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Evolution of extracellular enzyme activities during manure composting

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Aims: The objectives of this work were to determine the extracellular enzyme profiles during composting, relate the activities of these enzymes to the changes in microbial population and compare the enzyme profiles between two manures.

Methods and Results: API ZYM^{TM} assay was used to monitor the activities of 19 extracellular enzymes during poultry and pig manure composting. Results showed an overall increase in diversity and relative abundance of enzymes present. The relative abundance and activities of enzymes were higher in poultry manure than in pig manure. Among the 19 enzymes tested, esterase, valine amino-peptidase and α-galactosidase were the most abundant enzymes in poultry manure, whereas it was N-acetyl-β-glucosaminidase for the pig manure. A number of these enzymes correlated with change in numbers of different microbial groups during composting.

Conclusions: The composting process represented a combined activity of a wide succession of environments, as one enzyme/microbial group overlapped the other and each emerged gradually due to the continual change in temperature and progressive breakdown of complex compounds to simpler ones.

Significance and Impact of the Study: The results presented here show the applicability of the API ZYM^{TM} test not only in monitoring the quantitative and qualitative fluctuation of the available substrate during composting, but also in revealing differences in composts and compost maturity.

INTRODUCTION

Composting is a biological decomposition of organic matter by micro-organisms (Golueke 1972, 1992; Rynk *et al*. 1992; Beffa *et al*. 1996; Tiquia *et al*. 1996). During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a role (Garcia *et al*. 1992, 1993; Vuorinen 1999, 2000). For example, the mineralization of organic N, which involves the release of N from non-peptide C–N bonds in amino acids and urea, is mediated by enzymes such as amidohydrolases and dehydrogenases (Garcia *et al*. 1992; Tabatabai *et al*. 1994). Specific examples of enzymes important in soil microbiology include: cellulases, which degrade the polymer cellulose into smaller components; nitrogenase, which converts dinitrogen gas into biologically available ammonia; sulphatases, which release protein and certain organic compounds; and phosphatases, which remove phosphate groups from organic compounds (Burns 1978; Tate 1995; Nannipieri *et al*. 1996). Enzymes that catalyse the degradation of polymeric substances, such as cellulose, hemicellulose and lignin, are extracellular because the polymer is too large to be transported across the cellular membrane (Priest 1984). However, once the polymer has been reduced to its smaller units, subsequent catabolism may proceed intracellularly (Skujins 1976). Intracellular and extracellular enzymes cannot be distinguished in soil and compost suspensions. However, after a brief incubation, the extracellular groups of enzymes can be assigned readily to which a large portion of enzymes in soils and composts belong (Vuorinen 1999, 2000).

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Characterizing and quantifying the enzymatic activity 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 the composted product. While the change of physico-chemical properties (temperature, bulk density, CEC, N, C:N ratio, pH, organic C etc.) during the composting process has been extensively studied (Harada and Inoko 1980; Garcia *et al.* 1991; Mathur *et al.* 1993; Flynn and Wood 1996; Day *et al.* 1998; Tiquia *et al.* 1998a, b; Tiquia and Tam 2000a; Tiquia 2001), information on the biological properties and, in particular, enzymatic activities is rare, especially with regards to the assessment of compost maturity. The respiratory activity, CO_2 production and O_2 consumption rates, and microbial biomass, have been successfully employed to understand the composting process and to assess compost maturity (Iannotti *et al.* 1994; Insam *et al.* 1996; Tiquia *et al.* 1996; Epstein 1997). Community level physiological profiles have also been used for compost maturity testing (Belete *et al.* 2001). Enzyme activities during composting have been studied in the past (Godden *et al.* 1983; Garcia *et al.* 1992, 1993; Vuorinen 1999, 2000). However, most of these studies have been restricted to monitoring the changes of total enzyme activities (intracellular and extracellular) during composting. Very few researchers have attempted to assess the changes in extracellular enzyme activities and link these activities with varying composting conditions, changes in other important composting parameters (i.e. microbial properties), and compost maturity.

The present report addresses the study of an array of enzymes during the composting process. A novel approach based on the use of the API ZYM^{TM} kit was employed to evaluate the enzyme profiles during composting of manures. API ZYM^{TM} is a semi-quantitative micro-method designed for systematic and rapid study of 19 enzymatic reactions. It consists of a series of microcupules containing dehydrated chromogenic substrates of 19 different enzymes and one control (a microcupule containing no enzyme substrate). These enzymes include three phosphatases (alkaline phosphatase, acid phosphatase and phosphohydrolase), three esterases (lipase, esterase-lipase and esterase), three amino-peptidases (leucine amino-peptidase, valine amino-peptidase and cystine amino-peptidase), two proteases (chymotrypsin and trypsin), and eight glycosyl-hydrolases (β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-glucosidase, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase). The kit has been successfully used for the study of enzyme activities of micro-organisms and cell suspensions (Jain *et al.* 1991; Bidochka *et al.* 1999; Khan *et al.* 1999; Garcia-Martos *et al.* 2000). The specific objectives of this study were to (i) determine the extracellular enzyme profiles at different stages of composting, (ii) relate the activities of these enzymes to changes in microbial population numbers of the manure composts, and (iii) compare the enzyme profiles from two different manures (poultry and pig manure).

