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1 Use of recalcitrant agriculture wastes to produce biogas and feasible biofertilizer.

2

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29 **Abstract**

30 In the ongoing work, the digestion process of recalcitrant agricultural wastes (olive wastes and
31 citrus pulps) mixed in different proportions with, livestock manures, milk serum and maize silage
32 for biogas production was studied. Additionally, the chemical composition and the phytotoxicity of
33 the digestates (each separated in liquid and solid fraction) were evaluated with the purpose of being
34 used as organic fertilizer in agriculture. The results demonstrated that animal manure and
35 recalcitrant agricultural wastes, if properly mixed, produced high percentage of biogas. The
36 digestate chemical compositions differed and varied in respect to the kind of feedstock, and the ratio
37 of their mixing to feed the digesters. The digestate from the digester named Fattoria, mainly
38 powered with animal manures (poultry, cow and sheep), contained less phenols and more active
39 microbial biomass than the digestate from the digester Uliva, mainly fed with olive waste and citrus
40 pulp and in minor extent with animal manure and maize silage. Our data showed that the digestate
41 composition depended on the mix of biomass input. Additionally, the effects of digestate were
42 plant specie-specific and a positive correlation between the amount of phenols and the phytotoxic
43 effects of digestate on plants was also well evident. These results evidenced that each single
44 digestate has a own chemical feature, suggesting that the sustainable disposal of digestates requires
45 a preliminary screening to select the one which better fits the demands of a particular species for
46 optimizing crop production.

47 *Key words:* anaerobic digestion; antioxidant system; biogas; digestate; phytotoxicity; seed
48 germination.

49 **Introduction**

50 Agricultural activities, waste management, and use of energy from fossil fuels, all contribute to
51 global warming and climate change. [1,2], Against these background it is necessary to strengthen
52 waste management activities in the context of climate change, promoting alternative energy derived
53 from natural sources. Biogas technology, also known as anaerobic digestion (AD) technology, can

54 be considered a competitive process for reducing the rate of climate change and global warming
55 managing biodegradable waste streams to produce renewable energy and nearly stable residue
56 (digestate), in a sustainable way [3-5]. The energy produced in the form of biogas, is a
57 mixture of methane (45-75%), carbon dioxide (25-55%) and minor amounts of H₂S and H₂ and the
58 actual proportion is dependent on the feedstock (substrate) used, and on the processes employed.
59 While biogas represents an ascertained useful source of renewable energy, the digestate ever-
60 increasing production induces problems related to its sustainable disposal. Consequently, research
61 on agriculture valorization routes to reduce its environmental impact and to improve the economical
62 profitability of AD plants are of great interest [6]. Depending on their chemical features, some
63 digestates can have negative impact on environment or on plant growth [7], while some others may
64 influence soil fertility and plant health positively. However, a digestate cannot be considered
65 positive or negative *in toto*, therefore it is necessary to chemically and biologically characterize
66 each digestate for finding an adequate utilization. Despite all, the use of digestate as fertiliser is
67 legally limited in many countries due to unfamiliarity of the product and insufficient confidence in
68 its quality and safety [8]. Quality assurance is an important prerequisite for increasing market
69 confidence in digestate and for enhancing its use as fertilizer [9]. In many European Union Nations,
70 anaerobic digestion technologies and processes are a widely accepted practice that aims to increase
71 the profitability of dairy farmers and the food processing industry by utilizing organic wastes for
72 better meeting the needs of environmental regulators [10]. Currently, in Italy, issues such as
73 demand for renewable energy, landfill tax on organic wastes, demand for organic fertilizer,
74 pollution of the environment and legislation relating to the treatment and disposal of organic wastes
75 are all important factors influencing investments in AD [11]. Calabria (Southern Italy) is an
76 agricultural land with predominant production of citrus, oil and livestock [12] to produce milk and
77 cheese. Agriculture wastes and livestock manures are highly polluting and difficult to dispose of,
78 with a high cost for farmers [13]. Thus, their anaerobic digestion could be a reliable way to use
79 refuse as resource producing economic benefit [14-17]. This research, in cooperation with two

80 cooperatives **Fattoria della Piana** soc. Agricola, and **Uliva Srl soc. Agricola**, owners respectively
81 of two biogas plant each with 998 kWel of installed power, has the aim to evaluate the digestion
82 process of olive wastes and citrus pulps mixed with other organic biomass and animal manure, for
83 biogas production. The specific objectives were: 1) to compare the output and the composition of
84 biogas, obtained from the two plants fed with recalcitrant agriculture wastes (olive wastes and citrus
85 pulps), mixed in different proportions with livestock manures, milk serum and maize silage; 2) to
86 chemically characterize the two digestates, each separated in liquid and solid fractions; 3) to test *in*
87 *vitro* the effects of the liquid and solid fractions of the two digestates on seed germination, seed
88 performance and antioxidant system of model plants (cucumber, watercress and lettuce).

89

90 **Materials and Methods**

91 Biogas plants: process temperature and retention time

92 Each biogas energy plant has an installed power of 998 kWel. The two biogas plants were
93 differently supplied: the first one named **Fattoria (F)** was powered with animal manures (poultry,
94 cow and sheep), milk serum, maize silage and in minor amount with olive waste and citrus pulp.
95 The second one named **Uliva (U)** was mainly powered with olive waste and citrus pulp and in
96 minor amount with animal manure and maize silage.

97 The time of residence of the feedstock inside the digester (retention time), at constant process
98 temperature, influences the digestate quality. Retention times are quoted as hydraulic retention time
99 (HRT) and as minimum guaranteed retention time (MGRT). HRT is the nominal time that feedstock
100 remains inside the digester at the process temperature. HRT is usually expressed in days and it
101 depends, to a large extent, on the digestibility of the feedstock mixture.

102 $HRT [h \text{ or days}] = \text{Digester volume } [m^3] / \text{the influent flow rate } [m^3/h \text{ or days}]$.

103 Combinations of thermophilic or mesophilic process temperatures and MGRT can provide pathogen
104 reduction in the digestate obtained, equivalent to the EU sanitation standard of 70°C for 1 hour and
105 are thus allowed, depending on the feedstock mixtures. Biogas plant operators have selected process
106 temperatures and retention times which are appropriate for the feedstock that had to be digested.

107 **Fattoria:** process temperature: 40 °C, pH 7.8, total volume of the two digesters: 7500 m³ (2500
108 DIG.1 + 5000 DIG.2), total volume loaded per day: 120 m³/day, hydraulic retention time (HRT):
109 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C.

110 **Uliva:** process temperature: 40 °C, pH 8.0, total volume of the two digesters: 7420 m³ (3180 DIG.1
111 + 4240 DIG.2), total volume loaded per day: 120 m³/day, hydraulic retention time (HRT) 60 days,
112 minimum guaranteed retention time (MGRT) 16 h at 40°C.

113 The digestates coming from both plants were separated in liquid and solid fractions (Solid Uliva,
114 **SU**; Liquid Uliva, **LU**; Solid Fattoria, **SF**; Liquid Fattoria, **LF**, and analyzed for chemical and
115 biological characteristics.

116 Chemical analysis

117 Chemical parameters were determined in three replicates. Dry matter (dm) content was determined
118 at 105°C until the mass loss of the sample during 24 h was lower than 0.5% of its weight [18];
119 moisture content, after drying to constant weight at 105 °C; volatile solids, reflect the content of
120 OM which can be decomposed by combustion at 550 °C for 24 h up to constant weight; pH was
121 measured in distilled water using a 1:2.5 (digestate/water) suspension; organic carbon was
122 determined by the Walkley–Black procedure [19], and it was converted to organic matter by
123 multiplying the percentage of carbon by 1.72; total nitrogen was measured by Kjeldahl method
124 [20]; electric conductivity was determined in distilled water by using 1:5 digestate:water
125 suspension, mechanically shaken at 15 rpm for 1 hour to dissolve soluble salts, and then detected
126 by Hanna instrument conductivity meter. Available P was determined by the Bray II method [21].

