STUDY OF BIOCHEMICAL AND MICROBIOLOGICAL PARAMETERS DURING COMPOSTING OF PINE AND EUCALYPTUS BARK

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ABSTRACT

To study the possibility of some residues from pulp and paper industry being used as substrates to produce seedlings in containers, three composting experiments were carried out using: eucalyptus bark, pine bark and a mixture (60:40, v:v) of pine bark + eucalyptus bark. Biochemical parameters studied were: acid and alkaline phosphatases, lipase (C10), protease, urease, β-glucosidase and total cellulases. The microbiological populations of total aerobic bacteria, total fungi, actinomycetes, nitrifying bacteria, cellulolitic bacteria and fungi were also evaluated. At the end of the process physico- chemical characterisation of composts was also performed. Results showed that in general the highest microbiological populations as well as for enzymatic activities occurred during the thermophilic phase (>40 ºC) of process. According to the physico-chemical characteristics of composts pine bark seems to be the one that can be used more successfully in the formulation of substrates to produce plants in containers.
Keywords: *composting, pine and eucalyptus bark, enzymatic activities*

INTRODUCTION

In Portugal peat is the widely used substrate component to produce seedlings in containers. Peat is imported from the Nordic countries that make it a finite and very expensive resource. One of the possible uses of residues from the pulp and paper processing industry is the formulation of substrates to produce plants in containers. However, these residues should be formerly composted in order to guarantee high quality substrates.

Biochemical transformations of organic matter during composting are catalysed by enzymes, thus the degradation of the labile substrates of organic materials can be followed by studying specific hydrolases (Ayuso et al., 1996), such as total cellulases, β-glucosidase, lipases, proteases and urease which are involved in the carbon and nitrogen cycles, and play an important role in those transformations. Cellulases and β-glucosidase are involved in the degradation of carbon compounds and provide energy for microorganisms. Lipases are responsible for breakdown of plant and animal fats and waxes as well as microbial cell membranes (Finnerty, 1989). Proteases and urease hydrolyse long chain peptides into polypeptides and ammonia. Furthermore, phosphatases make organic phosphorous sources available for uptake by plants and microorganisms and are considered a general microbial activity indicator (Speir and Ross, 1978). Characterising and quantifying the enzymatic activities during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations, and may provide information about the maturity of composted products (Tiquia, 2002).

This study aims to follow several biochemical and microbiological parameters, which are frequently utilized to understand changes during the aerobic biodegradation process.

MATERIALS AND METHODS

Composting experiments - Three composting experiments were carried out using pine bark, eucalyptus bark and a mixture (60:40, v:v) of pine bark + eucalyptus bark, over six months, in aerated piles with mechanical turning. In order to balance C/N ratio of initial biomass urea was added at rates equivalent to 1, 0.55 and 1.4 % (w/w), respectively for pine bark, eucalyptus bark and pine + eucalyptus bark.

Sampling was performed at the beginning of the process (T1), at thermophilic phase (T2) – after 7 days, at mesophilic phase (T3) – after 65 days, at curing phase (T4) – after 130 days and at the ending of the composting process (T5) after 180 days.

Analytical methods - Determination of enzymatic activities was performed on aqueous compost extracts prepared according to Herrmann and Shann (1993). Acid and alkaline phosphatases, and β-glucosidase activity were assayed according to Tabatabai (1982). Lipase (C10) activity was determined according to Cunha-Queda (1999). Protease activity was evaluated according to Ladd and Butler (1972). Urease activity was determined according to Tabatabai and Bremner (1972). Total Cellulase activity was assayed according to Hope and Burns (1987). Microbial populations (i.e. total aerobic bacteria, actinomycetes, filamentous eumycetes, aerobic cellulolytic fungi and bacteria, autotrophic nitrifying bacteria) were quantified according to Pochon and Tardieux (1962). Moisture content was determined by weight loss at 105 °C. Organic matter content was quantified by weight loss ignition in a furnace at 550 °C. Electrical conductivity and pH measurements were performed on aqueous suspensions of the compost samples (1:6 v/v) (Jonhson, 1980).