MATERIALS AND METHODS

Composting set-up and sampling

The manures used in this study were: (i) poultry manure (a mixture of poultry manure, waste feed, wood shavings, and feathers and yard trimmings); and (ii) pig manure (a mixture of partially-decomposed pig manure and sawdust) disposed from the pig-on-litter system (Tiquia and Tam 1998). Three piles with forced aeration (Tiquia and Tam 2000b) were set-up for each composting experiment. Each pile was pyramidal in shape and weighed approximately 2000 kg. Before piling, the water content of the composts was adjusted to 65% (w/v) and then weekly during the composting trial. The composting trials lasted for 91 days. Temperature and samples were taken from three different locations of the compost piles: top (85 cm from the base of the pile), middle (50 cm from the base of the pile) and bottom (30 cm from the base of the pile). Triplicate samples were collected from each location of the piles at day 0, 7, 14, 35, 63 and 91.

Chemical analysis

Water extracts of the poultry and pig manure samples were prepared by shaking the fresh sample with distilled water at 1:10 w/v using a horizontal shaker for 1 h, and then filtered. Concentrations of water-extractable carbon (C) were measured using a Shimadzu TOC-500 analyser (Shimadzu, Kyoto Japan), those of NH_4^+-N and NO_x^--N by a colorimetric method (Mulvaney 1996), and extractable phosphorus (P) and potassium (K) by atomic absorption spectrometry.

Microbial counts

During composting, quantitative estimations of the populations of total aerobic heterotrophs, actinomycetes, and fungi in the compost samples were determined by direct plating on appropriate media (Parkinson 1994; Wellington and Toth 1994; Zuberer 1994). The serially-diluted suspension was inoculated on the agar using the plate frequency technique (Tiquia *et al.* 1998a). Each agar plate was divided into eight sections and about 0·1 ml of the compost suspension was dropped on each of the sections. After incubation, any visible growth observed in any of the eight sections was scored positive. The total number of sections with positive growth at each dilution was counted, and the population of micro-organisms in the sample was estimated using the Most Probable Number (MPN) method (Woomer 1994).

Table 1 Substrate composition, pH, and expected results from the test

Enzyme assayed for	Substrate	pH	Result	
			Positive	Negative
1. Control	–	–	Colourless or pale yellow	Colourless or pale yellow
2. Alkaline phosphatase	2 naphthyl-phosphate	8·5	Violet	Colourless or pale yellow
3. Acid phosphatase	2 naphthyl-phosphate	5·4	Violet	Colourless or pale yellow
4. Phosphohydrolase	Naphthyl AS-BI-phosphate	8·5	Blue	Colourless or pale yellow
5. Lipase	2 naphthyl-myristate	7·5	Violet	Colourless or pale yellow
6. Lipase-esterase	2 naphthyl-caprylate	7·5	Violet	Colourless or pale yellow
7. Esterase	2 naphthyl-butyrate	6·5	Violet	Colourless or pale yellow
8. Leucine amino-peptidase	L-leucyl-2-naphthylamide	7·5	Orange	Colourless or pale yellow
9. Valine amino-peptidase	L-valyl-2-naphthylamide	7·5	Orange	Colourless or pale yellow
10. Cystine amino-peptidase	L-cystyl-2-naphthylamide	7·5	Orange	Colourless or pale yellow
11. Chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamine	7·5	Orange	Colourless or pale yellow
12. Trypsin	N-benzol-DL-arginine-2-naphthylamide	8·5	Orange	Colourless or pale yellow
13. α-galactosidase	6-Br-2-naphthyl-α-D-galactopyranoside	5·4	Violet	Colourless or pale yellow
14. β-glucosidase	6-bromo-2-naphthol-α-D-galactopyranoside	5·4	Violet	Colourless or pale yellow
15. N-acetyl-β-glucosaminidase	1 naphthyl-N-acetyl-βD-glucosaminide	5·4	Brown	Colourless or pale yellow
16. α-glucosidase	2 naphthyl-2-D-glucopyranoside	5·4	Violet	Colourless or pale yellow
17. β-galactosidase	2 naphthyl-βD-galactopyranoside	5·4	Violet	Colourless or pale yellow
18. β-glucoronidase	Naphthyl-AS-BI-βD-glucuronide	5·4	Blue	Colourless or pale yellow
19. α-mannosidase	6-bromo-2-naphthyl-2-D-mannopyranoside	5·4	Violet	Colourless or pale yellow
20. α-fucosidase	2 naphthyl-αL-fucopyranoside	5·4	Violet	Colourless or pale yellow

API ZYM™ assay

The enzyme extracts were prepared by mixing 5 g from each sample with 50 ml sterile water. The solution was shaken for 10 min using a stomacher (Stomacher 400, Tekmar, Cincinnati, OH, USA) and allowed to settle for 10 min, and the supernatant fluid was used for enzyme analysis. API ZYM™ strips (BioMerieux, Marcy l' Etoile, France) consist of 20 microcupules containing dehydrated chromogenic substrates of 19 different enzymes (Table 1) and a control (a microcupule that does not contain any enzyme substrate). The enzyme substrates in the system are shown in Table 1. After the enzyme extracts had been prepared, an aliquot (65 μl) of the extract supernate was dispensed into each of the 20 microcupules. The API ZYM™ strips were then covered and incubated at 37°C for 4 h. After incubation, 30 μl of each reagent (ZYM A and ZYM B; BioMerieux) were added to all microcupules. After 5 min, a numerical value of 1–5 was assigned to each microcupule according to the colour chart provided by the manufacturer. For the purposes of this study, the results were reported as reactions of low intensity (1), moderate intensity (2–3) and high intensity (4–5).

