SJSS
SPANISH JOURNAL OF SOIL SCIENCE

Liquid digestate from organic residues as fertilizer: carbon fractions, phytotoxicity and microbiological analysis

Digestato líquido procedente de residuos orgánicos como fertilizante: fracciones de carbono, fitotoxicidad y análisis microbiológico
Digerido líquido proveniente de resíduos orgânicos como fertilizante: frações de carbono, fitotoxicidade e análises microbiológicas

AUTHORS

Yagüe M. R.[@]
mariarosa.yague@madrid.org

Lobo M. C.

[@] Corresponding Author

Departamento de Investigación Agroambiental, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA). Finca El Encín, Autovía A-2, km 38,2. Alcalá de Henares, E-28805 Madrid, Spain.

Received: 02.03.2020 | **Revised:** 27.10.2020 | **Accepted:** 09.11.2020

ABSTRACT

The circular economy promotes the use of renewable fuels as an alternative to natural gas. Anaerobic digestion for waste management produces methane, carbon dioxide and a residue-the digestate-which must be recovered. This residue can be separated into two parts, namely the liquid and solid fractions, the former characterized by its large volume, presence of nutrients in mineral forms, and highly variable composition. Here we studied the fertilizing capacity of the liquid fractions obtained from the waste derived from artichoke canning (LF-Ar), orange juice manufacturing (LF-Or) and pig slurry (LF-Sl). To this end, we examined the physical-chemical parameters, carbon fractions, phytotoxicity and presence of pathogens in these fractions. The liquid fraction derived from fruit and vegetables had a low nutrient content compared to that of slurry (~ 1.0 kg total-N m^{-3} *vs.* 5.6 kg total-N m^{-3} respectively). The NH_4^+-N content of the fractions ranged between 70-93% of total N. Given the permissible dose in non-vulnerable areas, LF-Sl, LF-Ar and LF-Or would provide 0.9-1.0 t of organic matter ha^{-1} and 134, 128 and 98 kg of C ha^{-1} from the total humic extract, respectively. The proportion of humic acids in the total humic extract was 59%, 51% and 34% respectively. The slurry digestate showed phytotoxicity probably due to high salinity, so it should be diluted based on the needs of the crop. On the basis of our findings, the characterized liquid fractions could be recovered in agricultural soils in line to circular economy principles.

RESUMEN

La estrategia de economía circular actualmente promueve el uso de combustibles renovables como alternativa al gas natural. La digestión anaeróbica para el tratamiento de residuos produce metano, dióxido de carbono y un residuo, el digestato, que debe valorizarse. Este residuo se puede separar en fracciones, líquido y sólido, caracterizándose la fracción líquida por su gran volumen, presencia de nutrientes en formas minerales y una composición muy variable. Se evaluó la capacidad fertilizante de tres fracciones líquidas obtenidas de diferentes sustratos de restos de conserva de alcachofas (LF-Ar), residuos de vegetales procedentes de empresas de zumos de naranja (LF-Or) y purines de cerdo (LF-Sl), analizando parámetros físico-químicos, fracciones de C, fitotoxicidad y presencia de patógenos. Las fracciones líquidas de frutas o verduras presentaron bajos contenidos en nutrientes en comparación con la fracción líquida de purines (~1,0 kg total-N m^{-3} vs. 5,6 kg total-N m^{-3} respectivamente). El contenido de

DOI: 10.3232/SJSS.2020.V10.N3.08

$N-NH_4^+$ osciló entre 70-93% del N total. Considerando la dosis permitida en áreas no vulnerables, LF-Sl, LF-Ar y LF-Or aportarían 0,9-1,0 t de materia orgánica ha^{-1} y 134, 128 y 98 kg de C en el extracto total húmico ha^{-1}, respectivamente. Las proporciones de ácidos húmicos respecto del extracto húmico fueron del 59%, 51% y 34%, respectivamente. El digestato de purines mostró fitotoxicidad que podría estar asociada a su elevada salinidad, por lo que se debería aplicar diluído de acuerdo con las necesidades del cultivo. En general, las fracciones líquidas caracterizadas podrían ser valorizadas en suelos agrícolas en línea con los principios de la economía circular.