Statistical analysis - Data were analysed using two-way ANOVA design using the software Statistica 5.0. The Newman-Keuls test was used to separate the means.

RESULTS AND DISCUSSION

Table 1 and 2 show the results of enzymatic activities and microbiological populations evolution during the three composting experiments. For every composting experiment the highest values of acid phosphatases were found at the beginning of the process. By contrast, for alkaline phosphatases the highest values were found for samples T2 and T3, except for the mixture with eucalyptus and pine bark. This evolution is consistent with the pH values for all trials carried out (data not shown).

In general the greatest values for lipase activity were found for samples at thermophilic (T2) and mesophilic phase (T3). When correlations between enzymatic activities and microbiological populations were established (data not shown) there was a high significant (α=0.05) and positive correlation between actinomycetes and lipase activity for all composting experiments. As for protease activity the maximum values were found for samples T2 and T3 with eucalyptus bark. A similar behaviour was observed for urease activity, although the maximum value was detected before the maximum of protease activity, which may be a result of the urea added to balance the C/N ratio of initial biomass.

Total cellulase and β-glucosidase activities were higher for eucalyptus bark than to the others biomass essayed; however, similar pattern was not observed for total aerobic cellulolytic bacteria neither for total aerobic cellulolytic fungi, which may be a result of microbiological technique used for the detection of those populations.

According to the physico-chemical characteristics of composts obtained (Table 3) results show that eucalyptus bark compost has a very high value of electrical conductivity (adequate

range: 0.301-0.500 mS cm^{-1}) and pH (adequate range: 5-6) to be used in the formulation of organic substrates (Miner, 1994).

Table 1. *Evolution of enzymatic activities during the composting experiments*

Enzymatic activities	Biomass	T1	T2	T3	T4	T5
Acid phosphatases (μmol *p*-nitrophenol g^{-1} dw h^{-1})	Eucalyptus bark	4.16 a*	3.04 b	2.17 cd	2.73 be	3.42 fg
	Pine bark	3.31 f	2.54 e	2.87 b	1.60 h	2.80 be
	Pine+Eucalyptus bark	4.51 i	1.30 j	2.03 ck	2.22 d	3.61 gk
Alkaline phosphatases (μmol *p*-nitrophenol g^{-1} dw h^{-1})	Eucalyptus bark	1.01 a	5.10 b	5.07 b	5.09 b	4.13 c
	Pine bark	1.34 d	2.33 e	2.63 fg	1.28 d	1.13 a
	Pine+Eucalyptus bark	2.91 h	0.75 i	2.74 f	2.49 g	2.72 f
Lipase (μmol *p*-nitrophenol g^{-1} dw h^{-1})	Eucalyptus bark	3.93 a	15.49 b	30.34 c	11.63 d	11.11 de
	Pine bark	8.20 fg	26.16 h	24.20 i	5.83 jk	4.51 aj
	Pine+Eucalyptus bark	7.03 fk	20.25 l	21.74 l	9.32 eg	9.92 deg
Protease (µmol tyrosine g^{-1} dw 2 h^{-1})	Eucalyptus bark	0.27 ab	7.10 c	8.89 d	0.60 ef	2.17 g
	Pine bark	0.78 e	0.29 ah	0.00 a	0.00 a	0.00 a
	Pine+Eucalyptus bark	0.10 a	1.54 i	0.77 e	0.48 bfh	0.16 a
Urease (µmol N-NH_4^+ g^{-1} dw $2h^{-1}$)	Eucalyptus bark	19.12 ab	63.58 c	14.64 d	19.23 adf	13.55 ad
	Pine bark	25.51 bg	29.62 eg	14.14 ad	13.03 ad	5.17 h
	Pine+Eucalyptus bark	3.91 h	21.62 bf	37.53 i	22.98 bf	25.29 beg
Total cellulase (µmol glucose g^{-1} dw 16 h^{-1})	Eucalyptus bark	6.32 ab	35.04 c	41.59 d	26.06 e	16.28 f
	Pine bark	10.92 g	15.80 fh	13.27 gh	8.71 aij	5.37 b
	Pine+Eucalyptus bark	6.96 abi	24.04 e	16.31 fh	14.15 fh	11.32 gj
â-glucosidase (µmol *p*-nitrophenol g^{-1} dw h^{-1})	Eucalyptus bark	1.71 ab	2.70 c	3.13 d	1.80 a	1.72 ab
	Pine bark	0.60 e	1.19 f	0.15 g	0.15 gh	0.05 g
	Pine+Eucalyptus bark	1.77 ab	1.54 b	0.46 ei	0.35 hi	0.53 ei

