MICROBIOLOGY OF COMPOSTING EUCALYPT BARK

by

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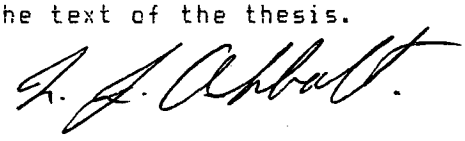

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Abbreviations

° - degree centigrade

mA - milliamphere

AOV - analysis of variance

ATCC - American Type Culture Collection

ATP - adenosine triphosphate

b - beta

B. - *Bacillus*

C - carbon

CFU - colony forming unit(s)

C_1 - exoglucanase

cm - centimeter

CMC - carboxymethylcellulose

CMCase - carboxymethylcellulase

C:N - carbon to nitrogen ratio

d - day(s)

m-DAP - meso-2,6-diaminopimelic acid

df - degrees of freedom

E. - *Eucalyptus*

ed. - editer

F - variance ratio

g - gram

% (G+C) or % GC - percent guanine plus cytosine

GC - gas chromatography

µg - microgram

h - hour

IBDU - isobutylidene diurea

kg - kilogram

L - litre

LigA - lignin agar

LSD - least significant difference

M - molar

m - meter

m^2 - square meter

m^3 - cubic meter

m.c. - moisture content(s)

mL - millilitre

mm - millimeter

µm - micron

min - minute

MS - mean square

N - nitrogen

NO_x - nitrogen oxide(s)

nov. spec. - novel species

ODW - oven dry (105°) weight

p - para

$p <$ - probability less than

PCA - principal component anaylysis

pers. comm. - personal communication

PVC - polyvinyl chloride

PVP - Polyvinylpolypyrrolidone

q or quinone - para-benzoquinone

r - correlation coefficient

RBBR - Remazol brilliant blue r dye

SEM - scanning electron microscopy/microscope

S_{sm} - simple matching coefficient of Sokal and Michener (1958)

sp(p). - species

TA - tannin agar

TB - tryptone liquid medium

T_{opt} - temperature optimum

T_{max} - temperature maximum

T_{min} - termperature minimun

TSA - tryptic soy agar

kV - kilovolt

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Abstract

The main aims of this study were to examine the optimal conditions for composting and factors influencing microbiological changes during the composting of *Eucalyptus* bark in the production of a plant growth medium.

A bench-scale composter was designed to provide strict control over air composition, moisture content, temperature and mixing. The composter consisted of six 4-L capacity gas-tight units of PVC plastic, each of which was provided with a mixing paddle coupled to a common drive. A natural temperature rise was simulated by having the units immersed in a water bath, with the temperature increased at rates consistent with those observed in large-scale compost heaps. This provided a comparatively inexpensive versatile system, with rates of CO_2 and CH_4 production and O_2 consumption automatically monitored by gas chromatography. Levels of volatilized NH_3 and nitrogen oxides (NO_x) were manually monitored from acid traps. The reproducibility of the system was as good as the best reported.

Optimal conditions for the bench-scale composting of eucalypt bark were considered to be a temperature of 55°, an aeration rate of at least 20 mL min^{-1} and an initial C:N ratio of 25-30, depending on the availability of nitrogen. Nitrogen, in decreasing order of availability, was provided in the form of urea, isobutylidene diurea (IBDU), fish wastes or sewage cake. Both respiratory activity and nitrogen loss data were considered to be of value in determining the economic as well as the microbiological optimal C:N ratio of bark compost. No further amendment other than water (giving an initial moisture content of 114% d.w. basis) was found to be necessary.

Ammonification and NH_3 volatilization occurred during the first sixteen days of composting while volatilization of NO_x was substantial during times of undesirable nitrogen availability. Delaying ammonification in the urea amended

composts (by either the addition of quinone or urea's replacement with IBDU) increased ammonia volatilization. Net nitrification followed peak net ammonification, but nitrate appeared to be produced largely chemically rather than biologically. Volatilization of NO_x was greatest from compost prepared using sewage cake.

Up to five peaks of CO_2 output were observed over a 30 day run, three occurring during the transition to thermophilic conditions and one or two peaks occurring during a plateau temperature of 55°. The predominant flora comprised *Bacillus spp.* during the mesophilic and early thermophilic phases (*B.brevis* and *B.sphaericus* followed by *B.circulans* and *B.brevis* then *B.circulans, B.sphaericus or B.stearothermophilus*). *Bacillus spp.* continued to predominate throughout the composting of sewage-bark and most of the fish-bark composts. However, actinomycetes (*Streptomyces spp.* and *Thermomonospora spp.*) and coryneforms predominated at latter stages of urea-bark composts. Strictly anaerobic bacteria appeared to be unimportant during the composting of bark. The predominant flora isolated during the mesophilic phase were not inhibited by compost components of any age, while members of the climax flora were inhibited by fresh compost components. Cellulase activity was not correlated with peaks in CO_2 output, but showed a slow increase or decrease, depending on the initial C:N ratio, over 30 days composting. However, lipase activity correlated with the peak in CO_2 output at about day sixteen in a fish-bark compost.

The identification of thermophilic *Bacillus spp.* was aided by a study of their esterase mobilities and the use of numerical taxonomy. Phenolic compounds present after 30 days composting were phytotoxic. However, levels of residual ammonium could largely account for the phytotoxicity exhibited by water extracts from most of these composts. Eucalypt bark composts had a higher density than pine-bark composts or peat moss, but were as good as or better than the latter materials with regard to their water characteristics and particle-size distribution.

1 - LITERATURE REVIEW

1.1 The Microbiology of Composting

1.1.1 Historical

Composting, defined as the microbial degradation of organic solid material under controlled conditions to a state in which it will not adversely affect the environment (Golueke, 1977), has been practiced since biblical times (Crawford, 1983). It was not until the turn of the century that the microbial transformation of plant and animal residues to a uniform, dark coloured mass, known as humus, was first studied (Hebert, 1893; Deherain and Demoussy, 1896). However, these initial studies on the microbiology and chemistry of composting failed to show any correlation between the changing microbial population of the compost and the chemical transformations involved (Russell and Ritchards, 1917; Ruschmann, 1928).

The 1920's saw the development of large-scale composting processes such as the Indore process in India and the Beccari process in Italy. These processes were largely anaerobic, resulting in problems related to putrefaction and survival of pathogens (due to the low temperatures achieved). It was not until the substantial work of Waksman and co-workers, on the physical conditions for, and microbial successions in, composting straw , that a clearer understanding of the microbiology of composting began to evolve (Waksman, *et.al.*, 1939a, 1939b). The results of this work showed that the media (favouring eubacteria) and incubation temperatures (favouring mesophiles) used by previous workers, severely limited the growth of the predominant compost flora, which comprised of mesophilic actinomycetes, fungi and, most importantly, their thermophilic counterparts. They also demonstrated that the major chemical changes occurred during the

thermophilic stage of composting (Waksman and Gerretsen, 1931; Waksman, *et.al.*, 1939b). Despite this and other more recent work, some workers have persisted with inappropriate temperatures for the isolation of compost microflora. For example, in an attempt to include both mesophilic and thermophilic microorganisms on the same isolation medium Deschamps, *et.al.* (1980a) used a single incubation temperature of 37°.

The need for nitrogen, phosphorus and potassium and the use of soil as inoculum in the windrow composting of plant material was noted by Rodale (1945). Realizing a potential for organic fertiliser, several commercial companies developed mechanized composting systems, making use of forced aeration to reduce the composting time. Examples include the VAM (Vuil-Arvoer-Mast) process in Holland, the Dano process in Denmark and the Frazer process in the United States (Golueke, 1977). All of these systems however, were developed by engineers with little knowledge of the microbiology and biochemistry essential to the process. It was not until the 1950's that the necessary research was undertaken at the University of California, and later by the U.S. Public Health Service. The publications of Golueke, *et.al.* (1954), Gotaas (1956), Hart (1970), McGauhey (1971), and Jeris and Regan (1973a) describe this work in detail. In brief, forced aeration and special additives were found to be unnecessary in ordinary windrow composting. Oxygen diffusion, aided by occasional turning, was shown to provide sufficient aeration. Given organic material with sufficiently available nutrients, no added chemicals were beneficial and special inocula were shown to be worthless.

Studies on the composting of straw, municipal wastes and sewage sludge have been reviewed by Updegraff (1972) and Finstein and Morris (1975). Only recently have microbial studies turned to aspects of bark composting (Nordstrom, 1974; Ross and Corden, 1975; Grant, 1976; Bagstam, 1977, 1978; Toskov *et.al.*, 1979; Deschamps, 1982; Solbraa, *et.al.*, 1983; Solbraa, 1984). The more recent findings and general aspects of composting relevant to the microbiology of bark composting are reviewed here.

1.1.2 Microbial Successions in Compost

Composting is a slow process depending on a sequential change in the microbial flora. The precise nature of the succession and the number of organisms present at each stage depends on the nature of the composting material and upon the preceding organisms in the succession (Crawford, 1983). The changes in temperature during aerobic composting have a fundamental influence on these microbial successions, first detailed by Waksman (1939a, 1939b). Three stages may be delineated; an initial mesophilic stage during which there is a rapid temperature rise over a few days, a thermophilic stage of at least ten days (or considerably longer if the material is not finely ground) and finally, following a decline in temperature, a maturing mesophilic stage of several weeks (Golueke, 1977).

The initial rapid temperature increase in moist material is considered to be due to bacterial activity (Dye and Rothbaum, 1964), with acid producers and Gram positive cocci predominating in hay compost (Gray and Biddlestone, 1976; Fesenstein, *et.al.*, 1965). During the composting of spruce bark, Bagstam (1978) found that after two days bacteria represented about 70% of the "total" estimated microbial numbers of 8.3×10^{10} organisms g^{-1}, and 90-100% during the later thermophilic stage. Fungi on the other hand are primarily found in the cooler outer regions of compost and are not present above 65° (Alexander, 1977). Furthermore, Finstein and Morris (1975) suggested that the rapid temperature rise and high temperature of the first two composting stages were not conducive to fungal growth.

The bacterial flora of various composts has been found to change from one dominated at low temperatures by species of *Achromobacter* (now *Alcalagenes*), *Bacillus, Flavobacterium, Micrococcus and Pseudomonas* (Anon, 1955; Niese, 1959) to one dominated by sporeforming *Streptomyces* and *Bacillus* at 45-50° (Lacey, 1973; Strom, 1978) and *Thermoactinomyces, Micropolyspora, Thermomonospora* and *Bacillus* species up to 65° (Lacey, 1973; Stutzenberger, *et.al.*, 1970; Strom, 1978) with *Bacillus* species at

the higher temperatures (Niese, 1959).

Despite the predominance of bacteria other that actinomycetes during the thermophilic stage of composting, few studies have been made on the species involved. Strom (1978) , however, in reviewing the role of bacteria in composting municipal wastes, identified fifteen species, ten of which belong to the genus *Bacillus*. In decreasing order of frequency he isolated *B. circulans, B.stearothermophilus, B. coagulans, B. licheniforma, B. brevis, B. sphaericus,* and *B. subtilis*. *Bacillus* dominated all of his samples taken at compost temperatures from 50° to 65°, although *Streptomyces, Thermoactinomyces*, two genera of nonsporing bacteria, and the fungus *Aspergillus fumigatus* were also isolated.

Information on the presence of anaerobic bacteria during composting is very limited. With high rates of microbial activity, particularly during the first week of composting, anaerobic micro-sites would be expected despite active aeration. Glathe (1934) demonstrated the presence of *Clostridium* in compost, however, few workers have since identified the anaerobes in compost. Gregory, *et.al.* (1963) found no anaerobic bacterium in moulding hay until after two days of composting, and then "total" estimated numbers of anaerobes were at least three orders of magnitude below the "total" aerobes (greater than 10^8 organisms per gram). Muller and Ritter (1972) similarly found "total" estimated numbers of anaerobes to be 1-2 orders of magnitude below those of "total" aerobes in mushroom compost. At temperatures above 70° they demonstrated that the anaerobic flora primarily comprised of anaerobic spore-forming bacteria.

Thermophilic fungi have been isolated from a number of high temperature environments including mushroom compost (Fergus, 1964), wheat straw compost (Chang and Hudson, 1967), municipal compost (Stutzenberger, *et.al.*, 1970; Kane and Mullins, 1973; Millner, *et.al.*, 1977) and self-heated bark/woodchip piles (Tansey, 1971; Smith and Ofosu-Asiedu, 1972; Flannigan and Sagoo, 1977). The relative importance of fungi during thermophilic composting is likely to be slight given their poor heat tolerance

(Hulme and Stranks, 1976; Ogundero and Oso, 1980) and low relative abundance (Hankin, *et.al.*, 1976; Bagstam, 1978), although at temperatures below 50° fungi are abundant with the thermoduric *A. fumigatus* being the most common (Flannigan and Sagoo, 1977; Moubasher, *et.al.*, 1982), being isolated (at 45°) even from antarctic soils (Ellis, 1980).

Little information exists on the microbial recolonization during the temperature descent and maturing stage of composting (Finstein and Morris, 1975). Recolonization of wheat straw compost by fungi was demonstrated by Chang and Hudson (1967) to depend on many factors in addition to the maximum temperature reached and its duration. Hankin, *et.al.* (1976) found that "total" estimated numbers of bacteria (other than actinomycetes) increased after the thermophilic stage in a leaf compost as the temperature dropped from 50° to 10°, while Bagstam (1978) observed an increase in actinomycetes and a decrease of other bacteria as the temperature dropped from 45° to a steady 20° in a bark compost. Van Klopotek (1962) studied both fungal and bacterial recolonization of compost and found that "numbers" of fungi increased with time with "total" counts of actinomycetes and fungi being greater than those found in rich soils. The importance of this active mesophilic flora with regards to the control of plant pathogens (Hoitink, 1980) is reviewed later (1.1.3.3.).

Rodale, *et.al.* (1975) stated that nitrogen-fixing bacteria may invade maturing compost if sufficient lime, air, and humus are present. This seems unlikely considering the low C:N ratio of a well made compost (30 or below) and the presence of mineral nitrogen both of which inhibit nitrogenase induction or activity (Quispel, 1974; Golueke, 1977). Likewise, thermophilic nitrogen fixation during composting is unlikely. Also, thermophilic nitrogen-fixing bacteria have only been isolated from alkaline thermal springs (Wickstrom, 1984).

1.1.3 Biodegradation of Bark Components

Bark contains cellulose, hemicellulose and lignin in about equal amounts (Srivastava, 1964) and various extractable substances (Hillis, 1962). Bark also contains pectic substances (ca. 7% in hardwoods) much of which may be soluble and associated with starch, with the remainder being generally associated with the hemicellulose component (Kertesz, 1951). Mixed cultures of microorganisms are more efficient degraders of cellulose (Enebo, 1949; Alexander, 1977) and lignin (Sundman and Nase, 1971, 1972) than are pure cultures. Han and Callihan (1974) have indicated the importance of pretreating plant material for increased microbial decomposition. Work on the microbial decompostion of cellulose has been reviewed by Keilish, *et.al.* (1970) and Bisaria and Ghose (1981), that of lignin by Kirk (1971) and Crawford and Crawford (1980), phenolics by Dagley (1967) and wood by Rossell, *et.al.* (1973), and Bisaria and Ghose (1981).

Only recently have microbial studies been undertaken on aspects of bark biodegradation (Nordstrom, 1974; Updegraff and Grant, 1975; Wilhelm, 1976; Deschamps, 1982), despite the several hundreds of thousands of tonnes of bark composted every year in the U.S.A. and Europe (Hoitink, 1980; Mach, 1983).

1.1.3.1 Cellulose Biodegradation

Cellulose, a linear homopolymer of anhydroglucose units linked by b(1->4)-glucosidic bonds and is always associated with a variety of polysaccharides, (hemicellulose, starch and pectin) and also lignin (Bisaria and Ghose, 1981). The initiation and completion of cellulose degradation in compost material is attributable to fungi (Waksman, *et.al.*, 1939a; Burman, 1961; Updegraff, 1972) since they are known to have a maximum growth temperature below 55° (Cooney and Emerson, 1964; Romanelli, *et.al.*, 1975). It is the thermophilic stage of composting, however, that is considered the most important period for

cellulolysis (Poincelot, 1974). Hankin, *et.al.* (1976) in their study of leaf composts showed that the increase in fungal cellulase production occurred as the cellulose content had already begun to decline, during the middle to late thermophilic stage (54-51°) and they established that actinomycetes were the major agents of cellulolysis. Stutzenberger (1971, 1972) found that the actinomycete *Thermomonospora curvarta* was the major agent of cellulolysis in a municipal compost (containing about 50% cellulose). He showed that the maximum carboxylmethylcellulase (CMCase) activity of clarified compost extracts occurred consistently at pH 6.0 and at 65°. Contrary to the findings by Stutzenberger, the compost microflora of another municipal waste (Jeris and Regan, 1973a) or of hardwood or softwood bark composts (Cappaert *et.al.*, 1976a; Bagstam, 1978) exhibited optimal cellulose degradation between 40-50°. Recently Deschamps, *et.al.* (1980a) isolated nine cellulolytic strains of bacteria from bark compost and soil using an incubation temperature of 37°. Six strains were identified as *Bacillus spp.*, two as *Cellulomonas spp.*, and one as a *Pseudomonas sp.*. Numerous workers have shown a decrease in cellulolysis at temperatures above 65° (Obrist, 1966; Regan and Jeris, 1970) although anaerobic cellulolytic bacteria are known to be active up to about 75° (Waksman *et.al.*, 1939b). Chino, *et.al.* (1983) in their study of composting sewage with rice hulls also found that aerobic bacteria predominated throughout all composting stages. They did not however find an increase in numbers of actinomycetes during the time of high cellulase activity, which was not until 23d after the thermophilic stage.

The identification of thermophilic cellulolytic anaerobic bacteria present during composting has only been reported by Deschamps (1982), who isolated thermoduric *Cellulomonas spp.* from bark compost at 37°. Actual identification of strictly anaerobic cellulolytic bacteria may be difficult as mixed cultures are often required for activity (Enebo, 1951; Brandon, 1979). Several thermophilic, anaerobic , cellulolytic bacteria have been described (Mc Bee, 1950; Lee and Blackburn, 1975) and Ng, *et.al.* (1977) made one of the first studies on the physiology and cellulase complex of *Clostridium thermocellum*. They found the

optimal pH for two glucanases to lie between pH 5.2-5.4, with an optimal temperature between 64-65°. Both of these cellulases were oxygen and thermally stable at 70° for 45 minutes. This cellulase complex appeared to be different from all other bacterial cellulases in that the proteins were combined with a carbohydrate, possibly to protect against proteolytic degradation (Ait, *et.al.*, 1979). Recent interest in *C.thermocellum* and other thermophilic anaerobes has increased due to their favourable conversion of cellulose to fuel ethanol (e.g. Ng, *et.al.*, 1981; Saddler, *et.al.*, 1981).

1.1.3.2 Hemicellulose Biodegradation

The hemicelluloses are polymers of galactose, mannose, xylose, arabinose, other sugars and their uronic acids. They are mainly concentrated in the primary and secondary cell wall layers, where they are closely associated with cellulose and lignin (Bisaria and Ghose, 1981). Xylan or mannitol are typically used for hemicellulose biodegradation studies.

The biodegradation of hemicellulose occurs at a faster rate than that of cellulose under both aerobic and anaerobic conditions (Acharya, 1935; Kirk and Highley, 1973). Optimal conditions for hemicellulases and cellulases from the one organism are similar (Keilich, *et.al.*, 1970) and similar rates of activity of these enzymes in various litters have also been demonstrated (Caldwell, *et.al.*, 1979; Spalding, 1980). In general cellulases (Reese and Mandels, 1963) and mannanases (Reese and Shibata, 1965) are inducible while xylanases are largely constitutive (Reese and Mantels, 1963). Dekker and Richards (1976) have reviewed the occurrence, purification, physico-chemical properties and modes of action of the hemicellulases.

The few studies on hemicellulases in composts generally agree with the concept of concurrent activity with cellulases. Equal degradation of hemicellulose and cellulose in bark composts by both mesophilic fungi (Wilhelm, 1976) and thermophilic

microorganisms (Bagstam, 1979) has been demonstrated. Different microorganisms however, may be responsible for the biodegradation of the hexoses and pentoses. Waksman *et.al.* (1939b) found hemicellulolytic spore-forming bacteria in compost at 75° when no cellulolytic bacteria were present. Also, most of the thermotolerant bacteria isolated by Deschamps (1982) which produced xylanases could not produce cellulases and vice-versa. Numerous thermophilic or thermotolerant bacteria can produce xylanases, including *Bacillus spp.* (Gordon, *et.al.*, 1973; Deschamps and Lebeault, 1980; King, *et.al.*, 1980) *Arthrobacter sp., Corynebacterium spp., Klebsiella sp., Micrococcus sp., Pseudomonas sp., Sporocytophaga sp., Streptomyces sp.*, (Deschamps and Lebeault, 1980) and *Clostridium thermohydrosulphuricum nov. spec.* (Anon, 1979). Fungi rather than bacteria have been shown to be the most significant degraders of xylan at temperatures of 45°, with *Aspergillus fischeri* showing the greatest utilization (Basu, 1980).

1.1.3.3 Lignin and Tannin Biodegradation

Lignin is a highly polydispersed polyphenolic macromolecule of nine-carbon phenylpropane units linked by C-C and C-O-C bonds (Hall, 1980). The bark lignin from hardwood is similar to that of the corresponding wood, but with a lower syringyl content in the guaiacyl-syringyl moieties (Adler, 1977). Bland and Menshun (1971) have examined the chemistry of eucalypt lignins. Lignin acts as a physical barrier to enzymes that attack cellulose and hemicellulose and the rate of decomposition in litter attributable to such enzymes is inversely proportional to the lignin content (Lindeberg, 1949).

Enzymes considered to be important in fungal lignolytic activity are intracellular oxygenases and extracellular laccases and peroxidases which produce partial fragmentation of the polymer (Crawford and Crawford, 1980). Hall (1980) however has proposed an alternative hypothesis that the enzymes responsible for lignolytic activity do not interact with the polymer itself, but generate reactive diffusible species, such as superoxide radical anions

which in turn attack the macromolecule.

The lignolytic system is considered inducible, with nitrogen starvation being the principle inducer with some fungi (Keyser, *et.al.*, 1978; Fenn, *et.al.*, 1981). As with cellulose decompostion, the rate of lignin decompostion increases with increasing temperature, and enrichment cultures of thermophilic bacteria degrade the lignin of finely ground wood in a relatively short period (Alexander, 1977). The work of Odier and colleagues indicated that several strains of bacteria can rapidly utilize more than 55% of the lignin supplied in a mineral medium (Odier and Monties,1977; Odier, *et.al.*, 1981). Contrary to the proposal of Kirk, *et.al.* (1976), that lignin does not serve as an energy source for fungi, Odier and Monties (1978) found that glucose suppressed bacterial lignin degradation. Forney and Reddy (1979) also showed that glucose suppressed bacterial lignolytic activity, but complex carbohydrate may be required for an energy source (Ander and Eriksson, 1975). Xylose has been shown to facilitate depolymerization of lignin by some fungi and prevent polymerization of low-molecular-size fractions of the lignocarbohydrate complex (Milstein, *et.al.*, 1983).

Only recently has a sensitive and unequivocal ^{14}C-label method for the assay of lignolytic activity emerged (Kirk, *et.al.*, 1975; Crawford and Crawford, 1976), as there is little evidence to suggest a correlation between ability to degrade single-ring aromatic or lignin model compounds and ability to degrade polymeric lignin (Crawford and Crawford, 1980). Recently, Haars, *et.al.* (1982) have developed a simple and inexpensive means of detecting the occurrence of lignin breakdown, not requiring complete decomposition to CO_2, by using fluorescein labelled lignin. Using the ^{14}C-label technique, lignolytic strains of *Nocardia* and *Pseudomonas* species were isolated by Haider *et.al.* (1978), of a *Bacillus megaterium* by Robinson and Crawford (1978), and of three *Streptomyces* strains by Crawford (1978). The degradation products of a lignolytic *Streptomcyes sp.* may be important in the production of surfactants and adhesive precursors (Crawford, *et.al.*, 1983).

Lignin has long been considered inert in the absence of oxygen, hence the formation of peat and coal (Zeikus, 1980). However, anaerobic lignolytic activity has been demonstrated by a *Xanthomonas* strain in the presence of nitrate and glucose (Odier and Monties, 1978), by a filamentous bacterium (Akin, 1980) and by mixed cultures with the (^{14}C)-lignin technique (Colberg and Young, 1982; Benner, *et.al.*, 1984). Evans (1977) has reviewed the anaerobic catabolism of aromatic compounds, including the possible role of nitrate respiration.

The importance of lignolytic fungi in compost is unknown. A number of studies on the well-known mesophilic, lignolytic, white rot fungi have recently been carried out using the (^{14}C)-lignin technique (Ander and Eriksson, 1978; Kirk, *et.al.*, 1978), but only one thermotolerant lignin degrader, *Phanerochaete chrysosporium* has been isolated (Kirk, *et.al.*, 1976). Tansey, *et.al.* (1977) were unable to show clearing of lignin agar by a number of thermotolerant fungi isolated from various habitats including wood and bark chip piles. An inadequate technique for the assay of lignin (by the "sulfuric acid" method) puts in doubt the reported lignolytic activity of thermophilic *Thermomyces sp.* (Waksman and Cordon, 1939), *Paecilomyces sp.* and *Allescheria spp.* (Eslyn, *et.al.*, 1975).

As lignin and tannins contain phenolic groups, their biodegradation by fungi is thought to occur by similar enzyme systems (Kirk, 1981), and the role of phenol oxidases (peroxidases and laccase) in lignin degradation by fungi have already been mentioned (Adler and Eriksson, 1976; Kirk, *et.al.*, 1977). Also, the characterizaton of wood-rotting Basidiomycetes as lignin-degrading (white) or brown-rotting types, by the Bavendamm (1928) test, makes use of a gallic or tannic acid containing medium. Cellulose degradation may also be involved in lignin biodegradation, as Westermark and Eriksson (1974) have indicated the necessity for a quinone group (formed by laccase activity in lignin degradation) in the co-degradation of lignin and cellulose by some white rot fungi. In the absence of the cellobiose dehydrogenase proposed by Westermark and Eriksson (1974), phenol

oxidase-catalyzed condensation reactions in lignin could aid in humus formation (Flaig, 1977). Two other phenol active enzymes, peroxidase and tyrosinase, may also be directly involved in humification (Flaig, *et.al.*, 1975), but there does not appear to be any correlation between lignolytic activity of fungi and their ability to form humic acid (Martin and Haider, 1971; Jain, *et.al.*, 1979). The pathways for phenolic acid degradation to carbon dioxide or humic acid production are discussed by Shindo and Kunatsuka (1975) and Martin and Haider (1980). The production of humic compounds from straw is greater under aerobic than anaerobic conditions and their mineralization is accompanied by multiplication of *Nocardia* and *Arthrobacter* under aerobic and of members of the *Micromonosporaceae* under anaerobic conditions (Tepper, *et.al.*, 1981).

Research on the degradation of lignin by bacteria only partly supports the concept of joint lignin and tannin decomposition. Of eleven lignin degrading bacteria isolated by Deschamps (1982), only six could assimilate tannic acid, while some 30 strains were isolated that degraded tannic acid. The greatest lignolytic activity was observed with a *Bacillus sp.* which was also able to degrade tannin. Bacterial delignification of lignocellulosic waste has not been demonstrated with pure cultures, but mixed cultures of a lignolytic *Bacillus sp.* and a cellulolytic *Cellulomonas sp.*, was shown to result in up to 44% delignification of bark chips (Deschamps, *et.al.*, 1981). This simultaneous depolymerization of lignin and cellulose during the degradation of lignocellulose by white rot fungi has also been observed (Setlif and Eudy, 1980). Crawford, *et.al.* (1982) recently demonstrated that chemical changes in lignin degradation of bark chips by *Streptomyces viridosporus*, were very similar to those reported for white-rotted lignins. It is also of interest to note that some extractives from *Eucalyptus* woods are natural inhibitors of enzymes (Hart and Hillis, 1974) and in general, tannins inactivate enzymes by complexing with them as well as with other components to make them highly resistant to microbial attack (Benoit, *et.al.*, 1968; Van Summere, *et.al.*, 1975). Also, as the degree of tannin polymerization increases, their toxicity decreases (Rudman, 1964).

Some flavanoids however, have been shown to stimulate rather than inhibit basidiomycetes in the decomposition of leaf litter (Lindeberg, *et.al.*, 1980). Phenolic compounds from barks are also highly toxic to plants (Krogstad and Solbraa, 1975; Still, *et.al.*, 1976; Yazaki and Nichols, 1979) so their destruction is vital in the production of bark compost.

Tannins are separated into two groups; 1) the hydrolysable tannins (esters of sugar,usually glucose, with at least one trihydroxybenzenecarboxylic acid) and 2) the condensed tannins (derivatives of flavanols) (Haslam, 1979). The important hydrolysable tannins in eucalypt bark and wood are ellagitannins (which hydrolyse to ellagic and gallic acids) (Seikel and Hillis, 1970) while leucoanthocyanins and catechin are the predominant condensed tannins (Hathway, 1962). In an examination of fungi able to degrade eucalypt leaf litter, Macauley (1977) found species of *Coelomycetes* and *Moniliales* as the primary invaders to be subsequently replaced by other species of *Moniliales*,in particular *Trichoderma, Penicillium* and *Mucorales spp.* . Tannin degrading bacteria isolated from compost and soil include *Bacillus, Klebsiella, Moraxella, Pseudomonas* and *Staphylococcus* strains (Buswell and Clark, 1976; Evans, 1977; Deschamps, *et.al.*, 1980b). Thermophilic *Bacillus spp.* have also been shown to catabolise protocatechuate by a unique 2,3-dioxygenase pathway (Crawford *et.al.*, 1979). Deschamps, *et.al.* (1980b) found that most strains examined assimilated the hydrolysable tannins, gallic or protocatechuic acids, but only a few could degrade the condensed tannins like catechin. Bacteria involved in the utilization of condensed tannins include *Azotobacter vinelandii, Escherichia coli* and *Pseudomonas fluorescens* (Basaraba, 1966). Microbial attack of the condensed tannins of bark have only recently been investigated. The first report of the degradation of pure condensed tannins was made by Grant (1976), who demonstrated the degradation of these tannins from *Pinus radiata* by *Penicillium adametzi*. Grant and McMurtry (1978) attempted to quantify the effects of these pine tannins on bacteria, algae and protozoa. They demonstrated that Gram negative bacteria and protozoans could grow in the presence of at least 0.05% tannins whereas Gram

positive bacteria and algae were very sensitive to these condensed tannins. Yeasts, too, are generally sensitive to tannins (Jacob and Pignal, 1975), although some show potential for the removal of resin acids from mill effluents (Spencer, *et.al.*, 1974).

1.1.4 Nitrogen Transformations

A clear succession in the forms of nitrogen has been observed during the composting of stable manure, with temperature having the most significant influence on these transformations (Waksman, *et.al.*, 1939b). These workers demonstrated that under conditions of active decomposition, soluble forms of nitrogen were first immobilized followed by the decomposition of simple carbohydrates. Next proteins, present both in the raw materials and microbial cells, were decomposed, liberating mineral nitrogen, primarily as ammonium. The completion of rapid decomposition (after about 33 days) was followed by nitrification. If rapid decomposition was delayed from the outset, either through too high or too low a temperature, then nitrogen was lost by volatilization. Similary, above 65° less nitrification occurred and ammonia-nitrogen was lost through volatilization. The most pronounced transformations of nitrogen within organic matter take place during the first two weeks of composting (Aldag and Rochus, 1981). Apart from the effects of temperature, nitrification and to a lesser degree ammonification are also suppressed by tannins (Basaraba, 1964; Rice and Pancholy, 1973), various oxidized lignin degradation products (Bundy and Bremner, 1974), salt (0.44% or more) (Laura, 1977), pesticides (Vlassak, *et.al.*, 1977; Ramakrishna and Sethunathan, 1983), heavy metals (Wilson, 1977) and xanthates (Ashworth, *et.al.*, 1980).

1.1.4.1 Ammonification and Ammonia Volatilization

The biochemistry of ammonification and the processes by which organic nitrogenous compounds are enzymatically transformed to

ammonium, have been reviewed by Ladd and Jackson (1982). The volatilization of ammonia during thermophilic composting may be high due to rapid ammonification (Alexander, 1977), increased air movement, rapid diffusion rates or a high pKa NH_3 (increasing with higher temperatures and pH) (Nelson, 1982). Various enzyme inhibitors have been examined to reduce ammonia volatilization from soil. Urease inhibitors have been studied in greatest detail because of urea's rapid rate of decomposition and its wide use as a fertilizer, or as an amendment for the composting of bark (Hoitink, 1980; Solbraa, *et.al.*, 1982) or leaves (Hankin, *et.al.*, 1976) where complete degradation of urea occurred within eight days. Bremner and Douglas (1971) examined over 100 compounds as urease inhibitors in soil and found these to be in the following (decreasing) order of effectiveness: catechol > phenylmercuric acetate > hydroquinone > various benzoquinones (Bundy and Bremner, 1973). Mulvaney and Bremner (1977) studied three antimetabolites patented as inhibitors of urease; pyridine-3-sulfonic acid, desthiobiotin and oxythramine chloride. These inhibitors were shown to be ineffective, even at levels above those recommended. Also, nitrification inhibitors such as 4-amino-1,2,4-triazole (ATC) and N-serve (2-chloro-6-methylpyrimidine), have no effect on urea hydrolysis (Guthrie and Bomke, 1981). The use of ammonification or nitrification inhibitors during composting has not been reported. Chemical ammonia fixation to phenolics and quinones at pH's above 7 may be important in the conservation of nitrogen in compost (Nommik and Vahtras, 1982).

1.1.4.2 Nitrification

The topic of nitrification and the microorganisms responsible for it have been reviewed by Verstraete (1975), Focht and Verstraete (1977) and Schmidt (1982). Biological nitrification occurs optimally at about 25° and is considered to be inhibited at temperatures above 50° (Evans, *et.al.*, 1982). Nitrification in thermophilic environments, however, has been demonstrated at temperatures of up to 60° (Waksman *et.al.*, 1939b; Ishaque and

Cornfield, 1974). The production of ammonia and the high pH during thermophilic composting (Golueke, 1977) is likely, however, to inhibit autotrophic nitrification (Anthonisen, *et.al.*, 1976). Although work with gamma-irradiated soils has indicated microbial thermophilic nitrification in some soils (Ishaque, *et.al.*, 1971), chemical nitrification under thermophilic conditions has also been observed (Cawse and White, 1969). Heterotrophic nitrification is another as yet unexamined possibility during composting, since heterotrophic nitrifiers have been isolated from many harsh environments (Ishaque and Cornfield, 1974; Remacle and Froment, 1972; Focht and Verstraete, 1977; Tate, 1977). Wiechers (1975) regards nitrification during the first stage of composting essential if very high (80°) temperatures are to be obtained during composting (by the reaction of nitrates and organic substances). The autotrophic nitrification inhibitors acetylene (Hynes and Knowles, 1978), ethyl urethane and thiourea (Quastel and Scholefield, 1949; Thomazeau, 1980) have been used to examine heterotrophic nitrification. Even though most heterotrophic nitrifiers are weak nitrifiers, Castignetti and co-worker (Castignetti and Gunner 1981; Castignetti and Hollocher, 1982) have recently reported a potent nitrifying *Alcaligenes sp.* which is also capable of denitrification. It is of interest to note that obligate methylotrophs are also capable of heterotrophic nitrification (Romanovskaia, *et.al.*, 1977). Representatives of various fungal genera (*Aspergillus spp, Cephalosporium sp. Penicillium sp., Chlorella sp., Ankistrodesmus sp.* and one bacterial genus (*Arthrobacter spp.*) are the only heterotrophic nitrifiers that have demonstrated oxidation of ammonium to nitrate (Focht and Verstraete, 1977). Golovacheva (1975) reported the first thermophilic nitrifying bacterium belonging to the genus *Nitrosomonas*. This bacterium, which oxidized ammonium at 40-70°, resembled *N. europaea*. Despite the apparent symbiotic association for nitrification with a *Thermus sp.* and a *Bacillus sp.* these heterotrophs, in pure culture, could not oxidize ammonium (Golovacheva, 1975), while the *Thermus sp.* was found to be able to reduce nitrite and nitrate (Golovacheva, 1976). No report appears to exist on the microorganism(s) responsible for

nitrification during thermophilic composting, or of the possibility of thermophilic chemonitrification in compost. Considering the formation of nitrite (Yates and Rogers, 1981) and nitrate (Knuth, 1970) during thermophilic composting (40-50°) of solid wastes, but not in thermophilic liquid composting (Thaer, *et.al.* (1975), thermophilic actinomycetes have been suggested as the active nitrifiers (Focht and Verstraete, 1977). Chemical oxidation of ammonium has been observed by Laudelout (1977) to occur between 25-32° with nitrite as the end product. Non-microbial transformation of nitrite to nitrate is possible at low pH (Bremner and Nelson, 1968) or following a corresponding reduction in manganese oxides (Bartlett, 1981).

1.1.4.3 Denitrification

Denitrification has been reviewed by Focht and Verstraete (1977), Firestone (1982) and Knowles (1982) with 23 genera of denitrifiers being detailed by these workers. Denitrification is defined by Firestone (1982) as the reduction of NO_3^- or NO_2^- to a large volume of N_2 and/or N_2O only under anaerobic conditions to distinguish it from gaseous N possibily produced during nitrification or other N transformations. Except for *Propionibacterium* (anaerobic to aerotolerant) all other denitrifiers are aerobic bacteria (Firestone, 1982). Unlike nitrification, denitrification may occur optimally at about 65°, and continue up to about 75° due to the activity of *Bacillus spp* (Keeney, *et.al.*, 1979), although more than 85% of the nitrogen evolved at 70° was nitrous oxide. At temperatures above 50° more nitrogen was recovered as gaseous N than was initially present in the system as nitrate-nitrogen and no nitrite- plus nitrate-nitrogen remained after four days incubation. From these findings and work with sterilized soil Keeney, *et.al.* (1979) suggested that the commonly-stated optimum temperature may be too high for true biological denitrification and that at temperatures above 50°, nitrite, produced at least by thermophilic *Bacillus spp.*, could react with oxidized nitrogen

functional groups to form nitrogenous gases. The use of the acetylene-inhibition of nitrous oxide reductase to quantify denitrification in soil is well established (Yoshinari and Knowles, 1976; Klemedtsson, *et.al.*, 1977; Kaspar and Tiedje, 1981), but interference by chemical denitrification, as just described, would limit its use to mesophilic temperatures. There is also an excellent positive correlation between mesophilic denitrification and the level of organic carbon in soil (Burford and Bremner, 1975), although assimilative nitrate reduction is also increased by the presence of readily oxidizable organic matter (Buresh and Patrick, 1978). The conservation of nitrate-nitrogen by assimilation in the presence of a complex carbon source is, however, only slight (below 5%) (Buresh and Patrick, 1978). Loss of nitrogen through volatilization of ammonia during thermophilic composting is well known, but no mention is made regarding the potential loss of nitrogen from compost through reactions such as nitrite with lignin components at high temperatures (Stevenson and Kirkman, 1964), heterotrophic nitrification-denitrification (Focht and Verstraete, 1977) or chemodenitrification (Keeney, *et.al.*, 1979; Smith and Chalk, 1980a, 1980b).

1.1.5 Assay of Microbial Activity and Biomass

1.1.5.1 Microbial Activity

Problems relating to the assay of microbial activity in compost, or other natural environments, stem from the inherent variability of the decay process. This variability can result from the changes in the environmental factors (pH, moisture, aeration, nutrient supply) and the composition of the decaying material as it interacts with the microbial flora (Alexander, 1977). Because of this variability, extensive replication of assay is necessary, despite the fact that many assay-parameters used are already averages of microbial activity (Grossbard, 1979). Parameters used to assay the microbial activity of straw and other organic matter

include loss of weight, shear strength, nitrogen content, respiratory activity (Nannipieri, *et.al.*, 1978; Grossbard, 1979) enzyme activity (Domsch, *et.al.*, 1979) and microcalorimetry (Sparling, 1981) but each has its own limitation(s). Assay of weight loss may be misleading as it is not practicable to separate microbial mass from the remaining tissue (Grossbard, 1979). Determinations of shear strength, enzyme activity and nitrogen content do have an advantage in that any sample of material can be used, replacing the constraints imposed by the use of nylon bags for weight loss assay. All of these assays are, however, destructive, requiring a considerable initial bulk if sequential sampling is undertaken. Respiratory activity on the other hand can be monitored automatically and nondestructively, with CO_2 evolution being a more sensitive assay than O_2 uptake, especially when ^{14}C labelled substrate is available (Stotzky, 1965; Grossbard, 1979). Also the specificity of O_2 uptake is lessened by interference due to chemical oxidation reactions (Wang and Ferng, 1978). The assay of CO_2 evolution is however not entirely satisfactory as it reflects the activity of all organisms present, not just microbial. Problems associated with the assay of $^{14}CO_2$ due to interference from ^{14}C labelled microorganisms and/or their metabolites can reduce the usefulness of the label technique (Paul and Van Veen, 1978). Other workers who have not demonstrated good correlations between microbial counts and respiratory activity attribute these differences to non-biological activity (chemical decarboxylation or free carbonate) (Stotzky, 1965) or by the "priming effect" (an increase in microbial activity by the addition of an energy material) (Laura, 1977). Nevertheless, Bunnell, *et.al.* (1977a, 1977b) have successfully developed a model relating microbial respiration to temperature, moisture, O_2 and substrate loss in bench-scale experiments, accounting for 70-90% of the weight loss of litter bags in the field.

Criticisms of a CO_2-free atmosphere used in some respirometry assays (Warburg experiments) (Parkinson, *et.al.*, 1971) are not valid in systems using flow through aeration or BaO_2 (which releases a mole of O_2 for each mole of

CO_2 absorbed) (Cornfield, 1961). In the assay of microbial activity in compost, flow through systems to assay respiratory activity are used almost exclusively (Jeris and Regan, 1973a; Bagstam, *et.al.*, 1974; Cappaert, *et.al.*, 1976a; Clark, *et.al.*, 1977; Deschamps, *et.al.*, 1979; Mote and Griffis, 1979), although Schulze (1961) and Nell and Wiechers (1978) used microcalorimetry in conjunction with respirometry. The simplicity of the assay of respiratory gases and ease in automating a respiratory system, particularly when gas chromatography is used, are also important reasons for the widespread use of respiratory activity as an indicator of degradation. The recent developments of radio gas chromatographic assay of (^{14}C)-labelled compounds (Tykva and Seda, 1975) and of a very sensitive assay of inert gases by ultrasonic detector GC (Blackmer and Bremner, 1977) have further stimulated the use of respirometry.

