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Bioresource Technology 81 (2002) 71–76

BIORESOURCE TECHNOLOGY

Microbial, chemical and physical aspects of citrus waste composting

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Received 3 January 2000; received in revised form 20 February 2001; accepted 20 March 2001

Abstract

Citrus waste supplemented with calcium hydroxide and with a C/N ratio of 24:1, pH of 6.3 and moisture content of 60% was composted by piling under shelter. With regular turning over of the pile and replenishment of moisture, the thermic phase lasted for 65–70 days and composting was completed after 3 months. Compost thus prepared had an air-filled porosity of 14%, water-holding capacity of 590 ml l^{-1}, bulk density of 1.05 g cm^{-3} and conductivity of 480 mS m^{-1}. Phosphorus content (in mg l^{-1}) was 15, potassium 1170, calcium 362, magnesium 121, sodium 32, chloride 143, boron 0.31, and water-soluble nitrogen and organic matter 126 and 4788, respectively. Total carbon amounted to 8.85% and total nitrogen to 1.26% of the dry weight, giving a C/N ratio of 7. Mature compost showed some, but acceptable, levels of phytotoxicity. Raw citrus waste was predominantly colonised by mesophilic yeasts. Thermophilous microorganisms present during the thermic phase mainly comprised the bacterial species *Bacillus licheniformis*, *B. macerans* and *B. stearothermophilus* and, to a lesser extent, fungi such as *Absidia corymbifera*, *Aspergillus fumigatus*, *Emericella nidulans*, *Penicillium diversum*, *Paecilomyces variotii*, *Rhizomucor pusillus*, *Talaromyces thermophilus* and *Thermomyces lanuginosus*. Bacteria prevalent in the final product included *B. licheniformis*, *B. macerans*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. luteola* and *Serratia marcescens*, whereas fungi isolated most frequently comprised *Aspergillus puniceus*, *A. ustus*, *E. nidulans*, *Paecilomyces lilacinus*, *T. lanuginosus*, yeasts and a basidiomycetous species, probably *Coprinus lagopus*.

Keywords: Bacteria; Citrus waste; Composting; Fungi; Nutrient status; Physical characteristics; Thermophiles; Yeasts

1. Introduction

Citrus fruits contain between 35% and 55% juice (Sinclair, 1984). With extraction of the juice, the remainder of the fruit, i.e., peel, membranes, juice vesicles and seed, are discarded as waste. In South Africa alone, waste generated by citrus processing plants averaged 250 000 t per annum between 1990 and 1997. Traditionally, the waste has been disposed of by dumping or by selling it as fodder. Dumping obviously creates an environmental hazard, whereas marketing of the waste as fodder requires that the waste be dried, which is a costly process. Citrus processors therefore consider other options for utilising the waste more cost-effectively.

Citrus waste has many applications (Sinclair, 1984), e.g., as fibre (Braddock and Graumlich, 1981), pectin (Ma et al., 1993) and flavonoid source, binding agent in food technology (Lo Curto et al., 1992), fermentation substrate for single-cell protein (Vaccarino et al., 1989), silage (Ashbell and Weinberg, 1988) and mosquito repellant (Anaso et al., 1990). However, most of these conversions require advanced technology or facilities. In Portugal, Correia Guerrero et al. (1995) increased the production of fresh and dry matter of lettuce planted two weeks after enriching the soil with dried orange pulp and peel wastes. They concluded that these wastes can be used as organic fertilizer, provided the physical and chemical soil and waste properties are known, and the amounts and types of wastes added to the soil are defined according to the requirements of the plants produced. As the bulk and consistency of citrus waste do not facilitate cost-effective transport to distant sites, the main target for the waste as organic fertilizer would be crops grown in the proximity of the processing plant, which in South Africa comprise mainly citrus. Such a practice obviously could have disastrous consequences due to the dispersion and maintenance of citrus pathogens by the waste.

