Compost Science & Utilization, (2002), Vol. 10, No. 2, 150-161

Microbial Population Dynamics and Enzyme Activities During Composting

Sonia M. Tiquia[1], Judy H.C. Wan[2] and Nora F.Y. Tam[3]
1. Environmental Sciences Division,
Oak Ridge National Laboratory, Oak Ridge, Tennessee
2. Department of Biology, Hong Kong Baptist University,
Kowloon Tong, Kowloon, Hong Kong, People's Republic of China
3. Department of Biology and Chemistry, City University of Hong Kong,
Kowloon Tong, Kowloon, Hong Kong, People's Republic of China

The changes in population size of different microbial groups (total aerobic heterotrophs, actinomycetes, fungi, fecal coliforms, ammonium- and nitrite-oxidizing bacteria, and denitrifying bacteria) and the activities of 19 different enzymes (three phosphatases, three esterases, two proteases, three amino-peptidases, and eight glycosyl-hydrolases) were examined during cocomposting of poultry litter (a mixture of poultry manure, waste feed, feathers, and wood shavings) and yard trimmings (a mixture of grass clippings, leaves, and wood barks). Three piles with forced aeration were established by mixing 2:1 (v/v) ratio of poultry litter and yard trimmings. During composting, samples were taken at three different locations (top, middle, and bottom) of the forced aeration piles for microbial and enzyme analyses. Results demonstrated that population size of different microbial groups was not a limiting factor in this composting process as the microorganisms in the poultry litter + yard trimmings compost are in great abundance. Although the numbers of these microbial groups were reduced by high temperature, their populations multiplied rapidly as composting progressed. Fecal coliforms were eliminated by day 49, suggesting that the poultry litter + yard trimmings compost showed an overall increase in diversity and relative abundance of extracellular enzymes present as composting progressed. The population of fungi and actinomycetes (microorganisms active in degradation of cellulose, hemicellulose, and lignin) were positively correlated with esterase, valine amino-peptidase, α-galactosidase, β-glucosidase, and lipase. Of all 19 enzymes examined, ß-galactosidase (enzyme involved in the hydrolysis of lactose) had the most significant positive correlation with microbial populations, such as total aerobic heterotrophs, ammonium- and nitrite-oxidizing bacteria, denitrifying bacteria, and fecal coliforms. Cystine amino peptidase, chymotrypsin, and trypsin showed no evidence of activity during the entire period of composting. This composting process represented a combined activity of a wide succession of environments in the compost pile as one microbial group/enzyme overlapped the other and each emerged gradually as a result of the continual change in temperature as well as moisture content, O_2 and CO_2 level, and progressive breakdown of complex compounds to simpler ones.

Introduction

Much of the research in composting has focused on the changes in physicochemical parameters, primarily in an effort to find a simple and reliable indicator of compost maturity, and to improve the efficiency of the process (Garcia *et al.* 1992a; Harada *et al.* 1981; Inbar *et al.* 1993; Namkoong *et al.* 1999; Tiquia and Tam 1999; Warman 1999; Wu *et al.* 2000). However, very little information is available on the microbiota, which determine the rate of composting and affect the quality of the end product (Epstein 1997). As a biological process, composting involves a myriad of microorganisms. These microorganisms, their composition and magnitude are impor-

tant components of the composting process. The indigenous microbes in the compost material can decompose the organic matter, and transformation N component through oxidation, nitrification and denitrification (Atkinson *et al.* 1996; Diaz-Ravina *et al.* 1989; Golueke 1992; Tiquia and Tam 2000a). However, if active microorganisms in the compost incorporate minerals from the waste into biomass (Golueke 1992), the depletion of any of these nutrients could limit the composting process. Nonetheless, composting may also involve sequential growth and degradation of subpopulations, where no significant change in overall levels of microorganisms or inorganic nutrient requirement occurs. To understand the composting process, the changes of different microbial populations such as bacteria, actinomycetes, and fungi would be helpful. While some of these organisms may be beneficial to plant growth and soil fertility, others may be pathogenic to humans and animals. Therefore, the levels of pathogens and their reduction during the composting process are also important criteria that should be evaluated.

It is also well known that all biochemical reactions during composting are catalyzed by enzymes (Ayuso *et al.* 1996; Garcia *et al.* 1992b; Godden *et al.* 1983; Vuorinen 1999, 2000). For instance, the mineralization of organic N during composting, which involves the release of N from nonpeptide C–N bonds in amino acids and urea is mediated by enzymes such as amidohydrolases and dehydrogenases (Tabatabai 1994). Alkaline and acid phosphatases are important enzymes in organic P mineralization and plant nutrition (Speir and Ross 1978). Reports (Epstein 1997) concerned with the ecology of the composting process have been mainly restricted to the enumeration and identification of microfloral populations, with the purpose of determining the dominating microbial populations during the various stages of composting (Beffa *et al.* 1996; Epstein 1997; Strom 1995). The role of free enzymes and their activities during composting have been subjected to a limited number of reports. Godden *et al.*(1983) found that cellulase, invertase, and alkaline phosphatase activities increased during early days of composting of cattle manure, and remained constant during the thermophilic sand curing period. On the contrary, Ayuso *et al.* (1996); Garcia *et al.* (1992b), and Diaz-Burgos *et al.* (1993) observed that the activities of phosphatases, urease and proteases decrease during sewage sludge composting.

