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Hydroponically Grown Tomato (Solanum
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New Uses of Treated Urban Waste Digestates on Stimulation of Hydroponically Grown Tomato (*Solanum lycopersicon* L.)

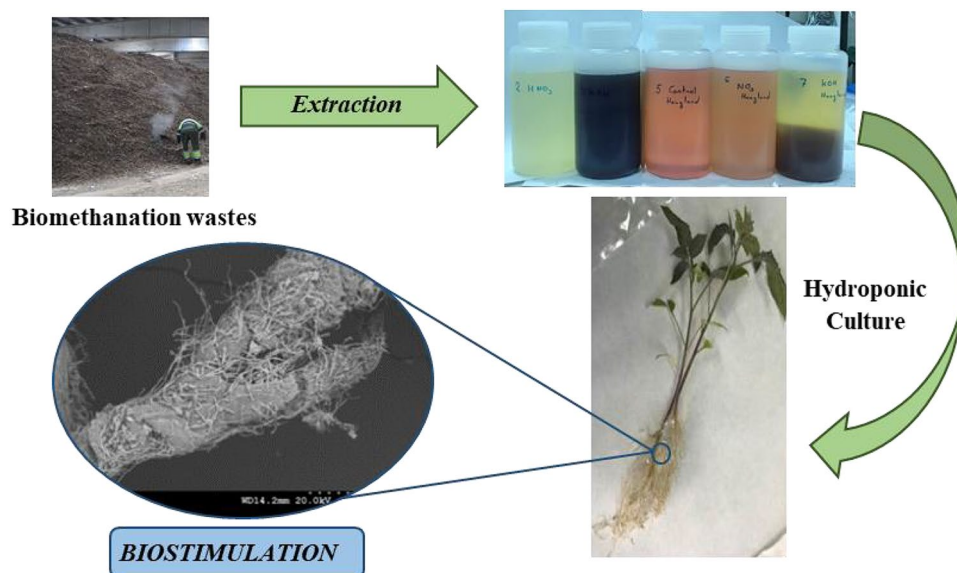
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Abstract

One strategy to re-use solid urban wastes is the production of energy by anaerobic digestion. This process also generates high volume of digestates, which are frequently disposal in landfills. The aim of this work is to assess anaerobic digestates as agricultural inputs. Three different bimethanation wastes from different plants were collected. Firstly, a complete physico-chemical characterization of the wastes was done according to the Spanish regulation, showing that the materials had the 90% of the particles below 25 mm, high values of pH, electric conductivity, organic matter, humic acids and soluble nutrients such as NO_3^- , SO_4^{2-} , Ca^{2+} , Mg^{2+} , PO_4^{3-} and K^+ . Total concentrations of heavy metals and microbiological parameters were below the threshold levels allowed for agricultural use. The wastes were then treated with a strong acid and a strong base having two different solutions (ATr and BTr, respectively) which were evaluated as biostimulants for tomato plants in hydroponic culture. Those liquid extracts, ATr and BTr, demonstrated their biostimulant ability towards root system of tomato enhancing the hair root density and plant biometric parameters including plants weight and chlorophyll content. This work demonstrates the re-use feasibility of treated digestates in agriculture as fertilizers and more over as feedstock for biostimulants production.

Graphic Abstract



Keywords Municipal solid wastes · Anaerobic digestate · Waste treatment · Nutrient recycling · Biostimulant

Extended author information available on the last page of the article

Statement of Novelty

The biomethanation wastes are being landfilled because of their lack of utility. This manuscript demonstrate the feasibility of digestates from municipal solid wastes anaerobic digestion to be used in agriculture and more over to produce biostimulators to enhance plant growth. This article presents the novel biostimulant production using digestates as unique feedstock. With the concepts of circular economy and waste valorization, this new strategy of digestate management prevents landfill disposal and valorize this waste. Hence, we estimate high scientific impact and interest for this article.

Introduction

Sustainable municipal solid wastes (MSW) management is a critical issue for cities all over the world, especially for mega-cities that generate millions of tons annually. Historically, landfill disposal was deemed to be the most conventional way to deal with collected MSW. However, it consumes and pollutes a considerable amount of land, and sometimes causes leachates and gas emission [24]. Anaerobic digestion (AD) is a widely used technique for the treatment of various organic biodegradable waste to produce energy in the form of biogas and nutrient-rich product, namely digestate [47]. Another use of municipal waste made-up of household and other yard waste is in agriculture. The re-use of these materials minimizes wastes, kills pathogens and reduces weeds germination in cultivated lands, leading to enhanced crop productivity [4]. Application of the organic fraction of MSW in agriculture is gaining popularity due to its positive effect on biological and physico-chemical properties of soil [53]. In the same way, the direct application of the liquid fraction extracted from this waste is a suitable strategy to achieve high yields in crops [40]. This also would reduce transport costs due to the lower volume the liquid fraction has compared to the solid fraction and its addition to soil is easier [38]. Taking into account that energy recovery (biogas) from these solutions is always low, new approaches must be studied to enhance its recovery as a nutrient resource [29, 30].

