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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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18 Organic matter characterization and phytotoxic potential assessment of a solid anaerobic

19 digestate following chemical stabilization by an iron-based Fenton reaction

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## 41 Abstract

42 Digestates, the by-product of the anaerobic bioconversion of organic wastes for the production of 43 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 44 45 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<sub>2</sub>O<sub>2</sub> and 46  $Fe^{2+}$ . In Fenton-treated samples the phytotoxic potential (determined on a test plant), total phenols 47 48 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 49 50 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 51 stabilization were achieved with a  $H_2O_2/Fe^{2+}$  ratio between 0.02 and 0.03. 52

- 53
- 54 Keywords: biomass valorization; elemental analysis; FT-IR spectroscopy; humic-like compounds;
  55 odor impact; post-digestion treatment; TG analysis; phenols.
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#### 58 Introduction

59 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 60 devoted to the anaerobic digestion (AD) technology, which was originally developed for treating 61 biodegradable wastes and sewage sludge.<sup>1,2</sup> The AD process relies upon the biological conversion of 62 63 organic by-products under low oxygen content for the production of a biogas, rich in methane (50-64 80% v/v, together with carbon dioxide and other contaminant gases (i.e. H<sub>2</sub>S). Biogas can be used directly as a fuel, in combined heat and power gas engines, or upgraded to natural gas-quality 65 biomethane.<sup>3</sup> Besides biogas, the AD process leads to the production of a partially degraded, nutrient-66

rich by-product currently named digestate.<sup>4</sup> Owing to its chemical composition and content of 67 essential plant nutrient elements - especially N-reduced forms and soluble potassium - anaerobic 68 69 digestate has the potential as soil conditioner to improve physical properties, and release soluble plant nutrient elements thus reducing the supply of synthetic fertilizers.<sup>5,6</sup> Moreover, it can increase soil C 70 storage<sup>7</sup> and contribute to soil pest control.<sup>8</sup> Nevertheless, a complete exhaustion of the AD process 71 is rarely achieved and, unless digestates undergo a proper post-processing treatment including 72 composting,<sup>9–11</sup> soil incorporation of yet unstable or immature by-product can negatively affect the 73 soil-plant system.<sup>10</sup> Most known undesirable features are: high levels of heavy metals,<sup>12</sup> phytotoxic 74 compounds,<sup>13</sup> pathogenic bacteria,<sup>14</sup> bad odor emission, unbalanced nutrient content and excess 75 inorganic N forms. As well as this, recent European<sup>15</sup> and National<sup>16</sup> regulations together with local 76 77 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 78 need to be fully stabilized and characterized for their residual phytotoxicity and chemical composition 79 80 before their safe use in agricultural systems.

Although known for more than one century, the Fenton reaction (first described by H.J.H. Fenton<sup>17</sup> 81 and based on  $Fe^{2+}$ -catalyzed  $H_2O_2$  decomposition in strongly oxidizing hydroxyl radicals) was not 82 83 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)<sup>18,19</sup> and is widely used 84 85 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, 86 organic solvents, pharmaceuticals, domestic chemicals, etc.<sup>20-24</sup> Popularity and widespread 87 88 applications of the Fenton oxidation process are due to the following features: 1) it works at near-89 ambient temperature and pressure conditions, 2) it requires cheap, relatively reactive and easy-to 90 handle reagents, 3) it is rapid and effective, and 4) it can be easily integrated in more sophisticated 91 chemical technologies for the treatment of a broad range of hazardous wastes, including solid 92 agricultural matrices such as digestates from AD plants. Although few studies have recently

approached the chemical stabilization of the anaerobic digestate,<sup>25–27</sup> they primarily focused on 93 94 dewaterability and changes in the chemical characteristics of treated organic wastes. Whereas no 95 attempt has been made to assess the phytotoxic potential and investigate specific changes in 96 spectroscopic features of digestate-derived humic-like compounds, which are key to restoring the 97 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 98 99 technology to stabilize the solid fraction of an anaerobic digestate, reduce its phytotoxic potential and 100 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 101 investigate various combinations of reduced  $H_2O_2$  and  $Fe^{2+}$  dosages on physical (bad odor emission), 102 chemical (total phenols, organic matter, electrical conductivity) and phytotoxic properties of a solid 103 anaerobic digestate. Fenton-treated digestates and their extracted humic and fulvic fractions were also 104 characterized by elemental analysis, FT-IR spectroscopy and TG analysis. Findings from the research 105 represent the first step towards Fenton process optimization in real plants for AD by-products 106 107 stabilization.

108

109 Materials and Methods

110 Anaerobic digestate

111 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 112 113 component, together with milk serum, maize silage and, in minor amount, olive waste and citrus pulp. 114 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 + 115 116 5000 m<sup>3</sup> tank reactor 2), operating under mesophilic conditions (40 °C). The total volume loaded per 117 day was 120 m<sup>3</sup>, the hydraulic retention time (HRT) 60 days, and the minimum guaranteed retention 118 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<sup>4,12</sup> 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 elemental analyzer CN628 LECO (LECO Corporation, USA). NH4<sup>+</sup>-N and NO3<sup>-</sup>-N contents in 2 M 124 KCl extracts (1:10 v/v) were determined colorimetrically by using a Flow Injection Analysis System 125 126 (FIAS 400 PerkinElmer, Inc., CT, USA) equipped with an AS90 Autosampler (PerkinElmer) and linked to an UV/Vis spectrophotometer Lambda 25 (PerkinElmer). Total K, S, Ca, Mg, Cl, F, Mn, B, 127 Cd, Cr, Pb, Ni, Hg, Cu and Zn were determined by inductively coupled plasma mass spectrometry 128 129 (ICP-MS ICAP-Q Thermo Scientific, CA) after microwave (Ethos up, Milestones srl, I) acid 130 digestion with HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (7:1 v/v). Enzymatic (alkaline phosphatase, dehydrogenase and hydrolysis of fluorescein diacetate) activities were determined colorimetrically.<sup>28</sup> All analyses were 131 132 carried out in triplicate.