Enzymes in composts can be classified as intracellular (enzymes inside viable cells) or extracellular (enzymes outside the cells) (Vuorinen 1999, 2000). Intracellular enzymes are enzymes that catalyze biochemical reactions occurring within the cells (Nannipieri *et al.* 1990). Conversely, extracellular enzymes are enzymes purposely released exterior to cells, generally to catalyze the degradation of polymeric substances (i.e. plant polymers, cellulose, hemicellulose, and lignin) too large to cross the cellular membrane (Furhmann 1999). The intracellular and extracellular enzymes cannot be distinguished in compost suspensions. However, after a brief incubation period, the extracellular groups of enzymes can be assigned more readily to which a large portion of enzymes in soils and composts belong (Vuorinen 1999, 2000).

In this study, changes in population size of different microbial groups (total aerobic heterotrophs, actinomycetes, fungi, fecal coliforms, ammonium- and nitrite-oxidizing bacteria, and denitrifying bacteria) and the activities of 19 different enzymes (three phosphatases, three esterases, two proteases, three amino-peptidases, and eight glycosyl-hydrolases) were examined during cocomposting of poultry litter and yard trimmings in forced aeration piles to evaluate the dynamics of the composting process, and characterize the microbial and biochemical properties of poultry litter + yard trimmings compost. The possible relationship between microbial populations and extracellular enzyme activities was also evaluated.

Materials and Methods

Composting Set-Up

Poultry litter (a mixture of poultry manure, waste feed, feathers, and wood shavings) and yard trimmings (grass clippings, leaves, and wood barks) were cocomposted at the Ta Kwu Ling Pig Breeding Center, New Territories of Hong Kong using the forced-aeration system (Epstein et al. 1976; Wan 2000; Tiquia and Tam 2000b). Three piles were built on perforated pipes connected to an air pump (Regenair^{TM} R1102). Each pile contained poultry litter and wood shavings) and yard trimmings at a ratio of 2:1 (v/v; poultry litter:yard trimmings). The piles were triangular in shape, about 2 m in width at the base and 1.5 m in height, and weighed approximately 2000 kg. To insulate the piles and minimize water loss, the piles were covered with a 5–cm layer of mature compost. The cover also acted as a biofilter to minimize odor emission. During composting, the air was pumped at a rate of 634 l min^{-1} for 24 h once a week The moisture content of the mixture was adjusted to 65% (w/v) at the beginning, and then weekly during the composting trial, which lasted for 91 days.

Sampling and Measurements

Temperature and poultry litter +yard trimmings 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). Subsamples collected from three different locations of the compost pile were mixed homogeneously to give one composite sample. Triplicate composite samples were collected at day 0 and then weekly until day 91.

The poultry litter +yard trimmings samples were analyzed for the following parameters: moisture content (105°C for 24 h); pH (1:10 w/v compost:water extract) using a pH electrode; total organic matter and C using loss on ignition (Allison 1965); and total N (Bremmer 1996), P (APHA 1995) and K (atomic absorption spectrometry). Quantitative estimation of total aerobic heterotrophs, fungi, actinomycetes, fecal coliforms, and ammonium- and nitrite-oxidizing bacteria during composting was determined by direct plating on appropriate selective media. The serially-diluted compost suspension was inoculated on the agar using the plate frequency technique (Tiquia *et al.* 1998). Each agar plate was divided into 8 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 considered positive growth. The total numbers of positive growth were counted and the population of microbial organisms in the sample was estimated using a Most Probable Number (MPN) computing package (Woomer 1994). The denitrifying bacterial population was determined by inoculation of serially-diluted compost suspension in tubed liquid media (Tiedje 1994) and the population was estimated using the MPN method. The incubation conditions and media used for the selective culture of the seven microbial groups are summarized in Table 1.