Although respiratory activity is useful as an indication of general microbial activity, other assay techniques are required for the assessment of specific groups of microorganisms, such as the cellulolytic or lipolytic bacteria. There are basically two techniques employed, those that inhibit particular groups and those that selectively assay a group without disrupting the system. Examples of inhibition techniques are the use of acetylene for the assay of heterotrophic nitrification (Hynes and Knowles, 1978) or heterotrophic denitrification (Yeomans and Beauchamp, 1978) and the use of antibiotics to remove the influence of particular groups of microorganisms (Sparling, *et.al.*, 1982). Because of their disruptive nature, inhibition techniques are generally valid only over a short period (Anderson and Domsch, 1978). For longer term studies, label techniques have been shown to be most useful (Paul and Van Veen, 1978). If sub-sampling is possible then the simultaneous sampling for estimation of numbers of the microorganisms of interest and an assay of their enzyme activity in the sample is preferable over estimation of microbial numbers alone (Hankin, *et.al.*, 1976). This is because the presence of a particular degradative organism, even in large numbers, does not necessarily correlate with active metabolism of a substrate in question. For example, peak rates of

CO_2 evolution may commonly preceed peak biomass production by 24 h (Nannipieri, *et.al.*, 1978).

1.1.5.2 Microbial Biomass

In an effort to understand organic matter transformations it is now common practice to assay the biomass involved rather than just the presence of reaction products (Jansson and Persson, 1982). As a consequence there is a need to define the microbial biomass, its energy requirements, functions and metabolic processes. It is a common feature of composting that the "total" estimated numbers of microorganisms remain about the same throughout composting, but there is a succession of flora (Robinson, 1974; Bagstam, 1978) which may well change the magnitude of the biomass. Actual determinations of the microbial biomass in compost has only recently been reported (Thouvenot *et.al.*, 1979; Sparling, *et.al.*, 1982; Sparling and Eiland, 1983).

The following assay techniques have been utilized for the determination of microbial biomass in composts:-

- Microbial activity:
    * dehydrogenase (Benefield, *et.al.*, 1977)
    * respiration following chloroform fumigation and reinoculation (Jenkinson and Powlson, 1976) or following glucose amendment (Anderson and Domsch, 1978)
    * microcalorimetry (Sparling, 1981)
    * ATP (Oades and Jenkinson, 1979; Lethokarl, *et.al.*, 1983)
- Microbial components:
    * chitin (Swift, 1973)
    * lipid phosphate (McKinley and Vestal, 1984)
    * lipopolysaccharide (Jorgensen *et.al.*, 1979)
    * muramic acid (King and White, 1977)
- Estimates of numbers:

* dilution plating (Sparling, *et.al.*, 1982)
* direct (Trolldenier, 1973; Sparling, *et.al.*, 1982)
* direct viable (fluorescein diacetate vital staining (Soderstrom, 1979a, 1979b) or its stained hydrolysis products (Swisher and Carroll, 1980). Acridine orange vital staining (Ramsay and Bawden, 1983).)

Of these, the classical dilution plate method underestimates numbers observed by direct microscopic examination by at least 50% (Sparling, *et.al.*, 1982). Non-viable cells may give an overestimate of active biomass by the chitin (for fungi), muramic (for bacterial) or direct staining methods (Ineson and Anderson, 1982). Assay for ATP activity however may give a more reliable indication of the active biomass as ATP rapidly dissipates after cell death and levels are dependent on the physiological state of the organism (King and White, 1977). In a comparison of ATP-luciferase assay and biomass assay by fumigation or glucose amendment, Sparling and Eiland (1983) reported that the level of ATP was very variable and depended on the extractant used. Respiration following glucose amendment suggested a biomass some 20% greater than that calculated from the fumigation assay. In another study Sparling *et.al.* (1982) obtained very similar results by direct counts, fumigation, glucose amendment and microcalorimetry, but some 54% more biomass was indicated over other assay techniques by the ATP assay.

1.1.5.3 Effect of Composting on Pathogens

There are two types of pathogens present in composting wastes, those that are introduced with the raw materials such as sewage (Dudley, *et.al.*, 1980) and those that develop during the composting process. The latter group is of lesser significance since it includes only a few opportunistic pathogenic microorganisms. Examples of these are the fungus *Aspergillus fumigatus* (Millner, *et.al.*, 1977; Marsh, *et.al.*, 1979), certain

actinomycetes such as *Thermoactinomyces vulgaris* and *Micropolyspora faeni* which cause farmer's lung disease (Festenstein, *et.al.*, 1965; Lacey, 1975) and airborne Gram negative bacteria (Lundholm and Rylander, 1980). Control of these opportunistic pathogens may be possible by maintaining a high moisture level in the compost and adequately protecting compost workers.

Most attention has however been paid to the introduced pathogens and parasites. The die-off of these organisms during composting is dependent upon temperature, time, antagonistic microbial activity and nutrient availability (Diaz, *et.al.*, 1977). Numerous data are presented in the literature giving the thermal death times (not in compost) of most pathogens, parasites and indicator organisms (Gotaas, 1956; Golueke, 1977). Most (*Salmonella spp., Shigella spp., Escherichia coli, Staphylococcus aureus, Streptococcus pyrogenes, Corynebacterium diptheriae, Nector americanus, Ascaricus lumbricoides* eggs, *Entamoeba histolytica* cysts, *Brucella saginata,* and *Trichinella spiralis* larvae) were killed within one hour at 55°. *Mycobacterium tuberculosis* were killed within 20 min. at 65° and poliovirus type 1 within one hour at 60° (Golueke, 1977). Pathogen survival has been observed in windrow (Burge *et.al.*, 1978) and forced aeration composting of sewage sludge (Epstein, *et.al.*, 1976) due to inclement weather or insufficient heating in the outer compost surfaces. Heat alone however is not entirely responsible for the elimination of pathogens during composting. Microbial antagonism (Diaz, *et.al.*, 1977; Lindgren and Clevstrom, 1978; Makawi, 1980) is also important. Maximal microbial antagonism is attained under optimal conditions of moisture and nutrient content (Krogstad and Gudding, 1975). The only animal pathogens reported to survive the composting process for more than a few days are *Salmonella cairo* and *Bacillus anthracis*. Knoll (1961) found that complete destruction of heat resistant *S.cairo* took some 6-7 days at 50°. The sporeformer, *B.anthracis* was generally eliminated after 6-11 days at 34-65° in a Dano drum composter.

Most plant pathogens including species of *Botrytis, Erwinia,*

Phytophthora, Pythium and *Rhizoctonia* are also destroyed during composting at 40-60° for 10-13 weeks (Hoitink, *et.al.*, 1976). Virus diseases of lettuce were however resistant to temperatures < 70° (Martin, 1966) and tobacco mosaic virus is completely resistant to composting (Hoitink, *et.al.*, 1982). Overheating during the composting of hardwood bark (> 60°) however, results in a loss of the microflora (e.g. *Trichoderma spp.*) which are antagonistic to plant pathogens in mature compost (Hoitink and Kuter, 1983). Hoitink and Kuter (1984) recently showed that *T.harizanum* in composted hardwood bark containing media continued to suppress root-rot fungi long after the degradation of the naturally occurring fungicides in that bark (e.g. ethyl esters of hydroxylated oleic acids).

In searching for a suitable indicator organism for pathogen survival in compost Burge, *et.al.* (1981) found coliphage F2 to be considerably more heat resistant than enteric pathogens, including viruses, bacteria, protozoan cysts and helminth ova. Nevertheless, a 15 log decline in coliphage F2 occurs in 2.5d at 55°. Coliphage F2 is also more resistant to NH_3 (an antiviral agent in sludge (Ward and Ashley, 1976, 1977)) than polioviruses, it does not replicate in sewage and is easily identified (Cramer, *et.al.*, 1983). As a consequence they proposed it as an indicator, with a time-temperature relationship based upon that required to inactivate 15 logs of coliphage F2 indicating satisfactory pathogen kill (Burge, 1983).

1.1.6 The Thermophilic Microflora

1.1.6.1 Definitions

Microorganisms have been arbitrarily placed into at least three groups depending on their cardinal temperatures, that is their minimum (T_{min}), optimum (T_{opt}) and maximum (T_{max}) growth temperatures and the history of these classification schemes is given by Ljungdahl (1979). For the present study the following definitions of Tansey and Brock (1978) are used:

Microorganisms that grow at 0° are described as psychrophiles, from 10° to 55° as mesophiles and above 55° as thermophiles. Three other divisions are also mentioned: psychrotrophs being organisms with their T_{opt} at mesophilic temperatures but also capable of growth at 0°, thermodurics being organisms that may grow above 55° but have their T_{opt} in the mesophilic stage and T_{min} below 35° for bacteria and 20° for fungi and extreme thermophiles (or caldoactive organisms) which are thermophiles with a T_{opt} above 65° (Williams, 1975).

1.1.6.2 The Range of Thermophilic Organisms

There have been several reviews of thermophilic actinomycetes (Cross, 1968; Cross and Goodfellow, 1973), other thermophilic bacteria (Williams, 1975), thermophilic fungi (Cooney and Emerson, 1964; Crisan, 1973) and thermophilic microorganisms (Brock, 1978a; Tansey and Brock, 1978). Brock (1978a) has compiled extensive data on well characterized, thermophilic procaryotic and eukaryotic microorganisms, quoting their cardinal growth temperatures. There are however, still problems in the classification of thermophiles because of similarities of some thermophilic strains with certain mesophilic strains and the pleomorphism of other strains at high temperatures. Only by the use of methods such as DNA hybridization, isoenzyme analysis (Sharp, *et.al.*, 1980), pyrolysis gas-liquid chromatography (GLC) (Wolf and Sharp, 1981) or assay of cell wall components (Kandler and Hippe, 1977; Hippchen, *et.al.*, 1981; Pask-Hughes and Shaw, 1982) have clear taxonomic differentiation been possible for some of these organisms.

There are only 23 thermophilic actinomycetes listed by Tansey and Brock (1978) for which the highest T_{max} reported is about 75°. Genera containing thermophilic species include *Actinobifida, Microbispora, Micropolyspora, Pseudonocardia, Streptomyces, Streptosporangium, Thermoactinomyces* and *Thermomonospora*.

Of the other 65 procaryotic species reported to grow above 55° most fall into four groups: photosynthetic (*Chloroflexus, Chromatium, Cyanobacterium, Mastigocladus* and *Synechococcus*), aerobic or facultative sporeformers (*Bacillus*), anaerobic sporeformers (*Clostridium*, and *Desulfotomaculum nigrificans*), and the Gram-negative non-sporeforming aerobes (*Thermus* and *Thermomicrobium*). There are a few other thermophiles, scattered amongst several genera, with most isolates being generally obtained from anaerobic environments. These belong to the genera *Acetogenium* (Leigh, *et.al.*, 1981), *Desulfovibrio* (Rosanova and Khudiakova, 1974), *Halobacterium* (Tansey and Brock, 1978), *Hydrogenbacter* (Kawasumi, *et.al.*, 1984), *Lactobacillus* (Rogosa, 1974), *Methanobacterium, Methanosarcina* (Zinder and Mah, 1979), *Streptococcus* (Deibel and Seeley, 1974), *Sulfolobus* (Zillig and Holz, 1981), *Thermoanaerobacter* (Wiegel and Ljungdahl, 1981), *Thermoanaerobium* (Lamed and Zeikus, 1980), *Thermobacteroides* (Ben-Bassat and Zeikus, 1981), *Thermoplasma* (Brock, 1978b), *Thermodesulfobacterium* (Zeikus, *et.al.*, 1983) and *Thermoproteus* (Woese *et.al.*, 1978). Recently mycelia-forming organisms from submarine volcanic areas, which equivocally grow at 250° (at 265 atms.), have been isolated and placed into the new genus *Pyrodictium* (Baross and Deming, 1983; Fischer, *et.al.*, 1983; Stettter, *et.al.*, 1983).

A similar number of thermophilic or thermoduric fungi are listed by Tansey and Brock (1978). However, only some twenty species were reported to grow above 55° and the T_{max} for fungi was stated to be 60-62°. The thermoduric and thermophilic fungi are mainly scattered amongst the Deuteromycetes (seventeen genera) and Ascomycetes (ten genera) with a few genera in the Zygomycetes, Basidiomycetes and Mycelia Sterilia.

1.1.6.3 Nomenclature of Thermophilic *Bacillus*

The genus *Bacillus* is reported to represent the predominant, if not the total flora isolated during the composting of wastes (Snell, 1960; Finstein and Morris, 1975; Morris, 1975; Poincelot,

1975; Strom, 1978). Its classification will therefore be discussed in some detail in this report.

Thermophilic aerobic sporeformer bacteria capable of growth at 73° were first isolated by Miquel (1888) and named *B. calfactor* by Miehe (1907). However, since 1920 most thermophilic *Bacillus* capable of growth at 65° have been lumped into the *B. stearothermophilus* group with the type strain (ATCC 12980) as described by Donk (1920). An excellent review of the taxonomy of thermophilic *Bacillus* was recently reported by Wolf and Sharp (1981). The type strain of *B. stearothermophilus* (ATCC 12980) grows with a T_{min}, T_{opt} and T_{max} of 29°, 60° and 75° respectively. In 1928 Hussong and Hammer proposed *B. caldolactis* and Prickett (1928) proposed the name *B. kaustophilus* for two isolates from milk which were capable of growth at 75°. Smith (1948) in the sixth edition of Bergey's Manual gives descriptions of twenty thermophilic species of *Bacillus* capable of growth at 55°. These were classified largely on sporangial morphology and several species were recognized by Bergey including those mentioned above. Subsequent work by Gordon and Smith from 1949 to 1974 (Gordon and Smith, 1949; Gordon, *et.al.*, 1973) formed the basis for the description of the genus in the seventh (Breed, *et.al.*, 1957) and eighth (Gibson and Gordon, 1974) editions of Bergey's Manual. Gordon, *et.al.* (1973) concluded that virtually all previously described *Bacillus* thermophiles conveniently fell into either *B. coagulans* or *B. stearothermophilus* (Table 1). Numerous other taxonomic studies on the thermophilic *Bacillus* are reviewed by Wolf and Sharp (1981). This review largely reflects the work of Walker and Wolf (1971) who divided the strains into three distinct major groups, two of which were further divided into minor subgroups (Table 2). These groupings have been more recently supported by examination of thermophilic *Bacillus* esterases, although subdivisions within groups 1 and 3 were less evident (Baillie and Walker, 1968; Sharp, *et.al.*, 1980). Heinen and Heinen (1972) differentiated three caldoactive strains from *B. stearothermophilus* on the basis of their temperature optima, fatty acid patterns and sub-microscopic structure. Sharp *et.al.* (1980) have also compared seven strains of *B. stearothermophilus* and

another three caldoactive strains on the basis of biochemical properties, DNA base composition, bacteriophage and bacteriocin sensitivities, esterases, constitutive enzyme production and antibiotic resistance and analysed the data by numerical taxonomic methods. In their view the caldoactive strains could be classified into Walker and Wolf's (1971) groups along with most other thermophilic *Bacillus spp.* (Table 3). However, *B. caldolyticus* and *B. stearothermophilus* 262 showed features common to groups 1 and 3. In conclusion, on the taxonomy of the thermophilic *Bacillus*, Wolf and Sharp (1981) suggested that the group 1 organisms were best designated as a new species designated *B. kaustophilus*. The other two groups were distinct but group three possibly contained several elements best segregated to themselves. Further support for the Walker and Wolf (1971) scheme and the proposal for a number of distinct species was recently demonstrated by a numerical taxonomic survey of 57 thermophilic *Bacillus* isolates from soil (Garcia, *et.al.*, 1982). They found 75% of the strains in 12 major phenons of which two were related to the first group (*B. kaustophilus*) and two to the third group (*B. stearothermophilus*) of Walker and Wolf (1971). *B. thermocatenulatus* (Golovacheva *et.al.*, 1975) is one isolate sufficiently different from previously described thermophilic rods to be considered as a distinct species. The distinguishing features of this yellow pigmented rod are the negative reactions on starch and gelatin and its ability to grow anaerobically and to reduce nitrate to gas. It is interesting to note that Heinen *et.al.* (1982) have recently isolated a yellow pigmented *Bacillus* which fits the description of *B. thermocatenulatus*, but they have named it *B. flavothermus*. And finally, *B. schlegelii* (Schenk and Aragno, 1979) is the first chemolithoautotroph to be comprehensively described. With a T_{opt} of 70° this strict aerobe oxidizes hydrogen in the presence of O_2 and CO_2 and can also grow heterotrophically.

TABLE 1

Differentiation of *B. stearothermophilus* from *B. coagulans* [1]

	B.stearothermophilus	B.coagulans
Growth at pH 5.7	-	+
pH Optimum	> 6	< 6
Growth in azide (0.2%)	-	+
Growth in sulphadiazine (5ug/ml)	-	+
Growth temperature (°C):		
Maximum	65-75	55-60 [2]
Minimum	30-45	15-25
Anaerobic growth	-	+
Spore survival (min. at 120°)	4-5	0.1
% G+C	44-53	47-56

[1] From Wolf and Sharp (1981).

[2] Maximum growth temperature, some strains of *B.coagulans* grown at 65° and pH 6.2.

TABLE 2

Reactions Characterizing the Principal Groups of *Bacillus stearothermophilus* [1]

	Group 1	Group 2	Group 3
No. of strains	127	40	63
Morphology of spores	oval to cylindrical	oval	oval to cylindrical
Swelling of sporangium	slight to definite	definite	definite
Growth in 3% (W/VO NaCl broth	-	+	-
Hydrolysis of:			
Gelatin	+-	-	+-
Casein	-	-	+-
Starch	R	-	++
NO_2^- from NO_3^-	+	-	+-
Gas from NO_3^-	+	-	-
Tomato-yeast milk	unchanged or reduction, or reduction & weak curd	unchanged	acid, clot, reduction
Litmus milk	unchanged or slight red.	unchanged	unchanged or acid, clot
Growth in glucose anaerobically	poor or -	-	+
Acid from:			
Arabinose	+-	-	-
Cellobiose	+-	+	+-
Lactose	-	-	+-
Mannitol	+-	+	-
Rhamnose	+-	+	+-
Xylose	+-	+	-
Subgroup division on	T_{min}, T_{max} nitrate to gas fermentations	-	limus milk lactose ferm.

[1] Data from Walker and Wolf (1971); +, positive; -, negative; +-, variable; ++, strongly positive; R, restricted.

TABLE 3

Classification of Recognized *Bacillus* Thermophiles

After Walker and Wolf (1971) [1]

GROUP 1	GROUP 2	GROUP 3
B. kaistophilus ATCC 8005 (Prickett, 1928)	*B. stearothermophilus* (Daron, 1967)	*B. stearothermophilus* NAC 1503, ATCC 7954
B. thermodenitrificans	*B. stearothermophilus* RS93 (Sharp *et.al.*, 1980)	*B. stearothermophilus* NAC 15118, ATCC 7953 (Donk, 1920)
B. stearothermophilus ATCC 12016	*B. stearothermophilus* (Epstein & Grossowicz, 1969)	*B. caldolactis* (Galesloot & Labots, 1959)
B. calsotenax (Heinen & Heinen, 1972)		*B. calidolactis* (Grinsted & Clegg, 1955)
B. caldovelox (Heinen & Heinen, 1972)		*B. thermoliquefaciens* (Galesloot & Labots, 1959)
		B. stearothermophilus NAC 1356
		B. stearothermophilus NAC 1492
		B. stearothermophilus NAC 26

[1] Wolf and Sharp (1981).

ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.;

NAC, National Canners Association, Washington D.C., U.S.A.

1.2 Composting of Bark

1.2.1 Methods of Composting

Composting is achieved either aerobically or anaerobically, with both methods having advantages and disadvantages. Aerobic methods result in temperatures of up to 80°, with 60-70° commonly held for three weeks in municipal wastes (Alexander, 1977) to several months in bark composts (Hoitink, 1980). Aerobic conditions are necessary for the removal of animal and plant pathogens (Krogstad and Gudding, 1975; Hoitink, *et.al.*, 1977; Kawata, *et.al.*, 1977), malodours (Parr, *et.al.*, 1978), weed seeds (Bollen, 1969) and for the reduction or elimination of phytotoxins (Still, *et.al.*, 1976). Anaerobic composting has the advantage of requiring less nitrogen and practically no nitrogen is lost (Rodale, 1975). Anaerobic composting is however slow and generally will not achieve high temperatures (Kaulander and Lindfors, 1976; Vogtmann and Besson, 1978).

1.2.1.1 Bench-scale Composting

A variety of bench-scale composters for the control and evaluation of composting have been described. These are of two basic designs: rotating drums generally of sufficient volume to allow development of thermophilic conditions (Jeris and Regan, 1968; Galler and Davey, 1971; Bagstam, *et.al.*, 1974) and smaller-scale static systems where temperature levels are supplied from an external source (Cappaert, *et.al.*, 1976a; Clark, *et.al.*, 1977; Deschamps, *et.al.*, 1979). Both designs incorporate a means of forced aeration and water replacement or retrieval (using cooled condensers). Reproducibility of results in both designs has frequently been poor as a result of balling of compost material in rotating drums and development of differential aeration in static systems as a result of gas tracking (Clark,

For large-scale composting of most bark, the bark is first hammermilled, mixed with nutrients then nearly always composted by a windrow method. Various recommendations have been made for turning the compost; from very little (Kawata, 1978), in response to high temperature (Koranski and Hanza, 1978) or every three days for the first 21 days (Solbraa, 1979c). Work on the composting of hardwood bark in Ohio has been by a mechanical method (Hoitink, 1980). The method used is based on the Metro system (Harding, 1968) in which the compost is aerated by fans through a perforated floor of a reactor bin in which an endless conveyor belt periodically mixes the contents.

1.2.2 Conditions for Composting

1.2.2.1 Temperature, Moisture and Aeration

The physical parameters of composting (temperature, moisture content (m.c.) and aeration) are all interrelated, since water is a product of aerobic respiration (the rate of which is dependent on m.c.) and evaporation is a function of aeration and temperature (Wiley and Pearce, 1957). Consequently, the notion that the hotter the compost the better is too simplistic, as the same temperature can be reached with a low m.c. poorly active compost as with a wet highly active compost. Also, cooling a hot compost (60-80°) by increasing the aeration (thus increasing evaporative cooling) will increase microbial activity, as long as the compost does not dry out (MacGregor, *et.al.*, 1981). A case in point is found in the composting of bark where the optimum temperature is 40-50° (Cappaert, *et.al.*, 1976b; Hoitink, 1980) but temperatures commonly reach 80° in bark compost windrows (Hoitink, 1980).

For optimal composting about 35% free air space (Jeris and Regan, 1973b) is required which means a m.c. of about 68% (wet weight) for bark (Cappaert, *et.al.*, 1976b). The calculations relating aeration, moisture loss and temperature for static pile composting are given by Haug and Haug (1977). Although the degree

of aeration required depends on the moisture loss and temperature levels, oxygen concentration should be within 5-12% (Hoitink, 1980). Forced aeration of a static pile of compost with a dividing flow is preferable to aeration by vacuum (combining gas flow) as forced flow gives a more uniform aeration, induces evaporative cooling at the most insulated region and transports heat out to the cooler edges. It also enhances convective updraft (set in motion by the temperature differential) and delivers air for less power (Finstein, 1980; Higgins, 1982). If ventilation is used to regulate temperature, as in the Rutgers Static Pile Composting Process (MacGregor, *et.al.* 1981) anaerobiosis is not a problem. In this latter process there is however the problem of non-uniform distribution of air through perforated piles within long windrows. Fortunately uniform air distribution can simply be achieved by using the program of Psarianos, *et.al.* (1983) which calculates the optimal perforation sizes and their distribution along the pipe.

1.2.2.2 pH and Chemical Amendments

Changes in pH have been observed in the aerobic batch composting of municipal solid wastes (Anon, 1955; Wiley, 1962; Stutzenberger, *et.al.*, 1970) and of poultry manure plus sawdust (Galler and Davey, 1971). Initially these composts were acidic (pH 4.5-6.0) and became more so during the first few hours, but within one to four days the pH rose to about 7.0. The final products were found to stabilize at, or slightly above, neutrality. In the composting of bark, pH is largely determined by the form of nitrogen used. When an ammoniacal form of nitrogen is used the pH behaves as just described, however the nitrate form results in a compost of pH 6 or below which restricts subsequent microbial activity during composting (Cappaert, *et.al.*, 1976a). Urea is reported to be the best form of N to add to unaerated windrows. However, with forced aeration part of the N added should be as ammonium nitrate or poultry manure to avoid a pH above 7.4 with resultant loss of ammonia (Hoitink, 1980). The correct mix of N is important as the

addition of sulphur (0.6 kg m^3) was ineffective in reducing the pH of the end product to the required 6.5 for plant growth (Hoitink, *et.al.*, 1978). Urea may be used to neutralize the low pH of bark (4.5), by producing a pH rise during the first few days of composting. Also, the addition of lime to composts may reduce the time to reach peak temperature by about one day (Wiley, 1962; Rose, *et.al.*, 1965) but, due to the resulting higher pH during the thermophilic stage, considerable quantities of NH_3-N can volatilise and thus lower the quality of the compost (Golueke, *et.al.*, 1954; Satriana, 1974).

Generally, chemical additives for composting bark are 1.1 kg of nitrogen m^3, with an additional 0.3 kg of phosphate m^3 for softwood bark (Cappaert, *et.al.*, 1976a; Solbraa, 1979b; Hoitink, *et.al.*, 1978). Organic forms of N may be better than inorganic due to their slower degradation, with a consequent reduced loss of NH_3 during the thermophilic stage (Gray and Biddlestone, 1976). Sewage sludge, which contains most of its N in an organic form (Sommers, 1977), has been used in bark and wood composts by a number of workers (Epstein, *et.al.*, 1976; Bagstam, 1977; Parr, *et.al.*, 1978; Taylor, *et.al.*, 1978). Sabey, *et.al.*, (1975) suggested that the problems of NO_3^--N and high moisture content in sewage sludge and the high C:N ratio in bark are cancelled out by combining the two. Also, detrimental effects of the elements B, Cd, Cu, Ni and Zn, possibly present in sewage sludge at levels toxic to animals or plants (Page, 1974; Keeney, *et.al.*, 1975; Mitchell, *et.al.*, 1978) are greatly reduced in the presence of insoluble organic matter (Barsdate, 1972) such as composted bark (Poonawala, *et.al.*, 1975; Henderson, *et.al.*, 1977). Bagstam (1977) found that composting spruce bark with dewatered fresh sewage sludge (max. water content 74%, wet weight) could be accomplished with the same results as composting bark with urea and phosphate. Some inorganic waste products have also been successfully incorporated with bark for composting. Michiels, *et.al.* (1981) demonstrated that in addition to 1.5% urea, the addition of blast furnace slag, lime-sludge or gypsum at a concentration of 10% resulted in a similar compost to that produced with just bark and urea.

1.2.3 Compost Maturity

The final or maturing stage of composting is primarily required to allow the possibly phytotoxic levels of NH_4^+, H_2S and volatile fatty acids (VFA) produced during the thermophilic stage, to decrease. Compost maturity or stability is recognised by a number of criteria: absence of NH_4^+ and H_2S (Spohn, 1978), reduction in starch and COD (Lossin 1970, 1971), pile temperature, degree of self heating on aeration, a C:N ratio of less than 20, amount of humus, growth rate plus number of fruiting bodies produced by the fungus *Chaetomium gracilis* (Jann, *et.al.*, 1960; Keller, 1961; Golueke, 1977), colour, total N > 2%, reducing sugars <35% (Sugahara, *et.al.*, 1979; Inoko, 1982), plant growth in extracts (Zucconi, *et.al.*, 1981), a $C:N_{organic}$ ratio of 5-6 in compost water extracts (Chanyasak, *et.al.*, 1982) and a decrease in cellulase activity (Chino, *et.al.*, 1983). Most of these criteria are to some degree dependent on the chemical nature of the materials composted and none as yet have been universally accepted (Inoko, 1982). Despite the claim of the universality of the $C:N_{organic}$ ratio 5-6 (Chanyasak, *et.al.*, 1982), it has yet to be used in other laboratories.

Presently the only guidelines used to assess the absence of inhibitors in bark compost are the onset of stabilization (lack of self-heating above 40°) and a pH 6.3-6.7 (Hoitink, *et.al.*, 1978).

1.3 Bark as a Plant Growth Medium

Hammermilled bark with particle sizes of 2 to 6 mm makes an excellent plant growth medium due to its resistance to packing, its low density, its high nutrient and water holding capacity and its low nutrient immobilization except for nitrogen (Bennett, *et.al.*, 1978). Hardwood bark mixtures have been used successfully to grow ornamentals (Klett, *et.al.*, 1972; Bekedorf, *et.al.*, 1977; Schusler, *et.al.*, 1977; Still, 1977) and vegetables (Nesterenko,

1976; Zhigalov, 1976). However, the highest yields have been obtained using composted hardwood bark mixed with peat or perlite (2:1 or 4:1) (Herr, *et.al.*, 1976; Reese, *et.al.*, 1978; Koranski, 1979).

The major adverse effects of bark as a plant growth medium are N-immobilization and phytotoxicity, with lesser problems of wettability and low available water content. Problems in wetting hardwood bark (Gartner, *et.al.*, 1973) and pine bark (Airhart, *et.al.*, 1978) have been encountered when these materials have been dried. Despite the high water holding capacity of hardwood bark, Spomer (1975a) found that only about 25% of this water was available to plants. Nevertheless, compared to plants grown in other media, plants in hardwood bark are more resistant to wilting (Spomer, 1975b). The chemical characteristics of hardwood bark as they relate to plant nutrition has been examined by Albrecht, *et.al.* (1983). They found little difference in plant nutrition as hardwood bark aged, except that an increase in humic acids may reduce the availability of micronutrients.

Little work has been undertaken on the potential of eucalypt bark for plant growth media. Eucalypt bark has been used composted, following mixing with other mill wastes (Ironside, 1976) and uncomposted, in compressed blocks combined with pine bark, sawdust and charcoal (Zeijlemaker and de Lasorde, 1976, 1977). The successful use of eucalypt bark compost has been demonstrated by the author (Ashbolt, 1979) and its suppression of plant pathogens by Sivasithamparam, *et.al.* (1982) as well as by a local nurseryman using ponded bark fines (Clark, V.S. *pers. comm.*).

1.3.1 Nitrogen-immobilization

N-immobilization in bark (or sawdust) results from the high C:N ratio, the ease with which some of the C compounds are decomposed in the presence of sufficient N (Bollen and Glennie, 1961; Allison and Murphy, 1962) and possibly from strong NH_4^+

adsorption (Allison and Jordan, 1973). Because microorganisms are more efficient in assimilating inorganic N than higher plants (Alexander, 1977) excess N over that required by the microflora must be added if plants are not to become N deficient (Ironside, 1976). This problem of N-immobilization may be overcome by adding a slow release N fertilizer (Gartner, *et.al.*, 1971), pre-extracting (by acid leaching) the bark (Goodwin, 1980) or by composting bark with added N (Sterrett and Fretz, 1977; Hoitink, *et.al.*, 1978; Gartner, 1981). Once composted, bark requires a different fertilizer regime for optimal plant growth than other soil-less media (Gillian and Smith, 1980).

1.3.2 Phytotoxicity of Bark

A number of workers have reported phytotoxicity of fresh softwood bark (Allison, *et.al.*, 1963; Anon, 1975; Hoitink and Pool, 1976; Bennett, *et.al.*, 1978) and of hardwood bark (Cappaert, *et.al.*, 1976a; Hoitink and Poole, 1977; Still, *et.al.*, 1976). In contrast to the claim of Lunt and Clark (1959), Gartner, *et.al.* (1968) and Klett, *et.al.* (1972) that reduced plant growth is primarily related to insufficient N, Hoitink and Pool (1977) and Still, *et.al.* (1976) suggested that phytotoxins may be the greatest limiting factor in the utilization of hardwood bark as a plant growth medium. This contradiction may be explained by the different bark mixes used and species and age of the test plants grown, considering that germinating seeds and seedlings are more sensitive to phytotoxins (Hoitink and Poole, 1977; Nichols, 1981).

Composting of bark has been shown to greatly reduce the level of phytotoxins but depending on the type of bark, at least 15-70 days composting are required to reduce or eliminate phytotoxins from hardwood bark (Still, *et.al.*, 1976; Cappaert, *et.al.*, 1977; Hoitink and Poole, 1977; Vleeschauwer, *et.al.*, 1981). The complete destruction of phytotoxins may not be beneficial as they are considered important in the antagonism of bark compost to plant pathogens (Krogstad and Solbraa, 1975; Hoitink, *et.al.*, 1977; Bennett, *et.al.*, 1978).

Phytotoxins from bark and wood have been broadly classed as polyphenols (Cappaert, *et.al.*, 1977; Kuwatsuka, *et.al.*, 1977). The few phytotoxins that have been isolated from bark include juglone from Walnut bark (*Juglans nigra*) (Davis, 1928), a tannic-acid-like compound from Silver Maple (*Acer saccharium* L.) (Still, *et.al.*, 1976) and catechin, 3,5,3',4'-tetrahydroxy stilbene, its glucoside and procyanidins B-1 and B-3 from ground *Pinus radiata* bark (Yazaki and Nichols, 1978, 1979). The phytotoxin in fresh *Eucalyptus camaldulensis* and *Eucalyptus regnans* sawdusts have been shown to be the ellagitannins D5, D6 and D13 (Yazaki and Nichols, 1979; Hillis, W.E., *pers. comm.*). In a study of the effects of thirteen naturally occurring phenolic acids, lactones and flavanoids on lettuce germination, Wolf, *et.al.* (1977) showed that the dihydroxy phenolic acids caffeic and chlorogenic and their flavanoid derivatives, quercetin and ratin were non-inhibitory, while the monohydroxy cinnamic acid derivatives and o-, m- and p-isomers of cumaric acid showed varying degrees of toxicity. The phytotoxicity of mono- and dihydroxy phenolic acid may be due to their different actions on indole acetic acid (IAA) oxidation (Henderson and Nitsch, 1962; Tomaszewski and Thimann, 1966). Differentiation of the phenolic acids is concentration dependent (Corcoran, 1971) although pH is unlikely to directly influence their phytotoxicity (Mayer and Evenari, 1953).

Phytotoxins may also be produced during the composting of bark. If anaerobic conditions develop then the build up of phytotoxic volatile fatty acids (VFA), H_2S and phenolics (such as p-hydroxybenzaldehyde, ferulic, syringic, vanillic, p-hydroxybenzoic and benzoic acids) may occur (Patrick, 1971; Wallace and Elliott, 1979; Harper and Lynch, 1982). De Vleeschauwer, *et.al.* (1981) demonstrated phytotoxic levels of acetic acid in refuse compost up to four months old. Ammonia at a concentration of over 0.15-0.20 mM was also phytotoxic (Bennett and Adams, 1970; Imai, 1977).

2 - Materials and Methods

2.1 Collection and Storage of Compost Materials

2.1.1 Bark

(A.) *Eucalyptus delegatensis* bark

Eucalyptus delegatensis bark was collected on 20/8/79 immediately after high pressure water debarking and prior to dumping (Plate 1) at Australian Newsprint Mills (A.N.M.), Boyer. The bark was air dried on a glasshouse floor and hammermilled (Ferguson Pty. Ltd.) through a 35mm screen. Material < 1mm was discarded and the remainder was stored at 10% moisture content (m.c.) [1] (Plate 2) until required.

(B.) Mixed eucalypt bark finings

Mixed eucalypt bark finings (0.5-10.0 mm dia.) were collected from A.N.M.,Boyer and delivered to "Millvale" nursery, Dromedary, on 8/3/79 for composting in large heaps. The species composition of this material was about 46% *E. regnans*, 44% *E. delegatensis* and 10% *E. obliqua*.

(C.) Compost inoculum

Compost inoculum was obtained from stabilized compost of mixed eucalypt bark and urea (1.19 kg m^3) located at

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1. All m.c. and elemental analyses are given on an oven-dry (105°) weight (ODW) basis.

Millvale Nursery, Dromedary (Plate 2). The material was stored in 20 litre sealed plastic bags at ambient temperature until required.

2.1.2 Sewage Cake

Urban sewage cake (not containing industrial wastes) was collected on 20/11/79 from the Glenorchy Sewage Depot, Derwent Park Rd., following anaerobic digestion at 37° and subsequent air drying on sand beds for six weeks. The material was stored at 224% m.c. at 4° in sealed 25 litre plastic drums until required.

2.1.3 Fish Waste

Flat-head fish heads, bones and guts were collected on 21/11/79 from Bass-Isle Seafoods, Glenorchy and weighed into plastic bags (1 kg) which were stored (327% m.c.) in a plastic drum at -18°.

2.2 Compost Mixes and Apparatus

2.2.1 Large-scale Composting

Two large-scale compost heaps (30m^3) of domed pyramidal shape (4m sides by 3m high) were constructed from mixed eucalypt bark and sewage cake or urea to give a C:N ratio of 35 and m.c. of 214% ODW (68% wet weight) (Plate 3). Mixing was partly by hand and partly by a front-end loader. The compositions of the bark, fish wastes and sewage cake used for large-scale and/or bench-scale composting are given in Table 4. Compost heaps were turned once after the second and nineteenth week of composting.

2.2.2 Bench-scale Composting

Either a constant initial content of bark (148g ODW) or a constant initial content of oven dry fish waste (16.5g), sewage cake (59.5g), isobutylidene diurea (IBDU) (3.75g) or urea (2.35g)

was placed in each compost unit. Composted mixed bark (10% w/w) was used as inoculum. The compositions and conditions used for each run of the composter are given in Appendix 1.

2.2.2.1 Constituents and conditions used for bench-scale composting.

The bench-scale composter is illustrated in Figures 1 and 2 and Plate 4. It consisted of six 4-L capacity gas-tight units of PVC plastic, each of which was provided with a mixing paddle coupled to a common drive. The compost was mixed for 15 min h^{-1} at 15 rpm. A natural temperature increase at rates consistent with those observed in the large-scale compost heaps (5° increase per day to 55° or 60°) was simulated by having the units immersed in a water bath. The compost was aerated by filtered, dry (through activated carbon and silica gel) compressed air at 20 mL per min unless otherwise mentioned. Gas flow rates through the units were checked daily with a ball flowmeter. The moisture content of each unit was maintained at about 214% m.c. by the use of a cooled (1°C) condenser which returned condensate from the effluent gas to the unit by gravity flow.

2.2.2.2 Gas chromatographic monitoring of compost mixes

Microbial activity was initially monitored daily by manual injection of effluent gas samples (1.0 mL) into a gas chromatograph (GC), but later injection was by an automatically operated series of solenoid valves (Figure 3) which sampled each unit every five hours. The GC (Perkin-Elmer Sigma 3B) was fitted with a hot wire thermal conductivity detector (300 mA) and glass-lined stainless steel columns, one of 2 mm by 1.5 m containing molecular sieve 5A 80-100 mesh, and the other of 2 mm by 1.5 m containing Carbosieve B. Columns were used in parallel with a temperature program from 80-120° to separate a range of possible compost gases (N_2, O_2, CO_2, CH_4, N_2O, H_2S and H_2O), or were run isothermally at 120° for CO_2 assay. Each treatment was duplicated over a 30 day

Plate 1

Mixed eucalypt bark dump at Australian Newsprint Mills, Boyer.

Plate 2

Hammermilled raw bark and composted bark inoculum.

Air-dried *Eucalyptus delegatensis* bark was hammermilled through a 35mm screen, sieved to remove particles < 1mm, then stored at about 10% moisture at ambient temperature.

Compost inoculum was obtained from stabilized compost of mixed eucalypt bark (*E.regnans, E.delegatensis and E.obliqua*) and urea (1.19 kg m^3) located at Millvale Nursery, Dromedary.

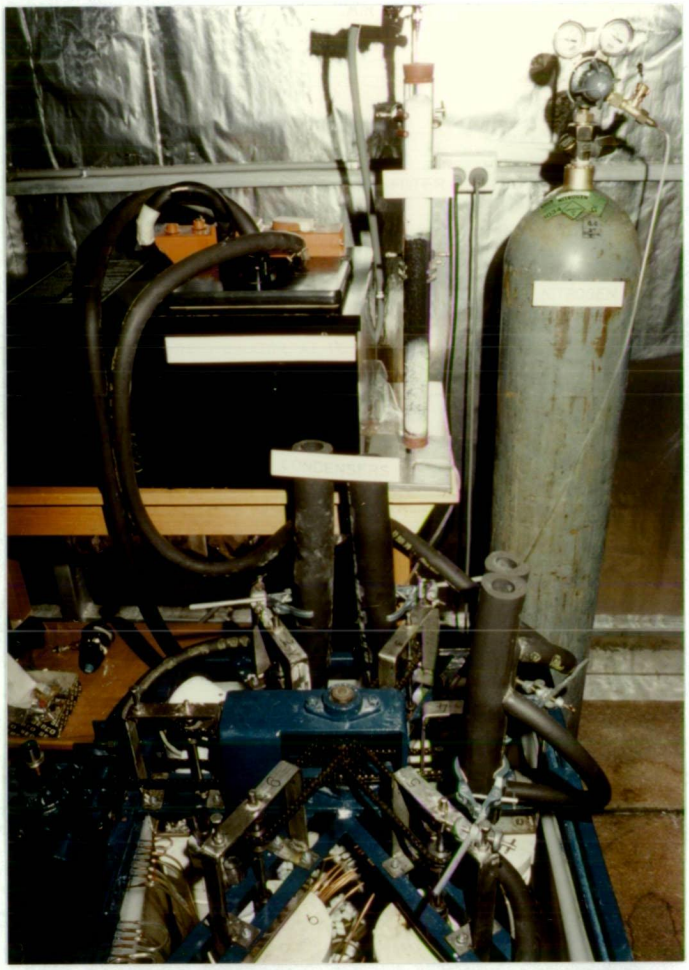

HAMMERMILLED
EUCALYPT BARK
COMPOSTED
EUCALYPT BAR

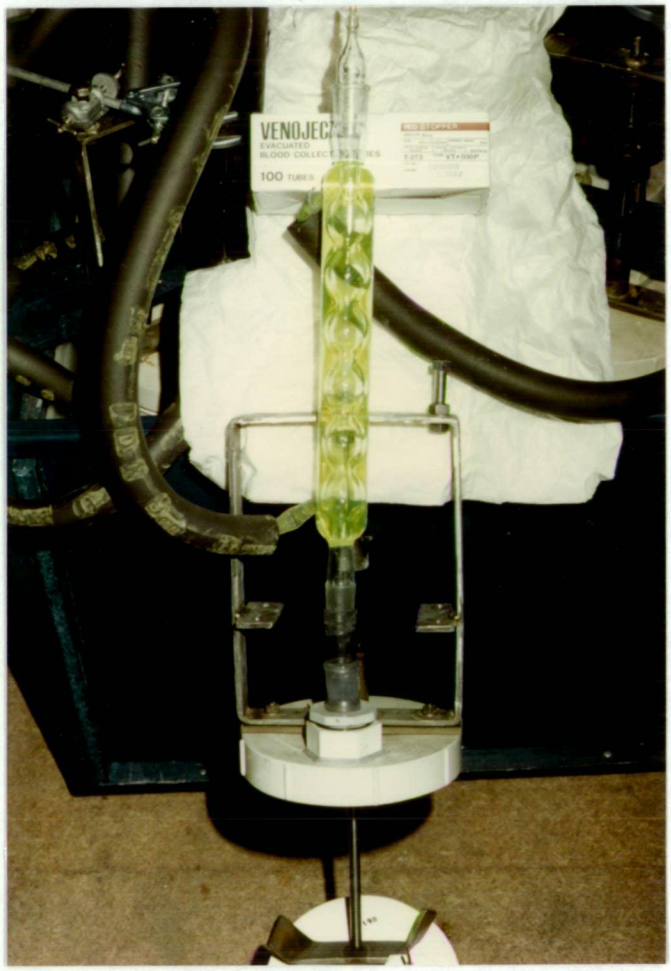
Figure 3

Automated gas sampling system.