127 Exchangeable K was extracted with 1 M NH₄OAc, and determined using a flame-photometer. The
128 NO₃-N was measured using a nitrate-ion selective electrode (USEPA, 2011), while NH₄-N was
129 determined by a colorimetric method based on Berthelot's reaction [22]. All values refer to material
130 dried at 105 °C for 24 h. The 5-day biochemical oxygen demand (BOD) was measured with a
131 respirometric Oxitop® IS 6 (WTW, Germany) based on pressure measurement, which is
132 automatically transformed into mg O₂ L⁻¹. In the Oxitop® system, cumulative oxygen consumption
133 measurements were made each day during a 5-day period. COD was determined by dichromate
134 oxidation of dried ground samples, according to an adaptation of the standard method described for
135 liquid samples [18] and using an automatic titration device (Metrohm Titrandosino device);
136 total water-soluble phenols were measured by using the Folin-Ciocalteu reagent, following the
137 Box method [23]. Tannic acid was used as a standard and the concentration of water-soluble
138 phenols was expressed as tannic acid equivalents (mg TAE/g dm) [24]. Fluorescein diacetate
139 hydrolysis (FDA) reaction was determined according to the methods of Adam and Duncan [25].
140 Briefly, to 2 g of digestate (fresh weight, sieved <2 mm) 15 ml of 60 mM potassium phosphate pH
141 7.6 and 0.2 ml 1000 µg FDA ml⁻¹ were added. The flask was then placed in an orbital incubator at
142 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol solution (2:1
143 v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3
144 min. The supernatant was filtered and the filtrates measured at 490 nm on a spectrophotometer
145 (Shimadzu UV-Vis 2100, Japan).

146 Germination test

147 The germination test on the Petri dish is a promising test in predicting the phytotoxicity [26].
148 Additionally, seed germination and germination index of model species [27-28], are recognized as
149 indicators particularly sensitive and may be adopted as test to determine the phytotoxicity of new
150 compounds /products [29-31].

151 The seeds of watercress, lettuce and cucumber were surface-sterilized for 20 min in 20% (v/v)
152 sodium hypochlorite, rinsed and soaked in distilled water (for a total of 1 h). Five 50-seed replicates
153 for germination test were carried out with different concentrations of solid and liquid digestate
154 fractions from Uliva and Fattoria. In the experiments five different concentrations of liquid fraction
155 of Fattoria and Uliva digestates were used (0, 10, 25, 50 and 100%); Uliva and Fattoria solid
156 digestate were extracted in water (1:5 w/v) for 24 h at room temperature in agitation and then
157 diluted with distilled water to have 5 concentrations (0, 10, 25, 50 and 100%). Fifty seeds of each
158 species were placed on filter paper in 9 cm Petri dishes containing 3 cm³ of each solution. The Petri
159 dishes were hermetically sealed with Parafilm to prevent evaporation and kept in a growth chamber
160 at a temperature of 25±1°C in the dark with a relative humidity of 70%. Seeds were considered
161 germinated when the radicle had extended at least 2 mm. Three replicates were analyzed for each
162 treatment.

163 Germination indexes. The number of seeds germinated was recorded daily for up to 7 d. From these
164 germination counts several germination attributes were calculated to characterize the phytotoxicity,
165 including total germination percentage (TG) (%) at 7 d, coefficient of velocity of germination
166 (CVG) [32], germination rate index (GRI) [33], and mean germination time (MGT) [33] as follow:

167
$$\text{CVG (\% day}^{-1}\text{)} = \frac{\sum Ni}{\sum (NiTi)} \times 100,$$

168
$$\text{GRI (\% day}^{-1}\text{)} = \frac{\sum Ni}{I},$$

169
$$\text{MGT (day)} = \frac{\sum (NiTi)}{\sum Ni}$$

170 Where N is the number of seed germinated on day i, Ti is the number of days from sowing and I is
171 the number of germinated seeds at 7 d. The CVG gives an indication of the rapidity of germination:
172 it increases when the number of germinated seeds increases and the time required for germination
173 decreases. The GRI reflects the percentage of germination on each day of the germination period.
174 Higher GRI values indicate higher and faster germination. The lower the MGT, the faster a
175 population of seeds has germinated.

176 Determination of enzyme activities

177 Seeds (0.5 g) that had been soaked for 3 d in the test solutions were ground using a chilled mortar
178 and pestle and homogenized in 0.1 M phosphate buffer solution (pH 7.0) containing 100 mg soluble
179 polyvinylpolypyrrolidone (PVPP) and 0.1 mM ethylenediamine tetra acetic acid (EDTA). The
180 homogenate was filtered through two layers of muslin cloth and centrifuged at 15000 g for 15 min
181 at 4°C. The resulting supernatant was used to evaluate the activity of catalase (CAT, EC 1.11.1.6),
182 peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione
183 reductase (GR EC 1.15.1.1). All enzyme activities were measured at 25°C by a UV: visible light
184 spectrophotometer (UV-1800 CE, Shimadzu, Japan).

185 CAT activity was determined by monitoring the disappearance of H₂O₂ at 240 nm, calculated using
186 its extinction coefficient (ϵ) = 0.036 mM⁻¹ cm⁻¹. The reaction mixture contained 1 mL potassium
187 phosphate buffer (50 mM, pH 7.0), 40 μ L enzyme extract and 5 μ L H₂O₂ [34].

188 APX activity was assayed according to Nakano and Asada [35]. The reaction mixture (1.5 mL)
189 contained 50 mM phosphate buffer (pH 6.0), 0.1 μ M EDTA, 0.5 mM ascorbate, 1.0 mM H₂O₂ and
190 50 μ L enzyme extract. The reaction was started by the addition of H₂O₂ and ascorbate oxidation
191 measured at 290 nm for 1 min. Enzyme activity was quantified using the molar extinction
192 coefficient for ascorbate (2.8 mM⁻¹cm⁻¹)

193 GR activity was assayed spectrophotometrically at 30 °C in a mixture containing 3 mL 100 mM
194 potassium phosphate buffer (pH 7.5), 1 mM 5,5'-dithio-bis (2-nitrobenzoic acid), 1 mM oxidized
195 glutathione (GSSG) and 0.1 mM NADPH. The reaction was initiated by the addition of 50 μ L of
196 enzyme extract. The rate of reduction of GSSG was followed by monitoring the increase in
197 absorbance at 412 nm over 2 min [36].

198 POX activity was measured on the basis of determination of guaiacol oxidation at 436 nm for 90 s
199 [37]. The reaction mixture contained 1 mL potassium phosphate buffer (0.1 M, pH 7.0), 20 μ L

200 guaiacol, 40 μL enzyme extract and 15 μL H_2O_2 . POX activity was quantified by the amount of
201 tetraguaiacol formed using its extinction coefficient (ϵ) = 25.5 $\text{mM}^{-1}\text{cm}^{-1}$.

202 For CAT, APX, GR and POX activities, the results were expressed as enzyme units (U) per mg
203 protein. One unit of enzyme was defined as the amount of enzyme necessary to decompose 1 μmol
204 of substrate per min at 25°C.

205 Total antioxidant capacity determination

206 Seeds treated with different salt solutions for 3 d were homogenized in a chilled mortar with
207 distilled water at a ratio of 1:4 (seeds/water; w/v) and centrifuged at 14000 g for 30 min. All steps
208 were performed at 4 °C. The supernatants were filtered through two layers of muslin cloth and were
209 used to determine the total antioxidant capacity by the spectrophotometric method of Prieto et al.
210 [38]. Aqueous extracts of the seeds were combined in Eppendorf tubes with 1mL of reagent
211 solution (0.6 M H_2SO_4 , 28 mM sodium phosphate, 4 mM ammonium molybdate mixture). The
212 tubes were incubated for 90 min at 95°C, then cooled to room temperature and the absorbance read
213 at 695 nm against a blank (mixture without seed extract). The assay was conducted in triplicate and
214 the total antioxidant activity expressed as the absorbance of the sample at 695 nm. The higher the
215 absorbance value, the higher the antioxidant activity [39].

216 Total phenolic content determination

217 Total phenolic content was determined with the Folin-Ciocalteu reagent according to a modified
218 procedure described by Singleton and Rossi [40]. Briefly, 0.50 mL of the aqueous extract of the
219 seeds was reacted with 2.5 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) for 4
220 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction
221 mixture. The absorbance readings were taken at 760 nm after 2 hours of incubation at room
222 temperature. Tannic acid was used as a reference standard, and the results were expressed as
223 milligram tannic acid equivalent (mg TAET/g fresh weight).

224 Statistical analysis

225 All data were analyzed by one-way analysis of variance (ANOVA). Separate ANOVAs were
226 performed for each of digestate fractions and concentrations. The response variables for these
227 ANOVAs were: seed germination, seedling growth, enzyme activities. Since the concentration of
228 each digestate fraction had five levels, on all significant ANOVAs we performed Tukey multiple
229 comparison tests to compare all pairs of means. The germination percentage data were previously
230 subjected to arcsine transformation but are reported in tables as untransformed values. All data
231 collected were statistically analyzed using SYSTAT 8.0 software (SPSS Inc.).

232

233 **Results**

234 *Biogas and Digestate composition*

235 No differences in the biogas composition between the two plants were observed (Table 1). In both
236 plants, the biogas production reached 440-450 m³/h with a methane content of ~60% (Table 1).