The enzyme activity was evaluated using API ZYM^{TM} strips (BioMerieux, Inc., Marcy-l' Tolle, France). API ZYM^{TM} is a semi-quantitative micro-method designed for systematic and rapid study of 19 enzymatic reactions. Each API ZYM^{TM} strip consists of a series of microcupules containing dehydrated chromogenic substrates of 19 different enzymes and one control (a microcupule containing no enzyme substrate). Prior to analysis, enzyme extracts were prepared by mixing 5 g from each poultry litter + yard trimming samples with 50 ml sterile distilled water. The solution was blended using a stomacher (blender), allowed to settle for 10 min, and then the supernatant was

TABLE 1.
Incubation conditions for microbial enumeration

Microbial Group	Culture Medium	Incubation Temperature (°C)	Incubation Time (days)
Total aerobic heterotrophs	Plate count agar	35–37	2
Actinomycetes	Starch casein medium	25	15
Fungi	Corn meal-rose bengal agar containing streptomycin and penicillin	25–26	7
Ammonium oxidizers	Ammonium sulphate-calcium carbonate-agar medium	24–26	21
Nitrite oxidizers	Sodium nitrite-calcium carbonate-agar medium	24–26	21
Denitrifying bacteria	Glycerol-peptone-KNO_3 liquid medium	24–26	7
Fecal coliforms	Difco m FC agar	35°C for 4–5 h; 44.5°C for 20 h	1

used for enzyme analysis. An aliquot (65 µl) of the extract supernate was dispensed into each of the 20 microcupules. The 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, Inc., Marcy-l' Tolle, France) were added to all microcupules. Following a waiting period for color development of about 5 min, a numerical value of 1–5 was assigned to each microcupule according to the color chart provided by the manufacturer. For the purposes of this study, the results were reported as reactions of low intensity (value of 1), moderate intensity (values of 2–4), and high intensity (value of 5).

Values for each location (top, middle, and bottom) were the result of a mean of three replicate piles. Pearson product-moment correlation coefficients were computed to show relationship between different microbial populations and enzyme activities. All statistical analyses were performed using SYSTAT statistical computing package (SYSTAT version 9.0).

Results and Discussion

Progression of the Composting Process

The peak temperatures occurred in the top and middle locations of the piles (72 and 70°C, respectively) were significantly higher than those recorded at the bottom (60°C) location. However, the time required to return to ambient temperature was similar in all three locations (Figure 1). Stentiford (1996) suggested that temperatures greater than 55°C maximize sanitization, that 45 to 55°C maximize the biodegradation rate, and that 35 to 40°C maximize microbial diversity

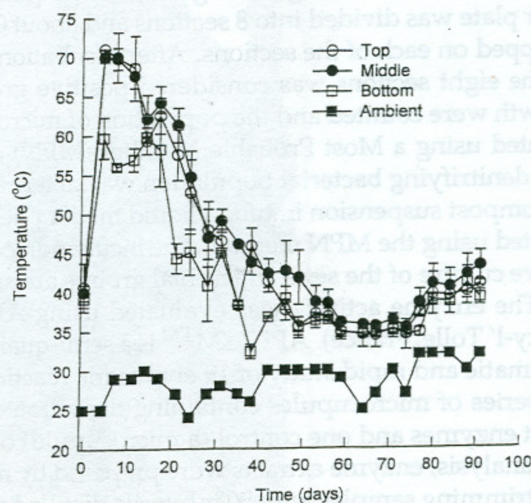

Figure 1. Pile and air temperature changes during composting.

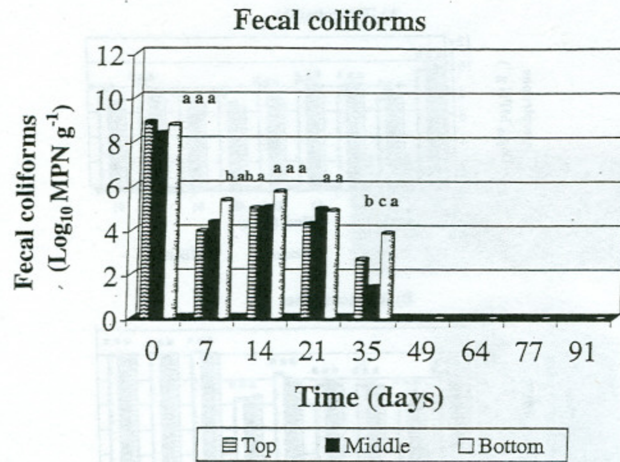

Figure 2. Log_{10} MPN counts of fecal coliform bacteria at different stages of composting. For each location, at each composting time, values represented by histograms with the same letter are not significantly different (p= 0.05).

in the composting process. In the present investigation, temperatures greater than 55°C were reached in all three locations of the forced-aeration piles. This temperature level, however, was not sustained at the bottom location (Figure 1). The temperature at the bottom of the forced aeration piles dropped to 44°C by day 21, while that of the top and middle maintained temperatures greater than 55°C for 21 days. Nevertheless, the sanitization at the bottom was comparable to the top and middle locations. Sanitization of the poultry litter + yard trimmings compost, as shown by the disappearance of fecal coliforms at all three locations (top, middle, and bottom) of the forced-aeration piles, was achieved within 49 days of composting (Figure 2). Our results indicate the thermophilic active composting process in the piles was capable of destroying populations of fecal coliform bacteria. For compost sanitization, Strauch (1987) recommended a limit of 5×10^2 CFU g^{-1} (fresh weight) for fecal coliforms. In our experiment, no detectable amounts of fecal coliforms were found in the poultry litter + yard trimming samples after 49 days of composting under forced aeration system. Thus, no secondary contamination or regrowth was detected after day 49 at all three locations.

