Anaerobic co-digestion of recalcitrant agricultural wastes: characterizing of biochemical parameters of digestate and its impacts on soil ecosystem.

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Abstract

Anaerobic digestion (AD) of organic wastes is a promising alternative to landfilling for reducing Greenhouse Gas Emission GHG and it is encouraged by current regulation in Europe.. Biogas-AD produced, represents an ascertained useful source of green energy, while its by-product (digestate) is a waste, that needs to be safely disposal. The sustainability of anaerobic digestion plants partly depends on the management of their digestion residues. This study has been focused on the environmental and economic benefits of co-digest recalcitrant agricultural wastes such olive wastes and citrus pulp, in combination with livestock wastes, straw and cheese whey for biogas production. The aim of this work was to investigate the effects of two different bioenergy by-products on soil carbon stock, on enzymes involved in nutrient cycling and microbial content. The two digestates were obtained from two plants differently fed: the first plant (Uliva) was powered with 60% of recalcitrant agricultural wastes, and 40% of livestock manure milk serum and maize silage. The second one (Fattoria) was fed with 40% of recalcitrant agricultural wastes and 60% of livestock manure, milk serum and maize silage. Each digestate was subsequently separated in liquid and solid fractions and each fraction was added to the soil in different concentrations. . Our results evidenced that mixing and type of input feedstock didn't affect the quality of biogas, while affected the composition of digestates. i 3 months after treatments, results showed that the changes in soilchemical and biochemical characteristics depended on the source of digestate, the type of fraction, and the concentration used. The mainly affected soil chemical and biochemical parameters were SOM, MBC, FDA, WSP and CAT that can be used as soil quality indicators to assess the digestate agronomical feasibility. Analysis of variance showed that the interactions of concentrations and fractions were less significant than factors individually. These results show that the co-digestion of olive waste and citrus pulp with other organic wastes produces high quality biogas independently from percentages and type of feedstock input. On the contrary, the agronomic quality of a digestate is strictly dependent on percentage and type of feedstocks that will be used to power the digester. .

Keywords: Anaerobic digestion; Biogas production; Carbon stock, Digestate quality; Soil ecosystem functioning;

1. Introduction

Organic waste removal has become an ecological problem, brought to light as a result of an increase in public health concerns and environmental awareness. Recently, the organic wastes have been recognized as a valuable resource that can be converted into useful products via microbially mediated transformations (Yu and Huang, 2009; Lesteur et al., 2010). There are various methods available for the treatment of organic wastes but the anaerobic digestion (AD) appears to be one of the most promising approach (Lee et al., 2009) for producing environmental and socio-economic benefit, in terms of renewable energy (biogas), reduction of organic wastes going to landfills and abatement of GHG emissions (Dennehy et al., 2016). While biogas-AD produced, represents an ascertained useful source of green energy, the residue , is a waste, that needs to be sustainably used for improving the economical profitability of AD plants (Iacovidou et al., 2013; Pivato et al., 2016).

. The digestate in comparison with undigested wastes has greater microbial stability and hygiene and higher amount of nitrogen in the form of ammonium (Alburquerque et al., 2012; Holm-Nielsen et al., 2009). Moller and Muller (2012) demonstrated that digestate contains high levels of macro and micro nutrients and as such represents an environmentally sound alternative to the mineral fertilisers, with the potential to improve soil fertility and quality. Wager Baumann, (2011) suggested also that digestate may reduce the need for irrigation by improving soil moisture retention properties. Agricultural wastes and livestock manures are highly polluting residues with a high cost of disposal for farmers (Panuccio et al., 2015), therefore their anaerobic digestion can represent a reliable and advantageous practice to convert refuse in resource.Anaerobic mono-digestion of animal manure and animal slurry is carried out in many Mediterranean areas with intensive animal production and high density of manure per hectare, as a sustainable option for manure treatment and manure management (Monou et al., 2009). The co-digestion of animal manure with organic wastes,

