH deformation of COOH at 1198 cm^{-1} was observed only in the samples Ctrl, B1, B2, B4, and B5 (Figure 2b). Furthermore, peaks at 1120 cm^{-1} and 1090 cm^{-1}, assigned to C–O stretching of aryl ethers and phenols, C–O stretching of secondary alcohols, increased in intensity in Ctrl and in Fenton treated A1, A2, B1, B2, B4, and B5 samples. The same peaks disappeared in A3, A4, A5, and B3 samples (Figure 2b).

TG analysis

Three different peaks were registered in DTG profiles in according to Wu et al.[32] The peak DTG1 at 60 °C was mainly associated to the dehydration of samples, whereas the peak DTG2 registered within the temperature range of 200-350 °C was attributed to the thermal degradation of readily degradable materials and semi-volatile compounds, such as carbohydrates, aliphatic structures, carboxylic groups, hemicellulose, cellulose, and microbial cell walls. The peak DTG3 observed within the range of 400-600 °C was associated to the thermal decomposition of aromatic and polynuclear structures of high molecular weight. The DTG2 and DTG3 peaks were corrected by subtracting the weight loss due to the residual water.

The corrected weight loss of DTG2 and DTG3 of reference untreated and Fenton-treated digestates is showed in Figure 3a. No difference between Dig and Ctrl samples was observed in DTG2 peak, while a slight increase (from 6.9% to 7.6%) in DTG3 peak was measured. DTG2 peak decreased in all treatments A and B except in A5 (the greatest H_2O_2/Fe^{2+} ratio), which showed a bigger peak than Dig (+18.3%) and Ctrl (+16.6%), and in B1 (no H_2O_2 addition) that remained unchanged respect to Dig and Ctrl. Conversely, DTG3 peak decreased as the H_2O_2/Fe^{2+} ratio was increased during the Fenton reaction, especially at lower Fe^{2+} dosage, until to reach 3.5% in A5. Whereas at the greater Fe^{2+} concentration (samples B) no peak DTG3 change was noticed except in B1 where the peak value increased (9.1%).

TG analysis of HA extracted from Fenton-treated digestates indicated that HA of Ctrl produced greater DTG2 and DTG3 peaks than HA from Dig (Figure 3b). No further increase of peak DTG2 was found in HA from Fenton-treated digestates, except in HA from treatments B4 and B5 (11.7 and 11.5%, respectively). DTG3 peak increased in HA extracted from A1 and remained nearly unchanged in other samples A respect to Ctrl. Samples B were also characterized by DTG3 peaks similar to those of Ctrl. Extracted FA produced thermograms having DTG2 peaks more pronounced than those of both Dig and Ctrl; whereas DTG3 peaks appeared strongly depleted in all treatments, including Ctrl that underwent only the thermal treatment without catalyst addition, and the A5 treatment (highest H_2O_2/Fe^{2+} ratio) which showed the lowest value (Figure 3c).

Phytotoxicity assay

The untreated solid anaerobic digestate (Dig) exerted a considerable phytotoxic potential as shown by the dose response curve (Figure 4). In particular, significant GI (%) inhibition (by about 11%) was noticeable already at low digestate extract dose (namely 1%) and this inhibiting effect increased along with the increasing extract concentration until reaching a 54% inhibition at the highest value (Figure 4). The ED_{50} value was 74.36%, thus confirming the phytotoxic potential of the aqueous extract from Dig. Interestingly, the phytotoxic response to the aqueous extract changed significantly in relation to

the H_2O_2/Fe^{2+} dosage used in the Fenton oxidation process. In details, the thermal treatment with no chemical reagent addition (Ctrl) decreased, at least partially, the phytotoxic potential of the residual biomass, which showed a slight stimulatory action at lower dosages and a phytotoxic response only at the highest concentration (Figure 5). No phytotoxic potential of the aqueous digestate extracts arose after the chemical treatment of solid digestate at the 846 mg L^{-1} Fe^{2+} dosage, despite the hydrogen peroxide concentration applied within the experimental range (Figure 5). Conversely, a stimulatory action was observed at 100% extract dosage: GI (%) values showed an increasing trend from 116.44 ± 7.5 (in treatment A2) to 152.65 ± 8.0% (in treatment A4) with an average value equal to 131.48 ± 22.5%. On the other side, the aqueous extracts from solid anaerobic digestate treated with an increased Fe^{2+} dosage (1692 mg L^{-1}) showed a clear phytotoxic action on seed germination and root growth of lettuce (Figure 5). Noticeably, there was an increased phytotoxic response to the treated matrix as long as the hydrogen peroxide concentration increased in the Fenton oxidation process, which became evident and extended over the entire dilution range when using a concentration of H_2O_2 larger than 18.9 mg L^{-1} (Figure 5). In the presence of the 100% extract the GI value ranged from 76.82% (treatment B2) to 94.37% (treatment B5).