Microbial Characteristics of the Composting Process

Total Aerobic Heterotrophs, Actinomycetes, and Fungi

Differences in pile temperatures at different locations of the forced-aeration piles did not significantly affect the changes in numbers of total aerobic heterotrophs, actinomycetes, and fungi of the poultry litter + yard trimmings compost (Figures 1 and 3). The population of these three microbial groups was similar in all three locations of the forced aeration piles during composting (Figure 3). The total aerobic heterotroph counts were highest (9.4–9.8 Log_{10} MPN g^{-1}) at the beginning of composting (Figure 3a). By day 7, their numbers dropped and then increased gradually as pile temperatures started to decline (Figures 1 and 3a). The actinomycete numbers dropped as the temperature began to peak and then increased as the pile temperatures started to decline (Figure 3b). Most of the fungi were eliminated at temperatures above 50° C (Figure 3c), and were recovered later when composting temperatures are moderate (<45° C). By the end of composting, the population of actinomycetes and fungi exceeded that of their initial numbers (Figures 3b and 3c). The exact role of fungi during composting is not clear. Although some of the fungi might be involved in the decomposition of cellulose and lignocellulosic compounds of the poultry litter + yard trimmings mixture, and provided a more readily available C for the bacteria. The increase in fungal population by the

end of composting might be due to the presence of cellulose and lignin. De Bertoldi *et al.* (1983) reported that fungi normally increase the remaining substrates are predominantly cellulose and lignin, which normally occurs during the process of cooling. The actinomycetes are thus well placed to exploit the compost environment as the piles cool in the immediate post peak heat phase. In the present study, the actinomycetes in the poultry litter + yard trimming piles became so numerous that their hyphal strands caused the compost surface to take on a gray or a white appearance during the cooling stage. Epstein (1997) reported that during the cooling stage of composting, actinomycetes actively degrade hemicellulose in the compost.

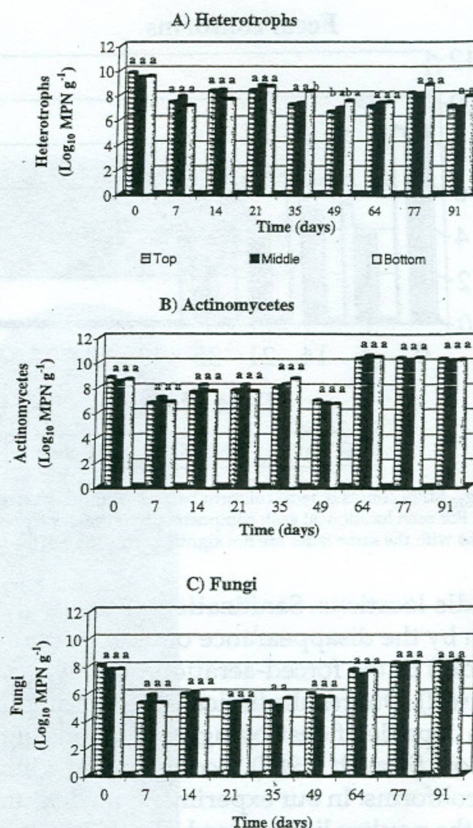

Figure 3. Log_{10} MPN counts of total aerobic heterotrophs, actinomycetes, and fungi at different stages of composting. For each location, at each composting time, values represented by histograms with the same letter are not significantly different (p= 0.05).

Ammonium and Nitrite Oxidizing Bacteria, and Denitrifying Bacteria

The ammonium- and nitrite-oxidizers were maintained at high population sizes during the decomposition process (Figures 4a and 4b), suggesting a rapid oxidation of NH_4^+-N to NO_3^--N. Ammonium oxidizing bacteria as dominated by *Nitrosomonas spp.* (Focht and Verstraete 1977; Wild 1988), and nitrite oxidizing bacteria as represented by *Nitrobacter* spp. (Wild 1988) were maintained at about 8 Log_{10} MPN g^{-1} during composting. The population sizes of denitrifying bacteria are highest at the beginning of composting (Figure 4c). The establishment of a large population of denitrifying bacteria suggests that some anaerobic microhabitats had existed within the piles. These microhabitats could have developed within the piles partially due to the initial high water content (65%) of the piles and partial-

