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*Review*

# Biotechnology of humified materials obtained from vermicomposts for sustainable agroecological purposes

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**The application of humic substances (HS) to plants stimulates their biochemical-physiological mechanisms, growth and development. Humified materials exhibit structural characteristics that allow interactions with heavy metal cations dissolved in aqueous environments. Due to their high availability, agriculture-derived vermicomposts (VC) (agro-materials) are excellent raw materials for obtaining HS. Based on these properties, it might be possible to develop biotechnology processes that use agro-materials for environmentally sustainable agriculture. The present article describes the application of a biotechnological working protocol that uses cattle manure vermicompost (CVC) as a raw material for HS. The suggested protocol includes six steps based on the structure-property-biological activity relationships of all the resulting agro-materials. Both the CVC and the resulting agro-materials were characterized using various chemical-physical and spectroscopic techniques. The agro-materials not only increased the agricultural production of different crops at the optimal sowing seasons but also increased production under water and saline stress conditions. The residues arising from the protocol processes were analyzed and found to be useful as decontaminants of heavy metals in aqueous liquid effluents. In addition, the use of such products resulted in increased profits for small- and medium-scale farmers. These studies suggest that agriculture-derived composted organic materials are viable for sustainable agroecological use.**

**Key words:** Vermicompost, humic substances, humic acids, stress.

## INTRODUCTION

Extensive research has revealed many of the properties and functions of humic substances (HS). Techniques for determining the structure of HS have been particularly useful for developing models that explain the various properties of HS (Nasir et al., 2011). The review performed by Schaumann and Bruhn (2011) explained that all structural models for HS are grounded on the common hypothesis that the formation of structures with superior chemical structural complexity (superstructures) is derived from interactions among small structural fragments. Recently, Nebbioso and Piccolo (2012) applied fractionation and structural analysis (known as *Humeomics*) to conclude that HS are composed of heterogeneous molecules that bind together as a function of their size, shape, chemical affinity, and hydrophobicity.

However, there is currently no single model that can explain the structural characteristics of HS. Rather,

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existing studies have focused on the structure-property-biological activity relationships of HS. Several studies reported that the hydrophobic properties of HS arising from non-polar (C-aromatic, C-aliphatic) structures in humic acids (HA) stimulate root growth in plants (Canellas et al., 2012). Additionally, the hydrophilic properties of HA arising from carbohydrate and lignin content might regulate bioactivity of plants (Nardi et al., 2007). According to several studies, structures with specific properties that are present in HA might act as substrates or precursors in the metabolic pathways of plants (Muscolo et al., 2007).

Current data indicates that HA is involved in the metabolic pathways of most plants. It has been shown that HA acts as stimuli for hormonal regulation associated with metabolism of carbon and nitrogen, anti-oxidative defensive systems, secondary metabolism, and protective mechanisms against stressors associated with salt and water levels (Nardi et al., 2002; Muscolo et al., 2007; Cordeiro et al., 2011; Aydin et al., 2012; Canellas et al., 2010; García et al., 2012a).

Therefore, a considerable number of HS-based products might be useful as environmental friendly alternatives in agriculture. Several such products can be applied to leaves or directly introduced into soil. These humified products are associated with stimulation of plant growth, increased crop yield, improved chemical-physical and biological soil conditions, increased nutrient availability, increased soil water retention, and improved quality of fruits and others products. Products with these characteristics are commercially available (http://humates.com/; http://www.humates.co.nz/; http://www.vanashreeagrotech.com).

The research group on Organic Matter and Biostimulants (Matéria Orgánica e Bioestimulantes (MOBI) at the Agrarian University of Havana, in collaboration with the Federal Rural University of Rio de Janeiro, has conducted studies lasting for two decades aimed at improving the sustainability of agriculture with humified solid and liquid products obtained from cattle manure vermicomposts (CVC). The present review compiles the results of these studies to demonstrate the viability of these products in terms of agriculture and the environment. We focus on studies that developed sustainable biotechnologies for the production and application of vermicompost humified materials (agro-materials) to promote ecological development and environmental sustainability at small and medium scales.