is less frequent, even if Al Seadi and Lukehurst, (2012) and Ebner et al. (2016) demonstrated that it produces more biogas with high methane percentage than manure alone, improving the profitability of biogas plants. Most existing studies on co-digestion have been based on biomass mixtures using either sewage sludge or a variety of animal manures together with materials such as food waste, energy crops or crop residues. Examples of key studies are: Kim et al., 2003, Koch et al., 2015 and Murto et al., 2004, who used sewage sludge mixed with residential or industrial food waste; Adelard and Poulsen, 2015, Ashekuzzaman and Poulsen, 2011, Lansing et al., 2010a, Lansing et al., 2010b, Li et al., 2015, Magbanua et al., 2001, Wang et al., 2012, Wang et al., 2013, Zarkadas et al., 2015, used mixtures of animal manure such as cow dung, pig manure or poultry manure in combination with food waste or crop residues (straw). Ogejo and Li,(2010), Owamah et al. (2014) Pagés-Díaz et al. (2015) and Rico et al. (2015) investigated the co-digestion of industrial wastes such as cheese whey, food waste, and slaughter house wastes combined with municipal solid wastes and animal manures (. No previous studies have been focused on the potential benefits of the co-digestion of animal manures, straw and cheese whey, with olive wastes and citrus pulp, recalcitrant pollutant wastes commonly produced in Mediterranean countries. This researchinvestigated on the benefit of co-digeste recalcitrant agriculture wastes (olive wastes and citrus pulps), mixed in different proportions with livestock manures, milk serum and maize silage in the production of more stable digestate with compatible soil use as fertilizer. The aim was to elucidate the effects of unprocessed digestates on soil ecosystem functioning. The impact of two digestates different in composition, each separated in liquid and solid fractions, was assessed 3 months after starting treatments, evaluating the effects on soil organic matter (SOM), nutrient cycling, microbial biomass C, enzyme activities and soil physic-chemical properties (pH, EC and water soluble phenols). The main aims were to relate the chemical composition of the digestates to the quality and percentage of the feedstock used, to evaluate differences in influencing belowground processes in respect to their attributes, and to test their capacity in maximizing the organic carbon for restoring soil fertility.

2. Materials and methods

2.1. Biogas plants: process temperature and retention time

This research was carried out in collaboration with two cooperatives Fattoria della Piana soc. Agricola, and Uliva Srl soc. Agricola, owners of biogas plants. 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 60% animal manures (poultry, cow and sheep), milk serum, maize silage and in minor amount with olive waste (20%) and citrus pulp (20%). The second one named **Uliva (U)** was mainly powered with olive waste 30%, and citrus pulp 30% and in minor amount (40%) with animal manure and maize silage (Panuccio et al., 2015).

Process temperatures and retention times are appropriate for the sanitation and are calibrated on the basis of 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.

As reported in Panuccio et al. (2015), 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)** a desirable upstream operation in the treatment process since dewatering the solid fraction reduces the cost of transport and facilitates its addition to soil (Holm-Nielsen *et al.*, 2009). The obtained fractions were analyzed for chemical and biological characteristics.

2.2. Digestate chemical analysis

Chemical parameters of the two digestates, each separated in liquid and solid fractions, were determined in three replicates as follow. 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 (AFNOR, 2001); 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 (Nelson and Sommers 1982), and it was converted to organic matter by multiplying the percentage of carbon by 1.72; total nitrogen was measured by Kjeldahl method (Bremner and Mulvaney, 1982); electric conductibility was determined in distilled water by using 1:5 digestate:water suspension, mechanically shaken 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 (Bray and Kurtz, 1945). Exchangeable K was extracted with 1 M NH_4OAc, and determined using a flame-photometer. The NO_3–N was measured using a nitrate-ion selective electrode (U.S. EPA, 2011), while NH_4–N was determined by a colorimetric method based on Berthelot's reaction (Sommer *et al.*, 1992). All values refer to material dried at 105 °C for 24 h. The 5-day biochemical oxygen demand (BOD) was measured with a respirometric Oxitop® IS 6 (WTW, Germany) based on pressure measurement, which is automatically transformed into mg O_2 L^{-1}. In the Oxitop® system, cumulative oxygen consumption measurements were made each day during a 5-day period. COD was determined by dichromate oxidation of dried ground samples, according to an adaptation of the standard method described for liquid samples (AFNOR, 2001) and using an automatic titration device (Metrohm Titrando-Dosino device); total water-soluble phenols were measured by using the Folin–Ciocalteau reagent, following the Box method (Box, 1983). Tannic acid was used as a standard and the concentration of water-soluble phenols was expressed as tannic acid equivalents (mg TAE/g dm) (Kuiters and Demnam, 1987). Fluorescein diacetate hydrolysis (FDA) reaction was determined according to the methods of Adam and Duncan (2001). Briefly, to 2 g of digestate (fresh weight, sieved <2 mm) 15 ml of 60 mM potassium phosphate pH 7.6 and 0.2 ml 1000 µg FDA ml^{-1} were added. The flask was then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol solution (2:1 v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3

149 min. The supernatant was filtered and the filtrates measured at 490 nm on a spectrophotometer
150 (Shimadzu UV–Vis 2100, Japan).

