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

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Abstract

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

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

Introduction

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

be considered a competitive process for reducing the rate of climate change and global warming managing biodegradable waste streams to produce renewable energy and nearly stable residue (digestate), in a sustainable way [3-5].[3-5]. The energy produced in the form of biogas, is a mixture of methane (45-75%), carbon dioxide (25-55%) and minor amounts of H_2S and H_2 and the actual proportion is dependent on the feedstock (substrate) used, and on the processes employed. While biogas represents an ascertained useful source of renewable energy, the digestate ever-increasing production induces problems related to its sustainable disposal. Consequently, research on agriculture valorization routes to reduce its environmental impact and to improve the economical profitability of AD plants are of great interest [6]. Depending on their chemical features, some digestates can have negative impact on environment or on plant growth [7], while some others may influence soil fertility and plant health positively. However, a digestate cannot be considered positive or negative *in toto*, therefore it is necessary to chemically and biologically characterize each digestate for finding an adequate utilization. Despite all, the use of digestate as fertiliser is legally limited in many countries due to unfamiliarity of the product and insufficient confidence in its quality and safety [8]. Quality assurance is an important prerequisite for increasing market confidence in digestate and for enhancing its use as fertilizer [9]. In many European Union Nations, anaerobic digestion technologies and processes are a widely accepted practice that aims to increase the profitability of dairy farmers and the food processing industry by utilizing organic wastes for better meeting the needs of environmental regulators [10]. Currently, in Italy, issues such as demand for renewable energy, landfill tax on organic wastes, demand for organic fertilizer, pollution of the environment and legislation relating to the treatment and disposal of organic wastes are all important factors influencing investments in AD [11]. Calabria (Southern Italy) is an agricultural land with predominant production of citrus, oil and livestock [12] to produce milk and cheese. Agriculture wastes and livestock manures are highly polluting and difficult to dispose of, with a high cost for farmers [13]. Thus, their anaerobic digestion could be a reliable way to use refuse as resource producing economic benefit [14-17]. This research, in cooperation with two

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

Materials and Methods

Biogas plants: process temperature and retention time

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

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

HRT [h or days] = Digester volume [m^3] / the influent flow rate [m^3/h or days].

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

Fattoria: process temperature: 40 °C, pH 7.8, total volume of the two digesters: 7500 m^3 (2500 DIG.1 + 5000 DIG.2), total volume loaded per day: 120 m^3/ day, hydraulic retention time (HRT): 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C.

Uliva: process temperature: 40 °C, pH 8.0, total volume of the two digesters: 7420 m^3 (3180 DIG.1 + 4240 DIG.2), total volume loaded per day: 120 m^3/day, hydraulic retention time (HRT) 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C.

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

Chemical analysis

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

127 Exchangeable K was extracted with 1 M NH_4OAc, and determined using a flame-photometer. The
128 NO_3–N was measured using a nitrate-ion selective electrode (USEPA, 2011), while NH_4–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 O_2 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 Titrando-Dosino device);
136 total water-soluble phenols were measured by using the Folin–Ciocalteau 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 cm^3 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 $CVG\ (\%\ day^{-1}) = \sum Ni / \sum (NiTi) \times 100,$

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

169 $MGT\ (day) = \sum (NiTi) / \sum Ni$

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 H_2O_2 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 µL enzyme extract and 5 µL H_2O_2 [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 µM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2 and
190 50 µL enzyme extract. The reaction was started by the addition of H_2O_2 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 $mM^{-1}cm^{-1}$)
193 GR activity was assayed spectrophotometrically at 30 ºC 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 µL 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 µL

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

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

Total antioxidant capacity determination

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

Total phenolic content determination

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

Statistical analysis

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

Results

Biogas and Digestate composition

No differences in the biogas composition between the two plants were observed (Table 1). In both plants, the biogas production reached 440-450 m^3/h with a methane content of ~60% (Table 1).