## THE BIOTECHNOLOGICAL WORKING PROTOCOL

The biotechnological protocol included six main steps, beginning with testing of the structure-property-biological activity relationships of both the starting CVC (in which case the tests also served as quality control) and the agro-materials (Figure 1). The CVC used as a raw material in the present protocol exhibited chemical-physical, mechanical, and spectroscopic characteristics that ensured the presence and richness of the organic, inorganic, and biological structures required for their use as starting materials for HS. The studies conducted with the CVC indicated the presence of nitrogen (1.7 to 2.6%), phosphorus (0.6 to 1.2%), potassium (0.3 to1.2%), calcium (4 to 10%), and magnesium (1 to 3%). The amount of organic matter varied from 21.8% to 44.5%. The pH was close to the neutral (pH ~7) (Ruiz, 1996; Del Pozo, 2008). The Fourier transform infrared (FTIR) spectroscopic characteristics of the CVC indicated the presence of O-H and N-H functional groups (3,433.6 cm$^{-1}$), $CH_2$ and $CH_3$ aliphatic structures (2,919.9 to 2,850.9 cm$^{-1}$), C=C olefin or aromatic structures, C=O amides I or quinones (1,642.1 cm$^{-1}$), N-H and C=N groups of amides II (1,514.0 cm$^{-1}$), deformation of O-H groups and C-O from phenols (1422,5 cm$^{-1}$), deformation of aliphatic $CH_2$ and $CH_3$ from salts of carboxylic acids (1,384.4 cm$^{-1}$), and polysaccharide structures or Si-O impurities (1,099 to 1,034 cm$^{-1}$) (Figure 2A).

Solid-state $^{13}$C- nuclear magnetic resonance (NMR) spectroscopy of the CVC showed that C-alkyl and O-alkyl structures were most abundant, representing 57.72%. The abundance of non-polar structures corresponded to 37.1%, whereas the abundance of polar compounds was 62.9% with a hydrophobicity index (HB/HI) of 0.59. The abundance of aromatic structures in the CVC was 11.34%, whereas the abundance of aliphatic structures was 88.25% (Figure 2B). A portion of the structural characteristics found by cross-polarization (CP)-$^{13}$C NMR spectroscopy were similar among different vermicompost (VC) types at different maturity stages (Aguiar et al., 2012).

The CVC induced increases in the yield of tomato crops when applied to soil at four t ha$^{-1}$, stimulating several biochemical-physiological indicators (García, 2006). CVC materials exhibited beneficial effects on foliar area and the weight of the aerial parts and roots of bell peppers, marigolds, cornflowers, and tomato plants and improved the quality of strawberries when used as a substrate, in organic aggregates, or combined with inorganic fertilizers (Bachman and Metzger, 2008; Atiyeh et al., 2001; Singh et al., 2008). VC also increased the colonization of *Sorghum bicolor* plants by arbuscular mycorrhizal fungi (AMF) (Cavender et al., 2003).

### Characteristics of agro-materials obtained from CVC

The agro-materials obtained from CVC exhibited properties (environmentally non-aggressive chemical composition, low production cost, and possible application to a wide range of crops) that allowed for their use in sustainable technologies. The characteristics, properties, and action of several products (BIOSTAN® and LIPLANT®, OPLANT+®, and CALDFER®) obtained in