Statistical analyses

To demonstrate relationships between different enzymes and microbial (total aerobic heterotrophs, actinomycetes and fungi) properties of the compost samples, Pearson product-moment correlation was performed. Principal components analysis (PCA) was carried out to describe the temporal dynamics of enzyme activities during the composting process. PCA was also computed to compare enzyme profiles of two manure composts during the final stage of composting. Statistical analyses were calculated using SYSTAT statistical computing package (SYSTAT Version 9·0, SPSS Inc., Chicago, IL, USA).

RESULTS

Temperature histories and chemical characteristics

The temperature poultry manure piles self-heated to 55°C in the first 2 days of composting, peaked at 60° and 71°C, and then declined to ambient level (Fig. 1a). In the pig manure piles, temperatures peaked at 61°C by day 7 (Fig. 1b) and remained stable until day 21. Thereafter, temperatures began to drop and then fluctuated within a narrow range (28–35°C) from day 56 until the end of composting (Fig. 1b).

Table 2 shows the changes in chemical properties of the compost extracts. The poultry manure compost had higher initial extractable C and K than the pig manure compost, but the initial concentrations of NH_4^+-N, NO_x^--N and extractable P were similar in both composts. During composting, the water-extractable C of the compost sampled

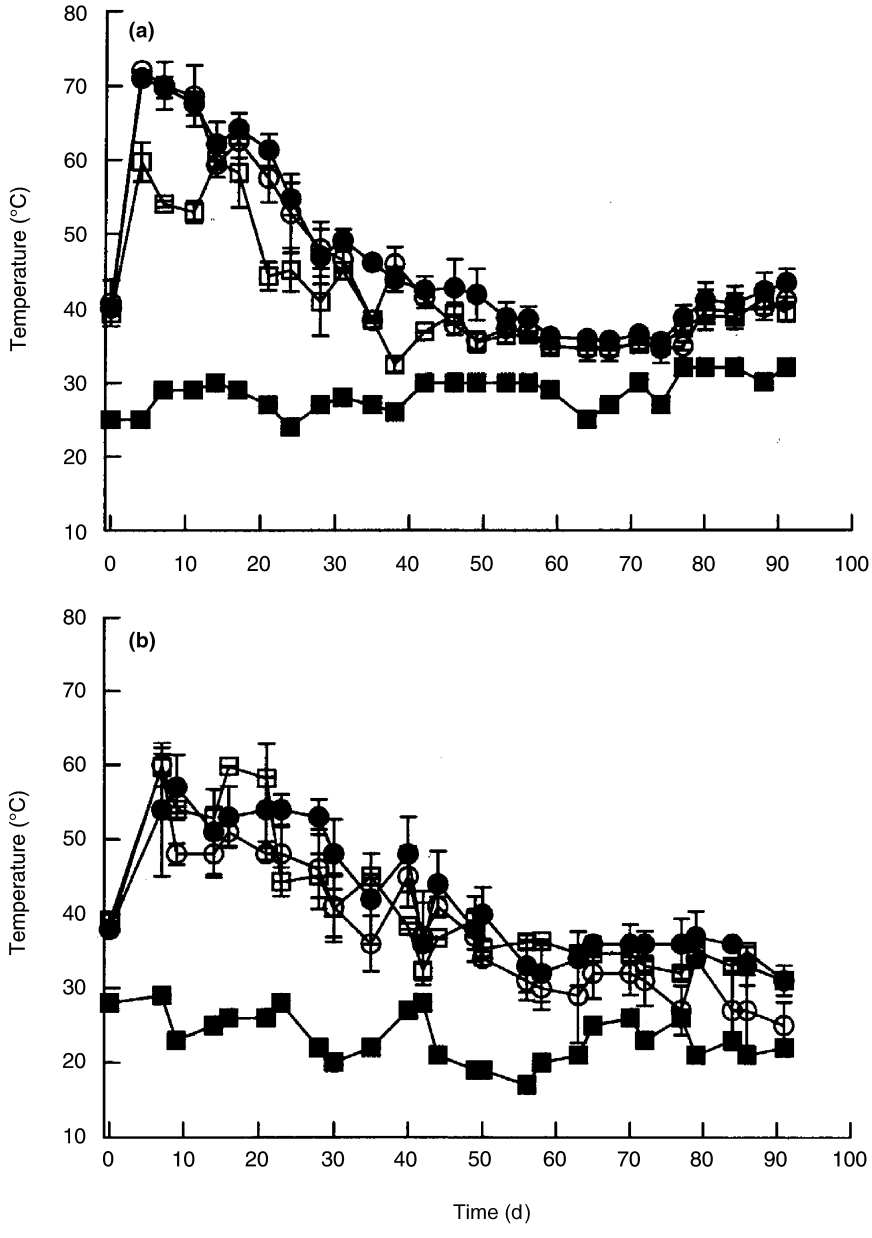

Fig. 1 Changes in air and pile temperatures during composting. (a) Poultry manure; (b) pig manure. Pile locations: (○) top; (●) middle; (□) bottom; (■) air temperature

decreased dramatically. Decreases in NH_4^+-N concentration in both composts were also observed which corresponded to increases in the concentration of NO_x^--N. Extractable P and K concentrations fluctuated in a very narrow range in the compost samples during composting (Table 2).

