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Use of chemometrics in the chemical and microbiological characterization of composts from agroindustrial wastes

M.A. Bustamante [a,*], F. Suárez-Estrella [b], C. Torrecillas [a], C. Paredes [a], R. Moral [a], J. Moreno [b]

[a] Departamento de Agroquímica y Medio Ambiente. Universidad Miguel Hernández de Elche. EPS-Orihuela, ctra. Beniel Km 3,2, 03312-Orihuela (Alicante), Spain
[b] Unidad de Microbiología, Departamento de Biología Aplicada, Escuela Politécnica Superior, Universidad de Almería, 04120 Almería, Spain

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ABSTRACT

Physico-chemical, chemical and microbiological parameters were studied in a set of fifteen different composts elaborated with agroindustrial wastes using two different composting systems (turning and static pile composting). To carry out the chemometric evaluation, multivariate statistical analysis techniques, such as hierarchical cluster analysis (HCA) and factorial analysis (FA) were used. Composts obtained showed suitable physico-chemical and chemical properties for their use as organic amendment and a good maturity degree. HCA allowed to classify the organic materials mainly in four groups: cluster A, cluster B, cluster C and unclustered composts; also, this statistical tool showed the lack of influence of the composting system in the final characteristics of these composts. On the other hand, through FA, it was possible to identify the principal variables associated to the composting of agroindustrial wastes in four factors that explained 72.3% of the variability.

1. Introduction

Composting is defined as the microbial degradation of heterogeneous solid organic materials under moist, self-heating and aerobic conditions to obtain a stable material that can be used as organic fertiliser (Amir et al., 2008). Therefore, composting constitutes a useful treatment to convert organic wastes into valuable final products with different applications, including the improvement of soil fertility and the suppression of certain phytopathogens (Nakasaki et al., 1998; Termorshuizen et al., 2006; Suárez-Estrella et al., 2007). However, the composting of organic wastes with different origin, e.g. livestock wastes or wastes from the agro-food industry, produces end-products that strongly differ in their characteristics and then, in their quality (Gómez-Brandón et al., 2008; Bustamante et al., 2008b; Tejada et al., 2009). On the other hand, the standards established for a safe use of compost usually only refer to sanitization criteria related to human pathogen contents and limit values for certain substances, such as heavy metals and/or organic pollutants (PCBs, PAHs) (Hogg et al., 2002). However, the use of these parameters is not enough to determine compost quality, which is generally based in two criteria: stability and maturity. Compost stability is strongly related to the rate of microbial activity in compost (Eggen and Vethe, 2001) and to the resistance of compost organic matter for further rapid degradation (Hue and Liu, 1995). Compost maturity refers to the suitability for plant growth, related to the degree of decomposition of phytotoxic compounds, and to the production of humic-like substances (Wu et al., 2000). In addition, the composting process involved a resident microbial population composed of a wide variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms (e.g. bacteria, yeasts and fungi), this microbial diversity being also considered a prerequisite for a satisfactory composting process, since the presence of certain microorganisms can reflect the quality of the maturing compost (Beffa et al., 1996; Takaku et al., 2006). Different authors have suggested numerous parameters to assess the maturity and stability degree of the composts obtained (Bernal et al., 1998, 2009; Wu et al., 2000; Eggen and Vethe, 2001; Said-Pullicino et al., 2007). However, compost stability and/or maturity are difficult to assess using a single parameter, mainly because of the great variety of raw materials and composting practices. Therefore, physical, physico-chemical, chemical and microbiological parameters are necessary to evaluate the maturity and/or stability of the final product obtained after composting, which is difficult and time-consuming. Chemometric methods have been used by different authors to obtain a classification of different organic materials (Campitelli and Ceppi, 2008; Bustamante et al., 2009) or to compare compost organic matter and naturally occurring organic matter (Zbytniewski et al., 2002). On the other hand, several researches are available regarding to the characteristics of composts from different materials, such as manures, municipal solid wastes or vegetal wastes (Bernal et al., 2009; Farrell and Jones,

* Corresponding author. Tel.: +34 966749655; fax: +34 966749711.
E-mail address: marian.bustamante@umh.es (M.A. Bustamante).

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2009; Som et al., 2009), but little is known about the microbial and chemical properties of composts from different agroindustrial wastes.

Therefore, the main objective of this study was to determine the physico-chemical, chemical and microbiological characteristics of the composts obtained from agroindustrial wastes, in order to evaluate, using multivariate techniques, the relationships among all the parameters studied and to classify the composts obtained.

2. Methods

2.1. Samples

In this study, a set of fifteen samples of mature compost were evaluated. Composts were obtained using manures, agricultural and agroindustrial wastes as raw materials, such as cattle and sheep manures, winery and distillery wastes (grape stalk, exhausted grape marc and vinasse), wastes from the orange juice and tomato-soap production, "alperujo" (solid waste obtained from olive oil production), almond peel, exhausted peat and spent mushroom substrate. Composts were prepared using different composting systems, the static pile composting system (Rutgers system) in four of the composts and the turning composting system in the rest. Table 1 shows the composition and proportion of the different composts, as well as the composting system used. In piles 11 and 12, a pH correction of the initial mixture was carried out using 0.1% CaO in order to avoid an initial inhibition of the thermophilic microorganisms (Sundberg et al., 2004).