151 *2.3. Experiments and soil sampling*

152 The soil (Haplic Kastanozem) used was taken from the agricultural farm of Mediterranea University
153 of Reggio Calabria, Italy, Each plastic pots (19 cm diameter) was filled with 350 g of soil, in order
154 to evaluate the effects of digestate on soil chemical and biochemical properties. Soil was amended
155 with the solid fractions of both digestates (Fattoria and Uliva) at the percentage of 0 (control), 25,
156 50 and 75%, and with the liquid fractions of both digestates (Fattoria and Uliva) at the
157 concentrations of 0 (control) 10, 20 and 30%. The soils were regularly watered to maintain 70% of
158 field capacity. The experiment was conducted in glass house, to protect soil from rainfall managing
159 the irrigation system to maintain 70% of field capacity. At the end of the experiments (3 months)
160 differently treated soils (3 replicates), were air-dried and sieved (<2mm) prior to the chemical
161 analysis. Soil samples for the biochemical determination (microbial biomass and enzyme activities)
162 were stored in the refrigerator at 4 °C for up to 24h until processing.

163 *2.3. Soil Chemical Analysis*

164 3 months after treatments soil samples, (0–20 cm) for each treatment, were taken, brought to the
165 laboratory on the same day of the collection, and kept in the refrigerator at 4 °C for up to 24 h until
166 processing. Prior to the soil analysis, except for enzymatic activities and MBC all the soil samples were
167 air-dried, sieved (<2 mm), and visible roots were removed. Organic C was estimated by the Walkley–
168 Black procedure (Nelson and Sommers, 1982) and was converted to organic matter by multiplying the
169 percentage of C by 1.72; total N was measured by the Kjeldahl method (Bremner and Mulvaney, 1982).
170 Humic substances were extracted with 0.1 N NaOH (solid:liquid ratio 1:10); the suspension was shaken
171 for 16 h at room temperature and centrifuged at 5,000 rpm for 30 min; the extract was dialyzed by
172 Wisking tubes against distilled water to pH 6.0. Subsequently, the solution was filtered through a
173 column of Amberlite IR 120 H^+. The fractionation of humic substances was carried out as follows:
174 aliquots of extracts were acidified to pH 2.0 with dilute H_2SO_4; the humic acids precipitated and were

removed by centrifugation, while the fulvic acids corresponded to the supernatants (Bettany et al. 1980). The C content of humic and fulvic acids was determined by dichromate oxidation (Nelson and Sommers, 1982). Phenols were extracted with distilled water (Kaminsky and Muller, 1977, 1978). Thirty grams of dry weight samples were mixed in 200 ml distilled water and shaken at 75 rev min)1 for 20 h at room temperature. Solutions were filtered through Whatman's No 1 paper. All samples were extracted in triplicate. Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin–Ciocalteau reagent, following the method of Box (1983). Tannic acid was used as standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (µg TAE g^{-1} D.W.).

2.4. Soil Biochemical Analysis

The amount of microbial biomass C (MBC) was determined by using the chloroform fumigation–extraction procedure (Vance et al., 1987) with field moist samples (equivalent to 20 g D.W.). The filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C using the methods of Walkley and Black (1934). MBC was estimated on the basis of the differences between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C (Vance et al., 1987).

Enzymatic assay: dehydrogenase (DH) activity was determined by the method of von Mersi and Schinner (1991). Briefly, to a sample of fresh soil equivalent to 1 g of oven dried (105° C) soil were added 1.5 ml of 1 M Tris–HCl buffer of pH 7.5 followed by 2 ml of 0.5% INT solution (Sigma product No I 8377), and the suspension was kept at 40 C for 1 h. Then 10 ml of extractant (methanol) was added and the samples were mixed using a vortex mixer, and then left in the dark for 10 min. Finally, the solids were filtered out (Whatman's no 40 paper), and the absorbance of the filtrate was determined at 490 nm.

