Science of the Total Environment xxx (2018) xxx-xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com

Use of biogas digestates obtained by anaerobic digestion and co-digestion as fertilizers: Characterization, soil biological activity and growth dynamic of *Lactuca sativa* L.

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ARTICLE INFO

Article history:
Received 28 May 2018
Received in revised form 30 July 2018
Accepted 30 July 2018
Available online xxx

Editor: Frederic Coulon

Keywords:
Onion waste
FT-IR
Poultry litter
Feed-lot manure
Pig slurry
C mineralization

ABSTRACT

Agro-industrial systems provide large quantities of organic wastes that could imply an important environmental risk. While manures can be easily treated by anaerobic digestion, horticultural fruit wastes generally cannot be processed alone and should be treated by co-digestion. To use organic wastes as fertilizers is fundamental to improve understanding of their impact on soil-plant systems. In this research, cattle manure, poultry litter, pig slurry and onion waste were collected. Animal manures were studied without treatment, treated by anaerobic digestion alone and in co-digestion with onion wastes. To study their effect on soil-plant systems, chemical and spectroscopic characterization of manures and their transformed products were combined with soil biological activity and growth dynamic of lettuce following wastes incorporation to the soil. Anaerobic digestion decreased the C/N ratio, whilst there was an increase in NH_4^+-N/N ratio and short-chain organic acids. The magnitude of these changes varied depending on the type of organic matter present in each material and the incorporation of onion wastes intensified them. However, the digestates presented similar structural characteristics to each other, independently of the material of origin. Digestate soil application produced a fast and short microbial stimulation (18–34 and 7–11 mg CO_2 during the first 6 h, digestates vs. rest of treatments). The digestate dosage should be done according to the content of NH_4^+-N given that the vegetal growth is related to it. Soils amended with digestates showed less CO_2 emission than soils amended with manures improving overall C balance.

1. Introduction

Agro-industrial intensive systems generate large volumes of organic wastes in relatively reduced areas, which, if it is not handled correctly, implies an important environmental risk (Kunz et al., 2009), potentially causing soil, water and air contamination through leached nutrient and greenhouse gases emission (Gómez-Brandón et al., 2013).

Animal manures can be easily treated by anaerobic digestion for the biogas production, because they provide adequate organic substrate. They contain the microbial groups involved in the process and generally have a high buffer capacity. On the other hand, horticultural fruit wastes have a big proportion of fermentable carbohydrates and a low buffer capacity, so, generally, they cannot be processed alone.

Onion is a traditional vegetable with high global importance, second in seeding area after tomato and a per capita consumption of 10.5 kg habitant^{-1} year^{-1} (Medina, 2013). The Latin-American production represents 9% of the total, and the most important producers are Mexico, Brazil, Argentina, Colombia and Chile (Galmarini, 1997). Argentina is among the top ten exporting countries with an average contribution of 3% of the global total (Ministerio de Agroindustria, 2016).

In Argentina, the onion production should be packaged and certified in region of origin. Consequently, each region count with sorter and packaging plants to process a local volume. These plants generate large amounts of slow degrading wastes that are accumulated and/or burned to reduce their volume generating environmental problems (odours, greenhouse gases emissions, leached nutrient, etc.).

In general, the onion present between 80 and 95% of water and a relatively high content of sugar (glucose, sucrose and fructose) which represents between 65 and 80% of the dry weight, and low pH (4–5). An important proportion of sulphur compounds produce their characteristic strong and penetrating odour and are responsible for their antimicrobial and antiparasitic properties (Corzo-Martínez et al.,

Abbreviations: AGR, absolute growth rate; CA, cover area; Cmin, carbon mineralization; DWL, dry weight of leaves; DWR, dry weight of roots; EC, electrical conductivity; CM, Feed-lot cattle manure; CMD, feed-lot digestate; FRCA, final real coverage area; LN, leaves number; MMCA, maximum modelled coverage area; MRCA, maximum real coverage area; N, nitrogen; OCMD, onion feed-lot digestate; OPF, onion pig digestate; OPLD, onion poultry litter digestate; PD, pig digestate; PL, poultry litter; PLD, poultry litter digestate; PS, pig slurry; RGR, relative growth rate; TKN, total Kjeldahl nitrogen; TS, total solid; VS, volatile solid; WHC, water-holding capacity; WWL, wet weight of leaves

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https://doi.org/10.1016/j.scitotenv.2018.07.444
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2007; Rose et al., 2005; Zohri et al., 1995). Lubberding et al. (1988) demonstrated that the anaerobic digestion of onions decreases the proportion of acetate (substrate for methanogenesis) while other acids accumulate reducing the pH.

Anaerobic co-digestion consists of the simultaneous digestion of a mixture of two or more substrates with complementary characteristics which allows an increase in the production of biogas and stabilizes the process (Mata-Alvarez et al., 2011). Achieving a successful combination of two or more different wastes requires careful management, because random testing or heuristic decisions on the relationship of waste incorporated into large-scale plants often produce alterations in processes, significant reductions in methane production (Zaher et al., 2009) and can even completely stop the process.

The agronomic reuse of any residue must be associated with some improvement in the soil-plant system, in order to justify its somewhat laborious and warrant adoption of farmers. The crop production depends on the complex interaction of the different component of the agro-ecosystem. Evaluating plant development through crop indicators in combination with microbiological activity may allow us to analyse the components as a whole.

The analysis of plant growth through the foliar area and weight of the different organs allows estimating fundamental processes that affect productivity, such as the rate of carbon fixation and the distribution of photoassimilates between the different organs of the vegetal (Di Benedetto and Tognetti, 2016). This analysis can be used to compare different managements.

The growth is defined as an irreversible increase in the extension of the plant. The accumulated dry biomass of the plant (or organ) increased slowly initially, in a positive acceleration phase; then increased rapidly, approaching an exponential growth rate and then declined in a negative acceleration phase until at zero growth, like a sigmoid curve (Poorter, 2002). The increase in plant biomass weight in leaf-crops is a consequence of the increase in expanded foliar area as a source of photo-assimilates (Cookson et al., 2005) and is directly related to the productivity (Di Benedetto and Tognetti, 2016). Lettuce growth estimation is a good indicator crop because of its rapid development and its sensitivity to toxic substances (Aruani et al., 2008; Montemurro et al., 2010; Rotondo et al., 2009).