The industrial plants that are part of the Valdemingómez Technology Park (VTP) are mechanical biological treatment (MBT) facilities that constitute the core of the municipal waste treatment service of the City of Madrid. The VTP separates, classifies and treats 1.2 Mt/year of these residues, in whose composition 35% of organic matter has been determined (wood and cellulose

not included). The management of biodegradable organic matter of this waste is a major challenge [34]. Among the recycling targets for municipal waste marked by the European Commission for the 2020 Horizon, the increase in the recycling of materials contained in this waste to a minimum of 50% by weight is included to transform the current economy, which is based on the intensive use of resources, into a new growth model based on efficient resource use and where waste is reincorporated into the production process for the production of new products or raw materials [17]. In this model of an efficient circular economy, the recycling of waste plays a significant role by allowing increased availability of resources for the industry, by reducing the environmental impact associated with waste management, and by promoting job creation and investment in the recycling sector. Using Eurostat sources and estimations, in the EU the average recycling of materials (paper and cardboard, glass, plastics, metal) is 30% and reaches 47% including the biological treatment of organic matter (composting and AD). In Spain, the figures are 17% and up to 33%, respectively. This means that the recycling of organic waste plays a key role in achieving these objectives.

According to the communication of the European Commission (2008), biological treatment (including composting and AD) can be classified as recycled when the compost (from the thermophilic aerobic digestion of organic matter or the digestate of the AD) is applied to the soil as an improver or used to produce growing substrates. This implies a wide range of uses for composted products and organic fertilizers produced from waste materials; so that the use of fossil peat is replaced, and the conservation of the soil is strengthened, avoiding at the same time CO₂ emissions. The legal framework for these products is still under development, in terms of legislation (RD 506/2013 on fertilizer products and Law 22/2011 on waste and contaminated soil), quality (EU Ecolabel for soil improvers (Decision 2006/799/EC) and growing medium (Decision 2007/64/EC) [39]. The bio-wastes, which are defined as “biodegradable waste from gardens and parks, food and kitchen waste from homes, restaurants, collective catering services and retail establishments; as well as, comparable waste from food processing plants” requires a special management (dealt with in article 24 of the EU Directive 2008/98/EC on waste). These practices including the use of compost produced from bio-waste, which is environmentally safe in applications regarding the agricultural sector, gardening or the regeneration of degraded areas, in substitution of other organic amendments and mineral fertilizers.

Among the biodegradable organic wastes collected in the European List of Waste (LOW, Decision 2014/955/EU) are those that are produced in the treatment of household waste or in the urban environment, including the organic

fraction of municipal waste. Due to this, the digestate of the anaerobic treatment of waste and sludge from the treatment of urban wastewater is also included.

The high concentration of mineral nitrogen (especially in the form of ammonium) and organic matter content that it is found in those digestates make them a useful feedstock for agriculture or the chemical industry [33]. They can contain also water, simple sugars (glucose, fructose and sucrose) and polysaccharides (pectin, cellulose and hemicellulose) [8]. However, their recycling in agriculture might be restricted by their Cu and Zn contents, salinity, biodegradability, phytotoxicity and hygiene characteristics [2]. It also must be considered that the feasibility of direct land application will be highly constrained by transport requirements [32]. The treatment of liquid digestate is needed to decrease its mass and increase nutrient concentrations [46]. Based on the stage of implementation, the technical performance, as well as financial aspects, struvite precipitation/crystallization, ammonia stripping and subsequent absorption using an acidic air scrubber were selected as best available technologies to be applied at full-scale for nutrient recovery as marketable fertilizer commodities [50].

Some studies have been carried out proving this and their results obtained indicated that subsurface injection of digestate and derived products at pre-sowing and topdressing, gave crop yields similar to those obtainable by the use of urea [41], meaning that the digestate can be used as an agricultural fertilizer because the nutrients present in the raw input material remain in it and are accessible for crops after the digestion process [27].

The goal of present work is to assess the usefulness of digestates obtained after anaerobic digestion of MSW from the Valdemingómez Technology Park (Madrid, Spain) as valuable input in agriculture. After a preliminary agronomical assessment of digestates, the possible biostimulant effect of liquid extracts derived from acid and basic treatment of digestates was assessed in hydroponically growth of tomato.

Materials and Methods

Materials

MSW were treated in two bio-methanation plants in Valdemingómez Technology Park (Madrid), Las Dehesas plant and La Paloma plant. After bio-methanation, the digestates were subjected to successive dehydration processes by means of different equipment (filter press, sieve and centrifuge). Solid and liquid fractions were generated in these processes. The solid fractions from Las Dehesas and La Polama bio-methanation plants and organic fraction of municipal solid waste (OFMSW) were mixed to favor the further bio-stabilization process through tunnels of aeration with controlled

temperature. Consequently, we collected the following three solid wastes that are produced in VTP:

LD: solid waste collected after dehydration process of digestate from Las Dehesas plant.

LP: solid waste collected after dehydration process of digestate from La Paloma plant.

BM: bio-stabilized mixture of LD + LP + OFMSW.

