ELSEVIER

Bioresource Technology 86 (2003) 59–64

BIORESOURCE TECHNOLOGY

Composting of the solid fraction of olive mill wastewater with olive leaves: organic matter degradation and biological activity

A. García-Gómez, A. Roig, M.P. Bernal *

Department of Soil and Water Conservation and Organic Waste Management, Centro de Edafología y Biología Aplicada del Segura, CSIC P.O. Box 4195, 30080 Murcia, Spain

Received 8 January 2002; received in revised form 14 April 2002; accepted 22 April 2002

Abstract

The flocculated solid fraction of olive mill wastewaters, obtained from two different olive oil extraction systems (FOMW1 and FOMW2) was composted, with olive leaves (OL) as bulking agent, by the static pile system (Rutgers). The dynamic of organic matter (OM) degradation during composting and its relationship with the basal respiration and fluorescein diacetate (FDA) hydrolytic activity, as indicators of biological activity, were studied. Two mixtures were prepared: C1, from 65% FOMW1 plus 35% OL; and C2, from 74% FOMW2 plus 25% OL and 1% urea. The biooxidative phase of composting in C1, which had a high initial C/N ratio, was long, leading to a high OM degradation, mainly of the lignocellulosic compounds. The water-soluble organic carbon content, C/N ratio and the urea supplied as a N source for the C2 compost make this mixture more adequate for composting, as it had a shorter composting time than C1, and developed a microbial population with a high metabolic activity. The results for basal respiration in C1 and C2 were correlated at a high probability level with those of FDA hydrolysis, and both parameters can be used for establishing the degree of biological stability of the composting material.

Keywords: Composting; Organic matter degradation; Biological activity; Basal respiration; Fluorescein diacetate hydrolysis

1. Introduction

The importance of the olive mill industry in Mediterranean countries is well known, as is the serious problem that the olive mill factories have in disposing of their by-products, mainly the olive mill wastewater (OMW) (Cabrera, 1994; Paredes et al., 1999). There are two main olive oil production systems: the three-phase centrifugation system, which produces OMW as the waste product, and a solid byproduct which is used for further oil extraction; and the two-phase centrifugation system, in which olive husks are left, together with a small amount of OMW from oil washing. OMW is a liquid waste highly contaminating and phytotoxic, due to the presence of polyphenols, salts and fats (Saviozzi et al., 1993; Paredes et al., 1999). Different methods have been used for its elimination or transformation. A new technology developed by Trainalba S.L. consists of a physico-chemical treatment to flocculate the organic matter (OM) of OMW with an organic commercial polyelectrolyte. This produces water, which can be used for irrigation, and a sludge as a waste, which is difficult to dispose of. This flocculated fraction of OMW (FOMW) is characterised by a slightly acid pH, due to the presence of fatty acids and alcohols from the OMW (Lanzani and Fedeli, 1986).

Composting has been shown to be a suitable method for recycling olive mill wastes, mainly OMW and olive husk, which need to be mixed with lignocellulosic materials to obtain adequate physical conditions for composting, due to their sticky texture (Madejón et al., 1998; Paredes et al., 2000). Preliminary studies of FOMW composting with different bulking agents, in a composting simulator, produced high OM degradation and a decrease in phytotoxicity when using straw or vine shoots as bulking agents (Negro and Solano, 1996). Composting produces degradation and stabilisation of OM, and degrades the phytotoxic organic substances. The composting process requires adequate conditions of

* Corresponding author. Tel.: +34-968-396-200; fax: +34-968-396-213.

E-mail address: pbernal@cebas.csic.es (M.P. Bernal).

0960-8524/03/$ - see front matter
PII: S0960-8524(02)00106-2

pH, temperature, moisture, oxygenation and nutrients, to allow the adequate development of the microbial population (De Bertoldi, 1992). Therefore, changes in these conditions during the process will affect the proliferation of certain microflora, having different enzymatic activities, which control the OM degradation.