Soil microbial communities are composed of a wide variety of species that adapt their abundance and activity to environmental factors (Pell et al., 2005). The biological activity of these communities plays an essential role in the geochemical transformations of organic matter and consequently, on soil fertility (Jenkinson and Ladd, 1981). Aerobic respiration is among the most used techniques to determine soil biological activity either through oxygen consumption or and CO_2 release, so it can be determined through these two indicators.

The hypothesis of this work is that agronomic use of digestates (as fertilizers), produced by anaerobic digestion and co-digestion, has a better performance than manures without process and similar to synthetic nitrogen fertilizers. To test the general hypothesis, different animal manures and onion waste were collected. The manures were treated by anaerobic digestion and co-digestion with onions wastes. Each manure and its products of digestion and co-digestion were characterized by basic chemical analysis and IR spectroscopy, and this information was integrated with soil biological activity and lettuce development to evaluate their fertilizing properties.

2. Materials and methods

2.1. Soil and wastes

The soil used for the incubation and pot trial was collected from rural area near the city of Bahía Blanca (Buenos Aires, Argentina) at the 0–0.15 m depth. It is a sandy-loam soil classified as Petrocalcic Paleustoll and its main characteristics were: pH (1:2.5 soil:water mass ratio), 7.9; electrical conductivity (EC, saturation extract), 0.44 ds m^{-1}, total organic carbon (C), 12.6 g kg^{-1}; NTK, 1.34 g kg^{-1}; NH_4^+-N, 5.6 mg kg^{-1}; and NO_3^--N, 4 mg kg^{-1}.

The cattle manure was obtained from a feed-lot located in Villarino, Buenos Aires province (geo-coordinates South 38°42′12″ West, 62°27′41″); the poultry litter, from an eggs production farm in Bahía Blanca, Buenos Aires province (geo-coordinates South 38°39′03″ West, 62°16′57″); and the pig slurry, from a pig farm in Coronel Pringles, Buenos Aires province (geo-coordinates South, 37°46′27″ West, 61°30′44″). The abbreviations utilized were: CM, PL and PS respectively. Additionally, the onion wastes was obtained from a sorter and packaging plant in Hilario Ascasubi city, Buenos Aires province (geo-coordinates South, 39°23′02″ West, 62°37′29″). It is composed of external cataphylls, damaged bulbs, oversized or small bulbs, and bulbs affected by fungal or bacterial diseases. Onion waste principal characteristics were: pH (1:10 onion waste:water mass ratio), 5.1; electrical conductivity (EC, 1:10), 3.1 ds m^{-1}, total organic carbon (C), 3.0 g kg^{-1} and TKN, 1,9 g kg^{-1} (C/N ratio: 158).

The CM consists of manure and remains of food (maize grain-based balanced feed with free access to oat grass hay as a source of fibre). The PL includes sawdust of *Eucalyptus saligna* from the litter, manure and food residues (maize grain-based balanced feed). The PS includes cleaning water, manure, urine and food debris (sorghum grain-based balanced feed).

Consequently, these materials, differ markedly from one another in terms of feeding, type of animals and collection form.

CM and PL were collected in 30 kg bags and air-dried before storage, while PS was collected in a 20 dm^3 plastic contained and was kept at 4 °C until use. Onion wastes were freshly collected in situ and cut in 1 cm pieces immediately before anaerobic digesters set-up.

The different manures have been anaerobically digested alone (CMD, PLD and PD) and in co-digestion with onion waste (OCMD, OPLD and OPD). Anaerobic digests were performed under laboratory conditions in 2 dm^3 batch-type digesters without inoculum, within the mesophyle range (22–25 °C) with manual agitation, and a hydraulic retention time of 60 days. The ratio 5:1 (*W*/W, manure:onion waste) was used for the co-digestion of manures with onion waste, according with proposed by Rinland (2016). During the biomethanization process biogas was burned in a Bunsen burner to verify that the gas was combustible.

2.2. Chemical and spectroscopy characterization of wastes

Both, in liquid samples (PS and digestates) and air-dried manures (CM and PL) the following parameters were determined: NH_4^+-N, NO_3^--N and total Kjeldahl nitrogen (TKN) by semi-micro-Kjeldahl method (Greensberg et al., 1992); total P by persulfate digestion method and ascorbic acid method (Greensberg et al., 1992), pH and EC (without dilution in liquid samples and 1:10 sample:water mass ratio in solid samples in both determinations).

For the S determination samples were microwave digested with percloric acid (MARS-5, CEM Corporation, USA) and the S content was measured in a high resolution multi-type ICP emission Spectrometer (ICP-AES, Shimadzu 9000).

Total C was determined by dry combustion (1500 °C LECO C Analyser). The solid samples were dried at 40 °C. A 4-cm^3 aliquot of each liquid sample was then applied on the LECO inert material absorbent (combustion aid for liquids, 501–427), and oven dried at 40 °C.

Total solid (TS) was determined by drying samples at 105 °C to constant weight. The ash content was determined by burning samples at 550 °C during 2 h. The volatile solid (VS) was calculated by subtracting the ash mass from the TS mass.

The liquid materials were centrifuged at 2764 RFC, and were subjected to a UV–Visible spectroscopic scanning between 180 and 665 nm (UV–Vis spectrophotometer PG instruments T60) and IR spectra were obtained within the mid IR range (4000-400 cm^{-1}) with 64 scans and 8 cm^{-1} of resolution (Nicolet iS50 FT-IR Thermo Scientific), according to the method proposed by Iocoli et al. (2017). Solid samples (solid wastes and effluent precipitates) were prepared as 1% Merck Uvasol potassium bromide tablets (1.8 mg dry sample in 180 mg KBr). The liquid samples were also recorded as pellets, which were obtained by incorporating 0.30 cm^3 into 180 mg KBr, in order to achieve a dry base concentration of 0.5–1.0%.