Characterization of Digestates

A characterization of the three wastes LP, LD and BM was performed including particle size, according to the Spanish regulations (RD 1110/1991). The granulometry was determined by mechanical compression and disaggregation at 70 °C oven dried organic materials before passing a series of standard steel mesh sieves. To quantify the different components of the fractions and thus be able to determine the percentage of impurities, they were separated into fractions > 2 mm of the components (arids: gravel, fragments of bricks, earthenware, tiles, mollusk shells). This fraction has been made equivalent to the fraction of gravels and stones referred to Organic Compost Amendment in Royal Decree 506/2013, on fertilizer products which establishes that the particles with a diameter greater than 5 mm, will not exceed 2%. Almost all the glass fraction that could be separated corresponded to fragments green, amber and transparent bottles. The Improper Fraction method used was similar to the one found in the European Committee for Standardization [16], which consists in a manual and heavy separation of the impurities in the fraction > 2 mm and the stones in > 5 mm. The quantitative determination of the impurities was made by separating the total of each of the materials in fractions 25–10 and 10–5 mm. In the 5–2 mm fraction, a quartering was carried out prior to separation minimizing the appearance of deviations on the data.

pH and electrical conductivity (EC) were determined in extract 1:5 weight:volume [48]. Oxidizable organic matter were measured by means of a sulfo-chromic solution, evaluating backward with Möhr salt. Total Carbon (C), and total Nitrogen (N) were determined according to UNE 77,321 (2003) in an elemental analyser (LECO CHNS-932). The samples were subjected to an alkaline extraction with sodium pyrophosphate 0.1 M—sodium hydroxide 0.1 M to obtain the total humic extract (HE) and then the humic acids were precipitated in this extract at pH 1 (RD 1110/1991). The ashes percentage was measured by total calcination at 540 °C using a Muffle furnace (P-Selecta Select-Horn) following the BOE 14 October 1981 (official methods of analysis of organic fertilizer products, plants, soil).

The measure of the self-heating (Rottegrade) was carried out in a Dewar vessel by measuring the temperatures over 48 h at least after a hydration with water. Biological oxygen

demand (BOD) with a method described for waste waters (UNE-EN 1899-2, 1998).

Soluble cations (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) and anions (Cl^- , NO_3^- , PO_4^- and SO_4^{2-}) were analysed in an extract 1:100 (w:v) by ionic chromatography (Metrohm 882 Compact C Plus) with cationic (Metrosep C4 250) and anionic (Asupp5-250) columns, respectively. Concentration of trace elements were analyzed after aqua regia extraction and determination by ICP-MS (NexION 300XX, Perkin-Elmer) according to UNE-EN 13650 [20]. Accuracy of trace elements analysis was evaluated using a certified reference material, sewage sludge LGC6181 from LGC Standards (UK). Chrome (VI) was measured by the method proposed by EPA 1992. Mercury was analyzed through the atomic absorption equipment AMA-254 of the LECO company.

To evaluate the possible phytotoxicity of BM, germination and phytotoxicity tests were carried out. The germination assay was carried out in Petri dishes, where seeds of *Lactuca sativa* were germinated in BM at 25 °C for 5 days. For the phytotoxicity test, 6 pots were prepared with different proportions of BM and coconut fiber: 100:0% BM; 80:20%; 60:40%; 50:50%; 20:80% and 0:100% coconut fiber as the control. All the pots were watered with solutions of the three materials in proportion 1:10 (wastes and distilled water) for 30 days.

Production and Evaluation of Liquid Fertilizers

Digestates (LP, LD and BM), were treated with nitric acid 20%, or potassium hydroxide 20% in proportion 1:1 (w/v). The extracts obtained for the three materials were mixed in proportion 1:1:1 to obtain two extracts. One extract derived from the acid treatment with nitric acid (ATr) and another extract derived from the basic treatment (BTr). Finally, the pHs of ATr and BTr were adjusted to 6.2–6.8 with calcium hydroxide and sulphuric acid, respectively.

A fertilization assay for 4 weeks was performed to assess the usefulness of ATr and BTr as fertilizers. Tomato plants (*Solanum lycopersicon* L.) were hydroponically cultivated in growth chamber model CCKF 0/16,985. The daily growth cycle was day: 16 h, 30 °C /50% relative humidity; and night: 8 h, 25 °C/70% relative humidity. Six different fertilization regimens were established:

DW: Deionized Water without fertilization.

ATr: fertilization with ATr extract (3.3 mL) diluted with water up to 1L to reach the optimum NO_3^- concentration for tomato according to Sonneveld et al. [45].

BTr: fertilization with BTr extract (25 mL) diluted with water up to 1 L to reach the optimum K^+ concentration for tomato according to Sonneveld et al. [45].

NS: fertilization with nutrient solution optimized for tomato according to Sonneveld et al. [45]. Macronutrients were in a concentration of (mmol/L): N 15, P 2, K 9, Ca 5,

Mg 1.5; and micronutrients (mg/L): Fe 2; Mn 1; Cu 0.1; Zn 4; B 0.5; Mo 0.05.

NS + ATr: fertilization with ATr extract (3.3 mL) diluted with nutrient solution up to 1L.

NS + BTr: fertilization with ATr extract (25 mL) diluted with nutrient solution up to 1L.

Fertilizer treatments BTr and NS + BTr were diluted 1:10 because of the high electric conductivity of the resulting nutrient solution. The number of replicates for each fertilization treatment was four.

For the design of nutrient solution, following Panreac analytical grade products were used: calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 99–103%), potassium dihydrogen phosphate (KH_2PO_4 98–100.5%), potassium sulfate (K_2SO_4 98%) and magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 99–100.5%) as macronutrients and ammonium molybdate tetrahydrate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 99–103%), copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 99%), zinc sulfate monohydrate ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 99–102%), boric acid (H_3BO_3 99.5%) and manganese sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 98–101%) as micronutrients. From the Merck brand potassium nitrate was used (KNO_3 100%) and of the brand Quimioprox the FeEDDHA.