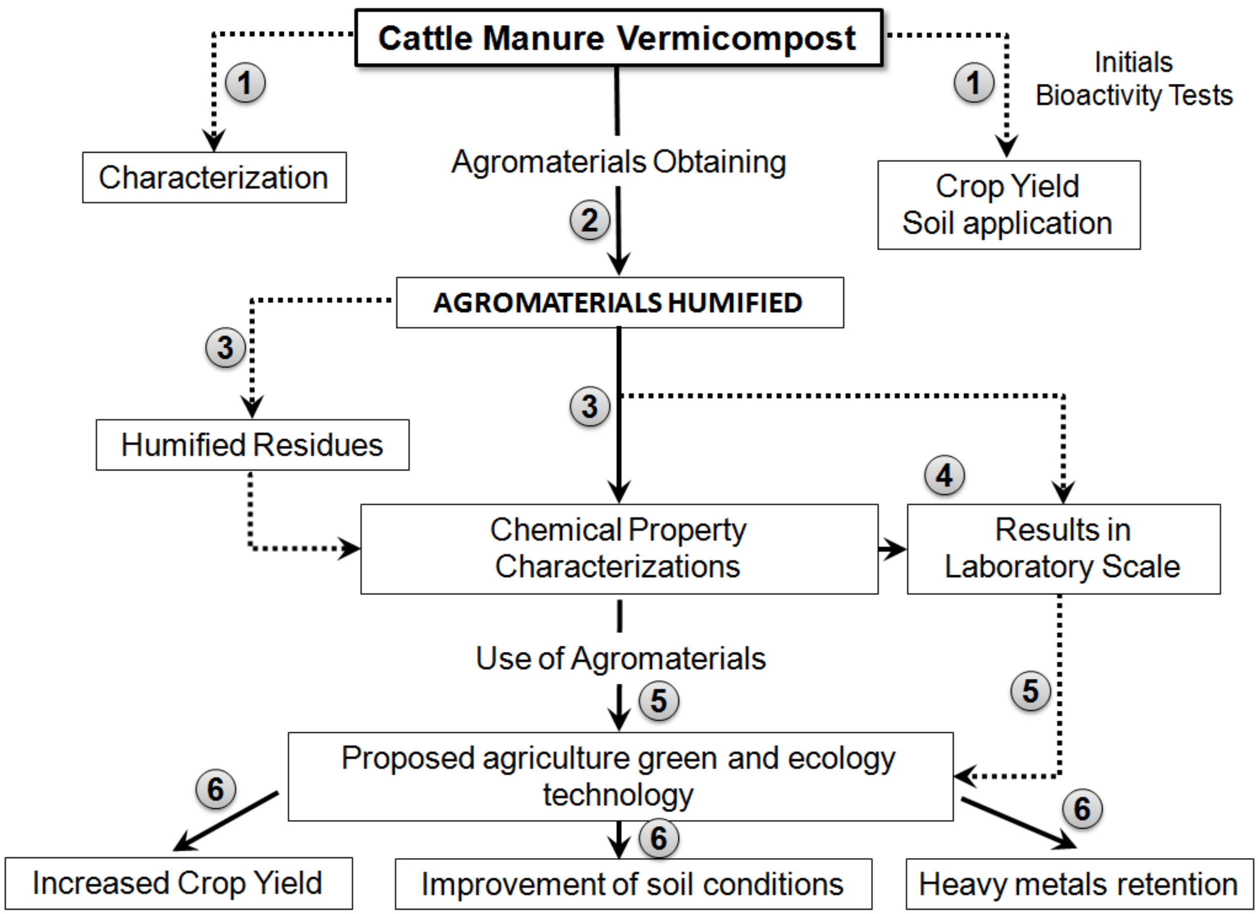


**Figure 1.** Overall working protocol applied by the MOBI research group. **Step 1:** Characterization and bioactivity testing in plants. **Step 2**: Procedures to obtain humified agro-materials. **Step 3**: Recovery of residual materials and characterization of their properties and activities. **Step 4:** Testing of material properties on a laboratory-scale. **Step 5:** Testing of material properties under agricultural conditions at the field-scale. **Step 6:** Attempted introduction of the technologies into agro-industrial and productive processes.

step 2 of the working protocol (Figure 1) are described in Table 1.

## Crop systems

Foliar application of 25 Kg $ha^{-1}$ of BIOSTAN® (Duncan, $p<0.001$; 0.005) in tomato crops induced positive effects on the nutritional status of the plants. This treatment also increased their growth, development, and agricultural yield compared to the control treatment and application at the beginning of blossoming and fructification (Alfonso et al., 2009; 2010). Combination of BIOSTAN® and AMF in tomato-maize association induced positive effects on the tomato crop at the non-optimal sowing seasons and reduced the effects of stress (Alfonso et al., 2006). The same effects were observed when BIOSTAN® ($p<0.001$) was combined with other organic fertilizers (Alfonso et al., 2007). Application of six mg $L^{-1}$ of liquid BIOSTAN® to a common bean crop (seven different varieties) resulted in increased growth and development of the plants, most particularly in regards to stalk length, number of pods per plant, number of grains per pod, and seed weight (González et al., 2003).

Use of LIPLANT® as an agro-material applied at a dilution of 1:40 v:v to the leaves of maize plants under laboratory conditions stimulated the activity of the membrane $H^{+}$-ATPase, increased the chlorophyll content, increased the net photosynthesis, and increased the nitrate reductase activity, all of which contributed to stimulation of growth and development of the plants (Martínez, 2006). Under field conditions, LIPLANT® at dilutions of 1:20 and 1:30 v:v increased the total agricultural yields of maize and cucumber crops, resulting in economic gains of up to 89% (Caro, 2004). Under field conditions, foliar application of LIPLANT® at a dilution of 1:80 v:v to tobacco crops increased the agricultural yield without altering the chemical characteristics of the leaves (De La Huerta et al., 2012). Other studies showed that LIPLANT® stimulated the growth, development, and yield