237 Both digestates had higher ammonium (NH₄⁺) to total nitrogen (N) ratios, decreased OM, total and
238 organic carbon (C) contents, reduced biological oxygen (O₂) demands (factor), elevated pH values,
239 smaller carbon to nitrogen ratios (C:N ratios), and a greater amount of nutrients than the respective
240 input materials (ingestate) (Table 2)

241 The two biogas digestates were chemically and qualitatively different one from the other. Fattoria
242 (**F**) had less total phenols, lower COD and BOD, but greater amount of K⁺, P, NH₄⁺, and NO₃⁻ than
243 Uliva (**U**) (Tables 3-4), **U** contained more Mg⁺⁺ and Ca⁺⁺. From a biological point of view, **F** had a
244 greater amount of bacteria, than **U**. Apart from the differences observed between the two digestates,
245 we detected chemical and biological differences, between the solid and liquid fractions of the same
246 digestate (Tables 3-4). In **Fattoria**, the solid fraction had less total phenols, total oil, saponifiable
247 fat and total hydrocarbon than the liquid one. Additionally the **SF** had greater amounts of bacteria,
248 K⁺, P, Mg⁺⁺ and Ca⁺⁺ and NO₃⁻ than **LF** (Table 3). In uliva digestate, the solid fraction had a lower
249 pollution load (BOD and COD) and contained less total phenols, total oil, saponifiable fat and total

250 hydrocarbons than **LU** (Table 4). T. Additionally, **SU** contained more P, NO_3^- and Ca^{++} and less K^+ ,
251 NH_4^+ and Mg^{++} with respect to **LU**.

252 *Seed germination*

253 Germination differed significantly among the species in respect to the type of fractions, to the
254 dilution levels, and to the combination of these factors. (Tables 5-6). Maximum germination
255 percentage (100%) was observed in water. **LF** at the lowest concentration, decreased (-20%) seed
256 germination percentage of lettuce and watercress. Higher LF concentrations, completely inhibited
257 the germination percentage of lettuce and watercress, while did not affect the total germination of
258 cucumber. Even if the germination percentage was reduced in seeds of lettuce and watercress
259 treated with 10% **LF**, there were no significant differences in germination rapidity (CVG), in
260 medium germination time (MGT) and in GRI, an index reflecting the percentage of germination on
261 each day (Table 5). These parameters were not affected in cucumber seeds by all **LF**
262 concentrations. **SF** at the lowest concentration speeded up the germination of lettuce and
263 watercress. Increasing its concentrations the germination percentage of these species decreased in a
264 concentration dependent manner. **SF** at all concentrations did not affect seed germination
265 percentage, germination rapidity (CVG), GRI and MGT of cucumber (Table 4). Lettuce and
266 watercress appeared the most sensitive species to the **F** treatments. Uliva digestate was more
267 detrimental than Fattoria on germination of all species assayed (Table 6). In presence of **LU**, no
268 germination was detected for lettuce and watercress and only a 50% of germination in presence of
269 the lowest **LU** concentration was observed for cucumber seeds. Increasing the **LU** concentrations,
270 the cucumber seed germination decreased accordingly, and no germination was observed with
271 100%**LU** (Table 6). **SU** was less detrimental than **LU** for all the three species. With the lowest **SU**
272 concentration, germinated only 40 % of lettuce and watercress and 59% of cucumber seeds.
273 Increasing the concentrations, the germination percentages decreased, much more for lettuce and
274 watercress than for cucumber. Also germination velocity, percentage of germination on each day,
275 and mean germination time were significantly affected by **SU** at all concentrations (Table 6). The

276 analysis of variance (Table 7) showed that the inhibitory effect on total germination of lettuce and
277 watercress was mainly due to the concentrations rather than to the type of fractions used.
278 Germination percentages of cucumber seeds were not affected by **F** digestate. Differently, **U**
279 digestate negatively affected TG of cucumber seeds and the effect was mainly due to the
280 concentrations rather than to the fractions used. The two digestates were responsible for the
281 significant changes on MGT (Table 7). In lettuce and watercress the effect was mainly due to the
282 fractions. In cucumber, the effect of **F** on MGT, was mostly dependent on the fraction and it was
283 much lower than that detected for lettuce and watercress (lower F-ratios). Differently, the effect of
284 Uliva, on cucumber MGT, was mainly due to the combinations of fraction x concentration in
285 comparison with changes induced by the two parameters individually considered.

286

287 *Enzyme activities, phenols and antioxidants*

288 In lettuce treated with the lowest concentration of **LF** no significant changes in enzyme activities in
289 respect to control were observed (Table 8). Increasing **LF** concentrations all enzyme activities were
290 inhibited. The lowest **SF** concentrations (10 and 25%) did not affect the enzyme activities, but
291 increasing its concentration, all the activities increased (Table 8). No activities were detected in
292 lettuce seeds treated with **LU**. Conversely, **SU** positively affected all the activities in a
293 concentration dependent manner. In watercress no enzyme activities were detected in seeds treated
294 with **LU** (Table 9). The two lowest concentrations of **SU** did not induce significant changes in the
295 enzymatic activities with respect to control, but when **SU** concentration increased all the activities
296 in watercress decreased. Differently, in cucumber **LU** and **SU** significantly increased the
297 antioxidant enzyme activities, linearly with the concentration (Table 10), while both Fattoria
298 fractions did not affect the enzyme activities even at the highest concentrations compared to control.
299 Regarding the non enzymatic antioxidants in lettuce and watercress, in presence of both liquid
300 fractions (Fattoria and Uliva), total antioxidant activity and total phenols were under the detection
301 limit (Table 11); the only exception was for seeds treated with **LF** at the lowest concentration,

302 where ToA and TP values were similar to the control. Conversely, in lettuce and watercress seeds,
303 increasing the concentrations of both solid fractions, the non enzymatic antioxidants increased
304 compared to control (Table 11). Cucumber showed a different trend, **LU** increased the
305 concentration of non enzymatic antioxidants with respect to the control (even if less than in
306 watercress and lettuce), except for the highest concentration that completely inhibited seed
307 germination. No significant differences with respect to the control were instead induced in the
308 amounts of ToA and TP by **SU**, **LF** and **SF** fractions at all concentrations (Table 11). It was found
309 a significant correlation between FDA and phenols of digestates and MGT (Table 12). A linear
310 inverse correlation was noted between the concentration of phenols and MGT in all the species
311 analyzed, while a linear positive correlation was observed between the amount of FDA and MGT
312 (Table 11).

313

314 **Discussion**

315 From an energetic point of view, our results evidenced that the two digesters differently fed,
316 produced the same amount of biogas with a high percentage of methane. Additionally, comparisons
317 between digested and undigested materials showed that the biomass have been transformed during
318 the digestion process and that the digestates have a higher content of plant-available nitrate,
319 ammonium, Ca, and Mg than indigested materials. Anaerobic digestion could therefore help
320 farmers to maximize the return of nutrients to the soil, reducing agricultural dependence from
321 inorganic fertilizer that are becoming increasingly expensive for their energy-intensive production
322 process, and responsible for the greenhouse-gas (GHG) emissions and water-pollution incidences
323 from agriculture. Even though olive and citrus wastes, which are used in a greater proportion to feed
324 the Uliva digester, contained major amounts of recalcitrant substances, such as lignocelluloses and
325 phenolic compounds [41] with the potential to reduce the activities of microorganisms [42-44],
326 unexpectedly, we didn't find an inverse relationship between the amount of biogas produced, and
327 the amount of phenols contained in the organic substrate utilized, as previously demonstrated by