Alkaline and acid phosphatase (AlPh, AcPh) activities were determined on 1 g (fresh weight) aliquots of soil, according to the method of Tabatabai (1982). Enzyme activities are expressed as µg *p*-nitrophenol produced by 1 g of dry soil in one hour (µg p-nitrophenol g^1 h^1).

FDA hydrolysis reaction was determined according to the methods of Adam and Duncan (2001). Briefly, to 2 g of soil (fresh weight, sieved <2 mm) 15 ml of 60 mM potassium phosphate pH 7.6 and 0.2 ml 1000 mg FDA ml^{-1} were added. The flask was then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3 min. The supernatant was filtered and the filtrates measured at 490 nm on a spectrophotometer (Shimadzu UV–Vis 2100, Japan). and expressed as µg fluorescein released per g of dry soil (Perucci, 1992).

Urease (URE) was determined according to the method of Kandeler and Gerber (1988). Soil (5 g fresh weight) was mixed with 2.5 ml of urea (80 mM) and 20 ml 0.1 M borate buffer pH (10.0). The mixture was allowed to react for 2 h in an orbital shaker at 37 °C. After incubation, pipette 2.5 ml of urea to the control, add 30 ml of KCl (2 M) to both sample and control, and shake for 30 min. Filter the contents of the flasks through folded filters. Aliquots of 1 ml of the filtered solution were mixed with 9 ml of distilled water, 5 ml of sodium/salicylate solution, and 2 ml of dichloroisocyanuric acid (Na^+ salt). The colour intensity of the solution was measured at 690 nm. Ammonium concentrations were determined by using a calibration curve of ammonium chloride standard solution.

Beta-glucosidase activity was detected according to the method of Valášková et al. (2007). Soil (1 g fresh weight) was placed into a plastic tube and treated with 4 mL of modified universal buffer (MUB, pH 6). The reaction mixture contains 0.16 ml of 1.2mM PNP-substrate (p-nitrophenyl-β-d-glucoside) in 50mM sodium acetate buffer (pH 5.0) and 0.04 ml of the sample. Reaction mixtures were incubated at 40°C for 20–120 min. After incubation the reaction was stopped and the yellow color from the *p*-nitrophenol was developed by the addition of 0.1 ml of 0.5M sodium carbonate, The *p*-nitrophenol was measured by absorption on a spectrophotometer at a wavelength of 400 nm and quantified by comparison with a standard curve.

225 Catalase activity (CAT) was determined by the method of Kuush (2001), measuring the absorbance
226 during the conversion of H_2O_2 to oxygen and water. The decrease in the absorbance was measured
227 at 240 nm, utilizing the extinction coefficient of 39.4 M^{-1} $cm^{-}1$.

228 *2.5. Data analysis*

229 Analysis of Variance was used to test the effects of the factors (treatments and concentrations) on
230 soil indexes for each case study separately. Treatment means were compared using Tukey's test
231 (Sokal and Rohlf, 1981). All statistical analyses were performed using Systat v. 8.0 software
232 package (SPSS Inc, Evanston, Ill, USA).

233

234 **3. Results**

235 The analyses indicated that changes in soil chemical characteristics under treatments depended on
236 the source of digestate, the type of fraction, and the concentration used. The solid fractions of both
237 digestates, increased the values of all soil parameters, except pH and total nitrogen, in comparison
238 to the control (Table 1). Solely the digestate Fattoria increased the HC percentage. These results
239 highlighted an increase in the stable fraction of organic matter that is more resistant to
240 decomposition and crucial for increasing soil carbon sequestration. WSP, belonging to the labile
241 fraction of SOM, was the only chemical parameter that, in treated soils, increased in a
242 concentration-dependent manner (Table 1). The lowest amount of WSP was detected in soils treated
243 with solid F at the concentrations of 25 and 50%. Solid fractions of both digestates increased also
244 the MBC concentration compared to the un-amended soil (Table 2). The greatest increment was
245 observed in presence of F at 50 %. Similar results were observed for FDA, dehydrogenase, urease,
246 catalase, β-glucosidase and acid phosphatase (Table 2). Solid Uliva, increased significantly only the
247 CAT activity while decreased the activities of β-glucosidase and urease. All the other enzymatic
248 activities didn't show significant differences in comparison to the control (Table 2).
249 Liquid fractions of both digestates increased the C/N ratio and the amount of WSP and SOM in
250 respect to the control (Table 3). The greatest WSP increment was observed with Uliva at the

highest concentration (30%). Fattoria greatly increased (Table 4) the activities of FDA, CAT and the MBC. U increased only the activity of CAT when applied at the highest concentrations (Table 4).

