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Enzymatic Activity as a Parameter for the Evaluation of Two-phase Olive Mill Wastes Composting

M. L. Cayuela[a,b], C. Mondini[b], M. A. Sánchez-Monedero[a] and A. Roig[a]
[a] *Department of Soil and Water Conservation and Waste Management. CEBAS-CSIC. Campus Universitario de Espinardo. Murcia. Spain.*
[b] *Experimental Institute for Plant Nutrition. ISNP-CRA. Gorizia. Italy*

Abstract
Two-phase olive mill waste (TPOMW) is a semisolid sludge generated during the extraction of olive oil by the two-phase centrifugation system. Although, at present, combustion is the most consolidate disposal option, composting is gaining interest as a sustainable strategy to recycle TPOMW for agricultural purposes. The quality of compost for agronomical use depends on the degree of organic matter stabilization, but despite several studies on the topic, no single method is available which alone can give a certain indication of compost stability. In addition, information on the biological and biochemical properties, including the enzymatic activity (EA) of compost, is rare. The aim of this work was to investigate the suitability of some enzymatic activities (β-glucosidase, arylsulphatase, acid-phosphatase, alkaline-phosphatase, urease and fluorescein diacetate hydrolysis (FDA)) as parameters to evaluate organic matter stability during the composting of TPOMW. These enzymatic indices were also compared to conventional stability indices. For this purpose two composting piles were prepared by mixing TPOMW with sheep manure and grape stalks in different proportions, with forced aeration and occasional turnings. The piles were sampled at four different stages (pile A: at 5, 18, 28 and 40 weeks of composting; pile B: at 5, 18, 23 and 34 weeks of composting), air dried and grounded for their chemical and enzymatic analysis. The composting of TPOMW followed the common pattern reported previously for this kind of material with a reduction of 40-50% of organic matter, a gradual increase in pH, disappearance of phyto-toxicity and formation of humic-like C. All enzymatic activities (EA) increased during composting except acid-phosphatase. FDA and alkaline-phosphatase underwent the greatest changes during the process and therefore, they are the most promising EA for the monitoring of TPOMW composting. Significant correlations were found between EA and some important conventional stability indices indicating that EA can be a simple and reliable tool to determine the degree of stability of TPOMW composts.

Keywords: olive mill wastes, composting, enzymatic activity, stability, organic matter

1 Introduction

Olive mill waste management is one of the major environmental challenges in all olive oil producing countries since they are produced in large quantities in short periods of time and their high phytotoxicity and antimicrobial properties adversely impact both soil and water qualities (Roig et al., 2006).

From the early nineties, the severe water restrictions in many Mediterranean countries has led to the creation and implementation of a new two-phase centrifugation system which has considerably reduced the amount of water needed and the wastes produced during the olive oil extraction. In spite of the environmental advantages of this new system, that has been called "ecological", olive mill waste disposal problems still remain unsolved. In fact, the semisolid sludge produced, known as two-phase olive mill waste (TPOMW), presents more disposal problems than the olive husk of the previous three-phase system and it demands for new environmental and economically viable management options.

One of the most promising alternatives for the recycling of TPOMW is through composting with other agricultural by-products (Cayuela et al., 2004; Roig et al., 2006). The peculiar physico-chemical properties of TPOMW, with limited porosity, high concentration of lignin, lipids and phenols, generate a particular composting process with high temperatures and a long thermophilic phase. Several composting experiments performed in the last years reveal the high quality of the composts obtained from TPOMW. Some physic-chemical properties (temperature, pH, CEC, organic C, NH_4^+, fats, phenols, humic-like substances) have been used to follow the evolution of the composting process. However, information on the changes in biological and biochemical properties during TPOMW composting, including the hydrolytic enzymatic activity (EA), is almost inexistent (Ntougias et al., 2006). Since composting is a microbial mediated process, dynamics of microbiological properties, including EA, are expected to give valuable information on the evolution of the process and the quality of the end products.