328 Battista et al. [45]. This could be the result of having used a multi-component mixture of wastes in
329 such proportions as to allow the easily degradable compounds they contained to act as buffer, or
330 release important micro- and macro-nutrients able to increase bacteria number and activity, thereby
331 overcoming the bacteriostatic effects of the phenolic compounds present in the starting biomass.
332 Surprisingly, even though the biomass input had no effect on the quantity and quality of the biogas
333 produced, it had a great impact on the quality and composition of the digestates. The different
334 mixture of biomass input, affected the composition of digestates from an agronomic point of view,
335 conditioning the content of phenols, a class of compounds with adverse effects on plant growth and
336 soil microorganisms [46-47]. Digestate Fattoria that was found to be more suitable for agronomic
337 purpose, contained less phenols than uliva, probably for the high amount of active bacteria (derived
338 from the livestock manure, the main waste that fed Fattoria digester) which used phenols as carbon
339 source for their own metabolic needs [48] and [46]. This is confirmed by the data of FDA (higher in
340 F digestate) that is a measure of total microbial activity and a marker of total enzyme activity [25],
341 [49], [50-51]. In general, Fattoria with high nutrients, lower phenols and pollutant load, was less
342 phytotoxic than U, evidencing a strong direct relationship between phenol content and phytotoxicity.
343 In presence of LU the fraction by far richer in phenols, no watercress and lettuce seeds
344 germinated. Data on antioxidant system confirmed that U mainly at the high concentrations,
345 represented a stress factor for lettuce and watercress. Conversely, cucumber seeds were able to
346 germinate in presence of LU 50% and SU 100% but with a low germination percentage and speed.
347 Both fractions of U, most at the highest concentrations, were phytotoxic, and the seeds protected
348 themselves from the stressful conditions, activating the antioxidant system to scavenge the ROS for
349 completing their germination [52]. Traditionally, reactive oxygen species (ROSs) in plants are
350 considered as by-product of aerobic metabolism and also as cellular indicators of stress and signals
351 for the activation of stress-response and defense pathways. The major defense systems against ROS
352 injury are based mainly on enzymes and antioxidant compounds that remove ROSs [53] since the
353 production of ROSs under stress conditions may negatively affect seed metabolism and in turn the

354 whole germination process [54-58]. In our study, to correlate, the chemical composition and the
355 biological effects of the single fractions a significant inverse linear correlation was found among the
356 concentration of phenols, MGT and antioxidant system activation, in all the species analyzed, while
357 the amount and activity of bacteria present in the digestates and MGT were positively correlated.
358 Among the three species investigated watercress was the most negatively affected by the digestates
359 showing the minimum germination and the maximum activation of the antioxidative system, while
360 cucumber resulted the species that better answered to the amendment with the digestates. These
361 results confirm the hypothesis of Fuchs et al. [59] in which they suggested that for obtaining
362 positive results, it is important not only to take into account the chemical characteristics of the
363 digestate but also to use it by leveraging its species-specificity.

364

365 **Conclusion**

366 This study demonstrated that animal and recalcitrant agriculture wastes represent a great resource in
367 producing biogas with high methane percentage. The results evidenced that digestate composition is
368 strictly dependent on the amount and kind of wastes used, and on the ratio in which they are mixed.
369 It is incorrect to generalize on the use of digestate as organic fertilizer, but it is imperative to test
370 preliminarily the digestate phytotoxicity every time new mixtures of biomass are used to feed the
371 digesters. In this study, a specificity between the kind of digestate and plant species was really
372 evident. Additionally an increase in antioxidant compounds (ToA and TP) in crops treated with **LU**
373 and **SU** were also identified. Even though, the digestate richer in phenols reduced crop productivity,
374 at the same time it increased the antioxidant content in plants. Thus, if used as fertilizer, it may
375 represent, an additional resource for agriculture to produce food with nutraceutical values.
376 Numerous ongoing studies continue to evidence that antioxidant rich foods or antioxidant
377 supplements reduce the risk of chronic disease and promote wellness. Thus the cultivation of
378 species in lands amended with digestate may provide enormous environmental and economic

379 benefits increasing the green economy when the species and the optimal cultivation conditions are
380 identified. Therefore, AD should not only considered a source of renewable energy, waste
381 management system, pollution-abatement technology, but also an opportunity for providing value-
382 added byproducts.

383

384 **Conflict of interest statement**

385 The authors declare they have no competing financial interests

386

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397 **References**

398 [1] Houghton, J.: Global warming. *Reports on Progress in Physics*. 68, 1343–1356 (2005).

399 [2] Mazzanti M., Zoboli, R.: Waste Generation, Incineration and Landfill Diversion. De-coupling

400 Trends, Socio-Economic Drivers and Policy Effectiveness in the EU. 13th Coalition Theory

401 Network Workshop organised by the Fondazione Eni Enrico Mattei (FEEM), held in Venice, Italy

402 on 24-25 January 2008 31 pages.

403 [3] Kothari, R., Tyagi, V.V., Pathak, A.: Waste-to-energy: A way from renewable energy sources to

404 sustainable development. *Ren. Sust. En. Rev.*14 (2010) doi:10.1016/j.rser.2010.05.005.

- 405 [4] Hublin, A., Schneide, D.R., Džodan, J.: Utilization of biogas produced by anaerobic digestion of
406 agro-industrial waste: Energy, economic and environmental effects. *Waste Manage Res.* 32(7),
407 626–633 (2014).
- 408 [5] Tambone, F., Terruzzi, F., Scaglia, B., Adani, F.: Composting of the solid fraction of digestate
409 derived from pig slurry: Biological processes and compost properties. *Waste Manage* 35, 55–61
410 (2015).
- 411 [6] Formowitz, B., Fritz, M.: Biogas digestates as organic fertilizer in different crop rotations. 18th
412 European Biomass Conference Exhibition (2010) Lyon France.
- 413 [7] Rigby, H., Smith, S.R.: Nitrogen availability and indirect measurements of greenhouse gas
414 emissions from aerobic and anaerobic biowaste digestates applied to agricultural soils. *Waste*
415 *Manage* 33, 2641–2652 (2013).
- 416 [8] Mangwandi, C., Tao, L.J., Albadarin, A.B., Allen, S.J., Walker, G.M.: The variability in nutrient
417 composition of Anaerobic Digestate granules produced from high shear granulation. *Waste*
418 *Manage* 33, 33–42 (2013).
- 419 [9] Seadi, T.A.L., Lukehurst, C.: Quality management of digestate from biogas plants used as
420 fertilizer. In: IEA Bioenergy edited by: David Baxter task Leader, European Commission, Joint
421 Research Centre, UK. (2012).
- 422 [10] Battini, F., Agostini, A., Boulamanti, A.K., Giuntoli, J., Amaducci, S.: Mitigating the
423 environmental impacts of milk production via anaerobic digestion of manure: Case study of a
424 dairy farm in the Po Valley. *Sci. Total Environ.* 481, 196–208 (2014).
- 425 [11] Neubauer, A.: Convergence with EU Waste Policies. Short guide for ENP partners and Russia,
426 Policy Guide: Waste Policy 2008 34 pages. ISBN number 978-92-79-08286-3 Catalogue
427 number KH-30-08-208-EN-C.
- 428 [12] Peris-Moll, E.M., Juliá-Igual, J.F.: Effects of Regulation (EEC) 2078/92 on citrus growing in
429 Calabria (Italy) and the Region of Valencia (Spain). *Span. J. Agric. Res.* 3(1), 34–42 (2005).

- 430 [13] Sabiiti, E.N.: Utilising agricultural waste to enhance food security and conserve the
431 environment. *Afr. J. Food Agric. Nutr. Dev.* 11, 1–9 (2011).
- 432 [14] Steinfeld, H., Gerber, P., Wasenaar, T., Castel, V., Rosales, M., de Haan, C.: Livestock’s long
433 shadow. *Environment Issues and Options*. Rome: LEAD and FAO, (2006) 408 pages
434 http://www.virtualcentre.org/en/library/key_pub/longshad/A0701E00.htm
- 435 [15] Bentsen, N.S, Felby, C.: Biomass for energy in the European Union - a review of bioenergy
436 resource assessments. *Biotech. Biofuels.* 5, 1-10 (2012.) doi:10.1186/1754-6834-5-25.
- 437 [16] EEA (European Environment Agency). EU bioenergy potential from a resource-efficiency
438 perspective. In *Environmental Production*, Printed by Rosendahls-Schultz Grafisk-
439 Environmental Management Certificate: DS/EN ISO 14001: 2004. Luxembourg: Publications
440 Office of the European Union (2013) ISBN 978-92-9213-397-9; ISSN 1725-9177;
441 doi:10.2800/92247.
- 442 [17] Mathias, J.F.C.M.: Manure as a Resource: Livestock Waste Management from Anaerobic
443 Digestion, Opportunities and Challenges for Brazil. *Int. Food Agr. Manage Rev.* 17, 87–110
444 (2014).
- 445 [18] AFNOR: NF T Qualite’ de l’eau—Determination de la demande chimique en oxygene (DCO)
446 90-101 (2001).
- 447 [19] Nelson, D.W., Sommers, L.E.: Total carbon, organic carbon, and organic matter. In: A.L. Page,
448 R.H Miller, D.R. Keeney (Eds) *Methods of soil analysis*. pp. 539–579 American Society of
449 Agronomy, Madison, (1982).
- 450 [20] Bremner, J.M., Mulvaney, C.S.: Nitrogen-total. In: Page AL, Miller RH, Keeney DR. editors.
451 *Methods of soil analysis*. pp. 595–624 American Society of Agronomy, Madison, (1982).
- 452 [21] Bray, R.H., Kurtz, T.: Determination of total, organic and available forms of phosphorous in
453 soils. *Soil Sci* 59, 39–45(1945).