Analysis of variance (Tables 5, 6), was carried out only on soil chemical and biochemical parameters that were effectively affected by the different treatments. The results (Table 5) confirmed that the positive significant effects on SOM were due solely to the solid fractions of both digestates; Fattoria had the greatest stimulatory effect (Table 5). WSP was affected only by both fractions of Uliva as displayed by the value of F-ratio. FDA and MBC were significantly affected solely by both fractions of Fattoria (Table 6).

Our results evidenced also that the effects of Fattoria on SOM were due to the concentrations, independently from the type of fraction used. Instead, the effects of Uliva on SOM depended on the type of fraction and on the concentration used (Table 6). For both digestates, the effects of the fractions, concentrations and their interactions on WSP were highly significant. The analysis of variance showed also that the interactions of the two factors, were less significant than the factors individually.

4. Discussion

The digestate generated as a by-product of co-digestion process of recalcitrant biomass is considered a potential fertilizer as, in general, no harmful effects were observed on soil ecosystem when it was used. To date, most of the previous studies have primarily been focused on evaluating the effects of digestate and its fractions on soil chemical parameters (Kouřimská et al., 2012; Chiew et al., 2015; Koszel and Lorencowicz, 2015) but little was yet known about how digestate fractions affects soil ecosystem functioning and carbon stock. We demonstrated that digestates have agronomic properties intermediate between fertilizers and amendments, being able to increase both chemical and biological soil properties. The work done and many returns of information collected on field experiments revealed that the two fractions of both digestates increased the content of soil organic matter at the same extent. What changed was the amount of stable (HC) and labile pool

fractions (phenolic compounds, microbial biomass, and enzymes) of organic matter, in respect to the digestate used. Only Fattoria (both fractions) increased the stable part of SOM (humic acids), bringing to the soil organic matter of good quality and with slow potential mobility of C in the soil system (Zagal et al., 2009). Translated this means that humic acids contained in the digestate provide a way of storing the various nutrients increasing CEC, water holding capacity (Gümüş and Seker, 2015) and stimulate overall plant growth by increasing microbial like activity by up to 2000 times in just a few weeks (Trevisan et al., 2010). Numerous works (Vallini et al., 1997; Vacca et al., 2005; Steinberg et al., 2006;Tikhonov et al., 2010) evidenced the influence of humic acids of different origins on the growth of soil bacteria, suggesting that when the living organisms came into contact with HSs, the cells used more efficiently the energy generated by them, favoring an increase in the number of colonies in soil (Visser, 1985). Our results agree with these findings as we found a greater MBC content in soil with a greater amount of HC. Melero et al. (2006) and Muscolo et al. (2014; 2015) showed also that MBC, WSP, and FDA were highly correlated to the SOM and noted that organic management had a positive indirect effect on soil organic matter. This leaded to an improvement of soil quality and productivity, through the increase in the microbial biomass responsible for the cycle of the main nutrients which in turn contribute to the maintenance of long-term agricultural sustainability. Consistent with a number of studies on the effects of organic amendments (Bastida et al., 2008; Fuchs et al., 2008; Saha et al., 2008; Reeve et al., 2012), our results showed a positive increase in soil enzyme activities. The great increase in presence of Fattoria, highlighted that the fertilizer power was strictly related to the feedstock used to feed the digester, showing a specificity between feedstock used and chemical composition of digestates rather than feedstock used and quality of biogas (Panuccio et al., 2015). Numerous works evidenced that there is a wide range of anaerobic digestates whose composition and aspect depend upon the type of biomass inputs (feedstock) and the configuration of the digester (Provenzano et al., 2011 Teglia et al., 2011; Furukawa & Hasegawa 2006; Voća et al., 2005; Rivard et al., 1995; Möller et al., 2008). In this work, the observed increase in the activities of catalase, enzyme related to the metabolic processes of aerobic organisms and used as indicator of soil fertility (Trasar-Cepeda et

al., 2007), and of dehydrogenase which is mainly associated with the oxidative activity of soil microbes (García-Gil et al., 2000; Masciandaro et al., 2000; Zhang et al., 2009; Muscolo et al. 2015) proved that Fattoria applied at high rate (50% for solid, and 30% for liquid fraction, respectively) had favorable effects on soil ecosystem stimulating microbial activities. Uliva did not stimulate soil biological properties as Fattoria did but, at the same time, it did not have negative effects on soils, increasing soil organic matter amount too. It was also noted that FDA and CAT were the enzymes whose activity correlated with the greatest number of soil properties affected by the amendment. Thus we can suggest to use FDA and CAT as bio-indicators for the quantitative assessment of soil quality when subject to treatment with organic amendments.

