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Farmers' and academia's views"

Comparative characterization of humic substances obtained from anaerobic digestate of horticultural residues

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Abstract

Currently, agriculture has as one of its main objectives the reduction of the use of agrochemicals, since their constant application causes damage to the environment and human health. Among the products that have been used to increase crop yields are bio-stimulant products. Humic substances (HS) have been recognized for their bio-stimulant action and direct impact on plant physiology. One of the ways of obtaining the HS is from the anaerobic digestate obtained by anaerobic digestion. Compared to commercial products, the HS from anaerobic digestate contain a wider variety of organic substances, more lipids, more nitrogen and a lower degree of oxidation. In this study, the HS obtained from anaerobic digestate of horticultural crop residues for their use in agriculture were characterized. The anaerobic digest samples were subjected to a basic treatment with sodium hydroxide (NaOH) at a concentration of 0.1 mol/L and a solid-liquid ratio of 1/10, which allowed the separation of humic and humin substances by centrifugation. Then, the separation of humic acid (HA) and fulvic acid (FA) by acid extraction with hydrochloric acid (HCl) was performed. The physical-chemical characterization of the HS showed the high potential fertilizer value due to their contents of N, P, K, and micronutrients. However, the proportions of N-P-K in both HS were widely variable; meanwhile, the micronutrients were below the recommended limits for the concentrations of potentially toxic elements. In conclusion, the HS obtained from the anaerobic digestate showed substantial differences in terms of nutrients and physicochemical characteristics. Future perspectives indicate that nutrient variability in bio-based fertilizers will be one of the greatest challenges to address in the future utilization of these products.

Keywords: Anaerobic digestate, horticultural residues, humic substances

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Introduction

In the last two decades, agriculture has aimed to reduce the use of agrochemicals because the constant application causes damage to human, animal and environmental health (1). Therefore, environmentally friendly alternatives with low production costs have been developed. Among the products that have been used to increase crop yields are biostimulant products (2). When biostimulant products are applied to plants or culture media, the potential to modify the physiology, promote growth and increase plant production has been demonstrated. The HS are recognized for their biostimulant action and direct impact on plants. Direct effects are understood as actions that are not mediated by soil characteristics or nutrient availability, but involve regulation of cellular activity, metabolic changes, altered gene expression, and hormonal action (3). One of the ways to obtain HS is from the anaerobic digestate produced by anaerobic digestion (AD). The HS, including HA and FA, are the main components of the organic substances of the anaerobic digestate. Compared to commercial HS, the humic substances from anaerobic digestates have a wider variety of organic substances, more lipids and nitrogen, as well as a lower degree of oxidation. As a consequence, the HA and FA could be extracted from anaerobic digestates as a new source of liquid organic fertilizer. (4). Therefore, the objective of this research was to characterize the HS extracted from the anaerobic digestate of horticultural residues.

Material and Methods

Completely mixed anaerobic bioreactors of 500 mL total volume were used in three times, with a hydraulic retention time (HRT) of 45 days. As a substrate for AD, a mixture of crop residues (leaves, stems and roots) from tomato, lettuce, onion, Chinese cabbage, carrot, beet and cabbage was used. One hundred milliliters of inoculum and 300 mL of the horticultural waste mixture were poured into each bioreactor. As inoculum for the batch reactors was used an anaerobic sludge from a biodigester that treat pig manure located in a near farm. The bioreactors were stirred at 50 rpm at intervals of 2-4 h per day. After the working time of the bioreactors, the settled sludge obtained was considered as the anaerobic digestate. The anaerobic digestate was extracted and washed three times with distilled water to extract the non-humic and water-soluble substances (proteins and sugars), in a 3:4 (m:v) ratio, in order to avoid interference between these compounds and the humic compounds (5). Thirty grams of sample were weighed and subjected to a basic treatment with sodium hydroxide (NaOH) with a concentration of 0.1 mol/L and a solid-liquid ratio of 1/10. After 24 h, the sample was centrifuged for 10 minutes at 3200 rpm, obtaining humin as a precipitate and HS as a supernatant. Subsequently, hydrochloric acid (HCl) was added to the supernatant, at a concentration of 1 mol/L, until reaching a pH between 1 and 2, and it was left to stand for another 24 h. Subsequently, the sample was centrifuged again for 10 minutes at 3200 rpm, obtaining HA as a precipitate and FA as a supernatant. Total elemental concentrations of HA and FA were determined by microwave-assisted digestion in hydrochloric acid using inductively coupled plasma optical emission spectroscopy (ICPOES; Thermo Fisher iCAP™ 7600). The methods used in the determination of each element are shown in table 1.

Results and Discussion

Table 1 shows the results of the chemical characterization of HA and FA obtained in the study. The total contents of the chemical elements in the HA were higher than those observed in the FA, except for Ca. However, similar values were obtained for the content of K in both samples of compounds, indicating that the forms in which the elements are found in ions in the anaerobic digestate are soluble in aqueous medium. The solubility of these ions is important for crop physiology, since it provides favorable characteristics for the use of HA and FA as biofertilizers.

