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Changes in the level of alkaline and acid phosphatase activities during green wastes and sewage sludge co-composting

Remy Albrecht *, Jean Le Petit, Virgile Calvert, Gérard Terrom, Claude Périssol

Aix-Marseille Université, Institut Méditerranéen d'Ecologie et de Paléoécologie, UMR CNRS IRD, Ecologie Microbienne et Biotechnologies, Faculté des Sciences et Techniques de Saint-Jérôme, Case 452, 13397 Marseille Cedex 20, France

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ABSTRACT

The aim of this work was to study the activity level of alkaline and acid phosphatases during the composting of green wastes and sewage sludge and to determine relationships between biotic and abiotic properties of compost and phosphatase activities. This study has revealed a noticeable separation of phosphomonoesterase activities into two distinct phases during composting. Alkaline and acid phosphatase activities were high during the first month of composting and then declined up to the end of the process. Both phosphatase activities were significantly correlated with a group of variables which are clearly related to advancement of maturity during the composting process: C/N, respiration, cellulase, protease, phenoloxidase activities, HA and Global Index of Composting Evolution (GICE) resulting from 14 biological and chemical parameters. This study has also revealed the complexity of factors regulating turnover of P in compost process since phosphatase activities appeared constrained across two thresholds: 60/70 and 100 μg of inorganic P g^{-1} DM for activation and repression, respectively.

1. Introduction

Composting of organic wastes is a biooxidative process involving the mineralization and partial humification of organic matter, leading to a stabilized final product, free of phytotoxicity and pathogens and with certain humic properties (Zucconi and De Bertoldi, 1987) and that can be utilized as agricultural fertilizers. During the first phase of the process, simple organic carbon compounds are easily mineralized and metabolized by the microorganisms, producing CO_2, NH_3, H_2O, organic acids and heat (Albrecht et al., 2009). During the second phase, the composting process results in the production of biologically mature, stable, and chemically complex organic compounds resembling soil humic substances (Plaza et al., 2005). In green waste and sewage sludge composting, sludge contains different macronutrients and one of the essential element is phosphorus. Transformation of organic phosphorus through enzymatic reactions (Eivazi and Tabatabai, 1977; Tate, 1984) and the immobilization of phosphorus in the biomass itself play a fundamental role in P cycling. According to Lima et al. (1996), only a small portion of sludge total P is in the inorganic form (PO_4^{3-}) and can be assimilated by plants or microorganisms (Rao et al., 1996), while approximately 70% of P is in the organic form. To be assimilated, organic P present in compost must be previously mineralized into orthophosphate ions. Only enzymes produced by plants and microorganisms are able to hydrolyse organic P into phosphates (Criquet and Braud, 2008). This process is catalyzed by phosphatases, which hydrolyse both esters and polyphosphates. According to Tabatabai (1994) and Criquet et al. (2004), among these enzymes, acid and alkaline phosphomonoesterases are considered the predominant phosphatases in most types of soil and litter. Investigations on the changes of the level of phosphatases activities could contribute to our understanding of P cycling during aerobic degradation process, which could allow more efficient use of P fertilizer in agricultural systems. Changes in the level of phosphatases activities in natural environment, especially in composts, are realized at two levels. First, regulation of their synthesis can be studied for a pure strain but not for a community of microorganisms because the mechanism of regulation is very complex and often very different from one microorganism to another. Second, changes in the level of phosphatase activities are also dependent of the nature of each phosphatase, since certain ones are repressible by inorganic phosphate and another not. So, in natural environments, phosphatase activities can be considered as an overall measure. They represent the resultant of their regulation synthesis in each microorganism and the regulation of the activities of the phosphatases released by the microorganisms present in the environment studied. To this aim, we studied the levels of alkaline and acid phosphatase activities during a common aerobic degradation process, the composting of green wastes and sewage sludge in windrows. Numerous studies have highlighted changes in the levels of phosphatase activities in soil and litter

* Corresponding author. Tel.: +33 4 91 28 85 31; fax: +33 4 91 28 81 90.
E-mail address: remy.albrecht@gmail.com (R. Albrecht).

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(Juma and Tabatabai, 1977; Criquet et al., 2004; Nèble et al., 2007; Criquet and Braud, 2008) but to our knowledge few in the composting field (Cunha-Queda et al., 2007).

The objectives of this work were (i) to investigate the presence and the changes in the level of acid and alkaline phosphatases activities during composting and (ii) to determine relationships between biotic and abiotic properties of compost and phosphatase activities.

2. Methods

2.1. Experimental materials

Composts are produced by the company Biotechna (Ensues, Bouches du Rhône, France) and were obtained from dewatered digested sewage sludge, green wastes and pine bark at a 1:1:1 v/v ratio (Table 1). The mixture was composted for 20 days in boxes with forced aeration, and then stored in windrows of 100 m^3 on a composting platform for 6 months. The heaps were mixed several times during process to promote organic matter humification. Composts were sieved (<20-mm mesh) to remove large bark pieces. The final product was certified conform to the French standards for composts made with materials of water treatment origin (NFU44-095, 2002).

2.2. Sampling design

Approximately 1 kg of homogeneous compost was collected from each windrow corresponding to nine different stages of composting (4, 31, 40, 67, 84, 101, 114, 128 and 146 days) with four replicates, *i.e.* 36 samples in total. Each compost sample is a homogeneous replicate and a representative mixture of the heterogeneity for each heap. All 36 samples were sieved (<20-mm mesh), ground with a Cyclotec® 1093 mill (FOSS) to 1-mm size then stored at 4 °C and sub-sampled for the analysis.