Composting converts organic matter into a stable substance which can be handled, stored, transported and

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0960-8524/02/$ - see front matter
PII: S0960-8524(01)00058-X

applied to the field without adversely affecting the environment. Proper composting effectively destroys pathogens and weed seeds through the metabolic heat generated by microorganisms during the process (Crawford, 1983). Such composts are not only suitable for use as a soil conditioner and fertiliser, but can also suppress soil-borne and foliar plant pathogens (Hadar and Mandelbaum, 1992; Zang et al., 1998). This paper describes how citrus waste can be composted, thus converting it into a value-added commodity without specialised equipment or facilities.

2. Methods

2.1. Raw material

Fresh citrus waste with a C/N ratio of 24:1 (10.97:0.456 g/100 g fresh weight), moisture content of 60%, pH of 4.1 and containing 6% crude protein (Kjeldahl N $\times$ 6.25), 3% ether-extractable crude fat and 10% crude fibre with a total digestible nutrient content of 74%, was provided by Letaba Citrus Processors, Tzaneen, Northern Province, South Africa.

2.2. Composting

3 m^3 of the waste was supplemented with 15 kg m^{-3} calcium hydroxide and piled to a height of approximately 1.2 m under shelter at the University of Pretoria Experiment Farm. The pile was turned over manually when the moisture content dropped below 40% on days 15, 25, 40, 55 and 75, and the moisture level then adjusted to about 60%.

2.3. Physical and chemical parameters

Temperatures in the pile were recorded every fifth day at depths of 5 and 30 cm from the surface at four opposite sides of the pile. At the same time, samples of 10 g each were collected from similar positions as above for determination of pH and moisture content. Maturity of the compost was assessed according to the cress seed germination assay (Baca et al., 1990) using 1:15 (w/v) aqueous suspensions prepared from samples taken at 0, 10, 25, 35, 60 and 90 days. Two weeks after the final turning-over, four samples of 250 g each were collected at a depth of approximately 20 cm from opposite sides of the pile. A composite of the four samples was submitted to Outspan Laboratories, Centurion, South Africa, for chemical and physical analysis.

2.4. Microbial analysis

Separate samples of 20 g each were collected 5 and 30 cm from the surface at four opposite sides of the pile on days 3, 15, 32 and 65, and 20 cm within the pile on days 0 and 90. 10 g of each of the eight or four samples collected on each occasion was suspended in 90 ml sterile 0.1% water agar and agitated for 20 min on a reciprocal shaker at ambient temperature. A serial dilution of each suspension was plated in duplicate on wort agar, water–yeast agar, standard 1 agar containing 300 mg l^{-1} cycloheximide, and potato-dextrose agar supplemented with 250 mg l^{-1} chloramphenicol for enumeration of yeasts, actinomycetes, unicellular bacteria and filamentous fungi, respectively. One set of plates was incubated at 25°C and the other at 45°C to facilitate separation of mesophilic and thermophilous (i.e., thermophilic and thermotolerant) taxa. Representative colonies of bacteria and filamentous fungi were isolated and identified. Identification of bacteria was based on API systems 20 NE and 50 CHB, whereas fungi were identified according to sources contained in Hawksworth et al. (1995).

3. Results

The temperature of the calcium hydroxide-amended citrus waste increased rapidly after piling and reached 60°C and 49°C within 5 days at a depth of 30 and 5 cm, respectively (Fig. 1). This high level was maintained for about 2 months despite periodic declines related to drying-out of the waste, although temperatures consistently increased again after turning-over and watering of the pile. The last peak of the thermic phase was recorded on day 65, whereafter temperatures gradually decreased, notwithstanding maintenance of a 40–55% moisture