TABLE 2
General properties of poultry+yard trimmings

Properties	Yard Trimmings	Chicken Litter	Chicken Litter And Yard Trimmings ('1:2 ratio)
Total organic matter (g kg^{-1})	517	509	566
Total C (g kg^{-1})	483	491	434
Total N (g kg^{-1})	5.1	33.9	14
C:N ratio	95:1	14:1	31:1
Total P (g kg^{-1})	ND	ND	10.9
Total K (g kg^{-1})	ND	ND	10.3
pH	ND	8.3	7.2

ND= not determined

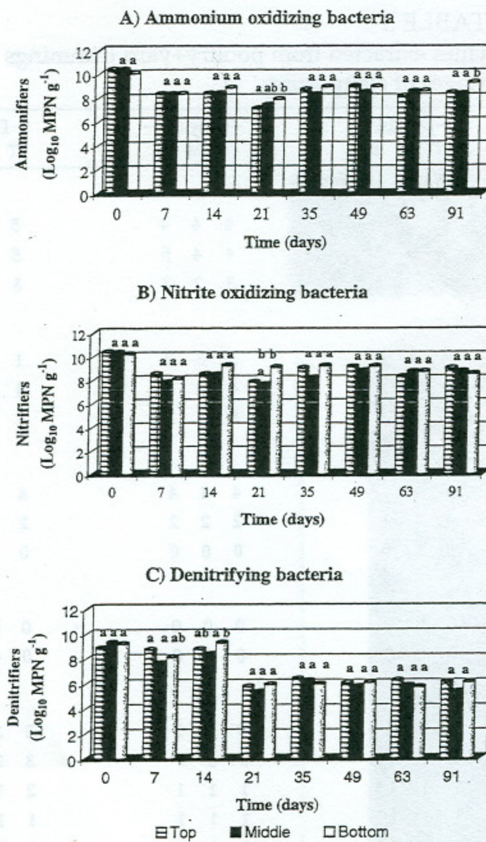

Figure 4. Log_{10} MPN counts of ammonium and nitrite oxidizing, and denitrifying bacteria at different stages of composting. For each location, at each composting time, values represented by histograms with the same letter are not significantly different (p= 0.05).

ly because of the rich contents of organic matter and nitrogen present in the poultry litter + yard trimming compost (Table 2), which promote microbial activity to the extent of causing depletion in O_2 content in isolated pockets within the piles. Moreover, some species of denitrifying bacteria may be facultative and grow aerobically (Firestone 1982). Some microbial genera capable of denitrification are *Bacillus, Flavobacterium,* and *Pseudomonas*. These microbial groups have been found not only in soils (Firestone 1982), but also during composting (Epstein 1997). It seems that as the composting proceeded, the denitrifying bacteria became smaller (Figure 4c), indicating that very little denitrification took place once the air was blown in the pile on a regular weekly basis. The air diffusion, oxygen availability, and redox potential should be measured in future studies to understand the dynamics of denitrifying bacterial population during composting.

Evolution of Enzyme Activities During Composting

Microbes in the compost pile cannot directly metabolize the insoluble particles of organic matter. Rather, they produce hydrolytic extracellular enzymes to depolymerize the larger compounds to smaller fragments that are water-soluble (Hankin *et al.* 1976). At this point, microbes transport the substrates across the cytoplasmic membrane to complete the degradation process (Tabatabai 1994). Analysis of API ZYM™ testing of poultry litter + yard trimmings compost showed an overall increase in diversity and relative abundance of enzymes present as composting progressed (Table 3). The alkaline and acid phosphatase and leucine amino-peptidase activities were high at the beginning of composting, reaching maximum activity at day 14. The high activity of these enzymes was probably due to the high organic matter and nutrient content of the poultry litter + yard trimmings (Table 2), which stimulated growth of total aerobic bacteria and subsequent phosphatase and peptidase synthesis. Phosphatases are the enzymes that catalyze the hydrolysis of organic P esters to orthophosphate (Tabatabai 1994), whereas leucine-amino peptidase, is an enzyme that catalyzes the hydrolysis of proteins into individual amino acids (Wagner and Wold 1999). These are key reactions in soils and composts (Garcia *et al.* 1992b; Tabatabai 1994; Vuorinen 1999). Phosphohydrolase showed a moderate level of activity at the beginning of composting and re-

TABLE 3
Relative activity of extracellular enzymes extracted from poultry+yard trimmings at different stages of composting

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

T=top; M=middle; B=bottom. Value of 1= low activity; 2–4=moderate activity; value of 5= high activity.

mained at this level until the end of composting (Table 3). Overall, moderate levels of lipase, esterase-lipase, and esterase activity were observed at the beginning 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 (Table 3). Cystine amino peptidase, chymotrypsin, and trypsin showed no evidence of activity during the entire period of composting (Table 3). 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 3). Higher values of β-glucosidase activity were observed the beginning of composting (Table 3), where the populations of fungi and actinomycetes were higher (Figure 3b and 3c). Hayes (1986) has found fungi to be the primary source of β-glucosidase in soil.