RESUMO

A estratégia de economia circular promove atualmente o uso de energias renováveis como alternativa ao gás natural. A digestão anaeróbia para o tratamento de resíduos produz metano, dióxido de carbono e um resíduo, o digerido, que deve ser valorizado. Este resíduo pode ser separado em duas frações, líquida e sólida, caracterizando-se a fração líquida pelo seu grande volume, presença de nutrientes em formas minerais e uma composição muito variável. Neste trabalho foi avaliada a capacidade fertilizante das frações líquidas obtidas a partir de resíduos de conservas de alcachofra (LF-Ar), da produção de sumo de laranja (LF-Or) e de chorumes de suínos (LF-Sl) analisando os parâmetros físico-químicos, frações de carbono, fitotoxicidade e presença de patogénicos nessas frações líquidas. As frações líquidas de fruta e vegetais apresentaram baixa concentração em nutrientes em comparação com a fração líquida dos chorumes (~ 1,0 kg total-N m^{-3} vs. 5,6 kg total-N m^{-3}, respectivamente). A concentração de $N-NH_4^+$ variou entre 70-93% do N total. Considerando a dose permitida em áreas vulneráveis, LF-Sl, LF-Ar e LF-Or forneceriam 0,9-1,0 t de matéria orgânica ha^{-1} e 134, 128 e 98 kg de C no extrato total húmico ha^{-1}, respetivamente. A proporção de ácidos húmicos nos extratos húmicos totais foi de 59%, 51% e 34% respetivamente. O digerido de chorume apresentou fitotoxicidade que poderá estar relacionada com a sua elevada salinidade, pelo que se deveria aplicar diluído de acordo com as necessidades da cultura. No geral, as frações líquidas caracterizadas poderiam ser valorizadas em solos agrícolas, estando assim em linha com os princípios da economia circular.

KEYS WORDS
Anaerobic digestates, humic acids, germination index, organic fertilization, nitrogen fractions, pathogens.

PALABRAS CLAVE
Digestatos anaeróbicos, ácidos húmicos, índice de germinación, fertilización orgánica, fracciones de nitrógeno, patógenos.

PALAVRAS-CHAVE
Digeridos anaeróbios, ácidos húmicos, índice de germinação, fertilização orgânica, frações de nitrogénio, patogénicos.

1. Introduction

In line with the circular economy strategy, the recycling of organic material derived from agriculture and the food industry into fertilizers is an environmental option that involves the recovery of nutrients and carbon (C). Anaerobic digestion is a method of treating biodegradable mono- or co-substrates (for example, livestock slurry, and the by-products of agriculture and food-processing plants) in the absence of oxygen. This process releases methane to provide heat and power and produces a raw digestate.

Biogas production through anaerobic digestion is growing rapidly in the agricultural sectors of many European countries (Monlau et al. 2015). The use of the liquid fraction of this digestion as fertilizer is the most popular option for its disposal (Webb et al. 2013). However, applications for this fraction are hampered by its highly variable composition (Coelho et al. 2018). Such variation is attributable to the inoculum composition, operating conditions of the anaerobic digestion (i.e. pH, temperature, retention time, organic loading, moisture, C/N ratio) (Náthia-Neves et al. 2018), changes during storage such as emission losses (Monlau et al. 2015), and characteristics of the original substrates (Akhiar et al. 2017).

Anaerobic digestion for biogas production leads to several alterations in the composition of the resulting digestates compared to the original feedstock (NH_4^+-N content, pH, C/N ratio, etc.) and these changes are relevant for plant availability of macro- and micro-nutrients after field application (Möller and Müller 2012; Nkoa 2014). Organic matter undergoes several changes during anaerobic digestion. In this regard, humic acids (HAs) and fulvic acids (FAs) can be used by anaerobic microorganisms, and degradation is determined by the nature of the organic substances present (Huan et al. 2017).