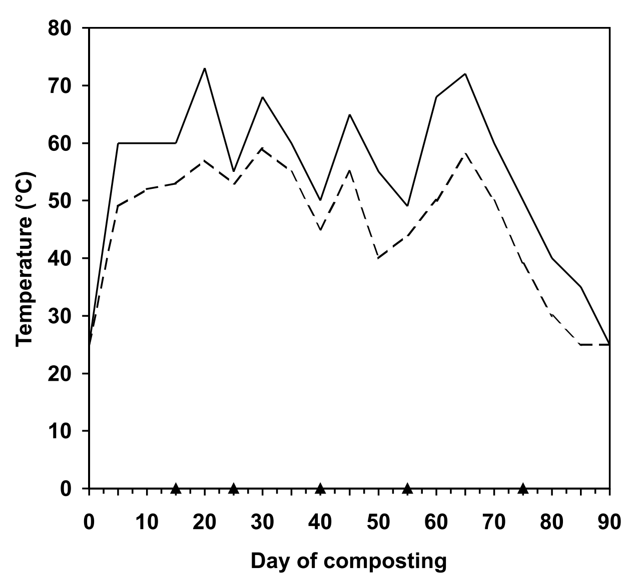

Fig. 1. Temperature fluctuations during composting of citrus waste: (- - - -) 5 cm within pile; (——) 30 cm within pile; (▲) turning-over of pile (each value is the mean of four recordings).

level (Fig. 2), and at 90 days stabilised at ambient temperature, about 25°C. The pH of the calcium hydroxide-amended citrus waste was 6.3 at piling and increased to around 9 between days 45 and 50 (Fig. 3). Turning-over and wetting of the pile on days 15, 25 and 40 was followed by a drop in pH at a depth of 30 cm, but from day 55 onwards this periodic decline was evident at both 30 and 5 cm. The pH stabilised at 7.2 on day 80.

Raw citrus waste was predominantly colonised by mesophilic yeasts (Table 1), but also contained low numbers of thermophilous bacteria and filamentous fungi, e.g. *Bacillus licheniformis, B. stearothermophilus, Aspergillus fumigatus* and *Talaromyces thermophilus*, as well as a few mesophilic actinomycetes (included under bacteria in Table 1). Mesophilic yeast populations remained constant at a depth of 30 cm on day 3, but increased more than 11-fold in the outer 5 cm and then declined progressively at both depths. Onset of the thermic phase was characterised by an increase in thermophilous organisms, particularly bacteria in the deeper, more anaerobic layers and fungi closer to the surface, although bacteria comprised the dominant thermophilous microbial group in mature compost. Mesophilic bacteria consistently outnumbered mesophylic filamentous fungi during composting.

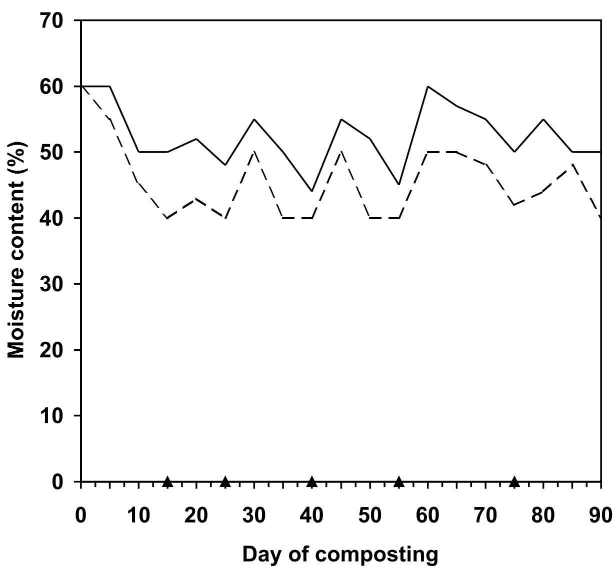

Fig. 2. Changes in moisture content of citrus waste during composting: (----) 5 cm within pile; (—) 30 cm within pile; (▲) turning-over of pile (each value is the mean of four samples).