In the present investigation, β-glucosidase, which is involved in the hydrolysis of cellobiose, showed significant positive correlation with fungal (r = 0.98) and actinomycete (r = 0.77) populations. However, correlation between β-glucosidase and fungi was higher (Table 4). Fungi and actinomycete populations also had significant positive correlation with β-galactosidase (enzyme involved in the hydrolysis of lactose)

TABLE 4
Coefficient of determination (r^2) for the relationship between microbial parameters and enzyme activities

Enzyme	Hetero	Ammoni	Nitri	Denitri	Actino	Fungi	Coliforms
Phosphatases							
Alkaline phosphatase	-0.72**	-0.46	-0.46	-0.07	-0.21	-0.49	-0.23
Acid phosphatase	-0.64*	-0.31	-0.29	-0.50	0.39	0.13	-0.53
Phosphohydrolase	-0.02	0.21	0.25	-0.18	0.30	0.44	-0.13
Esterases							
Lipase	0.16	-0.16	-0.17	0.44	-0.65*	-0.79**	0.30
Esterase-lipase	-0.80**	-0.89**	-0.90***	-0.53	0.25	-0.25	-0.73**
Esterase	-0.58*	-0.55	-0.59*	-0.98***	0.95***	0.69*	-0.94***
Amino-peptidases							
Leucine amino-peptidase	-0.50	-0.53	-0.52	-0.28	0.14	-0.16	-0.44
Valine amino-peptidase	-0.58*	-0.55	-0.59*	-0.98***	0.95***	0.67*	-0.94***
Cystine amino-peptidase	0.00	0.00	0.00		0.00	0.00	0.00
Proteases							
Chymotrypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycosyl-hydrolases							
α-galactosidase	-0.58*	-0.55	-0.59*	-0.98***	0.95***	0.69*	-0.94***
β-glucosidase	0.16	0.31	0.26	-0.51	0.77**	0.98***	-0.28
n-acetyl-β-glucosaminidase	-0.45	-0.08	-0.20	-0.38	0.42	0.33	-0.42
α-glucosidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-galactosidase	0.82**	0.94***	0.94***	0.62*	-0.34	0.19	0.81**
β-glucuronidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00
α-mannosidase	-0.47	-0.10	-0.28	-0.49	0.39	0.32	-0.42
α-fucosidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Hetero=total aerobic heterotrophs; Ammoni= ammonium oxidizing bacteria; Nitri=nitrite oxidizing bacteria; Denitri=denitrifying bacteria; Actino= actinomycetes; Coliforms= total coliform bacteria.

(Table 4). Fungi and actinomycetes are microorganisms involved in the decomposition of polymeric substances such as cellulose, hemicellulose, and lignin (Epstein 1997). In the present investigation, the population of fungi and actinomycetes were positively correlated with esterase, valine amino-peptidase, α-galactosidase, β-glucosidase, and lipase. Of all 19 enzymes, α-galactosidase (another enzyme involved in the hydrolysis of lactose) had the most significant positive correlation with microbial populations, such as total aerobic heterotrophs, ammonium and nitrite oxidizing bacteria, denitrifying bacteria, and fecal coliforms (Table 4).

API ZYM^{TM} characterization and quantification of enzymatic activities of poultry litter + yard trimmings reflected the dynamics of the composting process and may provide information about the biochemical fertility of the composted product. 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 at different stages of composting, since these enzymes are substrate-inducible enzymes.

Summary

The population size of different microbial groups was not a limiting factor in this composting process as the microorganisms in the poultry litter + yard trimmings compost are greatly in abundance. The population size of total aerobic heterotrophs,

actinomycetes, fungi, ammonium and nitrite oxidizers, and denitrifying bacteria were reduced by high temperature, but multiplied rapidly during the cooling phase. The numbers of fecal coliform bacteria were significantly reduced, suggesting that the poultry litter + yard trimmings was relatively free of such potential pathogens after forced aeration composting. Extracellular enzyme activities generally increased as composting progressed, indicating that the microorganisms are able to effectively and rapidly synthesize enzymes required for degradation of polymeric substances in the poultry litter + yard trimmings such as cellulose, hemicellulose, and lignin. Increases in the numbers of fungi and actinomycetes (microorganisms actively involved in the degradation of cellulose, hemicellulose and lignin) corresponded to increases in esterase, valine amino-peptidase, α-galactosidase, β- glucosidase, and lipase activities. Results of this study represented a combined activity of a wide succession of environments in the compost piles as one microbial group/enzyme overlapped the other and each emerged gradually as a result of continual change in temperature as well as moisture content, O_2 and CO_2 level, and progressive breakdown of complex chemical compounds to simple ones.

Acknowledgements

The authors thank the Central Matching Fund (Post-doctoral Fellowship) of City University of Hong Kong, and the University Grant Council (UGC) for the financial assistance provided for this project. This paper was presented at the International Conference on Microbiology of Composting and Other Biodegradation Processes held in Innsbruck, Austria on September 18-20, 2000.