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#### 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 together with the Fenton reagents:  $Fe^{2+}$  (as the catalyst) provided in form of high purity epta-hydrate 138 139 ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O, 99.5% purity) (VEBI Istituto Biochimico s.r.l., Italy) and analytical 140 grade hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>, 30% w/w) (Panreac Applichem, Spain) (as the oxidant), which was properly diluted to the desired final concentration. Reagent dosage and  $H_2O_2/Fe^{2+}$  ratio of 141 142 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 145 internal pressure gradient of -100 mbar, respect to ambient pressure, was maintained throughout the 146 entire process so as to allow the sample to become completely dry. The pH mixture was allowed 147 declining to pH 3.0 and maintained until the reaction had completed. The reaction took as long as 8 148 hours to complete, when any repelling odor was no longer appreciable and the mixture reached a 149 complete dryness. To sum up, ten chemically and thermally treated (A1-A5, B1-B5) and one 150 thermally treated samples with no reagent addition (Ctrl) were produced during the Fenton 151 stabilization process, and then immediately stored in air-tight bags before any further analysis. 152 Untreated solid anaerobic digestate was oven-dried at 105 °C until constant weight and taken as a 153 reference treatment (Dig).

154

## 155 Extraction of humic-like fractions

Fulvic (FA) and humic (HA) acids were extracted and fractionated as described previously.<sup>29</sup> Briefly, 156 an amount of the organic matrix equivalent to 2 g dry weight was transferred into a 250-mL Teflon 157 158 centrifuge tube containing 100 mL of 0.1 M NaOH and 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solution. The headspace of each tube was flushed with N<sub>2</sub> to create an anaerobic environment, and then the tubes were shaken 159 (80 rpm) at 65 °C for 24 h. The suspension was centrifuged at 6000 rpm for 20 min and filtered 160 161 through a 0.80 µm membrane filter. An aliquot of 25 mL of the filtered extract was acidified to pH 2 with diluted H<sub>2</sub>SO<sub>4</sub> (1:1 in water) to separate HA from FA. Following centrifugation (6000 rpm, 20 162 min), pelleted HA were collected, while the supernatant containing FA was further purified by 163 passing it through a 10 cm<sup>3</sup> of an insoluble polyvinylpyrrolidone resin (Fluka Analytical) equilibrated 164 165 with 0.005 M H<sub>2</sub>SO<sub>4</sub>. The eluate containing the non-humified fraction was discarded, while the 166 retained fraction was eluted with 0.5 M NaOH and collected. HA and FA fractions were freeze-dried 167 and stored at -20 °C before any further characterization.

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#### 169 **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).

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## 176 Chemical analyses

The electrical conductivity (EC) of Fenton-treated and control samples was measured in a 177 biomass/water (1:10, w/v) mixture; whereas the total phenolic compounds (TPC) content was 178 determined as described before.<sup>30</sup> Briefly, a suitable amount (0.500 g) of the organic matrix was 179 180 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 181 methanol. Content of TPC in the extracts was determined by using the Folin-Ciocalteu reagent (Sigma, 182 Italy). Briefly, 20 µL of the digestate extract was added with distilled water (830 µL), the Folin-183 Ciocalteu reagent (50 µL) and, after 3 minutes 100 µL of 6% (w/v) NaOH. Following 1 h incubation, 184 the content of TPC was determined spectrophotometrically at 725 nm. Analytical readings were 185 186 expressed as mg of catechol equivalent per kg dry weight. The determination of organic matter (OM) 187 content of Fenton digestates was done by loss on ignition according to the method 5A described in the Kellogg Soil Survey Laboratory Methods Manual.<sup>31</sup> Elemental C and N contents in Fenton 188 digestates and their extracted humic-like fractions were determined by an automatic elemental 189 analyzer CN628 LECO (LECO Corporation, USA). All the chemical analyses were carried out in 190 191 triplicate.

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## 193 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<sup>-1</sup> by

using a Spectrum One FT-IR spectrometer (PerkinElmer, CT, USA). One milligram of each organic
sample was ground up with 400 mg KBr (FT-IR grade) and homogenized in an agate mortar. KBr
pellets were compressed under vacuum, under a pressure of 10000 kg cm<sup>-2</sup> for 10 min. Thirty-two
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 Analyser (STA) 6000 PerkinElmer (PerkinElmer, CT, USA) operating within the range 30 - 900 °C 204 at a heating rate of 15 °C min<sup>-1</sup>, under synthetic air atmosphere ( $21 \pm 1\%$  O<sub>2</sub> and  $79 \pm 1\%$  N<sub>2</sub> at a 100 205 mL min<sup>-1</sup> flow rate). TG profiles and differential thermogravimetry (DTG) curves were obtained in 206 207 terms of the percentage of weight loss of the sample and from the first derivative of TG profiles representing the rate of weight loss, respectively. In order to obtain the real organic matter content 208 (C<sub>corr</sub>), the weight loss attributed to the residual water was subtracted from DTG peaks (DTG2 and 209 210 DTG3) as follows:

$$C_{corr} = 100 \cdot \left(\frac{C_i}{100 - C_{water}}\right)$$

(1)

where  $C_i$  is the weight loss of peak DTG2 or DGT3 registered in the DTG profiles (as an example, the DTG profile of Dig is reported in Figure S2), and  $C_{water}$  is the first DTG peak (DTG1) occurring to 60 °C and corresponds to the dehydration of the residual water content.<sup>32</sup>