The separation of raw digestate produces a liquid and a solid product, which need to be handled and stored separately (Möller and Müller 2012), commonly on-site. The characteristics of these products differ greatly. The solid fraction (SF) often comprises approximately 20-25% of the total digestate fresh volume and has a high dry matter (DM) content. The liquid fraction (LF) is characterized by a high volume and low DM (< 6.5%) and high N (76-94%) content (Tambone et al. 2017). Between 45-80% of the total N in the LF is present as NH_4^+-N (Möller and Müller 2012).

The evaluation of the physico-chemical properties and fertilizing capacity of LFs allows farmers to devise a fertilization strategy adjusted to crop requirements. In addition, the phytotoxicity of LFs should be studied before application. Seed germination can be affected by organic immaturity (toxins), the presence of NH_4^+, an indeterminate number of substances, either alone or combined (Zucconi et al. 1985), and/or high electrical conductivity (Coelho et al. 2018). The Zucconi phytotoxicity test is an effective and economical bioassay to assess the toxicity of an organic substance before use (Luo et al. 2018). The presence of pathogens such as *Salmonella spp* and *Escherichia coli* are indicators of health risks associated with the use of wastewater for agricultural purposes (e.g. sprinkle irrigation risk, WHO 2006).

The purpose of this work was to study the composition of liquid fractions of digestates from three different sources and assess the feasibility of using them as fertilizers. To this end, we examined their physico-chemical parameters and phytotoxicity, and also determined the presence of pathogens.

2. Materials and Methods

2.1. Source of liquid digestates

The first LF (LF-SI) was obtained from a digestate produced from a mixture of tobacco powder, cereal powder (pre-cleaned maize residue, broken kernels) and slurry from a pig fattening farm. The digestate was passed through a 750-µm sieve to obtain FL-SI. The original proportion of solids in the anaerobic digestion comprised 67% tobacco powder and 33% cereal powder, to which 20 m^3 of pig slurry from commercial plants was added. The digestion temperature was kept constant at approximately 35 ºC (mesophilic range) during the process (between 70-80 days) with stirring/agitation.

The other two LFs were obtained by mixing inoculum and fruit waste (LF-Or), and inoculum and vegetable waste (LF-Ar). The former comprised (expressed in weight) 98% inoculum and 3% orange juice remains (orange peel) and the latter 60% inoculum, 35% water and 5% canned artichoke remains (peduncle, leaves and stem). In both LFs, the inoculum was obtained from maternity pig slurry and shovels of prickly pears residue (weight ratio 7:3 respectively). The digestion temperature was kept constant at approximately 38 ºC (mesophilic range) during the process (73 days) with stirring. These last LFs were sourced from the Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CITYTEX), where they use sequential anaerobic digestion to produce a raw digestate with a very low DM content (< 1%, non-separated fractions), equivalent to conventional LFs derived from anaerobic digestion.

The origin and composition of the mixing materials and the procedure used in the anaerobic digestion for preparing the LFs were always the same. The composition of each LF was stable between batches over time, thereby making the product obtained relatively stable and homogeneous. Three replicates of each LF were sampled. Samples were stored under refrigeration (4 ºC) until analysis.

2.2. Physicochemical parameters

We studied the following chemical parameters of the LFs: pH and electrical conductivity (EC) in fresh samples; total-N (Kjeldahl method); dry matter (DM, 105 °C); and organic matter (OM, 580 °C) (MAPA 1984). Organic-N was calculated by the difference between total-N and mineral-N (as the sum of NH_4^+-N, ureic-N, and NO_3^--N). The contents of NH_4^+-N, ureic-N, NO_3^--N, total-P and P were extracted with water and citrate (AOAC 2010). Exchangeable cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) were determined by MAPA (1994).

Organic carbon (OC) was fractionated using the method described by Dabin (1971), which allows the separation of humic acids (C-HA) from the total humic extract (C-THE). The percentage of C from each fraction was determined by oxidation using potassium dichromate (Walkley and Black 1934), assuming that 77% of total C was oxidizable.