Both digestates had higher ammonium (NH_4^+) to total nitrogen (N) ratios, decreased OM, total and organic carbon (C) contents, reduced biological oxygen (O_2) demands (factor), elevated pH values, smaller carbon to nitrogen ratios (C:N ratios), and a greater amount of nutrients than the respective input materials (ingestate) (Table 2)

The two biogas digestates were chemically and qualitatively different one from the other. Fattoria (**F)** had less total phenols, lower COD and BOD, but greater amount of K^+, P, NH_4^+, and NO_3^- than Uliva (**U)** (Tables 3-4), **U** contained more Mg^{++} and Ca^{++}. From a biological point of view, **F** had a greater amount of bacteria, than **U**. Apart from the differences observed between the two digestates, we detected chemical and biological differences, between the solid and liquid fractions of the same digestate (Tables 3-4). In **Fattoria,** the solid fraction had less total phenols, total oil, saponifiable fat and total hydrocarbon than the liquid one. Additionally the **SF** had greater amounts of bacteria, K^+, P, Mg^{++} and Ca^{++} and NO_3^- than **LF (**Table 3**)**. In uliva digestate, the solid fraction had a lower pollution load (BOD and COD) and contained less total phenols, total oil, saponifiable fat and total

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

Seed germination

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

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

Enzyme activities, phenols and antioxidants

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

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

Discussion

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

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

Conclusion

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

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

Conflict of interest statement

The authors declare they have no competing financial interests

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References

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

[2] Mazzant,i M., Zoboli, R.: Waste Generation, Incineration and Landfill Diversion. De-coupling Trends, Socio-Economic Drivers and Policy Effectiveness in the EU. 13th Coalition Theory Network Workshop organised by the Fondazione Eni Enrico Mattei (FEEM), held in Venice, Italy on 24-25 January 2008 31 pages.

[3] Kothari, R., Tyagi, V.V., Pathak, A.: Waste-to-energy: A way from renewable energy sources to sustainable development. Ren. Sust. En. Rev.14 (2010) doi:10.1016/j.rser.2010.05.005.

[4] Hublin, A., Schneide, D.R., Džodan, J.: Utilization of biogas produced by anaerobic digestion of agro-industrial waste: Energy, economic and environmental effects. Waste Manage Res. 32(7), 626–633 (2014).

[5] Tambone, F., Terruzzi, F., Scaglia, B., Adani, F.: Composting of the solid fraction of digestate derived from pig slurry: Biological processes and compost properties. Waste Manage 35, 55–61 (2015).

[6] Formowitz, B., Fritz, M.: Biogas digestates as organic fertilizer in different crop rotations.18th European Biomass Conference Exhibition (2010) Lyon France.

[7] Rigby, H., Smith, S.R.: Nitrogen availability and indirect measurements of greenhouse gas emissions from aerobic and anaerobic biowaste digestates applied to agricultural soils. Waste Manage 33, 2641–2652 (2013).

[8] Mangwandi, C., Tao, L.J., Albadarin, A.B., Allen, S.J., Walker, G.M.: The variability in nutrient composition of Anaerobic Digestate granules produced from high shear granulation. Waste Manage 33, 33–42 (2013).

[9] Seadi, T.A.L., Lukehurst, C.: Quality management of digestate from biogas plants used as fertilizer. In: IEA Bioenergy edited by: David Baxter task Leader, European Commission, Joint Research Centre, UK. (2012).

[10] Battini, F., Agostini, A., Boulamanti, A.K., Giuntoli, J., Amaducci, S.: Mitigating the environmental impacts of milk production via anaerobic digestion of manure: Case study of a dairy farm in the Po Valley. Sci. Total Environ. 481, 196-208 (2014).

[11] Neubauer, A.: Convergence with EU Waste Policies. Short guide for ENP partners and Russia, Policy Guide: Waste Policy 2008 34 pages. ISBN number 978-92-79-08286-3 Catalogue number KH-30-08-208-EN-C.

[12] Peris-Moll, E.M., Juliá-Igual, J.F.: Effects of Regulation (EEC) 2078/92 on citrus growing in Calabria (Italy) and the Region of Valencia (Spain). Span. J. Agric. Res. 3(1), 34–42 (2005).

[13] Sabiiti, E.N.: Utilising agricultural waste to enhance food security and conserve the environment. Afr. J. Food Agric. Nutr. Dev. 11, 1–9 (2011).

[14] Steinfeld, H., Gerber, P., Wasenaar, T., Castel, V., Rosales, M., de Haan, C.: Livestock's long shadow. Environment Issues and Options. Rome: LEAD and FAO, (2006) 408 pages http://www.virtualcentre.org/en/library/key_pub/longshad/A0701E00.htm

[15] Bentsen, N.S, Felby, C.: Biomass for energy in the European Union - a review of bioenergy resource assessments. Biotech. Biofuels. 5, 1-10 (2012.) doi:10.1186/1754-6834-5-25.