- 454 [22]. Sommer, S.G, Kjellerup, V., Kristjansen, O.: Determination of total ammonium nitrogen in pig
455 and cattle slurry: sample preparation and analysis. *Acta Agric Scand B Soil Plant Sci.* 42, 146–
456 15(2006).
- 457 [23] Box, J.D.: Investigation of the Folin–Ciocalteu reagent for the determination of polyphenolic
458 substances in natural waters, *Water Res.* 17, 511–525(1983).
- 459 [24] Kuiters, A.T., Denneman, C.A.J.: Water-soluble phenolic substances in soils under several
460 coniferous and deciduous tree species. *Soil Biol. Biochem.* 19, 765–769 (1987).
- 461 [25] Adam, G., Duncan, H.: Development of a sensitive and rapid method for the measurement of
462 total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.*
463 33, 943–951(2001).
- 464 [26] Kamara, A., Kamara, A., Mansaray, M.M, Sawyerr, P.A.: Effects of Biochar Derived from
465 Maize Stover and Rice Straw on the Germination of their seeds. *Am. J. Agr. For.* 2(6), 246–249
466 (2014).
- 467 [27] Solaimam, Z.M., Murphy, D.V., Abbott, L.K.: Biochars influence seed germination and early
468 growth of seedlings. *Plant Soil* 353, 273–287 (2012).
- 469 [28] Bargmann, J., Rillig, M.C., Buss, W., Kruse, A., Kuecke, M.: Hydrochar and Biochar Effects on
470 Germination of Spring Barley. *J. Agron. Crop Sci* 199(5), 360–373 (2013).
- 471 [29] Warman, P.R.:Evaluation of seed germination and growth test for assessing compost maturity.
472 *Compost Sci. Util.* 7, 33–37 (1999).
- 473 [30] Araujo, A.S.F., Monteiro, R.T.R.: Plant bioassays to asses toxicity of textile sludge compost.
474 *Sci. Agr.* 622, 86–290 (2005).
- 475 [31] Mitelut, A.C., Popa, M.E.: Seed germination bioassay for toxicity evaluation of different
476 composting biodegradable materials. *Rom. Biotech. Letters* 16, 121–129 (2011).
- 477 [32] Kader, M.A., Sutzi, J.C.: Effects of thermal and salt treatments during imbibition on germination
478 and seedling growth of sorghum at 42/19°C. *J. Agron. Crop Sci.* 190, 35–38 (2004).

- 479 [33] Kader, M.A.: A comparison of seed germination calculation formulae and the associated
480 interpretation of resulting data. Journal and Proceeding of the Royal Society of New South
481 Wales 138, 38: 65–75 (2005).
- 482 [34] Beaumont, F., Jouve H.M., Gagnon, J., Gaillard, J., Pelmont, J.: Purification and properties of a
483 catalase from potato tubers (*Solanum tuberosum*). Plant Sci. 72, 19–26 (1990).
- 484 [35] Nakano, Y, Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in
485 spinach chloroplasts. Plant Cell Physiol 22, 867–880 (1981).
- 486 [36] Gomes-Junior, R.A., Moldes, C.A., Delite, F.S., Pompeu, G.B., Gratão, P.L., Mazzafera, P.,
487 Lea, P.J., Azevedo, R.A.: Antioxidant metabolism of coffee cell suspension cultures in
488 response to cadmium. Chemosphere 65, 1330–1337 (2006).
- 489 [37] Panda, S.K., Chaudhury, I., Khan, M.H.: Heavy metals induce lipid peroxidation and affects
490 antioxidants in wheat leaves. Biol. Plant. 46, 289–294 (2003).
- 491 [38] Prieto, P., Pineda, M., Aguilar, M.: Spectrophotometric quantitation of antioxidant capacity
492 through the formation of a phosphomolybdenum complex: specific application to the
493 determination of Vitamin E. Anal. Biochem. 269, 337–341(1999).
- 494 [39] Prasad, K.N., Yang, B., Yang, S.Y., Chen, Y.L., Zhao, M.M., Ashraf, M.: Identification of
495 phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi
496 (*Litchi sinensis Sonn.*) seeds. Food Chem. 116, 1–7 (2009).
- 497 [40] Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-
498 phosphotungstic acid reagents. Am J Enol. Vit. 16, 144–158 (1965).
- 499 [41] Rincon, B., Sanchez, E., Raposo, F., Borja, R., Travieso, L., Martin, M.A., Martin, A.: Effect of
500 the organic loading rate on the performance of anaerobic acidogenic fermentation of two phase
501 olive mill solid residue. Waste Manage 28, 870–877 (2008).
- 502 [42] Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.P.: Towards new indicators for the prediction of
503 solid waste anaerobic digestion properties. Water Sci. Technol. 53, 233–241 (2006).

- 504 [43] Levén, L., Nyberg, K., Korkea-Aho, L., Schnürer, A.: Phenols in anaerobic digestion processes
505 and inhibition of ammonia oxidising bacteria (AOB) in soil. *Sci. Total Environ.* 364, 229-238
506 (2006).
- 507 [44] Obied, H.K., Song, Y., Faley, S., Loughlin, M., Rehman, A., Mailer, R., Masoul, T., Ayboola,
508 S.: Biophenols and antioxidant properties of Australian canola meal, *Journal of Agric. Food*
509 *Chem.* 38, 9176–9184 (2013).
- 510 [45] Battista, F., Fino, D., Ruggeri, B.: Polyphenols Concentration's Effect on the Biogas Production
511 by Wastes Derived from Olive Oil Production. *Chem. Eng. Trans.* 38, (2014) 373–378 DOI:
512 10.3303/CET1438063.
- 513 [46] Muscolo, A., Panuccio, M.R., Sidari, M.: Respiratory enzyme activities during germination of
514 *Pinus laricio* seeds treated with phenols extracted from different forest soils. *Plant Growth*
515 *Regul.* 35, 31–35 (2001).
- 516 [47] Muscolo, A., Sidari, M.: Seasonal fluctuations in soil phenolics of a coniferous forest: effects on
517 seed germination of different coniferous species. *Plant Soil* 284, 305–318 (2006).
- 518 [48] Chowdhury, M.A.Z., Mahin, A.A., Fakhruddin, A.N.M.: Degradation of phenol by
519 *Pseudomonas putida* when supplied as sole carbon source and in the presence of glucose.
520 *Bangladesh J. Microbiol.* 23, 29–33 (2006).
- 521 [49] Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G.: Comparison of microbial numbers and
522 enzymatic activities in surface soils and subsoils using various techniques. *Soil Biol. Biochem.*
523 34, 387–401(2002).
- 524 [50] Muscolo, A., Panuccio, M.R., Mallamaci, C., Sidari, M.: Biological indicators to assess short-
525 term soil quality changes in forest ecosystems. *Ecol. Ind.* 45, 416–423 (2014).
- 526 [51] Muscolo, A., Settineri, G., Attinà, E.: Early warning indicators of changes in soil ecosystem
527 functioning. *Ecol. Ind.* 48, 542–549 (2015).
- 528 [52] Romanova, E.V., Gins, M.S., Plushikov, V.G., Zargar, M.: Productivity and antioxidant activity
529 of plant *Brassica chinensis* L. *Int J Biosci* 4 (2014) 162-167. <http://www.innspub.net>

- 530 [53] Bailly, C.: Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* 14, 1–15
531 (2004).
- 532 [54] Solecka, D.: Role of phenylpropanoid compounds in plant responses to different stress factors.
533 *Acta Physiol. Plantarum* 19(3), 257–268 (1997). <http://dx.doi.org/10.1007/s11738-997-0001-1>
- 534 [55] Amarowicz, R., Weidner, S.: Biological activity of grapevine phenolic compounds, In: K.A.
535 Roubelakis-Angelakis (Eds.). *Grapevine molecular physiology and biotechnology*. Dordrecht:
536 Springer Netherlands 2009 pp. 389–405. <http://dx.doi.org/10.1007/978-90-481-2305-6>.
- 537 [56] Meiado, M.V., Albuquerque, LSC, Rocha, EA, Rojasarchiga, M, Leal, I.R.: Seed germination
538 responses of *Cereus jamacaru DC. ssp. jamacaru (Cactaceae)* to environmental factors. *Plant*
539 *Sp. Biol.* 25 (2010)120–128. DOI: 10.1111/j.1442-1984.2010.00274.
- 540 [57] Lattanzio, V., Cardinali, A., Linsalata, V.: Plant Phenolics: A Biochemical and Physiological
541 Perspective In: V. Cheynier, P. Sarni-Manchado, S. Quidean (Eds), *Recent advances in*
542 *polyphenol research*. Oxford 3 (1) (2012) pp 1-39 Wiley-Blackwell.
- 543 [58] Ribeiro, R.C., Matias, J.R., Pelacani, C.R., Dantas, B.F.: Activity of antioxidant enzymes and
544 proline accumulation in *Erythrina velutina* Willd. seeds subjected to abiotic stresses during
545 germination. *J. Seed Sci.* 36(2), 231–239 (2014).
- 546 [59] Fuchs, J.G., Berner, A., Mayer, J., Schleiss, K.: Concept for quality management to secure the
547 benefits of compost use for soil and plants International Symposium “Organic Matter
548 Management & Using Compost in Horticulture 4-7 April 2011, University of Adelaide,
549 Australia.
- 550

551 **Table 1.** Composition of biogas from Fattoria (F) and Uliva (U) plants.