Discussion

According to most researchers,[18,41,42] Fenton chemistry encompasses the activation of hydrogen peroxide (H_2O_2) by ferrous (Fe^{2+}) ions to generate hydroxyl radicals ($HO^{\bullet}$) under acidic conditions *via* a complex reaction sequence, following the chain initiation reaction (Eq. 2):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \quad (k_1 = 63–76 \text{ mol } L^{-1} s^{-1}) \qquad (2)$$

In the presence of an organic substrate (R-H) the hydroxyl radical abstracts a hydrogen atom from R-H and generates an organic radical ($R^{\bullet}$), which then undergoes a series of chemical transformations to form various oxidation products (Eq. 3):

$$R\text{-}H + HO^{\bullet} \rightarrow H_2O + R^{\bullet} \quad \text{(chain propagation)} \qquad (3)$$

The hydroxyl radical is the main reactant of the Fenton oxidation process, capable of reacting with organic substrates via oxidation. Even though the use of excess concentration of Fe^{2+} and H_2O_2 should theoretically allow a complete conversion of all organic compounds into CO_2 and water, the occurrence of various competitive processes due to non-specific reactivity of $HO^{\bullet}$ towards both organic and inorganic substrates negatively affects the organic oxidation process, thus leading to chain termination (Eq. 4-6):

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \qquad (k_2 = 3.0 – 3.2 \times 10^8 \text{ mol L}^{-1} \text{ s}^{-1}) \qquad (4)$$

$$H_2O_2 + HO^{\bullet} \rightarrow HO_2^{\bullet} + H_2O \qquad (k_3 = 2.7 – 3.3 \times 10^7 \text{ mol L}^{-1} \text{ s}^{-1}) \qquad (5)$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \qquad (k_4 = 5.9 \times 10^9 \text{ mol L}^{-1} \text{ s}^{-1}) \qquad (6)$$

Although H_2O_2 is rapidly regenerated though Eq. (6), scavenging of $HO^{\bullet}$ by Fe^{2+} catalyst (Eq. 4) may reduce the overall oxidation efficiency. It is also true that the hydroperoxyl radical $HO_2^{\bullet}$ produced in reaction (Eq. 5) can reduce Fe^{3+} to Fe^{2+}, but because k_3 (Eq. 5) is an order of magnitude lower than k_2 (Eq. 4), Fe^{2+} catalyst is being decreased during the reaction. Thus, profiling the concentration of applied H_2O_2 and Fe^{2+} catalyst as well as their H_2O_2/Fe^{2+} ratio is crucial to maximize the efficiency of the process depending on the organic waste to treat.

H_2O_2 represents the dominant source of hydroxyl radicals, limitations in its concentration can severely affect the process efficiency, and most contributes to increase the treatment costs. Whereas Fe^{2+} catalyst requires dosages that strongly vary with the type and amount of waste to treat and has major advantages like natural abundance, environmental compatibility, low-toxicity, high reactivity and reduced commercial cost.[21,43] As to assess the feasibility of the efficiency of the post-digestion Fenton treatment of solid anaerobic digestate very small dosages of H_2O_2 (ranging from 6,03 to 24,12 mg L^{-1}) with relatively large amounts of catalytic Fe^{2+} (precisely 846 and 1692 mg L^{-1}) were here investigated. Most promising results were obtained when considering a hydrogen peroxide concentration not less than 18,09 mg L^{-1} with iron dosage not less than 846 mg L^{-1}, thus giving a H_2O_2/Fe^{2+} ratio larger than 0.02. Contrarily to what reported for chemically treating organic wastes dispersed in aqueous systems,[22,23] larger amounts of catalytic Fe^{2+} were here requested for the process