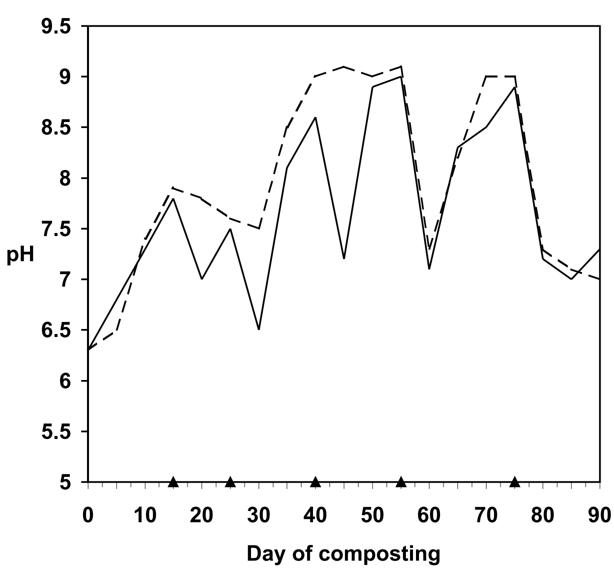

Fig. 3. Changes in pH of citrus waste during composting: (----) 5 cm within pile; (—) 30 cm within pile; (▲) turning-over of pile (each value is the mean of four samples).

Besides the species referred above, thermophilous filamentous fungi isolated during the composting of citrus waste comprised *Absidia corymbifera*, *Emericella nidulans*, *Penicillium diversum*, *Paecilomyces variotii*, *Rhizomucor pusillus*, *Thermomyces lanuginosus* and *T. ibananensis*, whereas thermophilous bacteria also included *B. macerans*, *Enterobacter cloacae*, *Serratia marcescens* and *Coryneform* species. Bacteria consistently isolated from plates prepared from composting citrus waste and incubated at 25°C were *B. licheniformis*, *B. macerans*, *B. stearothermophilus* and coryneforms. *B. brevis* and *Staphylococcus saprophiticus* were isolated only during the initial phases of composting, whereas *Alcaligenes denitrificans*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Serratia marcescens* occurred mesophilically only in the mature product. Actinomycetes, *B. circulans*, *B. subtilis*, *Pseudomonas cepacia*, *P. luteola*, *P. maltophila*, *P. paucimobilis* and *Staphylococcus xylosus* were isolated infrequently. The succession of mesophilic filamentous fungi was less clearly defined. *Aspergillus flavus, A. fumigatus, A. niger* and *A. ustus* were isolated on more than one occasion during composting, *A. parasiticus*, *A. terreus*, *Cladosporium cladosporioides*, *Eupenicillium cinnamopurpureum*, *Penicillium purpurogenum* and *P. sublateritium* occurred only once. *A. aericomus*, *A. caespitosus*, *A. puniceus*, *Fennellia nivea*, *Fusarium solani*, *Memnoniella echinata*, *Paecilomyces lilacinus* and a sterile basidiomycetous species were present only on plates prepared from mature compost. The latter species probably was *Coprinus lagopus*, which copiously produced basidiomata on the pile during and after the final phases of composting.

The cress seed germination index increased with maturation of the compost from zero on days 0, 10 and 25 to 77% at day 90 (Fig. 4), thus indicating a decrease in phytotoxicity with time. On day 60, the samples collected at a pile depth of 30 cm inhibited germination to a greater extent than samples taken at 5 cm.

Chemical and physical analyses showed the mature composted citrus waste to have an air-filled porosity of 14%, water-holding capacity of 509 ml l^{-1}, bulk density

Table 1
Population densities of microorganisms in citrus waste at various stages of composting

Microbial group	Log propagules g^{-1} dry weight									
	Fresh waste	30 cm within pile				5 cm within pile				Mature compost
		3 d[a]	15 d	32 d	65 d	3 d	15 d	32 d	65 d	
Thermophilous bacteria	0.20	2.51	2.01	0	0.86	2.43	3.15	0.28	0.58	3.61
Thermophilous fungi	0.08	0	1.38	0.04	0.36	0	3.46	2.54	1.99	1.03
Mesophilic bacteria	0.28	3.40	1.80	0.18	0.08	4.99	4.92	3.44	3.54	4.89
Mesophilic fungi	0	0	1.11	0	0	0	3.78	1.76	1.64	1.25
Mesophilic yeasts	3.58	3.61	0.99	0	0	4.64	2.89	2.11	1.01	1.58

[a] D = day.