Constituents	Units	Fattoria	Uliva
Biogas production	cm/h	440	450
Methane	Vol %	59	61
Ethane	Vol %	0	0
Propane	Vol %	0	0
Butane	Vol %	0	0
Pentane	Vol %	0	0
Carbon Dioxide	Vol %	41	39
Nitrogen	Vol %	0-2	0-2
Hydrogen	Vol %	0	0
Hydrogen Sulphide	ppm	~50	~50
Ammonia	ppm	~100	~100
Carbon monoxide	ppm	0	0
Volatile Organic Compounds	Vol %	0	0

552

553 **Table 2** Chemical and biological characteristics of biomass (ingestate) used to feed **Fattoria and**
 554 **Ulivo plants**. Values are means \pm SE (n=4). Different letters in the same **row** indicate significant
 555 differences $P \leq 0.05$

556

Parameters	Units	Ingestate Fattoria	Ingestate Ulivo
Total solids	%	40 ^b \pm 3	48 ^a \pm 2
Volatile Organic Compounds	Vol%	21 ^a \pm 2	13.5 ^b \pm 1
Moisture	%	80 \pm 4	85 ^b \pm 5
COD	mg/L	80000 ^b \pm 24	180000 ^a \pm 41
BOD	mg/L	25000 ^b \pm 34	50000 ^a \pm 33
Fluorescein diacetate hydrolysis	$\mu\text{g fluorescein g}^{-1} \text{ dm}$	1.1 ^a \pm 0.5	0.74 ^b \pm 0.05
Bacteria	CFU	90 x10 ^{3a} \pm 3	15 x10 ^{3b} \pm 1
Total phenols	mg/L	514 ^b \pm 6	1424 ^a \pm 4
Total oil	mg/L	400 ^b \pm 11	600 ^a \pm 9
pH		6.1 ^a \pm 0.4	5.5 ^a \pm 0.5
Conductivity	$\mu\text{S/cm}$	1640 ^a \pm 14	1326 ^b \pm 12
Total Carbon	% dm	144 ^a \pm 5	130 ^b \pm 3
Organic matter	% dm	248 ^a \pm 4	224 ^b \pm 4
Total nitrogen	% dm	6.0 ^b \pm 1	6.5 ^a \pm 2
C/N		24 ^a \pm 2	20 ^b \pm 3
K ⁺	mg/L	840 ^a \pm 5	340 ^b \pm 8
K ₂ O	mg/L	1340 ^a \pm 9	952 ^b \pm 7
P	mg/L	631 ^a \pm 8	560 ^b \pm 12
P ₂ O ₅	mg/L	1340 ^a \pm 47	1378 ^a \pm 38
NO ₃ ⁻	mg/L	112 ^b \pm 4	190 ^a \pm 7
NH ₄ ⁺	mg/L	149 ^a \pm 6	54 ^b \pm 6
Ca ⁺⁺	mg/L	1300 ^b \pm 14	1800 ^a \pm 15
Mg ⁺⁺	mg/L	149 ^b \pm 11	230 ^a \pm 13

557 **Table 3** Chemical and biological characteristics of solid and liquid digestate fractions from
 558 **Fattoria** Plant. Values are means \pm SE (n=4). Different letters in the same row indicate significant
 559 differences $P \leq 0.05$.

Parameters	Units	Liquid fraction	Solid fraction
Total solids	%	93 ^a \pm 5	75 ^b \pm 6
Volatile Organic Compounds	Vol%	63 ^b \pm 3	79 ^a \pm 5
Moisture	%	93 ^a \pm 5	75 ^b \pm 6
COD	mg/L	50000 \pm 121	-
BOD	mg/L	8500 \pm 12	-
Total phenols	mg/L	395 ^a \pm 12	325 ^b \pm 9
Total oil	mg/L	200 \pm 6	-
Fat (saponifiable)	mg/L	180 \pm 6	-
Total hydrocarbons	mg/L	33 \pm 1	-
pH		8.3 ^a \pm 0.6	8.4 ^a \pm 0.5
Fluorescein diacetate hydrolysis	$\mu\text{g fluorescein g}^{-1} \text{ dm}$	1.68 ^b \pm 0.5	2.45 ^a \pm 0.4
Bacteria	CFU	110 $\times 10^{3b}$ \pm 5	140 $\times 10^3$ ^a \pm 7
Conductibility	$\mu\text{S/cm}$	1879 ^{ab} \pm 10	1707 ^a \pm 11
Total Carbon	% dm	39.5 ^a \pm 4	43 ^a \pm 5
Organic matter	% dm	69 ^a \pm 2	74 ^a \pm 5
Total nitrogen	% dm	4.9 ^a \pm 2	5.3 ^a \pm 3
C/N		8.1 ^a \pm 3	8.1 ^a \pm 2
K ⁺	mg/L	480 ^b \pm 9	960 ^a \pm 8
K ₂ O	mg/L	576 ^b \pm 11	1152 ^a \pm 7
P	mg/L	290 ^b \pm 9	560 ^a \pm 12
P ₂ O ₅	mg/L	664 ^b \pm 16	1282 ^a \pm 11
NO ₃ ⁻	mg/L	140 ^b \pm 11	1500 ^a \pm 17
NH ₄ ⁺	mg/L	340 ^a \pm 12	30 ^b \pm 6
Ca ⁺⁺	mg/L	600 ^b \pm 11	900 ^a \pm 15
Mg ⁺⁺	mg/L	9 ^b \pm 2	100 ^a \pm 13

560

Total solid	%	8 ^b ± 3	40 ^a ± 6
Volatile substances	%	73 ^b ± 5	85 ^a ± 4
Moisture	%	92 ^a ± 8	60 ^b ± 6
COD	mg/L	94000 ± 16	-
BOD	mg/L	16000 ± 16	-
Total phenols	mg/L	940 ^a ± 12	502 ^b
Total oil	mg/L	230 ± 10	-
Fat (saponifiable)	mg/L	200 ± 13	-
Total hydrocarbons	mg/L	36 ± 6	-
pH		8.3 ^a ± 1	8.4 ^a ± 1.5
Fluorescein diacetate hydrolysis	µg fluorescein g ⁻¹ dm	1.18 ^a ± 0.5	2.15 ^a ± 0.6
Bacteria colonies	CFU	30 x 10 ^{3b} ± 2	55 x 10 ^{3a} ± 3
EC	µS/cm	1438 ^a ± 3	1298 ^b ± 5
Total Carbon	% dm	37.5 ^b ± 2	42.9 ^a ± 2.5
Organic matter	% dm	65 ^b ± 4	74 ^a ± 3
Total nitrogen	% dm	4.7 ^a ± 0.5	5.5 ^a ± 0.6
C/N		7.97 ^a ± 0.9	7.8 ^a ± 0.8
K ⁺	mg/L	660 ^a ± 15	300 ^b ± 11
K ₂ O	mg/L	792 ^a ± 8	360 ^b ± 5
P	mg/L	250 ^b ± 7	450 ^a ± 5
P ₂ O ₅	mg/L	573 ^b ± 12	1030 ^a ± 25
NO ₃ ⁻	mg/L	100 ^b ± 5	400 ^a ± 15
NH ₄ ⁺	mg/L	260 ^a ± 15	40 ^b ± 3
Ca ⁺⁺	mg/L	700 ^b ± 8	1400 ^a ± 17
Mg ⁺⁺	mg/L	150 ^a ± 7	50 ^b ± 5

561 **Table4** Chemical and biological characteristics of solid and liquid digestate fractions from **Uliva**

562 Plant. Values are means ± SE (n=4). Different letters in the same row indicate significant

563 differences P≤0.05.