453 efficiency, since lower concentrations of catalytic Fe^{2+} produced no stabilization effect. We
454 hypothesize that in our system constituted by a partially hydrated solid matrix of individual highly
455 heterogeneous millimetric-sized organic particles the Fenton process was mainly controlled by the
456 diffusion rate and persistence of the catalysts into the biomass, thus requiring larger Fe^{2+} ion
457 concentrations.
458 The efficiency of a classical Fenton reaction depends also on pH, temperature, and reaction time.[21]
459 Even though contrasting conclusions have been reached, he optimum pH range for effective waste
460 treatment appears to be 2.5-3.0,[19] as maintained in the present study. In fact, acid pH leads to the
461 dissolution of ferric ions and hydroxyl radical, allowing the H_2O_2 stabilization and resulting in
462 prolonged activity.[43] The treatment efficiency can be improved by increasing the temperature, which
463 can provide more energy to overcome the reaction activation energy. Generally, 25-40 °C represents
464 the most used temperature range for the Fenton process; however, higher temperatures (> 40 °C) can
465 be also applied depending on the nature of the waste material to treat.[44] Our previous investigations
466 on solid-state organic matrices (i.e. sewage sludge) have shown that 70 °C provided optimum
467 conditions for the oxidative treatment. A reaction time ranging from 20 to 60 min is required in most
468 cases even though prolonged reaction times (up to 240 min) were experienced for efficient treatments
469 of recalcitrant organic wastes (i.e. olive-oil mill waste).[43] In the present experiment a reaction time
470 as long as 8 hours is actually the combination of an expected shorter reaction time, due to the nearly
471 solid state consistency of the treated material, and the need to allow the material to reach a complete
472 dryness as well as absence of any repelling odor emission. In fact, shorter reaction times would
473 produce materials retaining the original repelling odor, or which become gradually bad smelling after
474 re-hydration of the final products. Confirming that application of a higher gradient of negative
475 pressure during the reaction can help the sample reach complete dryness, but not an optimal
476 stabilization.
477 Under our experimental conditions, TPC in Fenton-treated digestates decreased considerably, and
478 this decline, apparently linked to increasing the catalyst dosage, became particularly evident when

the H_2O_2/Fe^{2+} ratio was > 0.01. This finding confirms what previously reported[45] that the oxidation of organic contaminants increases at increasing H_2O_2/Fe^{2+} ratios. Interestingly, neither the total C nor the total N varied during the process, suggesting that non-intense oxidation conditions occurred during the process. Further inside, TG analysis showed that samples treated with increasing H_2O_2 dosage at 846 mg L^{-1} Fe^{2+} (samples A, greater H_2O_2/Fe^{2+} ratio) the recalcitrant fraction (DTG3 peak) declined as also previously observed.[46] On the other hand, at the greatest Fe^{2+} dosage (samples B, lower H_2O_2/Fe^{2+} ratio), the recalcitrant fraction was not affected. This finding suggests that rather than inducing more oxidative conditions, a major catalytic iron concentration could have led to a "scavenging effect" of the $HO^{\bullet}$ radical (Eq. 4) with a consequent reduced efficiency of the oxidative process, as also postulated by Wu et al.[46] Interestingly, samples treated only with Fe^{2+} catalyst evidenced an increase of the recalcitrant fraction (DTG3 peak; Figure 3), especially at the greatest dosage where ferrous ions could have become complexed with humic substances. In fact, at acidic pH, Fe^{2+} is adsorbed onto carboxylic groups of humic fractions.[47,48] Unlike extracted humic fractions– as reported below, no differences arose in FT-IR spectra of Fenton-treated digestates with no alteration of aliphatic groups, aromatic, ketone and quinone groups, and polysaccharides after the oxidation process, in contrast with Quina et al.[25], though they adopted more intense oxidative conditions (i.e. H_2O_2 > 5 g kg^{-1} total solid).