2.3. Microbiota activation and soil C mineralization

Biological activity was assessed by the release of CO_2 captured in a solution of NaOH and back titration with HCl (Zibilske, 1994). Briefly, fresh soil was sieved (5 mm), 100 g of soil were placed in 750 cm^3 glass vessels (microcosms), water was added to 50% of water-holding capacity (WHC) and they were pre-incubated for a week. Then, the nine wastes and urea (treatments) were added at a rate of 100 mg kg^{-1} of TKN (equivalent to 280 kg hm^{-2}) in three replicates in a completely randomized design, in addition to an un-fertilized soil as control; the moister was corrected to 60% of WHC. Additionally 3 vessels without soil were added as blanks. A trap with 30 cm^3 of NaOH solution (0.25 M) was placed in each vessel; traps were restored 12 times during the incubation (6, 18, 42, 66, 90, 114, 138, 162, 210, 264, 355, 475 and 644 h). Each time, the amount of CO_2 in the vessel was determined by back titration of the base solution with HCl (0.25 M) in an excess of $BaCl_2$ saturated solution (1.5 M). The initial determination (6 h) indicate the presence of simple labile organic compounds that can be rapidly used by a wide range of microorganisms prior to biomass increase (Höper, 2005).

The CO_2-C was calculated by multiplying the CO_2 production by the coefficient 0.273 (C proportion in CO_2 molecule). The mineralization of the organic C from the wastes (Cmin) was calculated as the differences between CO_2-C evolved in the amendment soil and CO_2-C produced in the un-amended soil. This was expressed as percentage of the C added with the wastes. This assessment assumes that there is no priming effect. Each Cmin curve was adjusted to a single exponential model using the Sigma Plot 10 software (Systat Software Inc., Chicago, Illinois, USA) with the following equation:

$$\mathrm{Cmin} = \mathrm{C_0} * (1 - \exp(-\mathrm{k} * \mathrm{x}))$$

2.4. Dynamic of growth of lettuce

The different organic amendments and urea were added at a rate of 100 mg kg^{-1} of TKN (equivalent to 280 kg hm^{-2}) in 4 replicates in pots (3 dm^3 containers) previously filled with soil. In addition three replicates without amendment were used as a control. Each pot was manually mixed and placed in a green-house (geo-coordinates South, 38°41′41″ West, 62°15′08″; Departamento de Agronomía, Universidad Nacional del Sur). Three days later a lettuce plant (*Lactuca sativa* L. yellow-butter-head variety) was transplanted to each pot. Each pot was manually watered daily to keep soil at 60% of WHC. The pots were rotated randomly weekly and an aerial image of each plant was taken on a ring of 0.25 m^2, to determine vegetal cover area (with a digital camera Samsung ES28/VLUU ES28, 1024 × 768 pixels of resolution and captured in JPG format). The harvest was made 60 days post-transplant. The leaves were cut at ground level and the roots were divided from the soil with a combined water-sieving separation. Wet and dry weight of leaves (WWL and DWL, respectively), humidity content (%H), dry weight of roots (DWR) and leaves number (LN) were determined. The leaves and roots were oven dried at 70 °C for 48 h to determine their dry weight.

The digital images taken as described earlier were analysed using the CobCal v2.1 software (Ferrari et al., 2006) to determine vegetal coverage area (CA, cm^2). To analyse the vegetal growth the equations proposed in bibliography (Di Benedetto and Tognetti, 2016) were adapted and used for the CA. Absolute growth rate (AGR) represent an increment in CA per unit of time (t) and it was calculated with this formula:

$$\mathrm{AGR} = \mathrm{dCA}/\mathrm{dt} \ \left(\mathrm{cm}^2\ \mathrm{d}^{-1}\right)$$

Relative growth rate (RGR) represents an increment in CA per unit of CA and per unit of time (t), which was calculated using the following formula:

$$\mathrm{RGR} = 1/\mathrm{CA} * \mathrm{dCA}/\mathrm{dt} \ \left(\mathrm{cm}^2\ \mathrm{cm}^{-2}\ \mathrm{d}^{-1}\right)$$

Each growth curve was adjusted to a 4 parameter sigmoidal model using the Sigma Plot 10 software (Systat Software Inc., Chicago, Illinois, USA) with the following equation:

$$\mathrm{f} = \mathrm{y_0} + \mathrm{a}/\left(1 + \exp\left(-\left(\mathrm{x} - \mathrm{x_0}\right)/\mathrm{b}\right)\right)$$

In addition to analyse vegetal growth the following indicators were used: maximum modelled coverage area (MMCA, the maximum value of coverage area of the model); maximum real coverage area (MRCA, the maximum value of coverage area of the growth curve), and time elapsed (days) to reach the MRCA (TMCA, sampling day with largest coverage area).

2.5. Experimental design and statistical analyses

In both assays completely randomized designs were utilized. Data of CO_2-C dynamics and coverage area were subjected to split-plot in time analysis of variance (ANOVA, main plot assigned to treatment and subplot, to sampling date). In case a significant interaction was detected, one-way ANOVA was applied within each sampling date. Data of accumulated emission of CO_2 and the vegetal variables determined at harvest were subjected to a one-way ANOVA. Fisher's least significant difference test (LSD) was employed to determine statistical differences (0.05 probability level) wherever statistical significance of ANOVA was observed. Software InfoStat (Di Rienzo et al., 2017) was used to run analyses.