[16] EEA (European Environment Agency). EU bioenergy potential from a resource‐efficiency perspective. In Environmental Production, Printed by Rosendahls-Schultz Grafisk-Environmental Management Certificate: DS/EN ISO 14001: 2004. Luxembourg: Publications Office of the European Union (2013) ISBN 978-92-9213-397-9; ISSN 1725-9177; doi:10.2800/92247.

[17] Mathias, J.F.C.M.: Manure as a Resource: Livestock Waste Management from Anaerobic Digestion, Opportunities and Challenges for Brazil. Int. Food Agr. Manage Rev. 17, 87–110 (2014).

[18] AFNOR: NF T Qualite´ de l'eau—Determination de la demande chimique en oxygene (DCO) 90-101 (2001).

[19] Nelson, D.W., Sommers, L.E.: Total carbon, organic carbon, and organic matter. In: A.L. Page, R.H Miller, D.R. Keeney (Eds) Methods of soil analysis. pp. 539–579 American Society of Agronomy, Madison, (1982).

[20] Bremner, J.M., Mulvaney, C.S.: Nitrogen-total. In: Page AL, Miller RH, Keeney DR. editors. Methods of soil analysis. pp. 595–624 American Society of Agronomy, Madison, (1982).

[21] Bray, R.H., Kurtz, T.: Determination of total, organic and available forms of phosphorous in 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–Ciocalteau 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).

[33] Kader, M.A.: A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales 138, 38: 65–75 (2005).

[34] Beaumont, F., Jouve H.M., Gagnon, J., Gaillard, J., Pelmont, J.: Purification and properties of a catalase from potato tubers (*Solanum tuberosum*). Plant Sci. 72, 19–26 (1990).

[35] Nakano, Y, Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22, 867–880 (1981).

[36] Gomes-Junior, R.A., Moldes, C.A., Delite, F.S., Pompeu, G.B., Gratão, P.L., Mazzafera, P., Lea, P.J., Azevedo, R.A.: Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. Chemosphere 65, 1330–1337 (2006).

[37] Panda, S.K., Chaudhury, I., Khan, M.H.: Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves. Biol. Plant. 46, 289–294 (2003).

[38] Prieto, P., Pineda, M., Aguilar, M.: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal. Biochem. 269, 337–341(1999).

[39] Prasad, K.N., Yang, B., Yang, S.Y., Chen, Y.L., Zhao, M.M., Ashraf, M.: Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinensis Sonn*.) seeds. Food Chem. 116, 1–7 (2009).

[40] Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol. Vit. 16, 144–158 (1965).

[41] Rincon, B., Sanchez, E., Raposo, F., Borja, R., Travieso, L., Martin, M.A., Martin, A.: Effect of the organic loading rate on the performance of anaerobic acidogenic fermentation of two phase olive mill solid residue. Waste Manage 28, 870–877 (2008).

[42] Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.P.: Towards new indicators for the prediction of 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, Rojasaréchiga, 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 ± SE (n=4). Different letters in the same row indicate significant
555 differences P≤0.05

556

Parameters	Units	Ingestate Fattoria	Ingestate Ulivo
Total solids	%	40^{b} ± 3	48^{a} ± 2
Volatile Organic Compounds	Vol%	21^{a} ± 2	13.5^{b} ±1
Moisture	%	80± 4	85^{b} ± 5
COD	mg/L	80000^{b} ± 24	180000^{a} ± 41
BOD	mg/L	25000^{b} ± 34	50000^{a} ± 33
Fluorescein diacetate hydrolysis	µg fluorescein g^{-1} dm	1.1^{a} ± 0.5	0.74^{b} ± 0.05
Bacteria	CFU	90 $x10^{3a}$ ±3	15 $x10^{3b}$ ±1
Total phenols	mg/L	514^{b} ± 6	1424^{a} ± 4
Total oil	mg/L	400^{b} ± 11	600^{a} ± 9
pH		6.1^{a} ± 0.4	5.5^{a} ± 0.5
Conductivity	µS/cm	1640^{a} ± 14	1326^{b} ± 12
Total Carbon	% dm	144^{a} ± 5	130^{b} ± 3
Organic matter	% dm	248^{a} ±4	224^{b} ± 4
Total nitrogen	% dm	6.0^{b} ±1	6.5^{a} ± 2
C/N		24^{a} ± 2	20^{b} ± 3
K^{+}	mg/L	840^{a} ± 5	340^{b} ± 8
K_20	mg/L	1340^{a} ± 9	952^{b} ± 7
P	mg/L	631^{a} ± 8	560^{b} ±12
P_20_5	mg/L	1340^{a} ± 47	1378^{a} ±38
NO_3^{-}	mg/L	112^{b} ± 4	190^{a} ± 7
NH_4^{+}	mg/L	149^{a} ± 6	54^{b} ± 6
Ca^{++}	mg/L	1300^{b} ± 14	1800^{a} ± 15
Mg^{++}	mg/L	149^{b} ± 11	230^{a} ± 13