Conversely, thermal treatment, catalysts dosage and the H_2O_2/Fe^{2+} ratio clearly affected yields and properties of both HA and FA extracted from Fenton-stabilized digestates. In fact, following the thermal treatment (Ctrl) an increased amount of both HA and FA was extracted, that continued to grow up by increasing the H_2O_2/Fe^{2+} ratio during the reaction. This behavior could be explained by the combined thermal treatment and complexation of Fe^{2+} onto HA and FA, that leads to a stable humic fraction.[47,48] However, as long as the oxidative condition increased – due to the increasing H_2O_2/Fe^{2+} ratio – lesser N was being incorporated into the humic-like fractions particularly FA. We surmise that the greater abundance of ferric ions generated by the large amount of H_2O_2 could have acted as a Lewis acid, thus promoting the catalytic cleavage of peptide bonds, release of amino acids

505 and major loss of N, which was not incorporated into the final FA product. In a few words, yields and
506 properties of humic-like fractions were the result of a combination of oxidative and hydrolytic
507 reactions determined by both highly reactive hydroxyl radicals and catalytic ferric ions.
508 The major impact of the Fenton process became clear analysing the FT-IR spectra of extracted HA.
509 In fact, the signals attributed to alkyl structures, amides and polysaccharides appeared reduced, and
510 also those of carboxylic groups resulted depleted after treatment. Conversely, FT-IR spectra showed
511 an increase of aromatic structures and groups as aryl ethers, phenols, and secondary alcohols thereby
512 indicating HA from Fenton-stabilized digestates as more stable products. This behavior was also
513 confirmed by the TG analysis. In fact, the recalcitrant fraction, associated to the thermal
514 decomposition of aromatic structures within the range of 400-600 °C, enlarged respect to HA from
515 non-treated digestate (Dig). In according to the theory of the supramolecular structure of humic
516 substances proposed by Piccolo,[49] the presence of Fe^{2+} promoted thermodynamically stable
517 associations due to the complexation with acidic functional groups of humic molecules, thus losing
518 their previous weakly-held conformations.[49]
519 Conversely, FA extracted appeared depleted in aromatic structures but enriched in aryl ethers and
520 phenols, and secondary alcohols, as shown in FT-IR spectra and confirmed by TG analysis. In other
521 words, the more recalcitrant fractions (DTG3 peaks) strongly decreased respect to Dig, whereas the
522 more labile fraction (DTG2 peaks) increased. During the Fenton stabilization process, when both
523 oxidative and hydrolytic reactions occurred, recalcitrant compounds could be transformed in more
524 labile ones: Nuzzo et al.[48] found that Fe addition leads to the formation of highly hydrated and poorly
525 associated fulvic molecules.
526 Contrasting results have been reported as regards digestates phytotoxicity, ranging from absence[50] to
527 strong[51] phytotoxic responses on germination and growth of *Lepidium sativum.* In accordance with
528 latter results, our Dig inhibited the lettuce germination at concentrations ≥ 1% reaching a ~50% of
529 inhibition at the largest concentration. As suggested by several authors, the GI inhibition could be
530 attributed to a high content of organic matter[52,53] and soluble salts as they can negatively affect the

water uptake needed for germination.[54] However, since the EC significantly increased in all the treated digestates, including those which were not phytotoxic, the influence of an unfavorable osmotic potential on seed germination and seedling growth was here considered negligible. On the other hand, phenolic compounds, alone or in complex mixture, can exert phytotoxic effects on both germination and seedling establishment, even at low concentrations.[55] As seen before, TPC strongly varied with reagent dosage and their combination, suggesting that the differences in phytotoxic responses can be strictly correlated with the TPC of the organic matrix. Interestingly, a stimulatory effect on the GI parameter was also observed, especially at the lowest concentrations. This phenomenon, known as hormesis, is generally induced by the phenolic acids, which show a dualistic behaviour, being stimulatory at low and inhibitory at high concentrations.[56,57] Both the TPC and phytotoxic potential of Fenton-treated digestates declined at the lowest Fe^{2+} catalyst dosage and when the H_2O_2/Fe^{2+} ratio increased, while a larger HA content was observed. We hypothesize that increased humic-like substances could have contributed to stimulate the germination and seedlings growth of lettuce, also as reported before.[58]

To sum up, this lab scale study showed that Fenton post-digestion treatment has great potential for large-scale application in increasing stability and detoxication of solid anaerobic digestates, whose agricultural valorization as soil amendment is severely constrained by residual phytotoxic potential and instability. We found it out that a reduced H_2O_2 dosage between 18.9 and 24.12 mg L^{-1} and an amount of Fe^{2+} catalyst slightly large but not exceeding 846 mg L^{-1}, at pH 3.0 and 70 °C for 8 h under continuous stirring at 50 rpm, represent the optimum conditions to produce an effective biomass stabilization. The resulting H_2O_2/Fe^{2+} ratio ranging from 0.02 and 0.03, which is certainly small when compared to those used in wastewater treatment, is in agreement with that suggested by Nieto et al.[59] Given the properties of the matrix to treat, this reagent ratio has been found suitable for decreasing the phytotoxic potential and total phenols, and increasing the stability of the product as well as the content and the structure of chemically-induced formation of humic-like fractions, which can be beneficial to soil properties. Nevertheless, it is also worth noting that the C/N ratio of the stabilized

product remains somewhat high (> 25) thus requiring additional inorganic N supply for avoiding microbial N immobilization when the stabilized material is being used as soil amendment.