We studied the following physical parameters of the LFs: bulk density (weight per unit volume occupied in a graduated cylinder) and particle size distribution, by filtration through metal sieves (2000, 1000 and 200 µm) and a nylon filter (20 µm). The trays were washed with distilled water between each sieve filtration to ensure that all particles smaller than the sieve pore size were collected in the filtrate. The filters were cleaned as soon as the flow ceased. When the flow rate decreased, indicating pore-plugging, the sieve was discarded. The total solids in each sieve were determined by the dry bulk weight at 105 °C.

2.3. Phytotoxicity test and microbial analysis

The phytotoxicity test was carried out using the method described by Zucconi et al. (1985), utilizing cress seeds (*Lepidium sativum*). A 1:10 water solution (liquid fraction: hot distilled water 65 °C, 30 min.; w:v) was added to Petri dishes and 20 seeds were incubated in the dishes at 25-26 °C for 48 h in the dark. A control using distilled water was used. This procedure was carried out in triplicate for each LF. After that, germinated seeds were counted and the radicles length were measured to calculate a germination index **[GI, Eq.1]**:

$$GI = \left[\left(\frac{GS}{GC}\right) * \left(\frac{LS}{LC}\right) \right] * 100 \qquad \textbf{[Eq. 1]},$$

where GS corresponds to germinated seeds in the LF treatment, GC to germinated seeds in the control treatment (with distilled water), LS to radicle length in the LF treatment, and LC to radicle length in the control treatment. A GI > 80% indicates the disappearance/absence of phytotoxins in the material under study (Zucconi et al. 1985).

With respect to the risk of pathogens in the LFs, we checked for the presence of *Escherichia coli* (most probable number; MPN; ISO-7251:2005) and *Salmonella spp* (UNE-EN ISO 6579-1:2017).

2.4. Statistical analysis

Duncan's test was performed to determine whether the treatments showed significant differences (p < 0.05) based on analysis of variance (ANOVA). The statistical analysis was performed using the SAS software, version 9.3 (SAS Institute 1999-2001).

3. Results and Discussion

The pH values (**Table 1**) in the LFs proceeding from vegetables and fruits (LF-Ar and LF-Or) and from pig slurry (LF-Sl) were similar. However, the EC was significantly higher in LF-Sl (25.2 dS m^{-1}) than in LFs from fruits-vegetables (10.5 and 9.0 dS m^{-1} for LF-Ar and LF-Or, respectively).

Concerning DM, OM, nutrients and density, the values were very low in LF from fruits-vegetables (LF-Ar and LF-Or) compared to LF-Sl (**Table 1**). This finding indicates the low fertilization capacity per fresh unit volume of LF-Ar, and particularly of LF-Or. The ratio organic-C/total-N was low and similar in all the FLs (< 3). These data are in agreement with the findings of other studies (Coelho et al. 2018; Tambone et al. 2017).

The NH_4^+-N was the main form of N present in the LFs, accounting for between 70 to 93% of total N (**Table 1**). This form is available to crops and susceptible to soil loss (Webb et al. 2013). The ureic-N and NO_3^--N forms were unappreciated for use as a fertilizer. The ratio of major nutrients (expressed as N-P_2O_5-K_2O) referring to fresh digestate for fertilization use was 1-0.3-0.5, 1-1-1.1, and 1-0-0.8 for LF-SI, LF-Ar and LF-Or, respectively.

Based on an application rate of 210 kg total-N ha^{-1} yr^{-1} (the maximum field N rates permitted for the application of organic sources in non-vulnerable zones, Council Directive 91/676/EEC), soil fertilization with LF-SI, LF-Ar, and LF-Or would correspond to applications of 38 m^3 ha^{-1}, 198 m^3 ha^{-1} and 280 m^3 ha^{-1} respectively.

The three LFs provided between 1.0 and 0.9 t OM ha^{-1} (equivalent to 0.5-0.6 t OC ha^{-1}), according to rates calculated on a normative basis. These values involves the addition of 134, 128 and 98 kg OC ha^{-1} from C-THE by LF-SI, LF-Ar and LF-Or, respectively, and 59%, 51% and 34% of C-THE from C-HA.