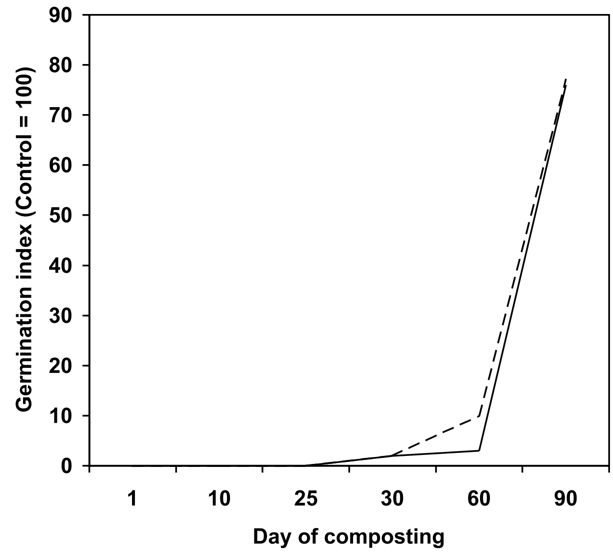

Fig. 4. Germination index of cress seed exposed to citrus waste samples collected at various stages of composting on days 0, 10, 25, 35, 60 and 90 days: (- - - -) samples taken 5 cm within pile; (——) sample taken 30 cm within pile (each value is the mean of four samples).

of 1.05 g cm^{-3} and conductivity of 480 mS m^{-1}. The compost contained (in mg l^{-1}) phosphorus (15), potassium (1170), calcium (362), magnesium (121), sodium (32), chloride (143), boron (0.31) and water-soluble nitrogen (126). Water-soluble organic matter was 4788 mg l^{-1}, total carbon 8.85% and total nitrogen 1.26% of the dry weight, thus giving a C/N ratio of 7.

4. Discussion

Rapid and entire humification of a substrate essentially depends on it initially having a C/N ratio which should be between 25 and 35 and a pH of 6–7.5 (Golueke, 1992). The citrus waste used here had a C/N ratio of 24, which is close to the range for optimal composting, but its pH of 4.1 had to be adjusted to a more appropriate level. Calcium hydroxide was selected for this purpose because preliminary studies showed it to be more suitable for composting citrus waste than calcium carbonate. A possible reason for this is that calcium hydroxide reacts exothermally when introduced into an acid substrate such as citrus waste, thus promoting thermophilic microbial activity which is inherent to composting (Poincelot, 1974). Furthermore, alkaline pre-treatment operates on the lignin–cellulose bonds, making the latter more available to attack by cellulolytic enzymes produced by microorganisms. Alkalis also partially extract and hydrolyse hemicelluloses and pectins, releasing readily-utilisable pentoses and hexoses (Vaccarino et al., 1989).

Composting of citrus waste exhibited a classical temperature pattern, commencing with a brief latency phase followed by a rapid increase in temperature lasting for about 2 months and culminating in a phase of gradual cooling for a further month. Similar patterns have been reported for a variety of waste products, e.g., hardwood bark (Hoitink et al., 1977), sewage sludge (Nakasali et al., 1985) and grape pulp (Faure and Deschamps, 1990), and are indicative of effective humification. Changes in pH observed during the composting process also conformed to accepted norms (Faure and Deschamps, 1990). In this regard, amendment with calcium hydroxide evidently had a significant effect as it increased the pH of raw citrus waste to the same level as that of more conventional composting materials, thus rendering the waste less conducive to fermentation by yeasts and more suitable for activities of humifying organisms. Accelerated heating of the waste due to the exothermic reaction of calcium hydroxide probably also expedited thermal inactivation of yeast cells. Elimination of yeast populations in citrus waste by amendment with 1% calcium hydroxide has previously been reported by Ashbell and Weinberg (1988), whereas preliminary studies (Authors, unpublished) showed citrus waste not amended with calcium hydroxide to maintain a pH below 5 and an active yeast population for at least a month. Since preparation of the compost reported here, various semi-commercial piles of calcium hydroxide-amended citrus waste, ranging in size from 50 to 300 m^3 have been humified successfully, although completion of the composting process took longer.