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Abbreviations used

AD, anaerobic digestion; AOP, advanced oxidation process; BSI, bad smell index; CSTR, continuously stirred tank reactor; DTG, differential thermogravimetry; EC, electrical conductivity; FT-IR, Fourier-transform infrared spectroscopy; FA, fulvic acids; GI, germination index; HA, humic acids; HRT, hydraulic retention time; MGRT, minimum guaranteed retention time; OM, organic matter; TG, thermogravimetric; TPC, total phenolic compounds

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736 **Figure captions**

737 **Figure 1.** FT-IR spectra of the solid anaerobic digestate before and after the iron-based Fenton
738 stabilization process carried out using different dosages of Fenton reagents (treatments as reported in
739 Table 2).

740

741 **Figure 2.** FT-IR spectra of humic (HA) (a) and fulvic acid (FA) (b) fractions extracted from the solid
742 anaerobic digestate before and after the iron-based Fenton stabilization process carried out using
743 different dosages of Fenton reagents (treatments as reported in Table 2).

744

745 **Figure 3.** Changes in corrected weight losses obtained from Eq. (1) corresponding to DTG2 and
746 DTG3 of a) solid anaerobic digestate and its b) humic and c) fulvic fractions before and after the iron-
747 based Fenton stabilization process carried out using different dosages of Fenton reagents (treatments
748 as reported in Table 2).

749

750 **Figure 4.** Dose-response curve of the germination index [GI (%)] (mean ± SE, n = 5) of seeds of
751 *Lactuca sativa* L. exposed for 48 h under controlled growth conditions (darkness, 25±1 °C) to
752 increasing aqueous extract concentrations (namely 0, 0.1, 1, 5, 10, 20, 80 and 100%) of untreated
753 solid anaerobic digestate (Dig). Different lowercase letters indicate significant differences among
754 treatments ($P < 0.05$). Fitting of the non-linear regression model to estimate the ED_{50} value (i.e. the
755 percent extract dose determining a 50% reduction of the maximum GI response) was at a significant
756 value of $P< 0.001$.

757

758 **Figure 5.** Variation of the germination index [GI (%)] (mean ± SE, n = 5) of seeds of *Lactuca sativa*
759 L. exposed for 48 h under controlled growth conditions (darkness, 25±1 °C) to increasing aqueous
760 extracts concentrations of solid anaerobic digestate before and after the iron-based Fenton

761 stabilization process carried out using different dosages of Fenton reagents (treatments as reported in
762 Table 2). The significant effect due to the aqueous extract concentration (C) is shown as *F*-value and
763 level of significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, not significant) estimated by a one-
764 way ANOVA.
765

766 **Table 1. Chemical, biochemical and biological characterization of a solid anaerobic digestate**
767 **from a medium-scale biogas producing plant[a]**

Parameter	Value
pH[b]	8.45 ± 0.03
EC (dS m^{-1} at 25°C)[c]	1.48 ± 0.01
Dry matter (% fresh weight)	33.5 ± 1
Ash (%)	10 ± 1
Volatile solids (%)	90 ± 1
Total C (g kg^{-1})	467.6 ± 0.8
Total N (g kg^{-1})	12.2 ± 0.1
C/N	38.3 ± 3.2
C_{HA} (%)	10.98 ± 0.16
C_{FA} (%)	6.88 ± 1.28
NH_4^+-N (g kg^{-1})	1.44 ± 0.13
NH_4^+-N (% Total N)	11.8 ± 1.7
NO_3^--N (g kg^{-1})	0.017 ± 0.001
BOD_5 (mg L^{-1})	1850 ± 25
Total K (g kg^{-1})	9.06 ± 0.09
Total S (g kg^{-1})	2.00 ± 0.04
Cd (mg kg^{-1})	<0.1
Cr_{VI} (mg kg^{-1})	2.48 ± 0.04
Pb (mg kg^{-1})	1.57 ± 0.06
Ni (mg kg^{-1})	1.44 ± 0.06
Hg (mg kg^{-1})	<1.5
Cu (mg kg^{-1})	20.60 ± 0.06
Zn (mg kg^{-1})	90.13 ± 0.06
Salmonella spp.	Absent
Escherichia coli (CFU g^{-1})	Absent
Dehydrogenase *activity* (µg INTF g^{-1} 2 h^{-1})	35.7 ± 1.1
FDA-hydrolase activity (µg fluorescein g^{-1} h^{-1})	261.0 ± 2.1
Alkaline phosphatase activity (µg *p*-NP g^{-1} h^{-1})	172.2 ± 6.2