5. Conclusions

In short, our results confirm that it is possible to co-digest olive waste and citrus pulp with animal sludge for producing a good quality biogas and also digestate with good agronomic properties. Solid fractions of both digestates can be disposal in soil, with positive consequence for long term fertility. Even if the liquid fractions of digestates were less effective than solid ones on biological soil fertility, they could be used for agriculture purpose, representing nutrient rich water that if used instead of fresh water for crop irrigation can have enormous environmental benefits . Additionally, using digestate instead of synthetic or manufacturing organic fertilizers, a notable reduction in the environmental impact of CO_2 and CH_4 emissions, coming from industril production process, can be also reached.

We can also expect an increase in the economic sustainability of small/middle size biogas plants due to the reduction of digestate treatment costs and, in some countries, additional income from the use of the heat generated by biogas production. The valorization of digestate into green fertilizers will lead to marketable products with a high economic value. The investment costs of anaerobic digesters are moderate and the potential of self-help is relatively high (even though planning requires skilled labour and expert design). The use of digestate as fertilizer thus can create added value, making biogas production interesting also from an economic point of view.

Acknowledgments

This study is financed by PSR-2007-2013- Calabrian Region Scientific Research Projects Mis. 124.

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Table 1**.** Chemical characteristics of sandy-loam soil treated with different concentrations of Fattoria and Uliva solid digestates (**S.D**.). pH, soil organic Matter (SOM %); Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols (WSP µg TAE g^{-1} dry soil). Numbers denote the standard errors (n=9) Means with the same letters, in the same column, are not significantly different (Tukey's test. $p \leq 0.05$)

S.D.	Treatmei	pH	SOM	HC	FC	N	C/N	WSP
Control	**0**	7.6±0.2^{a}	1.56±0.2^{b}	0.60±0.2^{b}	0.40±0.2^{b}	0.12±0.007^{a}	7.5±0.9^{d}	41±2.5^{e}
Fattoria	**25%**	7.6 ±0.1^{a}	2.34±0.2^{a}	0.76±0.2^{a}	0.61±0.3^{a}	0.12±0.006^{a}	11.3±0.3^{a}	50±3.0^{f}
	50%	7.4 ±0.2^{a}	2.35±0.2^{a}	0.75±0.1^{a}	0.66±0.1^{a}	0.12±0.005^{a}	11.4±0.5^{a}	63±2.3^{e}
	75%	7.3 ±0.3^{a}	2.40±0.3^{a}	0.73±0.1^{a}	0.67±0.2^{a}	0.14±0.006^{a}	10.0±0.2^{b}	82±2.5^{c}
Uliva	**25%**	7.6 ±0.1^{a}	2.30±0.1^{a}	0.65±0.2^{b}	0.58±0.2^{a}	0.13±0.009^{a}	10.2±0.5^{b}	75±3.5^{d}
	50%	7.5 ±0.2^{a}	2.33±0.3^{a}	0.62±0.1^{b}	0.60±0.2^{a}	0.13±0.007^{a}	10.4±0.2^{b}	96±2.9^{b}
	75%	7.6 ±0.3^{a}	2.36±0.2^{a}	0.63±0.1^{b}	0.68±0.1^{a}	0.15±0.005^{a}	9.2±0.5^{c}	158±2.9^{a}

Table 2**.** Microbial Biomass (MBC µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein released, µg g^{-1} dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg *p*-nitrophenol g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1}) β-glucosidase (β-GLU µg *p*-nitrophenol g^{-1} h^{-1}) Catalase (CAT, mg H_2O_2 g dry soil $^{-1}$) activities, in sandy soil treated with different concentration of Fattoria and Uliva solid digestates **(S.D**.). Numbers denote the standard errors (n=9) Means with the same letters, in the same column, are not significantly different (Tukey's test. $p \leq 0.05$)