Regular Soil and Plant Analyzer Development (SPAD) readings were taken with a chlorophyll meter (Minolta SPAD-502). SPAD index and plant fresh weight (shoot and root) were used as growth indicators. Scanning Electron Microscopy Hitachi S-3000N equipped with an Energy dispersive X-ray INCAx-sight detector (SEM–EDX) was used for studying the morphology and elemental analyses after preparation of samples following the glutaraldehyde method [6].

Statistical Analysis

The data were analyzed using a statistical package, SPSS version 20.0. One-way analysis of variance was carried out after performing a Levene variance homogeneity test. The statistical significance among treatments was judged by Tukey or Games-Howell post hoc test (according to variance homogeneity) at $p < 0.05$.

Results

Characterization of the Wastes

The granulometric analysis showed that the materials LP and LD had a high percentage of particles with diameters smaller than 5 mm (97 ± 12 and 73 ± 7 , respectively), being the glass particles in a large proportion. BM presented a significant amount of impurities of cellulosic materials. The threshold values for fertilizers indicate that the impurities (metals,

Table 1 Characterization of the municipal waste digestates (LP, LD) and bio-stabilized waste (BM) (mean ± Standard deviation, n=4)

	pH (1:5 v/v)	EC (dS m ⁻¹)	OM (%)	C (%)	N (%)	HE (%)	C/N	AP (%)
LP	8.6 ± 0.1	5.7 ± 0.2	32.5 ± 0.9	24 ± 1	1.7 ± 0.1	3 ± 1	14 ± 1	61 ± 1
LD	8.4 ± 0.1	4.6 ± 0.3	29 ± 0.5	25 ± 3	1.3 ± 0.1	5 ± 1	19.2 ± 0.7	66.1 ± 0.4
BM	8.1 ± 0.1	3.3 ± 0.1	32 ± 1	36 ± 2	1.8 ± 0.2	3.4 ± 0.2	20 ± 3	–

EC electrical conductivity, OM organic matter, HE humic extract, AP ashes percentage

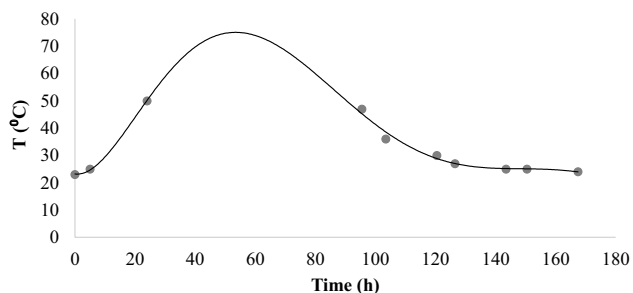


Fig. 1 Self-heating experiment for the sample BM in 7 days. The assay was done in a cylindrical dewar flask (KGW Isotherm); measuring the temperature with a HOBO MX2303 sensors data logger (Onset). The adjustment model used was polynomial grade

glasses and plastics) eventually present in diameter greater than 2 mm, will not exceed 1.5% [42]. According to the data of the granulometry and composition of the fractions, all the materials complied with 90% of the granulometry below 25 mm. BM presented a high content of impurities (3%) which meant an excess in the limit established [31]. The other materials, LD and LP, met the quality criteria established in granulometry and composition. The plastic fraction was composed by small pieces of plugs, CD cases, pens, etc. The cellulose fraction is not usually mentioned as impurities, although they are artificial and not easily biodegradable. Residues of tissues or reinforced cellulose (wipes, etc.) were very abundant in the compost and were practically absent in the dehydration residues of the digestate. The metal fraction was composed by fragments, above all, of crushed and agglomerated aluminum foil.

Table 1 shows the characterization of the urban digestates (LP, LD) and the bio-stabilized mixture of wastes (BM). The pH of dehydrated digestes (LP and LD) were basic (> 8.4) and BM, slightly lower but still alkaline (Table 1). Electric conductivity values were higher than the optimum value of 1.5 dS m⁻¹.

Table 2 Heavy metals content (mg kg⁻¹) of the urban waste digestates (LP, LD) and bio-stabilized waste (BM) (average values from 4 replicates ± SD)

	Cd	Cu	Ni	Pb	Zn	Cr	Hg	Mn
LP	1.08 ± 0.04	107 ± 10	28.2 ± 0.1	58 ± 5	341 ± 9	60 ± 3	0.41 ± 0.02	300 ± 5
LD	0.94 ± 0.07	192 ± 2	46 ± 5	76 ± 4	245 ± 3	70 ± 11	0.50 ± 0.07	328 ± 2
BM	1.7 ± 0.2	238 ± 13	36 ± 3	75 ± 16	360 ± 38	58 ± 8	0.56 ± 0.27	168 ± 12

Organic matter content of the three wastes were in the range of 29–32.5%, therefore they did not reach the minimum that would be 35% to be used as organic amendment (Royal Decree 506/2013). Despite the similar content of organic matter BM showed higher %C than LP and LD. Total N were between 1.3 and 1.8%. Resulting C/N values of the samples followed the order LP < LD < BM. The C/N ratio values of LD and BM were far from the ideal (15) [18]. For the last material (BM) the self-heating assay demonstrated that the waste was not stabilized (Fig. 1). The humic extract (HE) of the three samples was between 3 and 5%. After the acidification of the HE to pH 1, the humic acids were obtained with values of 0.2% for LP, 0.5% LD and 0.5% BM.