A knowledge of the dynamic of OM degradation and biological activity aspects is required to further understand FOMW composting, but literature concerning this particular topic is scarce. Indices of microbial activity include measurement of basal respiration rate ($C–CO_2$ evolved), which indicates the total metabolic activity of all microbiological processes that occur during OM degradation. This has been used as a measure of potential OM degradation in soil and during composting (Haynes, 1999; Wu et al., 2000). Measurement of fluorescein diacetate (FDA) hydrolysis has been used frequently to evaluate the total enzymatic activity of the microbial population present in soil and in organic substrates (Schnurer and Rosswall, 1982; Craft and Nelson, 1996; Haynes, 1999). This determination can be useful for studying the evolution of the biological activity during composting, however it has been seldom used for this purpose.

In the present work, the composting of FOMW was studied by mixing it with olive leaves (OL), which are usually harvested with the olives and separated from them in the mills before starting the oil extraction procedure. The aim was to study the mineralisation process of the OM during composting of FOMW with OL as a bulking agent, and its relationship to the basal respiration and FDA hydrolysis, used as indicators of the biological activity.

2. Methods

2.1. Composting procedure

By-products from the olive mill industry were supplied by Trainalba S.L. the solid fraction of the OMW from two mills (FOMW1 and FOMW2), obtained by flocculation of OMW through the process developed by Trainalba S.L. (patent no. P9401934), and OL were selected for composting (Table 1). FOMW1 came from OMW produced during the olive oil cleaning procedure in a two-phase extraction system, while FOMW2 came from OMW of the three-phase system, and contained olive pulp, giving it a more solid texture than FOMW1. The fresh OL (free of branches) used were separated from the harvested olives on arrival at the mill. The mixtures were prepared as follows:

C1: 65% FOMW1 + 35% OL (dry weight), equivalent to 80% FOMW1 + 20% OL (fresh weight).
C2: 74% FOMW2 + 25% OL + 1% urea (dry weight), equivalent to 91.5% FOMW2 + 8.0% OL + 0.5% urea (fresh weight).

The proportion of FOMW in the mixture C2 was increased with respect to C1 in order to recycle as much waste as possible, also an additional source of urea was used to increase the initial N concentration and lower the C/N ratio of the mixture to a more adequate value for composting than in C1 (i.e. about 30).

About 2500 kg of each mixture were composted in trapezoidal piles 1.5 m high with a 2×3 m base, by the Rutgers static composting system (Finstein et al., 1985). Air was blown from the base of the pile through the holes of three PVC tubes, 3 m in length and 12 cm in diameter. The timer was set for 30 s ventilation every 15 min, which meant alternative periods of air blowing (30 s), and 14 min 30 s without ventilation. Moreover, this system maintained a temperature ceiling in the pile at 55 °C, through the on-demand removal of heat by pressure-forced aeration (feedback). When $T > 55$ °C the forced ventilation system was connected automatically for continuous air blowing until $T \leqslant 55$ °C (Paredes et al., 2000). Ventilation demand was measured as the time of ventilation necessary to maintain the temperature below 55 °C. This encourages a high decomposition rate, since high temperatures inhibit and slow down the OM de-

Table 1
Main chemical characteristics of the original wastes used, the solid fraction of OMW (FOMW1 and FOMW2), the OL, the initial mixtures C1 and C2 (i), and the mature composts (m) (dry weight basis)

	pH	OM (%)	TOC ($g\,kg^{-1}$)	HOC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)	C/N	Lignin[a] (%)	Cellulose[a] (%)	Hemicellulose[a] (%)	Fat (%)
FOMW1	5.63	85.24	626.3	nd[b]	11.6	54.2	44.9	9.7	39.3	13.8
FOMW2	5.80	95.02	551.5	nd	15.1	36.5	40.0	22.6	40.0	10.2
OL	6.27	73.39	391.6	nd	11.9	33.0	30.4	19.3	25.4	6.2
C1-i	5.89	65.14	434.0	19.1	10.8	40.1	36.9	13.7	26.3	15.2
C2-i	6.15	92.69	528.5	33.9	19.0	27.8	38.6	26.0	36.3	7.1
C1-m	8.46	42.33	248.6	15.8	17.6	14.1	14.7	5.5	6.9	1.1
C2-m	7.75	88.31	468.4	22.5	30.2	15.5	23.6	16.2	13.4	1.1