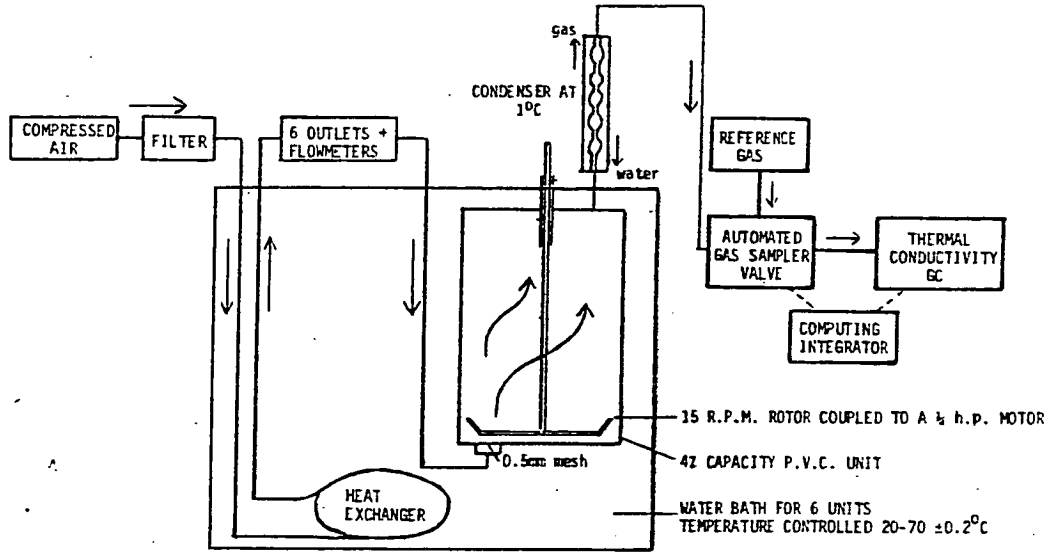
[1] Three-way solenoid valve, the arrow indicates the direction of gas flow in the normal (unenergized) state. Every five hours the time event switcher initiated the following sequence:

> lines A and B were energized, diverting helium from the sample loop to a direct vent enabling exhaust gas from the first unit to purge the sample loop for two minutes. Simultaneous signals through lines A, B and C returned the compost exhaust to vent, flushed the sample loop with helium into the GC and initiated a twelve minute run with the GC/computing integrator. This cycle was repeated for the remaining five units then followed by a three hour wait.




VENOJECT
EVACUATED
100 TUBES

SCHEME OF THE BENCH-SCALE COMPOSTING APPARATUS



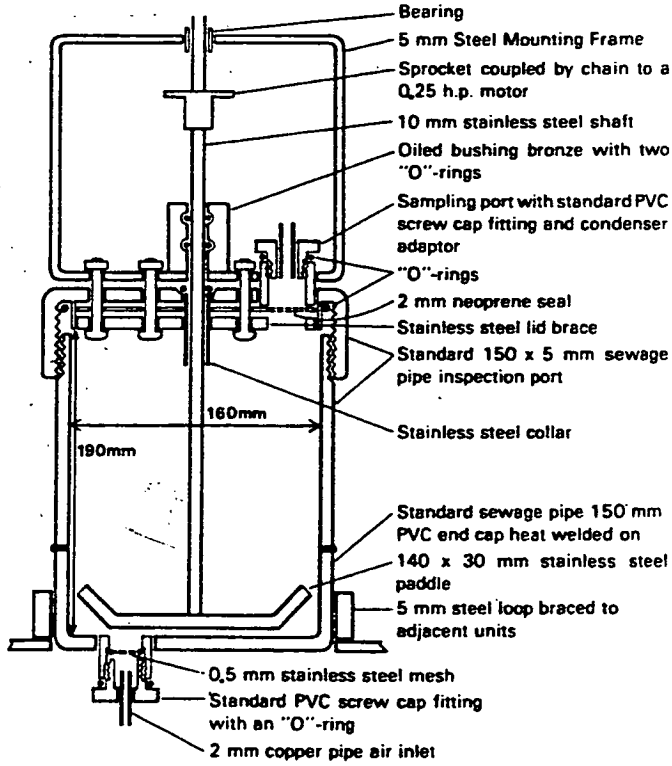


Fig. 2—Polyvinyl chloride composter unit.

Figure 1

Scheme of the bench-scale composting apparatus.

Compressed air was filtered through activated carbon and silica gel, heated to the temperature of the composter units and the flow rate was controlled by individual needle values to six parallel PVC units (Figure 2). A natural temperature increase at rates consistent with those observed in the large-scale compost heaps (5° increase per day to 55° or 60°) was simulated by having the units immersed in a water bath. The contents of each unit was mixed for 15 min h^{-1}. The moisture content of each unit was maintained at about 214% m.c. by the use of a cooled (1°C) condenser which returned condensate from the effluent gas to the unit by gravity flow. Effluent gas (1.0 mL) was sampled either manually pr automatically (Figure 3) for respiratory gases by gas chromatography.

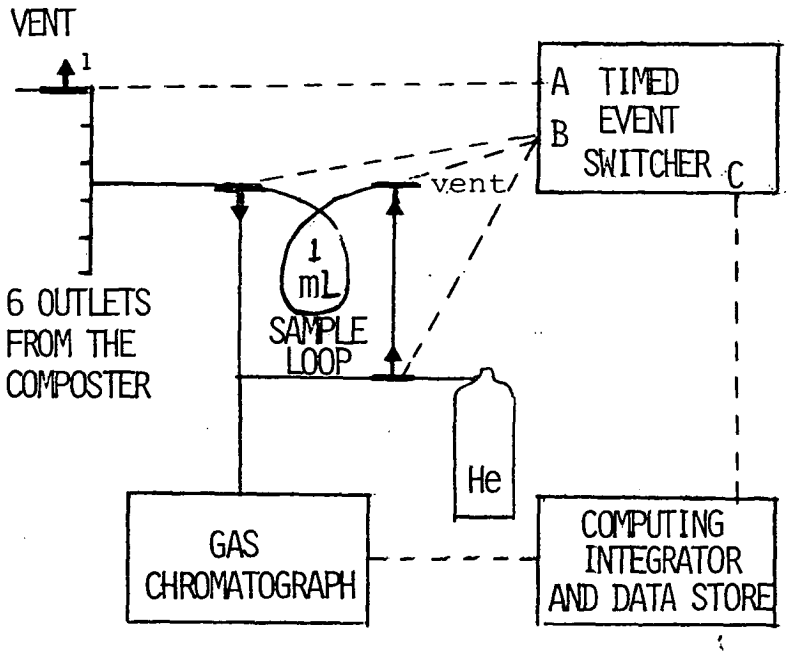
Figure 2

Polyvinylchloride composter unit.

Plate 3

Large-scale sewage-bark compost heap.

The large-scale heap was constructed from mixed eucalypt bark and sewage cake to give a C:N ratio of 35 and m.c. of 214%. Mixing was partly by hand and partly by front-end loader. The composition of components are given in Table 4. The heap is shown after the first turning (second week) and was turned again at week nineteen. A pile of raw eucalypt bark is shown in the background.



Automated gas sampling system.

Table 4

Composition of bark, fish wastes and sewage cake used in compost mixes [1]

% Composition	*Eucalyptus delegatensis* bark	Fish waste	Sewage cake
Total C	53.9	19.3	39.5
Total N	0.35	8.03	3.04
Ammonium-N	30 ppm	0.2	0.9
Nitrate-N	n.d.	0.01	0.03
Total P	10 ppm	1.3	0.5
Total K	80 ppm	1.7	0.07
Total Ca	3.8	7.8	2.7
Ash	2.0	23.0	32.9
Cellulose	38.1	(6.7)	n.t.
Hemicellulose	31.0	(10.4)	n.t.
Lignin	21.6	0.0	n.t.
Lipid	1.0	8.0	n.t.
Protein	2.2	50.2	n.t.
Soluble carbohydrate	4.1	1.7	n.t.
"Tannins"	44.8	10.3	7.3
Initial pH	4.5	6.2	5.8

[1] Determinations were made as follows:-

Organic carbon by the titrimetric method (Allison, 1965), total nitrogen by the micro-Kjeldahl method (Bremner, 1965a), mineral-N by distillation of 2N KCl extracts (Bremner, 1965b); total levels of other elements were determined after an initial nitric perchloric acid digestion with P measured in vanadomolybdophosphoric-yellow in a nitric acid system (Chapman and Pratt, 1961a), K by flame photometry (Chapman and Pratt, 1961b) and Ca by atomic absorption spectrophotometry (AAS) (Pye Unicam Ltd., 1972); ash, cellulose, hemicellulose, lignin, lipid, protein and soluble carbohydrate by proximate analysis (Allen, 1974); "tannins" were estimated after extraction in boiling water and addition of tungsto- and molybdophosphoric acids by spectrophotometry against a tannic acid standard curve (American Public Health Assoc., 1971); and pH in a 1:5 suspension of 1N KCl.

Note. Only *E.delegatensis* bark was assayed, large scale heaps also included *E.regnans* and *E.obliqua*.

Values in parenthesis only represent residues determined gravimetrically. n.d. - not detected. n.t. - not tested.

(d) run of the composter. For the first two runs, four replicates of each treatment were assayed so each treatment was also replicated over the 30d period. Treatments were randomly placed between the six PVC units. All compost mixes were brought to 214% moisture by the addition of distilled water.

2.3 Monitoring of Compost Activity and Conditions

Microbial activity in the large-scale compost heaps was monitored for changes in temperature, carboxymethyl cellulase (CMCase) activity, pH and estimated "total" numbers of bacteria. Assays were performed on subsamples obtained after bulking ten snap samples (10g each) taken at random during each sample period. Temperature was automatically monitored for the three weeks of composting at six hourly intervals by a chart temperature recorder (Grant Instruments Ltd., Toft, Cambridge, England) with three thermocouples placed at 0.3, 1.0 and 2.0 m depth from the centre top of each heap. For the remaining composting period temperature was monitored manually at weekly, then monthly intervals. CMCase activity was assayed using 1g subsamples as described below (2.3.1). The pH was determined with a glass electrode in a 2N KCl extract of freshly sampled compost (20g wet weight in 100 mL KCl, shaken for 1h). Bacteria were enumerated on tryptic soy agar (2.5.1.1).

During bench-scale composting compost respiratory activity was assayed by GC. Anaerobic bacterial activity was indicated by the presence of volatile fatty acids (VFA) by GC of acidified ether extracts of compost samples (2g wet weight) (VPI, 1974). The GC was run isothermally at 160° using a hot wire thermal conductivity detector and 4mm by 2m glass column packed with SP1220 (Supelco). Mineralization of nitrogen was determined by steam distillation of NH_4^+, NO_3^- and/or NO_2^- present in 2N KCl extracts of freshly sampled compost (2 g wet weight in 10mL KCl) (Bremner, 1965b). Urea was

assayed in 2N KCl-phenylmercuric acetate extracts by the colorimetric method of Douglas and Bremner (1970). Compost pH was determined with a glass electrode using the KCl extracts. Volatilized nitrogen was collected in 0.1M H_2SO_4 traps (50 mL) and assayed for NH_4^+-N and NO_2^--N (from N-oxides) by steam distillation (Bremner, 1965b) every 2-3d. Moisture content of compost materials was determined after 24 h at 105°. Temperature within the units was periodically monitored via a temperature probe passed through the condenser to the PVC units. Humification was periodically assayed by the absorbance at 550nm (Kaila, 1956) and ratio of absorbance at 440 and 640nm (Kononova, 1966) in tetra-sodium pyrophosphate (0.025 M) extracts of compost.

2.3.1 Compost Enzyme Activity

The CMCase activity in compost was assayed by a modified viscometric method of Braid and Line (1980). Ten mL 0.4% NaCMC in 0.15 M phosphate buffer pH 6.0 was incubated with 0.5g (wet weight) of compost at 65° for 1 h. Aliquots (1.0mL) of filtered solution were then placed in a Wells-Brookfield microviscometer which was operated at 60 revs min^{-1} at 30° C. The percentage reduction in viscosity was calculated using the formula:

$$(C-S)/(C-W) \times 100$$

where C= viscosity of NaCMC control sample,
S= viscosity of sample being assayed,
W= viscosity of water.

Lipase activity was assayed by the method of Deploey, *et.al.* (1981), but modified by incubating 0.5g (wet weight) of compost with the tween 20 at 65° for 1 h. One unit of lipase was defined as the release of 0.10 mM of fatty acid h^{-1}. Laccase activity was assayed spectrophotometrically at 525 nm with 0.1 % syringaldazine (Harkin, *et.al.*, 1974).

2.3.2 Estimation of Microbial Biomass in Compost

Microbial biomass in 30 d compost (at 55°) was estimated using the chloroform fumigation technique (Jenkinson and Powlson, 1976) with a k-factor (proportion of CO_2 released in 10d compared to an unfumigated control) of 0.45. The chloroform was purified and stored as described by Jenkinson and Powlson (1976).

2.4 Chemical Analyses

Organic carbon was estimated by the Walkley-Black titrimetric method (Allison, 1965), with the correction factor (f=1.109) being calculated from the carbon yield from tannic acid. Total nitrogen determinations were made by the micro-Kjeldahl method (Bremner, 1965a) while NH_4^+-N and NO_3^--N + NO_2^--N were determined by steam distillation (Bremner, 1965b). For other elements, materials were initially digested in nitric-perchloric acid (Piper, 1950) then total phosphate was determined by the vanadomolybdophosphoric-yellow assay using a Hitachi Perkin-Elmer 139 U.V./Vis. spectrophotometer (Chapman and Pratt, 1961a). Total potassium was determined by flame emission spectroscopy on an EEL flame photometer (Evans Electroselenium, Ltd., Essex) (Chapman and Pratt, 1961b), and total calcium by A.A.S. (Pye Unicam Ltd., 1972).

Cellulose, hemicellulose, lignin, lipid and ash were determined by proximate analysis (Allen, 1974) and "total" tannins were estimated by a colorimetric method following extractions from bark in boiling water (Amer. Pub. Health Assoc., 1971). Individual phenolics were assayed quantitatively by high pressure liquid chromatography (HPLC) of methanol extracts (see 2.3.2.1).

2.5 Isolation and Enumeration of Microorganisms

Samples of compost (5.0g w.w.) were macerated (Stomacher lab-blender 400) for 4 min. in sterile 95 mL volumes of 0.01% (w/v) peptone (Board and Lovelock, 1975) and 0.1mL aliquots of ten-fold serial dilutions spread, in triplicate, onto solid media. Plates were incubated at 28° for one week for mesophiles or at 55° for 4d for thermophiles.

2.5.1 Microbiological Media

Unless otherwise stated all media were sterilised by autoclaving (121° for 15 min). Filter-sterilization was performed where indicated using 0.45um Millipore filters.

2.5.1.1 For Total Estimated Numbers & Identification of Microorganisms

Bacteria were cultured on the modified Tryptic Soy Agar (TSA) of Martin (1975) containing (g/L dist. water): Gibco Diagnostic soy broth, 3.0; yeast extract, 0.1; L-cysteine hydrochloride (for anaerobic culture only), 0.5; agar, 20. Fungi were cultured on Difco potato dextose agar (PDA).

2.5.1.2 Estimated Numbers and Assay of Microorganisms Utilizing Complex Wood Components

Bacteria utilizing complex polysaccharides or lignin were estimated using double-layer agar plates. The mineral salts phosphate buffered (pH 7.2) medium of Hankin and Anagnostakis (1977) formed the basal layer. This contained (g/L dist. water): Na_2HPO_4, 6.0; KH_2PO_4, 4.0; $(NH_4)_2SO_4$,1.0; sodium thioglycollate, 0.50 (for anaerobic culture only); $MgSO_4$, 0.20; $CaCl_2$, 0.001; $FeSO_4.7H_2O$, 0.001; $ZnSO_4$, 7×10^{-5}; $CuSO_4$, 5×10^{-5}; H_3BO_3, 1×10^{-5}; MoO_3, 1×10^{-5}; yeast extract, 1; agar, 20.0. The overlay comprised the same medium amended as follows:-

- For **cellulolytic bacteria**, carboxymethylcellulose (No 7, Herculies, U.S.A.) (0.5%) (CMCA);
- For **lignolytic bacteria**, acid-swollen cellulose (0.3%) (Whatman CC41, W. & R. Balston, England) (Rantela and Cowling, 1966) and 0.1% filter-sterilized Indulin AT (Kraft pine lignin, Westvaco Co., Charleston, SC, USA) (purified as per Westermark and Eriksson, 1974) (LigA);
- For **pectolytic bacteria**, citrus pectin (0.5%) (Sigma Chemicals., St. Louis, U.S.A.) (PA);
- For **phenolic-acid decomposing bacteria**, either 0.05% gallic, ellagic or tannic acid (Sigma Chem., St. Louis, U.S.A.); or
- For **xylanolytic bacteria**, Larch xylan (0.5%) (*Larix sp.*, Sigma Chem., St. Louis, U.S.A.) (XA).

Cellulolytic bacteria were also enumerated on the medium of Teather and Wood (1982) in which cellobiose, 1.0% was the only C source. **Microaerophilic cellulolytic bacteria** were enumerated in tubes (30mL) containing a slope of the CMC agar which was overlain with a semi-solid (0.5% agar) and inoculated with compost dilutions in mineral salts phosphate buffered (pH 7.2) medium (Hankin and Anagnostakis, 1977). Most probable numbers were calculated from five replicate tubes over five 10-fold dilutions (5x5 tubes). **Cellulolytic bacteria and fungi** were assayed by release of dye from Remazol Brilliant Blue R (RBBR) cellulose as follows:-

- For bacteria the medium contained dye prepared as described by Leisola, *et.al.* (1975), containing (g/L dist. water): Whatman CC41 dye-cellulose suspension (100g/L with 1% aqueous RBBR dye), 20g; $NH_4H_2PO_4$, 2g; KH_2PO_4, 0.6g; K_2HPO_4, 0.4g; $MgSO_4.7H_2O$, 0.5g; $CaCl_2.2H_2O$, 0.1g; $MnSO_4.H_2O$, 0.005g; yeast extract, 1g; agar, 5g.
- For fungi, a modified Nilsson's (1973) BV11 medium containing (g/L dist. water): $(NH_4)_2SO_4$, 0.5g; KH_2PO_4, 1.0g; KCl, 0.5g; $MgSO_4$, 0.2g; $CaCl_2.H_2O$, 0.1g; yeast extract, 0.2g; agar, 15g; acid-swollen cellulose, 2.5g. Whatmans CC41 (W. & R. Balston, England) microgranular cellulose powder was swollen with ortho-phosphoric acid according to the method of Walseth (1952). The swollen cellulose was dialysed (Union Carbon 50mm dialysis tubing) with water before repeated decantation and resuspension.

Cellulolytic activity was also assayed in microcrystalline cellulose and NaCMC media. The RBBR-dyed cellulose in the medium described above was replaced with either 1g/L of Whatman CC41 cellulose or 5g/L NaCMC. Fifty mL of the medium was added to 250mL plugged and capped Erlenmyer flasks.

Bacteria were screened for **lignolytic activity** on the medium of Sundman and Nase (1971) which contained (g/L dist. water): glucose, 5; malt extract, 1; ammonium tartrate, 5; indulin AT (as prepared above), 0.5; and the mineral salts phosphate buffered (pH 7.2) medium of Hankin and Anagnostakis (1977).
A quantitative assay of lignolytic activity was obtained by the method of Janshekar, *et.al.* (1981) using the following medium (g/L dist. water):- K_2HPO_4, 1.60; KH_2PO_4, 0.50; $(NH_4)_2SO_4$, 1.25; NH_4NO_3, 1.00; $MgSO_4.H_2O$, 0.50; NaCl, 0.25; $FeCl_3.6H_2O$, 0.025; $CaCl_2$, 0.010; yeast extract, 0.10.

2.5.1.3 Estimated Numbers & Assay of Lipolytic Bacteria

The lipid agar of Hankin, *et.al.* (1979) was used, containing (g/L dist. water): peptone, 10; NaCl, 5; $CaCl_2.H_2O$, 0.1; yeast extract, 0.1; agar, 20; and tween 20, 20 m; with pH adjusted to 7.4.

2.5.1.4 Most Probable Number (MPN) and Assay of Nitrifiers

(a) Autotrophic Nitrifiers

Two media were used in 5x5 tube (5mL bijoux bottle) MPN's for autotrophic nitrifiers.

1. The medium of Soriano and Walker (1968) as modified by Belser and Schmidt (1978) containing (g/L dist. water): $(NH_4)_2SO_4$, 0.5; KH_2PO_4, 0.2; $CaCO_3$, 7.5; $CaCl_2.2H_2O$, 0.04; $MgSO_4.7H_2O$, 0.04; Fe^{3+}citrate, 0.0005. After autoclaving the pH was adjusted to 7.4 with 5% $NaCO_3$.
2. The medium of Golovacheva (1975) contained (g/L dist. water): $(NH_4)_2SO_4$, 2.0; K_2HPO_4, 1.0; $CaCO_3$, 10; $MgSO_4.7H_2O$,

0.5; NaCl, 0.5; Fe EDTA, 0.005; trace metals 0.5mL. The pH was adjusted to 7.6 with 5% Na_2CO_3 and the medium modified by the addition, after autoclaving, of 0.1% penicillin G (Sigma Chem.) to inhibit Gram positive bacteria.

(b) Heterotrophic Nitrifiers

Heterotrophic nitrification was assayed using three media in 5x5 MPN tubes.

1. The medium of Hirsch, *et.al.* (1961) containing (g/L dist. water): glucose, 0.9; $(NH_4)_2HPO_4$, 1.65; K_2HPO_4, 1.4; KH_2PO_4, 0.8; $MgSO_4.7H_2O$, 0.5; $CaCl_2$, 0.1; $FeSO_4.7H_2O$, 0.001; $ZnSO_4.7H_2O$, 0.001; $NaCl_2$, 0.01; $MnCl_2.4H_2O$, 0.01; MoO_3, 0.01. Filter-sterilized glucose and $(NH_4)_2HPO_4$ were added to the medium after autoclaving and the pH adjusted if necessary to 7.2.
2. The medium of Gunner (1963) contained (g/L dist. water): NH_4Cl , 2.0; KH_2PO_4, 1.0; K_2HPO_4, 1.5; $MgSO_4.7H_2O$, 0.6; Fe EDTA, 0.005; $ZnSO_4.7H_2O$, 0.03. After autoclaving the pH was adjusted to 7.0 with 0.05M succinic acid/NaOH buffer.
3. The medium of Verstraete and Alexander (1972a), containing (g/L dist. water): KH_2PO_4, 8.2; NaOH, 1.6; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $CaSO_4.2H_2O$, 0.0005; $CuSO_4.5H_2O$, 0.0005; $FeCl_3.6H_2O$, 0.0005; $ZnSO_4.H_2O$, 0.0005. The pH was adjusted to 7.0 prior to autoclaving. Filter-sterilized solutions of Na acetate, 16.9 and $(NH_4)_2SO_4$, 4.7 were then added.

2.5.1.5 Medium used in Cell Wall and Esterase Assays

The tryptone medium (TB) of Sargeant, *et.al.* (1971) was used, containing (g/L dist. water): Bacto tryptone, 20; yeast extract, 10; sucrose, 10; K_2SO_4, 1.3; $NaH_2PO_4.2H_2O$, 3.2; $MgSO_4.7H_2O$, 0.27; $MnCl_2.4H_2O$, 0.015; $FeCl_3.H_2O$, 0.007; citric acid, 0.32. The pH was adjusted to 7.1 with 6N KOH.

2.5.1.6 Screening for Nuclease Activity

The screening for DNase producers was performed using DNase test agar (Difco Lab. Detroit U.S.A.). Arella and Sylvestre's (1979) medium was used to screen for RNase producers. The medium contained (g/L dist. water): Difco trypticase soy powder, 23; agar, 15; yeast ribonucleic acid, 0.0002. The pH was adjusted to 7.4 with 0.05M tris(hydroxymethyl)aminomethane-HCl buffer (Tris-HCl). For the examination of isoenzymes of RNase the following medium was used (g/L dist. water): sucrose, 5.0; tryptone, 10.0; NaCl, 2.0; yeast extract, 0.5; with yeast RNA, 0.5 in the overlay of double layered plates. The pH was adjusted to 7.2 with 0.05M Tris-HCl.

2.5.2 Anaerobic Techniques

Dilution plating and isolations were carried out as described above, except that all diluents and media were pre-reduced (with 0.01% cysteine hydrochloride) and stored in an anaerobic chamber (Kaltec, South Australia) for at least one day. All microbiological manipulations were conducted within the anaerobic chamber. An anaerobic atmosphere within the chamber of about 80% N_2, 10% H_2 and 10% CO_2, was maintained with the palladium catalyst being replaced weekly with freshly regenerated palladium. Mesophilic anaerobes were incubated in the chamber (28°) while the thermophiles were placed in a gas tight jar (containing palladium) and transferred to a 55° incubator.

2.6 Detection Methods Used for Assaying Microorganisms

2.6.1 Detection Methods used for Assaying Utilization of Complex Carbon Sources

2.6.1.1 Utilization of Cellulose

After 4-6d growth on CMC media (2.5.1.2), plates were flooded with 0.04% congo red to stain undegraded NaCMC (Teather and Wood,

1982). Regions of cellulase activity were preserved for several weeks by a subsequent washing with 0.1N HCl. With the medium of Teather and Wood (1982), plates were overlain with the CMC agar (2.5.1.2) after 4-6d growth, incubated a further 16 h then stained as above. Relative cellulolytic activity was determined by the viscometric method previously described (2.3.1) after 14d growth in the microcrystalline cellulose or CMC medium without agar (50mL in 250mL flasks).

2.6.1.2 Utilization of Lignin

Possible utilization of lignin was demonstrated by the production of laccase on LigA (2.5.1.2) after 4-8d growth. Syringaldazine (0.1%) was poured over the incubated plates, with a red colour development indicating a positive test (Harkin, *et.al.*, 1974). Lignolytic bacteria were also screened on the medium of Sundman and Nase (1971). After 10d growth bacteria were scraped off plates and residual lignin was stained with $FeCl_3$-$K_3(Fe(CN)_6)$. A quantitative assay of lignolytic activity was obtained by the method of Janshekar, *et.al.* (1981). After one month's growth in liquid lignin medium (2.5.1.2) phenolic in cell-free supernatants were assayed against dioxane:water (1:1, v/v) at 281nm (Hitachi Perkin-Elmer UV/Vis spectrophotometer model 139, Japan).

2.6.2 **Detection of Nitrification**

Broths were assayed at intervals (following 6-12 weeks incubation) for NO_3^- and NO_3^- using the sulfanilic acid-alpha-naphthylamine spot test (Skerman, 1969).

2.6.3 **Estimated Numbers of Faecal Indicator Bacteria**

(a) Estimated numbers of Faecal Coliforms

Yellow colonies on suitable dilution plates of Lactose Teepol agar (LTA) were tested for indole and acid production, Voges-Proskauer reaction and citrate utilization

as described by Mara (1974).

(b) Estimated numbers of Faecal Streptococci

Following enumeration of red colonies on suitable dilution plates of m-enterococcus agar, representative colonies were streaked onto MacConkey agar. Confirmation of faecal streptococci was indicated by minute red colonies of Gram positive catalase-negative (in 5% H_2O_2) chains of cocci (Mara, 1974).

2.7 Identification of Isolates

2.7.1. Identification of Bacterial Isolates

Over 500 isolates representing the predominant flora and present in numbers in excess of 10^5 g^{-1} compost were selected from dilution plates and assessed for the ability to degrade various substrates (cellulose, pectin, lignin, lipid and xylan). Isolates were identified to genus using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The following tests were undertaken as described by Gordon, *et.al.* (1973): Gram reaction, cell morphology, position and size of spore, motility after 24 h, aerobic and anaerobic growth, acid and gas from glucose, arabinose, mannitol and xylose, growth in 7% salt, growth at pH 5.7, 28°, 55°, 60°, 65° and 70° and ability to hydrolyse starch and reduce nitrate. The production of catalase and oxidase were assayed as described by Skerman (1967). Extracellular DNase and RNase were assayed by the agar plate (2.5.1.6) methods of Arella and Sylvestre (1979). Flagella position was determined by transmission electron microscopy (2.7.1.5).

The identification of actinomycetes was aided by cell wall anaylsis (2.7.1.1) and decomposition of casein, tyrosine and xanthine was assessed by the methods of Staneck and Roberts (1974). Some coryneforms and actinomycetes were also assayed for

the mol percent guanine+cytosine (%G+C) (2.7.1.2) and type of pigment present (2.7.1.3). The morphology of bacteria growing *in situ* on agar was examined at various ages by light and scanning (2.7.1.4) microscopy using the methods of Cure and Keddie (1973).

Assistance with the identification of *Bacillus spp.* was provided by the use of the keys of Gordon, *et.al.* (1973), and by comparison of esterase patterns from thermophilic strains (*B.brevis* B636, *B.caldolyticus* B697, *B.coagulans* B666, *B.licheniformis* B691 and *B.stearothermophilus* B1518) supplied by Dr. Lindsay (C.S.I.R.O., Food Res., North Ryde N.S.W., Australia). Thermophilic *Bacillus spp.* were also analysed using the Clustan 1C program (Wishart, 1968) on a Burroughs B6800 computer. The simple matching coefficient (S_{sm}: Sokal and Michener, 1958) was applied with sorting by the unweighted pair-group method average (UPGMA) algorithm (Sneath and Sokal, 1973).

2.7.1.1 Cell Wall Analysis

The method of Staneck and Roberts (1974) was modified by growing cultures in 250 mL of TB (2.5.1.5) in 500 mL flasks. Cells were washed once in water then in 95% ethanol, dried then hydrolysed in acid. Ascending TLC was then performed (using drops of extracts) on 100° 1h activated glass plates precoated with 0.1 mm cellulose, no. 5552; Merck. The method allows the detection of the sugars arabinose, galactose, glucose, mannose, rhamnose, and ribose and stereoisomers of diaminopimelic acid (DAP).

2.7.1.2 Estimated %(G+C) Ratio

Cells were cultured in TB (2.5.1.5) for 1-2d to give 2-3g wet packed cells. The method of Gibson and Ogden (1979) was used to isolate and purify DNA for the determination of Mol %(G+C) using the thermal denaturation method of Marmur and Doty, (1962). Spectrophotometry at 260 nm was performed using a SP8-200 UV/Vis spectrophotometer fitted with a SPX 876 series 2 temperature programme controller (Pye Unicam, Holland). The %(G+C) was

calculated by the formula of Mandel and Marmur (1968) using *Nocardia cellulans* as a standard bacterium :-

$$\%GC_x = \%\ GC_{std} + 2.44\ (Tm_x - Tm_{std})$$

x = unknown sample of DNA
std = *N.cellulans* UQM [2] DNA (% GC = 72.7 moles %)
Tm = temperature at which half the absorbance at 260 nm has occurred

2.7.1.3 Pigment Analysis

Bacterial pigments were extracted by shaking 0.5g of washed packed cells in 10mL methanol for 3 min followed by centrifugation of cell debris at 10,000g. The absorption maxima of the methanol extracts were observed over the range 450-700 nm using a Pye Unicam scanning spectrophotometer.

2.7.1,4 Scanning Electron Microscopy (SEM) of Bacterial Isolates

Thin sections of agar cultures of bacteria (2 d at 55°) were fixed onto poly L-lysine coated glass coverslips in an osmium tetroxide atmosphere for 2 h followed by 4% glutaraldehyde in sodium phosphate buffer (0.1M, ph 7.2) for 4 h. Dehydration was carried out in a graded ethanol series using three changes of each alcohol concentration (30-50-60-70-80-90-95-100% alcohol in dist. water), infiltrated with acetone (50-100% in ethanol, two changes of each) then critical point dried from CO_2 (Polaron E-3000 critical point dryer, Polacon Equipment Pty. Ltd., Watford, England). Specimens were then mounted on copper stubs, coated with carbon and gold with a Dynavac sputter coater (model SC150, Dynavac High Vacuum Pty. Ltd., Victoria, Australia; at 20mA for 3 min at 0.3 Torr) and examined by SEM (Phillips 505) at 75kV. All photomicrographs were recorded on Polaroid type 52 polaplan film.

2.7.1.5 Transmission Electron Microscopy

Flagella arrangement and the presence of spores were determined by electron microscopy. Formvar coated copper grids

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2. Univ. Queensland Dept. Microbiology culture collection *N.cellulans* strain UQM2589.

were placed on drops of TB (2.5.1.5) cultures for 10-20 seconds, removed and placed on a drop of 0.5% uranyl acetate for 10 seconds. Excess stain was then removed and the grids air dried and examined using a Hitachi-H300 transmission electron microscope operating at 72kV.

2.7.1.6 Isoenzyme Patterns

(a) Preparation of protein extracts.

One day old broth cultures (TB, 2.5.1.5, 100mL) of bacterial isolates were centrifuged at 10,000g for 30 min at 4° (MSE). The supernatant was used for the assay of extracellular enzymes and the residue was further processed for the extraction of intracellular enzymes by the method of Sharp, *et.al.* (1980). The residue was washed twice in 0.15M NaCl, resuspended in the saline (3g wet cells/15mL) and sonicated on ice (5 times for 30 sec. 15kHz, MSA ultrasonic disintegrator). The final slurry was centrifuged at 48,000g for 1h at 4° to remove the cell debris. Both extra- and intracellular extracts were concentrated 25 fold (minicon B15 concentrator, Amicon) and stored at -18°.

(b) Preparation of electrophoresis gels.

The polyacrylamide gels contained 100 mL 0.15M pH 8.7 Tris-citrate (or Tris-NO_3 for lipases) buffer: acrylamide, 7.5g; N,N-methylene bisacrylamide, 0.1875g; N,N,N',N'-tetramethyl ethylene diamine, 0.33µmL; ammonium persulphate, 0.1g; and in some cases either yeast RNA, 0.01g; tributyrin, 0.2 mL; or tween 20, 40, 60 or 80, 0.2 mL. Gels were developed on a horizontal tank at 4° using a discontinuous borate buffer (0.1M, pH 8.7) at an initial standard current (15mA/5cm wide gel) until bromophenol blue indicator added at the loading site, had migrated 5cm.

(c) Staining of gels.

Patterns of esterase activity were developed and stained at 20° by the method of Baillie and Norris (1963) using the

following solution: 2mL of 0.1% fast naphthanol diazo B salt (Calbiochem, USA) and 1% naphthyl acetate in 50% acetone in water (stored at 4°) was mixed with 50 mL 0.1M Tris-maleate buffer pH 6.4 immediately prior to use. Patterns of lipase activity were developed by incubating the gels at 60° for 16 h in 0.15M phosphate buffer (one of a series pH 5.0, 6.0. 7.0, 8.0, 8.5, 9.0) with 0.01% $CaCl_2$. If tributyrin or tween was not included in the gel one was added to the buffer (tributyrin, 0.2 mL, tween 20, 40, 60 or 80, 0.2 mL). Regions of lipase activity were evident as clear zones with tributyrin, or white zones with the tweens. Patterns of RNase activity were developed by incubating the gels at 60° for 2h in one of a series of 0.15M phosphate buffer (pH 5.0, 6.0, 7.0 or 8.0). Gels were then stained with acridine orange (0.05% in 15% acetic acid) for 2h then destained with 1% acetic acid (with several changes). All stained gels were photographed using high contrast film (Kodak).

2.7.2 Identification of Fungal Isolates

Fungal isolates were identified to genus using "The Genera of Hyphomycetes from Soil" (Barrow, 1968). Colony morphology was directly observed under low power and the nature of the sporing structures and mycelium was observed under high power after staining with lacto-phenol cotton blue.

2.8 Toxins in Bark and Compost

2.8.1 Antibacterial Components in Composts

Compost samples (5g w.w.) were oven dried at 80° for 24h then ground (Laboratory Mill size 8", McPhersons Ltd., Australia) to pass through a 1.0mm mesh screen. A suspension of 10% ground compost in TSA was poured into 90mm Petri dishes and allowed to solidify at 25° from the horizontal. Plates were then irradiated with UV light (260nm) for 5 min, left for 30 min then overlayed with a further 10 mL of TSA as illustrated in Figure 4.

Predominant members of the compost microflora were then streaked across the plate covering a thin to thick layer of compost suspension.

2.8.2 Plant Bioassay of Phytotoxins

A modification of Still's, *et.al.* (1976) water extraction was used. The materials (20g samples) were ground (Laboratory Mill size 8", McPhersons Ltd., Australia) to pass a 1.0mm mesh screen, mixed with 250 mL of distilled water, homogenized for three min. in a Waring blender, filtered through three layers of cheesecloth and the extracts centrifuged at 5000g for ten min. The supernatant was condensed to 50 ml in a vacuum flask evaporator below 35° (Rotavapor-EL, Buchi, Switzerland), then used immediately or stored at 3° in the dark. Volumes (25mL) of the water extract were also shaken twice with 2.0g of water-insoluble polyvinylpolypyrrolidone (PVP) (Sigma Chem., U.S.A.) for three min each followed by centrifugation for ten min at 5000g and either used immediately or stored as above.

Ten lettuce seeds (*Lactuca sativa* cultivar Penlake, Yates) were placed on Whatman No.3 filter paper in 90mm Petri dishes. To each test batch of seeds was added 2.0 mL of a sterile 15mM potassium phosphate buffer (pH 6.0) and 5.0 mL of water extracts or PVP-treated extracts. Water extracts from fish mixes were filter sterilized to reduce microbial growth during the assay. Growth of twenty root radicles was assessed after three days at 21° in the dark.

2.8.2.1 Chemical Assay of Compost Extracts

Individual phenolics were qualitatively assayed by high pressure liquid chromatography (HPLC) following methanol extraction. Dry finely ground samples (0.5g) were shaken with 5mL of methanol for 15 min, solid material was removed by centrifugation at 10,000g for 10min and any remaining suspended material was removed by filtering (0.45um Millipore filter). The

liquid was dried by vacuum evaporation at 40° then redissolved in 0.2 mL of ethanol and stored at 0° in the dark or immediatly assayed by HPLC.

The liquid chromatograph (Waters Associates, U.S.A.) was fitted with dual pumps (Waters 660), a Waters automated gradient controller, a 280nm absorbance detector (Waters 440) and a C_{18} Radpak column (Waters Associates). A dual solvent system was used with solvent A consisting of 1% propionic acid in distilled water and solvent B of 50% ethanol in distilled water. A constant solvent flow rate of 2.00 mL min^{-1} was used throughout a 50 min run. The liquid carrier initially consisted of 100% of solvent A, at 25 min a linear mix of 1:1 of solvents A and B were used for the next 15 min followed by a final linear mix of 3:7 of A:B. Standards (made each day of assay at 10ppm in ethanol) were eluted as follows (time in min): gallic acid, 3.9; catechol, 6.0; protocatechuic acid, 6.6; p-hydroxybenzoic acid, 9.6; catechin, 12.1; p-coumaric, 18.5; phloridzen, 28.0.

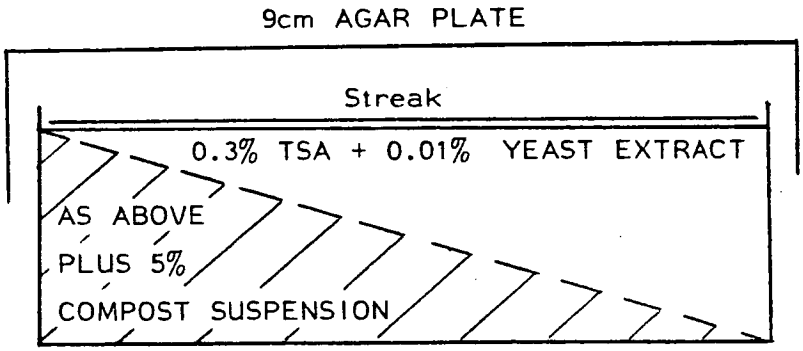
Figure 4

Microbial assay of compost toxicity.

Compost samples (5g w/w) were oven dried at 80° for 24h then ground (Laboratory Mill size 8", McPhersons Ltd., Australia) to pass through a 1.0mm mesh screen. A suspension of 10% ground compost in TSA was poured into 90mm Petri dishes and allowed to solidify at 25° from the horizontal. Plates were then irradiated with UV light (260nm) for 5 min, left for 30 min then overlayed with a further 10 mL of TSA as illustrated.

Predominant members of the compost microflora were streaked across the plate covering a thin to thick layer of compost suspension.