3. Results and discussions

3.1. Chemical and spectroscopic characterization of amendments

Anaerobic digestion decreased the C/N ratio (Table 1) as a consequence of the loss of C as CO_2 and CH_4 in biogas production. In contrast, anaerobic degradation of organic matter produced an accumulation of NH_4^+-N, resulting in an increase in the NH_4^+-N/N ratio. These observations coincide with numerous works cited in the review of Möller and Müller (2012). The magnitude of the mentioned changes varies depending on the type of organic matter present in each material. CMD and PLD presented a low NH_4^+-N/N ratio due to the higher content of lignified compounds (cereal straw in CMD and sawdust in PLD) that are only partially degradable under anaerobic conditions, in concordance with Tambone et al. (2009). On the other hand, PD presented a high NH_4^+-N/N ratio because it has easily biodegradable compounds, which is consistent with results presented in the review of Möller and Müller (2012). The co-digestion with onion increased the relation NH_4^+-N/N in all the cases. Onion waste contributes with a large amount of soluble sugars that improve the anaerobic degradation conditions, thus a greater proportion of organic matter of manures can be degraded, releasing more NH_4^+-N. In addition, approximately 40% of the total nitrogen of the onion corresponds to free amino acid (Aremu et al., 2011) that are rapidly degraded, contributing with NH_4^+-N. These results are in line with Zethner et al. (2002), Pötsch et al. (2004) and Emmerling and Barton (2007), who worked with co-digestion of manures with topinambour (*Helianthus tuberosus* L.). Additionally, the low ratio NH_4^+-N/N of CMD, OCMD and PLD coincides with a higher VS content, indicating a higher proportion of residual organic matter. The onion co-digestion decreased VS contents for OCMD and OPLD compared to CM and PL respectively. This last effect was not observed for OPD respect PS because it also has high proportions of easily degradable compounds and lower fibre content. The addition of onion to PS could generate an excess of soluble carbohydrates and antimicrobial products. The incorporation of onion did not generate variations in the content of S, possibly due to pH and anaerobic conditions that favour its volatilization as H_2S in the biogas mixture (Straka et al., 2007).

Anaerobic digestion tended to neutralize the pH, increasing it in the case of PS and onion waste and reducing it in the case of CM and PL.

All the liquid materials presented a high electrical conductivity, however much of this EC is the result of the nutrients concentration and its value should not be evaluated as the quality of water for irrigation (Möller, 2015).

The IR spectra of the amendments used show similar absorption zones (Table 2). The greatest differences were observed among the spectra of untreated materials (CM, PL and PS) (Fig. 1). An intensity change was observed of the band at 1650 cm^{-1} (PL ≈ PS > CM) and for the PS a widening of the band, overlapping at 1590 cm^{-1}; the presence of a wide band at 1457 cm^{-1}, and shoulder bands around 1505 cm^{-1} associated with lignin content (Pandey, 1999; Sun et al., 2001; Uçar et al., 2005) and 1426 cm^{-1}, absent in PS and reduced in CM; an intense and sharp band at 1397 cm^{-1} present only in PS, probably due to free ammonium and volatile fatty acids; a change in band intensity at 1030 cm^{-1} (CM > PL > PS) and a widening in this band in PS with two peaks at 1115 and 1074 cm^{-1}; and finally, two peaks at 870 and 850 cm^{-1} present only in PL. Additionally, in the PL spectra a shoulder band at 2500 cm^{-1} and a peak at 1450 cm^{-1} was observed, which confirms the presence of R-NH_3^- groups.

Anaerobic digestion produced marked changes in the functional groups present in the manures. Independently of the material of origin the anaerobic digestate showed greater uniformity among themselves.

After anaerobic digestion in CM, a reduction in shoulder band was observed at 1723 cm^{-1} and the band at 1645 cm^{-1}; an acute and well-defined peak at 1628 cm^{-1} and a broad-band centred on 1584 cm^{-1}; a strong band at 1397 cm^{-1}, a reduction in the band centred at 1030 cm^{-1}; and a band at 834 cm^{-1}(Fig. 1.a).

When comparing PL with PLD, a reduction in the band at 1648 cm^{-1} was observed; an intense and acute peak at 1625 cm^{-1} and a broad-band centred at 1596 cm^{-1}; an intense band at 1400 cm^{-1}; a reduction in the big band centred at 1030 cm^{-1}; a reduction in the band centred at 871 cm^{-1}, the disappearance of band around 856 cm^{-1}; and a band at 831 cm^{-1} (Fig. 1.b).

Anaerobic digestion generated smaller changes on PS (PD) than those observed in CM and PL. The reduction of bands at 1648 and 1597 cm^{-1} and the increase in the sharp band at 1628 cm^{-1} was observed; no major changes were observed in the band at 1397 cm^{-1}; an increase in peaks at 1346 and 1308 cm^{-1}; a reduction in the band centred at 1115 and 1074 cm^{-1}; no differences were observed in the band

Table 1
Chemical characterization of amendments.

Chemical properties	Units[a]	CMD	OCMD	PLD	OPLD	PD	OPD	PS	Units[b]	CM	PL
NH_4^+-N	mg dm^{-3}	181	690	508	1487	1011	2199	2240	mg kg^{-1}	515	1841
NO_3^--N	mg dm^{-3}	28.5	10.8	8.5	55.4	14.6	20.0	47.7	mg kg^{-1}	25.8	42.8
TKN	mg dm^{-3}	970	2134	1337	2361	1358	2695	3083	mg kg^{-1}	10,060	13,140
N	mg dm^{-3}	999	2145	1345	2416	1373	2715	3131	mg kg^{-1}	10,086	13,183
C	mg dm^{-3}	8870	5090	8680	5710	2160	3740	28,820	g kg^{-1}	304	343.3
P	mg dm^{-3}	40.8	14.4	52.0	50.0	25.2	56.4	460.0	g kg^{-1}	5.3	19.4
S	mg dm^{-3}	212.4	204.8	234.4	206.8	159.6	201.6	323.6	mg kg^{-1}	3200	18,100
NH_4^+-N/N		0.18	0.32	0.38	0.62	0.74	0.81	0.72		0.05	0.14
C/N		8.88	2.37	6.45	2.36	1.57	1.38	9.21		30.14	26.04
N/P		24.48	148.97	25.87	48.32	54.48	48.14	6.81		1.90	0.68
pH		8.06	7.55	7.65	7.73	7.63	7.64	5.1		9.4	9.3
EC	ds m^{-1}	21.40	20.76	19.12	20.57	12.35	19.62	23.15	ds m^{-1}	5.7	5.00
TS	g dm^{-3}	25.69	21.29	33.44	18.38	6.65	10.66	72.54	%	81.51	59.12
VS	g dm^{-3}	10.26	8.97	14.64	7.47	3.05	5.1	55.15	–	–	–

Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

[a] Wet weight basis.

[b] Dry weight basis.

Table 2
Location of principal indicator bands and their assignment to functional groups.