When nutrient contents are very low and high rates of LF-Ar and LF-Or application are required, conventional road transport would be questionable because of its economic and associated environmental costs. In this context, the irrigation option by a direct injection irrigation system could then be an interesting alternative. However, the effects of spreading LFs via irrigation on N emissions (NO_X, NH_3) should be evaluated, as well as the costs of connecting an irrigation system to LFs, and the availability of field plots near plants where digestates are produced.

This option might increase the window of time for organic residue application (e.g. maize), using NH_4^+-N mainly for crops. Other factors to consider would be the requirement to solve physical, chemical and biological aspects of the composition of LFs, which may clog irrigation systems (i.e. nozzle-sprinkler and drip irrigation) due to large particle sizes (mainly organic forms) and pH (mixed water and digestate). The particle size of LFs from wastewater should be taken into consideration, as well as the irrigation system and filter. Further precautions should also be taken when considering the LFs for agricultural purposes, including sprinkle installation of granular filters or strainers, and enlargement of the diameters of the nozzles to preferably no less than 5 mm in diameter (Pescod and Alka 1985). The LF-SI contained 45% total solids, the particles of which were up to 200 µm in diameter, while such particles accounted for only 8% and 5% of total solids in LF-Ar and LF-Or, respectively. The pH value of the LFs was around 8, and their cation content should be evaluated to avoid clogging by salt precipitates according to the quality of irrigation water.

Neither *Salmonella spp*. nor *E. coli* was detected in the three LFs obtained in the mesophilic process. Pathogens are rapidly inactivated by heat; this can be achieved by a combination of short time and high temperature. Other authors have observed the pathogens reduce or absence in mesophilic range (Rajagopal et al. 2019). Furthermore, other parameters such as an acidic or alkaline pH, ammonia concentration, and presence of volatile fatty acids were found to enhance *Salmonella* elimination during anaerobic digestion (Jiang et al. 2018; Seruga et al. 2020). Other authors have reported *E. coli* inactivation in mesophilic processes (37 ºC) of > 40 days (Pandey and Soupir 2011).

This anaerobic digestion therefore contribute to producing safe products that can be handled and used as fertiliser, particularly in sprinkle or pivot irrigation application.

In relation to phytotoxicity, the Zuconni test ($EC_{1:10}$, w:v), indicated that the LF-SI fully suppressed germination (**Figure 1**). This digestate showed high EC (**Table 1** and $EC_{1:10}$: 3.30 dS m^{-1}) compared with the other two (**Table 1** and $EC_{1:10}$: 1.12 and 0.91 dS m^{-1} for LF-Ar and LF-Or respectively). When a digestate suppresses seed germination because of high EC (Coelho et al. 2018), its phytotoxic effect during early growth (germination) is caused mainly by salinity (Alburquerque et al. 2012). However, other factors, such NH_4^+-N concentration and volatile fatty acid content (Salminen et al. 2001; Tambone et al. 2017) and/or hydrosoluble toxic substances produced during the anaerobic process (Zucconi et al. 1985), could contribute to inhibiting germination. Seed germination can be increased by diluting the digestate (Möller and Müller 2012), and a further dilution may solve the problem, i.e. if the digestate is applied together with irrigation water.

Table 1. Characterization of liquid digestates (fresh samples): physicochemical parameters, carbon fractions, phytotoxicity index and pathogens present. Mean values ± standard deviation