MICROBIAL ASSAY OF COMPOST TOXICITY



2.9 Physical Properties of Bark Compost

Bulk density, particle size distribution and moisture characteristics were determined by the methods described by Prasad (1979). Bulk density was determined by oven drying 5L of material at 102° for 24h. Particle size was determined using oven-dried material placed on the topmost sieve of a column of sieves (of 2mm to 0.1mm mesh diameter) shaken for 5 minutes at 180 shakes $min.^{-1}$.

Moisture characteristics were determined using evenly packed (0.5 g cm^{-2}) samples, in rings on a porous plate, at suctions of 10, 31, 50 or 100 cm (pF 1.0, 1.5, 1.7 and 2.0). Samples were weighed after equilibrium (normally 24-48 h). Total porosity (TP) was defined as the moisture content at zero suction. Air space (AS) was defined by the volume of water between TP and moisture at 10 cm suction. Easily available water (EAW) was the volume of water released when the suction was increased from 10 to 50 cm. Water-buffering capacity (WBC) was the volume released from 50 to 100 cm suction. Difficultly available water (DAW) was determined on a high pressure plate apparatus being the moisture between pF 2 and 4.2 .

3 - RESULTS

3.1 Large-Scale Composting

3.1.1 Monitoring of Temperature

Temperatures recorded at different levels through the centre of each pile are illustrated in Figure 5 (Appendix 2.1). Generally in the early stages of composting similar temperatures were found at depths of 1.0 and 2.0m, these being about 10° warmer than at 0.3m. After 75d the upper two probes in the urea-bark heap gave similar and lower readings than the probe at a depth of 2m. Temperatures in the sewage-bark heap, however, generally maintained a difference of about 10° between the top probe and that at 2m.

In the urea-bark pile, peak temperature (73°) was not reached until 16d, two days after the first turning. Mean (of the three probes) temperatures of over 55° were, however, reached after 8d and held for a further 40d. After the second turning (136d) the urea-bark pile held a mean temperature of over 55° for a further 54d.

Temperatures within the sewage-bark pile were lower than those in the urea-bark pile. A peak temperature of 63° was reached on days 10, 15 and 20. A mean temperature of over 55° was held for parts of days 10-12, 16 and 18-48. After the second turning a further peak of activity was observed (mean peak activity 52°), but in contrast with the urea-bark pile this activity was not maintained.

3.1.2 Monitoring of Cellulase Activity, pH and Moisture Content

The pattern of relative carboxymethylcellulase (CMCase) activity in the urea- and sewage-bark heaps was similar (Figure 6), although activity was consistently greater in the urea-bark heap. After the second turning of the heaps the urea-bark heap

igure 5

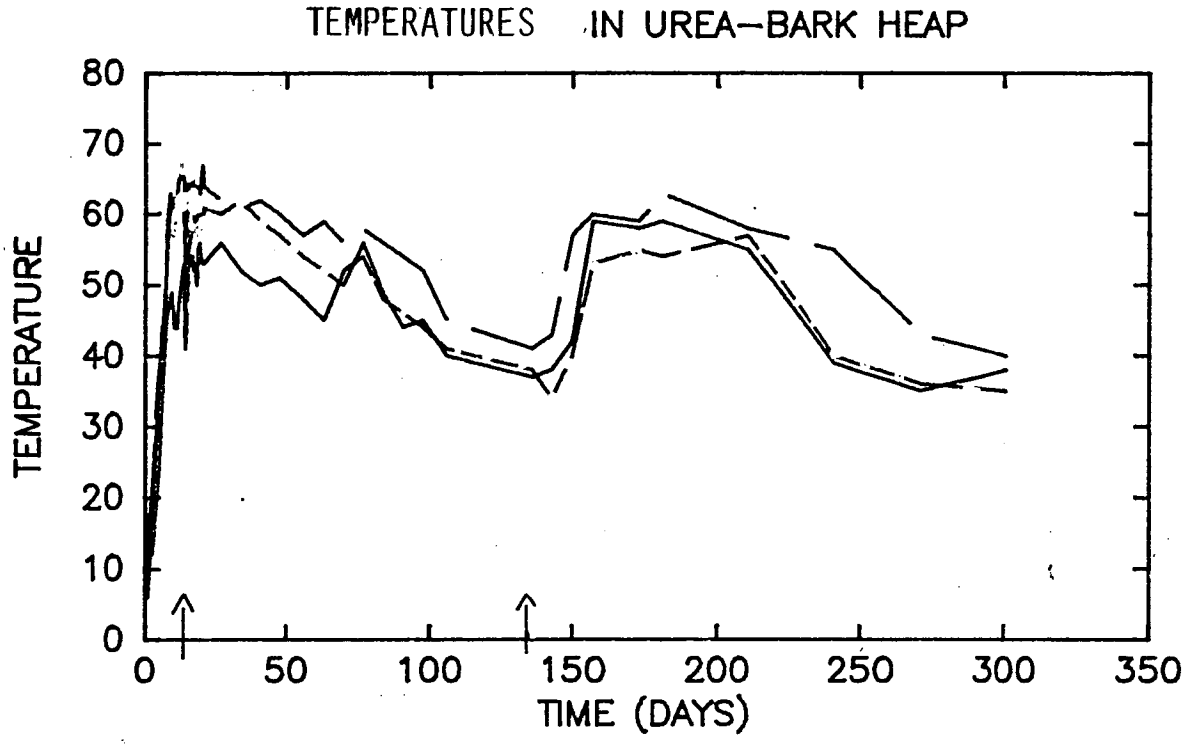
emperatures in large-scale compost heaps during 300d of
mposting urea- and sewage-bark mixes.

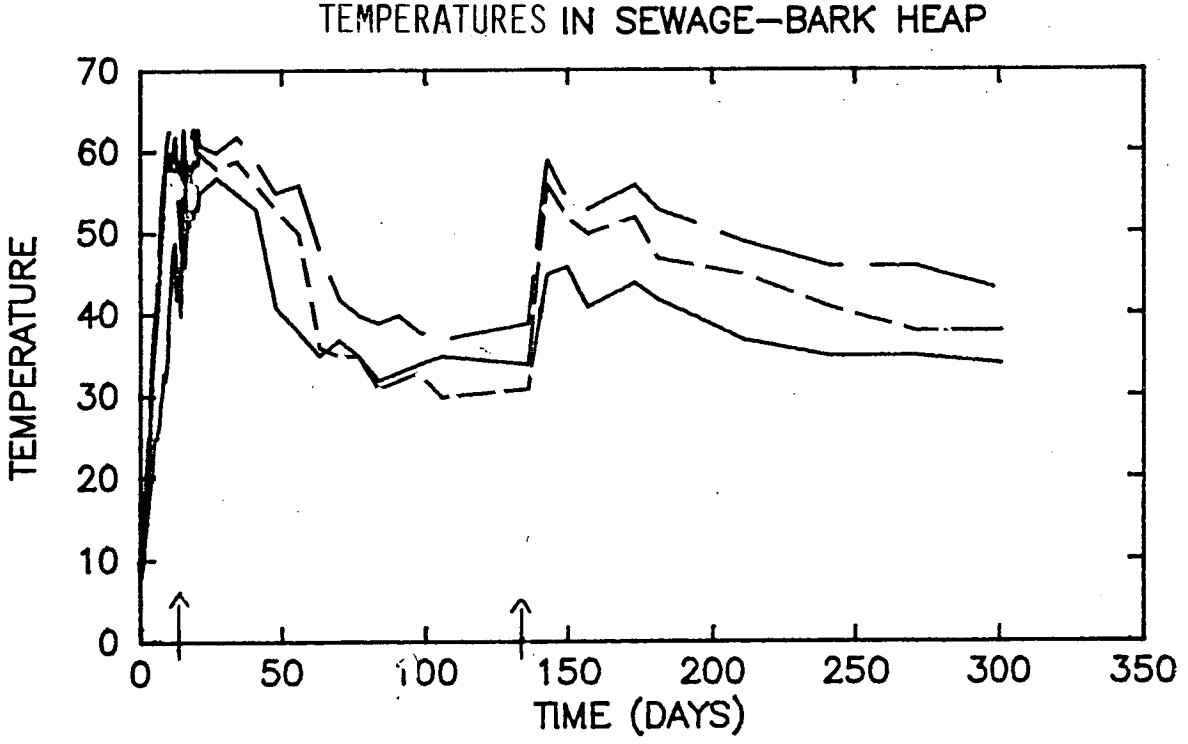
emperatures within each heap were monitored via three
hermocouples placed at 0.3, 1.0 and 2.0 m depth from the
entre top of the heaps.

rrows indicate time each compost heap was turned.

——— 0.3 m probe
— —— 1.0 m probe
- — — 2.0 m probe

ata for Figure 5 are shown in Appendix 2.1


TEMPERATURES IN UREA–BARK HEAP
TEMPERATURE
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
TIME (DAYS)


TEMPERATURES IN SEWAGE–BARK HEAP
TEMPERATURE
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
TIME (DAYS)

igure 6

oisture content, pH and carboxymethylcellulase activity during
omposting of urea- and sewage-bark in large-scale heaps.

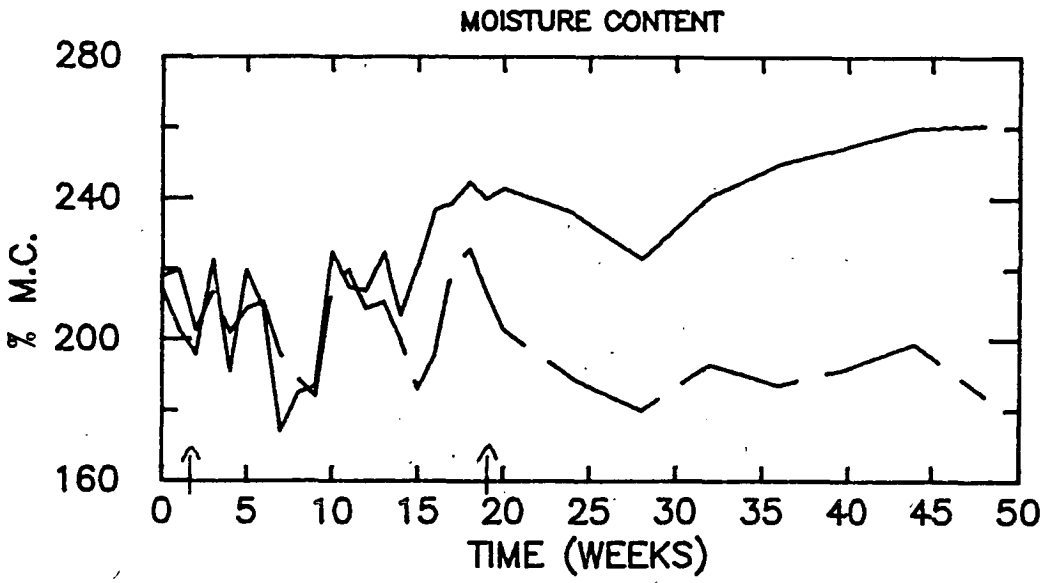
ompost samples (1g) were oven dried (105°C, 24h) for m.c.;
haken in a 1:5 suspension with 2N KCl to determine pH; or
ncubated with 20mL 0.4% NaCMC in 0.05 M phosphate buffer, pH
.0, for 1h at 65°C to determine the CMCase activity by
icroviscometry.

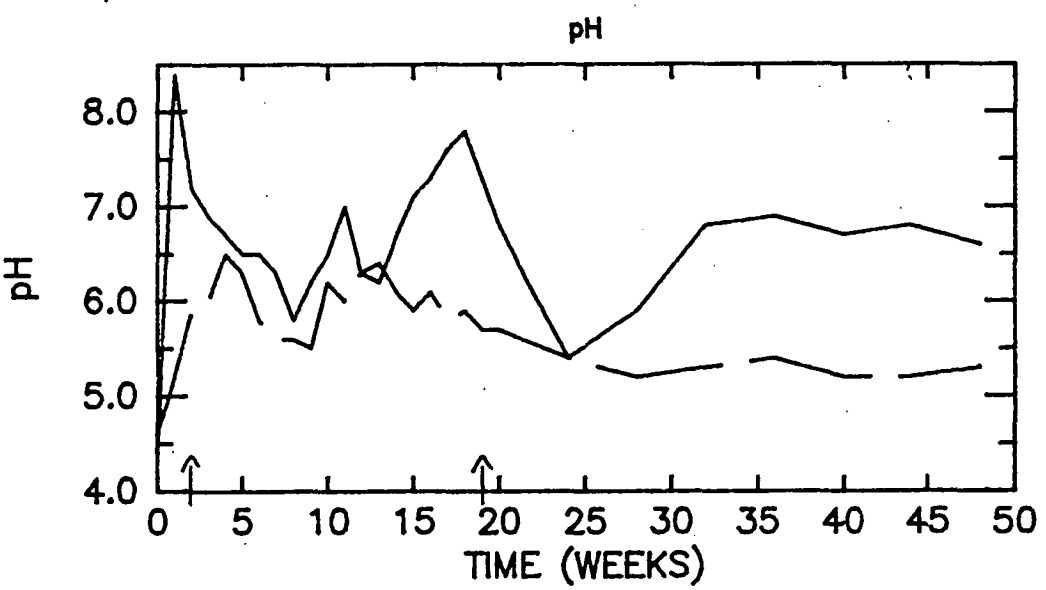
ach determination was the mean of duplicate samples.

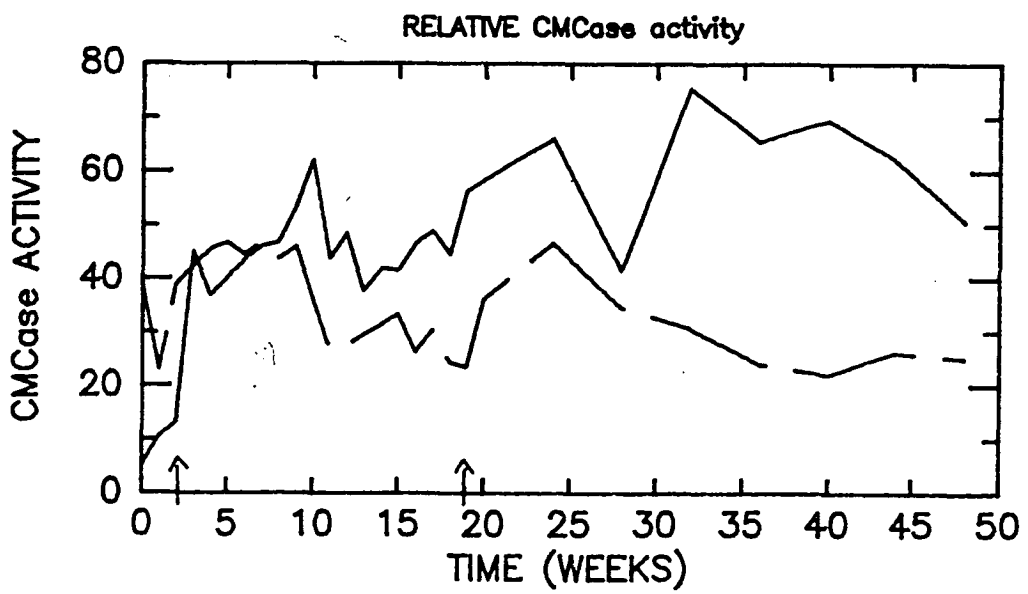
rrows indicate time each compost heap was turned.

——— Urea-bark, Initial C:N=35
— — Sewage-bark, Initial C:N=35

ata for Figure 6 are shown in Appendix 2.2


MOISTURE CONTENT
280
240
200
160
% M.C.
0
5
10
15
20
25
30
35
40
45
50
TIME (WEEKS)


pH
8.0
7.0
6.0
5.0
4.0
pH
0
5
10
15
20
25
30
35
40
45
50
TIME (WEEKS)


RELATIVE CMCase activity
80
60
40
20
0
CMCase ACTIVITY
0
5
10
15
20
25
30
35
40
45
50
TIME (WEEKS)

exhibited a significantly ($p < 0.01$) greater CMCase activity for the remainder of the experiment. This difference was unlikely to be an effect of pH or moisture content, because although these factors differed markedly in the two heaps, in each case optimal activity (Figure 6, Appendix 2.2) lay mid-way between the extremes observed.

3.1.3 Enumeration of Bacteria

Numbers of colony forming units (CFU) of mesophilic and thermophilic bacteria are illustrated in Figure 7. Total estimated numbers of bacteria were relatively constant at 10^7-10^8 g^{-1} wet compost while estimated numbers of mesophilic and thermophilic bacteria reflected the change in temperatures below or above about 45° (Appendix 2.2). Numbers of thermophilic CFU were well correlated with compost temperature in both heaps ($r^2=0.75$) while there was a poorer negative correlation between mesophiles and temperature ($r^2=-0.31$).

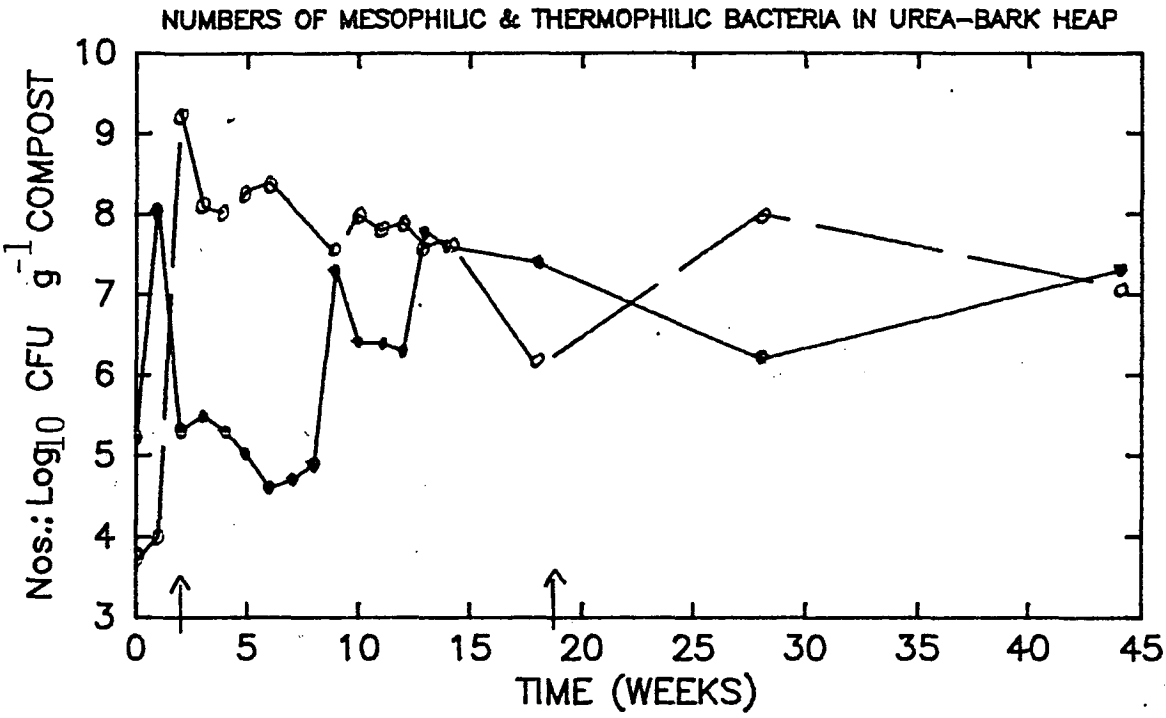
igure 7

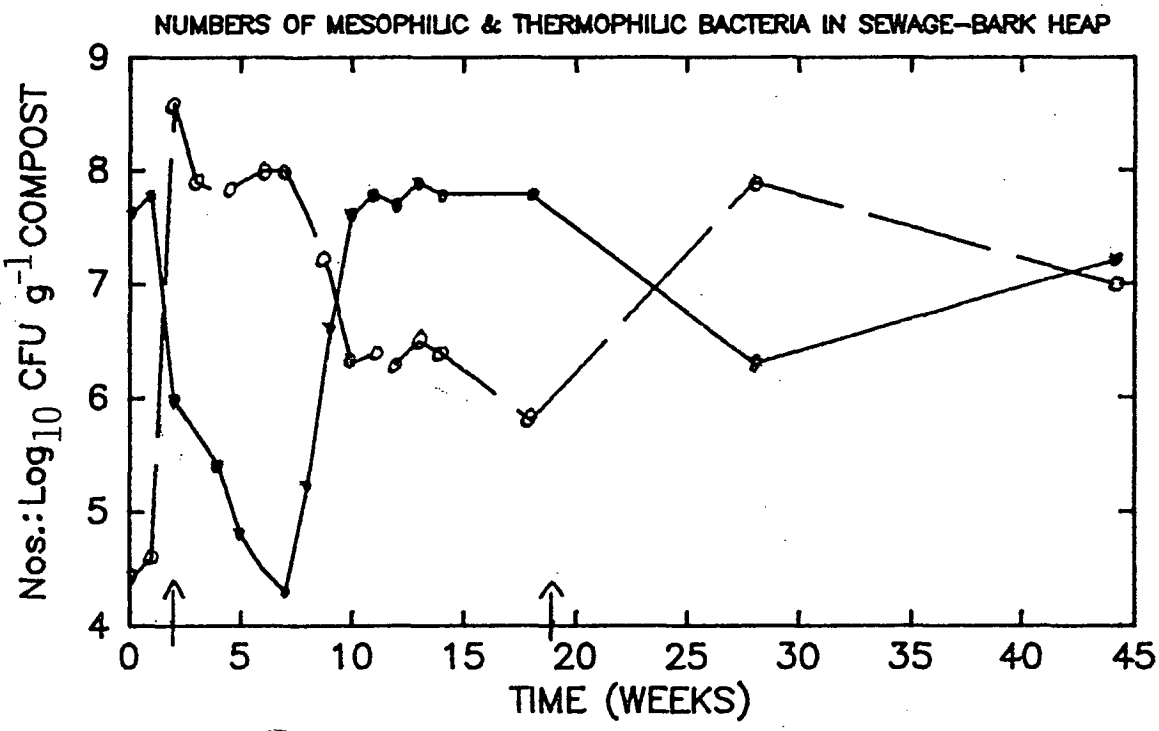
stimated numbers of bacteria during large-scale composting of
rea- and sewage-bark mixes.

acteria were enumerated on 0.3% tryptic soy agar plus 0.01% yeast
xtract (2.5.1.1). Serial dilutions were carried out in 0.01%
eptone and plates were incubated at 28° or 55° C for 3-5d. Each
alue was the mean of duplicate plates.

rrows indicate time each compost heap was turned.

——— Mesophiles
— — Thermophiles
ata for Figure 7 are shown in Appendix 2.2


NUMBERS OF MESOPHILIC & THERMOPHILIC BACTERIA IN UREA–BARK HEAP
Nos.: Log_{10} CFU g^{-1} COMPOST
10
9
8
7
6
5
4
3
0
5
10
15
20
25
30
35
40
45
TIME (WEEKS)


NUMBERS OF MESOPHILIC & THERMOPHILIC BACTERIA IN SEWAGE–BARK HEAP
Nos.: Log_{10} CFU g^{-1} COMPOST
9
8
7
6
5
4
0
5
10
15
20
25
30
35
40
45
TIME (WEEKS)

3.2 Bench-Scale Composting

3.2.1 Evaluation of the Bench-scale System and of the Parameters Used to Monitor the Composting of Bark

3.2.1.1 Reproducibility of the Results Achieved by the Bench-scale System

Consecutive runs, R1 and R2, in which the system was loaded with essentially the same fish-bark mixtures were undertaken to determine the reproducibility of the results obtained. There was no significant ($p < 0.05$) difference between the two runs or between the six replications [1] of the nine variables monitored (Figures 8-10). The cumulative coefficients of variation for levels of CO_2 output, O_2 uptake, CMCase activity, m.c., NH_4^+-N, NO_3^--N, pH plus the two indices of humification (absorbance in pyrophosphate extracts at 550 and 440/660 nm) were 15, 15, 4, 3, 9, 4, 3, 7 and 10 respectively. The data compiled from these two runs and the split-plot analysis of variance (AOV) on these results are given in Appendices 4 and 5 respectively.

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1. The data was also analysed as a completely randomized block design to isolate the effect of replications (in the split-plot design, replication sums of squares are included in the main effect's residual term).

Figure 8

Mean CO_2 output, O_2 uptake and CMCase activity over time for two fish-bark composts.

Samples (1 mL) of effluent compost gas were analysed by thermal conductivity gas chromatography for CO_2 and O_2 at 4h intervals over the 30d period. Compost samples (0.5g) were incubated with 10mL 0.4% Na carboxymethylcellulose (in phosphate buffer, pH 6.0 for 1h at 65°C) to determine the CMCase activity by microviscometry every two days.

Each determination was the mean obtained for samples from triplicate units over duplicate runs of the bench-scale composter.

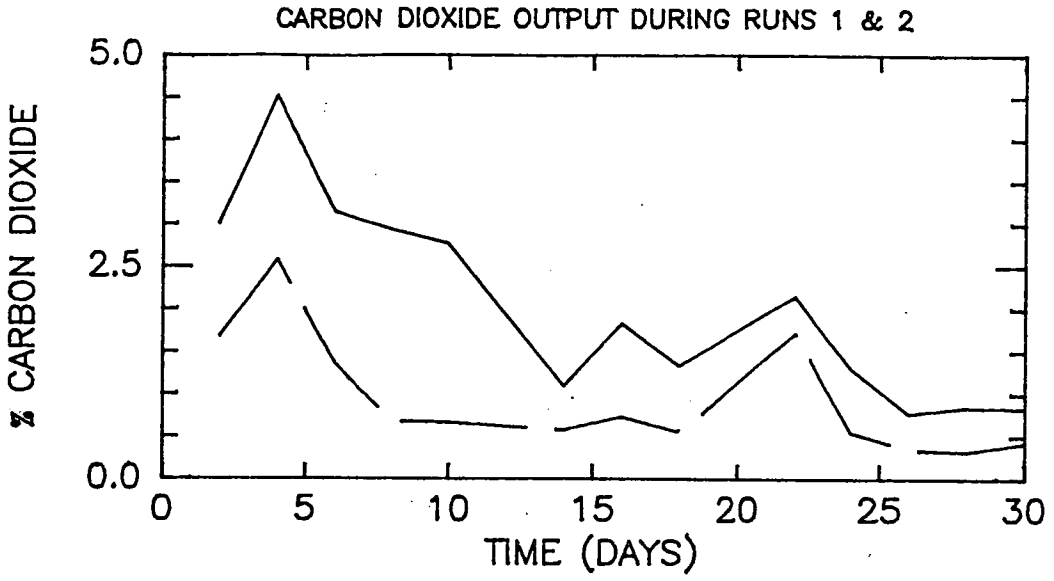
Each unit of the composter initially contained 148g of bark, 15g of composted inoculum, 16.01 or 8.34g of thawed fish waste (to give a C:N ratio of either 45 or 65) and distilled water to give a moisture content of 214%.

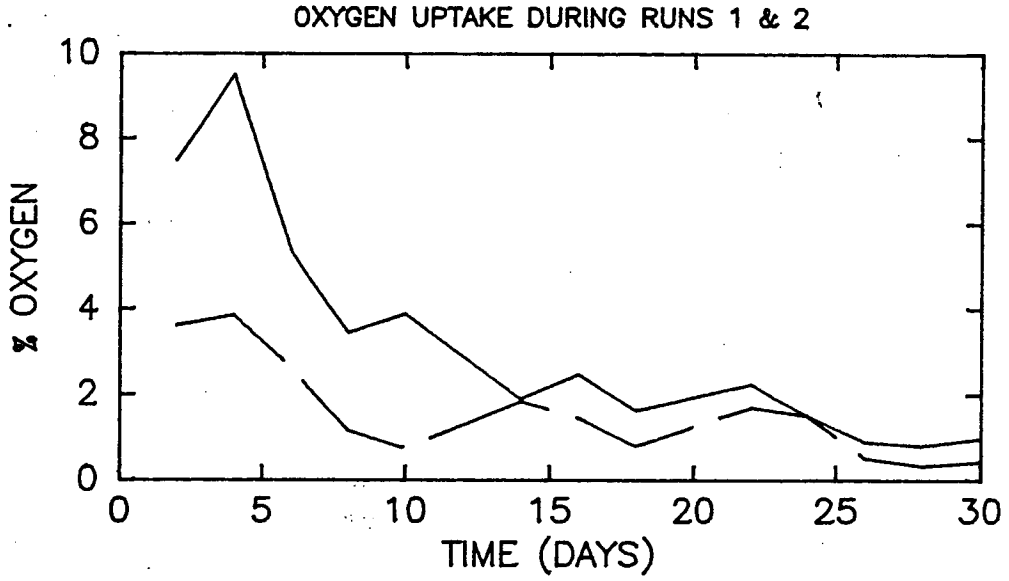
Data for Figure 8 are shown in Appendices 3 and 4 (R1 & R2) and results from the AOV are given in Appendix 5.1-5.2

$LSD_{(0.01)}$ between two means at :-	CO_2	O_2	CMCase
A) different times in one treatment:	0.50	0.87	5.88
B) any time or treatment :	0.48	1.15	8.67

——— Fish-bark, Initial C:N=45

— — Fish-bark, Initial C:N=65


CARBON DIOXIDE OUTPUT DURING RUNS 1 & 2
% CARBON DIOXIDE
5.0
2.5
0.0
0
5
10
15
20
25
30
TIME (DAYS)


OXYGEN UPTAKE DURING RUNS 1 & 2
% OXYGEN
10
8
6
4
2
0
0
5
10
15
20
25
30
TIME (DAYS)

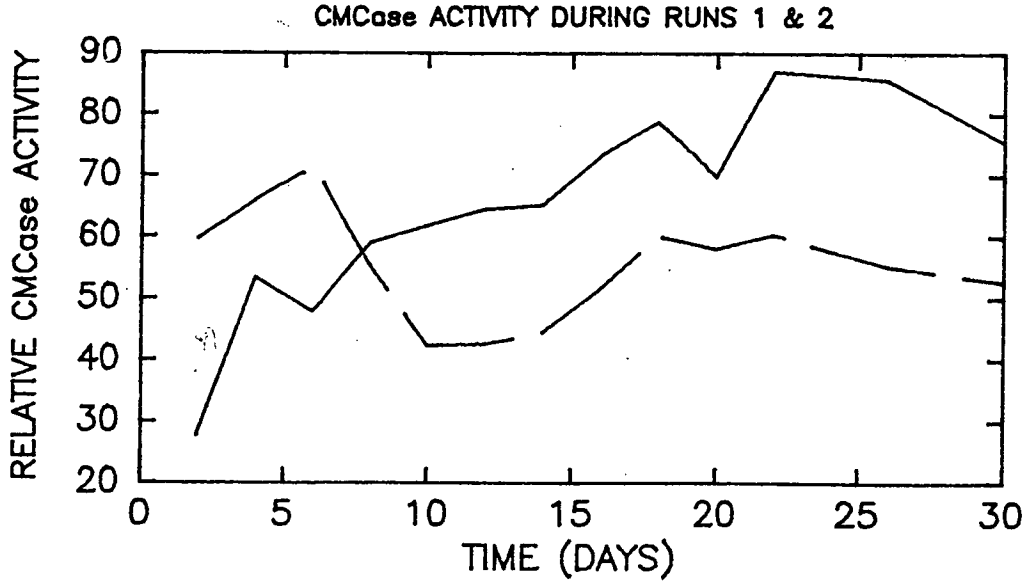

CMCase ACTIVITY DURING RUNS 1 & 2
RELATIVE CMCase ACTIVITY
90
80
70
60
50
40
30
20
0
5
10
15
20
25
30
TIME (DAYS)

Figure 9

Mean NH_4^+ and NO_3^- production and pH over time from two fish-bark composts.

Mineralized forms of nitrogen were determined by steam distillation of 2N KCl extracts of freshly sampled compost (2 g wet compost in 10 mL KCl). Compost pH was also determined using the same KCl extracts and a glass electrode.

Each determination was the mean obtained for samples from triplicate units over duplicate runs of the bench-scale composter.

Each unit of the composter initially contained 148g of bark, 15g of composted inoculum, 16.01 or 8.34g of thawed fish waste (to give a C:N ratio of either 45 or 65) and distilled water to give a moisture content of 214%.

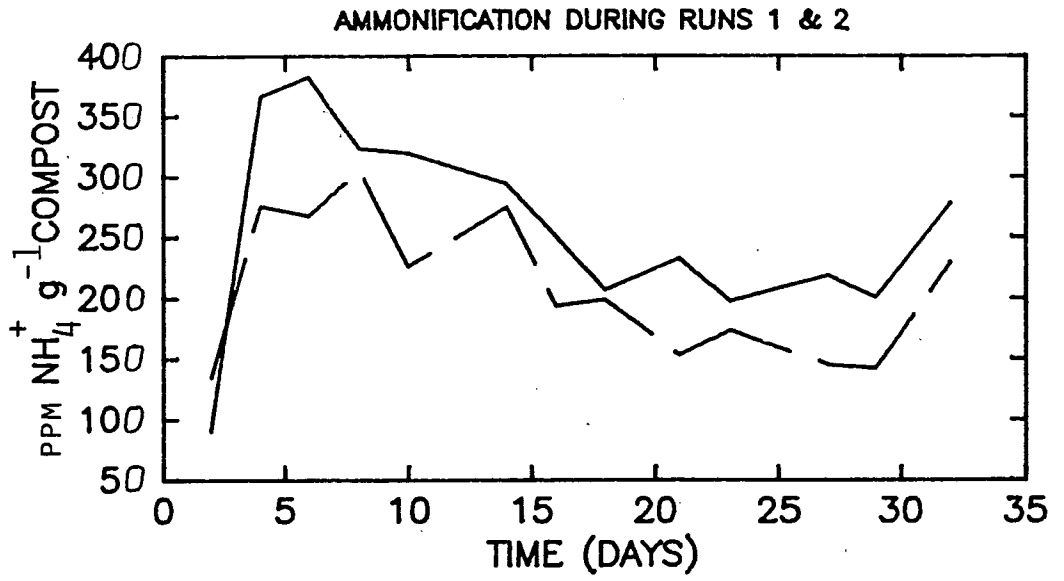
Data for Figure 9 are shown in Appendix 4 (R1 & R2) and results from the AOV are given in Appendix 5.1.

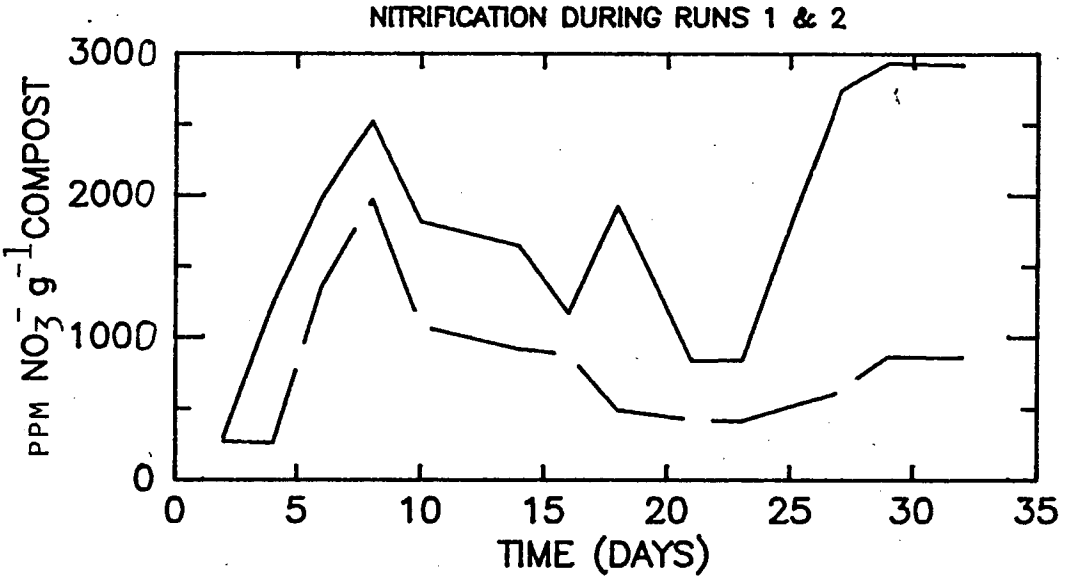
$LSD_{(0.01)}$ between two means at :-

	Ammonium	Nitrate	pH
A) different times in one treatment:	56.1	71.4	1.0
B) any time or treatment :	74.8	101.7	1.4

———— Fish-bark, Initial C:N=45

— — Fish-bark, Initial C:N=65


AMMONIFICATION DURING RUNS 1 & 2
PPM NH_4^+ g^{-1}COMPOST
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
TIME (DAYS)


NITRIFICATION DURING RUNS 1 & 2
PPM NO_3^- g^{-1}COMPOST
3000
2000
1000
0
0
5
10
15
20
25
30
35
TIME (DAYS)

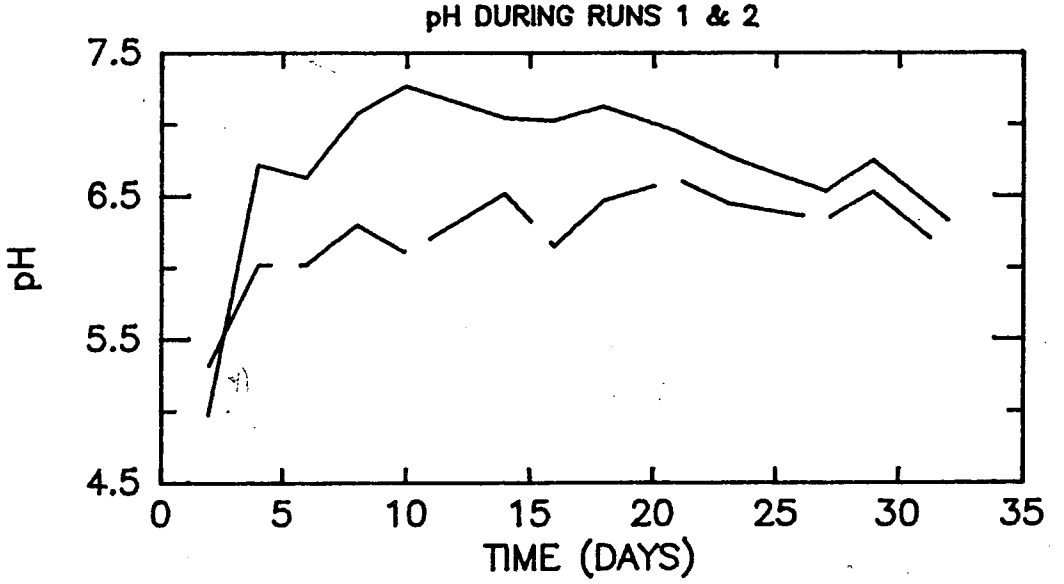

pH DURING RUNS 1 & 2
pH
7.5
6.5
5.5
4.5
0
5
10
15
20
25
30
35
TIME (DAYS)

Figure 10

Mean humification indices and moisture content over time from two fish-bark composts.

Humification indices were assayed by the absorbance at 550nm (pyrophosphate index) and ratio of absorbance at 440 and 660nm in tetra-sodium pyrophosphate (0.5g in 50 mL) extracts. Moisture content was determined after 24h at 105° C.

Each determination was the mean obtained for samples from triplicate units over duplicate runs of the bench-scale composter.

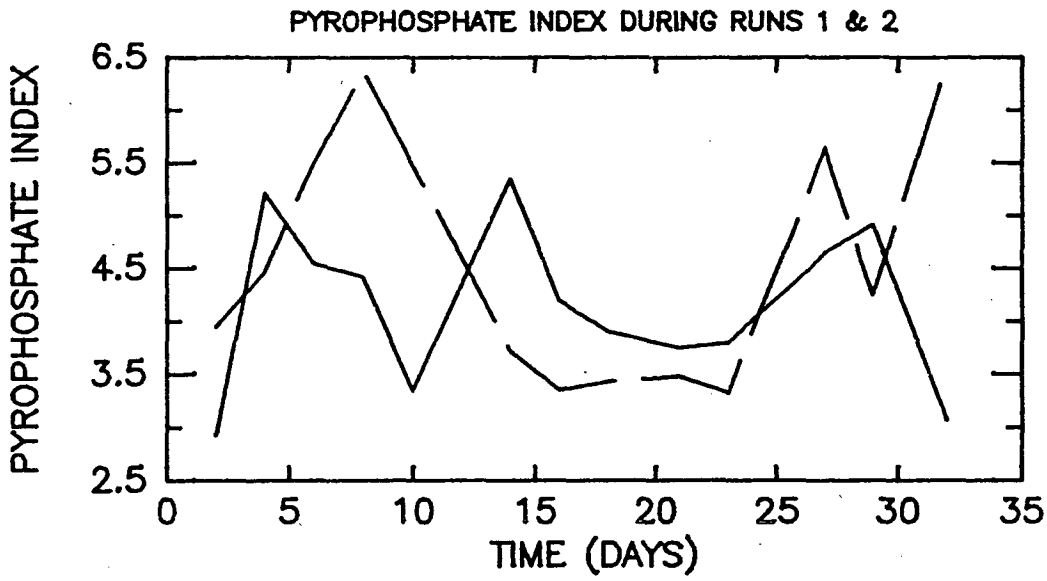
Each unit of the composter initially contained 148g of bark, 15g of composted inoculum, 16.01 or 8.34g of thawed fish waste (to give a C:N ratio of either 45 or 65) and distilled water to give a moisture content of 214%.

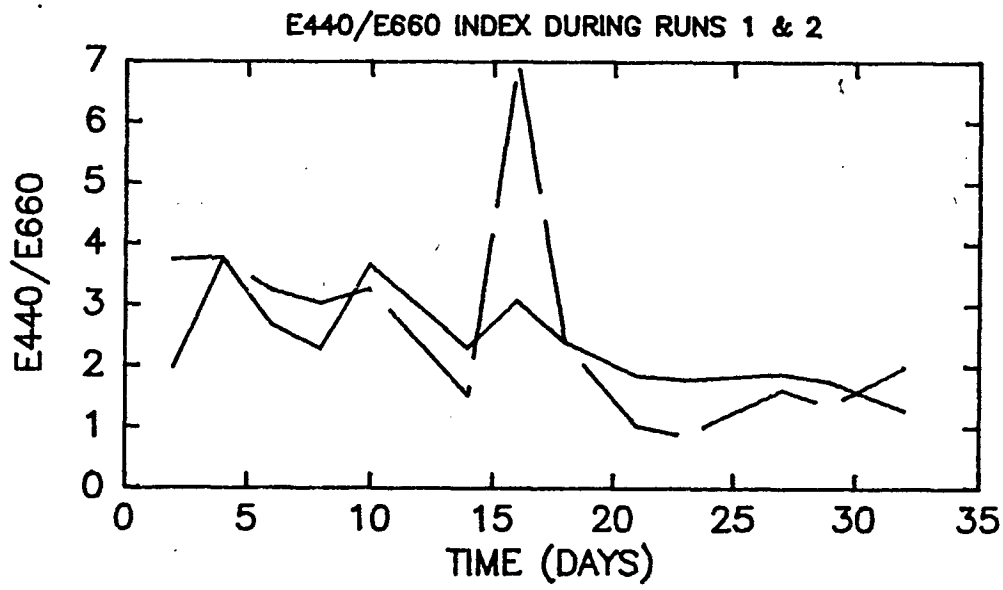
Data for Figure 10 are shown in Appendix 4 (R1 & R2) and results from the AOV are given in Appendix 5.1-5.2.