2.3. Enzymatic assays

Acid phosphatase activities were measured by reacting 1 g of compost, mixed with 5 mL of acetate buffer (0.1 M, pH 5), with 10 mM *p*-nitro phenyl phosphate at 50 °C, stirred for 1 h and then centrifuged 10 min at 16,800*g* and 4 °C (Eivazi and Tabatabai, 1977). One millilitre of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added and the flask swirled for a few seconds to stop the reaction. Samples were centrifuged 10 min at 16,800*g* and 4 °C to prevent interference by precipitates. The intensity of yellow colour (release of *p*-nitrophenol, $\varepsilon^M = 1.9 \times 10^4\ M^{-1}\ cm^{-1}$) was measured at 412 nm in a Kontron spectrophotometer Uvikon 860. We calculated the *p*-nitrophenol content by referring to a calibration curve obtained with standards containing 0, 10, 20, 30, 40 and 50 ppm of *p*-nitrophenol. Two controls, both without substrate and without compost, were also included. To measure alkaline phosphatase activities, acetate buffer was replaced by NaOH–glycine (0.1 M, pH 9) buffer (Eivazi and Tabatabai, 1977). Results for both acid and alkaline phosphatases were expressed in unit defined as μmol of *p*-nitrophenol released $min^{-1}\ g^{-1}$ DM.

Table 1
Biotic and abiotic characteristics of dewatered digested sewage sludge, green wastes and mature compost.

Parameter		Sewage sludge	Green waste	Compost
Moisture	%	76.1	25.1	34
pH		6.9	7.2	7.8
C/N		6.5	36.30	12.4
Organic matter	% DM	80.8	74.5	39.1
Cd	mg kg^{-1} DM	2	0.22	0.77
Cr	mg kg^{-1} DM	14	11.71	21.9
Cu	mg kg^{-1} DM	276	22.57	122
Hg	mg kg^{-1} DM	1.25	0.04	0.88
Ni	mg kg^{-1} DM	10	6.7	14.7
Pb	mg kg^{-1} DM	40	18.65	65
Zn	mg kg^{-1} DM	378	68.6	266

2.4. Chemical characteristics

2.4.1. Water extractable inorganic P (P_w) measurement

P_W was extracted using the method of Zhou et al. (2001). One gram of soil was agitated (150 rpm) in 25 mL of distilled water for 2 h at 25 °C. The mixture was filtered and the supernatant was analyzed for PO_4^{3-} by the molybdenum-blue method (Murphy and Riley, 1962) adapted by Alef and Nannipieri (1995). Extraction and measurement were carried out in triplicate for each sample. Results were expressed in μg $PO_4^{3-}\ g^{-1}$ of dry matter (μg $PO_4^{3-}\ g^{-1}$ DM).

2.4.2. Organic phosphorus P (P_{org}) measurement

Total phosphorus was determined after mineralization by acidification (7 N H_2SO_4) of compost extracts. $PO_4^{3-}\ g^{-1}$ formed were analyzed by the molybdenum-blue method (Murphy and Riley, 1962) adapted by Alef and Nannipieri (1995). Extraction and measurement were carried out in triplicate for each sample. Results were expressed in μg P g^{-1} of dry matter (μg P g^{-1} DM).

2.4.3. Statistical analyses

The relationships between biotic and abiotic factors were analyzed by the Pearson correlation coefficient (r). Significant correlations were retained for $P < 0.05$, $P < 0.01$ and $P < 0.001$. All statistical analyses were performed using Xlstat 2007.

3. Results and discussion

3.1. Relationships between alkaline and acid phosphatase activities and organic and inorganic P during composting

We measured alkaline and acid phosphatases in composting of green waste and sewage sludge during 146 days (Table 2). These enzymes exhibited high activities during the first month of composting. Then, both alkaline and acid phosphatase activities declined up to the end of the process, with, respectively, 70.5–44.8 mU g^{-1} DM and 23.4–11.3 mU g^{-1} DM between 40 and 146 days of composting. Several authors found the same trend during composting of various biowastes (municipal solid waste, manure, sewage sludge and pig slurry), with a maximum of phosphatase activity after 3 weeks of composting followed by a sudden decline (Tiquia et al., 2002; Ros et al., 2006; Raut et al., 2008). The high organic matter content and large quantity of nutrients in original compost stimulate growth of total aerobic bacteria and subsequent phosphatase and peptidase synthesis (Cunha-Queda et al., 2007). However, different patterns with gradual increase during composting have also been reported in manure composting (Tiquia et al., 2002; Cayuela et al., 2006) and in olive mill wastes composting. Alkaline phosphatase is here the main phosphatase during the composting process. The ratio of alkaline to acid phosphatase activities stayed stable with a mean value of three and no significant change during composting process. Plant roots are devoid of alkaline phosphatases, which are ascribed to soil bacteria and fungi (Burns, 1982). Indeed, unlike microorganisms, plants have been shown to produce only acid phosphomonoesterases (Guillemin et al., 1995). Consequently, we can deduce that phosphatase in our compost samples originated from microorganisms and not from green wastes, which comprise various components