Location wavenumber (cm^{-1})	Vibration	Functional group
3100–3600	O—H stretch	Bonded and non-bonded hydroxyl groups. Phenols, alcohols and carboxylic acids.
	N—H stretch	Primary and secondary Amides and amines.
2920, 2850	C—H stretch	Aliphatic hydrocarbon structures and alkyl substitute groups
1740-1720	C═O	Aldehyde, ketone, carboxylic acids and esters
1640	C═O	Amide*I*, carboxylates
	C═C	Aromatic skeleton
1600	N—H in plane	Amines
1600–1590	C═C	Aromatic skeleton
1570–1540	N—H in plane	Amides II
1515–1505		Higher weight and hydrophobic aromatic and olefinic compounds. Lignin.
1460	C—H in plane	Aliphatic groups
1425	COO^- stretch	Carboxylic acids
1384–1400	N—O stretch	Nitrates and nitrites
	NH4	Free ammonium
	C—H in plane	Methyls and methylenes
1270–1220	C—O stretch	Carboxylic acids, esters, phenols and alcohols.
	C—N stretch	Amide III
1250–900	C—O—C, C—O	Polysaccharides, esters and hydroxy compounds
	C—O—P	Phosphodiesterase
	C—H in plane	Aromatic and alkenes
875	C—O out of plane	Carbonate
850–750	NH_2 out of plane	Primary amine group
	N—O stretch	Nitrates and nitrites
	C—H out of plane	Substituted and condensed aromatic systems
750–700	N—H wag	Secondary amine group

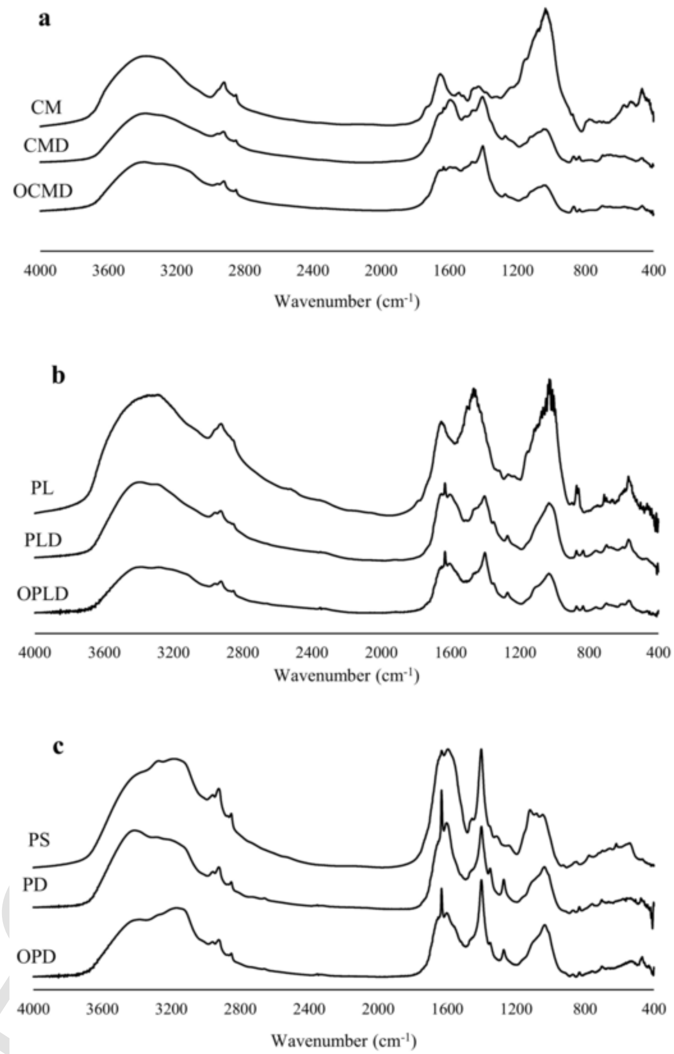

Fig. 1. IR spectra of wastes and their product of digestion and co-digestion. a) Cattle manure (CM, CMD and OCMD), b) poultry litter (PL, PLD and OPLD) and c) pig slurry (PS, PD, OPD).

at 1030 cm^{-1}, the band at 849 cm^{-1} disappeared and the definition of the two bands at 865 and 827 cm^{-1} increased (Fig. 1.c).

The analysis of the IR spectra, detailed in the preceding paragraphs, allows to infer that the anaerobic degradation generated: a reduction of the content of structural polysaccharide-type compounds (2920, 2850 and 1030 cm^{-1}, cellulose and hemicellulose type), aromatic compounds (1650 cm^{-1} band reduction) and high molecular weight olefinic compounds (1515–1505 cm^{-1}, lignin type); the increase of free ammonium (1625 and 1400 cm^{-1}), short-chain organic acids (1625 and 1397 cm^{-1}), amines and low molecular weight amides (1570–1600 cm^{-1} and 863 cm^{-1}). In three cases (CMD, PLD and PD), the largest proportion of aliphatic C were concentrated on the precipitation, indicating that they correspond to high molecular weight and/or insoluble compounds.

The co-digestion (OCMD, OPLD and OPD) produced little changes in comparison to digestion (CMD, PLD and PD) and they were similar in three materials (Fig. 1). These changes were: a reduction in bands at 1650 y 1584 cm^{-1}; an increase in the peak at 1630 cm^{-1}; a better definition of the band at 1397 cm^{-1}; and an increase in the peak at 865 cm^{-1}.

The overlap of bands mainly in the region 1500–1700 cm^{-1} (vibrations C═C and C═O, deformations N—H and O—H) and the displacement of bands produced by the complexity of the organic matrix impede the use of the absorbance relations 1650/2920 and 1650/2850 to estimate the degree of aromaticity, and the ratios 1034/2920, 1034/2850 and 1034/1540 to estimate the degree of polycondensation suggested by various authors for the study of humic acids (Amir et al., 2003).