Parameters	LF-SI	LF-Ar	LF-Or	Significance
Chemical				
pH¶	8.11±0.05	7.96±0.03	8.06±0.04	ns
Electrical conductivity (dS m^{-1})¶	26.1±0.3	10.5±0.1	9.0±0.1	***
Dry matter (%)¶¶	6.17±0.04a	0.89±0.05b	0.67±0.01c	***
Organic matter (g kg^{-1})¶¶	25.89±0.54a	4.54±0.18b	3.57±0.15b	***
Total-N (g kg^{-1})¶¶	5.56±0.03a	1.08±0.07b	0.77±0.01c	**
NH_4^+-N (g kg^{-1})	3.9	1.0	0.6	-
Ureic-N (g kg^{-1})	nd	nd	nd	-
NO_3^--N (mg kg^{-1})	7.0	0.8	0.5	-
Organic -N (g kg^{-1})	1.66	0.08	0.17	-
Total-P (g kg^{-1})	0.75	0.48	nd	-
P-ext water and citrate (g kg^{-1})	0.70	nd	nd	-
Cations exchange (g kg^{-1})				
K^+	2.28	0.96	0.54	
Ca^{2+}	0.43	0.09	0.05	-
Mg^{2+}	0.06	0.05	0.05	-
Na^+	0.28	0.17	0.08	-
Carbon fractions (g kg^{-1})¶¶				
Total humic extract	3.54±0.24a	0.64±0.11a	0.35±0.01a	***
Humic acids	2.08±0.20a	0.33±0.06a	0.12±0.01a	***
Microbiological				
Salmonella spp (CFU 25 g^{-1})	Absence	Absence	Absence	-
E. coli (NMP g^{-1})	Absence	Absence	Absence	-
Physical				
Density (kg m^{-3})¶¶	1041±5a	1001±2b	1002±2b	***
Size particles (% total solids)¶				
≥ 2000 µm	0.85±0.09a	0.01±0.01b	0.01±0.01b	***
< 2000-1000 µm	0.64±0.07a	0.02±0.01b	0.01±0.01b	***
< 1000-200 µm	1.26±0.11a	0.04±0.00b	0.02±0.00b	***
< 200-20 µm	3.41±0.11a	0.06±0.01b	0.06±0.01b	***
< 20 µm	-	0.71±0.01a	0.60±0.01b	***
∑Total solids	6.16±0.03	0.83±0.02	0.70±0.02	-

¶n=2; ¶¶n=4, number the replicates for each sample. Shadow determinations were analysed by an external laboratory. CFU: colony-forming units. MPN: most probable number. nd: not detectable. Different letters indicate significant differences between treatments $p < 0.05$, ns: not significant $p > 0.05$.

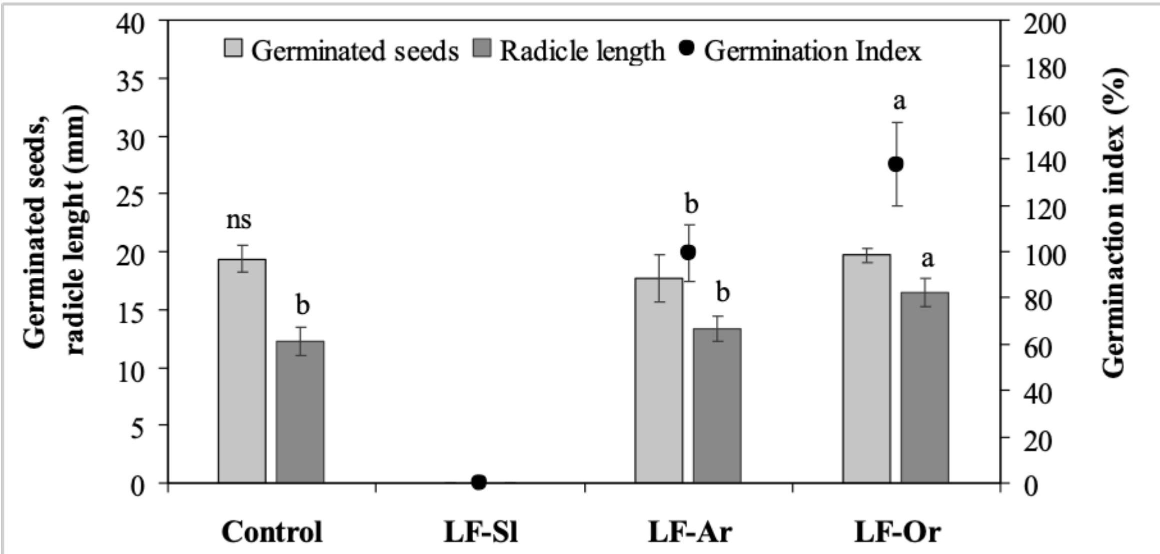

Figure 1. Mean values (n = 3±standard deviation) of phytotoxicity according to the parameters of the Zucconi test: seed germination, radicle length and germination index in *Lepidium sativum*. Different letters indicate significant differences between treatments $p < 0.05$; ns: not significant $p > 0.05$.