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

Parameters	Units	Liquid fraction	Solid fraction
Total solids **Parameters**	% **Units**	7 [b] ± 2 **Liquid fraction**	25 [a] ± 4 **Solid fraction**
Volatile Organic Compounds	Vol%	63 [b] ± 3	79 [a] ±5
Moisture	%	93 [a] ± 5	75 [b] ± 6
COD	mg/L	50000 ±121	-
BOD	mg/L	8500 ± 12	-
Total phenols	mg/L	395 [a] ± 12	325 [b] ± 9
Total oil	mg/L	200 ± 6	-
Fat (saponifiable)	mg/L	180 ± 6	-
Total hydrocarbons	mg/L	33 ± 1	-
pH		8.3 [a] ± 0.6	8.4 [a] ± 0.5
Fluorescein diacetate hydrolysis	µg fluorescein g^{-1} dm	1.68 [b] ± 0.5	2.45 [a] ± 0.4
Bacteria	CFU	110 $x10^{3b}$ ± 5	140 $x10^{3}$ [a] ±7
Conductibility	µS/cm	1879 [ab] ± 10	1707 [a] ± 11
Total Carbon	% dm	39.5 [a] ± 4	43 [a] ± 5
Organic matter	% dm	69 [a] ± 2	74 [a] ± 5
Total nitrogen	% dm	4.9 [a] ± 2	5.3 [a] ± 3
C/N		8.1 [a] ± 3	8.1 [a] ± 2
K^+	mg/L	480 [b] ± 9	960 [a] ± 8
K_20	mg/L	576 [b] ± 11	1152 [a] ± 7
P	mg/L	290 [b] ± 9	560 [a] ±12
P_20_5	mg/L	664 [b] ± 16	1282 [a] ±11
NO_3^-	mg/L	140 [b] ± 11	1500 [a] ± 17
NH_4^+	mg/L	340 [a] ± 12	30 [b] ± 6
Ca^{++}	mg/L	600 [b] ± 11	900 [a] ± 15
Mg^{++}	mg/L	9 [b] ± 2	100 [a] ± 13

560

Total solid	%	$8^{b} \pm 3$	$40^{a} \pm 6$
Volatile substances	%	$73^{b} \pm 5$	$85^{a} \pm 4$
Moisture	%	$92^{a} \pm 8$	$60^{b} \pm 6$
COD	mg/L	94000 ± 16	-
BOD	mg/L	16000 ± 16	-
Total phenols	mg/L	$940^{a} \pm 12$	502^{b}
Total oil	mg/L	230 ± 10	-
Fat (saponifiable)	mg/L	200 ± 13	-
Total hydrocarbons	mg/L	36 ± 6	-
pH		$8.3^{a} \pm 1$	$8.4^{a} \pm 1.5$
Fluorescein diacetate hydrolysis	µg fluorescein g^{-1} dm	$1.18^{a} \pm 0.5$	$2.15^{a} \pm 0.6$
Bacteria colonies	CFU	$30 \times 10^{3\,b} \pm 2$	$55 \times 10^{3\,a} \pm 3$
EC	µS/cm	$1438^{a} \pm 3$	$1298^{b} \pm 5$
Total Carbon	% dm	$37.5^{b} \pm 2$	$42.9^{a} \pm 2.5$
Organic matter	% dm	$65^{b} \pm 4$	$74^{a} \pm 3$
Total nitrogen	% dm	$4.7^{a} \pm 0.5$	$5.5^{a} \pm 0.6$
C/N		$7.97^{a} \pm 0.9$	$7.8^{a} \pm 0.8$
K^{+}	mg/L	$660^{a} \pm 15$	$300^{b} \pm 11$
K_20	mg/L	$792^{a} \pm 8$	$360^{b} \pm 5$
P	mg/L	$250^{b} \pm 7$	$450^{a} \pm 5$
P_20_5	mg/L	$573^{b} \pm 12$	$1030^{a} \pm 25$
NO_3^{-}	mg/L	$100^{b} \pm 5$	$400^{a} \pm 15$
NH_4^{+}	mg/L	$260^{a} \pm 15$	$40^{b} \pm 3$
Ca^{++}	mg/L	$700^{b} \pm 8$	$1400^{a} \pm 17$
Mg^{++}	mg/L	$150^{a} \pm 7$	$50^{b} \pm 5$