Microbial numbers

The total aerobic heterotroph counts of the compost samples were highest at the beginning of composting (Table 3). By day 7, their numbers dropped and then increased gradually when temperatures started to decline (Fig. 1). The actinomycete numbers dropped as pile temperatures began to peak by day 7, and then increased as the temperatures started to decline (Table 3 and Fig. 1). Fungal numbers declined dramatically at temperatures above 50°C but recovered later when compost temperatures were moderate (< 45°C) (Table 3 and Fig. 1).

Table 2 Changes in water-extractable components of poultry yard trimmings and spent pig litter at different stages of composting

	Chemical properties*														
	Ext. C (g kg^{-1})			NH_4^+-N (g kg^{-1})			NO_X^--N (g kg^{-1})			Ext. P			Ext. K		
Time (days)	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B
Poultry litter + yard trimmings															
0	67·69	72·16	104·06	6·77	6·26	6·98	0·05	0·33	0·28	4·18	4·11	5·26	12·98	10·14	13·95
14	71·65	60·04	47·88	4·82	4·51	4·44	0·82	0·02	0·72	4·23	3·96	4·46	12·88	10·35	12·03
35	58·18	41·53	28·87	2·90	1·84	0·98	1·88	1·00	1·04	4·35	3·92	3·99	11·51	8·72	12·20
63	3·01	4·39	4·61	2·14	0·38	0·40	1·44	0·79	0·89	4·41	3·84	3·68	10·89	14·71	11·76
77	4·78	3·83	3·19	0·10	0·09	0·21	1·32	1·07	1·98	3·96	4·73	3·76	11·08	16·96	11·88
91	3·46	4·83	3·67	0·72	0·06	0·06	2·47	1·97	2·03	4·23	4·22	4·96	12·22	13·84	12·22
Spent pig litter															
0	47·76	47·75	40·13	3·61	4·16	4·17	0·47	0·18	0·13	3·64	4·81	4·84	7·72	6·65	6·09
14	28·75	27·82	20·21	4·93	3·76	3·29	0·71	0·49	0·61	3·81	3·81	3·57	8·82	8·73	8·19
35	19·5	17·30	10·20	3·46	3·19	2·00	0·99	1·08	0·89	3·79	4·89	4·13	7·98	8·81	8·32
63	9·37	7·36	6·35	3·20	3·32	0·45	1·26	1·05	1·43	4·61	4·44	4·58	9·67	10·03	10·62
77	2·70	2·52	1·08	1·91	1·87	0·22	2·07	2·01	1·96	5·04	5·23	4·40	9·37	9·09	9·89
91	2·00	2·70	1·15	0·93	0·87	0·16	2·73	2·77	2·00	5·07	5·18	4·58	8·43	8·89	10·04

*T = top location of the pile; M = middle location of the pile; B = bottom location of the pile.

Table 3 Log_{10} MPN counts of total aerobic heterotrophs, actinomycetes, and fungi in poultry litter + yard trimmings and spent pig litter at different stages of composting

	Microbial numbers*								
	Heterotrophs (log_{10} MPN g^{-1})			Actinomycetes (log_{10} MPN g^{-1})			Fungi (log_{10} MPN g^{-1})		
Time (days)	T	M	B	T	M	B	T	M	B
Poultry litter + yard trimmings									
0	9·80	9·46	9·52	8·78	8·49	8·64	8·09	7·75	7·78
7	7·43	7·89	7·16	6·76	7·21	6·84	5·31	5·88	5·30
14	8·32	8·41	7·66	7·63	8·20	7·70	5·96	6·06	5·47
35	7·23	7·36	8·29	8·00	8·28	8·71	5·37	5·08	5·63
63	7·09	7·37	7·43	10·32	10·57	10·45	7·72	7·36	7·62
77	8·20	8·11	8·83	10·38	10·23	10·40	8·22	8·19	8·26
91	8·11	8·04	8·88	10·30	10·17	10·21	9·24	9·23	8·33
Spent pig litter									
0	8·55	8·06	8·22	9·14	8·97	8·88	7·39	7·10	6·92
7	6·79	5·77	6·79	8·15	7·50	7·33	4·05	4·11	4·10
14	6·07	7·34	6·80	7·46	7·64	7·80	4·23	3·31	2·94
35	8·03	7·79	8·12	8·82	8·61	8·73	5·52	5·40	5·07
63	7·23	7·19	7·14	9·77	9·72	9·83	6·33	6·37	6·91
77	7·73	7·15	7·85	10·13	9·66	9·90	7·33	7·02	7·10
91	7·88	8·26	8·23	10·45	9·78	9·63	8·31	8·50	7·95

*T = top location of the pile; M = middle location of the pile; B = bottom location of the pile.