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## 216 **Phytotoxicity assay**

The phytotoxic assay of either the native or Fenton-treated solid anaerobic digestate was carried out in accordance to previously described<sup>33</sup> adopting a completely randomized design with five replications. Briefly, Fenton-treated digestates were extracted with water (1:20, w/v, on dry weight basis) under shaking (120 rpm) at room temperature for 24 h. Then, the suspension was filtered

(Whatman<sup>®</sup> no. 42) and the extract was diluted with sterile distilled water so as to obtain the following 221 222 concentrations: 0, 0.1, 1, 5, 10, 20, 80, and 100%. Germination and root growth of lettuce (Lactuca 223 sativa L.) seeds exposed to the digestate aqueous extracts were evaluated. Briefly, lettuce seeds were 224 surface-sterilized by soaking in 15% (v/v) NaClO solution for 15 minutes and then rinsed with 225 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 226 then placed in the dark into a growth chamber at  $24 \pm 1$  °C and 70% relative humidity. After 48 hours, 227 228 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 229 multiplying the germination percentage by the root length percentage, divided by 100 as reported 230 231 before.<sup>34</sup>

232

#### 233 Statistical analysis

Chemical data shown in Tables 3 and 4 are reported as mean values (n = 3), and a Tukey's honest 234 significant difference (HSD) post hoc test was run to compare means at a P < 0.05 level of significance. 235 Data from the phytotoxic assay (Figures 4 and 5) were first tested for deviation from normality 236 237 (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 238 239 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 240 241 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<sup>35</sup> was used to estimate ED<sub>50</sub> values, 242 243 which represents the percent extract dose lowering the maximum GI (%) by 50%. The model 244 parameters were estimated by the least square method (TableCurve 2D v.4.0 software, Jandel 245 Scientific Ekrath, D) using the Levenburg-Marquardt algorithm for fitting non-linear equations. Non-246 linear regressions were repeated several times in order to minimize the sum of the square of deviation

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

274

## 275 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<sup>-1</sup> 276 277 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 278 levels: 846 and 1692 mg L<sup>-1</sup>. However, the higher the Fe<sup>2+</sup> catalyst, the lower the stability in terms of 279 odor emission as in the case of Fenton-treated digestates B2 and B3 vs A2 and A3 (Table 3). This 280 finding suggests that beside a proper single catalyst dosage, also a  $H_2O_2/Fe^{2+}$  ratio larger that 0.01 is 281 equally decisive for ensuring optimum conditions for the Fenton oxidation process. Expedectly, no 282 reduction in the BSI was found when one or both catalysts were omitted (Table 3). It is interesting to 283 note that this finding is consistent with the larger amount of HA and FA recovered in samples A4, 284 285 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<sub>2</sub>O<sub>2</sub> 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<sup>-1</sup>) (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<sub>2</sub>O<sub>2</sub> (from 0 to 24.12 mg L<sup>-1</sup>) rather than that of catalytic Fe<sup>2+</sup> (from 846 to 1692 mg L<sup>-1</sup>), which indeed did not bring about any TPC reduction (Table 3).

296 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).

303

## 304 Humic and fulvic fractions of digestates

305 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 306 a slight, but negligible, increase of the HA extraction yield. The same did not occur for FA where 307 308 yield raised from 0.9% of control native digestate (Dig) to 2.7% of thermally treated sample Ctrl 309 (Table 4). Moreover, increasing amounts of H<sub>2</sub>O<sub>2</sub> enhanced the HA yield, but this trend was more evident at the lowest  $Fe^{2+}$  dosage (846 mg L<sup>-1</sup>) and at a H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup> ratio larger that 0.01: HA yields in 310 311 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 312 and B4 treatments (Table 4). 313

314 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 315 316 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, 317 and B5) showed the smaller C content was observed when the larger  $Fe^{2+}$  catalyst dosage was applied 318 319 (Table 4). On the other hand, a declining trend of elemental N content of extracted HA was observed 320 in all Fenton-treated digestates, particularly in those from samples treated with the greater catalytic  $Fe^{2+}$  dosage (Table 4). 321

322 Conversely, elemental C and N contents in extracted FA followed a slightly different trend, and they 323 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).

328

## 329 FT-IR spectroscopy

The FT-IR spectra of Fenton-treated digestates and control samples are reported in Figure 1. Relevant 330 331 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 332 carboxylic groups (3300-3400 cm<sup>-1</sup>) and C-H stretching of alkyl structures (2847-2955 cm<sup>-1</sup>). The 333 absorbance bands at 1710-1772 cm<sup>-1</sup> and 1590-1686 cm<sup>-1</sup> were assigned to C=O stretching in 334 carboxyl groups, carboxylic acids and ketones and aromatic C=C, C=O in amides I, ketone and 335 quinone groups, respectively. In particular, the peak at 1738 cm<sup>-1</sup> raised by increasing both  $Fe^{2+}$  and 336 H<sub>2</sub>O<sub>2</sub> rate. The absorbance bands at 1508-1560 cm<sup>-1</sup> and 1444-1460 cm<sup>-1</sup> were assigned to N-H 337 stretching of amide II and C–H stretching in aliphatic structures, respectively. At 1370-1381 cm<sup>-1</sup> the 338 peak related to COO<sup>-</sup> antisymmetric stretching, C-H and bending of CH<sub>2</sub> and CH<sub>3</sub> groups of amide 339 III or aromatic ethers was observed (Figure 1). The absorbance band at 1100-1270 cm<sup>-1</sup> was assigned 340 to C–O stretching of aryl ethers and phenols, and to C–O stretching of secondary alcohols. The peak 341 at 1040 cm<sup>-1</sup> was attributed to C–O stretching of polysaccharides. In all spectra the absorption bands 342 at 874-896 cm<sup>-1</sup> were assigned to C–O bonds in the carbonate ion.<sup>32,38–40</sup> 343