4. Conclusions

This study reveals considerable differences between the anaerobic digestates from fruits-vegetables *vs.* pig slurry in terms of nutrients and physicochemical characteristics. The origin of the LFs had serious effects on composition variability from vegetables and fruits (LF-Ar and LF-Or) compared to pig slurry (LF-Sl). Given such high variability, we strongly recommend analysis of the digestates before use as fertilizers. Moreover, to establish safe application rates of these materials for crop agriculture, their phytotoxicity indices should be determined prior to use. On the basis of our findings, the characterized liquid fractions could be recovered both in terms of the effects on soil surface (e.g. deposit of organic materials) and on the crop (e.g. risk to agricultural soils), in line with circular economy principles taking into account their possible effects on different types of irrigation systems (e.g. clogging). The salinity of the liquid fractions should be evaluated before its use in surface irrigation.

5. Acknowledgements

The authors thank the staff of the Agroenvironmental soil laboratory at the Instituto Madrileño de Investigación y Desarrollo Rural (IMIDRA), the Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CITYTEX) and the Instituto Tecnológico Agrario de Castilla y León (ITACYL) for providing the digestates.

This work was funded by a NEIKER-IMIDRA contract in the framework FEDER-INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria), under the Project RTA 2015-00060-C04-04. Dr M.R. Yagüe was supported by a postdoctoral contract (DOC-INIA, grant number DOC 2015-021) funded by INIA and the European Social Fund.

REFERENCES

• Akhiar A, Battimelli A, Torrijos M, Carrere H. 2017. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Manage. 59:118-128. DOI: 10.1016/j.wasman.2016.11.005.

• Alburquerque JA, De la Fuente C, Ferrer-Costa A, Carrasco L, Cegarra JH, Abad M, Bernal MP. 2012. Assessement of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass Bioenerg. 40:181-189. DOI: 10.1016/j.biombioe.2012.02.018.

• AOAC. 2010. Association Official Analytical Chemists. Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition. Washington, DC.

• Council Directive 91/676/EEC of 12 December concerning the protection of waters against pollution caused by nitrates from agricultural sources. Off. J. Eur. Communities: L375. Brussels, Belgium.

• Coelho JJ, Prieto ML, Dowing S, Hennessy A, Casey I, Wookcock T, Kennedy N. 2018. Physical-chemical traits, phytotoxicity and pathogen detection in liquid anaerobic digestates. Waste Manage. 78:8-15. DOI: 10.1016/j.wasman.2018.05.017.

• Dabin B. 1971. Étuded'uneméthode d'extraction de la matière humique du sol. Science du Sol 1:47-63.

• Huan L, Youkang L, Chenchen L. 2017. Evolution of humic substances during anaerobic sludge digestion. Environ Eng Manag J. 16(7)1577-1584. DOI: 10.30638/eemj.2017.171.

• ISO 7251:2005 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of presumptive *Escherichia coli* -- Most probable number technique.

• Jiang Y, Dennehy C, Lawlor PG, Huc Z, Yang Q, McCarthy G, Pin Tan S, Zhan, Gardiner GE. 2018. Inactivation of Salmonella during dry co-digestion of food waste and pig manure. Waste Manag. 82:231-240. DOI: 10.1016/j.wasman.2018.10.037.

• Luo Y, Liang J, Zeng G, Chen M, Mo D, Li G, Zhang D. 2018. Seed germination test for toxicity evaluation of compost: its roles, problems and prospects. Waste Manage. 71:109-114. DOI: 10.1016/j.wasman.2017.09.023.