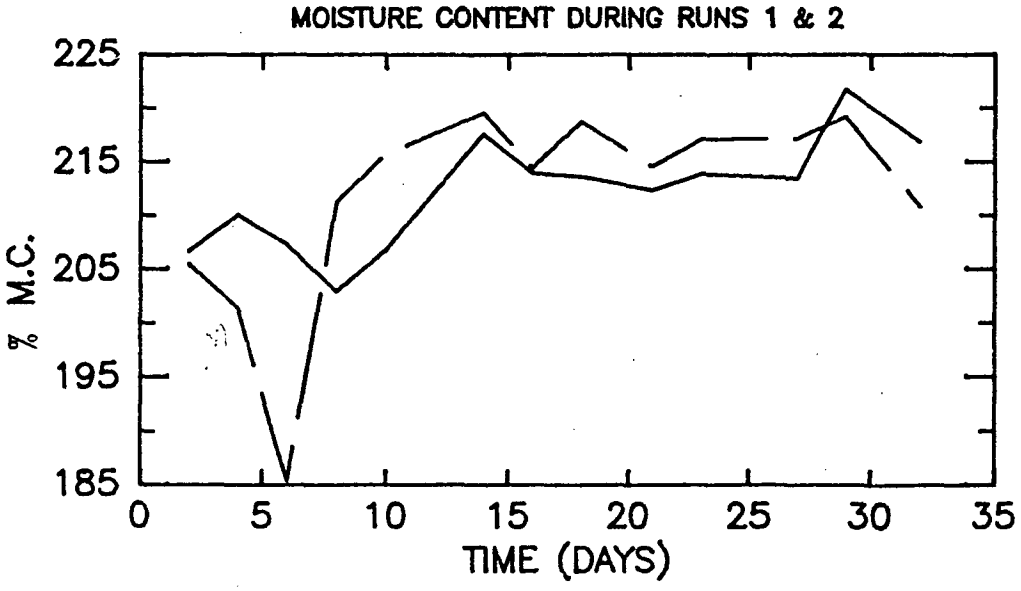
$LSD_{(0.01)}$ between two means at :-	550 nm	440/660nm	% M.C.
A) different times in one treatment:	0.73	0.27	27.9
B) any time or treatment :	0.97	0.96	28.4

———— Fish-bark, Initial C:N=45

— — Fish-bark, Initial C:N=65


PYROPHOSPHATE INDEX DURING RUNS 1 & 2
PYROPHOSPHATE INDEX
6.5
5.5
4.5
3.5
2.5
0
5
10
15
20
25
30
35
TIME (DAYS)


E440/E660 INDEX DURING RUNS 1 & 2
E440/E660
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
35
TIME (DAYS)


MOISTURE CONTENT DURING RUNS 1 & 2
% M.C.
225
215
205
195
185
0
5
10
15
20
25
30
35
TIME (DAYS)

3.2.1.2 Correlations Between the Parameters of Compost Activity

A study of the correlations between various parameters assayed was undertaken to identify those giving a similar measure of microbial activity during composting. The correlation matrix of the nine variables assayed during R1 and R2 and their correlations after principle component analysis (PCA) are given in Table 5. From the correlation matrix it is evident that all parameters except m.c. show at least two significant ($p < 0.001$) correlations with other variables. PCA was however, quite successful in reducing the artificial inflation of correlation coeficients due to neighbour effects (Sokal and Rohlf, 1969).

As expected the PCA and data on Figures 8-10 show a strong positive correlation between CO_2 output and O_2 uptake and between pH and ammonium levels. However a high negative correlation between respiratory activity and CMCase activity was also evident. Other notable correlations were between NH_4^+, NO_3^- and the humification index (abs_{550} nm) while these characters all showed a negative correlation with m.c. .

3.2.1.3 Degradation of Compost Components

The degradation of compost components after 35d composting in R2 were determined by proximate analysis and the mean losses are shown in Table 6. The mean percentage losses of the six components assayed were generally significantly ($p < 0.05$) greater in the lower C:N (45) fish-bark composts. However, the loss of lignin was greater from the higher C:N (65) composts and there was no significant ($p < 0.05$) difference between the losses of lipid and soluble carbohydrate at the two C:N ratios.

Table - 5

Correlation Matrix of Compost Variables Assayed During R1 and R2. [1]

r

	1-pH	2-m.c.	3-O_2	4-CO_2	5-CMCase	6-NH_4^+	7-NO_3^-	8-Abs_{550}	9-$Abs_{440/660}$
1	1.000								
2	0.148	1.000							
3	-0.302	-0.210	1.000						
4	-0.157	-0.244	0.879	1.000					
5	0.351	0.046	-0.640	-0.488	1.000				
6	0.300	-0.226	0.203	0.332	0.152	1.000			
7	0.154	-0.054	-0.528	-0.414	0.589	0.288	1.000		
8	0.075	-0.185	-0.055	-0.039	0.249	0.357	0.282	1.000	
9	-0.166	-0.136	0.401	0.355	-0.317	0.188	-0.091	0.008	1.000
	1	2	3	4	5	6	7	8	9

[1] See Appendices 3 & 4 for data. Critical values for r with 24 df were 0.388 & 0.496 (for $p < 0.05$ & 0.01 respectively).

Principle Component Analysis of Compost Variables Assayed During R1 and R2. [2]

Latent Roots	1	2	3	4	5
	3.17	1.91	1.08	0.82	0.46

(cut-off for varimax rotations)

Varimax Rotations

Variate	Rotations [3] 1	2	3
pH	0.1995	0.0120	-0.8753
m.c.	0.2435	0.6137	-0.3465
oxygen uptake	-0.9409	0.0120	0.0923
Carbon dioxide output	-0.9093	-0.0836	-0.0762
CMCase Activity	0.7047	-0.3610	-0.3077
Ammonification	-0.3055	-0.6402	-0.5600
Nitrification	0.5768	-0.5853	-0.1147
Humification (Abs_{550})	0.0793	-0.6962	-0.0835
($Abs_{440/660}$)	-0.5307	-0.2165	0.1137

[2] See Appendix 1 for composition of compost mixes. In PCA clouds of observations describe hyperellipsoids in a multidimensional space. These hyperellipsoids are dissected by principle axes with latent roots (variance along the principle axes) always at a maximum. So looking at successive axes (rotations 1, 2 etc.) smaller sources of variation are successively removed (Sokal & Rohlf,1969). See Appendix 3.1 and 4.1-4.3 for raw data.

[3] An absolute value of > 0.5 is taken as significant.

Table - 6

Mean Percentage Losses of Compost Components[1] after 35d of Composting.

Component	C:N ratio Initial=45 inal =34	Initial=65 Final =48
Ash	4.13	2.72 **
Cellulose	26.38	6.67 **
Hemicellulose	31.41	17.94 **
Lignin	0.51	3.60 **
Lipid	85.29	85.66 n*
Protein	9.20	0.43 **
Soluble Carbohydrate	94.52	89.70 n*
Total weight	24.42	14.28 **

[1] Losses shown are percentages of the initial dry weight of each component during bench-scale composting of fish-bark composts (run 2). Detailed results are given in Appendix 4.3.

** Significant ($p < 0.01$) difference between treatments;

n* No significant difference between treatments.

3.2.2 Optimization of the Bench-scale System

3.2.2.1 Optimal Temperature and Rate of Aeration for Composting

The effect of compost temperature was evaluated during the latter period of R5 using fish-bark mixes of initial C:N=45 and 55. The maximal respiratory activity was found to occur at 55° (Table 7 and Figure 11).

The optimal rate of aeration during composting was evaluated during R6 using a fish-bark mix of initial C:N ratio of 45. Respiratory data indicated that greatest activity ($p < 0.01$) occurred at the lowest rate of aeration (10 mL min^{-1}) (Table 8, data Appendix 3). However, poor resolution of peaks of microbial activity were obtained at this rate of aeration (Figure 12) and total microbial counts were lower (Table 13). Consequently, a level of 20 mL min^{-1} was used in subsequent runs.

Table - 7

Mean CO_2 Output at Various Temperatures in Fish-bark composts of Initial C:N=45 and 55. [1]

C:N ratio	Mean CO_2 Output (mg g^{-1} Compost h^{-1}) at: 55° ⟶[2] 50° ⟶ 55° ⟶ 60° ⟶ 55°
45	0.45 a[3] 0.26 c 0.39 ab 0.07 d 0.34 b
55	0.30 a 0.15 c 0.25 ab 0.04 d 0.20 bc

[1] Mean of duplicate units of R5 automatically assayed by GC every 5h after 450h composting and 48h after a change in temperature. See Appendix 1 for composition of R5 mix and Appendix 3 for data.

[2] The compost was stabilized for at least 4d at 55° between the changes to 50° and 60°.

[3] Values followed by the same letter were not significantly ($p < 0.01$) different using Duncan's new multiple range test (Steel and Torrie, 1960).

Table - 8

Mean CO_2 Output and % Loss of CO_2-C at 55° and Various Aeration Rates During 720 hours of Composting Fish-bark of Initial C:N = 45. [1]

Aeration Rate (mL min^{-1})	Mean CO_2 Output (mg g^{-1} Compost h^{-1})	% Loss of CO_2-C
10	1.58 a [2]	36.29 a
15	0.73 b	20.51 b
20	0.60 b	13.86 c
30	0.35 b	12.78 c
$LSD_{(0.05)}$	0.23	1.64
$LSD_{(0.01)}$	0.52	1.85

[1] Mean of duplicate units, automatically assayed by GC every 5h during R6 and manually assayed by GC every day during R3 (15 mL min^{-1}). See Appendix 1 for compositions of R3 & R6 and Appendix 3 for data.

[2] Means followed by the same letter were not significantly ($p < 0.01$) different (Appendix 5).

Figure 11

CO_2 output during the composting of fish-bark of initial C:N=45 or 55, aerated at 30 mL min^{-1} and incubated at 50°, 55° and 60° during latter stages.

Each determination was the mean of effluent gas from duplicate units automatically assayed by gas chromatography every 5h.

Each unit of the composter initially contained 134 or 148g of bark (to give a C:N ratio of 45 or 55), 15g of composted inoculum, 14.45g of thawed fish waste and distilled water to give a moisture content of 214%. Sodium azide (0.05%) was added to two units to determine the level of non-biological CO_2 production. The units were run at a constant temperature of 55° from 192h to 475h after which the temperature was adjusted to 50° for 100h, followed by 55° (100h), 60° (50h) and 55° to the termination of the run.

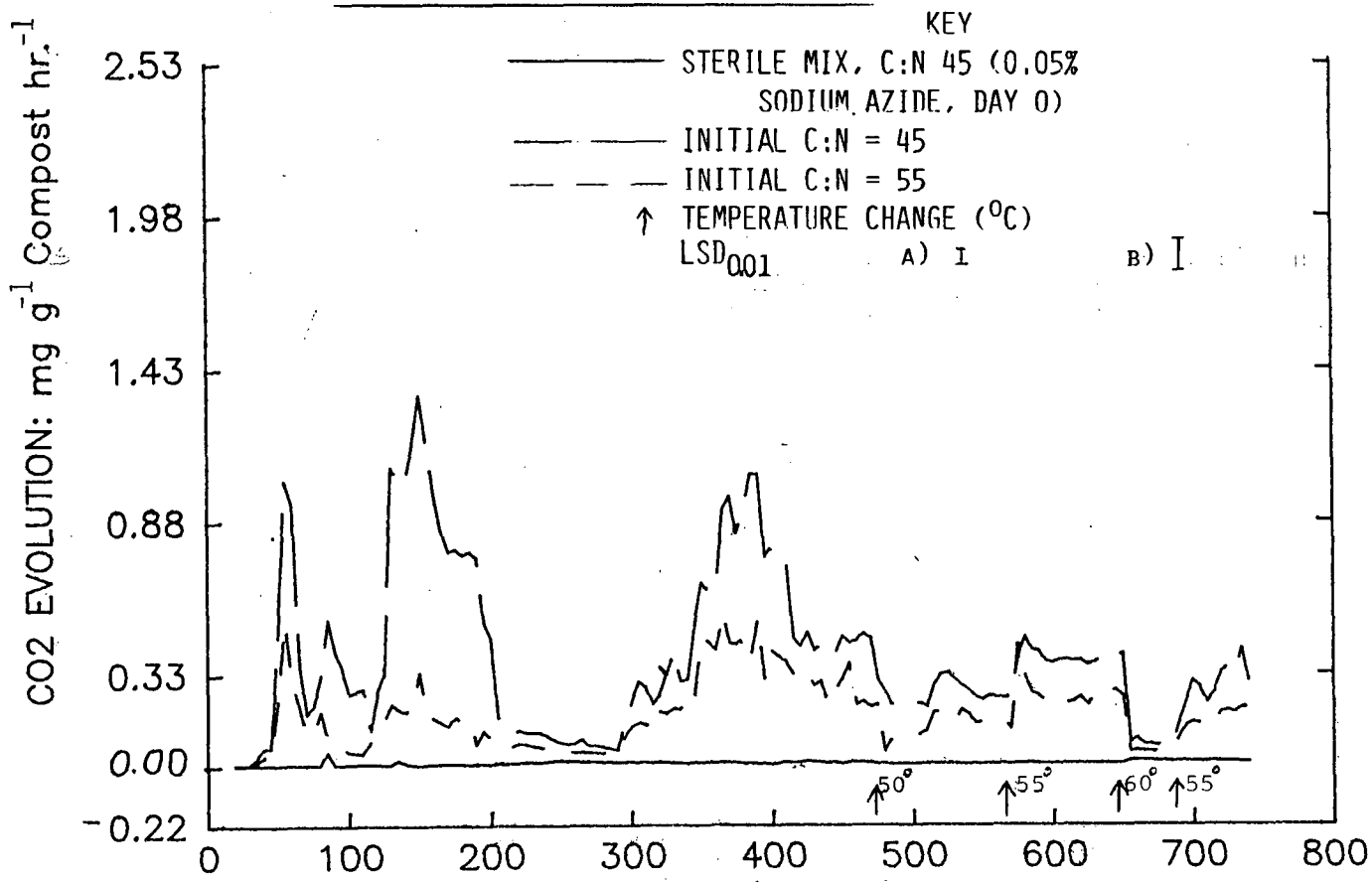
Data for Figure 11 are shown in Appendix 3.4 (R5) and AOV results are given in Appendix 5.5.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.17
B) any time or treatment : 0.38

FIGURE 11. RESPIRATORY ACTIVITY OF COMPOST MIXES:

R5) FISH-BARK, AERATION 30ML MIN^{-1}, INITIAL C:N = 45 OR 55 AND AT TEMPERATURES OF 50, 55 AND 60°C.



igure 12

O_2 output during the composting of fish-bark of initial
:N=45 aerated at 10, 20 or 30 mL min^{-1}.

ach determination was the mean of effluent gas from
uplicate units automatically assayed by gas chromatography
very 5h.

ach unit of the composter initially contained 134g of bark, 15g
f composted inoculum, 14.45g of thawed fish waste and distilled
ater to give a moisture content of 214%. Temperature was
ncreased from 20° to 55° at 5° per day.

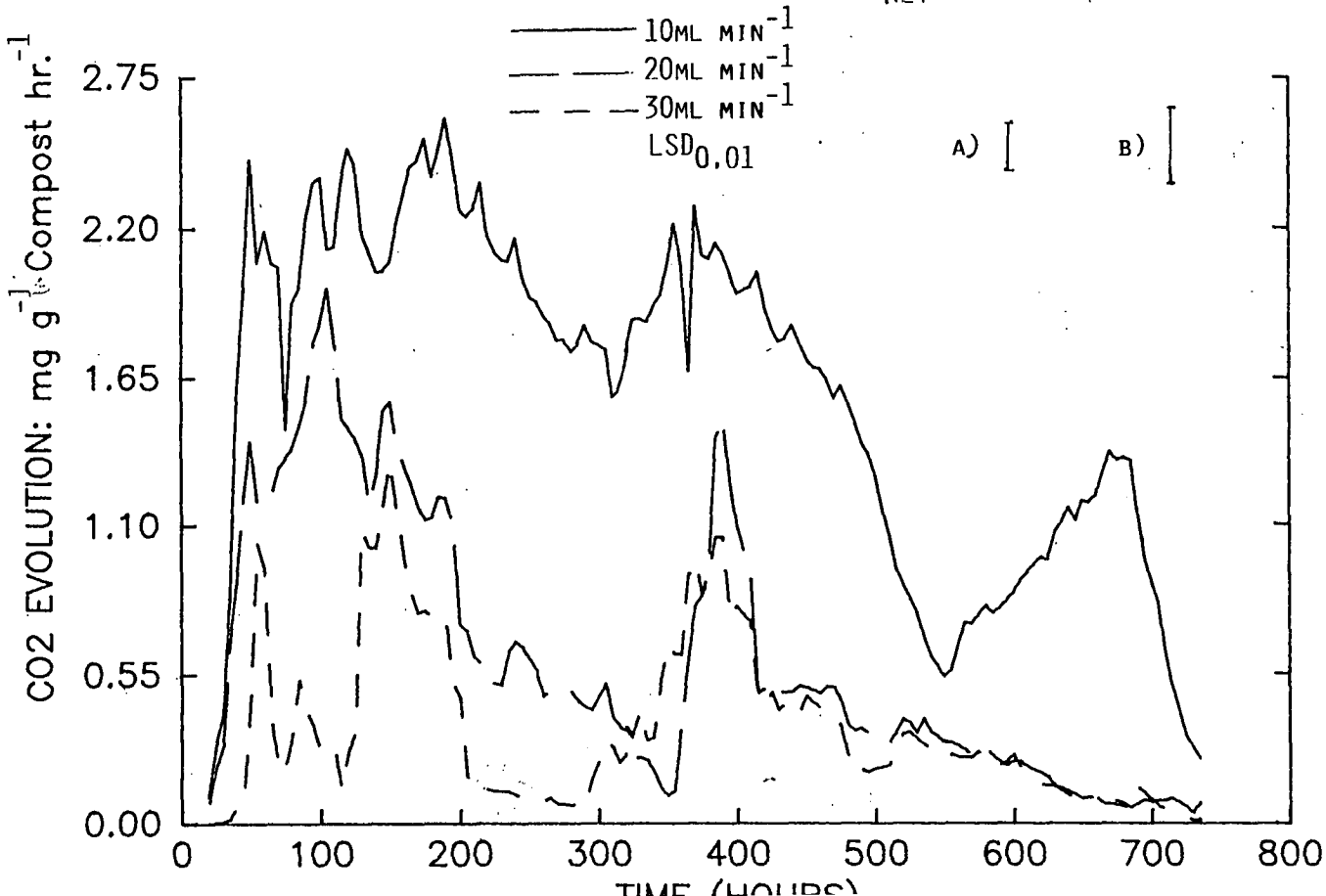
ata for Figure 12 are shown in Appendix 3.6 (R6) and AOV results
re given in Appendix 5.5.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.17
B) any time or treatment : 0.38

FIGURE 12 RESPIRATORY ACTIVITY OF COMPOST MIXES:

R6) FISH-BARK, AERATION AT 10, 20 OR 30 ML MIN^{-1}, AND INITIAL C:N = 45



3.2.2.2 Optimal Mix of Compost Components Based on Respiratory Activity

Runs seven, eight and nine of the composter were designed to determine the optimal C:N ratio for the four N additives:- fish, sewage, urea and IBDU, while during R3, R4 and R8 various additives ($CaCl_2$, thiourea and p-benzoquinone) were also investigated.

Mean % weight losses (as CO_2-C) from each treatment were calculated from the five hourly recordings of CO_2 output (Appendix 3) over 28d composting (Table 9). The results of CO_2 output versus time are given in Figures 13-17. The data indicate that the optimal C:N ratio for respiration is in the order of 25 for fish and sewage mixes and in the order of 35 for the urea-bark mix. No significant ($p < 0.05$) difference was found between the activities of the two lower C:N mixes, except for the sewage-bark mix which showed a significant difference at $p < 0.01$ (Figures 15 & 16).

Neither the addition of calcium (1.5mg g^{-1} compost) nor thiourea (56ug g^{-1} compost) made any significant ($p < 0.01$) difference to the composting of fish-bark (Figures 13 and 14). Substituting IBDU for urea significantly ($p < 0.01$) increased the total C loss, while treating the urea mix with the urease and nitrification inhibitor, p-benzoquinone, made no significant ($p < 0.05$) difference to the overall CO_2-C loss, but they did significantly delay the first peaks of microbial activity by 110 and 50h respectively (Figure 15).

At least three peaks in respiratory activity were apparent during composting (Figures 11-17), with the first two peaks (largest) generally occurring during the mesophilic stage of composting. In the fish-bark composts of initial C:N=45 these peaks in activity occurred at about 50, 100 and 390 h, while fish-, urea- and sewage-bark composts of lower C:N generally showed peaks in activity at 50, 110, 150 and 195 h. The exceptions were the sewage-bark composts which did not produce a peak in activity at 195 h. A peak in respiratory activity at

about 380 h was only shown by the fish-, urea- and sewage-bark composts with initial C:N ratios of 45, 35 and 25 respectively. The addition of quinone to urea-bark composts or the replacement of urea with IBDU delayed all peaks in respiratory activity until the thermophilic stage of composting (Figure 17). The urea-bark+quinone compost produced peaks in activity at 140, 280, 340 and 370 h, while the IBDU-bark compost showed an initial further delay with peaks at 190, 270, 340 and 370 h.

Table - 9

Mean % Weight Loss as CO_2-C [1] in Composts of Various C:N Ratios after 28d of composting.

Compost	% CO_2-C Loss over 28d Composting [2]			
C:N Ratio	Fish-bark	Urea-bark	IBDU-bark	Sewage-bark
25	15.55 a	15.64 a	-	6.52 b
35	15.04 a	16.78 a	19.48 d	4.22 c
" + Quinone	-	15.44 af	-	-
45 Sterile	0.17 e	-	-	-
"	13.86 f	-	-	-
" 30 mL min^{-1}	10.36 g	-	-	-
55 " " "	6.01 b	-	-	-

[1] Losses shown are relative to the initial total weight in each compost. Results are means from duplicate units in each run which were automatically assayed every 5h by GC and aerated at 20 mL min^{-1} unless otherwise noted in the table.

[2] Means followed by a different letter were significantly ($p < 0.01$) different. ($LSD_{(0.05\ \&\ 0.01)}$ = 1.64 & 1.85) (Appendix 5.10).

Figure 13

CO_2 output during the composting of fish-bark of initial C:N=45, aeration 15 mL min^{-1}.

Each determination was the mean of effluent gas from duplicate units manually assayed by gas chromatography every day.

Each unit of the composter initially contained 148g of bark 15g of composted inoculum, 8.34g of thawed fish waste and distilled water to give a moisture content of 214%. Temperature was initially 20° and increased at 5° per day to 55°.

Propylene oxide was added (indicated by arrow) at day 0 (5 mL) and day 9 (10 mL) to two units.

$CaCl_2$ was added (1.5mg g^{-1}) at day 0 to different duplicate units to determine the effect of calcium on the development of the thermophilic flora.

Data for Figure 13 are shown in Appendix 3.3 (R3) and AOV results are given in Appendix 5.3.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.14
B) any time or treatment : 0.32

FIGURE 13. RESPIRATORY ACTIVITY OF COMPOST MIXES:

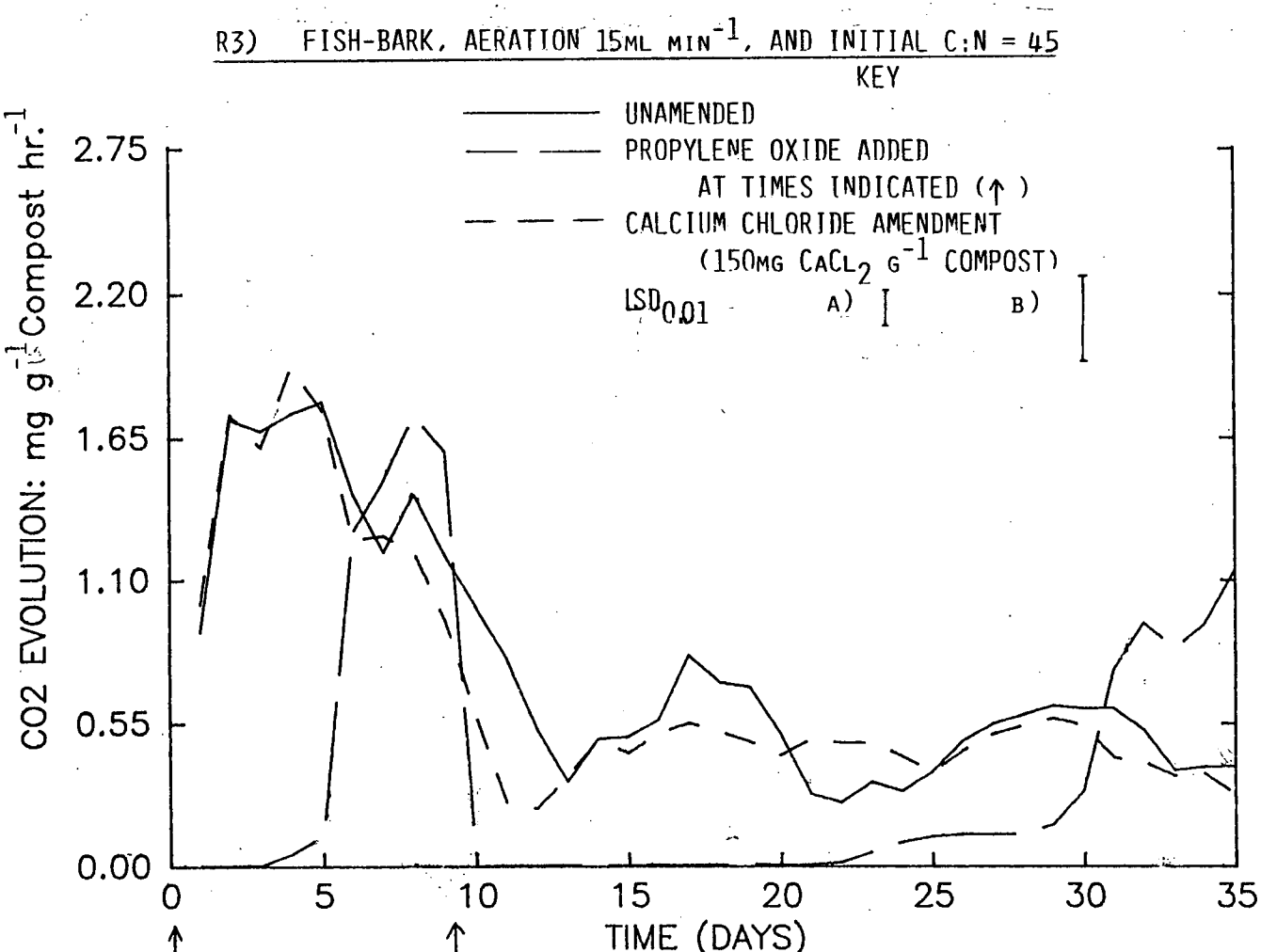


Figure 14

CO_2 output during the composting of fish- and urea-bark of initial C:N=45, aeration 10 mL min^{-1}.

Each determination was the mean of effluent gas from duplicate units manually assayed by gas chromatography every day.

Each unit of the composter initially contained 148g of bark 15g of composted inoculum, 16.0g of thawed fish waste or 2.81g urea and distilled water to give a moisture content of 214%. Temperature was initially 20° and increased at 5° per day to 55°.

Thiourea was added (10mg) at day 0 to duplicates of fish-bark to determine the effect of this nitrification inhibitor on the production of nitrate during composting.

Data for Figure 14 are shown in Appendix 3.3 (R4) and AOV results are given in Appendix 5.4.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.32
B) any time or treatment : 0.71

FIGURE 14 RESPIRATORY ACTIVITY OF COMPOST MIXES:

R4) FISH-AND UREA-BARK, AERATION 10ML MIN^{-1}, AND INITIAL C:N = 45

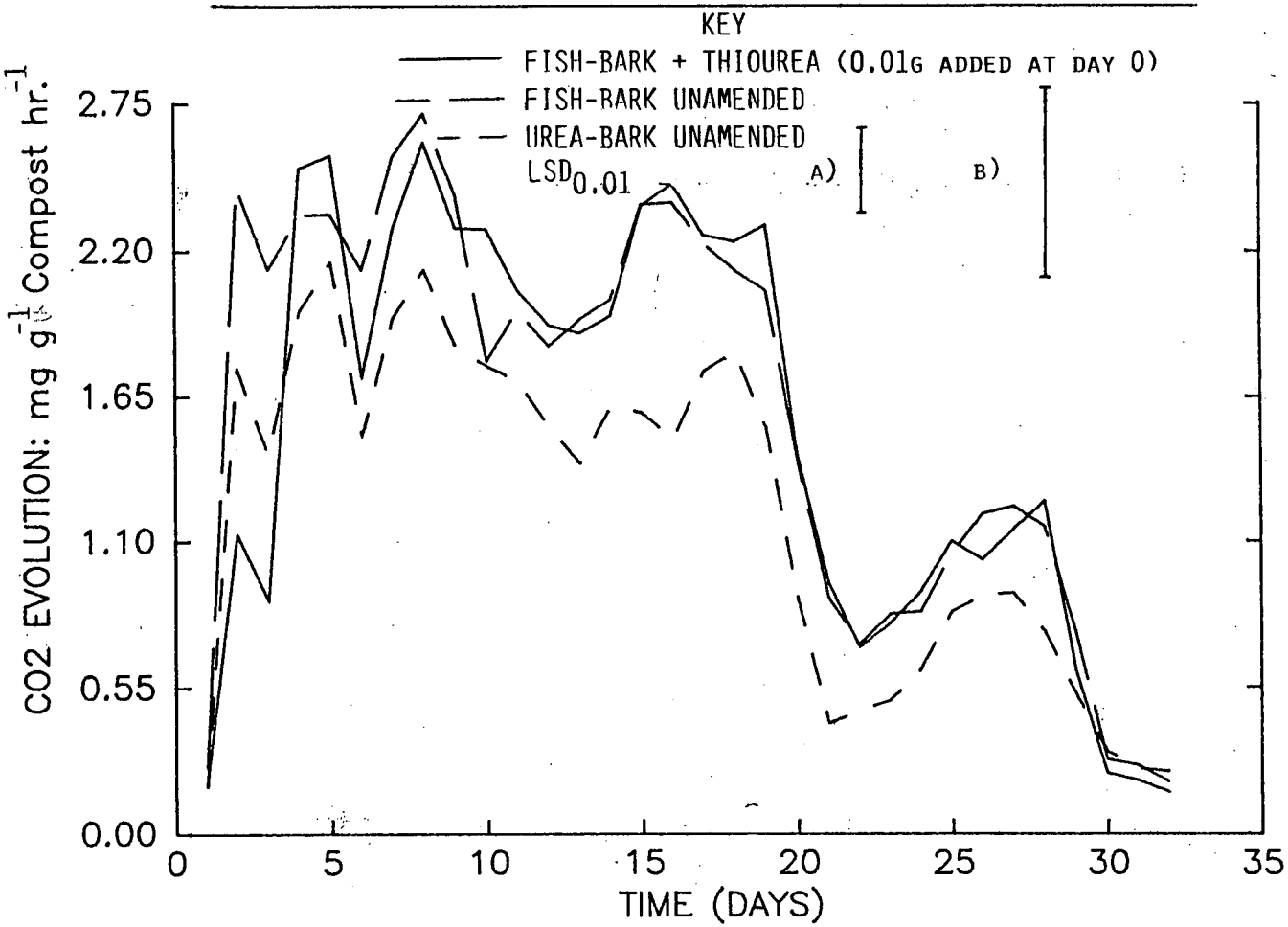


Figure 15

CO_2 output during the composting of fish-, urea- and sewage-bark of initial C:N=35, aeration 20 mL min^{-1}.

Each determination was the mean of effluent gas from duplicate units automatically assayed by gas chromatography every 5h.

Each unit of the composter initially contained 92, 105.2 or 91g of bark with 14.45g of thawed fish waste, 2.81g urea or 56.0g sewage cake respectively, 15g of composted inoculum and distilled water to give a moisture content of 214%. Temperature was initially 20° and increased at 5° per day to 55°.

Data for Figure 15 are shown in Appendix 3.7 (R7) and AOV results are given in Appendix 5.6.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.32
B) any time or treatment : 0.69

FIGURE 15. RESPIRATORY ACTIVITY OF COMPOST MIXES:

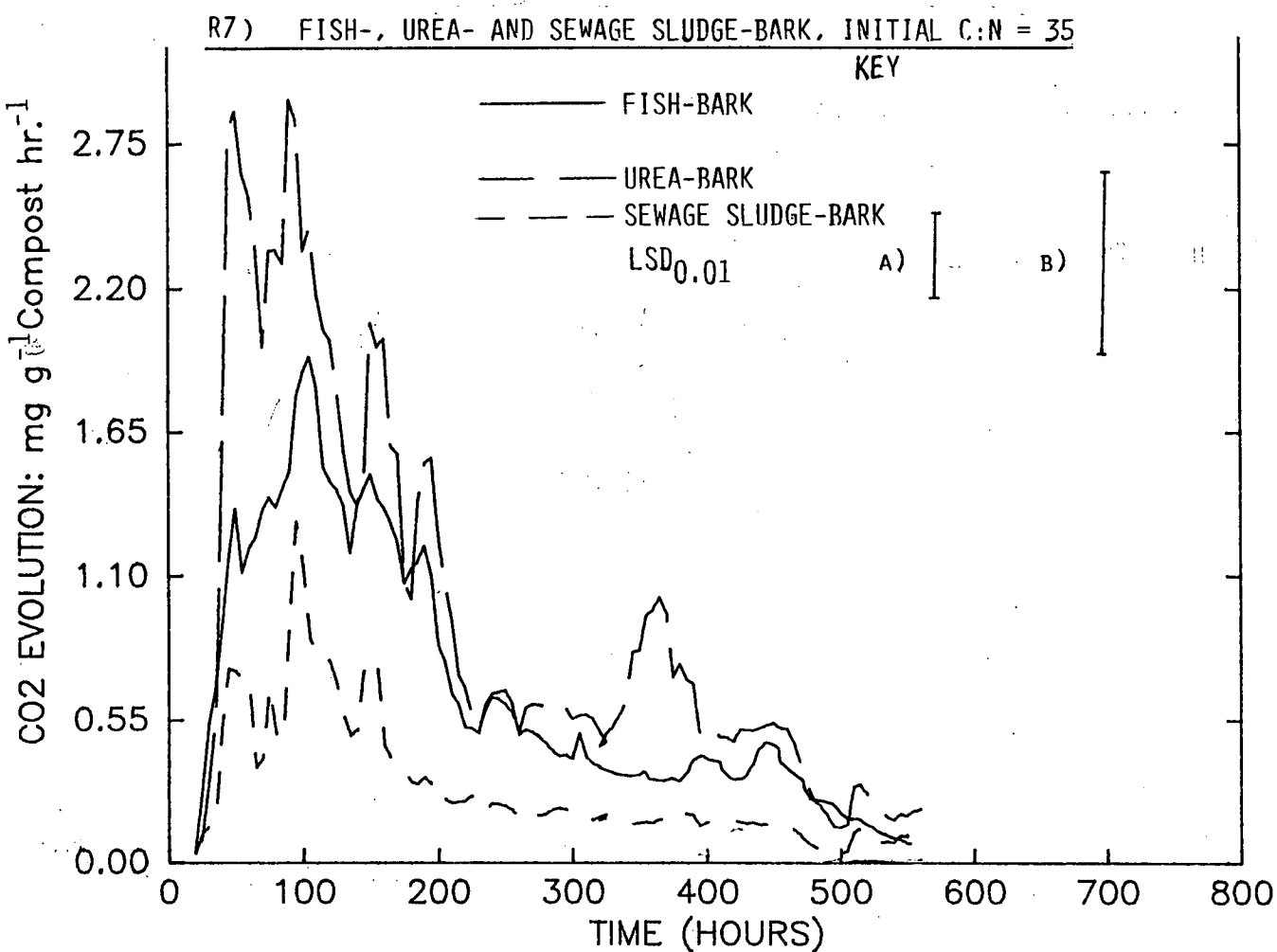


Figure 16

CO_2 output during the composting of fish-, urea- and sewage-bark of initial C:N=25, aeration 20 mL min^{-1}.

Each determination was the mean of effluent gas from duplicate units automatically assayed by gas chromatography every 5h.

Each unit of the composter initially contained 56.3, 42.4 or 65.2g of bark with 14.45g of thawed fish waste, 2.81g urea or 56.0g sewage cake respectively, 15g of composted inoculum and distilled water to give a moisture content of 214%. Temperature was initially 20° and increased at 5° per day to 55°.

Data for Figure 16 are shown in Appendix 3.9 (R9) and AOV results are given in Appendix 5.9.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.21
B) any time or treatment : 0.46

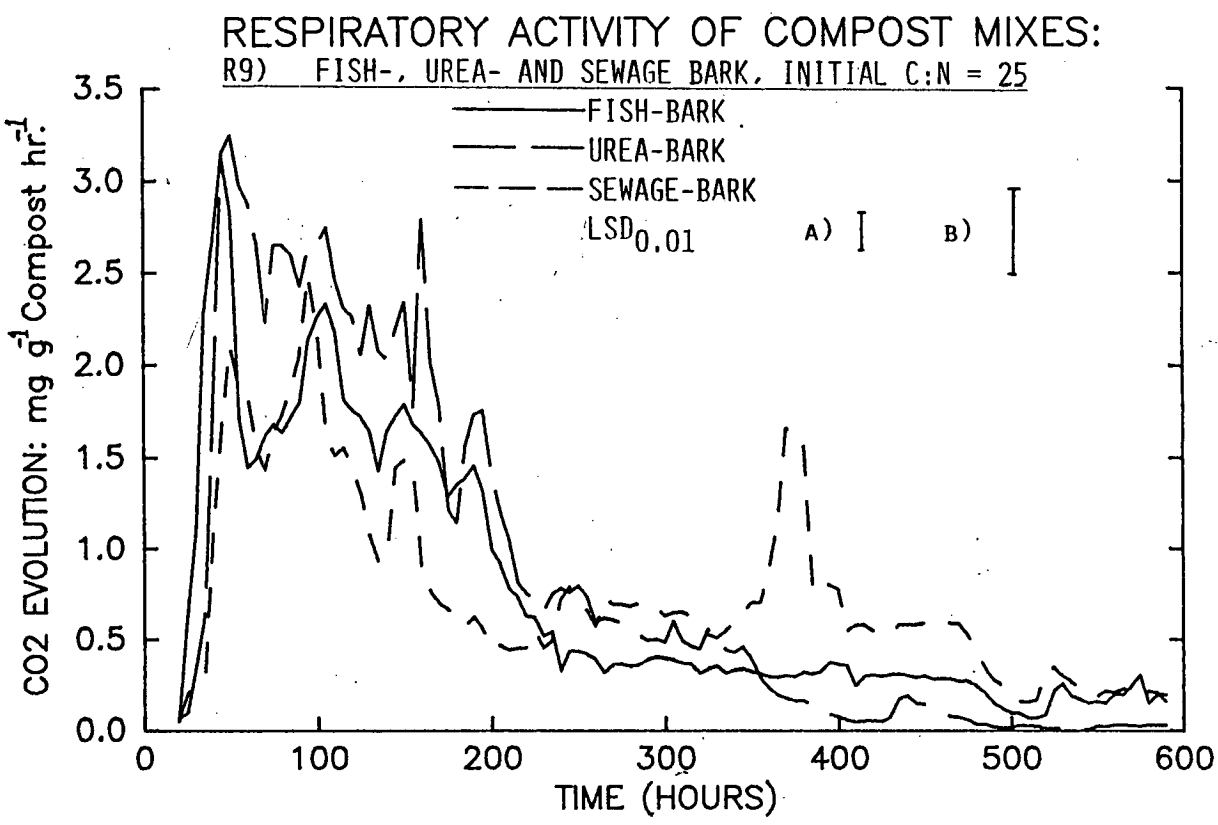

RESPIRATORY ACTIVITY OF COMPOST MIXES:
R9) FISH-, UREA- AND SEWAGE BARK, INITIAL C:N = 25
FISH-BARK
UREA-BARK
SEWAGE-BARK
LSD0.01
A)
B)
CO2 EVOLUTION: mg g-1 Compost hr.-1
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
TIME (HOURS)

Figure 17

CO_2 output during the composting of urea-, urea + p-benzoquinone- and IBDU-bark of initial C:N=35, aeration 20 mL min^{-1}.

Each determination was the mean of effluent gas from duplicate units automatically assayed by gas chromatography every 5h.