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<sup>-1</sup> and at 2954 cm<sup>-1</sup>, 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<sup>-1</sup> 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<sup>-1</sup> assigned to amide II of N–H stretching and C–H stretching of aliphatic structures, 350 respectively (Figure 2a). The peak at 1600 cm<sup>-1</sup> assigned to aromatic C=C, C=O in amides I, ketone, 351 and quinone groups was more intensive in HA extracted from Fenton treated samples (Figure 2a). In 352 the spectra the adsorption at 1198 cm<sup>-1</sup> assigned to C–O stretching and OH deformation of COOH<sup>38,40</sup> 353 354 appeared in the HA extracted from treatments B2 and B4. In addition, the peak at 1125 cm<sup>-1</sup>, corresponding to C–O stretching of aryl ethers and phenols, C-O stretching of secondary alcohols. 355 was more intensive in treatment A1, B1, B2 and B3. In particular, in sample B3 the signal drew two 356 bands (1120 cm<sup>-1</sup> and 1140 cm<sup>-1</sup>)<sup>32,38–40</sup> (Figure 2a). 357

As regards FT-IR analysis of FA, only FT-IR spectra of samples A1 and B1, where 846 and 1492 mg 358  $L^{-1}$  of Fe<sup>2+</sup> was applied in the Fenton oxidation, respectively, showed the peak at 1381 cm<sup>-1</sup> assigned 359 to COO<sup>-</sup> antisymmetric stretching, C-H and bending of CH<sub>2</sub> and CH<sub>3</sub> groups (Figure 2b). The C-O 360 stretching and OH deformation of COOH at 1198 cm<sup>-1</sup> was observed only in the samples Ctrl, B1, 361 B2, B4, and B5 (Figure 2b). Furthermore, peaks at 1120 cm<sup>-1</sup> and 1090 cm<sup>-1</sup>, assigned to C-O 362 363 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, 364 365 A4, A5, and B3 samples (Figure 2b).

366

#### 367 **TG analysis**

Three different peaks were registered in DTG profiles in according to Wu et al.<sup>32</sup> The peak DTG1 at 368 60 °C was mainly associated to the dehydration of samples, whereas the peak DTG2 registered within 369 the temperature range of 200-350 °C was attributed to the thermal degradation of readily degradable 370 371 materials and semi-volatile compounds, such as carbohydrates, aliphatic structures, carboxylic groups, 372 hemicellulose, cellulose, and microbial cell walls. The peak DTG3 observed within the range of 400-373 600 °C was associated to the thermal decomposition of aromatic and polynuclear structures of high 374 molecular weight. The DTG2 and DTG3 peaks were corrected by subtracting the weight loss due to 375 the residual water.

376 The corrected weight loss of DTG2 and DTG3 of reference untreated and Fenton-treated digestates 377 is showed in Figure 3a. No difference between Dig and Ctrl samples was observed in DTG2 peak, 378 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 379 Dig (+18.3%) and Ctrl (+16.6%), and in B1 (no H<sub>2</sub>O<sub>2</sub> addition) that remained unchanged respect to 380 Dig and Ctrl. Conversely, DTG3 peak decreased as the  $H_2O_2/Fe^{2+}$  ratio was increased during the 381 Fenton reaction, especially at lower  $Fe^{2+}$  dosage, until to reach 3.5% in A5. Whereas at the greater 382 383 Fe<sup>2+</sup> concentration (samples B) no peak DTG3 change was noticed except in B1 where the peak value 384 increased (9.1%).

TG analysis of HA extracted from Fenton-treated digestates indicated that HA of Ctrl produced 385 386 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 387 388 11.5%, respectively). DTG3 peak increased in HA extracted from A1 and remained nearly unchanged 389 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 390 both Dig and Ctrl; whereas DTG3 peaks appeared strongly depleted in all treatments, including Ctrl 391 392 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). 393

394

# 395 **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 400 4). The ED<sub>50</sub> 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 402 403 chemical reagent addition (Ctrl) decreased, at least partially, the phytotoxic potential of the residual 404 biomass, which showed a slight stimulatory action at lower dosages and a phytotoxic response only 405 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<sup>2+</sup> dosage, despite the hydrogen 406 peroxide concentration applied within the experimental range (Figure 5). Conversely, a stimulatory 407 action was observed at 100% extract dosage: GI (%) values showed an increasing trend from 116.44 408 409  $\pm$  7.5 (in treatment A2) to 152.65  $\pm$  8.0% (in treatment A4) with an average value equal to 131.48  $\pm$ 22.5%. On the other side, the aqueous extracts from solid anaerobic digestate treated with an 410 increased  $Fe^{2+}$  dosage (1692 mg L<sup>-1</sup>) showed a clear phytotoxic action on seed germination and root 411 growth of lettuce (Figure 5). Noticeably, there was an increased phytotoxic response to the treated 412 matrix as long as the hydrogen peroxide concentration increased in the Fenton oxidation process, 413 which became evident and extended over the entire dilution range when using a concentration of 414  $H_2O_2$  larger than 18.9 mg L<sup>-1</sup> (Figure 5). In the presence of the 100% extract the GI value ranged 415 from 76.82% (treatment B2) to 94.37% (treatment B5). 416