• MAPA. 1994. Métodos oficiales de análisis. Tomo III: Métodos oficiales de análisis de suelos y aguas para el riego. Madrid: Servicio de Publicaciones del Ministerio de Agricultura, Pesca y Alimentación. p. 205-285.

• Möller K, Müller T. 2012. Effects of anaerobic digestion on digestate nutrient and availability and crop growth: a review. Eng Life Sci. 12:242-257. DOI: 10.1002/elsc.201100085.

• Monlau F, Sambusiti C, Ficara E, Aboulkas A, Barakat A, Carrère H. 2015. New opportunities for agricultural digestate valorization: current situation and perspectives. Energ Environ Sci. 8:2600-2621. DOI: 10.1039/C5EE01633A.

• Náthia-Neves G, Berni, M, Dragone, G, Mussatto SI, Forster-Carneiro T. 2018. Anaerobic digestion process: technological aspects and recent developments. Int J Environ Sci Te. 15(9):2033-2046. DOI: 10.10007/s13762-018-1682-2.

• Nkoa R. 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agronomy for Sustainable Development 34(2):473-492. DOI: 10.1007/s13593-013-0196-z.

• Pandey PK, Soupir ML. 2011. *Escherichia coli* inactivation kinetics in anaerobic digestion of dairy manure under moderate mesophilic and thermophilic temperatures. AMB Express 1:18. DOI: 10.1186/2191-0855-1-18.

• Pescod MB, Alka U. 1985. Guidelines wasterwater reuse in agriculture. In: Pescod MB, Arar A, editors. Treatment and use of sewage effluent for irrigation. Proceedings of the FAO Regional Seminar on the treatment and use of sewage effluent for irrigation; 1985 Oct 7-9; Nicosia, Cyprus.

• Rajagopal R, Ghosh D, Ashraf S, Goyette B, Zhao X. 2019. Effects of low-temperature dry anaerobic digestion on methane production and reduction in dairy cow manure. Int J Environ Sci Te. 16:4803-4810. DOI: 10.10007/s13762-019-02291-w.

• Salminen E, Rintala J, Härkönen J, Kuiyunen, M, Högmander H, Oikari A. 2001. Anaerobically digested poultry slaughterhouse waster as fertiliser in agricultura. Biores Technol. 78:81-88.

• SAS Institute Inc. 1999-2001. SAS/TAT. Software V 8.2 Cary. NC.

• Seruga P, Kzywonos M, Paluszak Z, Urbanowska A, Pawlak-kruczek H, Niedzwiecki L, Pinkowsa H. 2020. Pathogen reduction potential in anaerobic digestion of organic fraction of municipal solid waste and food waste. Molecules 25:275. DOI:10.3390/molecules25020275.

• Tambone F, Orzi V, D'Imporzano G, Adani F. 2017. Solid and liquid fractionation of digestate: mass balance, chemical characterization, and agronomic and environmental value. Bioresource Technol. 243:1251-1256. DOI. 10.1016/j.biortech.2017.07.

• UNE-EN ISO 6579-1:2017. Microbiología de la cadena alimentaria. Método horizontal para la detección, enumeración y serotipado de Salmonella. Parte 1: Detección de *Salmonella spp*. (ISO 6579-1:2017).

• Walkley A, Black IA. 1934. An examination of the Degtjareff method for determining soil organic carbon matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.

• Webb J, Sørensen P, Velthof G, Amon B, Pinto M, Rodhe L, Reid J. 2013. An assessment of the variation of manure nitrogen efficiency throughout Europe and an appraisal of means to increase manure-N efficiency. Adv Agron. 119:371-442. DOI: 10.1016/B978-0-12-407247-3.00007-X.

• WHO. 2006. Wastewater use in agriculture. Guidelines for the safe use of wastewater, excreta and greywater, Vol. 2. Geneva: WHO.

• Zucconi F, Monaco A, Forte M, De Bertoldi M. 1985. Phytotoxins during the stabilization of organic matter. In: Gasser JKR, editor. Composting of agricultural and other wastes. Elsevier. p. 73-85.