[a] Percentages with respect to initial dry weight of pile, considering constant the ash content during the composting process.
[b] nd: not determined.

gradation. Temperature probes were situated inside the pile, halfway up, where the highest temperatures developed. When the temperature started to decrease, the pile was turned once in order to homogenise the mixture and stimulate the process. The biooxidative phase of composting (active phase) was considered finished when the temperature of the pile stabilised near to the ambient temperature (final stage). Air blowing was stopped and the mixtures were then allowed to mature over a period of two months (maturation phase). The moisture level of the pile was controlled weekly during the biooxidative phase of composting and adjusted by adding the necessary amount of water, using overhead sprinklers, to obtain values between 40% and 60% moisture content. One representative sample was taken at each sampling time by mixing six sub-samples from six sites of the pile, spanning the whole profile (from the top to the bottom of the pile). A sub-sample was air-dried and ground to 0.5 mm for chemical analysis, the rest of the sample was kept frozen (−20 °C) until being used for biological determinations.

2.2. Analytical methods

The samples were analysed for pH in a 1:10 (w/v) water-soluble extract, dry matter by drying at 105 °C for 12 h, and for OM content by 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 was the water-soluble organic carbon (HOC), after a 1:20 (w/v) extraction. Lignin and cellulose concentrations were determined by the American National Standard methods (ANSI/ASTM, 1977) and the hemicellulose concentration by subtracting the cellulose concentration from the total value for the delignified sample (holocellulose) obtained by Browning's method (Browning, 1967). The fat content was determined by diethyl-ether extraction (Soxhlet system). All analyses were done at least in duplicate. The relevant parameters of the original wastes and the initial and mature samples of the composting are shown in Table 1.

The proportion of OM-loss by mineralisation was calculated using the initial (X_1) and final (X_2) ash concentrations, according to the equation:

$$\text{OM-loss } (\%) = 100 - 100[X_1(100 - X_2)]/[X_2(100 - X_1)]$$

The results were fitted to a first-order kinetic equation by a non-linear least-square procedure (Marquardt–Levenberg algorithm, Table 2), described by the equation:

$$\text{OM-loss} = A(1 - e^{-kt})$$

where OM-loss represents the OM degraded (% of initial OM), t is the composting time (d), A the maximum degradation (% of initial OM) and k is the rate constant (d^{-1}).

Table 2
Parameters obtained from the fitting of the OM-loss curves to a first-order kinetic model: maximum OM degradation (A), rate constant (k) and mineralisation rate ($A \times k$)

Mixture	A (% OM)	k (d^{-1})	$A \times k$ (% OM d^{-1})	RMS	F
C1	77.1 (4.48)	0.010 (0.0012)	0.771	20.6	556.6***
C2	46.0 (8.67)	0.011 (0.0033)	0.506	24.8	85.9***

Statistical parameters: residual means square (RMS) and F factor. Standard deviation in brackets.
*** $P < 0.001$.

Basal respiration and FDA hydrolysis activity were determined on the frozen sub-samples, after defrosting, homogenisation and chopping in a mill. Samples were incubated at 20 °C for 24 h in order to reactivate the microbial biomass. Fresh samples, equivalent to 8 g dry matter, were incubated in 250 ml plastic flasks, at 26 °C for 10 d. The CO_2 produced was adsorbed into 10 ml of 2 M NaOH solution placed in a vial on the compost sample inside the plastic incubation flask. The CO_2–C evolved was determined by titration of the NaOH solution with 2 M HCl in an excess of $BaCl_2$. Basal respiration rate was expressed as mg C–CO_2 evolved per gram of dry material d^{-1}. The FDA hydrolysis was determined, by the modified method of Inbar et al. (1991) and You and Sivasithamparam (1994), on a sample equivalent to 1 g dry matter. The calibration curve was performed for each sample, using 1 g dry matter, to avoid any interference of the humified OM (Swisher and Carroll, 1980; Inbar et al., 1991).