Extracellular enzyme profiles at different stages of composting

High alkaline and acid phosphatase activities were high in the poultry manure piles from the beginning of the test, reaching maximum activity by the end of the testing period (Table 4). Phosphohydrolase activity was moderate at the onset of composting and then declined to a low level at the end of composting. Overall, a moderate level of lipase, esterase and esterase activities were observed at the begin-

Table 4 Relative activity of extracellular enzymes extracted from poultry manure at different stages of composting

	Day 0			Day 7			Day 14			Day 63			Day 91		
Enzyme*	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B
Phosphatases															
Alkaline phosphatase	4	4	4	4	4	4	5	5	5	4	4	4	5	5	5
Acid phosphatase	4	4	4	4	4	4	4	4	5	4	4	5	5	5	5
Phosphohydrolase	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3
Esterases															
Lipase	3	2	2	3	3	3	4	4	4	2	3	3	1	2	2
Esterase-lipase	2	2	2	3	3	3	4	4	4	4	3	4	4	4	4
Esterase	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
Amino-peptidases															
Leucine amino-peptidase	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Valine amino-peptidase	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
Cystine amino-peptidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteases															
Chymotrypsin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Trypsin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycosyl-hydrolases															
α-galactosidase	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
β-glucosidase	3	3	3	1	1	1	1	1	1	3	3	3	3	3	3
N-acetyl-β-glucosaminidase	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2
α-glucosidase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β-galactosidase	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
β-glucuronidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-mannosidase	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
α-fucosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Reactions of low intensity (value of 1), moderate intensity (values of 2–4), and high intensity (value of 5). T = top location of the pile; M = middle location of the pile; B = bottom location of the pile.

ning of composting. The activity of esterase-lipase and esterase increased as composting progressed, while lipase activity declined to a low level by the end of the testing period. Leucine-amino peptidase activity was moderate at day 0, reaching maximum activity by day 14 and maintaining this level throughout the composting trial. Cystine amino peptidase, chymotrypsin and trypsin showed no evidence of activity during the entire period of composting (Table 4). Of the eight glycosyl-hydrolases, only α-galactosidase, β-glucosidase and N-acetyl-β-glucosaminidase showed any significant activity, fluctuating between low and moderate activity. The activity of α-mannosidase was low, while β-galactosidase, β-glucuronidase and α-fucosidase remained undetected during the entire testing period (Table 4).

In the pig manure, alkaline and acid phosphatase activities were also high at the beginning of composting, reaching maximum activity at day 14 and maintaining at this level throughout the period of composting (Table 5). Phosphohydrolase activity was low at the onset of composting but reached moderate activity by day 63. This level of activity was sustained until day 91. Overall, a moderate level of lipase and esterase activity was found in turned or forced-aerated piles, whereas a high level of esterase-lipase activity was observed throughout the entire period of composting (91 days) (Table 5). Leucine amino-peptidase activity was moderate in both piles in the first 2 months and increased to high values from day 63 to day 91. In contrast, valine amino-peptidase and cystine amino-peptidase decreased from moderate to low (for valine amino-peptidase) and non-detectable (for cystine amino-peptidase) levels during composting (Table 5). Trypsin and chymotrypsin showed no evidence of activity at day 0, but activity began at day 14 (for trypsin) and day 63 (for chymotrypsin), and was then maintained at a low intensity for the rest of the composting period (Table 5). Of the eight glycosyl-hydrolases, α-mannosidase and α-fucosidase showed no evidence of activity throughout the duration of the composting trial in both piles. Activities of α-galactosidase, β-galactonidase, β-glucuronidase and N-acetyl-β-glucosaminidase were not detected during the initial stage of composting but

Table 5 Relative activity of extracellular enzymes extracted from spent pig litter at different stages of composting

	Day 0			Day 7			Day 14			Day 63			Day 91		
Enzyme*	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B
Phosphatases															
Alkaline phosphatase	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
Acid phosphatase	3	4	4	3	4	4	5	5	5	5	5	5	5	5	5
Phosphohydrolase	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2
Esterases															
Lipase	3	4	4	3	3	3	2	2	2	2	2	2	2	2	2
Esterase-lipase	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5
Esterase	3	4	4	3	4	4	4	4	4	4	4	4	4	4	4
Amino-peptidases															
Leucine amino-peptidase	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
Valine amino-peptidase	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Cystine amino-peptidase	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1
Proteases															
Chymotrypsin	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Trypsin	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Glycosyl-hydrolases															
α-galactosidase	0	0	0	0	0	0	0	0	1	2	2	2	2	2	2
β-glucosidase	1	1	1	1	1	1	2	2	2	5	5	5	5	5	5
N-acetyl-β-glucosaminidase	0	0	0	2	1	2	4	4	4	4	5	5	4	5	5
α-glucosidase	1	1	1	1	3	2	4	3	3	4	4	4	4	4	4
β-galactosidase	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2
β-glucuronidase	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4
α-mannosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Reactions of low intensity (value of 1), moderate intensity (values of 2–4), and high intensity (value of 5). T = top location of the pile; M = middle location of the pile; B = bottom location of the pile.

fluctuated from low to moderate as composting progressed. Low activities of α-glucosidase and β-glucosidase were detected at day 0 and, as composting proceeded, these increased continuously to reach a high intensity level (Table 5).

Multivariate statistical analysis

Figure 2 shows the ordinate plot of PCA for poultry and pig manure composts. The data plot includes samples taken at 0, 7, 14, 63 and 91 days of composting. The analysis paired the sample taken at different locations (top, middle and bottom) of the forced-aeration piles, and separated the compost samples taken at different stages of composting. PCA of poultry manure indicated that the older composts (day 63 and 91 composts) had greater extracellular enzyme activities (i.e. esterases, amino-peptidases, glycosyl-hydrolases and phosphatases) than the younger composts (day 0, 7 and 14 composts), since the older samples had higher PC 1 and PC 2 scores (Fig. 2a and Table 6). Data from the pig manure compost showed that the younger composts (day 7 and 14 composts) had greater enzyme activities (highest PC 1 and PC 2 scores) than the older composts (day 63 and 91 composts) (Fig. 2b). Compost taken at 7 and 14 days of composting had greater glycosyl-hydrolase, phosphatase, amino-peptidase and esterase activities than compost samples taken at 63 and 91 days of composting (Table 6).