Extracellular enzymes are known to be involved in the depolymerization of different constituents of organic wastes. Some important enzymes involved in the composting process include cellulases and β-glucosidases related to C mineralization, proteases and urease involved in N cycle and phosphatases and arylsulphatase related to P and S cycles (Mondini et al., 2004). Some authors have reported a decrease in enzymatic activity during the composting process related to the decline in microbial activity and available substrates (García et al., 1993; Benitez et al., 1999). On the other hand, the formation of stable humic-enzymatic complexes during composting explains the increase in EA found in other studies (Mondini et al., 2004; Benitez et al., 2005).

The aim of this work was to study the evolution of some important enzymatic activities during the composting of TPOMW and their comparison with other chemical and biological properties commonly used as indicators of compost quality and maturity.

2 Materials and Methods

Two composting piles were prepared by mixing TPOMW, sheep litter (SL) and grape stalks (GS) in the following proportions (dry weight basis): Pile 1: TPOMW (50 %) + SL (50%); Pile 2: TPOMW (45 %) + SL (45 %) + GS (10%). Both mixtures were composted in trapezoidal piles (1.0 m high and 2 × 3 m base) by the Rutgers system with occasional turnings (Cayuela et al., 2006). Water was regularly added to maintain appropriate moisture (40-60%).

Sampling was made at four different stages of the composting process by mixing five sub-samples from different locations of the pile:

-M: From the mesophilic phase (7 weeks of composting).
-T1: From the thermophilic phase (18 weeks of composting)
-T2: From the thermophilic phase (28 (pile A) and 23 (pile B) weeks of composting)
-F: From the final compost obtained (40 (pile A) and 34 (pile B) weeks of composting)

Chemical and enzymatic analyses were performed on air-dried samples. Total nitrogen (TN) and total organic carbon (TOC) were determined by automatic elemental microanalysis (NA 1500 Carlo Erba Instruments). The cation exchange capacity (CEC) was analysed with $BaCl_2$-triethanolamine. pH was determined in a 1:10 (w/v) water-soluble extract. Water soluble carbon (WSC) was determined in a 1:20 (w/ v) water extract. Extractable carbon (C_{ext}) was measured in a 0.1 M NaOH compost extract (1:20 w/ v) and the fulvic acid carbon (FAC) after precipitation of the humic acid at pH 2 in the supernatant solution using a TOC analyzer (Formacs^{HT} Skalar analyzer). The humic acid carbon content (HAC) was calculated by subtracting FAC from the C_{ext}. Phytotoxicity, expressed as germination index (GI), was assayed by the *Lepidium sativum* test (Zucconi et al., 1981). FDA was determined according to Schnurer and Rosswall (1982). The rest of EAs were determined according to Mondini et al (2004).

3 Results and discussion

The composting process underwent the common pattern previously described for this kind of material with a long thermophilic phase due to the presence of recalcitrant substances (lignin, fats) in TPOMW (Cayuela et al., 2004; 2006). Figure 1 shows the evolution of the TOC/TN ratio and the pH during the composting of both mixtures. The TOC/TN decreased gradually in both composting piles starting in 25 and reaching values around 15 in the final composts, showing a suitable degree of stability.

Regarding pH, a marked increase was observed until week 20, keeping a constant value afterwards. This sharp increase in alkalinity has been previously reported during the composting of TPOMW (Cayuela et al., 2004; Alburquerque et al., 2006) and described as an intrinsic outcome of the mineralization of these spe-

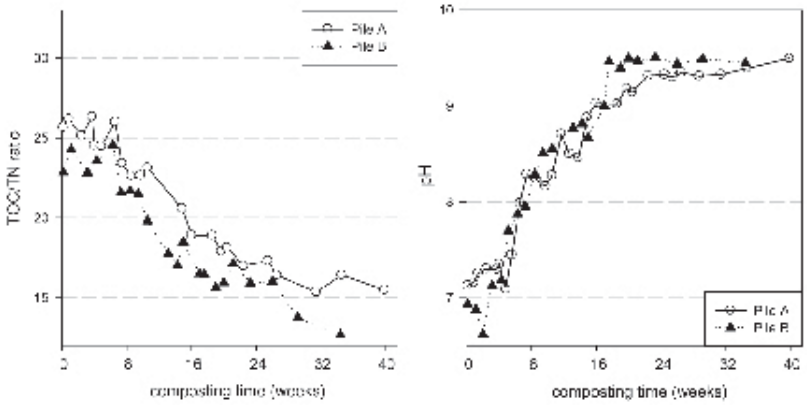


Fig. 1: Evolution of TOC/TN and pH during the composting of TPOMW in piles A and B.

cific materials. In fact, TPOMW is rich in organic acids of low-molecular weight (acetic, butiric, propionic) which degradation contribute to this marked increase in pH. Hence, the stabilisation of pH may be used as a stability index in TPOMW composts.