768 [a] Values are the mean ± SD (*n*=3) expressed on a dry matter basis.
769 [b] Biomass/H_2O, 3:50, w/v.
770 [c] Biomass/H_2O, 1:10, w/v.
771

Table 2. Dosages of Fenton reagents used in the chemical stabilization process of a solid anaerobic digestate from a medium-scale biogas producing plant. Reactions were carried out using a laboratory-scale air-tight glass reactor (as shown in Figure S1) operating at pH 3.0, internal pressure -100 mbar, temperature 70 °C, stirring speed 50 rpm, for 8 h

Treatment	Fenton reagent dosage		H_2O_2/Fe^{2+} ratio
	Fe^{2+} (mg L^{-1})	H_2O_2 (mg L^{-1})	
Dig[a]	0	0	0
Ctrl	0	0	0
A1	846	0	0
A2	846	6.03	0.0071
A3	846	12.06	0.0143
A4	846	18.09	0.0214
A5	846	24.12	0.0286
B1	1692	0	0
B2	1692	6.03	0.0036
B3	1692	12.06	0.0071
B4	1692	18.09	0.0107
B5	1692	24.12	0.0143

[a] Physically and chemically untreated solid anaerobic digestate was taken as a reference treatment.

Table 3. Selected physical and chemical properties of the solid anaerobic digestate before (Dig) and after the iron-based Fenton process carried out using different dosages of Fenton reagents[a]

Treatment[b]	BSI[c]	$EC_{1:10}$ (dS m^{-1})	TPC[d] (mg kg^{-1})	OM[e] (%)	C (%)	N (%)	C/N
Dig	3	1.48 ± 0.02 k	363.2 ± 17.9 a	91.7 ± 2.6	46.8 ± 0.3 a	1.22 ± 0.01 a	38.3 ± 0.3 c
Ctrl	3	1.43 ± 0.02 k	250.5 ± 9.1 b	91.3 ± 2.2	46.1 ± 0.9 a	0.99 ± 0.10 b	46.5 ± 1.2 b
A1	3	1.56 ± 0.02 j	183.0 ± 3.4 c	91.7 ± 1.7	47.2 ± 0.8 a	0.99 ± 0.06 b	47.7 ± 0.9 b
A2	2	1.84 ± 0.03 i	111.2 ± 1.9 d	91.1 ± 1.2	47.3 ± 0.4 a	0.99 ± 0.09 b	47.8 ± 0.8 b
A3	2	2.04 ± 0.02 h	95.6 ± 1.8 e	91.5 ± 1.7	47.1 ± 0.7 a	0.99 ± 0.04 b	47.7 ± 1.4 b
A4	1	2.48 ± 0.02 f	85.0 ± 1.6 f	90.8 ± 1.5	47.3 ± 0.3 a	1.01 ± 0.05 b	46.8 ± 0.9 b
A5	1	2.30 ± 0.02 g	45.3 ± 4.4 g	89.2 ± 1.2	47.4 ± 0.4 a	1.00 ± 0.05 b	47.6 ± 0.8 b
B1	3	2.66 ± 0.02 e	179.7 ± 3.3 c	88.8 ± 2.3	47.2 ± 0.5 a	0.97 ± 0.01 b	48.7 ± 0.6 a
B2	3	3.49 ± 0.03 c	117.8 ± 5.1 d	91.2 ± 1.0	47.3 ± 0.6 a	1.01 ± 0.05 b	46.4 ± 0.7 b
B3	3	3.23 ± 0.02 d	101.6 ± 7.3 e	90.7 ± 1.1	47.3 ± 0.1 a	1.02 ± 0.06 b	46.8 ± 0.6 b
B4	1	3.92 ± 0.02 b	69.3 ± 2.4 fg	90.0 ± 1.3	47.7 ± 0.3 a	0.96 ± 0.09 b	49.7 ± 1.0 a
B5	1	4.20 ± 0.02 a	43.8 ± 2.7 g	90.1 ± 2.0	47.3 ± 0.3 a	0.97 ± 0.09 b	48.8 ± 0.9 a