When the data from composted poultry and pig manure (day 91 composts) were compared, it became evident that the poultry manure compost was separated from pig manure compost (Fig. 3). The composted manure had higher PC 1 and PC 2 scores (Fig. 3) than the pig manure, indicating that the enzyme activities in composted poultry manure were greater than the composted pig manure.

DISCUSSION

This investigation has demonstrated that poultry and pig manure composts went through changes, including changes in temperature of the compost mass, microbial numbers and chemical components. API ZYM^{TM} testing of poultry and pig manure composts showed an overall increase in diversity

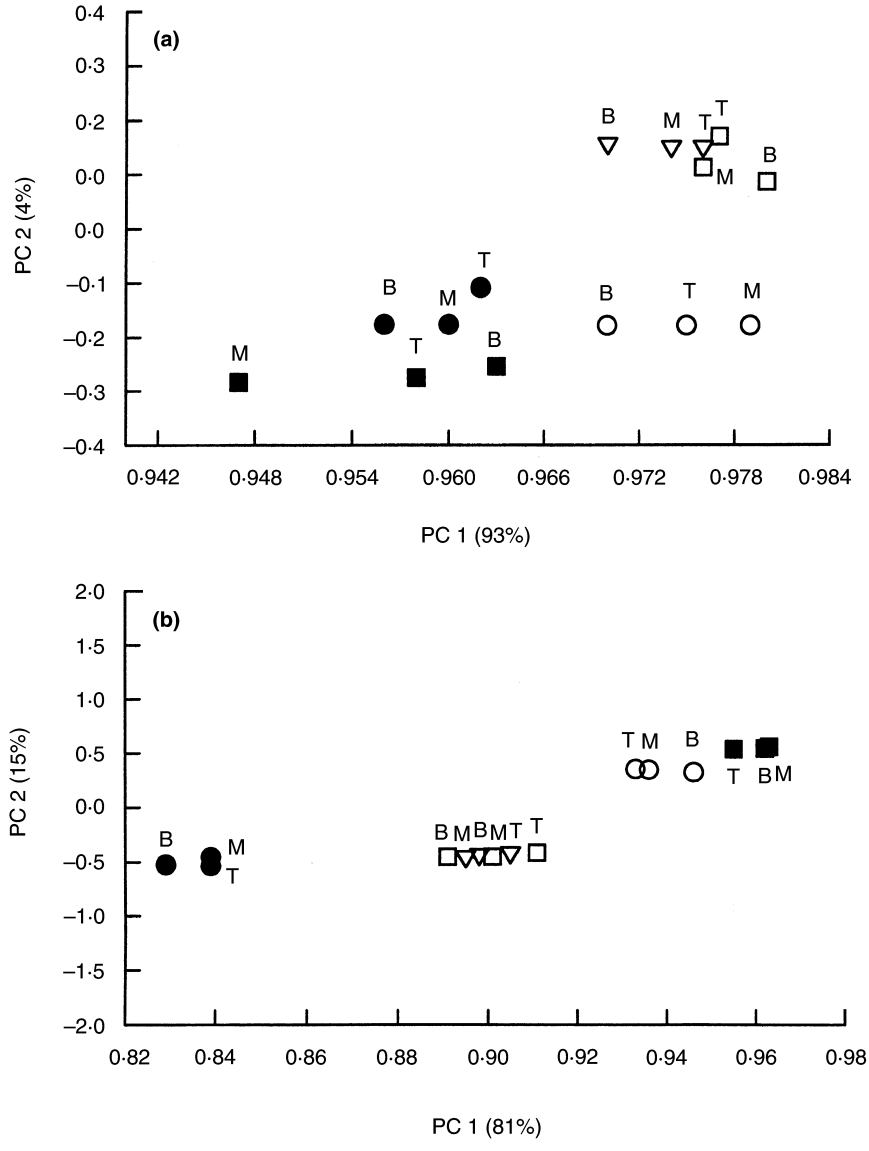

Fig. 2 Ordinate plot from principal components analysis of enzyme profiles of (a) poultry and (b) pig manure composts at different stages of composting. Letters on the graph indicate the different locations (T = top; M = middle; B = bottom) of the forced-aeration piles. Composting time: (●) day 0; (○) day 7; (■) day 14; (□) day 63; (▽) day 91

and relative abundance of the enzymes present. Results of the principal components analysis showed that the relative abundance and activities of the enzymes present differed between the two manures examined. Enzyme activities were increasingly more abundant in poultry manure than in pig manure. This is probably due to the fact that the poultry manure had a higher microbial population than the pig manure, especially during the later stage of composting (Table 3). When the enzyme profiles were compared at different stages of composting, different results were found between the two composts. In the poultry manure, greatest enzyme activity was observed in older composts (day 63 and 91 composts), whereas in pig manure, the younger composts (day 7 and 14 composts) showed greatest activity. This result could be attributed to differences in chemical composition and decomposition states of the two manures. The pig manure used in this study had been through partial decomposition for 13 weeks (Tiquia and Tam 1998) in the pig-on-litter (POL) system, prior to composting in windrows. The POL system is a pig production method by which pigs are raised on a litter bedding material (sawdust mixed with a commercial bacterial product) and the manure is decomposed *in situ* (Tiquia and Tam 1998). Therefore, a large portion of the soluble organic matter in the manure