3.2. Microbiota activation and soil C mineralization

The split-plot ANOVA presented interaction (ANOVA TxD; $F_{(120, 264)} = 64.16$; $p < 0.0001$), so each date was analysed independently (Table S1). The digestates showed a very intense CO_2 emissions in the first 6 h (Figs. 2 and 3), which indicate a faster microbiota activation. This is coincident with a previous study (Iocoli and Gómez, 2015). OPLD and OCMD generate lower CO_2 emissions than PLD and FLD respectively in this period ($p < 0.05$), whereas OPD did not differ to PD. This reduction of CO_2 emissions coincide with a reduction in C/N ratio, C content and VS value, and an increase in NH_4^+-N/N ratio proving that the lower CO_2 emissions was the result of the smaller contribution of organic matter (substrate for microorganisms). Other authors also reported a fast and short microbial stimulation after anaerobic digestate application to soil (Alburquerque et al., 2012; Grigatti et al., 2011; Marcato et al., 2009). After this short period, the emission was reduced to a value close to the control. The PS showed maximum activity between 18 and 42 h, while the CM and PL reached 90 h maintaining high values until the end of the assay. The rapid mineralization observed in PS versus CM and PL treatment could be due to: 1) the lower C/N ratio, similar to microorganisms C/N ratio (5) improving balance of energy of microorganisms; 2) presence of volatile fatty acids (described in IR characterization, section 3.1) that can be easily and rapidly degraded by microorganisms; and 3) higher inorganic N content (higher NH_4^+-N/N ratio) that can be rapidly assimilated by microorganisms improving organic matter degradation. In contrast, CM and PL contain a lignocellulosic fraction that is slowly degraded, consisting of cereal straw in the CM and sawdust in the case of PL.

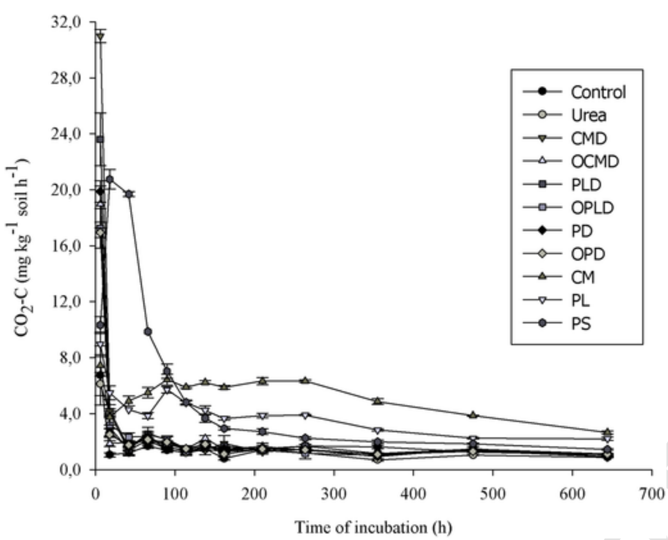

Fig. 2. Soil CO_2-C emission rate ($mg\,kg^{-1}\,soil\,h^{-1}$) during incubation. Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

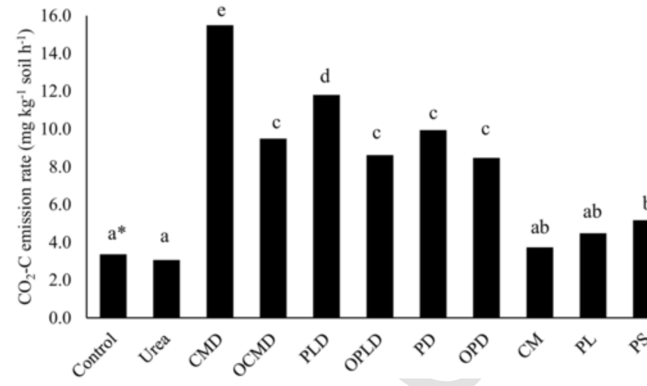

Fig. 3. CO_2-C emission rate ($mg\,kg^{-1}\,soil\,h^{-1}$) during the first 6 h of incubation. *different letters indicate significant statistical differences (LSD $p<0.05$) Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

At 644 h, CMD and PLD showed greater values than the control, probably as a result of the higher content of remaining organic matter (Fig. 4). The rest of digestates did not differ from the control. Unprocessed wastes produced the biggest CO_2-C release differing from each other and all the rest of amendments (1473, 1180 and 1014 mg CO_2-C kg^{-1} suelo for CM, PS and PL respectively; Fig. 4). The slighter PL CO_2-C release could be due to the lignificated structure of sawdust.

Analysing C mineralization kinetics by exponential model, unprocessed wastes (CM, PL and PS) were adjusted during the 644 h (Cmin: 68.7; 23.34 and 74.5% respectively), while the digestates were adjusted only during the first 210 h, when they reached the maximum value of Cmin (between 12 and 45%) (Table S2). The digestates, after 210 h showed less CO_2 emission with values close to the control and consequently, less Cmin. This allows suggest a possible C immobilization in the long term. In all digestates, the half-life was less than a day (0.2–0.65 days) confirming the presence of labile C, short chain organic acid type (in concordance with spectroscopic characterization) and a remnant of recalcitrant organic matter that was not degraded during incubation. Unprocessed wastes presented a half-life between 2 and 21 days (PS: 2; PL: 11 and CM: 21 days). The large differences between half-life in unprocessed wastes confirm their dif-

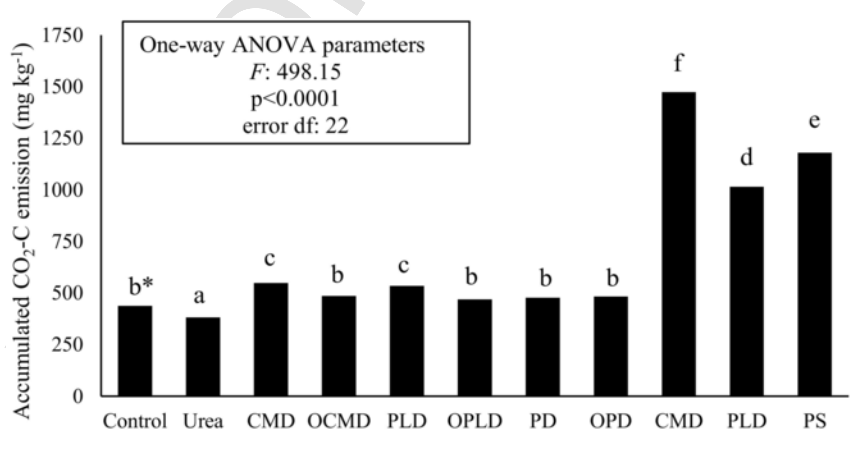

Fig. 4. Accumulated soil CO_2-C emission during 644 h of incubation with the different products. *different letters indicate significant statistical differences (LSD $p<0.05$) Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

ferent composition, while the similarity between half-life in the digestate notes the capacity of anaerobic digestion to generate more uniform products.