The BOD of LD and LP (with values of 40 ± 0 and 40 ± 14, respectively), showed the existence of biological activity with no differences between both digestates. Although bacteria have been identified in these residues, they did not present *Escherichia coli* above the required limits (less than 1000 most probable number per gram of processed product) and *Salmonella* was absent in 25 g of processed product, which were the microbiological parameters required for these studies according to Spanish legislation (Royal Decree 506/2013). For the sample BM, bacteria found in the sowings were: *Staphylococcus lentus*, *Oligella ureolytica*, *Staphylococcus cohnii* subsp. urealyticus; *Alcaligenes faecalis* subsp. faecalis; *Pseudomonas stutzeri* and *Staphylococcus cohnii* subsp. urealyticus.

The total concentrations of heavy metals (Table 2) of the three wastes were below the threshold levels allowed by the Spanish legislation (RD 506/2013) for agricultural re-use. However, there were light differences between the three wastes. LD presented higher concentrations of Cu, Ni, Pb and Mn than LP, which had higher concentration of Zn. The composted product BM showed clearly higher concentrations of Cd, Cu and Zn and lower concentration of Mn than LP and LD. Cr (VI) was not detected in LP, LD and BM.

Table 3 Nutrients and major elements content (g Kg⁻¹) of the urban waste digestates (LP, LD) and bio-stabilized waste (BM) (average values from 4 replicates ±SD)

	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Fe
LP	371 ± 3	3.1 ± 8	23 ± 1	70 ± 5	390 ± 10	270 ± 4	76 ± 3	19 ± 3	13.12 ± 0.09
LD	330 ± 20	4.6 ± 0.5	40 ± 5	71 ± 4	330 ± 20	220 ± 10	70 ± 3	22 ± 4	19.1 ± 0.3
BM	310 ± 10	14 ± 2	1.0 ± 0.1	300 ± 50	310 ± 10	270 ± 10	290 ± 30	35 ± 7	6.8 ± 0.5

Table 4 pH previous and before neutralization and electrical conductivity (EC) of the fertilization treatments

Solution	Initial pH	Adjusted pH	EC dS m ⁻¹
ATr	2.67	6.54	1.76
BTr	12.61	6.68	7.08
NS	6.03	6.03	2.51
ATr+NS	2.82	6.17	3.74
BTr+NS	12.56	6.71	7.71

The nutrients and major elements of the three wastes were in Table 3. The digestates LP and LD had similar concentrations of some soluble nutrients such as NO₃⁻, SO₄²⁻, Ca²⁺, Mg²⁺ and other soluble ions such as Na⁺ and Cl⁻. Nevertheless, LD had higher concentrations of soluble PO₄³⁻ but lower K⁺ than LP. BM showed higher concentrations of NO₃⁻, SO₄²⁻, Ca²⁺, Mg²⁺ than the above digestates. In contrast, the concentration of was PO₄³⁻ notably lower. No significant differences for Na⁺ and Cl⁻ concentrations were found between BM and the digestates LP and LD. The concentration of total Fe was higher in the digestates with respect to BM.

The germination test in the Petri dishes showed no growth of the *Lactuca sativa* seeds. In the phytotoxicity test with coconut fiber substrate the pots with a percentage of at least 20% of coconut fiber survived for 30 days. The plants in the pot with only BM as a growing substrate lasted 3 days.

Production and Evaluation of Liquid Extracts

After the addition of the nitric acid and potassium hydroxide in the proportion 1:1, most of the liquid was absorbed by the solid phase. The volume of extract obtained after the filtration were the 37% of the initial acidic and 35% of the alkaline solutions. The pH and electrical conductivity of the extracts were shown in Table 4. The values of pH obtained after acid and basic treatments were extremes (2.7 and 12.6 respectively) and not suitable for use in agriculture. Therefore, their pH was adjusted to adequate range for plants. However, this correction and the own nature of BTr produced high EC in this extract. Due to the high conductivity of solutions produced with the basic treatment (BTr and

Table 5 Fresh weight of stem and root of tomato plants after 4 weeks of treatment with deionized water (DW), fertilizer from acid extraction (ATr), fertilizer from basic extraction (BTr), nutrient solution (NS) and combinations (mean ± SD, n=6)

Treatment	Fresh weight (g)	
	Stem	Root
DW	0.6 ± 0.1 ^c	0.27 ± 0.03 ^b
ATr	1.0 ± 0.2 ^{bc}	0.4 ± 0.2 ^{ab}
BTr	0.9 ± 0.2 ^{bc}	0.5 ± 0.1 ^{ab}
NS	1.4 ± 0.2 ^{ab}	0.5 ± 0.2 ^{ab}
NS + ATr	1.8 ± 0.9 ^a	0.6 ± 0.5 ^{ab}
NS + BTr	1.4 ± 0.1 ^{ab}	0.8 ± 0.2 ^a

Different letters indicate significant differences between treatments (p ≤ 0.05)