3. Results and discussion

3.1. Organic matter degradation

Both piles were turned once during the full composting process, when the temperature first started to decrease (after 56 and 28 d in C1 and C2, respectively, Fig. 1b). After turning, the process was re-activated as shown by the increased temperature and ventilation demand (Fig. 1a and b). During turning, material at the exterior of the pile was incorporated into the pile, providing degradable substrate for the microbial biomass. The presence of urea in the C2 mixture could have retarded the development of high thermophilic temperatures, producing a long initial mesophilic phase, as shown by Sánchez-Monedero et al. (2001). The ventilation demand indicates the maximum microbial activity reached during composting (Jeris and Regan, 1973). Therefore, microbial activity was higher in both piles during the

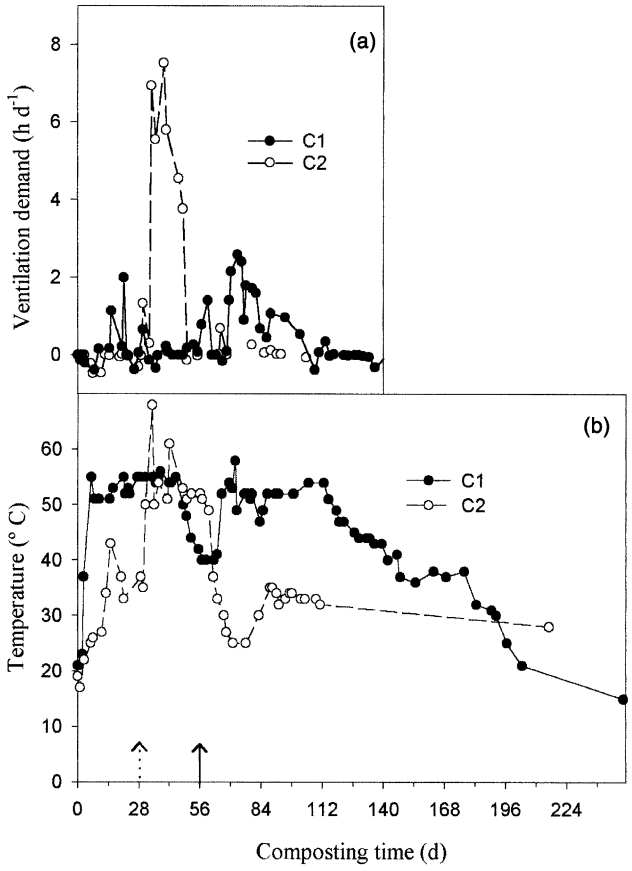

Fig. 1. (a) Ventilation demand required in piles C1 and C2 during the biooxidative phase of composting, to maintain the maximum temperature at 55 °C and (b) temperature profile during composting of the piles C1 and C2, (arrows indicate whirling).

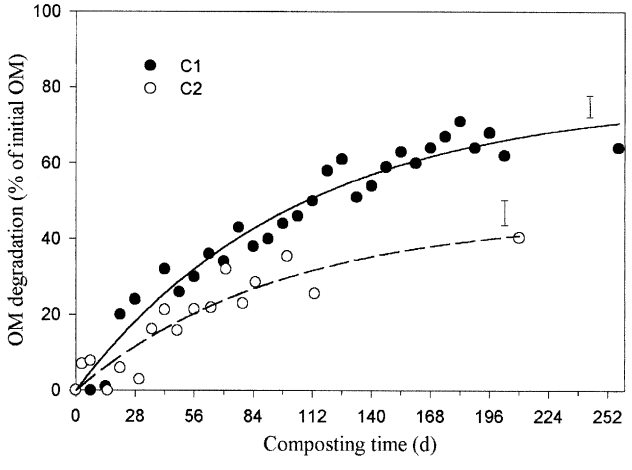

Fig. 2. Degradation of OM during composting of piles C1 and C2. Symbols represent the experimental results and lines the fitted curves. Bars indicate the least significant difference.

thermophilic phase. The high initial C/N ratio in C1 (Table 1) caused an excessively long composting time, with low ventilation demand in the thermophilic phase (Fig. 1).