The anaerobic digestates used in this experiment had a prolonged period of residence (high hydraulic retention time in the biodigestor) maximizing its degradation. In most cases reported in the literature, the digestates applied come from industrial processes, where the search for the highest energy efficiency reduces the residence time. Consequently, the degradation of the organic matter is incomplete (Abdullahi et al., 2008; Salminen et al., 2001) and an unstable digestate is obtained. This can cause storage problems, unpleasant smells (Smet and Van Langenhove, 1998) and unfavourable impacts on the soil-plant system as a result of incorporating unstable materials to the agricultural land (Abdullahi et al., 2008; Alburquerque et al., 2012; Salminen et al., 2001).

3.3. Growth dynamic of Lactuca sativa L.

The different N availability among amended soils resulted in differences in growth dynamic (Fig. 5 and Table 3). The split-plot ANOVA presented interaction (ANOVA TxD; $F_{(35,126)}=3,87$; $p<0.0001$), so each date was analysed independently (Table S3).

Table 3 shows maximum modelled coverage area (MMCA), maximum real (MRCA), final real (FRCA) and time elapsed (days) to reach the maximum coverage area (TMCA). Control, PLD and OPD reached the MRCA at 42 days; CMD, OCMD, OPLD and PL at 49 days; and Urea, PD and PS at 60 days. Additionally, Control, CMD, PLD, OPLD, CM and PL showed a reduction in the CA between the maximum CA moment and the final of the experiment. This reduction is a consequence of the senescence of the basal leaves which may indicate a nutrient deficiency or imbalance. Treatments that did not reduce coverage area (Urea, OCMD, PS, PD and OPD) were separately analysed (split-plot ANOVA) showing no interaction (ANOVA TxD; $F_{(28,105)}=1.09$; $p=0.3615$), which indicates that they produce similar dynamics of growth in amended lettuce.

In every case, the co-digestion with onion wastes, improved the nutrient availability.

Lower growth and an earlier time to MRCA in Control, CMD, PLD, OPLD, CM and PL, indicated N deficiency during a large experiment period, probably due to less available N input and/or N immobilization. N immobilization could be explained by a large proportion of C associated to lignocellulosic compounds. In addition manures without process also present a high C/N ratio. The rest of the treatments presented a little deficiency in the last part of the trial. In addition during plant development phytotoxic symptoms were not observed in any treatment.

The greatest changes in coverage area among treatments occurred between day 35 and 42. Significant differences among treatments were detected only from day 42 onwards (Table S3), coinciding with the maximum absolute growth rates (AGR) (Fig. 5.b).

CMD reached the maximum relative growth rate (RGR) at 14 days, PS and PL at 28 days and the rest of treatments at 21 days (Fig. 5.c). However, this parameter (RGR) was less adequate to compare among treatments than the other parameters (AGR and sigmoidal curve, Fig. 5.a and b) given that the changes in relative growth are minor and could not be related to the lettuce production.

The CA at 60 days (Table S3) presented a strong positive linear adjustment (R^2: 0.95, $p<0,001$) with wet weight at harvest (Table 4), confirming that this parameter is a good indicator of vegetal development.

All treatment differ from control in in wet weight of leaves (WWL) and dry weight of leaves (DWL, Table 4). The WWL was the

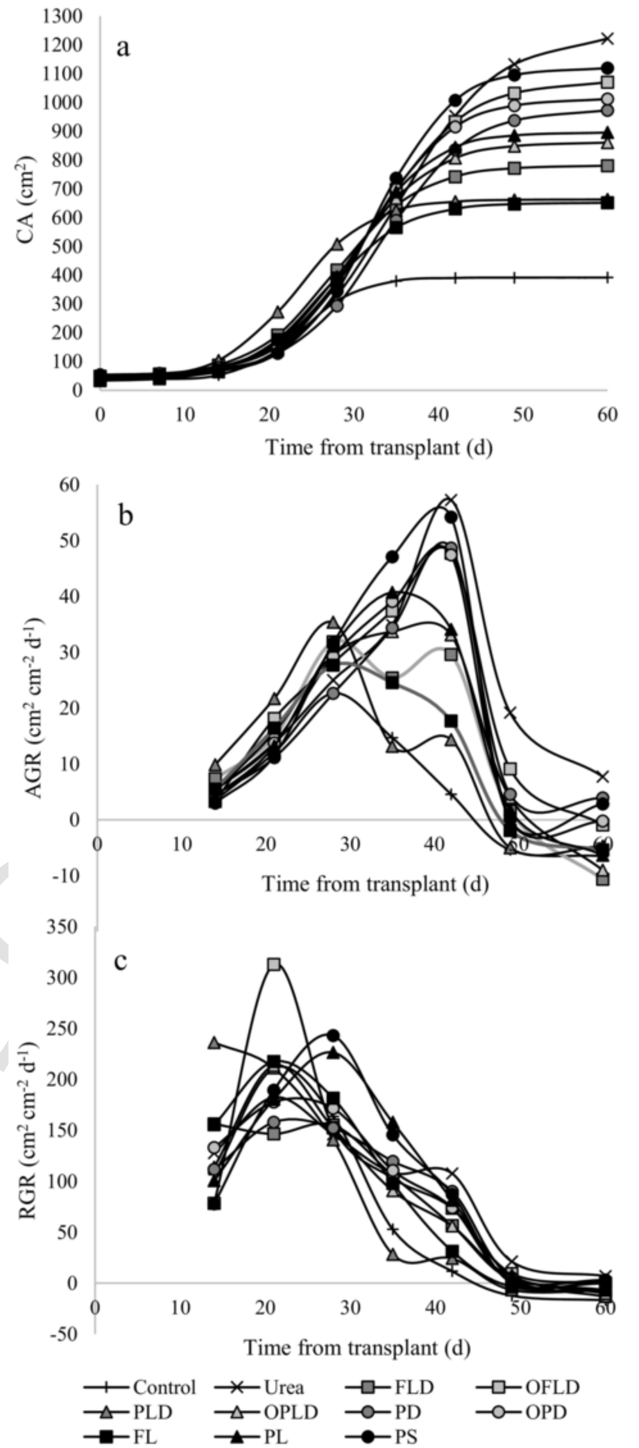

Fig. 5. Analysis growth of lettuce dynamic. (a) Sigmoidal curve fit of coverage area (CA, cm^2); (b) absolute growth rate (AGR, $cm^2\ d^{-1}$) and (c) relative growth rate (RGR, $cm^2\ cm^{-2}\ d^{-1}$). Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