417

#### 418 **Discussion**

419 According to most researchers,<sup>18,41,42</sup> Fenton chemistry encompasses the activation of hydrogen 420 peroxide (H<sub>2</sub>O<sub>2</sub>) by ferrous (Fe<sup>2+</sup>) ions to generate hydroxyl radicals (HO<sup>•</sup>) under acidic conditions 421 *via* a complex reaction sequence, following the chain initiation reaction (Eq. 2):

422 
$$\operatorname{Fe}^{2+} + \operatorname{H}_2O_2 \to \operatorname{Fe}^{3+} + \operatorname{HO}^{\bullet} + \operatorname{HO}^{\bullet} + \operatorname{HO}^{\bullet} (k_1 = 63 - 76 \text{ mol } \operatorname{L}^{-1} \operatorname{s}^{-1})$$
 (2)

- In the presence of an organic substrate (R-H) the hydroxyl radical abstracts a hydrogen atom from RH and generates an organic radical (R\*), which then undergoes a series of chemical transformations
  to form various oxidation products (Eq. 3):
- 426  $R-H + HO^{\bullet} \rightarrow H_2O + R^{\bullet}$  (chain propagation) (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<sup>•</sup> towards both organic and inorganic substrates negatively affects the organic oxidation process, thus leading to chain termination (Eq. 4-6):

433	$\mathrm{Fe}^{2+} + \mathrm{HO}^{\bullet} \longrightarrow \mathrm{Fe}^{3+} + \mathrm{HO}^{\bullet}$	$(k_2 = 3.0 - 3.2 \times 10^8 \text{ mol } \text{L}^{-1} \text{ s}^{-1}) $ (4)
434	$\mathrm{H_2O_2} + \mathrm{HO}^{\bullet} \longrightarrow \mathrm{HO_2}^{\bullet} + \mathrm{H_2O}$	$(k_3 = 2.7 - 3.3 \times 10^7 \text{ mol } \text{L}^{-1} \text{ s}^{-1})$ (5)
435	$\mathrm{HO}^{\bullet} + \mathrm{HO}^{\bullet} \longrightarrow \mathrm{H}_2\mathrm{O}_2$	$(k_4 = 5.9 \times 10^9 \text{ mol } \text{L}^{-1} \text{ s}^{-1}) \tag{6}$

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

H<sub>2</sub>O<sub>2</sub> represents the dominant source of hydroxyl radicals, limitations in its concentration can 442 443 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 444 445 major advantages like natural abundance, environmental compatibility, low-toxicity, high reactivity and reduced commercial cost.<sup>21,43</sup> As to assess the feasibility of the efficiency of the post-digestion 446 Fenton treatment of solid anaerobic digestate very small dosages of H<sub>2</sub>O<sub>2</sub> (ranging from 6,03 to 24,12 447 mg L<sup>-1</sup>) with relatively large amounts of catalytic Fe<sup>2+</sup> (precisely 846 and 1692 mg L<sup>-1</sup>) were here 448 449 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 450 451  $H_2O_2/Fe^{2+}$  ratio larger than 0.02. Contrarily to what reported for chemically treating organic wastes dispersed in aqueous systems,<sup>22,23</sup> larger amounts of catalytic Fe<sup>2+</sup> were here requested for the process 452

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.

The efficiency of a classical Fenton reaction depends also on pH, temperature, and reaction time.<sup>21</sup> 458 Even though contrasting conclusions have been reached, he optimum pH range for effective waste 459 treatment appears to be 2.5-3.0,<sup>19</sup> as maintained in the present study. In fact, acid pH leads to the 460 dissolution of ferric ions and hydroxyl radical, allowing the H<sub>2</sub>O<sub>2</sub> stabilization and resulting in 461 prolonged activity.<sup>43</sup> The treatment efficiency can be improved by increasing the temperature, which 462 can provide more energy to overcome the reaction activation energy. Generally, 25-40 °C represents 463 the most used temperature range for the Fenton process; however, higher temperatures (> 40 °C) can 464 be also applied depending on the nature of the waste material to treat.<sup>44</sup> Our previous investigations 465 on solid-state organic matrices (i.e. sewage sludge) have shown that 70 °C provided optimum 466 conditions for the oxidative treatment. A reaction time ranging from 20 to 60 min is required in most 467 cases even though prolonged reaction times (up to 240 min) were experienced for efficient treatments 468 of recalcitrant organic wastes (i.e. olive-oil mill waste).<sup>43</sup> In the present experiment a reaction time 469 470 as long as 8 hours is actually the combination of an expected shorter reaction time, due to the nearly solid state consistency of the treated material, and the need to allow the material to reach a complete 471 dryness as well as absence of any repelling odor emission. In fact, shorter reaction times would 472 473 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, and478 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<sup>45</sup> that the oxidation 479 of organic contaminants increases at increasing  $H_2O_2/Fe^{2+}$  ratios. Interestingly, neither the total C nor 480 the total N varied during the process, suggesting that non-intense oxidation conditions occurred 481 482 during the process. Further inside, TG analysis showed that samples treated with increasing H<sub>2</sub>O<sub>2</sub> dosage at 846 mg L<sup>-1</sup> Fe<sup>2+</sup> (samples A, greater  $H_2O_2/Fe^{2+}$  ratio) the recalcitrant fraction (DTG3 peak) 483 declined as also previously observed.<sup>46</sup> On the other hand, at the greatest Fe<sup>2+</sup> dosage (samples B, 484 lower  $H_2O_2/Fe^{2+}$  ratio), the recalcitrant fraction was not affected. This finding suggests that rather 485 486 than inducing more oxidative conditions, a major catalytic iron concentration could have led to a "scavenging effect" of the HO<sup>•</sup> radical (Eq. 4) with a consequent reduced efficiency of the oxidative 487 process, as also postulated by Wu et al.<sup>46</sup> Interestingly, samples treated only with Fe<sup>2+</sup> catalyst 488 evidenced an increase of the recalcitrant fraction (DTG3 peak; Figure 3), especially at the greatest 489 490 dosage where ferrous ions could have become complexed with humic substances. In fact, at acidic pH, Fe<sup>2+</sup> is adsorbed onto carboxylic groups of humic fractions.<sup>47,48</sup> Unlike extracted humic fractions-491 as reported below, no differences arose in FT-IR spectra of Fenton-treated digestates with no 492 alteration of aliphatic groups, aromatic, ketone and quinone groups, and polysaccharides after the 493 oxidation process, in contrast with Quina et al.<sup>25</sup>, though they adopted more intense oxidative 494 conditions (i.e.  $H_2O_2 > 5$  g kg<sup>-1</sup> total solid). 495