Five maturation indices widely reported in literature were selected for the evaluation of the composting process (Table 1).

Tab. 1: Selected maturation indices for the characterization of TPOMW composting process.

Pile	Composting phase	WSC/TN	GI (%)	C_{HA}/C_{EXT} (%)	C_{HA}/C_{FA}	CEC/ TOC
A	M	4.63	9	33.5	0.50	1.4
	T_1	3.60	0	27.7	0.38	2.1
	T_2	3.03	61	68.8	2.20	2.1
	F	1.72	74	73.1	2.71	2.4
B	M	4.31	5	36.3	0.57	1.8
	T_1	1.63	61	72.3	2.61	2.6
	T_2	1.63	98	73.5	2.77	2.7
	F	1.53	88	76.8	3.32	3.6

Both piles showed high initial values for the ratio water soluble C / total N (WSC/TN) that decreased during composting. The final values were in the range (between 1-2) reported by other authors for compost with good degree of stability, elaborated from olive mill wastes (Alburquerque et al., 2006) that, in general, are higher than those obtained for other materials (<0.5) such as municipal solid

waste or sewage sludge (Sánchez-Monedero et al., 2001). Very low germination indices (GI) were observed during the mesophilic phase in both piles, showing the high toxicity in the early composting stages. However, in both piles GI exceeded 50% during the thermophilic stage.

Stability was also confirmed by the remarkable increase in the indices based on humification processes such as the percentage of humic acids (C_{HA}/ C_{EXT}) the polimerisation degree (C_{HA}/ C_{FA}) and the humification degree (CEC/ TOC). In pile A the disappearance of toxicity and the formation of humic-like compounds were delayed respect to pile B, probably because, in the latest, the addition of grape stalks improved the structure and therefore the aeration of the mixture.

Changes in the six hydrolytic EAs evaluated throughout the composting of TPOMW are shown in figure 2. Both piles showed a parallel evolution of EA throughout the process. All the enzymes, but acid phosphatase, showed very low activity in the initial TPOMW samples and during the first stages of composting.

Fluorescein diacetate (FDA) hydrolysis increased with composting time, showing the highest increment during the thermophilic phase between T1 and T2 (Figure 2). Results obtained were in agreement with those reported by Ntougias et al. (2006) who observed an increase in FDA activity during the composting of olive press cake and olive leaves. FDA activity has been proposed in recent studies to estimate non-specific enzyme activity during the composting process (García-Gómez et al., 2003; Ntougias et al., 2006). It has been also used as a measure of microbial activity to predict suppressive capacity of compost on plant pathogens (Inbar et al., 1991). However there is controversy about the effectiveness of using this parameter for evaluating stability in compost since different trends have been found depending on the starting composting materials. In this study, FDA showed significant correlations with several maturation indices traditionally used in literature: TOC/ TN (r:-0.813); WSC/TN (r: -0.735); pH (r: 0.735); GI (r: 0.842); C_{HA}/ C_{EXT} (r: 0.811); C_{HA}/ C_{FA} (r: 0.832) ($P<0.05$), and therefore, it could be included as a stability index for TPOMW composting.

An increase in β-glucosidase was also observed (Figure 2). This is another important enzyme that catalyses the hydrolysis of cellobiose and thus plays a major role in the initial phases of decomposition of organic C compounds. Some authors have found a decrease of β-glucosidase activity throughout the composting of different materials that attributed to the decrease in available organic substrates (Benitez et al., 1999; García et al., 1993). Nevertheless, other studies have reported an increase of this enzymatic activity during composting explained by the stabilisation of the enzyme by humic complexes (Mondini et al., 2004; Benitez et al., 2005).