564

565 **Table 5** Germination indexes: Total germination (TG); Coefficient of germination velocity (CVG), Germination Rate Index (GRI) and Mean
 566 Germination Time (MGT) determined for lettuce, watercress and cucumber seeds treated with different concentration of Fattoria digestate.

Treatment	Lettuce				Watercress				Cucumber			567
	TG %	CVG %	GRI %	MGT days	TG %	CVG %	GRI %	MGT days	TG %	CVG %	GRI %	MGT days
Control	100	27.8	27.7	3.9	100	26.7	27.5	3.8	100	28.9	27.8	3.4 ⁵⁶⁸ 570
LF 10%	80	27.5	27.4	4.1	80	26.0	27.1	4.0	100	28.2	27.9	3.5 ⁵⁶⁹ 571
LF 25%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.9	28.4	3.5 ⁵⁷⁰ 572
LF 50%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.6	27.9	3.5 ⁵⁷¹ 573
LF 100%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.5	27.6	3.5 ⁵⁷² 574
SF 10%	100	29.8*	29.9*	3.4*	100	29.6*	31.1*	3.3*	100	28.9	27.8	3.5 ⁵⁷³ 575
SF 25%	70	28.5*	29.6*	5.1*	70	28.8*	28.6*	3.4*	100	28.8	27.7	3.5 ⁵⁷⁴ 576
SF 50%	48	14.3**	16.8**	19***	55	14.0**	19.8**	10***	100	28.7	27.7	3.5 ⁵⁷⁵ 577
SF 100%	20	14.2**	2.28***	15***	18	14.0**	2.12***	16***	100	28.4	27.3	3.7 ⁵⁷⁶ 578

580 ***p<0.001; ** p<0.01; *p<0.05; *p<0.1

581

582

583 **Table 6** Germination indexes: Total germination (TG); Coefficient of germination velocity (CVG), Germination Rate Index (GRI)
 584 and Mean Germination Time (MGT) determined for lettuce, watercress and cucumber seeds treated with different concentration
 585 of liquid and solid fractions of Uliva digestate.

	Lettuce				Watercress				Cucumber			
Treatment	TG %	CVG %	GRI %	MGT days	GP %	CVG %	GRI %	MGT days	GP %	CVG %	GRI %	MGT days
Control	100	28.8	27.7	3.7	100	28.7	27.5	3.6	100	28.9	27.8	3.4
LU 10%	nd	nd	nd	nd	nd	nd	nd	nd	50	26.5*	23.0**	4.4*
LU 25%	nd	nd	nd	nd	nd	nd	nd	nd	29	24.6**	20.8**	4.8*
LU 50%	nd	nd	nd	nd	nd	nd	nd	nd	10	15.5*	15.3**	30***
LU 100%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SU 10%	40	25.8*	21.4**	4.2*	35	25.6*	21.1**	4.5*	59	26.8*	23.4**	4.0*
SU 25%	20	19.6**	6.6***	5.1*	18	19.8**	6.4***	5.4*	35	21.6**	21.8**	4.4*
SU 50%	15	14.3**	0.28***	35***	10	14.0**	0.28***	35***	20	19.7*	18.6***	4.9*
SU 100%	8	14.2**	0.28***	35***	5	14.0**	0.28***	35***	10	15.7*	15.4**	28***

600 ***p<0.001; ** p<0.01; *p<0.05; *p<0.1

601 **Table7** Analysis of variance of different treatments of Fattoria and Uliva digestate fractions (solid and
 602 liquid) on total germination (TG) and mean germination time (MGT) of seeds of lettuce, watercress and
 603 cucumber

	Fattoria					
	Lettuce		Watercress		Cucumber	
	TG	MGT	TG	MGT	TG	MGT
R ²	0.999	0.995	1.00	0.995	0.437	0.784
Source of Variance: <i>F-ratio</i>						
Concentrations	5478.28***	183.53***	8740.86***	199,631***	n.s	7.00**
Fractions	4150.21***	1382.25***	6981.82***	1564.50***	n.s	30.73***
Conc x Fractions	647.66***	428.45***	1125.23***	472.95***	n.s	3.46*
	Uliva					
	Lettuce		Watercress		Cucumber	
	TG	MGT	TG	MGT	TG	MGT
R ²	0.999	0.998	1.00	0.999	0.999	0.998
Source of Variance: <i>F-ratio</i>						
Concentrations	4513.91***	378.47***	19193.37***	638.18***	6442.46***	732.76***
Fractions	955.862***	1914.50***	2376.56***	3053.74***	260.12***	10.11***
Conc x Fractions	157.61***	454,16***	487.19***	745.84***	21.04***	1554.10***

604 ***p<0.001; ** p<0.01; *p<0.05; *p<0.1

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606 **Table 8** Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase,
607 (POX) and catalase (CAT) enzymes in 7 day old lettuce seedlings under Solid (SU) and Liquid (LU)
608 Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means
609 \pm SE (n=4). Different letters in the same column indicate significant differences $P \leq 0.05$.

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Treatment	APX U mg ⁻¹ prot	GR U mg ⁻¹ prot	POX U mg ⁻¹ prot	CAT U mg ⁻¹ prot
Control	1.41 \pm 0.2 ^d	0.05 \pm 0.03 ^e	0.26 \pm 0.1 ^f	25 \pm 2.0 ^f
LU 10%	nd	nd	nd	nd
LU 25%	nd	nd	nd	nd
LU 50%	nd	nd	nd	nd
LU 100%	nd	nd	nd	nd
SU 10%	2.92 \pm 0.2 ^b	0.11 \pm 0.02 ^d	0.29 \pm 0.2 ^f	56 \pm 2.1 ^c
SU 25%	3.44 \pm 0.3 ^b	0.16 \pm 0.02 ^c	0.56 \pm 0.3 ^d	99 \pm 4.5 ^b
SU 50%	4.40 \pm 0.5 ^a	0.20 \pm 0.01 ^b	1.10 \pm 0.2 ^b	107 \pm 3.5 ^b
SU 100%	4.97 \pm 0.4 ^a	0.25 \pm 0.03 ^a	1.26 \pm 0.1 ^a	135 \pm 4.0 ^a
LF 10%	1.98 \pm 0.1 ^c	0.08 \pm 0.03 ^e	0.28 \pm 0.1 ^f	29 \pm 1.5 ^e
LF 25%	nd	nd	nd	nd
LF 50%	nd	nd	nd	nd
LF 100%	nd	nd	nd	nd
SF 10%	1.55 \pm 0.2 ^d	0.06 \pm 0.01 ^e	0.27 \pm 0.2 ^f	24 \pm 2.1 ^f
SF 25%	1.76 \pm 0.2 ^d	0.07 \pm 0.02 ^e	0.30 \pm 0.3 ^f	29 \pm 2.0 ^f
SF 50%	2.40 \pm 0.3 ^b	0.12 \pm 0.01 ^d	0.39 \pm 0.2 ^e	37 \pm 3.1 ^d
SF 100%	3.22 \pm 0.5 ^b	0.16 \pm 0.02 ^c	1.06 \pm 0.1 ^c	35 \pm 2.0 ^d

611 **Table 9** Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase,
612 (POX) and catalase (CAT) enzymes in 7 day old watercress seedlings under Solid (SU) and Liquid (LU)
613 Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means
614 \pm SE (n=4). Different letters in the same column indicate significant differences $P \leq 0.05$.

615

Treatment	APX U mg ⁻¹ prot	GR U mg ⁻¹ prot	POX U mg ⁻¹ prot	CAT U mg ⁻¹ prot
Control	2.21 \pm 0.5 ^c	0.09 \pm 0.03 ^d	0.32 \pm 0.1 ^d	27 \pm 2.0 ^f
LU 10%	nd	nd	nd	nd
LU 25%	nd	nd	nd	nd
LU 50%	nd	nd	nd	nd
LU 100%	nd	nd	nd	nd
SU 10%	2.42 \pm 0.2 ^c	0.16 \pm 0.02 ^b	0.33 \pm 0.2 ^f	67 \pm 2.1 ^c
SU 25%	2.74 \pm 0.3 ^c	0.16 \pm 0.02 ^b	0.36 \pm 0.3 ^d	108 \pm 4.5 ^b
SU 50%	4.00 \pm 0.5 ^a	0.25 \pm 0.01 ^a	1.4 \pm 0.2 ^b	109 \pm 3.5 ^b
SU 100%	4.38 \pm 0.4 ^a	0.28 \pm 0.03 ^a	1.76 \pm 0.1 ^a	144 \pm 4.0 ^a
LF 10%	1.77 \pm 0.4 ^c	0.06 \pm 0.03 ^d	0.33 \pm 0.1 ^d	27 \pm 2.5 ^d
LF 25%	nd	nd	nd	nd
LF 50%	nd	nd	nd	nd
LF 100%	nd	nd	nd	nd
SF 10%	1.23 \pm 0.2 ^d	0.07 \pm 0.01 ^d	0.29 \pm 0.2 ^d	27 \pm 2.1 ^d
SF 25%	1.76 \pm 0.2 ^c	0.09 \pm 0.02 ^d	0.31 \pm 0.3 ^d	31 \pm 2.0 ^d
SF 50%	3.00 \pm 0.3 ^b	0.15 \pm 0.01 ^c	0.47 \pm 0.2 ^e	33 \pm 3.1 ^d
SF 100%	3.42 \pm 0.5 ^a ^b	0.19 \pm 0.02 ^b	0.98 \pm 0.1 ^c	33 \pm 2.0 ^d

616 **Table 10** Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase,
617 (POX) and catalase (CAT) enzymes in 7 day old cucumber seedlings under Solid (SU) and Liquid (LU)
618 Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means \pm
619 SE (n=4). Different letters in the same column indicate significant differences $P \leq 0.05$.