Table 6 Correlation between principal components (PC 1 and PC 2) and single variables (enzymes) for the PCAs described in the text

Poultry manure			Pig manure		
Enzyme	PC 1	PC 2	Enzyme	PC 1	PC 2
Esterase	0·935		N-acetyl-β-glucosaminidase	0·951	
Valine amino-peptidase	0·935		Alkaline phosphatase	0·942	
α-galactosidase	0·935		Leucine amino-peptidase	0·942	
Lipase	0·755		α-glucosidase	0·942	
N-acetyl-β-glucosaminidase	0·678		β-glucosidase	0·933	
α-mannosidase	0·649		Acid phosphatase	0·893	
β-glucosidase	0·637		Esterase-lipase	0·869	
Alkaline phosphatase		0·777	Phosphohydrolase	0·867	
Acid phosphatase		0·735	Esterase	0·529	
Phosphohydrolase		0·543	α-galactosidase		0·524

may have already been used up by the indigenous micro-organisms (Tam and Tiquia 1996), so the spent pig manure removed from the system may have contained a lesser amount of soluble organic matter and more complex organic matter prior to windrow composting. The initial poultry manure, on the other hand, had relatively higher soluble chemical components compared with pig manure (Table 2). Therefore, the breakdown of complex or larger compounds to simpler compounds by micro-organisms was not necessary. In composting, the soluble organic matter in the starting material is initially assimilated by the micro-organisms (Rynk *et al.* 1992). Once the soluble organic matter is used up, micro-organisms produce hydrolytic enzymes to depolymerize the larger compounds (i.e. lignin, cellulose and hemicellulose) to smaller fragments that are water-soluble (Priest 1984; Tate 1995; Tiquia *et al.* 2002). The water-soluble components dissolve in the water and are finally assimilated by the micro-organisms. The micro-organisms in the pig manure may have used up a large quantity of soluble organic matter prior to composting in windrows and therefore, a very high extracellular activity was observed during the early stage of composting. Interestingly, the extracellular enzyme profiles of poultry and pig litter also differed in terms of the enzymes found. For instance, chymotrypsin, trypsin and β-glucuronidase were absent in the poultry manure compost during the entire composting trial, but were present in the pig manure compost. In contrast, α-mannosidase was absent in the pig manure but was detected in the poultry manure during composting (Tables 4 and 5).

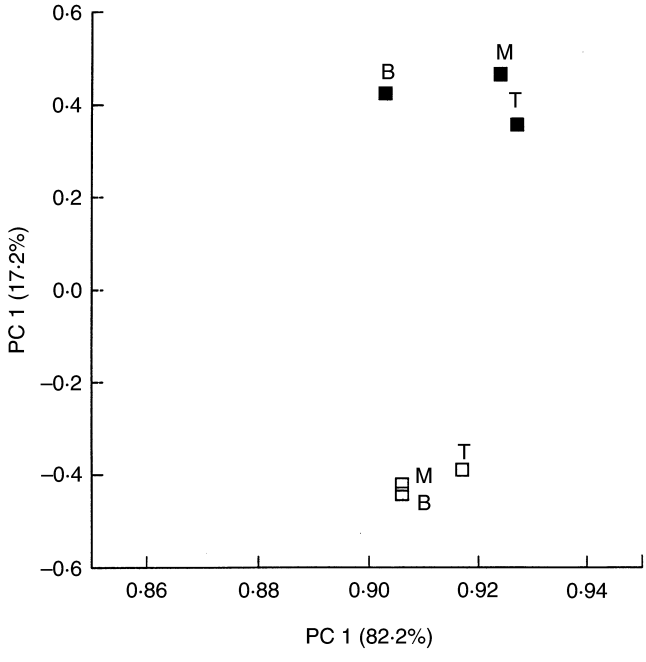

Fig. 3 Ordinate plot from principal components analysis of enzyme profiles of two manure composts at day 91. Letters on the graph indicate the different locations (T = top; M = middle; B = bottom) of the forced-aeration piles. Manure type: (■) poultry manure and (□) pig manure

Correlation analysis showed that typical enzymes such as esterase, N-acetyl-β-glucosaminidase, β-glucosidase, alkaline and acid phosphatase, and phosphohydrolase were closely related with PC 1 for both manure composts (Table 6), indicating that these enzymes were increasingly abundant in the composts. Among these enzymes, esterase, valine amino-peptidase and α-galactosidase were the most abundant (highest PC 1 scores) in the poultry manure, whereas N-acetyl-β-glucosaminidase was most abundant in the pig manure. Principal components analysis was able to separate different groups of compost very clearly. It is interesting to note that although the activities of the final composts from the two manures were different, the mature (stabilized) compost samples (day 63 and 91 samples) from the same manure grouped together (Fig. 3), suggesting that the enzyme profile of the composts from the same origin

Table 7 Coefficient of determination (r^2) for the relationship between microbial counts and enzyme activities in poultry litter + yard trimmings and spent pig litter piles