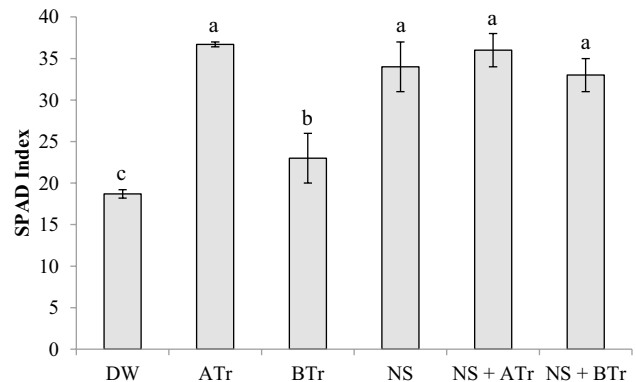


Fig. 2 SPAD index of the tomato leaves grown with deionized water (DW), fertilizer from acid extraction (ATr), fertilizer from basic extraction (BTr), nutrient solution (NS) and combinations (mean ± SD, n=4)

BTr + NS), both had to be diluted with water in the proportion (1:10).

The growth and nutrient status by SPAD index of tomato plants treated with nutrient solution or liquid fertilizers produced above are showed in Table 5 and Fig. 2, respectively. The highest shoot growth was obtained by fertilizer treatments with nutrient solution (NS, NS + ATr and NS + BTr). However, no significant differences were found between the application of ATr and BTr treatments and the fertilization treatment NS. All the fertilizer treatments had higher SPAD

value than DW without nutrient supply. The higher values of SPAD index were obtained by tomato plants fertilized with NS, with and without ATr or BTr and the treatment ATr. In contrast, the treatment BTr had lower value of SPAD index. The root development was affected by the fertilizer treatments. NS + BTr significantly promoted the root growth with respect to DW. The other fertilization treatments did not reach significant differences regarding DW root growth.

SEM analysis showed that the use of ATr or BTr made the roots to develop a higher number of root hairs with respect to NS (Fig. 2). The analysis of the elemental composition of those roots by EDX spectra, showed that the roots treated with the ATr presented higher levels of iron and silicon compared with the others.

Discussion

Characterization of Wastes and Agronomical Assessment

The possibilities of valorization of biodegradable wastes heavily relied on the final state they have and in meeting the quality criteria marked in the European and state regulations. The granulometric analysis showed that the materials were suitable in terms of physical pollution. From the particle size it can also be deduced that the microbial action can be promoted. The smaller the particle size, the greater the area in contact with the microorganisms, facilitating the degradation of organic matter, the homogenization and mixing of the materials, maintaining optimal temperatures during all stages of the process [26].

The bacterial colonies found in the BM sample did not correspond to *E. coli* or *Salmonella*; being some of them typical of urea degradation environments which were responsible of the ammonia production, which was in accordance with the alkaline pH measured in these materials [13]. There are numerous viable crops at the pHs measured for the samples, such as sugarcane, barley, wheat, and olive, which tolerate pH up to 8.5. However, at pH greater than 8, ferric chlorosis [23] and decreased availability of phosphorous [29, 30] could occur. Conductivity values could result in a problem if it is destined for plant growing substrate because it could lead to water stress which affected the multiplication and expansion of the cells during all growing stages [54]. It is not a marked requirement for organic amendment or soil improvers, but it is for growing substrates, so it would be necessary to dilute the substrate to reduce the EC. The percentage of ashes was high so that most of the particles in the sample were silica sands (glass, arids) and non-volatile materials. This corresponded to the large amount of glass present in the samples that with the combustion generated glassy materials including cenospheres, pleiospheres,

ferrospheres, and solid spheres [19]. This also represented the new trend in the urban waste where the percentage of paper and plastic is increasing [51].

Although the organic matter was low in the digestes, they complied, except for LD samples, the criteria of end of waste or soil improver. Those low percentages of total carbon were because the samples were residues of a bio-methanation, with part of the carbon been transformed into methane [28]. The centrifuge samples tended to give lower values, which agreed that this fraction contained largest number of inert components in the form of glass sand and other impurities than the BM fraction.

About the composition of the raw material (Table 1), the elements C and N were the most relevant to produce an adequate composting [3]. Generally, an ideal amendment should have a C/N ratio equal to 10, which is required for the normal microbial growth and the optimal process of humification [37]. If C/N is very high (LD and BM), the amount of N available for cell growth is limited at the expense of M.O., which inactivates or slows down the process [21]. The opposite accelerates the microbial growth and the decomposition of organic matter with a rapid depletion of O₂. The samples LP and LD came from wastes that have been subjected to anaerobic digestion while BM is the mixture of LP + LD + organic waste composted aerobically. This could be related with the result of the C/N of the bio-stabilized BM which corresponded with values for mature materials. This fact did not correspond with other indicators for the degree of maturity in this material like self-heating experiment (Fig. 1), indicating that it still could continue the composting process. Therefore, it was not stabilized. For the rest of the materials, the high C/N ratios could be caused by the important amount of impurities that contain the materials and that could contribute to excessively high C fractions that did not contribute in a real way to the metabolic processes.

The highest percentage of the EH corresponded to soluble organic substances, with complexing properties and that could exert positive effects in the soil–plant system [12]. Considering that the minimum percentage that a material must contain to be considered a humic amendment is 5% (BOE-A-2013-7540; Royal Decree 506/2013), the results obtained indicate that LD sample could meet the requirement with respect to this parameter.

