



# 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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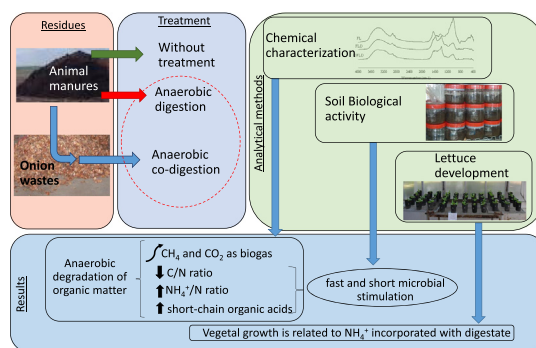
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## HIGHLIGHTS

- Anaerobic digestates showed similar or organic structural characteristics.
- Digestate soil application improved the overall C balance with respect to manures.
- Co-digestion with onion wastes improved nutrient availability for lettuce plants.
- The larger part of yield is the result of the initial content of inorganic nitrogen.
- The digestate dosage should be done according to the content of  $\text{NH}_4^+$ -N.

## GRAPHICAL ABSTRACT



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## 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  $\text{NH}_4^+$ -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  $\text{CO}_2$  during the first 6 h, digestates vs. rest of treatments). The digestate dosage should be done according to the content of  $\text{NH}_4^+$ -N given that the vegetal growth is related to it. Soils amended with digestates showed less  $\text{CO}_2$  emission than soils amended with manures improving overall C balance.

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**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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## 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  $10.5 \text{ kg habitant}^{-1} \text{ year}^{-1}$  (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., 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  $\text{CO}_2$  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),  $0.44 \text{ ds m}^{-1}$ , total organic carbon (C),  $12.6 \text{ g kg}^{-1}$ ; NTK,  $1.34 \text{ g kg}^{-1}$ ;  $\text{NH}_4^+-\text{N}$ ,  $5.6 \text{ mg kg}^{-1}$ ; and  $\text{NO}_3^--\text{N}$ ,  $4 \text{ mg kg}^{-1}$ .

The cattle manure was obtained from a feed-lot located in Villarino, Buenos Aires province (geo-coordinates South  $38^\circ 42' 12''$  West,  $62^\circ 27' 41''$ ); the poultry litter, from an eggs production farm in Bahía Blanca, Buenos Aires province (geo-coordinates South  $38^\circ 39' 03''$  West,  $62^\circ 16' 57''$ ); and the pig slurry, from a pig farm in Coronel Pringles, Buenos Aires province (geo-coordinates South,  $37^\circ 46' 27''$  West,  $61^\circ 30' 44''$ ). 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,  $39^\circ 23' 02''$  West,  $62^\circ 37' 29''$ ). 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),  $3.1 \text{ ds m}^{-1}$ , total organic carbon (C),  $3.0 \text{ g kg}^{-1}$  and TKN,  $1.9 \text{ g kg}^{-1}$  (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  $20 \text{ dm}^3$  plastic contained and was kept at  $4^\circ \text{C}$  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 digesters were performed under laboratory conditions in  $2 \text{ dm}^3$  batch-type digesters without inoculum, within

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