	Poultry manure			Pig manure		
Enzyme†	Hetero	Actino	Fungi	Hetero	Actino	Fungi
Phosphatases						
Alkaline phosphatase	−0·72**	−0·21	−0·49	0·38	0·05	0·13
Acid phosphatase	−0·64*	0·39	0·13	0·36	0·15	0·18
Phosphohydrolase	−0·02	0·30	0·44	0·66	0·34	0·45
Esterases						
Lipase	0·16	−0·65*	−0·79**	−0·24	0·08	0·20
Esterase-lipase	−0·80**	0·25	−0·25	−0·19	−0·13	−0·01
Esterase	−0·58*	0·95***	0·69*	−0·06	−0·14	−0·04
Amino-peptidases						
Leucine amino-peptidase	−0·50	0·14	−0·16	0·38	0·09	−0·13
Valine amino-peptidase	−0·58*	0·95***	0·67*	−0·03	0·31	0·12
Cystine amino-peptidase	0·00	0·00	0·00	−0·38	−0·09	0·13
Proteases						
Chymotrypsin	0·00	0·00	0·00	0·38	0·09	0·12
Trypsin	0·00	0·00	0·00	0·84***	0·56*	0·66**
Glycosyl-hydrolases						
α-galactosidase	−0·58*	0·95***	0·69*	0·80***	−0·51*	0·57*
β-glucosidase	0·16	0·77**	0·98***	0·78***	0·09	0·56*
N-acetyl-β-glucosaminidase	−0·45	0·42	0·33	0·32	−0·02	0·07
α-glucosidase	0·00	0·00	0·00	0·34	−0·01	0·16
β-galactosidase	0·82**	−0·34	0·19	0·84***	0·56*	0·66**
β-glucuronidase	0·00	0·00	0·00	0·84***	0·56*	0·66**
α-mannosidase	−0·47	0·39	0·32	0·00	0·00	0·00
α-fucosidase	0·00	0·00	0·00	0·00	0·00	0·00

†Hetero = total aerobic heterotrophs; Actino = actinomycetes.
***, ** and * indicate correlations significant at 0·001, 0·01 and 0·05 probability levels, respectively, Correlation analyses were based on 63 observations (3 pile locations × 3 replicate piles × 7 sampling dates).

would be relatively similar when they reached maturity/stability.

As a biological process, composting involves many micro-organisms (Golueke 1992; Beffa *et al.* 1996; Tiquia and Michel 2002). These micro-organisms, and their composition and magnitude, are important components of the composting process. Bacteria are usually involved in the self-heating during the initial stage of composting (Golueke 1992). They are by far the most important parameters during the most active stages of composting because of their ability to grow rapidly on soluble proteins and other readily available substrates (Golueke 1992; Epstein 1997). In the initial heating phase, bacteria utilize the simple, easily-degradable organic substances in the compost (Strom 1985). Bacteria may also attack more complex materials, or may exploit substances released from the less-degradable substances due to extracellular enzyme activities of other organisms (Epstein 1997). Fungi also play a part in the initial rise in compost temperature (De Bertoldi *et al.* 1983). Most fungi are eliminated by high temperatures (Epstein 1997) but commonly recover when temperatures are moderate (Tiquia *et al.* 2001) and the remaining substrates are predominantly cellulose or lignin (De Bertoldi *et al.* 1983). Like fungi, actinomycetes can also utilize complex organic material. They tend to grow in numbers in the later stages of composting and have been shown to attack polymers such as hemicellulose, lignin and cellulose (De Bertoldi *et al.* 1983). The population of these three microbial groups was positively correlated with a number of important enzymes tested in this study. Results of the correlation analysis indicated that populations of total aerobic heterotrophs, actinomycetes and fungi of the poultry and pig manure were positively correlated with a number of enzymes (Table 7). Fungal and actinomycete numbers of both manures were positively correlated with β-galactosidase (an enzyme involved in the hydrolysis of cellobiose) and β-galactosidase

(an enzyme involved in the hydrolysis of lactose) (Table 7). The total aerobic heterotrophs, on the other hand, correlated positively with α-galactosidase and β-galactosidase activities in both composts (Table 7).

Although this enzyme test is rather preliminary, the results seem to show a potential usefulness of enzyme activity measurements as indices of the course of the actual composting. The enzymes tested in the present study may also be used as a good index of qualitative and quantitative fluctuation of the amount of substrate during composting, since some of these enzymes are substrate-inducible enzymes. Enzymes such as esterase-lipase, valine amino-peptidase, α-galactosidase, β-glucosidase and N-acetyl-β-glucosaminidase were induced by the enzyme substrate. Results of this study also showed combined activity of a wide succession of environments in the compost pile, as one enzyme/microbial group overlapped the other and each emerged gradually as a result of the continual change in temperature and progressive breakdown of complex compounds to simpler ones.

The report presented here suggests the applicability of the API ZYM^{TM} test for revealing differences in composts. However, more methodological work is needed to make comparisons within one experiment, and to obtain more generally-applicable data. For example, more compost from different sources must be examined in order to determine the potential use of this assay as a means of testing compost maturity. Moreover, the effect of incubation time on the activities of these enzymes must be considered. So far, no investigation on the effect of incubation time has been made using the kit, which may be crucial for separating the intracellular from extracellular enzymes in compost samples.

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