**Table 1.** Characteristics, properties, and uses of some of the agro-materials obtained from the protocol.

| Agro-materials | State at RT | B.C | O.C | I.C | Application | Target-use |
|---|---|---|---|---|---|---|
| **BIOSTAN®** | solid | --- | HS (20-50%)<br>Hormones<br>Amino acids | -Na, K, Ca, Mg, Cr, Mn, Sr, Fe, Cu, Ni, Zn | Foliar: OD*<br>~20 $mgL^{-1}$ | Micro propagation of banana.<br>Maize<br>Common bean<br>Tomato |
| **LIPLANT®** | liquid | Bacteria Fungi Actinomyces | HS (20-50%)<br>Hormones<br>Amino acids | -Na, K, Ca, Mg, Cr, Mn, Sr, Fe, Cu, Ni, Zn, Co, Ti | Foliar: OD*<br>~1:40 v:v | Tomato maize<br>Common bean<br>Tobacco, Cucumber |
| **OPLANT+®** | liquid | --- | HS (20-65%)<br>Hormones<br>Amino acids | -Na, K, Ca, Mg, Cr, Mn, Sr, Fe, Cu, Ni, Zn, N | Foliar: OD*<br>~1:60 v:v | Vegetables<br>Common bean |
| **CALDFER®** | solid | --- | Organic matter | -Ca, Mg, Fe, Cr, Ni, Cu | Retention of cations from heavy metals | Ni, Pb, Cu, Co, Zn, Al |

B.C, biological components; O.C, organic components; I.C, inorganic components; OD, optimal dose; RT, room temperature.

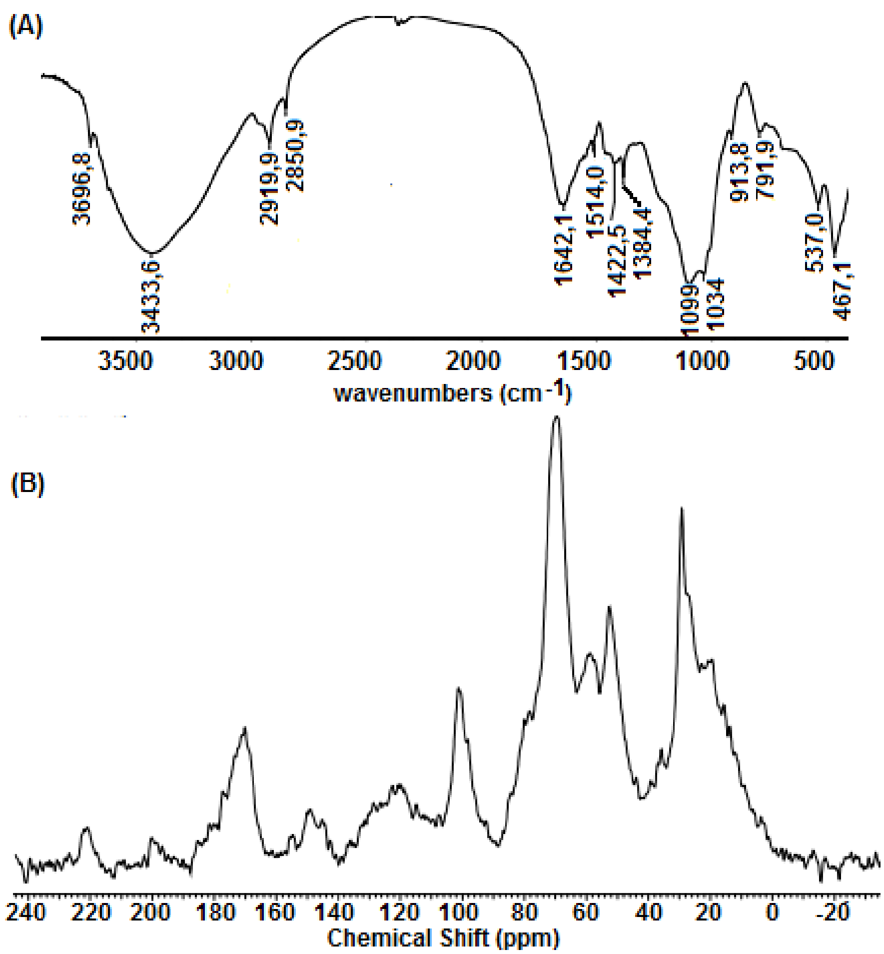


**Figure 2.** The FTIR and CP-$^{13}$C NMR spectra of CVC used as starting materials by the MOBI research group (unpublished data).

of tomato and lettuce crops, as well as of *Murraya paniculata* L. and *Morus alba* L (Mesa et al., 2005; Terry et al., 2012; Baños et al., 2009; Ruiz et al., 2009). All the statistical analyses were carried out at Duncan test and *p* value 0.05-0,001.