Conversely, thermal treatment, catalysts dosage and the  $H_2O_2/Fe^{2+}$  ratio clearly affected yields and 496 properties of both HA and FA extracted from Fenton-stabilized digestates. In fact, following the 497 thermal treatment (Ctrl) an increased amount of both HA and FA was extracted, that continued to 498 grow up by increasing the  $H_2O_2/Fe^{2+}$  ratio during the reaction. This behavior could be explained by 499 the combined thermal treatment and complexation of Fe<sup>2+</sup> onto HA and FA, that leads to a stable 500 humic fraction.<sup>47,48</sup> However, as long as the oxidative condition increased – due to the increasing 501 502  $H_2O_2/Fe^{2+}$  ratio – lesser N was being incorporated into the humic-like fractions particularly FA. We 503 surmise that the greater abundance of ferric ions generated by the large amount of H<sub>2</sub>O<sub>2</sub> could have 504 acted as a Lewis acid, thus promoting the catalytic cleavage of peptide bonds, release of amino acids and major loss of N, which was not incorporated into the final FA product. In a few words, yields and properties of humic-like fractions were the result of a combination of oxidative and hydrolytic 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 an increase of aromatic structures and groups as aryl ethers, phenols, and secondary alcohols thereby 511 512 indicating HA from Fenton-stabilized digestates as more stable products. This behavior was also confirmed by the TG analysis. In fact, the recalcitrant fraction, associated to the thermal 513 decomposition of aromatic structures within the range of 400-600 °C, enlarged respect to HA from 514 non-treated digestate (Dig). In according to the theory of the supramolecular structure of humic 515 substances proposed by Piccolo,<sup>49</sup> the presence of  $Fe^{2+}$  promoted thermodynamically stable 516 associations due to the complexation with acidic functional groups of humic molecules, thus losing 517 their previous weakly-held conformations.<sup>49</sup> 518

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.<sup>48</sup> 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<sup>50</sup> to 527 strong<sup>51</sup> phytotoxic responses on germination and growth of *Lepidium sativum*. In accordance with 528 latter results, our Dig inhibited the lettuce germination at concentrations  $\geq 1\%$  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<sup>52,53</sup> and soluble salts as they can negatively affect the

water uptake needed for germination.<sup>54</sup> However, since the EC significantly increased in all the 531 532 treated digestates, including those which were not phytotoxic, the influence of an unfavorable osmotic 533 potential on seed germination and seedling growth was here considered negligible. On the other hand, 534 phenolic compounds, alone or in complex mixture, can exert phytotoxic effects on both germination and seedling establishment, even at low concentrations.<sup>55</sup> As seen before, TPC strongly varied with 535 reagent dosage and their combination, suggesting that the differences in phytotoxic responses can be 536 strictly correlated with the TPC of the organic matrix. Interestingly, a stimulatory effect on the GI 537 538 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 539 stimulatory at low and inhibitory at high concentrations.<sup>56,57</sup> Both the TPC and phytotoxic potential 540 of Fenton-treated digestates declined at the lowest  $Fe^{2+}$  catalyst dosage and when the  $H_2O_2/Fe^{2+}$  ratio 541 increased, while a larger HA content was observed. We hypothesize that increased humic-like 542 substances could have contributed to stimulate the germination and seedlings growth of lettuce, also 543 544 as reported before.<sup>58</sup>

To sum up, this lab scale study showed that Fenton post-digestion treatment has great potential for 545 large-scale application in increasing stability and detoxication of solid anaerobic digestates, whose 546 547 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<sup>-1</sup> and an 548 amount of Fe<sup>2+</sup> catalyst slightly large but not exceeding 846 mg L<sup>-1</sup>, at pH 3.0 and 70 °C for 8 h under 549 continuous stirring at 50 rpm, represent the optimum conditions to produce an effective biomass 550 stabilization. The resulting  $H_2O_2/Fe^{2+}$  ratio ranging from 0.02 and 0.03, which is certainly small when 551 552 compared to those used in wastewater treatment, is in agreement with that suggested by Nieto et al.<sup>59</sup> 553 Given the properties of the matrix to treat, this reagent ratio has been found suitable for decreasing 554 the phytotoxic potential and total phenols, and increasing the stability of the product as well as the 555 content and the structure of chemically-induced formation of humic-like fractions, which can be 556 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.