variable that showed more clearly the differences between treatments. PS and Urea presented greater production, in concordance with Iocoli and Gómez (2015). In every case, the application of co-digestions products generated higher yields than digestion products

Table 3
Measured and estimated parameters from coverage area (CA). Maximum modelled coverage area (MMCA), final real coverage area (FRCA), maximum real (MRCA), time elapsed to reach the MRCA (TMCA), model coefficient (adj R^2) and standard error (SE).

	Control	Urea	CMD	OCMD	PLD	OPLD	PD	OPD	CM	PL	PS
MMCA	391.6	1222.3	780.5	1070.5	663.0	860.8	973.1	1012.3	651.4	895.6	1119.6
FRCA	325.8	1213.2	701.4	1037.0	619.0	791.4	963.4	981.4	603.5	840.2	1104.7
MRCA	434.9	1213.2	818.8	1047.1	706.1	890.6	963.4	984.1	676.7	910.1	1104.7
TMCA	42	60	49	49	42	49	60	42	42	49	60
adj R^2	0.9084	0.9520	0.8473	0.9597	0.9350	0.9005	0.9145	0.9618	0.8345	0.8174	0.9475
SE	47.54	102.62	125.42	84.59	65.12	110.59	114.31	78.75	111.17	167.85	105.47

Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

Table 4
Post-harvest determined vegetal variables.

	WWL (g)		DWL (g)		H (g)		DWR (g)		LN (leaves plant^{-1})	
Control	24.56	a*	2.14	a	91.32	ns	4.76	a	25	a
Urea	102.97	g	9.54	fg	90.71	ns	11.30	d	33	b
CMD	61.83	bcd	6.09	bcd	90.17	ns	6.20	ab	32	b
OCMD	89.01	f	8.17	efg	90.82	ns	7.49	abcd	34	b
PLD	57.05	bc	5.48	bc	90.34	ns	6.76	abc	33	b
OPLD	75.30	def	6.76	cde	91.15	ns	6.80	abc	32	b
PD	81.31	ef	7.51	de	90.82	ns	6.65	bcd	31	b
OPD	84.59	f	7.57	def	91.05	ns	5.95	ab	32	b
CM	50.45	b	4.71	b	90.71	ns	6.08	ab	32	b
PL	68.48	cde	6.87	cde	90.03	ns	9.05	bcd	31	b
PS	110.34	g	9.88	g	91.05	ns	10.48	cd	32	b

Control: unfertilized pots; Urea: synthetic fertilizer; CMD: cattle manure digestate; OCMD: onion cattle manure digestate; PLD: poultry litter digestate; OPLD: onion poultry litter digestate; PD: pig digestate; OPD: onion pig digestate; CM: cattle manure; PL: poultry litter; PS: pig slurry.

* Different letters indicate significant statistical differences (LSD $p < 0.05$).

(OCMD > CMD; OPLD > PLD and OPD > PD) and, CMD and PLD presented higher yields than CM and PL respectively. The yield increase probably was due to a larger proportion of inorganic nitrogen (NH_4^+-N/N ratio).

Regarding leaves number (LN), all treatments differed from the control, but not from each other, while no differences were observed in %H. The differences observed in DWR were less than those observed in DWL, probably because the treatments promoted principally the development of leaves. It is noteworthy that the pot condition limited the root's development.

The CA, WWL and DWL showed a strong positive linear adjustment to inorganic N input and the NH_4^+-N/N ratio of each amendment ($R^2 > 0.6$; $p < 0.001$). This suggests that the larger part of yield is a consequence of the initial content of inorganic nitrogen present in each amendment.

4. Conclusions

The manures (cattle manure, pig slurry and poultry litter) presented a complex composition and they differentiated between each other. Poultry litter and cattle manure presented a lower bio-degradability than pig slurry due to their larger proportion of lignificated compounds.

The anaerobic digestates (obtained by digestion and co-digestion) presented similar structural characteristics to each other, independently of the material of origin, characterized by a low C/N ratio, high NH_4^+-N/N ratio, greater proportion of short-chain organic acids and greater stability. The digestate dosage should be done according to the content of NH_4^+-N given that the vegetal growth is related to it. In contrast, the organic nitrogen is associated with recalcitrant organic structures and is not readily available for plants. Soils amended with anaerobic digestates showed less CO_2 emission than soils amended with manures and similar to the unamended soil.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.444.

Uncited reference

Parera i Pous et al., 2010

Acknowledgements

The authors are indebted to Olga Inés Pieroni (INQUISUR) for her collaboration on IR spectra interpretation, to Mariana Dennehy (INQUISUR) for allowing us to access her laboratory, to Pasdevicelli family for pig slurry, to Andrea Mairosser (EEA Ascasubi, INTA) for her collaboration on the onion waste collection and characterization, and to Gregorio Zabaloy for the revision of English grammar and syntax.

Funding

Financial support was provided by SGCyT, UNS: [Projects numbers PGI 24/A200 and PGI-TIR 24/TA12]; by Agencia Nacional de Promoción Científica y Tecnológica: [Project number PICT 2014-1760] and by Consejo Nacional de Investigaciones científicas y Técnicas [Project number P-UE 2016 CERZOS]. This research was part of GAI Ph.D. Thesis supported by a doctoral grant funded by the Consejo Nacional de Investigaciones Científicas y Técnicas: (CONICET-Argentina).

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