OPLANT+®, introduced by foliar application at a dilution of 1:60 v:v under laboratory conditions, exhibited positive effects on lettuce crops. This agro-material stimulated biochemical and physiological parameters of plant production, including nitrate reductase and photosynthetic pigment content, to increase growth and net assimilation rate. This effect allowed more than one harvest of crops to be performed within the same sowing season (Duncan test, $p<0.05$) (Hernández, 2010a). OPLANT+® also exhibited positive effects on common bean crops (Del Valle et al., 2012).

Several studies on the use of liquid humus obtained from several CVC have been conducted. Foliar application of 140 to 170 g $L^{-1}$ of liquid humus extracts from CVC induced the growth of *Sorghum bicolor* L. (Gutiérrez-Miceli et al., 2008). Foliar application of liquid humus obtained from CVC of plant organic residues induced improvement of the internal and external qualities of strawberries and stimulated the development of the plants (Singh et al., 2010).

**Protection against stress**

Abiotic stressors from water, salt, and heavy metals content induce production of reactive oxygen species (ROS) in plants that consequently cause oxidative stress. This results in severe crop yield losses. Several studies have investigated the protective effects of agro-materials on plants under stress conditions. Foliar and root application of liquid humus extracts obtained from the CVC used in the protocol described in Figure 1 showed protective effects on maize plants exposed to salt stress. Increases in free proline content and biomass accumulation in the stressed plants were observed when the humus was applied (Huelva et al., 2009). Foliar application of LIPLANT® at a dilution of 1:50 v:v to tomato crops sown on soil with high salt content over two sowing seasons increased the crop yield (Pérez et al., 2009). LIPLANT® applied to tomato crops under salt stress conditions restored fruit quality compared to control plants and improved the quality of plants compared to salt stressed plants without LIPLANT® application (Pérez et al., 2011).

Foliar application of HA from CVC at concentrations ranging from 30 and 40 mg HA $L^{-1}$ to rice plants sown on soil under conditions of induced water stress increased peroxidase (POX) activity. However, the growth and development of the stressed plants were similar to the non-stressed plants (Hernández, 2010b). Application of the same concentrations of HA to the roots of rice plants subjected to water stress increased POX activity, increased free proline content, protected against membrane permeability, and reduced the $H_2O_2$ content of the plant tissues (García et al., 2012b).

Recently, it was reported that HA applied to the roots of rice plants stimulated several enzymatic mechanisms associated with the anti-oxidative defensive system, as well as genes for aquaporins *(OsTIP 1;1, OsTIP 1;2, OsTIP 2;1,)*, which are proteins associated with the transport of water and $H_2O_2$. Thus, the potential of these humified materials to exert protective effects against abiotic stressors were demonstrated (García et al., 2012a). Foliar application of the same compounds to common bean plants sown in soil with high heavy metals content demonstrated protective effects mediated by the activation of anti-oxidative defensive mechanisms (Portuondo, 2010). Other plant defensive systems were also activated by HA application to both the leaves and the roots of rice plants. The activities of $\delta^1$ pyrroline-5-carboxylate reductase (P5CR) and phenylalanine ammonia-lyase (PAL) were stimulated, resulting in increased proline and phenolic content (García et al., 2012d).

HA with high molecular mass exerted effects on secondary metabolism associated with the synthesis of phenols (Schiavon et al., 2010). Application of HA to maize plants exerted an effect on the production of ROS and increased the activity of catalases (CAT) (Cordeiro et al., 2011). These protective effects of HA were also shown with common bean plants under salt stress conditions (Aydin et al., 2012).

**Substitutes for phytohormones and promotion of rooting**

*In vitro* micro-propagation of tissues for agricultural purposes represents an important alternative for increasing the availability of seedlings; however, the phytohormones used in this process are mostly synthetic, and their cost is high. HS exhibiting hormone-like activity might serve as natural and low-cost alternative. The HA isolated from the CVC used as starting materials in the present protocol could potentially be used to replace plant hormones for *in vitro* micro-propagation of bananas. HA added to culture media at doses of 10 to 50 mg $L^{-1}$ dramatically increased root length, number, and dry mass. The HA could completely replace phytohormones such as indole-3-acetic acid (IAA) and 6-benzylaminopurine (6-BAP) (Fernández, 2010) (Figure 3). Agro-materials, such as LIPLANT® and BIOSTAN®, increased the *in vitro* cell regeneration of rice, increased the inductor effects of phytohormone 2,4-dichlorophenoxyacetic acid (2,4-D), improved regenerative ability, and increased the number of roots when added to culture media (Godoy et al., 2006).