559

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565

## 566 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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735

## 736 Figure captions

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

740

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

744

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

749

Figure 4. Dose-response curve of the germination index [GI (%)] (mean  $\pm$  SE, n = 5) of seeds of *Lactuca sativa* L. exposed for 48 h under controlled growth conditions (darkness,  $25\pm1$  °C) to increasing aqueous extract concentrations (namely 0, 0.1, 1, 5, 10, 20, 80 and 100%) of untreated solid anaerobic digestate (Dig). Different lowercase letters indicate significant differences among treatments (P < 0.05). Fitting of the non-linear regression model to estimate the ED<sub>50</sub> value (i.e. the percent extract dose determining a 50% reduction of the maximum GI response) was at a significant value of P < 0.001.

757

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

- 761stabilization process carried out using different dosages of Fenton reagents (treatments as reported in762Table 2). The significant effect due to the aqueous extract concentration (C) is shown as *F*-value and763level of significance (\* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001; ns, not significant) estimated by a one-764way ANOVA.
- 765

Parameter	Value
pH <sup>b</sup>	$8.45\pm0.03$
EC (dS m <sup>-1</sup> at $25^{\circ}$ C) <sup>c</sup>	$1.48\pm0.01$
Dry matter (% fresh weight)	$33.5 \pm 1$
Ash (%)	$10 \pm 1$
Volatile solids (%)	90 ± 1
Total C (g kg <sup>-1</sup> )	$467.6\pm0.8$
Total N (g kg <sup>-1</sup> )	$12.2 \pm 0.1$
C/N	$38.3\pm3.2$
C <sub>HA</sub> (%)	$10.98\pm0.16$
C <sub>FA</sub> (%)	6.88 ± 1.28
$NH_4^+-N (g kg^{-1})$	$1.44 \pm 0.13$
NH <sub>4</sub> <sup>+</sup> -N (% Total N)	11.8 ± 1.7
$NO_{3}^{-}-N (g kg^{-1})$	$0.017 \pm 0.001$
$BOD_5 (mg L^{-1})$	$1850 \pm 25$
Total K (g kg <sup>-1</sup> )	$9.06 \pm 0.09$
Total S (g kg <sup>-1</sup> )	$2.00\pm0.04$
Cd (mg kg <sup>-1</sup> )	<0.1
Cr <sub>VI</sub> (mg kg <sup>-1</sup> )	$2.48\pm0.04$
Pb (mg kg <sup>-1</sup> )	$1.57\pm0.06$
Ni (mg kg <sup>-1</sup> )	$1.44\pm0.06$
Hg (mg kg <sup>-1</sup> )	<1.5
Cu (mg kg <sup>-1</sup> )	$20.60\pm0.06$
Zn (mg kg <sup>-1</sup> )	$90.13\pm0.06$
Salmonella spp.	Absent
Escherichia coli (CFU g <sup>-1</sup> )	Absent
Dehydrogenase activity (µg INTF g <sup>-1</sup> 2 h <sup>-1</sup> )	$35.7\pm1.1$
FDA-hydrolase activity ( $\mu g$ fluorescein $g^{-1} h^{-1}$ )	$261.0\pm2.1$
Alkaline phosphatase activity ( $\mu g p$ -NP g <sup>-1</sup> h <sup>-1</sup> )	$172.2\pm6.2$

766 Table 1. Chemical, biochemical and biological characterization of a solid anaerobic digestate 767 from a medium-scale biogas producing plant<sup>a</sup>

- <sup>*a*</sup> Values are the mean  $\pm$  SD (*n*=3) expressed on a dry matter basis. <sup>*b*</sup> Biomass/H<sub>2</sub>O, 3:50, w/v.
- 769
- <sup>c</sup> Biomass/H<sub>2</sub>O, 1:10, w/v. 770
- 771

768

772 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

Traatmont	Fenton reage	H <sub>2</sub> O <sub>2</sub> /Fe <sup>2+</sup> ratio	
	$Fe^{2+}$ (mg L <sup>-1</sup> )	$H_2O_2 (mg L^{-1})$	
$\mathrm{Dig}^{a}$	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

<sup>a</sup> Physically and chemically untreated solid anaerobic digestate was taken as a reference treatment.