References

APHA (American Public Health Association), 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. APHA, Washington D.C.

Allison LE., 1965. Organic carbon. In: Black, C.A., D.D. Evans, J.L. White, L.E. Ensiminger, F.E. Clark and R.C. Dinauer. (eds.). Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. SSSA, Madison, Wisconsin, pp. 1367–1378.

Atkinson, C.F., D.D. Jones and J.J. Gauthier. 1996. Biodegradability and microbial activities during composting of poultry litter. *Poultry Sci.*, 75: 608–617.

Ayuso, M., T. Hernandez, C. Garcia and J.A. Pascual. 1996. Biochemical and chemical-structural characterization of different organic materials used as manures. *Biores. Technol.*, 57: 201–207.

Beffa, T., M. Blanc, L. Marilley, J.L. Fischer, P.F. Lyin and M. Aragno. 1996. Taxonomic and metabolic microbial diversity during composting. In De Bertoldi M., P. Sequi, B. Lemmes and T. Papi. (eds.). *The Science of Composting*. Part I. Chapman and Hall, London, pp. 149–161.

Bremmer, J.M. 1996. Nitrogen-Total. In: Sparks, D.L. (ed.). Methods of Soil Analysis. Part 3– Chemical Methods. SSSA, Madison, Wisconsin, pp. 1085–1121.

Davis, C.L., S.A. Hinch, C.J. Donkin, and P.J. Germishuizen. 1992. Changes in microbial population numbers during composting of pine bark. *Biores. Technol.*, 39: 85–92.

De Bertoldi, M., Vallini, G., Pera, A. 1983. The Biology of Composting: A Review. *Waste Manage. Res.*, 1: 167–176.

De Bertoldi, M., Vallini, G., Pera, A. 1985. Technological aspects of composting including modeling and microbiology. *In*: Gasser, J.K.R. (ed.). *Composting of Agricultural and Other Wastes*. Elsevier Applied Science Publishers, New York, pp. 27–41.

Diaz-Burgos, M.A., B. Ceccanti and A. Polo. 1993. Monitoring biochemical activity during sewage sludge composting. *Biol Fertil Soils*, 16: 145–150.

Diaz-Ravina, M., M.J. Acea and T. Carballas. 1989. Microbiological characteization of four