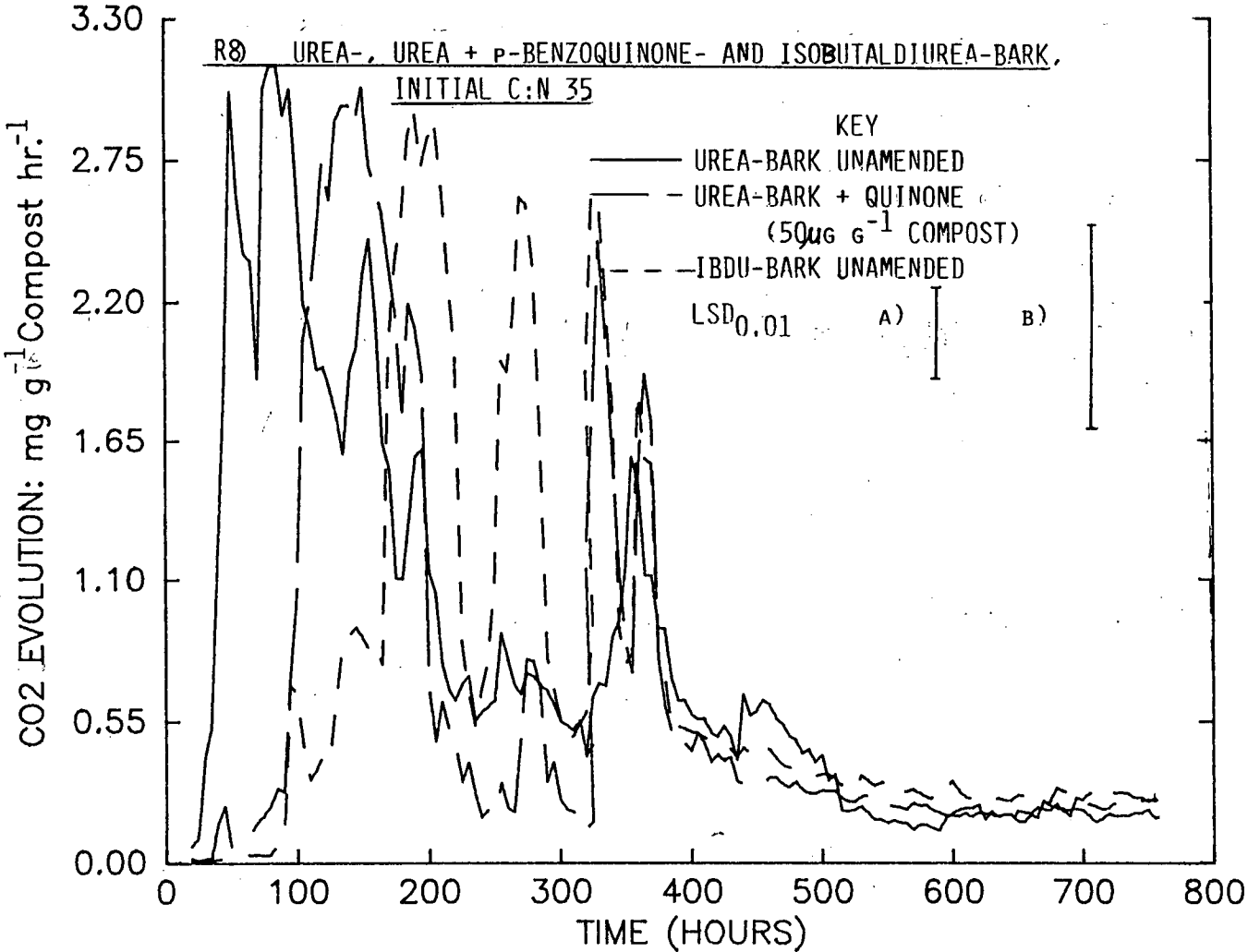
Each unit of the composter initially contained 105.2 or 91.0g of bark with 2.81g urea (+/- 5mL p-benzoquinone) or 3.75g IBDU respectively, 15g of composted inoculum and distilled water to give a moisture content of 214%. Temperature was initially 20° and increased at 5° per day to 55°.

Data for Figure 17 are shown in Appendix 3.8 (R8) and AOV results are given in Appendix 5.8.

$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.35
B) any time or treatment : 0.79

FIGURE 17. RESPIRATORY ACTIVITY OF COMPOST MIXES:



3.2.2.3 Estimated Numbers of Microorganisms in Relation to Optimization of Composting Conditions

Numbers of thermophilic bacteria present during composting of fish-bark of initial C:N=45 or 65 were estimated in R2, as shown in Table 10. Where estimates were $< 0.01 \times 10^6$ for both treatments at the one sample time, that time was not used in the AOV. If only one of the treatments gave a count $< 0.01 \times 10^6$ once, then a missing value < 0.01 was calculated by the Genstat statistical program (Genstat V, 1979). When all counts for two sample times were $< 0.01 \times 10^6$ no AOV was attempted.

Data on estimated numbers of bacteria during R2 supported the respiratory data in that there was significantly (generally at $p < 0.01$) larger populations detected in the composts of C:N=45 as compared with that of C:N=65 (Table 10). The same trend was also apparent in R4 between numbers of mesophilic and thermophilic bacteria and respiratory activity (Table 11 & Appendix 6). However, neither total estimated numbers nor estimated numbers of groups of particular hydrolytic bacteria were correlated (none were significant at $p < 0.05$) with the respiratory data itself for R2 or R4 (Table 12).

During R4 there was a statistical ($p < 0.01$) difference between the fish- and urea-bark treatments and between these treatments over time for estimated numbers of total aerobes. Mean counts of thermophiles were always significantly greater than for the corresponding group of mesophiles and there was also a significantly ($p < 0.01$) different count over time for both mesophiles and thermophiles (Appendix 6). The lower rate of aeration during R4 compared with that during R1 and R2 (10, 30 & 30 mL min^{-1} respectively) with units containing the same initial mix, indicated a significantly later development of the cellulolytic and pectinolytic flora at the higher rate of aeration (Table 13).

Table - 10

Mean Estimates of Numbers of Thermophilic Bacteria Isolated at Intervals During Composting of Fish-bark of Initial C:N = 45 or 65. [1]

Day, Temp Treat.	Total Estimates			% of Thermophiles being:			
	Eubacteria	Anaero.	Actino-mycetes	Cellulo-lytic	Ligno-lytic	Pectino-lytic	Lipo-lytic
2, 30°							
C:N 45	0.52a1	0.13a1	< 0.01	n.d.	n.d.	n.d.	100.0a1
C:N 65	0.49a1	< 0.01b1	< 0.01	n.d.	n.d.	n.d.	100.0a1
7, 50°							
C:N 45	11185.00a2	7.05a2	< 0.01	n.d.	n.d.	n.d.	6.9a2
C:N 65	166.50b2	4.85a2	1.17	n.d.	n.d.	n.d.	96.7b2
14, 55°							
C:N 45	15350.00a3	55.00a3	184.00	39.4	n.d.	17.3	96.7a3
C:N 65	165.50b2	0.61b3	1.00	13.0	n.d.	82.9	65.8b23
28, 55°							
C:N 45	760.50a4	< 0.01	57.03	36.8	n.d.	41.3	86.9a2
C:N 65	464.50b3	< 0.01	2.42	3.05	n.d.	93.8	14.1a3

[1] Bacteria (millions g^{-1} compost) were enumerated on 0.3% TSA (Gibsons) + 0.01 yeast extract (total count) plus L-cysteine hydrochloride (anaerobe count), on mineral salts agar plus 0.5% NaCMC, 0.3% Indulin AT + 0.1% NaCMC, 0.5% pectin; or on Tween 20 agar after incubation at 55° for 4d. Results were a mean obtained from duplicate units & of duplicate plates for each unit in R2. Composting conditions are given in Appendix 1 and detailed results in Appendix 6. Significant differences in numbers ($LSD_{0.01}$, or $LSD_{0.05}$ if underlined) between treatments at the one time (indicated by a different letter) and within a treatment between times (indicated by a different number) were calculated for each group on $\log_{10}$ transformed data or percentage of aerobic count data (Appendix 6). n.d. - not detected.

Table - 11

Mean Estimates of Numbers of Microorganisms During Composting of Fish- and Urea-bark of Initial C:N = 45 During R4. [1]

Day, Temp. Treat.	Total CFU/g Compost Eubacteria Aero.	Eubacteria Anaero.	Actinomycetes	Fungi	% of Total Flora being: Cellulolytic	Pectinolytic	Xylanolytic	Lipolytic
MESOPHILES								
7, 50°								
Fish-bark	82.9a1	< 0.1	< 0.1	< 0.1	38.6a1	59.0a1	0.1	n.d.a1
Urea-bark	356.5b1	0.5	17.0	1.0	15.1b1	15.1a1	2.6	0.3b1
14, 55°								
Fish-bark	93.5a1	< 0.1	< 0.1	< 0.1	84.5a1	86.1a1	n.d.	22.5a2
Urea-bark	63.2b2	< 0.1	< 0.1	29.0	23.5b2	1.8b2	23.6	23.5b2
28, 55°								
Fish-bark	18.0a2	< 0.1	< 0.1	< 0.1	n.d.a3	65.0a1	12.2	14.7a3
Urea-bark	78.5b2	< 0.1	< 0.1	57.5(y)	1.7a3	92.1b3	n.d.	73.3b3
THERMOPHILES								
7, 50°								
Fish-bark	230.0c1	4.6	< 0.1	< 0.1	66.8c1	66.8c1	1.0	34.1c1
Urea-bark	270.4c1	< 0.1	256.5	< 0.1	0.7d1	0.1d1	0.6	2.2a1
14, 55°								
Fish-bark	2839.0c1	72.5	1490.5	< 0.1	23.8b2	64.6c1	31.9	30.8c1
Urea-bark	1069.0b1	< 0.1	116.0	< 0.1	n.d.d1	0.5d1	0.5	19.8d2
28, 55°								
Fish-bark	103.5c1	< 0.1	25.1	< 0.1	7.9a3	70.1a1	n.d.	59.1c2
Urea-bark	62.0a1	< 0.1	1045.2	< 0.1	3.4a1	2.7c1	47.5	44.4d3

[1] Total colony forming units (millions g^{-1} compost) were enumerated on 0.3% TSA (Gibsons) + 0.01 yeast extract (total bacterial count) plus L-cysteine hydrochloride (anaerobe count), potato dextose agar (total fungal count), on mineral salts agar plus 0.5% NaCMC, 0.3% Indulin AT + 0.1% NaCMC, 0.5% pectin; or on Tween 20 agar after incubation at 55° for 4d. Results were a mean obtained from duplicate units & of duplicate plates for each unit. Composting conditions are given in Appendix 1 and detailed results in Appendix 6.

Significant differences ($LSD_{0.01}$) between treatments at the one time (indicated by a different letter) and within a treatment between times (indicated by a different number) were calculated for each group as either a percentage of total aerobic count or $\log_{10}$ transformed data (Appendix 6). (y) - yeast. n.d. - not detected.

Table - 12

Correlation Matrix of Microbial Counts and Respiration During R1 and R2. [1]

	r			
Counts	1.0000			
L-Counts	0.7052	1.0000		
CO_2 Output	0.2541	-0.2676	1.0000	
O_2 Uptake	-0.0342	-0.6112	0.8678	1.0000
	Counts	L-Counts	CO_2	O_2

[1]Critical values for r with 6 df were 0.707 & 0.834 ($p < 0.05$ & 0.01 respectively). L- Log_{10}.

Correlation Matrix of Microbial Counts and Respiration During R4. [1]

	r			
L-Mesophiles	1.0000			
L-Thermophiles	-0.0909	1.0000		
L-Total est.	0.4284	0.8195	1.0000	
CO_2 Output	0.6683	0.3191	0.6548	1.0000
	L-Meso.	L-Thermo.	L-Total	CO_2

[1]Critical values for r with 6 df are 0.707 & 0.834 ($p < 0.05$ and 0.01 respectively). L- Log_{10}.

Table - 13

Comparison of Bacterial Counts During Composting of Fish-bark (Initial C:N=45) at Two Rates of Aeration. [1]

Day, Temp. Treat.	Total Count Thermophilic Aero.	Anaero.	% of Thermophiles being: Cellulo-lytic	Pectino-lytic	Lipo-lytic
7, 50°					
10 mL min^{-1}	230.0a1	4.5a1	66.8a1	66.8a1	34.1a1
30 mL min^{-1}	11587.8b1	7.1a1	n.d.	n.d.b1	6.9a1
14, 55°					
10 mL min^{-1}	2839.0a2	72.5a2	23.9a2	33.8a2	30.8a2
30 mL min^{-1}	15534.0b1	55.0a2	39.2b1	17.3b2	96.7a2
28, 55°					
10 mL min^{-1}	128.6a3	n.d.a3	7.9a3	70.1a3	59.1a2
30 mL min^{-1}	817.5b2	n.d.a3	36.8b2	41.3a3	86.9b2

[1] Bacteria (millions g^{-1} compost) were enumerated on 0.3% TSA (Gibsons) + 0.01 yeast extract (total count) plus L-cysteine hydrochloride (anaerobe count), on mineral salts agar plus 0.5% NaCMC, 0.5% pectin; or on Tween 20 agar after incubation at 55° for 4d. Results were a mean obtained from duplicate units & of duplicate plates for each unit (R2 & R4). Composting conditions are given in Appendix 1 and detailed results in Appendix 6. Significant differences in numbers ($LSD_{0.01}$) between treatments at the one time (indicated by a different letter) and within a treatment between times (indicated by a different number) were calculated for each group on data either as a percentage of total aerobic count or log_{10} transformed data (Appendix 6).
n.d.- not detected.

Figure 18

Total Counts of Microorganisms Isolated During Peaks of Respiratory Activity in Various Compost Mixes, Initial C:N=35.

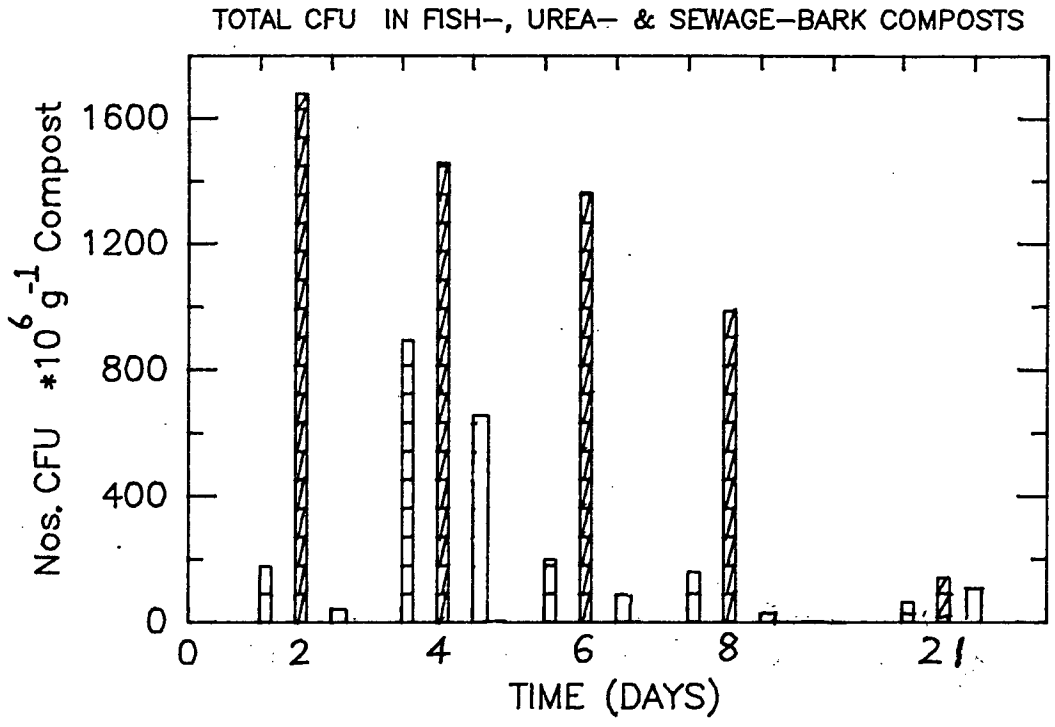
Each unit of the composter initially contained 92.0, 105.2 or 91.0g of bark with 14.45g fish, 2.81g urea or 3.75g IBDU (or 56.0g sewage cake) respectively, 15g of composted inoculum and distilled water to give a moisture content of 214%. The temperature of the compost was initially 20° and increased at 5° per day to 55°. The temperatures corresponding to each peak of activity during R8 are given below.

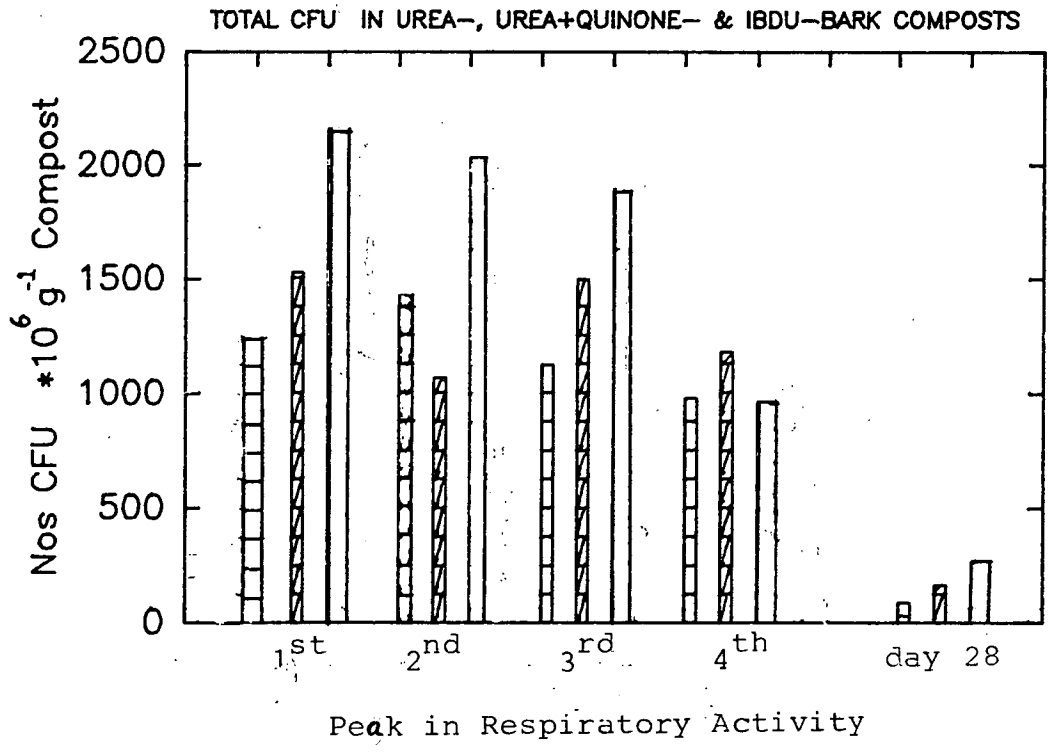
Peaks in respiratory activity occurred at days and temperatures:

Fish-bark  Urea-bark 2(25°), 3(30°), 6(45°) & 8(55°).

Urea-bark  Urea-bark + p-benzoquinone 6(45°), 8(55°), 14(55°) & 15(55°).

Sewage-bark  IBDU-bark 8(55°), 12(55°), 14(55°) & 15(55°).


TOTAL CFU IN FISH–, UREA– & SEWAGE–BARK COMPOSTS
Nos. CFU *10^6 g^-1 Compost
1600
1200
800
400
0
0
2
4
6
8
21
TIME (DAYS)


TOTAL CFU IN UREA–, UREA+QUINONE– & IBDU–BARK COMPOSTS
Nos CFU *10^6 g^-1 Compost
2500
2000
1500
1000
500
0
1st
2nd
3rd
4th
day 28
Peak in Respiratory Activity

Total numbers of microorganisms isolated at the temperature of the compost at periods corresponding to peaks in respiratory activity are shown in Figure 18 for R7 and R8. This change in sampling time and incubation temperature resulted in a considerable improvement in the correlation between respiration and microbial estimates (Table 14) over that obtained previously.

Table - 14

Correlation Matrix of Microbial Counts and Respiration During R7 and R8. [1]

	Run 7		
		r	
Counts	1.0000		
Log Counts	0.9211	1.0000	
CO_2 Output	0.9168	0.8951	1.0000
	Counts	Log-Counts	CO_2

	Run 8		
		r	
Counts	1.0000		
Log Counts	0.9415	1.0000	
CO_2 Output	0.7306	0.6513	1.0000
	Counts	Log-Counts	CO_2

[1] Critical values for r with 13 df were 0.514 & 0.641 ($p < 0.05$ & 0.01 respectively). Data were collected at days 2, 4, 6, 8 and 21 during R7 and days 2, 3, 6, & 8 (R8, urea-bark), 6,8,14 & 15 (R8, urea+quinone-bark) and 8, 12, 14 & 15 (R8, IBDU-bark).

In the composting of urea-bark, the addition of p-benzoquinone significantly ($p < 0.05$) reduced the CMCase activity for the first 16d, thereafter there was no significant difference in activity (Appendix 7.4). On the other hand, the IBDU-bark compost showed similar activity to the quinone treated urea-bark mix for the first 16d, but peaked in activity at 24d, with activity remaining significantly ($p < 0.01$) greater until termination of the run.

3.2.3.3 Detection of Other Enzymes During Composting

Lipolytic activities in samples of two fish-bark composts (R5) are illustrated in Figure 23 (Appendix 7.4). The peak in esterase activity at day 16 corresponded with the third peak in respiratory activity observed during R5 (Figure 12). Estimated numbers of lipolytic thermophiles in a similar compost (R4), also peaked at 16d (Table 13). Laccase activity was not detected in any compost sample over a 28d period.

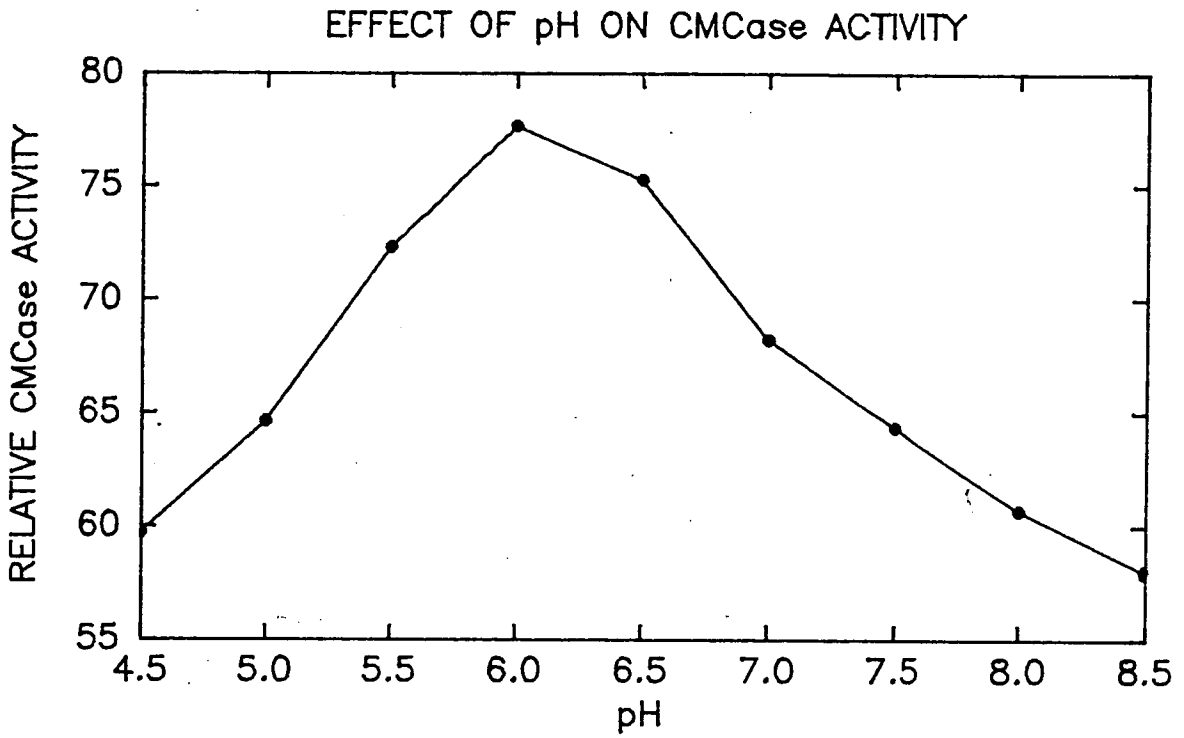
Figure 19

Effect of pH on the assay of CMCase activity in 20d old fish-bark compost (pH= 7.2, initial C:N=45).

Compost samples (0.5g) were incubated with 10mL 0.4% NaCMC in citrate-phosphate buffer (0.05M) of varying pH for 1h at 65°.

Each determination was the mean of duplicate assays.

See Appendix 7.1 for results.


EFFECT OF pH ON CMCase ACTIVITY
RELATIVE CMCase ACTIVITY
80
75
70
65
60
55
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
pH

Figures 20 and 21

Figure 20

Relative CMCase activity against temperature within fish-bark compost samples of various ages (initial C:N=45).

Figure 21

Relative CMCase activity in fish-bark compost of various initial C:N ratios.

Compost samples (0.5g) were incubated with 10mL 0.4% NaCMC in 0.05 M phosphate buffer (pH=6.0) at the indicated temperature or 65° for 1h. The temperature of the compost at day four was 35° and remained constant at 55° from day eight onwards.

Each determination was the mean of duplicate assays.
See Appendix 7.2-7.3 for results and AOV.

$LSD_{(0.01)}$ between two means at :-

	Figure 20	Figure 21
A) different times in the one treatment:	6.44	6.38
B) any time or treatment :	9.24	8.76

Assay Temperature		C:N Ratio
50	.	25
55	+	35
60	*	45
65	0	55
70	X	65
75	0	

FIGURE 20

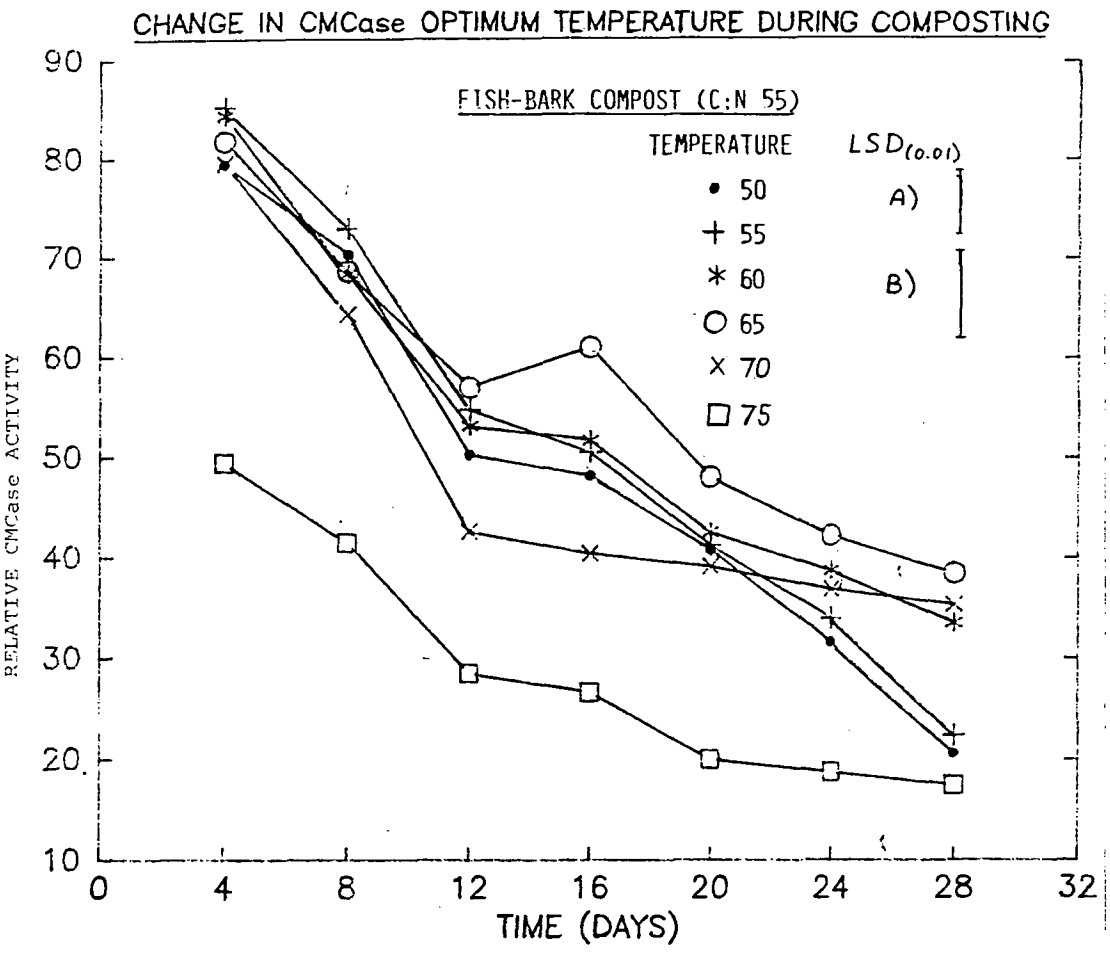


FIGURE 21

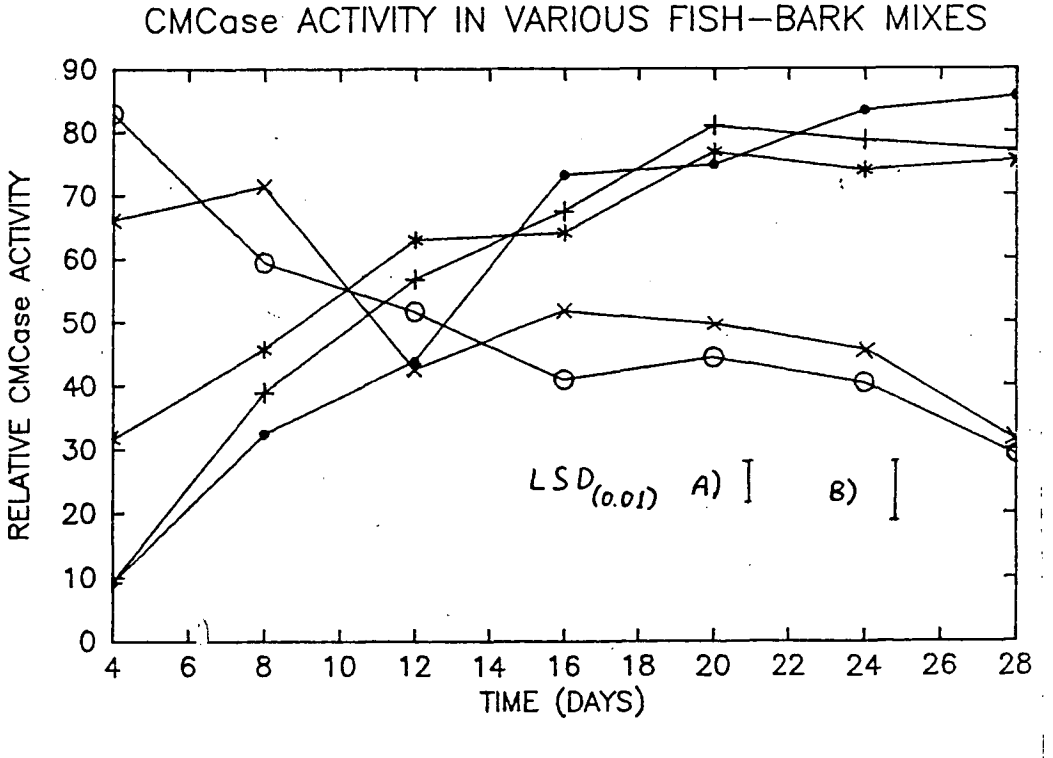


Figure 22

Relative CMCase activity in fish-, urea- and sewage-bark compost of initial C:N ratios 25 and 35.

Compost samples (0.5g) were incubated with 10mL 0.4% NaCMC in 0.05 M phosphate buffer (pH 6.0) at 65° for 1h. Scales of relative activity are the same for both C:N 25 and 35.

Each determination was the mean of duplicate assays.

See Appendix 7.3 for results and AOV.

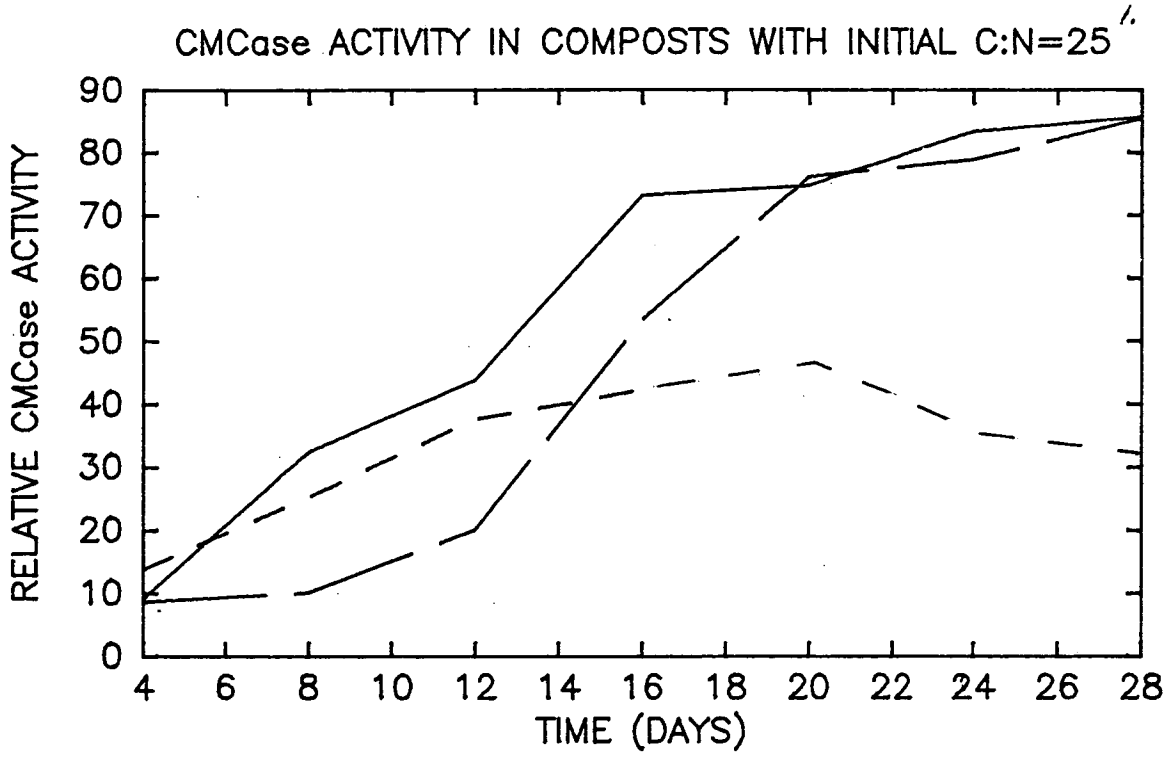
$LSD_{(0.01)}$ between two means at :-

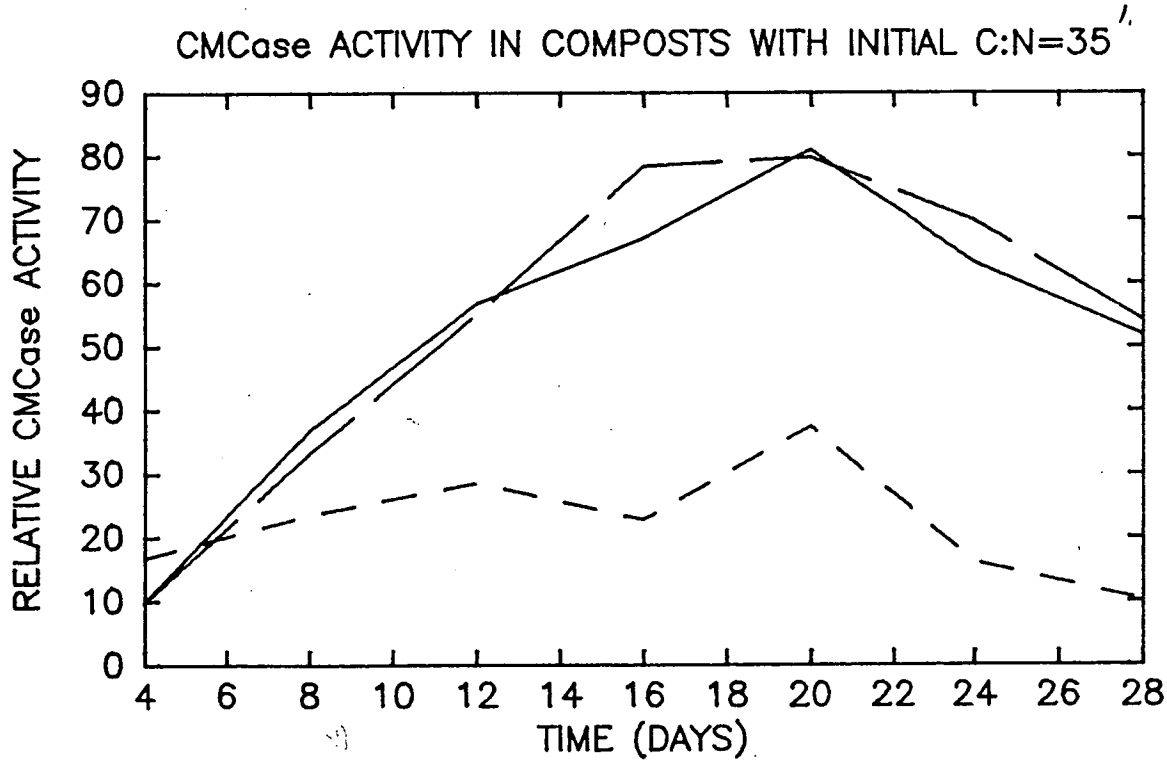
A) different times in the one treatment: 3.44
B) any time or treatment : 4.54

_____ Fish-bark

__ __ Urea-bark

__ __ __ Sewage-bark





$^{1}LSD_{(0.01)}$ A) I B) I

Figure 23

Lipolytic activity during the composting of fish-bark composts of initial C:N ratios 45 and 55.

Compost samples (0.4g) were incubated with 5mL 3.0% tween-20 in 0.05M citrate-phosphate buffer (pH 7.5) at 65° for 1h. The reaction was stopped with acetone-95% ethanol and the solution titrated to the original pH with 0.05M NaOH.

One unit of esterase = release of 0.1 mM of fatty acid h^{-1}.

Each determination was the mean of duplicate assays.

See Appendix 7.4 for results.

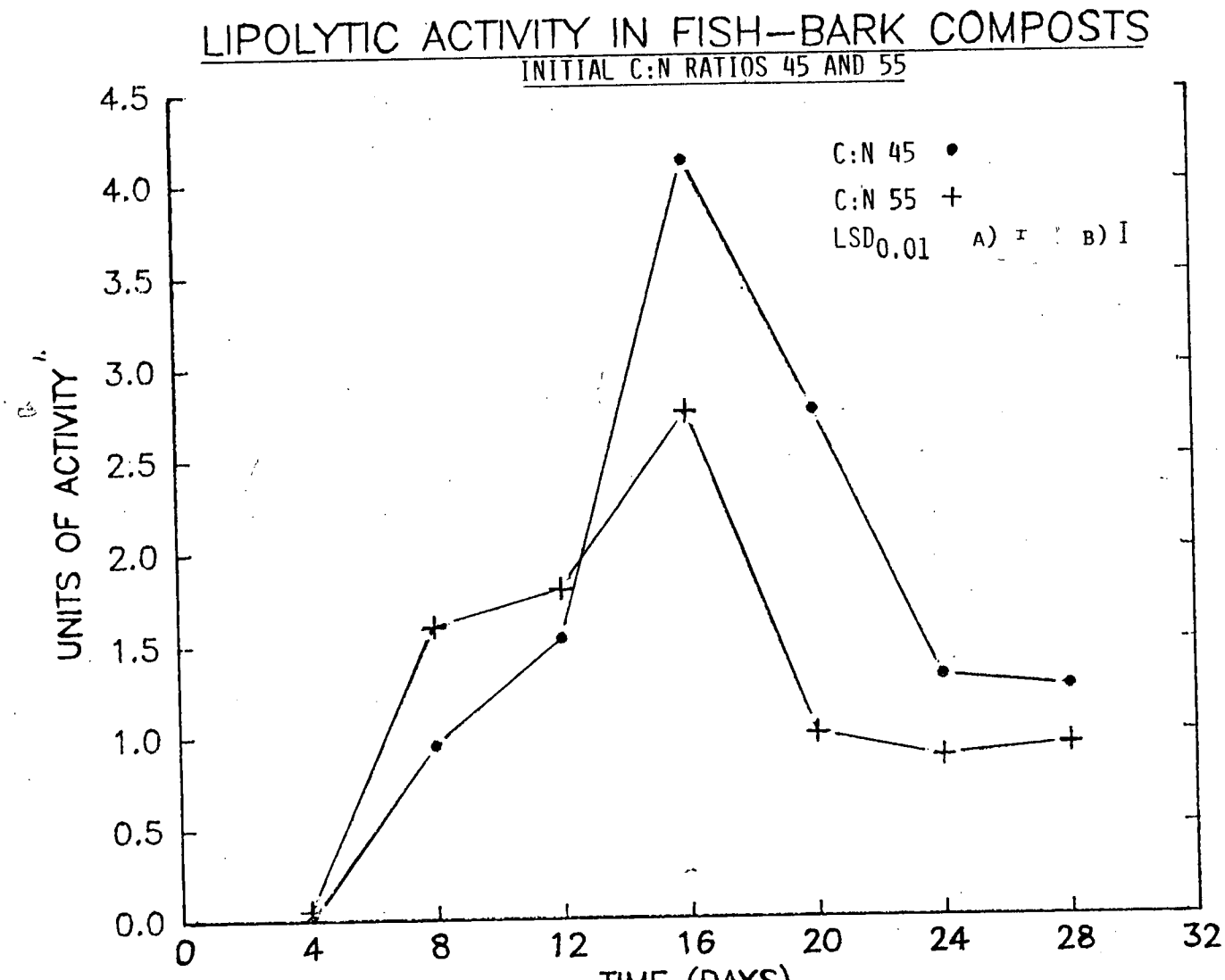
. C:N= 45

\+ C:N= 55

$LSD_{(0.01)}$ between means at :-

A) the same C:N ratios: 0.0245

B) different C:N ratios: 0.0784


LIPOLYTIC ACTIVITY IN FISH–BARK COMPOSTS
INITIAL C:N RATIOS 45 AND 55
C:N 45
C:N 55
LSD0.01 A) B)
UNITS OF ACTIVITY
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
28
32
TIME (DAYS)

3.2.4 Nitrogen Transformations During Composting

3.2.4.1 Ammonification and Nitrification

Levels of net ammonification and nitrification were followed during the composting of fish-bark mixes of initial C:N=25, 35, 45, 55 and 65 (Figure 24), and fish-, urea- and sewage-bark mixes of initial C:N=25 and 35 (Figures 25 & 26), (Appendix 5). It is important to stress that in the following data only net values were determined, not rates of production. For example, similar net levels of ammonification may result from rapid incorporation of ammonium into the biomass from a rapidly ammonifying population as compared to slow incorporation from an inactive microflora.