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 $P \leq 0.05$.
564

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

	Lettuce				Watercress				Cucumber			
Treatment	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
LF 10%	80	27.5	27.4	4.1	80	26.0	27.1	4.0	100	28.2	27.9	3.5
LF 25%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.9	28.4	3.3
LF 50%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.6	27.9	3.5
LF 100%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.5	27.6	3.6
SF 10%	100	29.8*	29.9*	3.4*	100	29.6*	31.1*	3.3*	100	28.9	27.8	3.7
SF 25%	70	28.5*	29.6*	5.1*	70	28.8*	28.6*	3.4*	100	28.8	27.7	3.5
SF 50%	48	14.3**	16.8**	19***	55	14.0**	19.8**	10***	100	28.7	27.7	3.7
SF 100%	20	14.2**	2.28***	15***	18	14.0**	2.12***	16***	100	28.4	27.3	3.7

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

Table 6 Germination indexes: Total germination (TG); Coefficient of germination velocity (CVG), Germination Rate Index (GRI) and Mean Germination Time (MGT) determined for lettuce, watercress and cucumber seeds treated with different concentration 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***

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

Table7 Analysis of variance of different treatments of Fattoria and Uliva digestate fractions (solid and liquid) on total germination (TG) and mean germination time (MGT) of seeds of lettuce, watercress and 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*: F-ratio*						
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*: F-ratio*						
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***

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

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 ± SE (n=4). Different letters in the same column indicate significant differences $P \leq 0.05$.

610

Treatment	**APX** U mg^{-1} prot	**GR** U mg^{-1} prot	**POX** U mg^{-1} prot	**CAT** U mg^{-1} prot
Control	1.41 ± 0.2^{d}	0.05 ± 0.03^{e}	0.26 ± 0.1^{f}	25 ± 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 ± 0.2^{b}	0.11 ± 0.02^{d}	0.29 ± 0.2^{f}	56 ± 2.1^{c}
SU 25%	3.44 ± 0.3^{b}	0.16 ± 0.02^{c}	0.56 ± 0.3^{d}	99 ± 4.5^{b}
SU 50%	4.40 ± 0.5^{a}	0.20 ± 0.01^{b}	1.10 ± 0.2^{b}	107 ± 3.5^{b}
SU 100%	4.97 ± 0.4^{a}	0.25 ± 0.03^{a}	1.26 ± 0.1^{a}	135 ± 4.0^{a}
LF 10%	1.98 ± 0.1^{c}	0.08 ± 0.03^{e}	0.28 ± 0.1^{f}	29 ± 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 ± 0.2^{d}	0.06 ± 0.01^{e}	0.27 ± 0.2^{f}	24 ± 2.1^{f}
SF 25%	1.76 ± 0.2^{d}	0.07 ± 0.02^{e}	0.30 ± 0.3^{f}	29 ± 2.0^{f}
SF 50%	2.40 ± 0.3^{b}	0.12 ± 0.01^{d}	0.39 ± 0.2^{e}	37 ± 3.1^{d}
SF 100%	3.22 ± 0.5^{b}	0.16 ± 0.02^{c}	1.06 ± 0.1^{c}	35 ± 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 ± 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 ± 0.5^{c}	0.09 ± 0.03^{d}	0.32 ± 0.1^{d}	27 ± 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 ± 0.2^{c}	0.16 ± 0.02^{b}	0.33 ± 0.2^{f}	67 ± 2.1^{c}
SU 25%	2.74 ± 0.3^{c}	0.16 ± 0.02^{b}	0.36 ± 0.3^{d}	108 ± 4.5^{b}
SU 50%	4.00 ± 0.5^{a}	0.25 ± 0.01^{a}	1.4 ± 0.2^{b}	109 ± 3.5^{b}
SU 100%	4.38 ± 0.4^{a}	0.28 ± 0.03^{a}	1.76 ± 0.1^{a}	144 ± 4.0^{a}
LF 10%	1.77 ± 0.4^{c}	0.06 ± 0.03^{d}	0.33 ± 0.1^{d}	27 ± 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 ± 0.2^{d}	0.07 ± 0.01^{d}	0.29 ± 0.2^{d}	27 ± 2.1^{d}
SF 25%	1.76 ± 0.2^{c}	0.09 ± 0.02^{d}	0.31 ± 0.3^{d}	31 ± 2.0^{d}
SF 50%	3.00 ± 0.3^{b}	0.15 ± 0.01^{c}	0.47 ± 0.2^{e}	33 ± 3.1^{d}
SF 100%	$3.42 \pm 0.5a^{b}$	0.19 ± 0.02^{b}	0.98 ± 0.1^{c}	33 ± 2.0^{d}