composted urban refuses. *Biol. Wastes,* 30: 89–100.
Epstein E., 1997. The Science of Composting. Technomic Publishing Company. Lancaster, Pennsylvania, 487 p.
Epstein, E., G.B. Willson, W.W. Burge, D.C. Mullen and N.K. Enkiri. 1976. A forced aeration system for composting wastewater sludge. *J. Wat. Pollut. Contr. Fed.,* 48: 688–694.
Firestone, M.K., 1982. Biological denitrification. *In:* Stevenson F.J. (ed.). *Nitrogen in Agricultural Soils.* ASA, CSSA, and SSSA, Inc. Madison, Wisconsin, pp. 289–326.
Focht, D.D. and W. Verstraete. 1977. Biochemical ecology of nitrification and denitrification. *Adv. Microbiol. Ecol.,* 1: 135–214.
Furhmann, J.J.C. 1999. Microbial metabolism. *In:* Sylvia, D.M., J.J.C, Furhmann, P.G. Hartel and D.A. Zuberer. (eds.). *Principles and Applications of Soil Microbiology.* Prentice Hall, New Jersey, pp. 189–217.
Garcia, C., T. Hernandez, F. Costa, and M. Ayuso. 1992a. Evaluation of maturity of municipal waste compost using simple chemical parameters. *Commun. Soil Sci. Plant Anal.,* 23: 1501–1512.
Garcia, C., T. Hernandez, F. Costa, B. Ceccati, and C. Ciardi. 1992b. Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes. *Can. J. Soil Sci.,* 72: 243–253.
Godden, B., M. Penninckx, A. Perard and R. Lannoye. 1983. Evolution of enzyme activities during composting of cattle manure. Euro. J. Appl. Microbiol. Biotechnol., 17: 306–310.
Golueke, C.G. 1992. Bacteriology of composting. *BioCycle,* 33: 55–57.
Hankin, L.H., R.P. Poincelot, and S.L. Anagnostakis. 1976. Microorganisms from composting leaves: Ability to produce extracellar enzymes. *Microb Ecol.,* 2: 296–308.
Harada, Y. A. Inoko, M. Tadaki, and T. Izawa. 1981. Maturing process of city refuse compost during piling. *Soil Sci and Plant Nutr.,* 27: 357–364.
Hayes, M.B.H., 1986. Soil organic matter extraction, fractionation, and effects on soil structure. In: Chen, Y. Avnimelech Y. (Eds.), The Role of Organic Matter in Modern Agriculture. Martinus Nijhoff, Boston, pp. 183–208.
Inbar, Y., Y. Hadar and Y. Chen. 1993. Recycling of cattle manure: The composting process and characterization of maturity. *J. Environ Qual.,* 22: 857–863.
Namkoong, W., E.Y. Hwangm J.G. Cheong and J.Y. Choi. 1999. A comparative evaluation of maturity parameters for food waste composting. *Compost Sci. Util.,* 7: 55–62.
Nannipieri, P., S. Greco and B. Ceccanti. 1990. Ecological significance of biological activity in soil. *In:* Bollag J.M. and G. Stotzky. (eds.) *Soil Biochemistry.* Marcel and Dekker, New York. Pp. 293–355.
Nodar, R., M.J. Acea and T. Carballas. 1990. Microbial populations of poultry sawdust litter. *Biol Wastes,* 33: 295–306.
Speir, T.W. and D.J. Ross. 1978. Soil phosphatase and sulphatase. *In:* Burns, R.G. (ed.). *Soil Enzymes.* Academic Press, New York, pp. 197–250.
Stentiford, E.I. 1996. Composting control: Principles and practice. In: De Bertoldi, M., P. Sequi, B., Lemmes and T. Papi. (eds.). *The Science of Composting. Part I.* Blackie Academic and Professional, Chapman and Hall, London, pp. 224–252.
Strauch, D., 1987. Microbiological specifications of disinfected compost. In: De Bertoldi, M., M.P. Ferranti, P. L'Hermite and F. Zucconi, F. (eds). *Compost: Production, Quality and Use.* Elsevier Science, London. pp. 210–229.
Strom, P.F. 1985. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Appl. Environ. Microbiol.,* 50: 899–905.
Tabatabai, M.A., 1994. Soil enzymes. *In:* Weaver, R.W., J.S. Angle and P.S. Bottomley. (eds.). *Methods of Soil Analysis. Part 2- Microbiological and Biochemical Properties.* Book Series No. 5. SSSA, Inc., Madison, Wisconsin, pp. 775–833.
Tiedje, J.M., 1994. Denitrifiers. *In:* Weaver, R.W., J.S. Angle, P.S. Bottomley. (eds.). *Methods of Soil Analysis. Part 2- Microbiological and Biochemical Properties.* Book Series No. 5. SSSA, Inc., Madison, Wisconsin, pp. 245–267.
Tiquia, S.M. and N.F.Y. Tam. 1999. Chemical parameters for maturity determination of pig manure disposed from the pig-on-litter (POL) system in Hong Kong. *In: Proceedings of the Conference on Manure Management '99.* Saskatoon, Saskatchewan pp. 449–463.
Tiquia, S.M. and N.F.Y. Tam. 2000a. Fate of nitrogen during composting of chicken litter.

Environ Pollut., 110: 535–541.

Tiquia, S.M. and N.F.Y. Tam. 2000b. Co-composting of spent pig litter and sludge with forced-aeration. *Biores. Technol.*, 72: 1–7.

Tiquia, S.M., N.F.Y. Tam and I.J. Hodgkiss. 1996. Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. *Biores. Technol.*, 55: 201–206.

Tiquia, S.M., N.F.Y. Tam and I.J. Hodgkiss. 1998. *Salmonella* elimination during composting of spent pig litter. *Biores. Technol.*, 63: 193–196.

Vuorinen, A.H. 1999. Phosphatases in horse and chicken manure composts. *Compost Sci. Util.*, 7, 47–54.

Vuorinen, A.H. 2000. Effect of bulking agent on acid and alkaline phosphomonoesterase and ?-D-glucosidase activities during manure composting. *Biores. Technol.*, 75: 113–138.

Wan, J.H.C. 2000. *Co-composting of Chicken manure and Green Waste under Turned and Forced Aeration Systems*. M. Phil. Thesis. City University of Hong Kong, Kowloon, Hong Kong, P.R. China.

Wagner, H.H. and D.C. Wolf. 1999. Carbon transformations and soil organic matter decomposition. *In*: Sylvia, D.M., J.J.C, Furhmann, P.G. Hartel and D.A. Zuberer. (eds.). *Principles and Applications of Soil Microbiology*. Prentice Hall, New Jersey, pp. 218–258.

Warman, P.R. 1999. Evaluation of seed germination and growth test for assessing compost maturity. *Compost Sci. Util.*, 7: 33–37.

Wild, A., 1988. *Russell's Soil Conditions and Plant Growth. 11th Edition.* Longman Group UK Ltd., Essex, 991 p.

Woomer, P.L. 1994. Most probable number counts In: Weaver, R.W., J.S. Angle and P.S. Bottomley. (eds.). *Methods of Soil Analysis Part 2. Microbiological and Biochemical Properties.* SSSA, Madison, Wisconsin, pp. 60–79.

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