However, the longer biooxidative phase in C1 led to a greater proportion of the OM fraction being degraded during composting, the values being 64% in C1 and 40% in C2 (Fig. 2). The curve of OM degradation fitted a first-order kinetic function (Table 2), as generally found during composting (Bernal et al., 1996; Paredes et al., 2000). The value for maximum OM degradation (A) of C1 was close to the range found for the composting of sewage sludge, animal manure, OMW and OMW sludge with different lignocellulosic wastes as bulking agents (55–68% OM) (Bernal et al., 1996; Paredes et al., 1996, 2000). But the value found for C2 was lower than the cited range, indicating that its OM was more resistant to degradation. This was due to the nature of the FOMW2, as the same OL was used in both piles. FOMW2 came from three-phase OMW, which contains olive pulp, mucilage, pectin, etc. (Paredes et al., 1999), while FOMW1 came from the two-phase OMW produced during the washings of the olive oil (free of olive pulp).

About 60% of the initial contents of lignin and cellulose were degraded during composting in C1 (Table 1), compared to 39% and 38% respectively in C2. Moreover, the high concentration of ammonium formed during hydrolysis of the added urea in C2 could have inhibited the activity of fungi responsible for degradation of lignocellulosic compounds (Ko et al., 1974). The degradation of fats is of special interest due to their high concentration in FOMW samples (Table 1). Fats give a particular texture to the composting mass that inhibit the oxygenation (gas exchange) of the pile, whilst their hydrophobic character makes difficult the absorption of water added to the pile in order to maintain an adequate moisture content. Most of the fat was degraded during composting (Table 1), as has been found also during composting of all olive mill wastes, such as olive marc (Estaún et al., 1985), olive husks with wheat straw (Madejón et al., 1998), and FOMW with grape marc and straw in composting simulator experiments (Negro and Solano, 1996).

3.2. Microbial activity

In C1 the basal respiration did not change significantly during composting, and only a slight increase in the FDA hydrolysis activity occurred after 84 d of composting (Fig. 3a and b). In C2, basal respiration and FDA activity increased during the thermophilic phase, reaching their maximum values on day 49, when both maximum temperature and ventilation demand occurred (Fig. 1a and b). The development of the microbial biomass needs a large quantity of available nutrients, particularly nitrogen, which was mainly supplied by urea hydrolysis in this pile. Later, microbial respiration activity and FDA hydrolysis decreased in

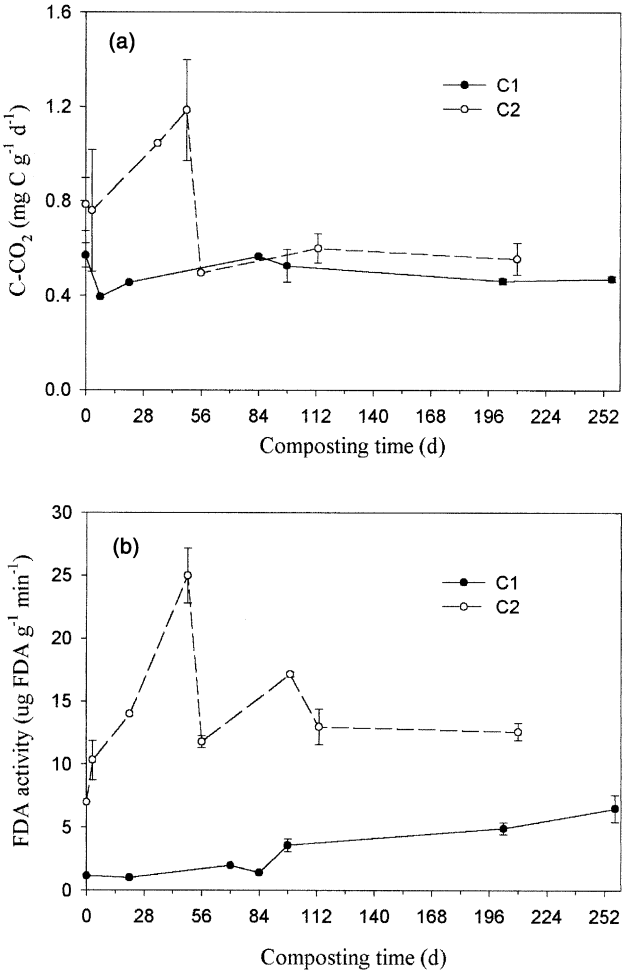

Fig. 3. (a) Evolution of basal respiration and (b) FDA hydrolytic activity during the composting process.