In a study on *in vitro* micro-propagation of banana clones, BIOSTAN® increased the survival and length of seedlings and increased the number of roots without altering the number of days needed to perform the first

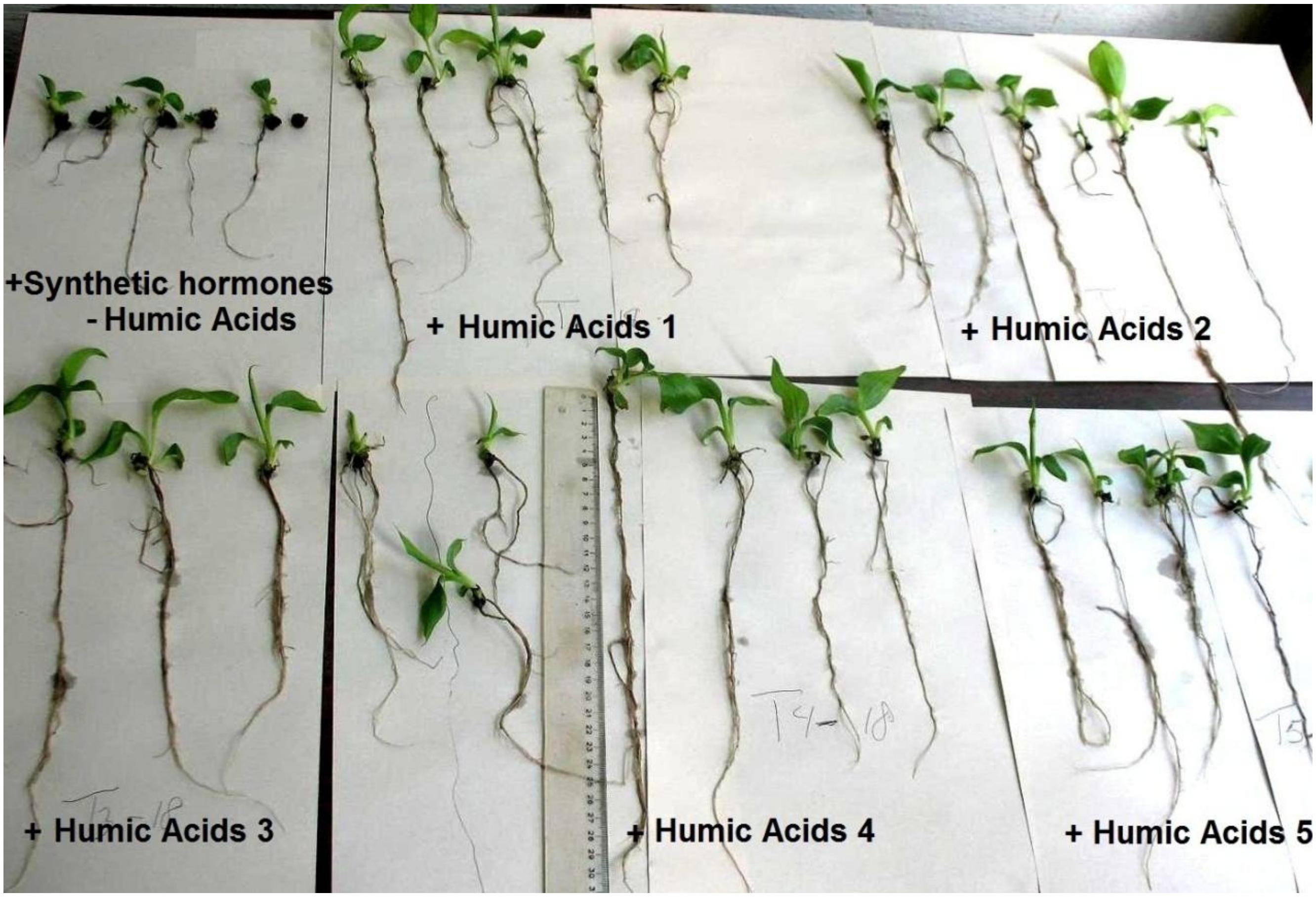


**Figure 3**. Image of banana seedlings treated with HA from CVC fully replacing synthetic phytohormones. **No Humic Acids**: control treatment according to the *in vitro* micro-propagation method; **Humic Acids1-4:** different concentrations of HA (Unpublished images supplied by Professor Márcia Beatriz Moya Fernández, Agrarian University of Havana - UNAH).

subculture when added to the culture media at the stage of establishment (Héctor et al., 2007).