From the AOV of the ammonification data, significant ($p < 0.01$) differences were observed between C:N ratios and the different N treatments. A significant difference was also shown within treatments over time and between different treatments over time (Appendix 85. Similar patterns were observed during each run of the composter (Appendix 5). However, with the exception of data from fish-bark compost of initial C:N=65, there was no significant ($p < 0.05$) difference in ammonification between other fish-bark mixes over time. This is apparent from Figure 24, as peaks in ammonification occurred at days 12 and 20 for all other fish-bark mixes. The bimodal pattern of ammonification was not observed with the urea- and sewage-bark mixes. In these mixes, at both C:N ratios, there was generally a single peak in ammonification at d12-16 for the urea-bark mixes and d20 for the sewage-bark mixes (Figures 25 & 26).

The AOV for the nitrification data, were similar to those just reported for ammonification (Appendices 5 & 8). The fish-bark mixes generally exhibited a unimodal pattern of nitrification with a peak at d16-20 for composts with C:N ratios of 25, 35, 45 and 55, or at d12 for other amendments, coinciding with peak ammonification. The sewage-bark mixes however, showed the reverse trend with peak nitrification apparently preceding ammonification by 8d.

Figure 24

Net ammonification and nitrification in fish-bark composts of various initial C:N ratios.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by titration following steam distillation.

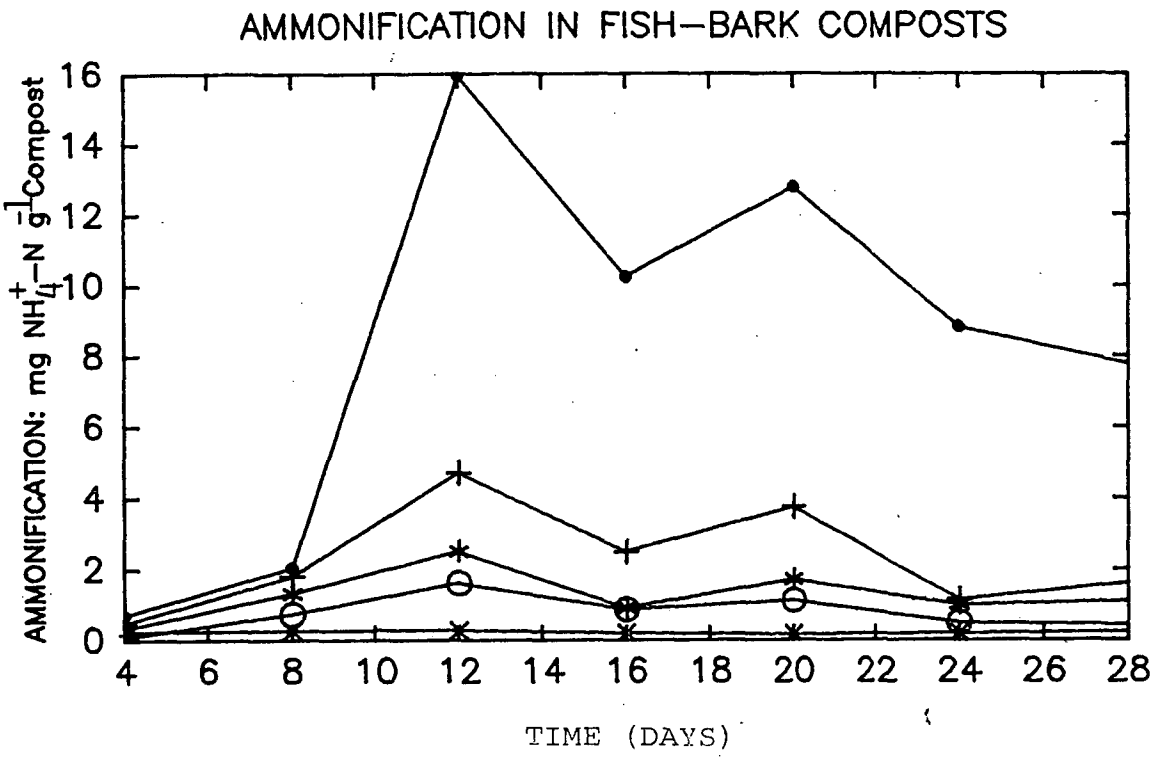
Data for Figure 24 are shown in Appendix 4 and AOV results are given in Appendix 8.1.

$LSD_{(0.01)}$ between two means at :-

	Ammonification	Nitrification
A) different times in the one treatment:	1.12	0.48
B) any time or treatment :	1.54	0.84

Fish-bark Mix of initial C:N :-

.	25
+	35
*	45
O	55
X	65


AMMONIFICATION IN FISH–BARK COMPOSTS
AMMONIFICATION: mg NH_4^+–N g^{-1}Compost
0
2
4
6
8
10
12
14
16
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

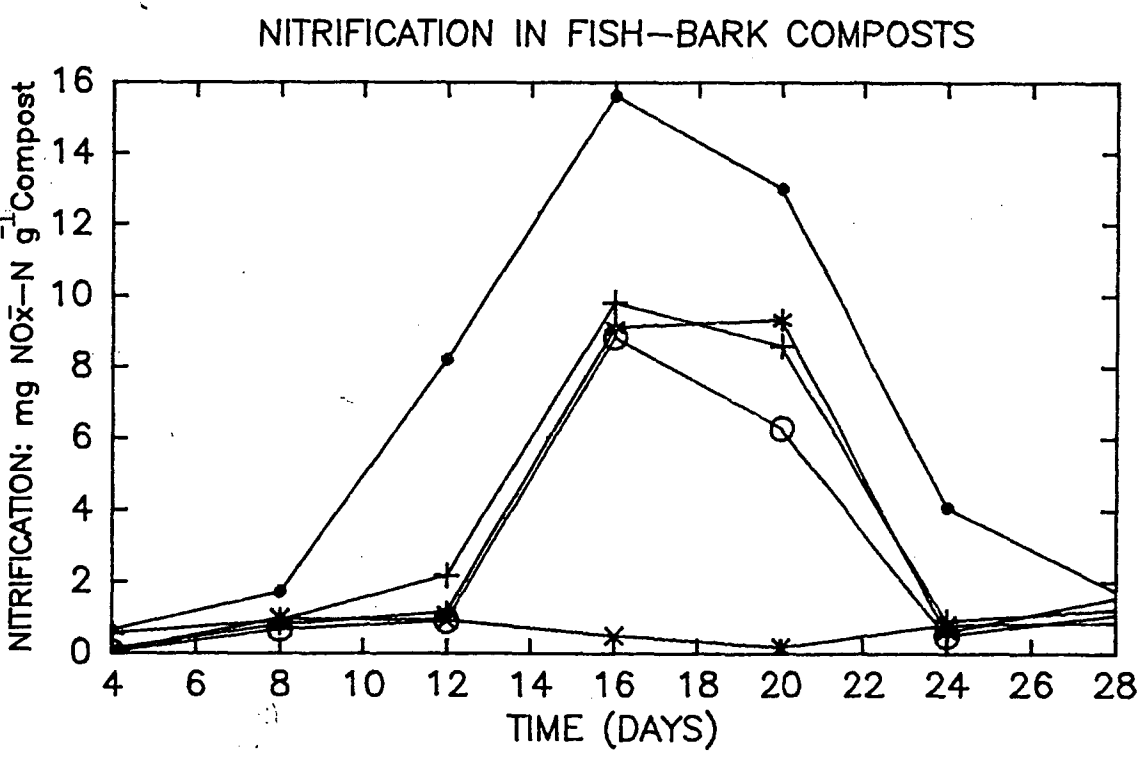

NITRIFICATION IN FISH–BARK COMPOSTS
NITRIFICATION: mg NOx^-–N g^{-1}Compost
0
2
4
6
8
10
12
14
16
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Figure 25

Net ammonification and nitrification in fish, urea- and sewage-bark composts of initial C:N=25.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by titration following steam distillation.

Data for Figure 25 are shown in Appendix 4 and AOV results are given in Appendix 5.9.

$LSD_{(0.01)}$ between two means at :-

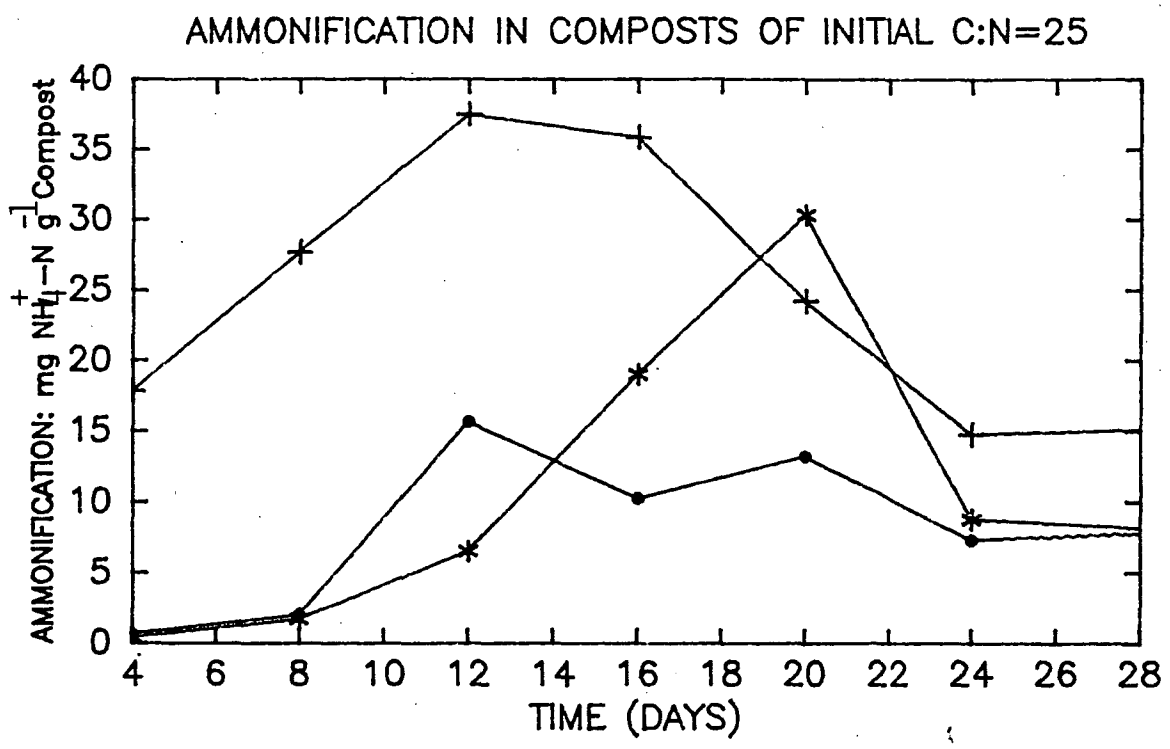
	Ammonification	Nitrification
A) different times in the one treatment:	4.17	2.16
B) any time or treatment :	8.41	5.00

Compost :

. Fish-bark

\+ Urea-bark

* Sewage-bark


AMMONIFICATION IN COMPOSTS OF INITIAL C:N=25
AMMONIFICATION: mg NH_4^+–N g^{-1}Compost
0
5
10
15
20
25
30
35
40
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

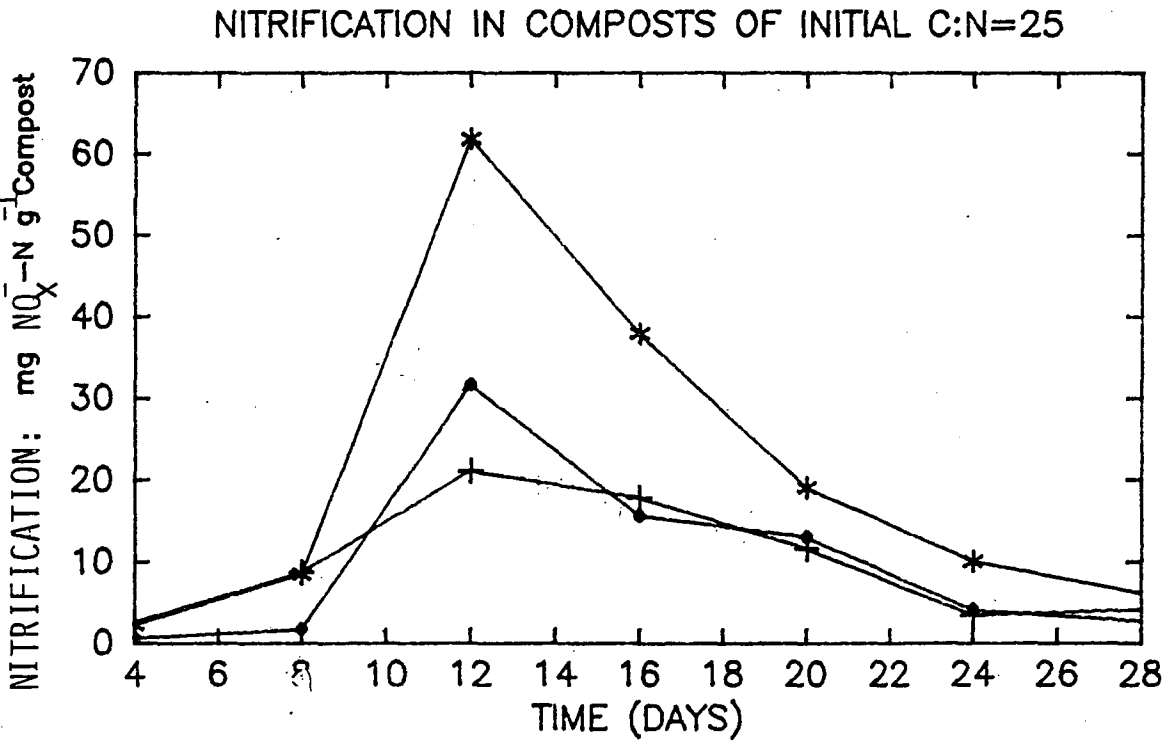

NITRIFICATION IN COMPOSTS OF INITIAL C:N=25
NITRIFICATION: mg NO_X^-–N g^{-1}Compost
0
10
20
30
40
50
60
70
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Figure 26

Net ammonification and nitrification in fish, urea- and sewage-bark composts of initial C:N=35.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by titration following steam distillation.

Data for Figure 26 are shown in Appendix 4 and AOV results are given in Appendix 5.8.

$LSD_{(0.01)}$ between two means at :-

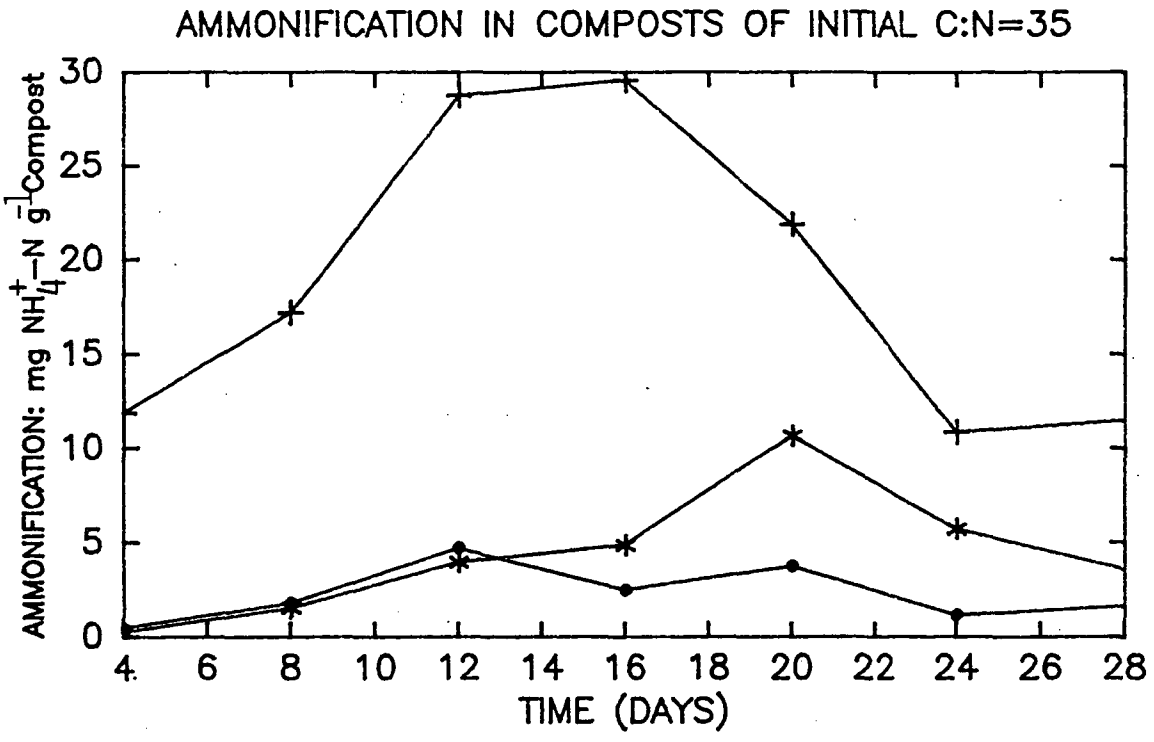
	Ammonification	Nitrification
A) different times in the one treatment:	4.88	2.89
B) any time or treatment :	10.29	6.08

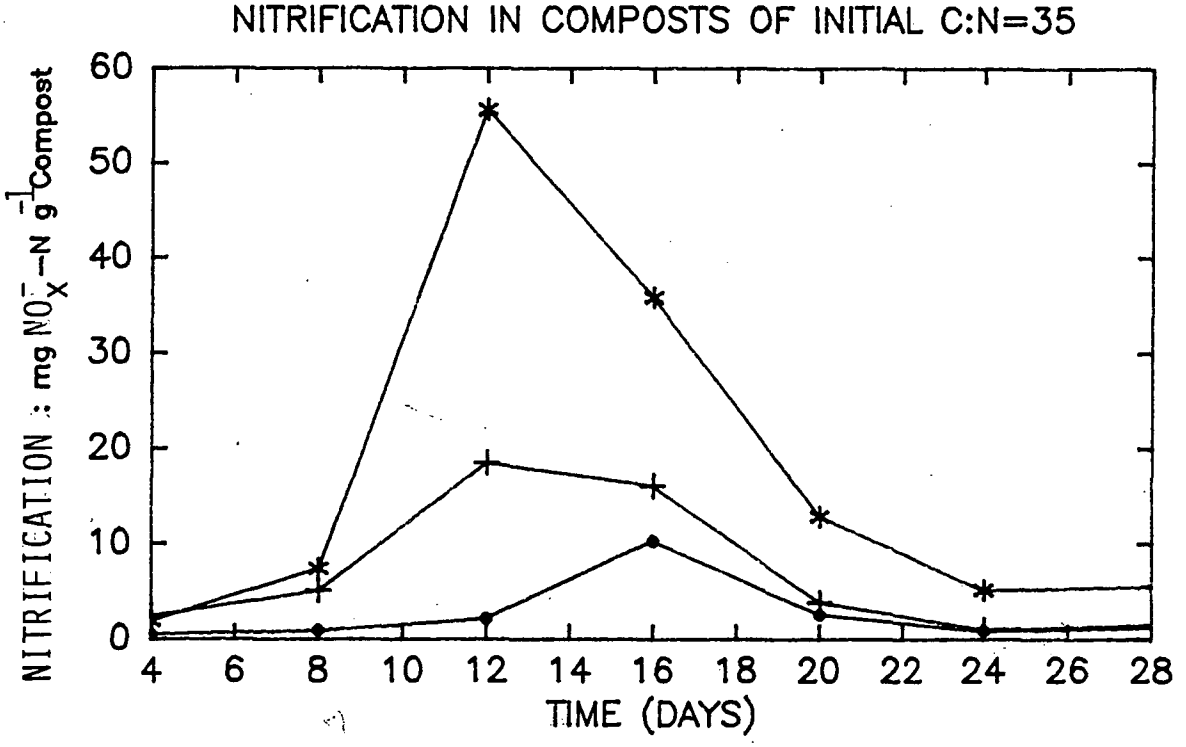
Compost :

. Fish-bark

\+ Urea-bark

* Sewage-bark


AMMONIFICATION IN COMPOSTS OF INITIAL C:N=35
AMMONIFICATION: mg NH_4^+–N g^{-1}Compost
0
5
10
15
20
25
30
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)


NITRIFICATION IN COMPOSTS OF INITIAL C:N=35
NITRIFICATION : mg NO_X^-–N g^{-1}Compost
0
10
20
30
40
50
60
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

During composting, significant correlations were observed between pH (Figure 27) and ammonification and to a lesser degree between both pH and CMCase with nitrate levels (Tables 5 & 15).

During R4 (fish- and urea-bark composts of initial C:N=45) N mineralization was followed by monitoring levels of ammonium, nitrite and nitrate ions (Figure 28). Accumulation of nitrite-N was mainly restricted to d12-16, although low levels were detected to d28 in the fish-bark composts. The concentration of nitrate-N after d8 was generally greater than that of the other two forms of mineral N.

Hydrolysis of urea during R8 was also examined in the urea- and IBDU-bark composts. Within 4d of commencement of composting nearly all urea in the urea-bark compost was hydrolysed, while considerable quantities of urea were still present in the p-hydroxybenzoquinone amended urea-bark and IBDU-bark composts, becoming undetectable after 20 or 24 days respectively (Figure 29).

Table - 15

Correlation Matrix of pH, Ammonium and Nitrate During 28 Days Composting of all Mixes. [1]

	r		
pH	1.0000		
Ammonium	0.5978	1.0000	
Nitrate	0.0556	0.3437	1.0000
	pH	Ammonium	Nitrate

[1]Critical values for r with 61 df were 0.250 & 0.325 ($p < 0.05$ & 0.01 respectively). Data was collected at days 4, 8, 12, 16, 20, 24 and 28 (Appendix 4).

Figure 27

pH levels in composts of various initial C:N ratios.

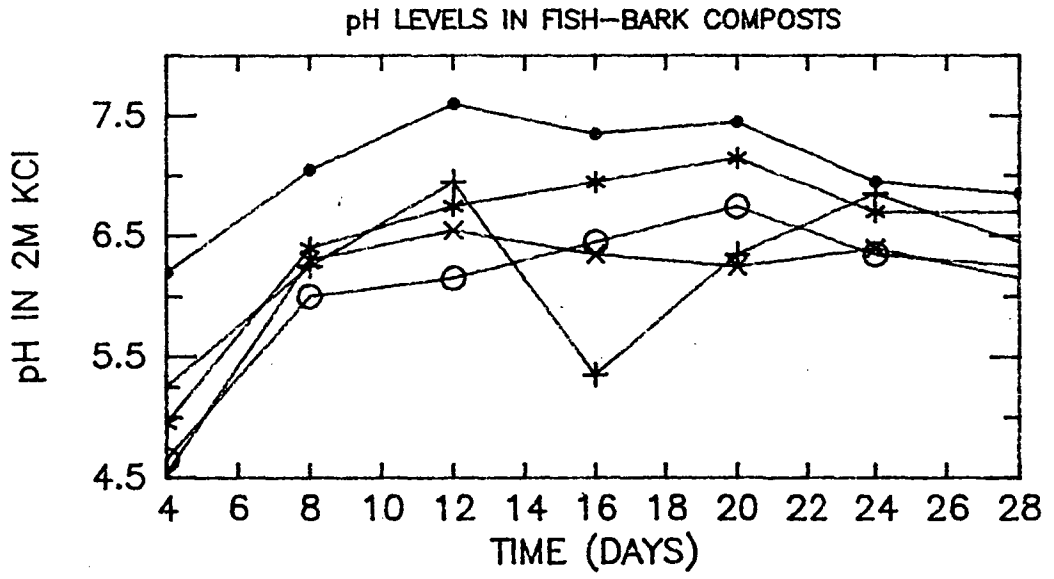
Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by a glass electrode.

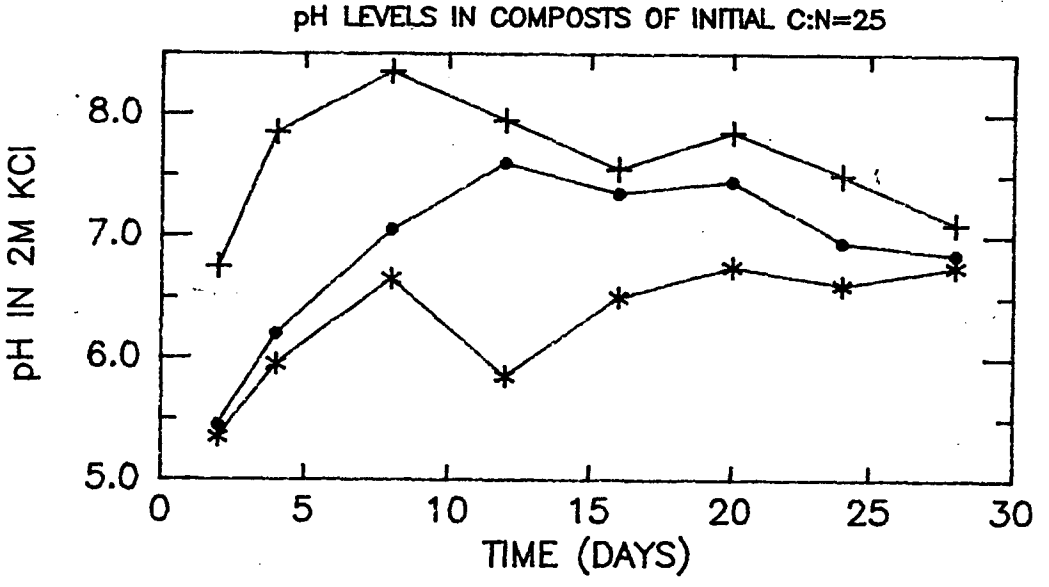
Data for Figure 27 are shown in Appendix 4 and AOV results are given in Appendix 8.2.

$LSD_{(0.01)}$ between two means at :-

	Composts	
	Fish-bark	C:N=25 & 35
A) different times in the one treatment:	0.5	0.4
B) any time or treatment :	0.3	0.3

Fish-bark Composts of initial C:N :		Composts of initial C:N=25 or 35 :	
.	25	.	Fish-bark
+	35	+	Urea-bark
*	45	*	Sewage-bark
0	55		


pH LEVELS IN FISH–BARK COMPOSTS
pH IN 2M KCl
7.5
6.5
5.5
4.5
4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (DAYS)


pH LEVELS IN COMPOSTS OF INITIAL C:N=25
pH IN 2M KCl
8.0
7.0
6.0
5.0
0 5 10 15 20 25 30
TIME (DAYS)

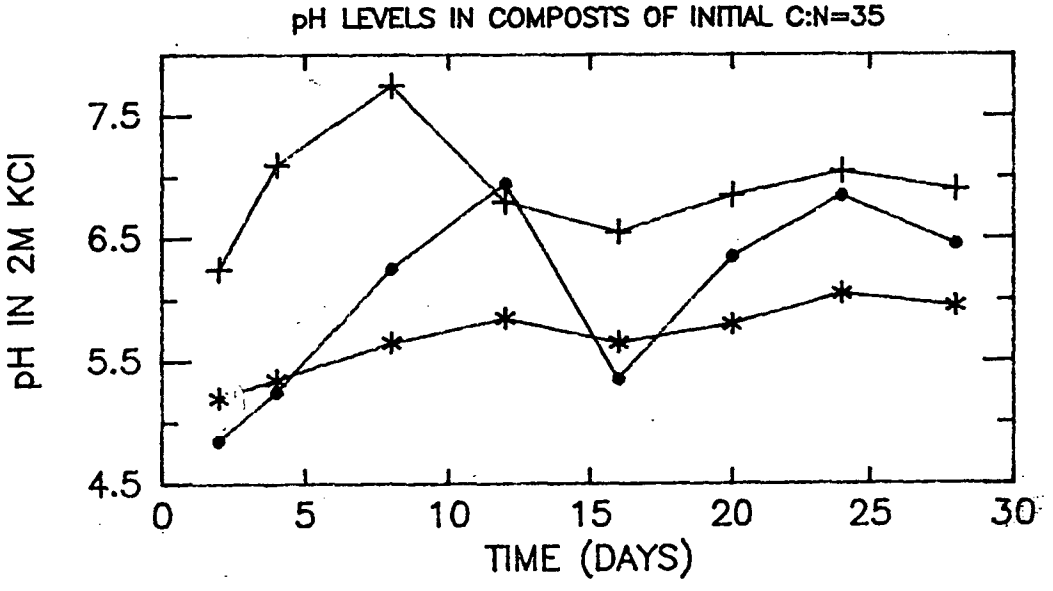

pH LEVELS IN COMPOSTS OF INITIAL C:N=35
pH IN 2M KCl
7.5
6.5
5.5
4.5
0 5 10 15 20 25 30
TIME (DAYS)

Figure 28

Net mineralization of N in fish- and urea-bark composts of initial C:N=45.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by titration following steam distillation.

Data for Figure 28 are shown in Appendix 4.5 (R4) and AOV results are given in Appendix 5.4.

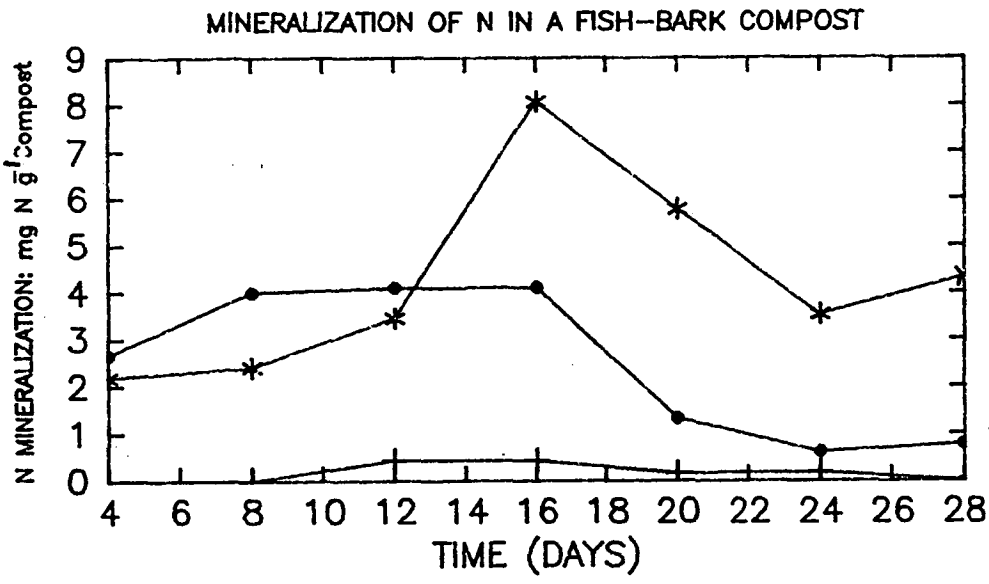
$LSD_{(0.01)}$ between two means at :-

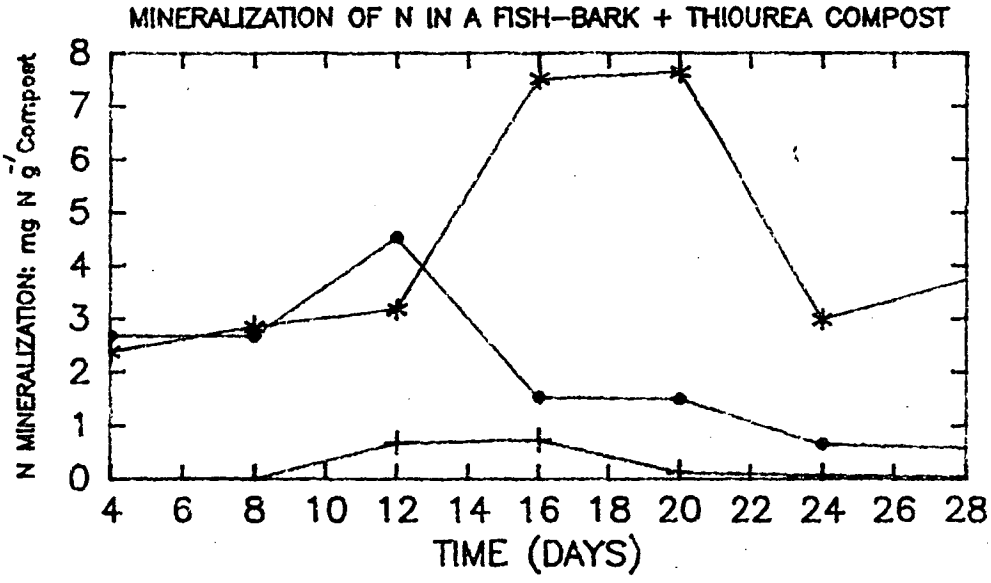
	NH_4^+	NO_2^-	NO_3^-
A) different times in the one treatment:	3.07	0.08	3.69
B) any time or treatment :	6.20	0.16	8.15

. NH_4^+

\+ NO_2^-

* NO_3^-


MINERALIZATION OF N IN A FISH–BARK COMPOST
N MINERALIZATION: mg N g⁻¹Compost
0 1 2 3 4 5 6 7 8 9
4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (DAYS)


MINERALIZATION OF N IN A FISH–BARK + THIOUREA COMPOST
N MINERALIZATION: mg N g⁻¹Compost
0 1 2 3 4 5 6 7 8
4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (DAYS)

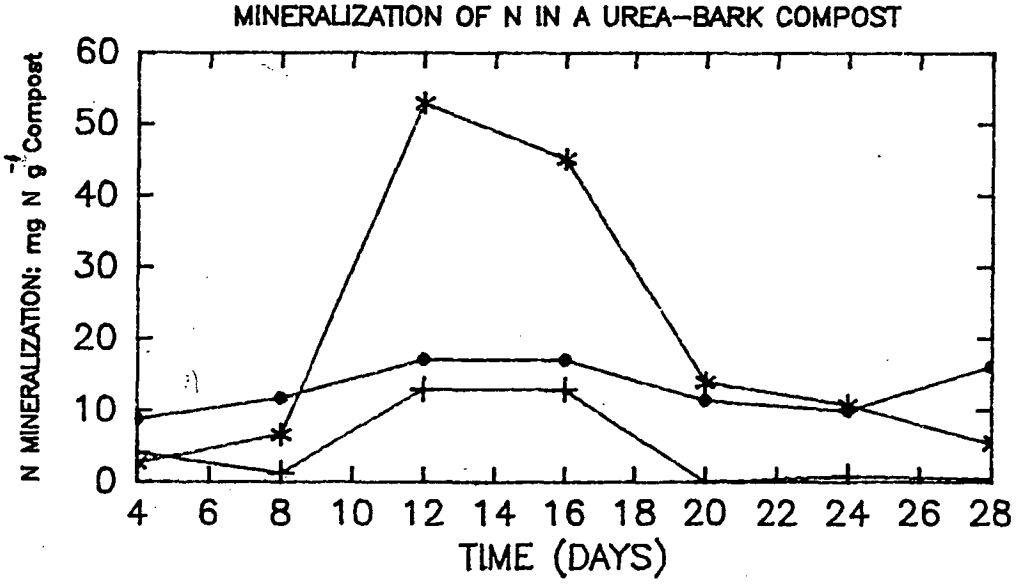

MINERALIZATION OF N IN A UREA–BARK COMPOST
N MINERALIZATION: mg N g⁻¹Compost
0 10 20 30 40 50 60
4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (DAYS)

Figure 29

Residual urea in urea- and IBDU-bark composts of initial C:N=35.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl-phenylmercuric acetate) assayed colorimetrically.

Data for Figure 29 are shown in Appendix 4.10 (R8) and AOV results are given in Appendix 5.8.

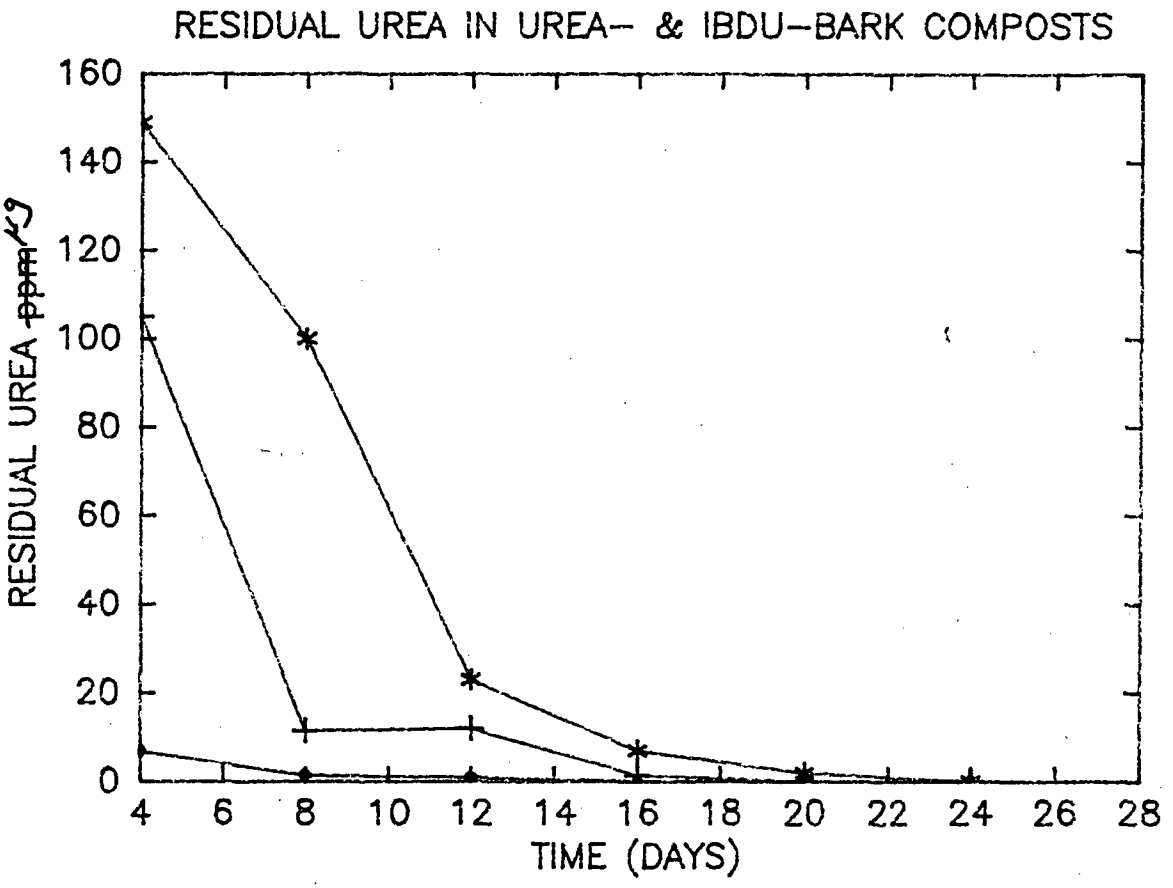
$LSD_{(0.01)}$ between two means at :-

A) different times in the one treatment: 0.22
B) any time or treatment : 0.46

. Urea-bark

\+ Urea-bark + p-benzoquinone

* IBDU-bark


RESIDUAL UREA IN UREA– & IBDU–BARK COMPOSTS
RESIDUAL UREA ppm/g
160
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

3.2.4.2 Non-biological N-Transformations

The use of various compost sterilants was considered to investigate the possibility of non-biological N-transformations occurring under thermophilic conditions. During R3, propylene oxide was shown to be an effective microbial inhibitor for only 5-10d (Figure 13), but sodium azide maintained very low CO_2 output and no detectable CMCase or lipase activity throughout the 28d of composting during R5 (Figure 11 & Appendices 3 & 4 respectively).

Data from R5 (Figure 30) showed that sterilization significantly ($p < 0.01$) reduced the level of ammonification at all sample times except days 4 and 16 when this situation was reversed. The level of ammonification in the C:N=55 compost was also greater than that of the sterile (C:N=45) compost, except for days 24 and 28 when there was no significant difference ($p < 0.05$) between the composts.

The presence of a peak (maximum at d16) in ammonification in the sterile compost was evidence for non-biological ammonification occurring during the thermophilic phase of composting. This non-biological flush in ammonification followed the biological flush by some four days (Figure 30).

Non-biological nitrification was also observed during the thermophilic phase of composting (Figure 30). There was generally no significant ($p < 0.01$) difference between the level of nitrate in the presence or absence of microbial inhibitors, although in the absence of a microbial inhibitor, a greater net level of nitrification was noted at d8 and 28 in the composts.

Figure 30

Net mineralization of N in sterile and non-sterile fish-bark composts of initial C:N=45.

Each determination was the mean of duplicate extracts (2g compost in 10mL 2M KCl) assayed by titration following steam distillation.

Data for Figure 30 are shown in Appendix 4.6 (R5) and AOV results are given in Appendix 5.5.