S.D.	Treatment	FDA	DH	β-GLU	URE	Ac. P	Ak. P	CAT	MBC
Control	**0%**	42.4±1.5^{c}	59.5±1.5^{b}	78±2.2^{a}	87.9±2.2^{b}	248±3.5^{c}	339±3.0^{a}	40.2±1.0^{c}	833±3.4^{d}
Fattoria	**25%**	67.0±2.2^{a}	69.0±1.4^{a}	80±2.3^{a}	109.0±2.0^{a}	351±3.0^{a}	345 ±3.6^{a}	50.1±2.1^{c}	995±3.0^{a}
	50%	68.9±2.0^{a}	73.5±1.5^{a}	85±3.0^{a}	110.1±2.1^{a}	375±3.0^{a}	348±3.2^{a}	61.0±1.5^{b}	1080±5.0^{a}
	75%	59.7±2.4^{b}	61.0±2.0^{b}	81±3.0^{a}	91±2.0^{b}	280±2.3^{b}	344±2.5^{a}	63.0±1.9^{b}	918±6.0^{b}
Uliva	25%	40.5±2.5^{c}	59.4±1.6^{b}	62±2.5^{b}	79.4±2.0^{c}	248±3.0^{c}	340 ±2.0^{a}	74.0±1.0^{a}	865±3.0^{c}
	50%	41.0±2.1^{c}	62.1±1.8^{b}	69±3.1^{b}	81.1±2.3^{c}	247±3.5^{c}	344±3.0^{a}	75.0±1.5^{a}	861±5.0^{c}
	75%	43.9±2.8^{c}	63.5±2.5^{b}	70±2.0^{b}	81.4±2.5^{c}	246±3.0 ^{c}	345±3.0^{a}	76.5±1.9^{a}	858±3.4^{c}

Table 3. Chemical characteristics of soil treated with different concentration of Fattoria and Uliva liquid digestates (**L.D**.). pH; soil organic Matter (SOM %); Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols (WSP µg TAE g^{-1} dry soil). Numbers denote the standard errors (n=9). Means with the same letters, in the same column, are not significantly different (Tukey's test. $p \leq 0.05$)

L.D.	Treatment	pH	SOM	HC	FC	N	C/N	WSP
Control	**0**	7.6 ± 0.2^{a}	1.56 ± 0.2^{b}	0.60 ± 0.2^{b}	0.40 ± 0.2^{b}	0.12 ± 0.007^{a}	7.5 ± 0.3^{c}	41 ± 2.5^{e}
Fattoria	**10%**	7.6 ± 0.1^{a}	2.30 ± 0.1^{a}	0.75 ± 0.2^{a}	0.58 ± 0.2^{b}	0.12 ± 0.09^{a}	11.1 ± 0.3^{a}	50 ± 3.5^{d}
	20%	7.4 ± 0.2^{a}	2.33 ± 0.3^{a}	0.72 ± 0.1^{a}	0.59 ± 0.2^{b}	0.14 ± 0.07^{a}	10.4 ± 0.4^{a}	56 ± 2.9^{d}
	30%	7.3 ± 0.3^{a}	2.36 ± 0.2^{a}	0.73 ± 0.1^{a}	0.62 ± 0.1^{a}	0.15 ± 0.05^{a}	9.2 ± 0.5^{b}	67 ± 2.9^{b}
Uliva	**10%**	7.6 ± 0.1^{a}	2.30 ± 0.1^{a}	0.67 ± 0.2^{b}	0.56 ± 0.2^{b}	0.12 ± 0.09^{a}	11.1 ± 0.5^{a}	55 ± 3.5^{d}
	20%	7.5 ± 0.2^{a}	2.33 ± 0.3^{a}	0.66 ± 0.1^{b}	0.60 ± 0.2^{b}	0.13 ± 0.07^{a}	10.4 ± 0.2^{a}	66 ± 2.9^{b}
	30%	7.6 ± 0.3^{a}	2.36 ± 0.2^{a}	0.67 ± 0.1^{b}	0.64 ± 0.1^{a}	0.15 ± 0.05^{a}	9.2 ± 0.5^{b}	98 ± 2.9^{a}

Table 4. Microbial Biomass (MBC µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein released, µg g^{-1} dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg *p*-nitrophenol g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1}) β-glucosidase (β-GLU µg *p*-nitrophenol g^{-1} h^{-1}) activities, in soil treated with different concentration of Fattoria and Uliva liquid digestates (**L. D**.) Numbers denote the standard errors (n=9) Means with the same letters, in the same column, are not significantly different (Tukey's test. $p \leq 0.05$)