[a] Values are mean ± SD (*n=3*) expressed on a dry matter basis. Lowercase different letters indicate significant differences among treatments ($P < 0.05$).
[b] Treatments as reported in Table 2.
[c] Bad odor index
[d] Total phenolic compound content, expressed as catechol
[e] Organic matter by weight loss on ignition.

785 **Table 4. Yield and elemental analysis of humic and fulvic acid fractions extracted from a**
786 **solid anaerobic digestate before (Dig) and after the iron-based Fenton stabilization**
787 **carried out using different dosages of Fenton reagents[a]**

Treatment[b]	Extraction yield (%)	C (%)	N (%)
Humic acids			
Dig	3.7	43.06 ± 0.19 a	5.97 ± 0.07 a
Ctrl	3.9	43.04 ± 0.18 a	5.92 ± 0.07 a
A1	3.0	41.44 ± 0.23 c	5.42 ± 0.10 b
A2	4.1	41.21 ± 0.40 c	5.29 ± 0.10 b
A3	5.6	42.26 ± 0.20 b	5.33 ± 0.25 b
A4	7.6	42.57 ± 0.07 b	5.26 ± 0.07 b
A5	6.5	42.59 ± 0.19 b	5.22 ± 0.22 b
B1	3.0	41.80 ± 0.28 c	4.72 ± 0.08 c
B2	3.8	37.98 ± 0.34 d	4.57 ± 0.12 c
B3	3.0	38.66 ± 0.39 d	4.75 ± 0.04 c
B4	4.9	41.23 ± 0.44 c	4.12 ± 0.07 d
B5	5.4	41.32 ± 0.35 c	4.10 ± 0.07 d
Fulvic acids			
Dig	0.9	40.50 ± 0.42 a	4.80 ± 0.14 a
Ctrl	2.7	33.25 ± 0.57 d	4.09 ± 0.03 b
A1	3.4	33.19 ± 0.37 d	3.38 ± 0.06 c
A2	3.7	33.40 ± 0.32 d	3.08 ± 0.05 d
A3	4.2	34.60 ± 0.21 c	2.88 ± 0.01 e
A4	4.6	35.42 ± 0.43 b	2.68 ± 0.01 f
A5	2.8	35.66 ± 0.24 b	2.63 ± 0.10 f
B1	2.7	33.05 ± 0.17 d	3.46 ± 0.07 c
B2	2.2	34.64 ± 0.13 c	3.41 ± 0.07 c
B3	2.3	35.91 ± 0.10 b	3.39 ± 0.08 c
B4	5.4	35.89 ± 0.23 b	3.23 ± 0.01 d
B5	3.4	35.80 ± 0.20 b	3.20 ± 0.01 d

788 [a] Values are mean ± SD (n=3) expressed on a dry matter basis. Lowercase different letters
789 indicate significant differences among treatments within the same extracted fraction ($P < 0.05$).
790 [b] Treatments as reported in Table 2.

791 **Table 5. Wavenumber and assignment of main absorbance bands in FT-IR spectra**

Wavenumber (cm^{-1})	Assignment
3300-3400	OH of phenols, alcohols and carboxylic groups
2847-2955	C–H stretching of alkyl structures
1710-1772	C=O stretching in carboxyl groups, carboxylic acids and ketones
1590-1686	Aromatic C=C, C=O in amides I, ketone and quinones
1508-1560	N–H stretching of amide II
1444-1460	C–H stretching in aliphatic structures
1394-1430	OH of phenols, COO^-, $-CH_3$
1370-1381	COO^- antisymmetric stretching, C–H and bending of CH_2 and CH_3 groups
1220-1240	amide III or aromatic ethers
1198	C–O stretching and OH deformation of COOH
1090-1270	C–O stretching of aryl ethers and phenols, C-O stretching of secondary alcohols
1034-1040	C–H stretching of polysaccharides
874-896	C–O bonds in the carbonate ion

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