The microbiology of citrus waste composting corresponded with that of grape marc humification (Streichsbier et al., 1982) in that both substrates initially were predominantly colonised by yeasts that were soon replaced by other organisms. Contrary to the results with grape marc reported by Streichsbier et al. (1982), but in accordance with Faure and Deschamps (1990), actinomycetes were virtually absent during the entire composting process. The predominance of bacteria over fungi once humification commenced nevertheless agrees with most other composting processes (Streichsbier et al., 1982; Faure and Deschamps, 1990), although the relatively high incidence of thermophilous fungi in the outer layers of the pile implicates their importance as aerobic decomposers of citrus waste. Most of the bacteria and fungi that were isolated during the composting of citrus waste, particularly the thermophilous taxa, are common inhabitants of composts and other organic substrates (Poincelot, 1974; Domsch et al., 1980; Streichsbier et al., 1982; Faure and Deschamps, 1990). Even *C. lagopus*, which sporulated abundantly on the compost and apparently also proliferated within the pile, appears to be one of only a few basidiomycetes capable of thermophilous existence (Cooney and Emerson, 1964), and hence adapted to a compost environment. It fell beyond the scope of this study to elucidate the contribution of the various organisms to the humification process, but it is evident that their presence was not merely incidental as virtually all the dominant species are capable of degrading at least one of the major polymer components in the waste; of cellulose, hemicellulose and pectin (Domsch et al., 1980; Sneath, 1986; Faure and Deschamps, 1990).

The C/N ratio of citrus waste decreased during humification in line with accepted principles. A C/N ratio below 20 is indicative of proper compost maturity, with a ratio of 15 or less being preferred (Poincelot, 1974; Jiménez and Garcia, 1989). The final C/N ratio of 7, as well as quotient (final ratio/initial ratio) of citrus waste compost were low, indicating that it should not induce immobilisation of mineral nitrogen in soil (Casale et al., 1995). Like C/N ratio, the phytotoxicity of citrus waste declined according to a pattern typical of composting, with the mature product still showing low but acceptable levels of toxicity (Jiménez and Garcia, 1989). This is in complete contrast to the lack of phytotoxicity towards lettuce of fresh orange pulp and peel wastes reported by Correia Guerrero et al. (1995).

Compared to the fresh product (Correia Guerrero et al., 1995), the mineral content of citrus waste compost was low, but nevertheless of the same order as that of similar chemically unenriched composted materials (Poincelot, 1974; Baca et al., 1990). The relatively high conductivity of the compost could promote salinity, but its air-filled porosity, water-holding capacity and bulk-density are within the limits conducive to plant growth (Handreck and Black, 1984). Indeed, in field experiments (Authors, unpublished), pre-plant incorporation of the compost into soil at 2, 4 and 8 kg m^{-2} improved the growth of citrus trees by as much as 25%, compared to control trees in non-amended soil.

Acknowledgements

We thank Letaba Citrus Processors, the Southern African Citrus Growers' Association, and the National Research Foundation for financial support.

References

Anaso, H.U., Ilouno, L.E., Onuorah, D., Umerie, S.C., 1990. Potency of orange peel as a mosquito fumigant. Biol. Wastes 34, 83–89.

Ashbell, G., Weinberg, Z.G., 1988. Orange peels: the effect of blanching and calcium hydroxide addition on ensiling losses. Biol. Wastes 23, 73–77.

Baca, M.T., Delgado, I.C., Sanchez-Raya, A.J., Gallardo-Lara, F., 1990. Comparative uses of cress seed germination and physiological parameters of *Helianthus annuus* L. to assess compost maturation. Biol. Wastes 33, 251–261.