C2, as did the temperature, indicating a decrease in the organic substrate degradable by the microflora. This was also found by Schwab et al. (1994), in a simulated solid waste using a pilot plant-scale composter. Both basal respiration and FDA activity remained constant during the maturation phase. The values of basal respiration after two months of maturation were very close to those found by Forster et al. (1993) (0.25–0.62 mg C–CO_2 g^{-1} d^{-1}) and Hue and Liu (1995) (0.06–0.63 mg C–CO_2 g^{-1} d^{-1}) in mature composts of different origin, which indicates the microbial stability reached after the composting process.

The results for basal respiration in C1 and C2 were correlated at a high probability level with those of FDA hydrolysis ($r = 0.749$, $P < 0.01$), thus both parameters indicated the total microbial activity during composting. Some studies have shown that the absorbance value of FDA can indicate the microbial biomass in organic substrates and soils amended with organic materials (Swisher and Carroll, 1980; You and Sivasithamparam, 1994; Sánchez-Monedero, 1997). According to Swisher and Carroll (1980), and Inbar et al. (1991), not all microorganisms show FDA activity. In fact, Schwab et al. (1994) found a good relationship between microbial biomass, measured by direct bacteria counting using microscopy, and FDA activity during composting of simulated solid waste, but the relationship was not found for fungi and actinomycetes. Similarly, You and Sivasithamparam (1994) did not find a significant correlation between total fungi counts and FDA activity in mixtures of manure and straw. Therefore, in C1, fungi may have been the dominant microflora developed during composting, which was able to degrade mainly polymers, such as cellulose and lignin, as sources of C and N, because of the initial conditions of the C1 mixture (high C/N ratio, low HOC and N concentrations, and high fats). However, further research is needed to confirm this.

Therefore, the FOMW can be composted, with OL as bulking agent, into a stabilized product. However, the mixture of wastes for C2 was better for composting than C1, as shown by the shorter composting time with higher microbial activity than C1. The high initial HOC and N concentrations, together with an adequate C/N ratio of C2 were responsible for the development of a microbial population with a high metabolic activity. The basal respiration and FDA hydrolysis activity are valid parameters for establishing the degree of biological stability of the composting material. As a general conclusion, it can be said that both the two- and three-phase olive oil extraction systems can become environmentally friendly technologies by integrating a composting system for transformation of the wastes into stabilised compost.

Acknowledgements

The authors wish to thank Dr. D.J. Walker for the English revision. This research was financed by the Spanish CICYT through the PETRI project no. 95-0234-OP-02-02.

References

ANSI/ASTM, 1977. Standard test methods for lignin wood. D 1103. American National Standard.

Bernal, M.P., Navarro, A.F., Roig, A., Cegarra, J., Garcia, D., 1996. Carbon and nitrogen transformation during composting of sweet sorghum bagasse. Biol. Fertil. Soils 22, 141–148.

Browning, B.L., 1967. Methods of Wood Chemistry. Interscience Publishers, New York, USA, p. 395.

Cabrera, F., 1994. The problem of the olive oil mill wastes in Spain: Treatment or recycling? In: Atti VII Congresso Internazionale: L'approccio Integrate della Moderna Biologia: Uomo, Territorio, Ambiente. Vieste, Italy, pp. 117–125.

Craft, C.M., Nelson, E.B., 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Appl. Environ. Microbiol. 62, 1550–1557.

De Bertoldi, M., 1992. The control of the composting process and quality of end products. In: Jackson, D.V., Merillot, J.M., L'Hermite, P. (Eds.), Composting and Compost Quality Assurance Criteria. Commission of the European Communities, pp. 85–93.

Estaún, V., Calvet, C., Pagés, M., Grases, J.M., 1985. Chemical determination of fatty acids, organic acids and phenols, during olive marc composting process. Acta Horticulturae 172, 263–270.

Finstein, M.S., Miller, F.C., MacGregor, S.T., Psarianos, K.M., 1985. The Rutgers strategy for composting: process design and control. EPA Project Summary (EPA 600/S2-85/059). US Environmental Protection Agency, Washington, DC.