Samples, three replicates for compost, were taken by mixing seven sub-samples from seven sites of the pile, from the whole profile (from the top to the bottom of the pile). Each sample was divided into two parts: one was air-dried and ground to 0.5 mm for analysis and the other was immediately frozen and kept for microbiological analysis.

Table 1
Characteristics of the composting piles and type of composting system used (percentages on dry weight basis).

Pile	Proportion of the raw materials	Composting time (days)[a]	Maximum temperature (°C)[b]	Composting system used
P1	20% GS + 80% CM	86	56	Turning pile
P2	40% GS + 60% CM	86	42	Turning pile
P3	60% GS + 40% CM	86	32	Turning pile
P4	80% GS + 20% CM	86	29	Turning pile
P5	60% EGM + 40% OJW	102	41	Turning pile
P6	60% EGM + 40% ALP	167	53	Turning pile
P7	60% EGM + 40% AW	102	64	Turning pile
P8	60% EGM + 40% EP	72	38	Turning pile
P9	60% EGM + 40% SPS	96	57	Turning pile
P10	60% EGM + 40% SPS + V	96	66	Turning pile
P11	60% EGM + 40% OJW	246	53	Static pile (Rutgers)
P12	60% EGM + 40% TSW	161	55	Static pile (Rutgers)
P13	60% EGM + 40% CM	154	54	Static pile (Rutgers)
P14	60% EGM + 40% SM	197	55	Static pile (Rutgers)
P15	50% EGM + 25% SM + 25% TSW	147	50	Turning pile

GS: grape stalk; CM: cattle manure; EGM: exhausted grape marc; OJW: orange juice waste; ALP: alperujo; AW: almond waste; EP: exhausted peat; SPS: spent mushroom substrate; V: vinasse; TSW: tomato soap waste; SM: sheep manure.

[a] The composting time includes both bio-oxidative and curing phase.

[b] Maximum temperature values reached during the bio-oxidative phase.

2.2. Physico-chemical and chemical methods

Compost samples were analysed for electrical conductivity (EC) and pH in a 1:10 (w/v) water-soluble extract. Dry matter of the samples was determined after 12 h at 105 °C. Organic matter (OM) was assessed by determining the loss-on ignition at 430 °C for 24 h (Navarro et al., 1993). Total nitrogen (TN) and total organic carbon (TOC) were determined by automatic microanalysis (Navarro et al., 1991), as were the 0.1 M NaOH-extractable organic carbon (Cex), water-soluble carbon (WSC) and fulvic acid-like carbon (Cfa), the latter after precipitation of the humic acid-like carbon (Cha) from the NaOH-extraction at pH 2.0 (Sánchez-Monedero et al., 1996). Cha was calculated by subtracting Cfa from Cex. Water-soluble phenols (POL) were determined by the modified Folin–Ciocalteu method in a 1:20 (w/v) water extract (Beltrán et al., 1999) and germination index (GI) was calculated using seeds of *Lepidium sativum* L. (Zucconi et al., 1981). The humification parameters (humification ratio (HR), percentage of humic acid-like carbon (PHA), humification index (HI), polymerisation ratio (PR)) were calculated according to Bustamante et al. (2008b). After $HNO_3/HClO_4$ digestion, P was assessed colorimetrically as molybdovanadate phosphoric acid, Na and K were determined by flame photometry and other nutrients (Ca, Mg, Fe, Cu, Mn, Zn) and toxic heavy metals (Cr, Ni, Cd, Hg, Pb) by atomic absorption spectrometry.

2.3. Microbiological determinations

Microbial populations (mesophilic and thermophilic aerobic bacteria, mesophilic and thermophilic actinomycetes and mesophilic fungi) in the compost samples were determined using agar plate dilution methods. Initial suspensions were prepared by the addition of 10 g (wet weight) of a compost sample to 90 ml of 0.9% (w/v) sterile saline solution in 250 ml Erlenmeyer flasks. The suspensions were shaken at 120 rpm for 20 min at room temperature and were serially diluted with sterile saline solution. Aliquots of 0.1 ml of each diluted suspension were spread on prepared agar plates. Three plates were used per dilution. Aerobic bacteria were enumerated on Nutrient Agar plates; mesophilic fungi were enumerated on Rose Bengal Chloramphenicol Agar and actinomycetes on Actinomycete Isolation Agar.

Incubation was at 30 °C for mesophilic microorganisms and at 60 °C for thermophilic microorganisms. After 24 h of incubation, mesophilic aerobic bacteria were enumerated. Thermophilic aerobic bacteria and actinomycetes were counted after 48 h and 72 h of incubation, respectively. The rest of microorganisms were enumerated after 5 days of incubation. The number of microorganisms was expressed as colony forming units (CFU) per gram of compost.

2.4. Multivariate statistical analysis

The chemometric analysis was carried out using hierarchical cluster analysis (HCA) and factorial analysis (FA). HCA is a technique used for classifying objects, which have been characterized by the values of a set of variables, into different groups. The clusters are formed by grouping objects according to similarity, and the results are presented in the form of dendograms, which allow visualizing the distances between objects (Gil et al., 2008). Data was clustered by the between-groups linkage or Unweighted Pair Group Method with Arithmetic mean (UPGMA) technique, which defines the distance between two clusters as the average of all the pairs of distances between elements of both clusters; similarities and dissimilarities were quantified by Square Euclidean dis-