Alkaline phosphatase was the enzymatic activity that showed the largest increase during TPOMW composting. The levels of alkaline phosphatase obtained for final TPOMW composts are in the range of those obtained by Mondini et al (2004) for composts made from cotton wastes.

Acid phosphatase activity was very high in TPOMW samples (4000-5000 μg

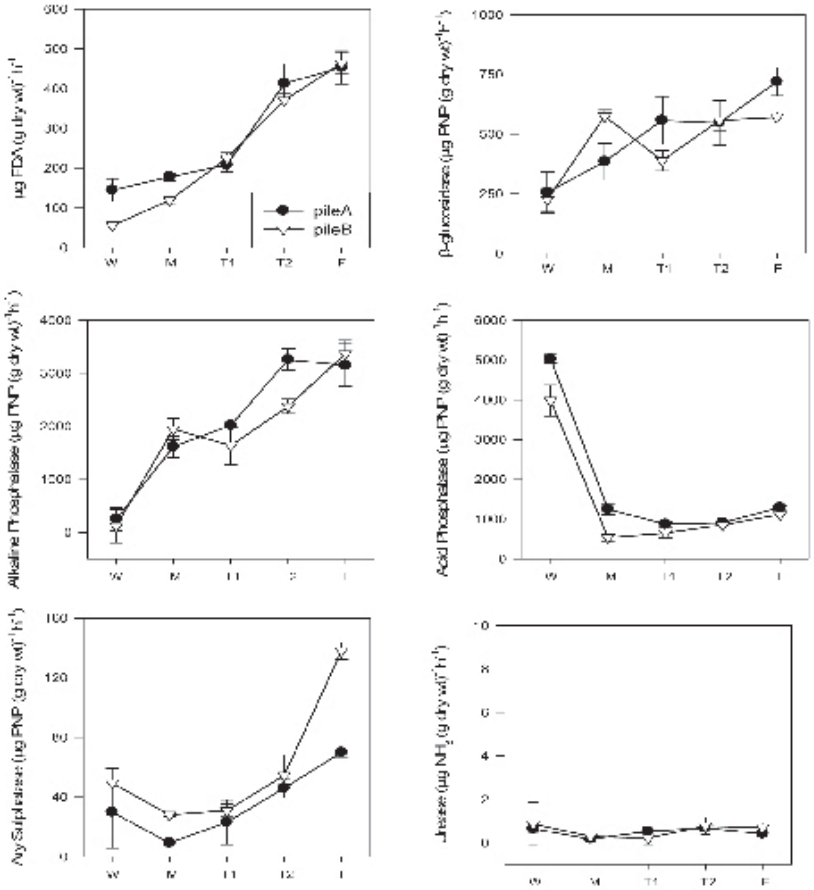


Fig. 2: Dynamics of enzymatic activity during the composting of TPOMW. W: TPOMW before composting; M: mesophile stage; T1, T2: termophile stage; F: final compost. Vertical bars represent standard deviation (n=3).

PNP (g dry weight)$^{-1}$ h^{-1}). However, values were very low during the composting process, and no significant changes were observed. The increase in pH with composting time, that reached values above 9, was much higher than the optimum (between 4 and 6.5) reported for this enzyme.

Arylsulphatase, that catalyses the hydrolysis of organic sulphate esters, showed a higher increase in pile A (without grape stalks). This EA also correlated with

important maturation indices: C_{HA}/ C_{FA} (r: 0.760) and CEC/ TOC (r: 0.887).

The urease activity, which catalyses the hydrolysis of urea to CO_2 and NH_4^+, was very low during all the experiment, probably because of the low concentration of N in TPOMW and the low degree of mineralisation.

4 Conclusion

Most of the enzymatic activities studied in the present work (FDA, β-glucosidase, alkaline phosphatase and arylsulphatase) increased during the composting of TPOMW. EA may increase during the degradation of wastes because of the stabilization of extracellular enzymes through the formation of humo-enzymatic complexes that protect them from physical and microbial degradation. Urease and acid phosphatase activities showed no significant changes during the composting of TPOMW because of the low level of N in the organic matrix and the high pH evolved during composting. FDA and arylsulphatase correlated with many important stability indices commonly used in literature and therefore they can be considered valid parameters for establishing the degree of biological stability of the composting material.

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