Forster, J.C., Zech, W., Würdinger, E., 1993. Comparison of chemical and microbiological methods for the characterization of the maturity of composts from contrasting sources. Biol. Fertil. Soils. 16, 93–99.

Haynes, R.J., 1999. Size and activity of the soil microbial biomass under grass and arable management. Biol. Fertil. Soils 30, 210–216.

Hue, N.V., Liu, J., 1995. Predicting compost stability. Compost. Sci. Util. 3, 8–15.

Inbar, Y., Boehm, M.J., Hoitink, H.A.J., 1991. Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Soil Biol. Biochem. 23, 479–483.

Jeris, J.S., Regan, R.W., 1973. Controlling environmental parameters for optimum composting: II. Moisture, free air space and recycle. Compost. Sci. 14, 8–15.

Ko, W.H., Hora, F.K., Herlicksa, E., 1974. Isolation and identification of a volatile fungistatic substance from alkaline soil. Phytopathology 64, 1398–1400.

Lanzani, A., Fedeli, E., 1986. Composition and utilization of olive-mill wastewaters. In: Tavola Rotonda: Lo smaltimento delle acque reflue dei frantoi (Accademia Nazionale dell' Olivo). Arti grafiche Panetto and Petrelli, Spoleto, Italy, pp. 15–28.

Madejón, E., Galli, E., Tomati, U., 1998. Composting of wastes produced by low water consuming olive mill technology. Agrochimica 42, 135–146.

Navarro, A.F., Cegarra, J., Roig, A., Bernal, M.P., 1991. An automatic microanalysis method for the determination of organic carbon in wastes. Commun. Soil Sci. Plant Anal. 22, 2137–2144.

Navarro, A.F., Cegarra, J., Roig, A., García, D., 1993. Relationship between organic matter and carbon contents of organic wastes. Biores. Technol. 44, 203–207.

Negro, M.J., Solano, M.L., 1996. Laboratory composting assays of the solid residue resulting from the flocculation of oil mill wastewater with different lignocellulosic residues. Compost. Sci. Util. 62–71.

Paredes, C., Bernal, M.P., Roig, A., Cegarra, J., Sánchez-Monedero, M.A., 1996. Influence of the bulking agent on the degradation of olive-mill wastewater and sludge during composting. Int. Biodeter. Biodegr. 38, 205–210.

Paredes, C., Cegarra, J., Roig, A., Sánchez-Monedero, M.A., Bernal, M.P., 1999. Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes. Biores. Technol. 67, 111–115.

Paredes, C., Roig, A., Bernal, M.P., Sánchez-Monedero, M.A., Cegarra, I., 2000. Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes. Biol. Fertil. Soils 32, 222–227.

Sánchez-Monedero, M.A., 1997. Compostaje de residuos orgánicos. Estudio del proceso y evaluatión agrícola de los composts. Ph.D. Thesis, Universidad de Murcia, Murcia, Spain.

Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P., 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of composting mixtures. Biores. Technol. 78, 301–308.

Saviozzi, A., Riffaldi, R., Levi-Minzi, R., Scagnozzi, A., Vanni, G., 1993. Decomposition of vegetation-water sludge in soil. Biores. Technol. 44, 223–228.

Schnurer, I., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 6, 1256–1261.

Schwab, B.S., Ritchie, C.J., Kain, D.J., Dobrin, G.C., King, L.W., Palmisano, A.C., 1994. Characterization of compost from a pilot plant-scale composter utilizing simulated solid waste. Waste Manage. Res. 12, 289–303.

Swisher, R., Carroll, G.C., 1980. Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. Microbial Ecol. 6, 217–226.

Wu, L., Ma, L.Q., Martinez, G.A., 2000. Comparison of methods for evaluating stability and maturity of biosolids compost. J. Environ. Qual. 29, 424–429.

You, M.P., Sivasithamparam, K., 1994. Hydrolysis of fluorescein diacetate in an avocado plantation mulch suppressive to *Phytophthora cinnamomi* and its relationship with certain biotic and abiotic factors. Soil Biol. Biochem. 26, 1355–1361.