Table 10 Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase, (POX) and catalase (CAT) enzymes in 7 day old cucumber seedlings under Solid (SU) and Liquid (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means ± 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 ± 0.5^{b}	0.11 ± 0.01^{c}	2.5 ± 0.1^{b}	31 ± 2.0^{e}
LU 10%	4.10 ± 0.5^{a}	0.15 ± 0.01^{b}	5.4 ± 0.7^{a}	47 ± 2.0^{d}
LU 25%	4.33 ± 0.5^{a}	0.17 ± 0.01^{b}	6.1 ± 0.5^{a}	56 ± 2.0^{c}
LU 50%	5.44 ± 0.7^{a}	0.28 ± 0.01^{a}	6.6 ± 1.0^{a}	61 ± 2.0^{b}
LU 100%	nd	nd	nd	nd
SU 10%	3.82 ± 0.2^{ab}	0.16 ± 0.02^{b}	5.1 ± 0.6^{a}	37 ± 2.1^{e}
SU 25%	$3.94 \pm 0.^{ab}$	0.19 ± 0.03^{b}	6.0 ± 0.9^{a}	48 ± 4.5^{b}
SU 50%	4.55 ± 0.5^{a}	0.26 ± 0.04^{a}	6.3 ± 0.5^{a}	69 ± 3.5^{a}
SU 100%	5.38 ± 0.4^{a}	0.29 ± 0.04^{a}	7.4 ± 1.2^{a}	74 ± 4.0^{a}
LF 10%	2.77 ± 0.4^{b}	0.09 ± 0.03^{c}	2.3 ± 0.5^{b}	26 ± 1.5^{e}
LF 25%	2.94 ± 0.5^{b}	0.08 ± 0.02^{c}	2.4 ± 0.3^{b}	31 ± 2.0^{e}
LF 50%	2.78 ± 0.7^{b}	0.11 ± 0.03^{c}	2.5 ± 0.5^{b}	33 ± 3.1^{e}
LF 100%	3.04 ± 0.5^{b}	0.10 ± 0.03^{c}	2.3 ± 0.5^{b}	33 ± 2.0^{e}
SF 10%	2.54 ± 0.7^{b}	0.07 ± 0.01^{c}	2.1 ± 0.2^{b}	30 ± 2.1^{e}
SF 25%	2.49 ± 0.65^{b}	0.08 ± 0.02^{c}	2.3 ± 0.4^{b}	31 ± 2.0^{e}
SF 50%	2.77 ± 0.4^{b}	0.11 ± 0.03^{c}	2.2 ± 0.2^{b}	30 ± 3.1^{e}
SF 100%	3.01 ± 0.5^{b}	0.09 ± 0.02^{c}	2.4 ± 0.3^{b}	31 ± 2.0^{e}

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

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

Table 12 Correlation coefficient between fluorescein diacetate hydrolysis (FDA) and total phenol (TP) 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

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