Braddock, R.J., Graumlich, T.R., 1981. Composition of fibre from citrus peel, membranes, juice vesicles and seed. Lebensm-Wiss Technol. 14, 229–231.

Casale, W.L., Minassian, V., Menge, J.A., Lovatt, C.J., Pond, E., Johnson, E., Guillermet, F., 1995. Urban and agricultural wastes for use as mulches on avocado and citrus and delivery of microbial agents. J. Hortic. Sci. 70, 315–322.

Cooney, D.G., Emerson, R., 1964. Thermophilic Fungi. W.H. Freeman, San Francisco.

Correia Guerrero, C., Carrasco de Brito, J., Lapa, N., Santos Oliveira, J.F., 1995. Re-use of industrial orange wastes as organic fertilizers. Biores. Technol 53, 43–51.

Crawford, J.H., 1983. Composting of agricultural wastes. A review. Process Biochem. 2, 14–18.

Domsch, K.H., Gams, W., Anderson, T.-H., 1980. Compendium of Soil Fungi. Academic Press, New York.

Faure, D., Deschamps, A.M., 1990. Physico-chemical and microbiological aspects in composting of grape pulps. Biol. Wastes 34, 251–258.

Golueke, C.G., 1992. Bacteriology of composting. Biocycle, January 1992, 55–57.

Hadar, Y., Mandelbaum, R., 1992. Suppressive compost for biocontrol of soilborne plant pathogens. Phytoparasitica 20, 113–116.

Handreck, K.A., Black, N.A., 1984. Growing Media for Ornamental Plants and Turf. New South Wales University Press, Sydney.

Hawksworth, D.L., Kiek, P.M., Sutton, B.C., Pegler, D.N., 1995. Ainsworth & Bisby's Dictionary of Te Fungi. CAB International, Wallingford.

Hoitink, H.A.J., Vandoren, D.M., Schmitthenner, A.F., 1977. Suppression of *Phytophthora cinnamomi* in a composted hardwood bark medium. Phytopathology 67, 561–565.

Jiménez, E.I., Garcia, V.P., 1989. Evaluation of city refuse compost maturity: a review. Biol. Wastes 27, 115–145.

Lo Curto, R., Tripodo, M.M., Leuzzi, U., Giuffrè, D., Vaccarino, C., 1992. Flavonoids recovery and SCP production from orange peel. Biores. Technol. 42, 83–87.

Ma, E., Cervera, Q., Sánchez, G.M., 1993. Integrated utilization of orange peel. Biores. Technol. 44, 61–63.

Nakasali, K., Sasaki, M., Shoda, M., Kubota, H., 1985. Characteristics of mesophylic bacteria isolated during thermophilic composting of sewage sludge. Appl. Environ. Microbiol. 49, 42–45.

Poincelot, R.P., 1974. A scientific examination of the principles and practice of composting. Compost Sci. 15, 24–31.

Sinclair, W.B., 1984. The Biochemistry and Physiology of the Lemon and Other Citrus Fruits. University of California, Division of Agriculture and Natural Resources.

Sneath, P.H.A., 1986. Endospore-forming gram-positive rods and cocci. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Hall, J.G. (Eds.), Bergey's Manual of Systemic Bacteriology, vol. II. Williams & Wilkens, Baltimore, pp. 1104–1207.

Streichsbier, F., Messner, K., Wessely, M., Röhr, M., 1982. The microbiological aspects of grape marc humification. Eur. J. Appl. Microbiol. Biotechnol. 14, 182–186.

Vaccarino, C., Lo Curto, R., Tripodo, M.M., Patané, R., Laganá, G., Ragno, A., 1989. SCP from orange peel by fermentation with fungi–acid-treated peel. Biol. Wastes 30, 1–10.

Zang, W., Han, D.Y., Dick, W.A., Davis, K.R., Hoitink, H.A.J., 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology 88, 450–455.