### Effects of agro-materials on some soil properties

Application of new agricultural technologies requires studies on environmental impact. Thus, the influence of some of these biostimulants on the chemical and biological properties of the soil was investigated. LIPLANT® includes biological composition groups and genera of microorganisms similar to those comprising the CVC and the soil. This treatment did not exhibit antagonistic effects when its interactions (number of microorganisms and microbial respiration) were isolated and studied *in vitro*. At the same time, application of LIPLANT® to the soil for more than one year improved the soil fertility (availability of phosphorus and processes of organic matter humification) (Arteaga et al., 2007). Studies that assessed the effects of humic extracts on soil for onion crops found that such extracts contributed small amounts of organic matter and phosphorus to the soil (Rebato et al., 2011).

This type of study was also used to investigate the bioactivity of maize crops exposed to humic extracts obtained from residual solid (step 3) under field conditions. Consecutive applications of residual humic extracts did not induce significant changes in the chemical-physical characteristics of the soil compared with the initial conditions (Pimentel, 2007).

## REUTILIZATION OF HUMIFIED RESIDUAL AGRO-MATERIALS

Step 3 of the biotechnological working protocol involves the study of processes for the recovery of humified materials that remain as residual materials. Such materials originate from the processes for production of liquid humus (BIOSTAN®, LIPLANT®, and OPLANT+®) from CVC. Their primary formats are damp and solid in appearance. Some studies subjected the residual solids to consecutive processes of purification to extract the HS and organic substances that were still soluble in the aqueous media. The resulting solids (Figure 4) were characterized using spectroscopic techniques (FTIR and