*dw, dry weight basis. *Means of each parameter designated by the same letter are not significantly different at α=0.05.*

Table 2. *Evolution of microbiological populations during the composting experiments*

Microbiological population	Biomass	T1	T2	T3	T4	T5
Total aerobic bacteria [log(CFU g^{-1} dw)]	Eucalyptus bark	9.32 ab*	9.30 ab	9.08 ac	8.94 cd	9.28 b
	Pine bark	7.17 e	8.92 cd	8.53 f	8.06 g	7.47 h
	Pine+Eucalyptus bark	8.26 gi	8.74 d	8.17 g	8.42 fi	7.86 j
Filamentous eumycetes [log(CFU g^{-1} dw)]	Eucalyptus bark	6.92 ab	-----	6.80 ab	-----	6.62 ab
	Pine bark	5.90 c	-----	6.63 ade	-----	5.92 c
	Pine+Eucalyptus bark	6.95 ad	-----	6.54 be	-----	6.23 f
Actinomycetes [log(CFU g^{-1} dw)]	Eucalyptus bark	3.49 a	-----	5.73 b	-----	4.67 c
	Pine bark	3.45 a	-----	5.65 b	-----	4.73 c
	Pine+Eucalyptus bark	3.47 a	-----	5.53 b	-----	4.64 c
Total aerobic cellulolytic bacteria [log(CFU g^{-1} dw)]	Eucalyptus bark	3.68 a	5.63 bc	5.29 b	5.55 bc	5.93 c
	Pine bark	4.60 d	5.85 c	2.65 e	4.44 df	2.27 g
	Pine+Eucalyptus bark	4.14 f	3.77 a	5.35 b	6.59 h	5.50 bc
Total aerobic cellulolytic fungi [log(CFU g^{-1} dw)]	Eucalyptus bark	6.74 ab	6.16 cd	6.77 ab	6.78 ab	6.51 ac
	Pine bark	5.82 de	5.29 fg	7.11 bh	6.29 cij	5.55 ef
	Pine+Eucalyptus bark	6.92 ah	5.03 g	6.84 ah	6.64 ai	6.01 dj
Autotrophic nitrifying bacteria [log(MPN g^{-1} dw)]	Eucalyptus bark	1.03	2.95	5.28	6.52	6.41
	Pine bark	3.13	6.59	5.87	5.58	5.40
	Pine+Eucalyptus bark	1.64	3.39	5.06	7.62	5.13

*dw, dry weight basis. *Means of each parameter designated by the same letter are not significantly different at α =0.05.*

Table 3. *Physico-chemical characterisation of the three composts*

Parameters	Eucalyptus bark compost	Pine bark compost	Pine+Eucalyptus bark compost
Moisture (%)	53.57	61.35	60.98
Organic matter (% dry weight basis)	79.70	82.30	82.00
pH (1:6 v:v)	6.80	4.50	4.70
Electrical conductivity (1:6 v:v) (mS cm^{-1})	1.45	0.22	1.19

Results obtained are consistent with those anticipated by the enzymatic activities for eucalyptus bark compost and to the final content of organic matter (OM) that was the lowest found for the three composts, suggesting a greater OM mineralisation during the process.

CONCLUSIONS

Results showed that in general the highest microbiological populations occurred during the thermophilic phase (>40 ºC) of composting process. Regarding the enzymatic activities results showed that the maximum values were also observed during the thermophilic phase as well as during the mesophilic phase (<40 ºC). Referring the different types of materials used for the composting trials, in general, higher enzymatic activities were found for the eucalyptus bark. The assessment of compost quality showed that compost obtained from pine bark is the one that can be used more successfully in the formulation of organic substrates to produce plants in containers.

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