Treatment	APX U mg ⁻¹ prot	GR U mg ⁻¹ prot	POX U mg ⁻¹ prot	CAT U mg ⁻¹ prot
Control	3.34 \pm 0.5 ^b	0.11 \pm 0.01 ^c	2.5 \pm 0.1 ^b	31 \pm 2.0 ^e
LU 10%	4.10 \pm 0.5 ^a	0.15 \pm 0.01 ^b	5.4 \pm 0.7 ^a	47 \pm 2.0 ^d
LU 25%	4.33 \pm 0.5 ^a	0.17 \pm 0.01 ^b	6.1 \pm 0.5 ^a	56 \pm 2.0 ^c
LU 50%	5.44 \pm 0.7 ^a	0.28 \pm 0.01 ^a	6.6 \pm 1.0 ^a	61 \pm 2.0 ^b
LU 100%	nd	nd	nd	nd
SU 10%	3.82 \pm 0.2 ^{ab}	0.16 \pm 0.02 ^b	5.1 \pm 0.6 ^a	37 \pm 2.1 ^e
SU 25%	3.94 \pm 0. ^{ab}	0.19 \pm 0.03 ^b	6.0 \pm 0.9 ^a	48 \pm 4.5 ^b
SU 50%	4.55 \pm 0.5 ^a	0.26 \pm 0.04 ^a	6.3 \pm 0.5 ^a	69 \pm 3.5 ^a
SU 100%	5.38 \pm 0.4 ^a	0.29 \pm 0.04 ^a	7.4 \pm 1.2 ^a	74 \pm 4.0 ^a
LF 10%	2.77 \pm 0.4 ^b	0.09 \pm 0.03 ^c	2.3 \pm 0.5 ^b	26 \pm 1.5 ^e
LF 25%	2.94 \pm 0.5 ^b	0.08 \pm 0.02 ^c	2.4 \pm 0.3 ^b	31 \pm 2.0 ^e
LF 50%	2.78 \pm 0.7 ^b	0.11 \pm 0.03 ^c	2.5 \pm 0.5 ^b	33 \pm 3.1 ^e
LF 100%	3.04 \pm 0.5 ^b	0.10 \pm 0.03 ^c	2.3 \pm 0.5 ^b	33 \pm 2.0 ^e
SF 10%	2.54 \pm 0.7 ^b	0.07 \pm 0.01 ^c	2.1 \pm 0.2 ^b	30 \pm 2.1 ^e
SF 25%	2.49 \pm 0.65 ^b	0.08 \pm 0.02 ^c	2.3 \pm 0.4 ^b	31 \pm 2.0 ^e
SF 50%	2.77 \pm 0.4 ^b	0.11 \pm 0.03 ^c	2.2 \pm 0.2 ^b	30 \pm 3.1 ^e
SF 100%	3.01 \pm 0.5 ^b	0.09 \pm 0.02 ^c	2.4 \pm 0.3 ^b	31 \pm 2.0 ^e

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623 **Table 11** Total Antioxidant Activity (ToA, $\mu\text{mol } \alpha\text{-tocopherol/ g FW}$) and Total Phenols (TP, μg
624 TAET/g DW) in 7 day old seedlings of lettuce, watercress and cucumber with Solid (SU) and Liquid
625 (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria digestates at different concentrations. Values are
626 means \pm SE (n=4). Different letters in the same column indicate significant differences $P \leq 0.05$.

Treatment	Lettuce		Watercress		Cucumber	
	ToA	TP	ToA	TP	ToA	TP
Control	0.65 \pm 0.02 ^c	209 \pm 10 ^d	0.73 \pm 0.02 ^c	231 \pm 11 ^b	0.77 \pm 0.01 ^c	244 \pm 14 ^c
LU 10%	nd	nd	nd	nd	1.91 \pm 0.10 ^b	555 \pm 25 ^b
LU 25%	nd	nd	nd	nd	2.62 \pm 0.03 ^a	521 \pm 10 ^b
LU 50%	nd	nd	nd	nd	2.99 \pm 0.02 ^a	625 \pm 20 ^a
LU 100%	nd	nd	nd	nd	nd	nd
SU 10%	2.06 \pm 0.4 ^b	275 \pm 10 ^c	3.13 \pm 0.15 ^{ab}	285 \pm 10 ^b	1.80 \pm 0.04 ^b	223 \pm 22 ^c
SU 25%	2.91 \pm 0.9 ^b	299 \pm 8 ^b	3.55 \pm 0.4 ^a	317 \pm 8 ^b	2.44 \pm 0.02 ^a	243 \pm 18 ^c
SU 50%	3.39 \pm 0.2 ^a	345 \pm 10 ^a	3.97 \pm 0.2 ^a	356 \pm 12 ^b	2.68 \pm 0.03 ^a	229 \pm 19 ^c
SU 100%	3.94 \pm 0.5 ^a	367 \pm 12 ^a	4.43 \pm 0.5 ^a	398 \pm 15 ^a	2.84 \pm 0.05 ^a	233 \pm 20 ^c
LF 10%	0.85 \pm 0.01 ^c	223 \pm 10 ^d	0.75 \pm 0.08 ^c	256 \pm 13	0.79 \pm 0.01 ^c	234 \pm 16 ^c
LF 25%	nd	nd	nd	nd	0.87 \pm 0.01 ^c	235 \pm 15 ^c
LF 50%	nd	nd	nd	nd	0.84 \pm 0.01 ^c	241 \pm 14 ^c
LF 100%	nd	nd	nd	nd	0.79 \pm 0.01 ^c	245 \pm 13 ^c
SF 10%	0.69 \pm 0.04 ^c	219 \pm 9 ^d	1.01 \pm 0.09 ^c	233 \pm 12 ^b	0.78 \pm 0.01 ^c	232 \pm 11 ^c
SF 25%	1.77 \pm 0.4 ^b	224 \pm 10 ^d	1.33 \pm 0.12 ^d	243 \pm 6 ^b	0.67 \pm 0.01 ^c	236 \pm 14 ^c
SF 50%	2.40 \pm 0.9 ^b	305 \pm 10 ^b	2.24 \pm 0.5 ^b	277 \pm 15 ^a	0.88 \pm 0.01 ^c	247 \pm 11 ^c
SF 100%	3.10 \pm 0.2 ^a	317 \pm 9 ^b	2.97 \pm 0.7 ^b	299 \pm 10 ^a	0.97 \pm 0.01 ^c	249 \pm 10 ^c

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629 **Table 12** Correlation coefficient between fluorescein diacetate hydrolysis (FDA) and total phenol (TP)
 630 on mean germination time (MGT) of lettuce, watercress and cucumber seeds.

		Fattoria					
		Lettuce		Watercress		Cucumber	
Fractions		SF	LF	SF	LF	SF	LF
FDA	r	0.957***	0.872*	0.959***	0.795	0.657**	0.696**
	R ²	0.915	0.761	0.921	0.631	0.432	0.485
TP	r	0.783**	0.901*	0.782***	0.837*	0.586*	0.693**
	R ²	0.613	0.811	0.611	0.700	0.344	0.480
		Uliva					
		Lettuce		Watercress		Cucumber	
Fractions		SU	LU	SU	LU	SU	LU
FDA	r	0.885***	-	0.882***	-	0.902***	0.900***
	R ²	0.783	-	0.778	-	0.813	0.810
TP	r	0.883***	-	0.880***	-	0.903***	0.899***
	R ²	0.780	-	0.775	-	0.816	0.808

631 ***p<0.001; ** p<0.01; *p<0.05; *p<0.1

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