The concentration of heavy metals (Table 2) was low according to the Spanish legislation and no further problems derived from their re-use in agriculture was expected. Despite the similar nature of LP and LD, significant differences of heavy metals concentrations indicated that the waste management had impact on the quality of this materials, being observed different levels depending of the waste analyzed [47]. The bio-stabilization of digestates with new organic wastes to generate the material labeled BM produced a clear modification of heavy metals concentration

with respect to LP and LD. The combined effect of the application of fresh organic waste and composting process that concentrate the metals because carbon lost [25], produced increments of Cd, Cu and Zn concentration and dilution of Mn in the final product BM with respect to the digestates.

The high amount of sodium and chlorides (Table 3) were not optimal for agricultural crops to present competition with essential nutrients such as potassium or nitrates, respectively, and produced high EC. Nutrients were high in urban waste and could be useful for soil amendment [49], justifying also the relatively high values of electrical conductivity. This fact may had a negative effect on the germination test, making germination of the seeds even impossible. This problem could be solved if mixed with other substrates or organic materials, diluting its salinity and stabilizing the waste [5]. The similar concentration of soluble nutrients, Na^+ and Cl^- of LP and LD could be because their common origin, MSW. The higher concentration of NO_3^- , SO_4^{2-} in BM with respect to digestate was because composting by aerobic process. Composting promotes de oxidation of organic N, ammonia and S to NO_3^- , SO_4^{2-} because of the action of aerobic microorganisms [52]. Similar trend was expected for PO_4^{3-} but the soluble forms of this nutrient decrease after composting because the high content of calcium and basic conditions that promoted the formation of precipitates of calcium phosphates. The higher concentration of Ca and Mg in BM with respect to digestates could be because the addition of fresh organic waste to the composting pile and the own composting process that produces concentration effect due to carbon emission, mainly CO_2 .

The phytotoxicity test in the Petri dishes showed that the material BM was not suitable for germination due to the absence of growth of the seeds. The high conductivity made the material BM not suitable. The mixture of BM with other substrates (coconut fiber) was viable with at least 20% of substrate. The effect of the coconut fiber was direct in the dilution of the conductivity, so the plants were able to develop properly.

Production and Evaluation of Liquid Extracts

The liquid extracts obtained by acid (ATr) and basic (BTr) treatments presented extreme pH values not adequate for agricultural purposes (Table 4). Hence, the resulting nutrient solutions were neutralized to reach pH around 6.5. The neutralization of BTr with H_2SO_4 and the high concentration of K^+ in the extract produced elevate EC not suitable for crops (Table 4). Doses of both extract, ATr and BTr, were diluted with water to adjust the NO_3^- and K^+ concentration, respectively, according to the nutrient solution optimized for tomato [7]. Despite this dilution, the electrical conductivity of BTr (and BTr + NS) was too elevate for its use in agriculture. Therefore BTr and BTr + NS had to be diluted 1:10

with deionized water. Thus the final nutrient content of BTr and BTr + NS were tenfold lower than was expected.

The visual observation of tomato plants after 4 week of hydroponic culture seemed right and no visual symptoms of nutrient deficiency was detected. The SPAD index (Fig. 2) reveled significant differences between fertilization treatments indicating differences into the chlorophyll content. The treatment NS demonstrate its adequacy for tomato plants. Therefore, NS was used as reference. ATr with NS (ATr + NS) did not incremented the SPAD index with respect to NS. Hence, the join use of ATr with additional fertilization did not improve the nutrient status of tomato. In contrast, ATr without additional fertilization reach similar SPAD index than NS and NS + ATr. Therefore, in this case, ATr extract was enough to supply nutrients for tomato and reach adequate chlorophyll content. In a similar way, NS + BTr that contains tenfold lower nutrients than NS and NS + ATr reached similar SPAD index than NS. However, the treatment BTr was not enough to reach SPAD index close to NS because the extract BTr had to be diluted 1:10 to reduce the EC. For all, ATr and BTr extracts improved the nutrient absorption by tomato in hydroponic culture with low nutrient content or act as plant biostimulats that minimize the negative effects of nutrient scarcity. Plant biostimulants which are defined as substances and/or microbial inoculants applied to plants with the aim to enhance plant resilience and also to improve nutrient uptake and translocation [14]. An example are the humic substances which are known to influence plant physiological processes, enhancing crop yield, plant growth and nutrient uptake [11]. They promote root growth, offering the potential to increase soil C inputs [36] and produces alleviation of stress in roots by reducing the reactive oxygen species [9].

NS + BTr was the unique treatment that produced significant increments of root weight with respect to DW, probably because tomato had not need to develop much root to absorb nutrients from solution. In addition, NS + BTr produced one of the biggest tomato shoot despite the low nutrients concentration with respect to NS and NS + ATr. Therefore BTr stimulate the growth of tomato shoot and root. Canellas et al. [10] reported increment of maize growth (root and shoot) by effect of humic acids extracted from vermicompost in hydroponic culture. The increment of growth was because humic acids can interfere with nutrient sensing, regulate plant growth and stress responses. Vermicompost leachate stimulated the growth of leaves and bulbs of the medicinal plant *Drimiopsis maculate* [15] demonstrating once again the positive effects of extract from organic materials on plant growth. *Arabidopsis thaliana* under treatment with humic substances enhanced protein and energetic metabolism to support a higher growth rate [11]. These authors corroborated that humic substances extracted from different sources do not elicit the same plant responses. In particular, the

relative abundances of aliphatic and aromatic compounds and the presence of carbohydrates and peptide components might be responsible for the different effects of the humic substances. At this respect, previous work demonstrate that the auxin-like effect of dissolved organic matter from digestates was because of the presence of both phenyl acetic acids and auxin [43].