L.D.	Treatment	FDA	DH	β-GLU	URE	Ac.P	Ak.P	CAT	MBC
Control	0%	42.4±1.5^{b}	59.5±1.5^{a}	78±4.2^{a}	87.9±2.2^{a}	248±3.5^{a}	339±3.0^{a}	40.2±1.0^{d}	860±3.4^{b}
Fattoria	10%	83.5±2.5^{a}	59.4±1.6^{a}	82±2.5^{a}	89.4±2.0^{a}	248±3.0^{a}	340 ±2.0^{a}	68.2±0.6^{a}	965±7.0^{a}
	20%	91.9±2.1^{a}	62.1±1.8^{a}	81±3.1^{a}	91.1±2.3^{a}	247±3.5^{a}	344±3.0^{a}	69.5±1.0^{a}	990±5.0^{a}
	30%	73.9±2.8^{a}	63.5±2.5^{a}	84±4.0^{a}	92.4±2.5^{a}	246±3.0 ^{a}	345±3.0^{a}	60.3±2.1^{b}	998±4.4^{a}
Uliva	10%	40.5±2.5^{b}	57.2±1.6^{a}	79±2.5^{a}	86.4±2.0^{a}	235±3.0^{a}	338 ±3.0^{a}	43.8±1.2^{d}	845±9.0^{b}
	20%	38.9±2.1^{b}	60.1±1.8^{a}	80±3.1^{a}	90.0±2.3^{a}	239±3.5^{a}	336±2.5^{a}	49.5±1.4^{c}	850±6.0^{b}
	30%	39.9±2.8^{b}	61.0±2.5^{a}	81±4.0^{a}	89.3±2.5^{a}	244±3.0 ^{a}	340±4.0^{a}	50.4±2.3^{c}	853±4.5^{b}

Table 5. Analysis of variance of the effects of Fattoria and Uliva digestate fractions (solid and liquid) on soil organic matter (SOM), total phenols (WSP), fluorescein diacetate (FDA), microbial biomass C (MBC) and catalase (CAT). F-ratio and R^2 are shown

		Fattoria				
		SOM	WSP	FDA	MBC	CAT
Solid						
	F-ratio	32.598	191.81	128.99	1062.31	118.88
	p value	0.000	0.000	0.000	0.000	0.000
	R^2	0.924	0.986	0.980	0.997	0.978
Liquid						
	F-ratio	0.067	40.43	270.77	518.56	336.70
	p value	0.976	0.000	0.000	0.000	0.000
	R^2	0.024	0.969	0.990	0.995	0.992
		Uliva				
		SOM	WSP	FDA	MBC	CAT
Solid						
	F-ratio	12.72	1104.33	1.33	98.97	18.42
	p value	0.002	0.000	0.33	0.000	0.001
	R^2	0.827	0.998	0.34	0.974	0.874
Liquid						
	F-ratio	0.067	247.79	0.82	3.13	28.73
	p value	0.976	0.000	0.52	0.088	0.000
	R^2	0.024	0.989	0.24	0.540	0.915

Table 6. Two-way ANOVA results for the effects of Fattoria and Uliva digestates (solid and liquid fractions) on soil organic matter (SOM), total phenols (WSP), fluorescein diacetate (FDA), microbial biomass C (MBC) and catalase (CAT)

	Fattoria				
	WSP	FDA	MBC	CAT	SOM
R^2	0.782	0.974	0.990	0.997	0.988
Source of variance: F-ratio					
Treatments	2.89	28.71***	259.04***	2.023	96.80***
Concentrations	10.06**	179.94***	396.59***	1368.51***	337.48***
Fractions x Conc.	8.08**	11.90***	33.70***	291.00***	60.03***
	Uliva				
	WSP	FDA	MBC	CAT	SOM
R^2	0.707	0.997	0.347	0.947	0.906
Source of variance: F-ratio					
Treatments	4.99*	4551.26***	2.25	31.36***	8.95**
Concentrations	6.23**	8070.42***	1.29	37.61***	45.57***
Fractions x Conc.	4.99*	938.93***	0.80	46.34***	2.66

Significance *p<0.05; **p<0.01; ***p<0.001