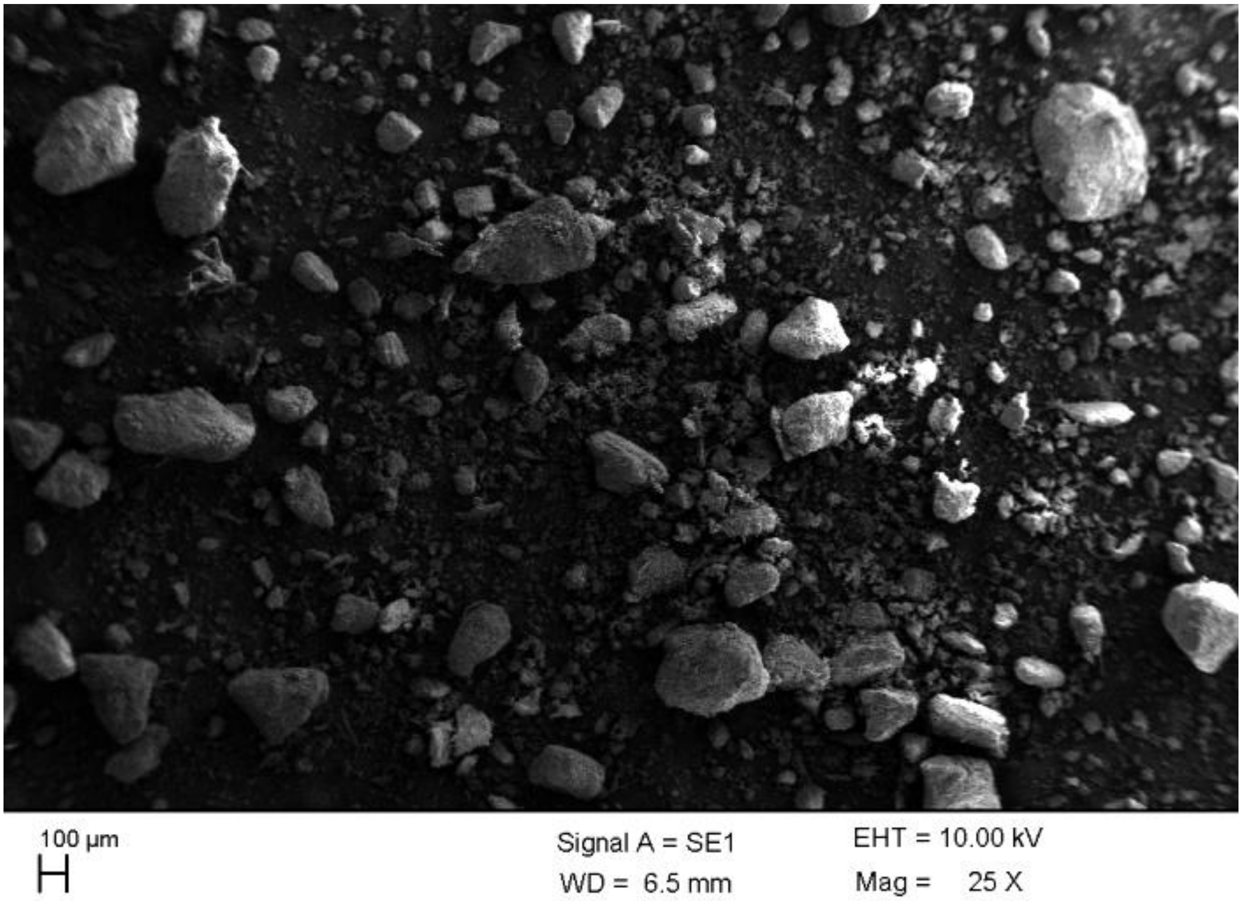


**Figure 4.** Image obtained by scanning electron microscopy (SEM) of CALDFER®, scale 100 µm.

atmospheric pressure chemical ionization-mass spectroscopy (APCI-MS)). These solids exhibited functional groups and structural fragments that could bind to metallic cations. Retention tests indicated that these solids retained more than 95% of added $Ni^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ (García et al., 2007).

García et al. (2012d) reported that in both mono- and multi-elemental systems, the residual solids could retain greater than 65% of added $Pb^{2+}$ and $Ni^{2+}$ cations. Based on FTIR spectroscopy, -OH, -COOH and -NH participated in the formation of bonds with the cationic metals. They also investigated the possibility of incorporating residual solids as organic aggregates into the soil. Studies conducted with maize plants showed that at a ratio of 1:4 m:m of soil to residual solid, the increase in plant growth and development was similar to CVC used as soil aggregates during the vegetative stage of cultivation.

While some studies focused on direct use of residual solids, others investigated methods to obtain HS from them. Humified extracts stimulated biochemical-physiological processes and increased the agricultural yield of maize crops under laboratory and field conditions (Pimentel, 2007). Other extracts from the residual solids that were obtained by means of different procedures contained HS. Such extracts improved in the main physiological indicators in radish crops (Rebato et al., 2011).

The results of studies conducted with residual solids extracted from humus from CVC will be useful as biotechnologies for agriculture where profit is also made from the residues. Residual solids from CALDFER® might be used to decontaminate industrial residues contaminated with heavy metals. Other extracts obtained from those solids might be incorporated into processes where BIOSTAN® and LIPLANT® are applied according to the requirements of crops.

## FUTURE STUDIES

The effort to introduce a biotechnology based on small- and medium-scale production and application of agro-materials resulted in products exhibiting properties with beneficial features for agriculture, livestock breeding, and the environment. However, other features should be subjected to rigorous scientific studies before such biotechnologies can be applied for actual production.

**Feature (i):** Although the full conditions (procedures and assessment techniques) needed for quality control of the raw material (CVC) are met, the CVC originate from varied sources and must therefore be standardized.
**Feature (ii):** Studies on the modes of action of HS agro-materials applied to both leaves and roots must be continued.
**Feature (iii):** Training of professionals for research on organic matter and HS must continue, as well as the preparation and participation of farmers in the field experiments.
**Feature (iv):** Studies should be conducted under different climatic conditions and in different biomes to establish wider application of these biotechnologies and investigate their global environmental sustainability.

## CONCLUSIONS

A biotechnological package able to operate in a sustainable manner would be highly useful, particularly to developing societies. Sustainability includes meeting the economic, social, cultural, and environmental needs of any society. Appropriate and rational use of natural resources, as well as reutilization of materials considered as refuse represent some of the more important routes leading to sustainable development. Humified organic matter is widely available and easily accessible as an environmental resource. Thus, it is possible to stimulate investment on research focusing on the production of this type of biotechnology. Application of CVC and liquid humus to plants might promote increased agricultural production and improve soil conditions. In addition, the refuge of such techniques might be easily used in agriculture and environmental decontamination.

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