SEM images of roots (Fig. 3) showed higher density of root hairs in ATr and BTr treatments than NS. Hence the dissolved organic matter of ATr and BTr showed again biostimulant effects on root growth. The higher density of root hairs could explain the higher nutrient uptake capacity of roots after application of biostimulants [14, 37]. Nevertheless, not only the root growth was affected by dissolved organic matter, the composition of root was modified too. The highest abundance of iron in roots treated with ATr could be because of humic substances are able to form stable complexes with micronutrients (i.e., Fe) [14] activating the molecular and enzyme network involved in nutrient root acquisition, transporters and activity of plasma membrane H^+ —ATP-ase [35, 37]. Secondly the extraction procedure with HNO_3 to produce ATr dissolved Fe from digestates. In contrast, the use of KOH to produce the extract BTr immobilize Fe in digestates and the extraction of Fe in this conditions was impeded. So the high amount of Fe in roots of ATr was not observed in BTr because the low concentration expected in BTr and because BTr treatment had to be diluted tenfolds. Hence the final concentration of Fe was very low. Abou Chehade et al. [1] reported positive effects of biostimulants from food processing by-products on tomato growth, yield and quality. However the mechanisms of action could not be define with certainty because the difficulty in standardizing the raw materials used for biostimulant production.

Although more research is needed, ATr and BTr are a promising process to convert the municipal waste digestates obtained from bio-methanation to plant biostimulant. Now, other authors have built a model that could be potentially applied for the monitoring of different biowaste-derived

samples from maturation processes [44]; been able to industrialized this process; making it profitable.

This study overall, has proved biological treatment of municipal organic matter is an effective way both to obtain energy and nutrients from a waste in terms of carbon cycle recovery. From a waste with no other utility more than be landfilled, new products have been developed than could be used to stimulate plant growth reintroducing a supposedly lost carbon in a useful carbon cycle. The acidic and alkaline treatment also implies a stabilization of the solid phases due to the significant reduction of microorganisms derived from the extreme acid and basic pHs. This will add an additional value to the AD process of the municipal wastes by enhancing the yield of resources recovered, minimizing the landfilling of biodegradable organic matter. Organic matter and nutrients recovery are recognized to be key factors for a credible circular flow of these materials in the future global circular economy [22].

Conclusions

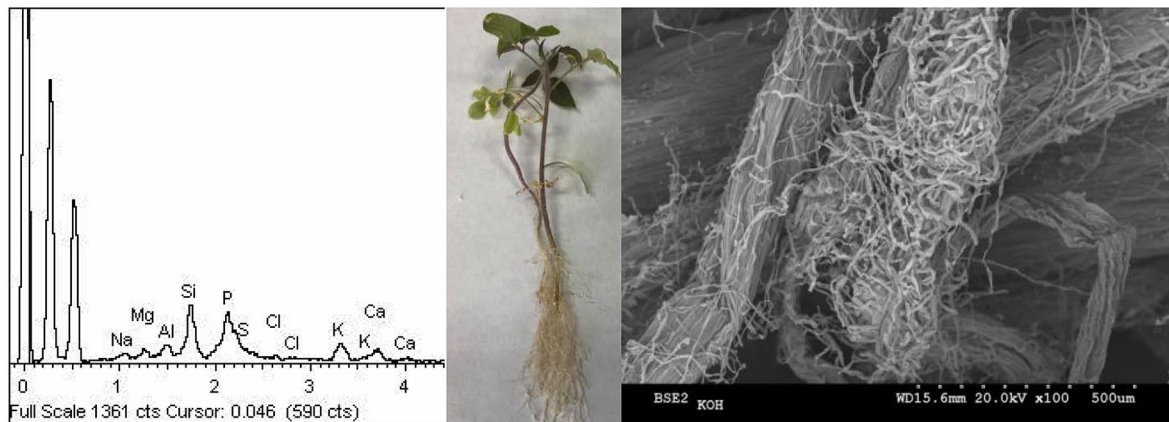
Digestates and bio-stabilized digestates are wastes adequate to be used in agriculture such as organic fertilizers and amendments with important contents of macro- and micronutrients. Their organic matter content and more over humic fraction are valuable characteristic of these materials to improve agricultural soil fertility. However the most important point of this work was the production of biostimulants. This work demonstrates the effectivity of acid and basic treatment of digestates from bio-methanation of municipal solid wastes to produce a valuable biostimulant for agricultural practices. Acid and basic extracts stimulate shoot and root growth of tomato plants and avoid reduction into chlorophyll content of leaves under nutrients scarcity. So, digestates from bio-methanation municipal solid wastes is a promising source of products for agriculture. Digestates have real possibilities of recycling that reduce the current landfill disposal.



Nutrient Solution (NS)



Acid treatment (ATr)



Basic treatment (BTr)

Fig. 3 Energy dispersive X-ray (EDX) analysis, visual and morphological (SEM) aspects of the tomato roots after irrigation with nutrient solution (NS), ATr or BTr, respectively

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