Both compounds presented low content of total N, which could be related to the elimination of proteins during the extraction process of HA and FA. However, the amounts of N present are slightly higher than those found in some types of soils used for intensive agriculture in Cuba. Meanwhile, the K and P contents in the HA were higher than those observed in the FA. However, these concentrations are interesting due to the relevance of these elements for the correct development of plants. The N, P, and K are important nutritional elements that are required for proper plant growth and development. Nitrogen has a metabolic and structural function, it constitutes part of proteins, chlorophyll molecules, and nucleic acids, which are fundamental in the physiological processes of ionic absorption, photosynthesis, respiration, and cellular synthesis. Meanwhile, potassium contributes to control the translocation and accumulation of sugars, maintains the balance of anionic charges at the cellular level and regulates the water status of the plant; for that reason it is important for understanding the physiology of crops (6). Likewise, phosphorus participates in the processes of phosphorylation, photosynthesis, respiration and in the synthesis and decomposition of carbohydrates, proteins and fats, which promotes the rooting and tillering of crops.

The content of secondary macronutrients (Ca, Mg and S) is considered significant in both samples analyzed. Sulfur was the main component of HA (1000 mg/kg DM), and the second in abundance in FA (250 mg/kg DM). These amounts are higher than those found in various natural fertilizers traditionally used in vegetable production in tropical conditions. The relative abundance of Ca in FA could provide an alkalinizing effect to the soil, desired in the management of various crops. The possibility of easily and economically supplementing macronutrients to agricultural production results in obtaining higher quality food, with better yields and at a lower cost (7).

Table 1. Chemical characterization of humic and fulvic acids from anaerobic digestate of horticultural residues.

Element	**Analytical method**	**Unit**	**Humic acid**	**Fulvic acid**
Total nitrogen (N)	DIN ISO 11261 (05.97)	% DM	0,309	0,031
Lead (Pb)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	0,35	0,10
Boron (Bo)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	1,7	1,1
Cadmium (Cd)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	0,020	0,016
Calcium (Ca)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	67	140
Chrome (Cr)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	0,66	0,24
Iron (Fe)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	320	27
Potassium (K)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	580	510
Cooper (Cu)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	20	0,42
Magnesium (Mg)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	96	29
Manganese (Mn)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	2,4	1,3
Molybdenum (Mo)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	2,8	0,020
Nickel (Ni)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	0,71	0,31
Phosphorus (P)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	200	72
Mercury (Hg)	DIN EN ISO 12846, 2012-08	mg/kg DM	0,015	<0,002
Sulphur (S)	DIN EN ISO 11885 (E22) 2009-09	mg/kg DM	1.000	250

The proportion in which the micronutrients (Fe, Cu and Mn) were found in both acids exceeds the requirements of several crops of economic importance. Iron content in HA compared to FA was significant. The concentration of Fe in HA could contribute to a better bioavailability of this element in the soil, thus contributing to improve agricultural yields in intensive farming conditions (2). Micronutrients play an important role in the resistance of plants to abiotic and biotic stresses, particularly in resistance to diseases and pests (8).

Both compounds showed low contents of heavy metals (Pb, Cd, and Hg), which is advantageous because it reduces the risk of contamination due to progressive bioaccumulation and allows the recycling of the organic material. In the case of HA, lead reached a concentration of 0.35 mg/kg DM, although at this level it is still not considered toxic (9).

Conclusions and Outlook

The highest concentration of chemical elements present in the anaerobic digestate was found in HA. Significant increases in macronutrients are obtained in HA compared to FA, except for potassium content. Meanwhile, the content of micronutrients in both acids exceeds that required by several economically important tropical crops. The humic substances of the anaerobic digestate of horticultural residues have low contents of heavy metals, which allows the recycling and application of organic material for organoponic crops.

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References

1. Nyamangara J, Kodzwa J, Masvaya E, Soropa G. Chapter 5 - The role of synthetic fertilizers in enhancing ecosystem services in crop production systems in developing countries: Academic Press; 2020.
2. Van. M, Pepe O, De Pascale S, Silletti S, Maggio A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture. 2017;4(5):1-12.
3. Du-Jardin P. Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae. 2015;196:3-14.
4. Huan L, Youkang L, Shuxin Z, Chenchen L. Extracting humic acids from digested sludge by alkaline treatment and ultrafiltration. Journal Mater Cycles Waste Management. 2013;4(23):1257-65.
5. Isolation of IHSS soil fulvic and humic acids, (2012).
6. Madrigal-Valverde A, Garbanzo-León G. Uso de residuos agroindustriales en previveros de palma aceitera (Elaeis guineensis, Arecaceae): crecimiento y absorción de nutrimentos. Cuadernos de Investigación UNED. 2020;10(2):257-66.
7. Tripathi DK, Singh VP, Chauhan DK, Prasad SM, Dubey NK. Role of macronutrients in plant growth and acclimation: Recent advances and future prospective. In: Media SSB, editor. Improvement of Crops in the Era of Climatic Changes. 2. New York2014. p. 197-216.
8. Sherefu A, Zewide I. Review Paper on Effect of Micronutrients for Crop Production. Journal of Nutrition and Food Processing. 2021;4(7):1-8.
9. Nurmesniemi M. Forest fertilizer properties of the bottom ash and fly ash from a large-sized (115 mw) industrial power plant incinerating wood-based biomass residues. Journal of the University of Chemical Technology and Metallurgy. 2012;47:43-52.