Treatment <sup>b</sup>	$\mathbf{BSI}^{c}$	EC <sub>1:10</sub>	$\mathrm{TPC}^d$	OM <sup>e</sup>	С	Ν	C/N
		$(dS m^{-1})$	$(mg kg^{-1})$	(%)	(%)	(%)	
Dig	3	$1.48\pm0.02\;k$	363.2 ± 17.9 a	$91.7\pm2.6$	$46.8 \pm 0.3$ a	$1.22 \pm 0.01$ a	$38.3 \pm 0.3$ c
Ctrl	3	$1.43\pm0.02\;k$	$250.5\pm9.1\ b$	$91.3\pm2.2$	$46.1\pm0.9~a$	$0.99\pm0.10~b$	$46.5\pm1.2\ b$
A1	3	$1.56\pm0.02\ j$	$183.0\pm3.4\ c$	$91.7\pm1.7$	$47.2\pm0.8~a$	$0.99\pm0.06\ b$	$47.7\pm0.9~b$
A2	2	$1.84\pm0.03\ i$	$111.2\pm1.9~d$	$91.1\pm1.2$	$47.3\pm0.4~a$	$0.99\pm0.09~b$	$47.8\pm0.8\ b$
A3	2	$2.04\pm0.02\ h$	$95.6\pm1.8\;e$	$91.5\pm1.7$	$47.1\pm0.7~a$	$0.99\pm0.04\ b$	$47.7\pm1.4~b$
A4	1	$2.48\pm0.02\ f$	$85.0\pm1.6\;f$	$90.8 \pm 1.5$	$47.3\pm0.3~a$	$1.01\pm0.05~b$	$46.8\pm0.9\ b$
A5	1	$2.30\pm0.02\ g$	$45.3\pm4.4~g$	$89.2\pm1.2$	$47.4\pm0.4~a$	$1.00\pm0.05\ b$	$47.6\pm0.8\ b$
B1	3	$2.66\pm0.02\;e$	$179.7 \pm 3.3$ c	$88.8\pm2.3$	$47.2\pm0.5~a$	$0.97\pm0.01~b$	$48.7\pm0.6~a$
B2	3	$3.49\pm0.03\;c$	$117.8 \pm 5.1 \text{ d}$	$91.2\pm1.0$	$47.3\pm0.6~a$	$1.01\pm0.05\ b$	$46.4\pm0.7\;b$
B3	3	$3.23\pm0.02\;d$	$101.6 \pm 7.3$ e	$90.7 \pm 1.1$	$47.3 \pm 0.1 \text{ a}$	$1.02\pm0.06~b$	$46.8\pm0.6\;b$
B4	1	$3.92\pm0.02\;b$	$69.3 \pm 2.4 \text{ fg}$	$90.0\pm1.3$	$47.7\pm0.3~a$	$0.96\pm0.09~b$	$49.7 \pm 1.0 \text{ a}$
B5	1	$4.20 \pm 0.02$ a	$43.8 \pm 2.7$ g	$90.1\pm2.0$	$47.3 \pm 0.3$ a	$0.97\pm0.09~b$	$48.8\pm0.9~a$

Table 3. Selected physical and chemical properties of the solid anaerobic digestate before (Dig) and after the iron-based Fenton process 778 carried out using different dosages of Fenton reagents<sup>a</sup>

<sup>*a*</sup> Values are mean  $\pm$  SD (*n*=3) expressed on a dry matter basis. Lowercase different letters indicate significant differences among treatments (*P* < 779

780 0.05).

777

b Treatments as reported in Table 2.
c Bad odor index 781

782

<sup>d</sup> Total phenolic compound content, expressed as catechol 783

<sup>e</sup>Organic matter by weight loss on ignition. 784

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 <sup>a</sup>

Treatment <sup>b</sup>	Extraction yield (%)	C (%)	N (%)
Humic acids			
Dig	3.7	$43.06 \pm 0.19 \text{ a}$	$5.97 \pm 0.07$ a
Ctrl	3.9	$43.04 \pm 0.18 \ a$	$5.92 \pm 0.07$ a
A1	3.0	$41.44\pm0.23\ c$	$5.42\pm0.10~\text{b}$
A2	4.1	$41.21\pm0.40\ c$	$5.29\pm0.10~\text{b}$
A3	5.6	$42.26\pm0.20\ b$	$5.33\pm0.25$ b
A4	7.6	$42.57\pm0.07~b$	$5.26\pm0.07~\text{b}$
A5	6.5	$42.59\pm0.19~b$	$5.22\pm0.22$ b
B1	3.0	$41.80 \pm 0.28$ c	$4.72\pm0.08~c$
B2	3.8	$37.98 \pm 0.34 \ d$	$4.57\pm0.12~\mathrm{c}$
B3	3.0	$38.66 \pm 0.39 \text{ d}$	$4.75\pm0.04~c$
B4	4.9	$41.23 \pm 0.44$ c	$4.12 \pm 0.07 \; d$
B5	5.4	$41.32 \pm 0.35$ c	$4.10 \pm 0.07 \text{ d}$
Fulvic acids			
Dig	0.9	$40.50 \pm 0.42$ a	$4.80 \pm 0.14$ a
Ctrl	2.7	$33.25\pm0.57\ d$	$4.09\pm0.03~b$
A1	3.4	$33.19\pm0.37~d$	$3.38\pm0.06~c$
A2	3.7	$33.40\pm0.32~d$	$3.08\pm0.05~\text{d}$
A3	4.2	$34.60 \pm 0.21 \text{ c}$	$2.88 \pm 0.01 \text{ e}$
A4	4.6	$35.42\pm0.43~b$	$2.68\pm0.01~f$
A5	2.8	$35.66\pm0.24~b$	$2.63\pm0.10~\mathrm{f}$
B1	2.7	$33.05 \pm 0.17 \text{ d}$	$3.46 \pm 0.07$ c
B2	2.2	$34.64 \pm 0.13$ c	$3.41 \pm 0.07$ c
B3	2.3	$35.91\pm0.10\ b$	$3.39\pm0.08~c$
B4	5.4	$35.89\pm0.23~b$	$3.23 \pm 0.01 \text{ d}$
B5	3.4	$35.80\pm0.20\ b$	$3.20\pm0.01$ d

788 <sup>*a*</sup> Values are mean  $\pm$  SD (*n*=3) expressed on a dry matter basis. Lowercase different letters

indicate significant differences among treatments within the same extracted fraction (P < 0.05).

790 b Treatments as reported in Table 2.

Wavenumber (cm <sup>-1</sup> )	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 <sup>-</sup> , -CH <sub>3</sub>
1370-1381	COO <sup>-</sup> antisymmetric stretching, C–H and bending of CH <sub>2</sub> and CH <sub>3</sub>
1220-1240	groups 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

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

