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Isolation by isoelectric focusing of humic-urease complexes from earthworm (Eisenia fetida)-processed sewage sludges

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Abstract Vermicomposting is an eco-biotechnological process that transforms energy-rich and complex organic substances into a stabilized humus-like product. In a laboratory experiment, Eisenia fetida (Sav.) earthworms were employed to process putrescible sewage sludges into a high-value biofertilizer, very rich in urease activity and humic-urease complexes (stabilized extracellular enzymes). Extracellular humic-urease complexes were extracted by a single 24-h extraction at 37°C using neutral pyrophosphate (0.1 M); then, the extracts were dialysed and characterized by means of an analytical isoelectric focusing technique. This technique gave a multiplicity of humic bands enzymatically active, with isoelectric points ranging from 4.8 to 5.6. The results demonstrated that, after an 18-week incubation period, sewage sludge had undergone a biochemical evolution, which caused a doubling of absolute urease activity and a six-fold increase in specific activity (activity with reference to the humic C fraction). The biochemical evolution of the vermicompost was evaluated also from the sharp decrease in pyrophosphate-extractable C and water-soluble C. Stabilization of organic C during vermicomposting and the activity of humic-urease complexes expressed at low pH values are of extreme importance when organic wastes are used in acid soils for biochemical restoration purposes.

Key words Sewage sludge · Vermicomposting · Extracellular urease · Humic substance · Isoelectric focusing

Introduction Vermicomposting is considered a feasible method for enhancing the value and detoxification of organic wastes, before their use as soil conditioners (Edwards and Neuhauser 1988; Nogales et al. 1999). Products with a relatively high content of humic-like compounds, active microorganisms and enzymes, greatly contribute to the enhancement of the biochemical fertility of soils degraded by intensive-cultivation, pollution or other natural causes (Perucci 1992; Dick 1992; Nannipieri 1994; Garcia et al. 1994). Enzymatically active humic fractions, extracted from vermicompost, have been found to be particularly efficient in mitigating soil salinity and in improving its physical structure when added in fertirrigation solutions, both in laboratory and field experiments (Garcia et al. 1995; Masciandaro et al. 1997). It is known that many extracellular enzymes can become bound to humic matter during a composting or a vermicomposting process, regardless of the type of organic matter used (Ceccanti and Garcia 1994; Ceccanti et al. 1997), but knowledge of the chemical and biochemical properties of such extracellular enzymes is very scanty. The study of these humic-enzyme complexes and the separation of enzymatically active from non-active humic matter can be done through the isoelectric focusing (IEF) technique (De Nobili et al. 1983; Ceccanti et al. 1986, 1989; Garcia et al. 1995). IEF electrophoretically separates amphoteric molecules according to their net surface charge density or isoelectric points (pI); pI is the pH value at which the net surface electrical charge is “zero”. Compounds with a comparable molecular weight and electrophoretic mobility, such as those in humic or fulvic fractions, but differing slightly in their electrical surface charge, may be sharply separated by IEF. The surface electrical charge of a humic-enzyme complex is of particular importance when predicting its stability and movement (solubility) in a given physical environment characterized by an amount of electrical charges similar to that existing in many soils. The purpose of this work was the extraction and quan-
tification of humic-urease complexes from vermicomposted sewage sludge and their purification through an analytical IEF technique.

Materials and methods

Sewage sludge

A 50% mixture of two different kinds of sewage sludges, an anaerobically digested sewage sludge produced from a wastewater treatment plant of a paper mill plant and an aerobically digested municipal sludge, was used as a substrate for the vermicomposting process. For the determination of the heavy metals content, the mixture was air dried and crushed to pass through a 2-mm sieve. In accordance with the United States Environmental Protection Agency (1983), HNO₃–HClO₄ and diethylenetriaminepentaacetic acid (DTPA) procedures were used for determining total and available heavy metals, respectively. Concentrations of metals are given in Table 1.

Vermicomposting

The mixture of sewage sludges was vermicomposted during an 18-week period. All characteristics of the process have already been described by Benítez et al. (1999). Samples were collected weekly during vermicomposting and stored in plastic vials at 4°C until all chemical analyses and urease tests were carried out.

Organic C extraction

Water-soluble C (WSC) was extracted from vermicompost with distilled water in a 1:10 solid:liquid ratio by mechanical shaking at 60°C for 1 h. WSC was analyzed in the supernatant after centrifugation. Pyrophosphate-extractable C (PEC) was extracted at 37°C for 24 h under shaking, using 100 g organic product suspended in 11 Na₂P₂O₇ (0.1 M, pH 7.1) as extractant. Then, the suspension was centrifuged at 18,000 rpm and filtered through a 0.22-μm Millipore membrane. The extract was dialysed against distilled water and, once dialysed, concentrated to the initial volume. WSC was analyzed in the supernatant after centrifugation. Pyrophosphate extractable C (PEC) was extracted at 37°C for 1 h.

C analysis

The C contents of WSC and PEC were determined by acid digestion with K₂Cr₂O₇ and H₂SO₄ at 160°C for 30 min. A spectrophotometric method was used to quantify the Cr₆⁺⁺ produced by the reduction of Cr₆⁺⁺ (λ = 590 nm) (Garcia et al. 1994).

Urease activity

Urease activity in PEC was determined using 0.5 ml dialysed extract, 2 ml of 6.4% urea as substrate and 2 ml phosphate buffer (0.1 M, pH 7.0). Controls were run as an enzyme test, but organic extract was added at the end of incubation, before the determination of NH₃. Enzyme tests and controls were incubated at 37°C for 2 h, then kept at 2°C for 15 min to stop the reaction. The NH₃ released into the solution from the hydrolytic reaction of urea was measured as gaseous NH₃ through a selective electrode (model 95–12; Orion, Cambridge, Mass.).

Statistical analysis

All the results reported are means of three replicates. To test the effect of time on chemical and biochemical parameters, ANOVA was conducted using Statgraphics Plus statistical software (Statistical Graphics, Princeton, N.J.).

Results and discussion

Evolution of extractable organic C

Figure 1 shows the decrease with time in the content of PEC and WSC of sludge. Both PEC and WSC decreased sharply during the first 2 weeks, reaching nearly 30% and 50% of their initial contents, respectively; thereafter, a further 18 weeks was required to further halve their concentration. WSC is considered as an index of mineralization of organic C mainly composed of labile and easily degradable compounds (Garcia et al. 1994).