$LSD_{(0.01)}$ between two means at :-

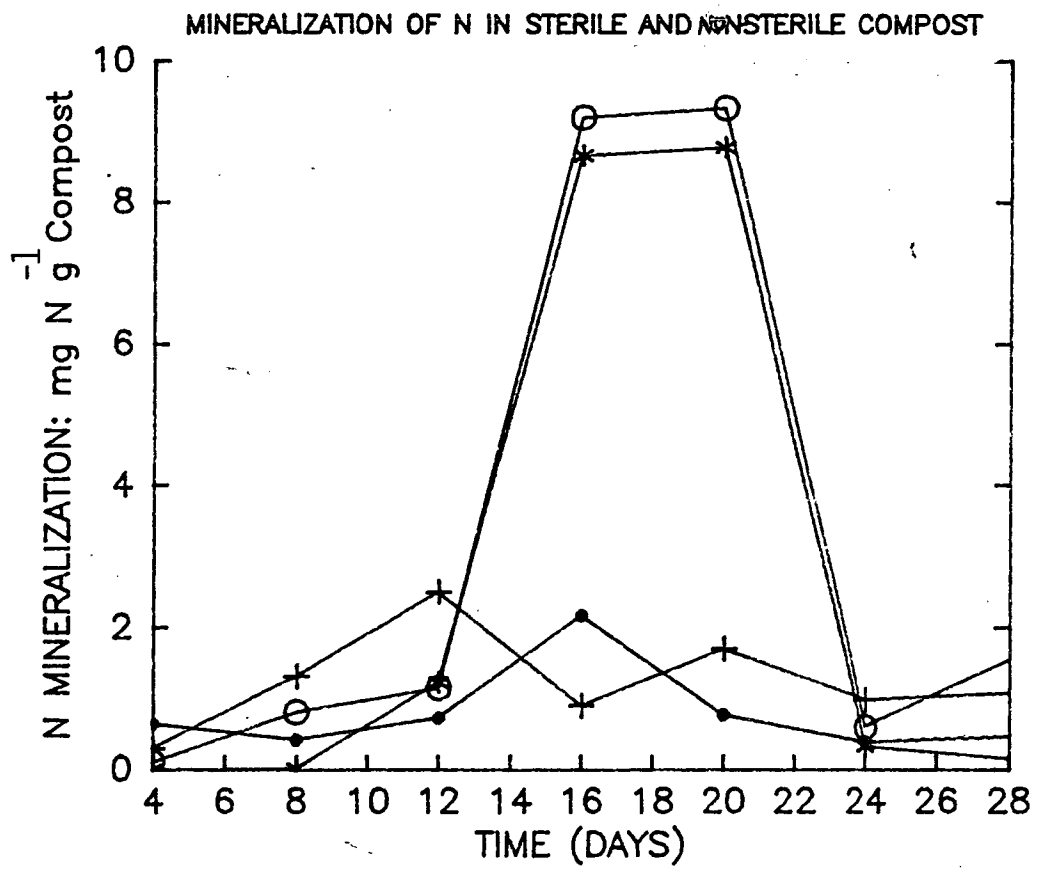
	NH_4^+	NO_3^-
A) different times in the one treatment:	0.31	0.36
B) any time or treatment :	0.64	0.58

. NH_4^+ in sterile compost

\+ NH_4^+ in non-sterile compost

* NO_3^- in sterile compost

0 NO_3^- in non-sterile compost


MINERALIZATION OF N IN STERILE AND NONSTERILE COMPOST
N MINERALIZATION: mg N g^{-1} Compost
0
2
4
6
8
10
4 6 8 10 12 14 16 18 20 22 24 26 28
TIME (DAYS)

3.2.4.2 Volatilization of Nitrogen During Composting

Percentage losses of N, via volatilization of ammonia and N-oxides from the various composts, are given in Table 16. Up to 25% loss of NH_3-N occurred in composts of urea-bark with this being some ten fold greater than the loss from equivalent fish- or sewage-bark composts. Levels of N-oxides volatilized were, however, less and in the reverse order to the losses of ammonia in fish-, urea- and sewage-bark composts (Figures 31-33).

The use of p-benzoquinone + urea or IBDU to slow down the release of urea resulted in an overall greater loss of N than that from unamended urea-bark (Table 16). The patterns of N loss resulting from these treatments are illustrated in Figure 34.

Statistical comparisons were made between N loss data from the fish-bark composts of initial C:N=25, 35, 45 and 55 and between each amendment at a C:N=25 and 35. From the AOV tables (Appendix 8) significant (at least at $p < 0.01$) differences were shown between treatments, within a treatment over time and between treatments over time.

Despite these overall differences in N loss resulting from different treatments, some common patterns of N loss were apparent (Figures 31-33). In fish-, urea- and sewage-bark composts with an initial C:N=25 there was a bimodal ammonia release, with peaks occurring at d8 and 12 in fish and urea composts and at d12 and 16 in the sewage composts. Also, all composts that exhibited some loss of N-oxides showed an increased loss at d6 or 8 and generally a smaller peak loss at d18 or 20.

Figure 31

Volatilization of ammonia and N-oxides from fish-bark composts of various initial C:N ratios.

Each determination was the mean of NH_3 and nitrogen oxides trapped from duplicate units in 0.1M H_2SO_4 and assayed by titration following steam distillation every 2-3d.

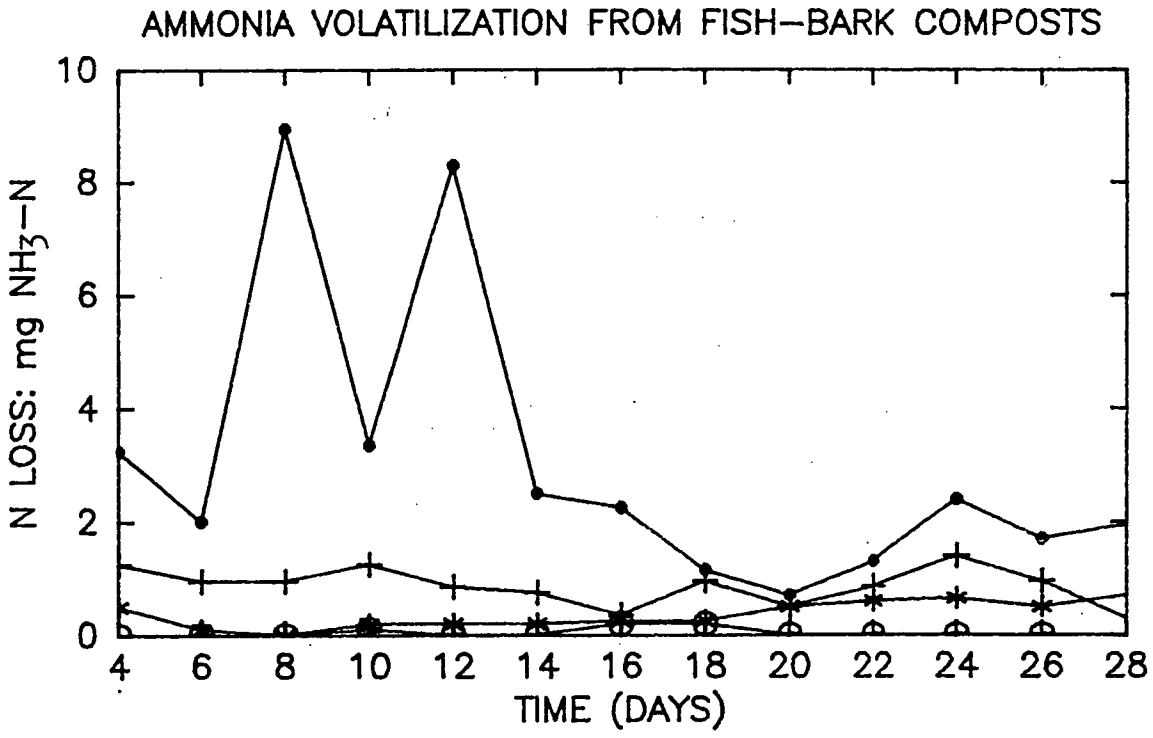
Data for Figure 31 are shown in Appendix 4 and AOV results are given in Appendix 8.1.

$LSD_{(0.01)}$ between two means at :-

	Ammonia	N-oxides
A) different times in the one treatment:	0.17	0.46
B) any time or treatment :	0.28	1.64

Fish-bark compost of initial C:N :-

.	25
+	35
*	45
0	55


AMMONIA VOLATILIZATION FROM FISH–BARK COMPOSTS
N LOSS: mg NH3–N
0
2
4
6
8
10
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

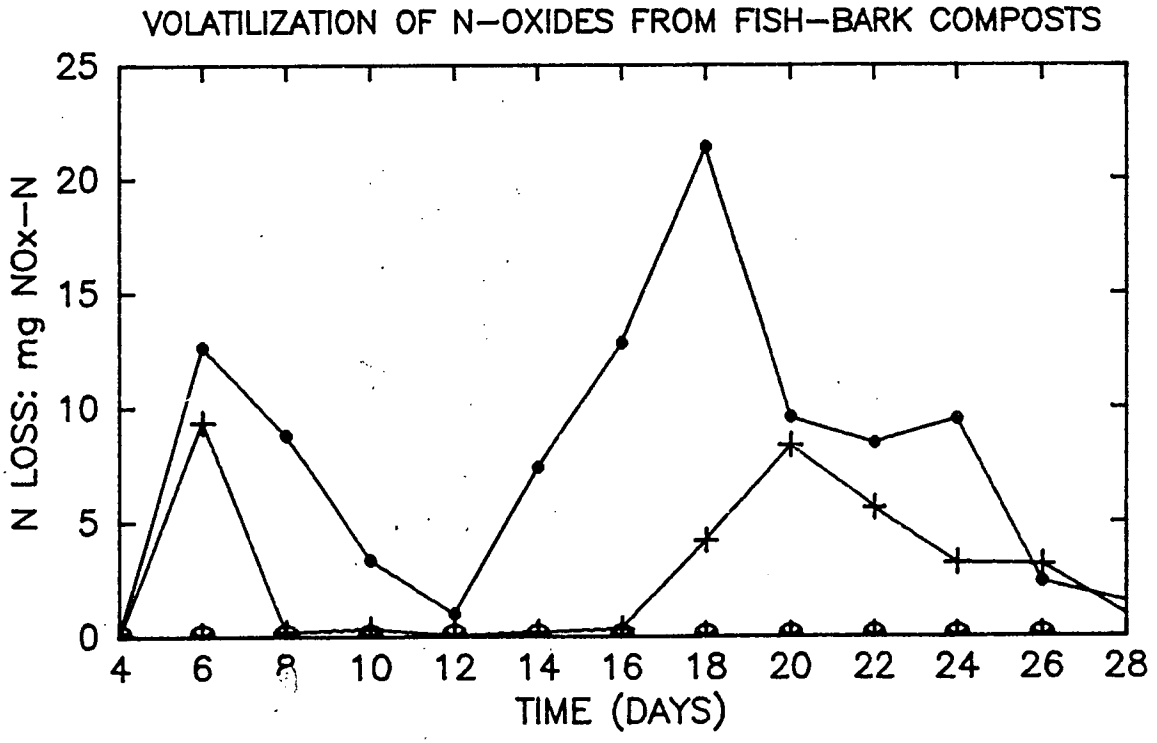

VOLATILIZATION OF N–OXIDES FROM FISH–BARK COMPOSTS
N LOSS: mg NOx–N
0
5
10
15
20
25
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Figure 32

Volatilization of ammonia and N-oxides from fish-, urea- and sewage-bark composts of initial C:N=25.

Each determination was the mean of NH_3 and nitrogen oxides trapped from duplicate units in 0.1M H_2SO_4 and assayed by titration following steam distillation every 2-3d.

Data for Figure 32 are shown in Appendix 4.11 (R9) and AOV results are given in Appendix 5.9.

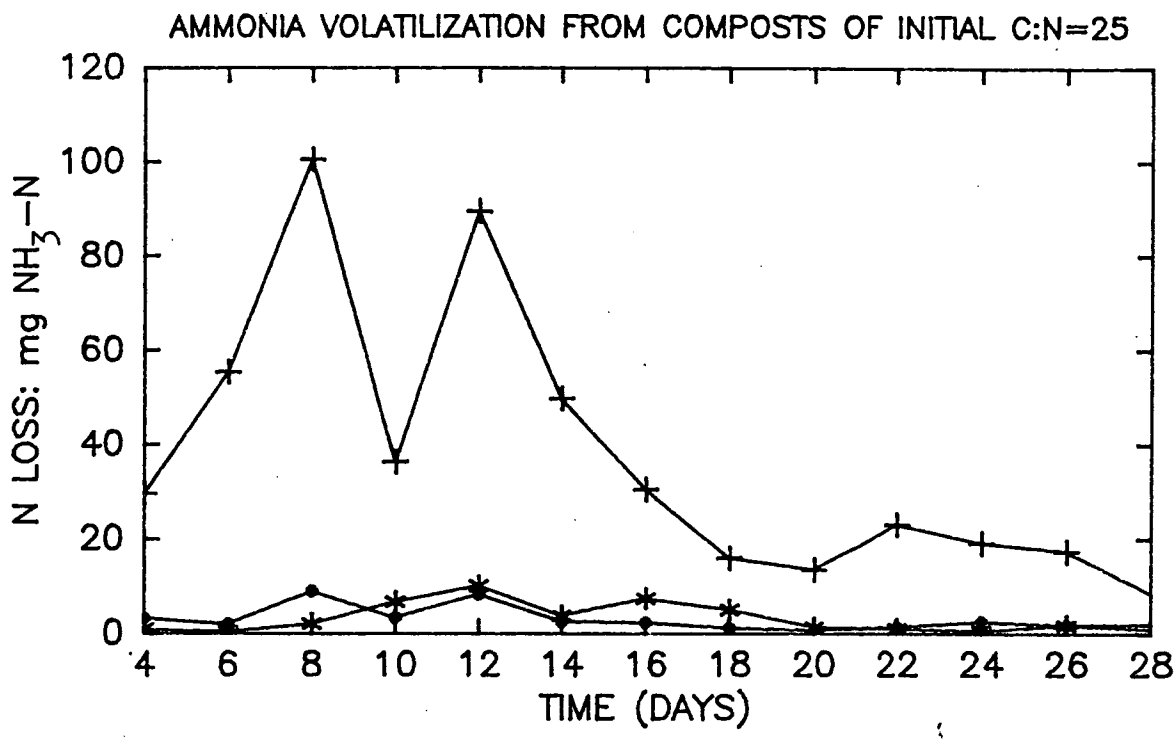
$LSD_{(0.01)}$ between two means at :-

	Ammonia	N-oxides
A) different times in the one treatment:	0.18	0.15
B) any time or treatment :	0.35	0.32

. Fish-bark compost.

\+ Urea-bark compost.

* Sewage-bark compost.


AMMONIA VOLATILIZATION FROM COMPOSTS OF INITIAL C:N=25
N LOSS: mg NH_3–N
120
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

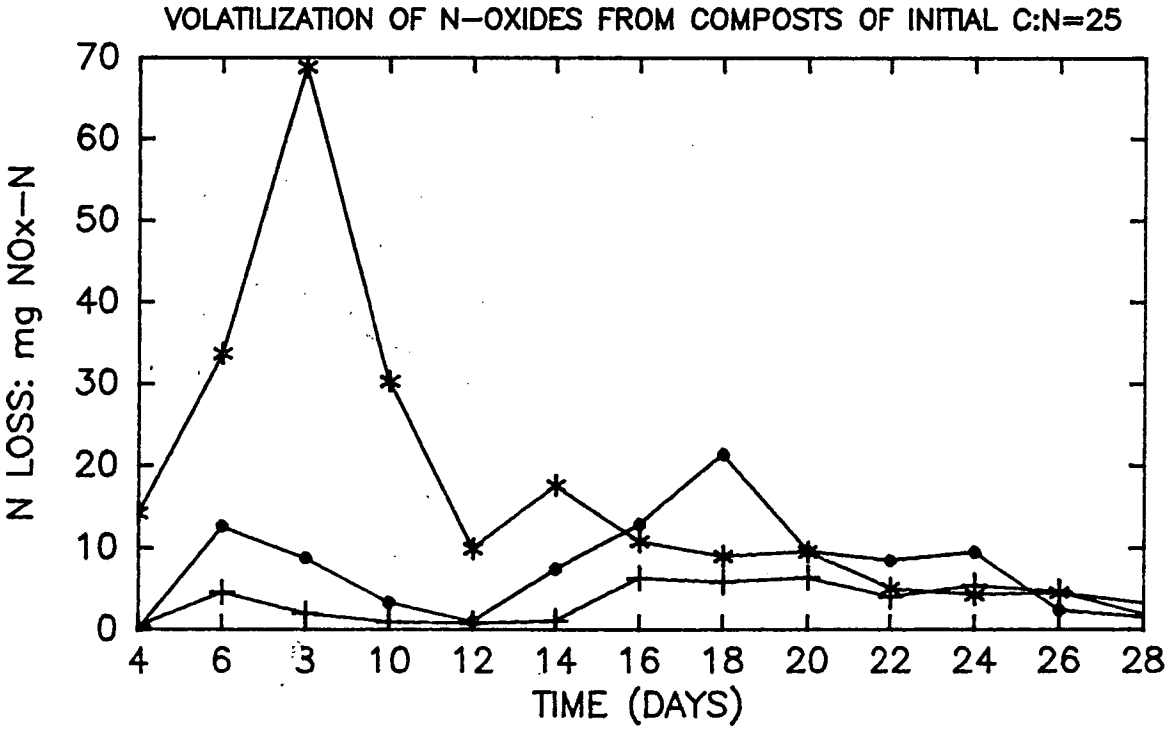

VOLATILIZATION OF N–OXIDES FROM COMPOSTS OF INITIAL C:N=25
N LOSS: mg NOx–N
70
60
50
40
30
20
10
0
4
6
3
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Figure 33

Volatilization of ammonia and N-oxides from fish-, urea- and sewage-bark composts of initial C:N=35.

Each determination was the mean of NH_3 and nitrogen oxides trapped from duplicate units in 0.1M H_2SO_4 and assayed by titration following steam distillation every 2-3d.

Data for Figure 33 are shown in Appendix 4.8 (R7) and AOV results are given in Appendix 5.6.

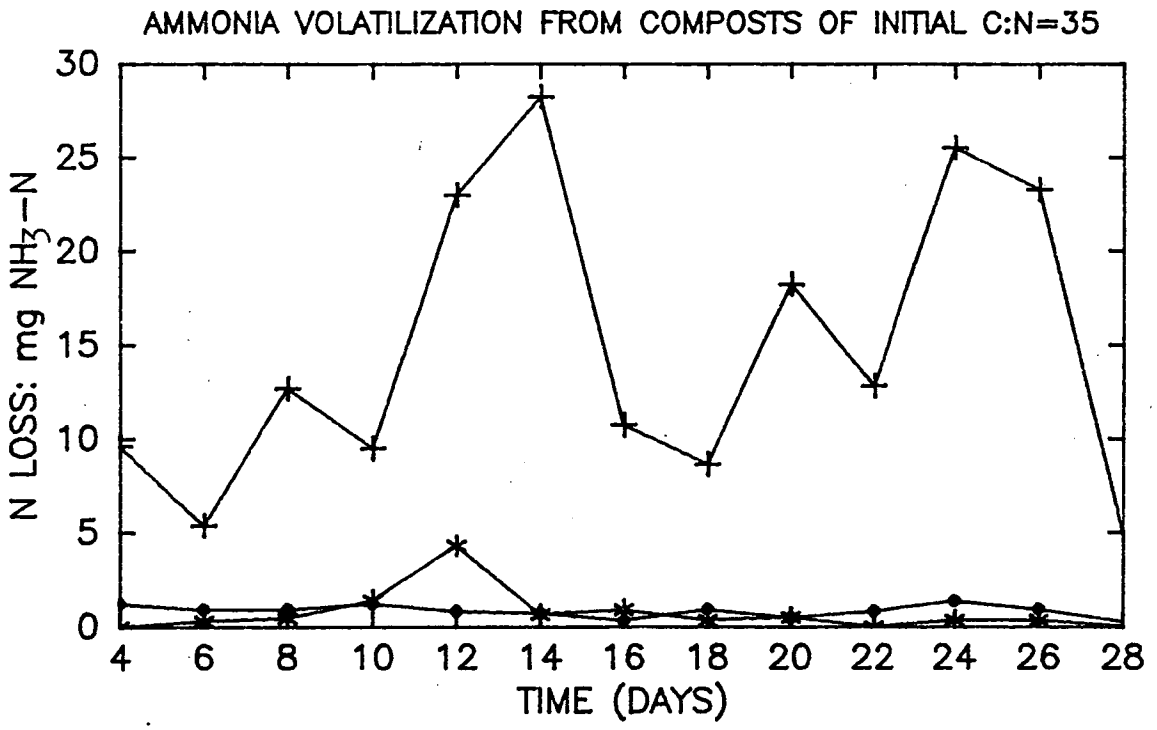
$LSD_{(0.01)}$ between two means at :-

	Ammonia	N-oxides
A) different times in the one treatment:	0.37	0.34
B) any time or treatment :	0.69	0.53

. Fish-bark compost.

\+ Urea-bark compost.

* Sewage-bark compost.


AMMONIA VOLATILIZATION FROM COMPOSTS OF INITIAL C:N=35
N LOSS: mg NH3-N
0
5
10
15
20
25
30
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

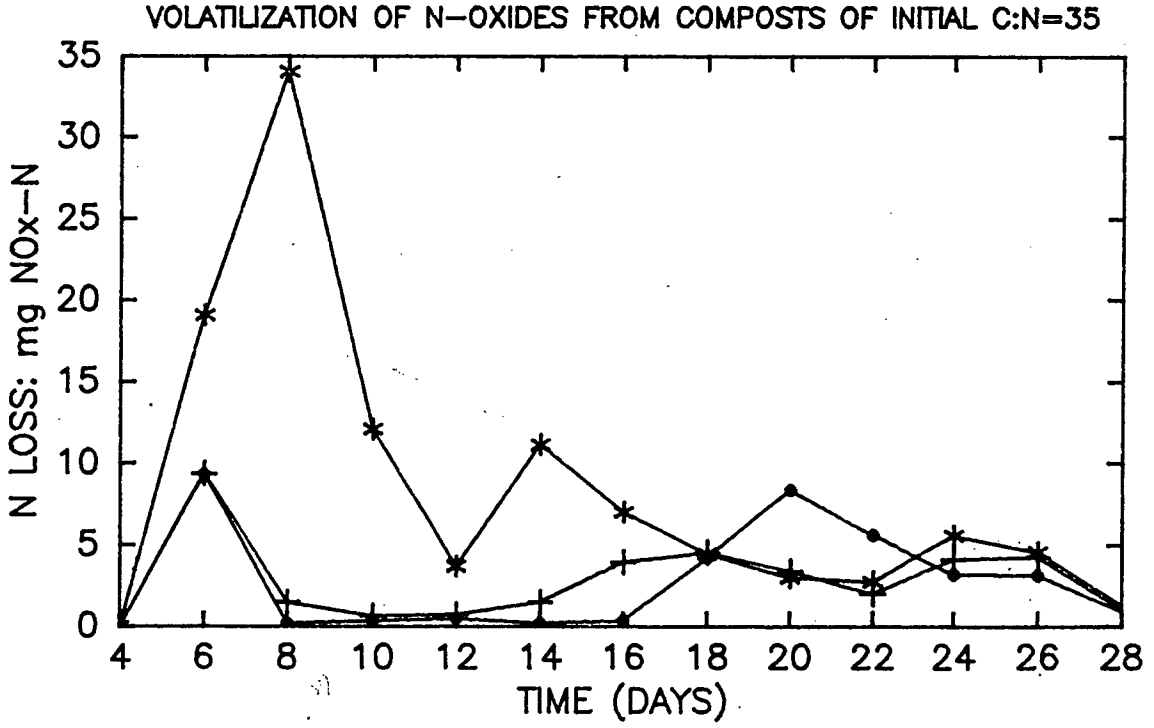

VOLATILIZATION OF N-OXIDES FROM COMPOSTS OF INITIAL C:N=35
N LOSS: mg NOx-N
0
5
10
15
20
25
30
35
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Figure 34

Volatilization of ammonia and N-oxides from urea-, urea + p-benzoquinone & IBDU-bark composts of initial C:N=35.

Each determination was the mean of NH_3 and nitrogen oxides trapped from duplicate units in 0.1M H_2SO_4 and assayed by titration following steam distillation every 2-3d.

Data for Figure 34 are shown in Appendix 4.10 (R8) and AOV results are given in Appendix 5.8.

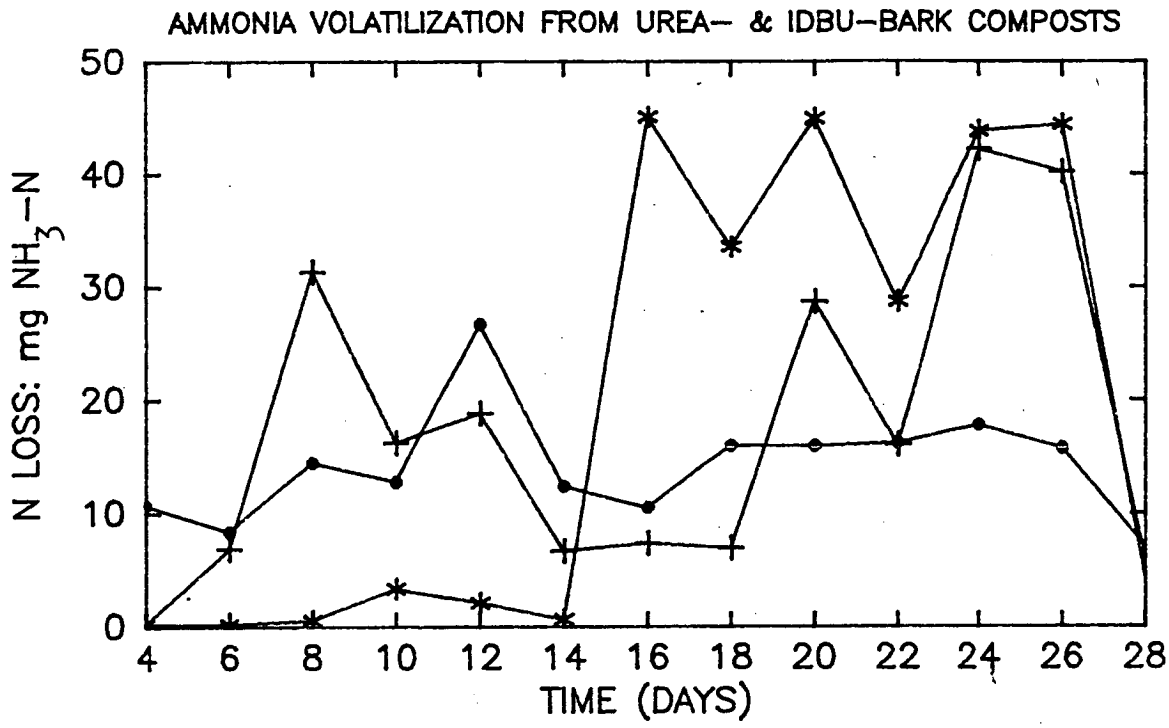
$LSD_{(0.01)}$ between two means at :-

	Ammonia	N-oxides
A) different times in the one treatment:	0.80	0.40
B) any time or treatment :	1.60	0.82

. Urea-bark compost.

\+ Urea-bark + p-benzoquinone compost.

* IBDU-bark compost.


AMMONIA VOLATILIZATION FROM UREA– & IDBU–BARK COMPOSTS
N LOSS: mg NH_3–N
0
10
20
30
40
50
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

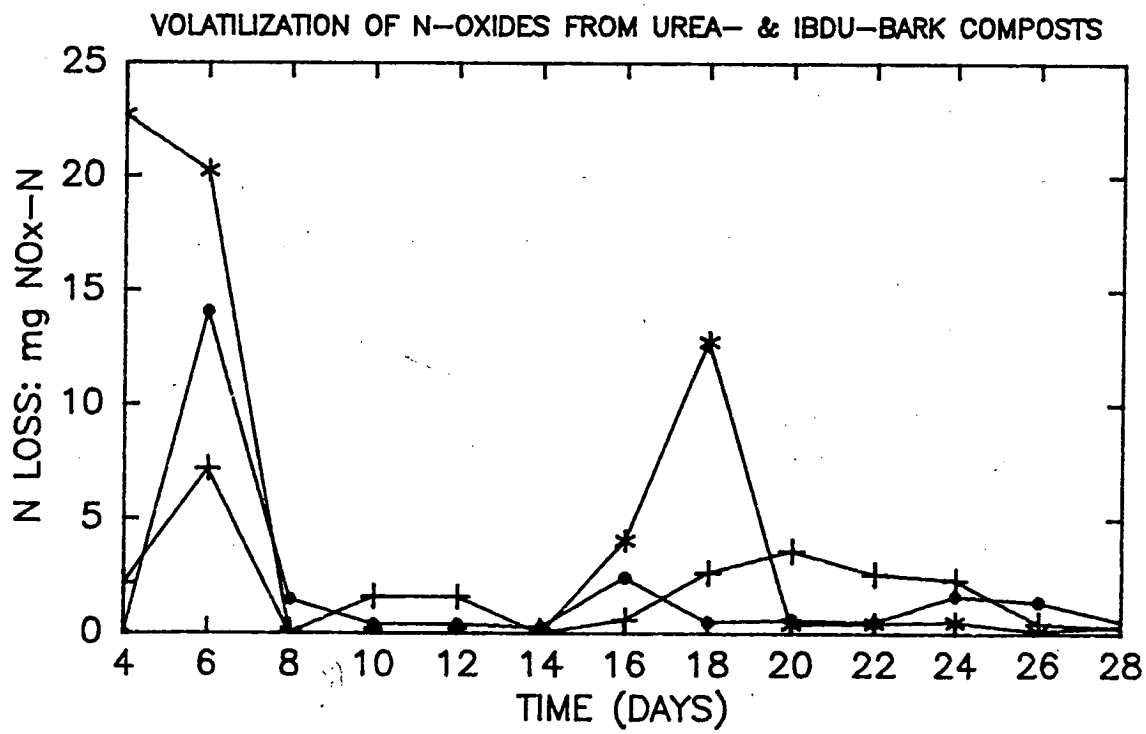

VOLATILIZATION OF N–OXIDES FROM UREA– & IBDU–BARK COMPOSTS
N LOSS: mg NOx–N
0
5
10
15
20
25
4
6
8
10
12
14
16
18
20
22
24
26
28
TIME (DAYS)

Table - 16

Mean Percentage Losses of Nitrogen by Volatilization after 28d of Composting. [1]

Compost	Form of Volatilized Nitrogen NH_3	NO_x
Fish-bark Mixes:		
C:N=25	2.686 a [2]	6.677 a
C:N=35	0.709 b	2.236 b
Sterile C:N=45	0.040 b	0.000
C:N=45	0.251 b	0.000
C:N=55	0.018 b	0.000
Urea-bark Mixes:		
C:N=25	25.277 c	2.223 b
C:N=35	11.193 d	2.125 b
+ quinone "	12.874 e	1.417 b
IBDU-bark "	16.081 f	3.607 c
Sewage-bark Mixes:		
C:N=25	2.650 a	13.400 d
C:N=35	0.597 b	6.756 a

[1] Percentage losses shown are relative to the initial weight of N in each compost. Volatilized N was continuously collected in dilute acid and assayed by titration following steam distillation every 2d, (Appendix 4).

[2] Means followed by a different letter in one column were significantly different ($LDS_{(0.01)}$ = 1.287 & 1.114 for NH_3 and NOx respectively).

3.2.5 Estimation of Compost Microbial Biomass

The microbial biomasses of composts produced from R7 and R8 after 30d composting were calculated by the fumigation method from the CO_2 output data reported in Appendix 3.2 (Figure 35) using a k-factor of 0.45 (Jenkinson *et.al.*, 1979). As observed by other workers (Anderson and Domsch, 1978) a net negative CO_2 output was indicated from all composts for the first 10h. Consequently, microbial biomasses were determined from CO_2 outputs between 10-200 h, and estimated to be 15.99, 15.97, 15.68 and 22.66 mg biomass g^{-1} for fish-, urea-, sewage- and IBDU-bark composts of initial C:N=35. Attempted determinations of microbial biomass in other composts (R8 & R9) were unsuccessful as some units developed water leaks.

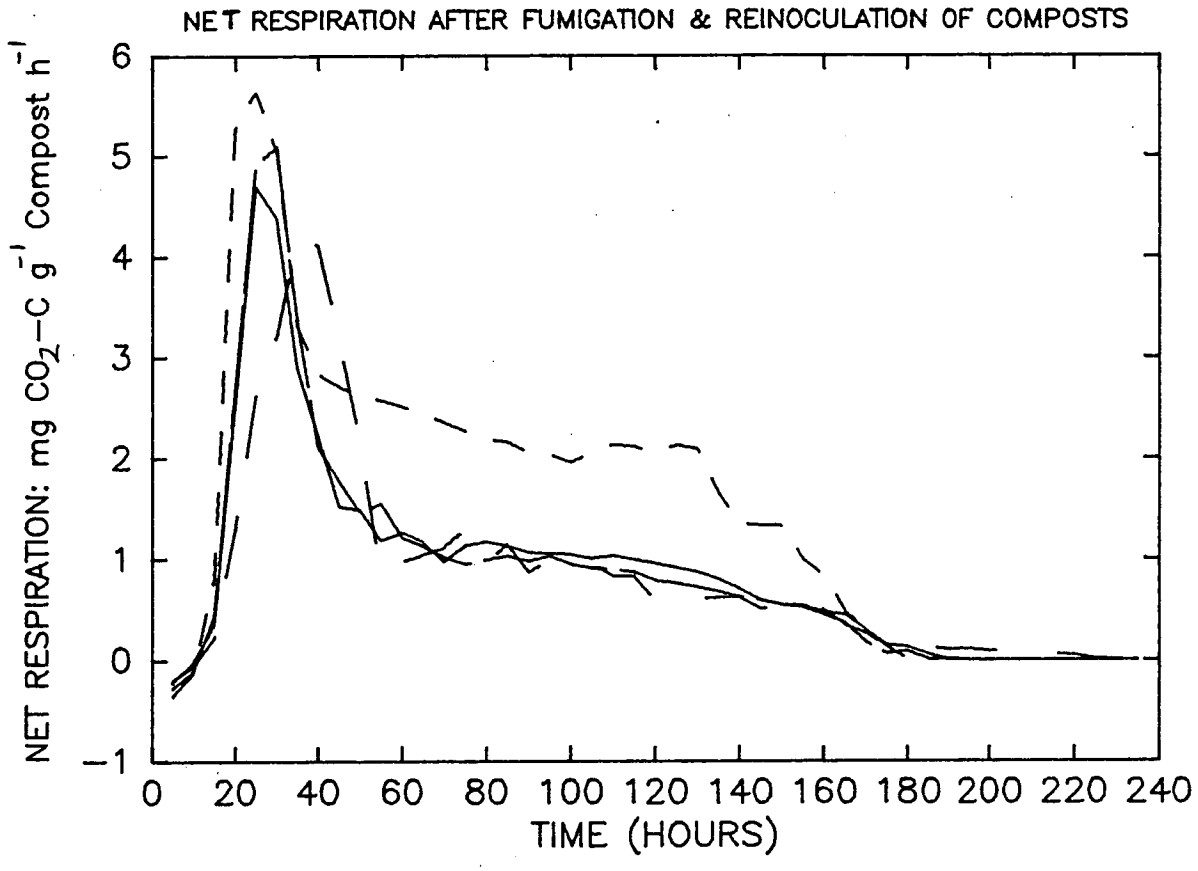
Figure 35

Net respiration curves showing the release of CO_2-C from $CHCl_3$-fumigated, reinoculated composts of initial C:N=35.

Net respiration data for 30d old composts were obtained from paired units, one fumigated the other not. Effluent from each unit was assayed automatically every 5h. The net respiration levels were obtained following subtraction of the respiration data from the matching unfumigated unit.

See Appendix 3.2 for data.

_____ Fish-bark compost.
_____ __ Urea-bark compost.
__ __ Sewage-bark compost.
_ _ _ IBDU-bark compost.


NET RESPIRATION AFTER FUMIGATION & REINOCULATION OF COMPOSTS
NET RESPIRATION: mg CO_2–C g^{-1} Compost h^{-1}
6
5
4
3
2
1
0
−1
0 20 40 60 80 100 120 140 160 180 200 220 240
TIME (HOURS)

3.3 Identification and Biochemical Properties of Compost Isolates

From an original total of 522 microbial isolates representing the predominant ($>10^5$ CFU g^{-1} compost) microbial flora at various stages of composting (R2 & R4), 212 representative strains were selected for characterization and identification (15 isolates from the original total were lost). Of these, 109 were identified as thermophilic *Bacillus spp.*, 24 as mesophilic *Bacillus spp.* and 79 were not identified. The distribution of isolates among taxa from R2 and R4 are given in Tables 17 and 18 and morphological and biochemical properties are given in Appendix 9.

Three strains of pink-pigmented Gram positive coccoid bacteria were the most numerous isolates on TSA or LigA from composting fish-bark of initial C:N=45 at 55°. The biochemical properties of these strains (123, 198 and 240) are given in Appendix 9, but the salient features were their oxidative metabolism, cell wall type 1 (ie. no *meso*-DAP nor arabinose), thermophilic nature (growth between 45-68° with optima at 60-63°) and predominant coccoid morphology with the occasional rod or filament (Plate 4). Rods or filaments were never observed in liquid or solid culture during early stages of growth (cultures were examined at 1, 2, 4, 6, 8, and 12h). The %(G+C) by T_m determined against *Nocardia cellulans* (%G+C = 72.9) was 79 moles %. The methanol extracts of the three strains showed three absorption maxima at 536, 575 and 600 nm being typical of carotenoid pigments. Considering these characteristics the three isolates could not be assigned to any recognized genus, but they did fit the general description for coryneform bacteria.

The 109 thermophilic *Bacillus spp.* isolated during R2 and R4 were compared on 30 morphological, physiological and biochemical characteristics. The group average (UPGMA) method of cluster analysis on these bacteria resulted in groupings (phenons) with

the closest agreement with the identifications obtained using the key of Gordon, *et.al.* (1973). Seventy-eight percent (85 strains) could be grouped into 12 phenons at a taxonomic distance of 0.22 (Figure, 36). Strains identified as *B.brevis* were clearly a diverse group, spanning phenons 1, 2, 5, 7 and 8. Isolates similar to *B.coagulans* were not placed in any phenon while *B.stearothermophilus* isolates belonging to groups 1 and 3 described by Walker and Wolf (1971) were always placed in a different phenon to isolates belonging to group 2. Four out of eight unidentified isolates were placed into one of the twelve phenons shown in Figure 36.

Isolates obtained during R7 and R8 were generally identified to generic level largely on morphology and Gram reaction (Table 19). *Bacillus spp.* dominated all stages of the composting of fish-bark mixtures and for most stages of the composting of sewage-bark mixtures with an initial C:N=35. However, the urea- and IBDU-bark composts supported quite different microbial successions. In these composts, *Bacillus spp.* only dominated during the period between first and second peaks of respiratory activity, after which a succession of *Streptomyces*, *Thermomonospora* and then other *Streptomyces* dominated the microflora (Table 19). The addition of quinone or the replacement of urea with IBDU delayed the peaks in respiratory activity and the estimated numbers of *Bacillus spp.* had dramatically declined at the time of the second peak of respiratory activity.

late 4

.E.M. of isolate 240, pink-pigmented Gram positive coryneform.

hite bar = 5 μm.

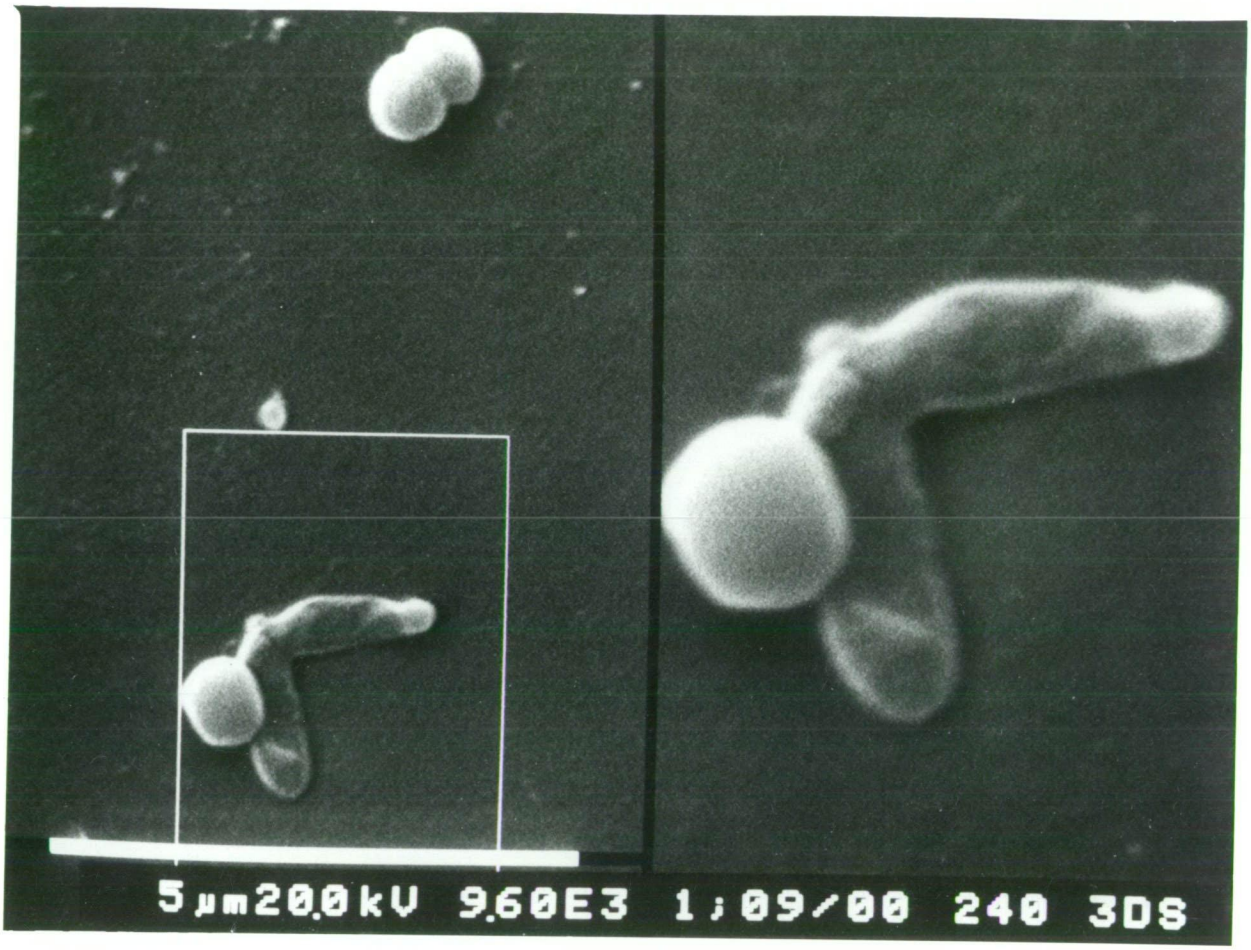

5µm20.0kV 9.60E3 1;09/00 240 3DS

Figure 36

Dendrogram showing the relationship between thermophilic *Bacillus* isolates.

Thirty morphological, physiological and biochemical characteristics were analysed by the Clustan 1C program (Wishart, 1968) using the simple matching coefficient (Sokal and Michener, 1958) and UPGMA sorting algorithm (Sneath and Sokal, 1973).

See Appendix 9 for data.

Bre- *Bacillus brevis*
Cir- *B.circulans*
Coa- *B.coagulans*
Meg- *B.megaterium*
S.1- *B.stearothermophilus* group 1
S.2- *B.stearothermophilus* group 2
S.3- *B.stearothermophilus* group 3
Sph- *B.sphaericus*
?- *B.sp.*

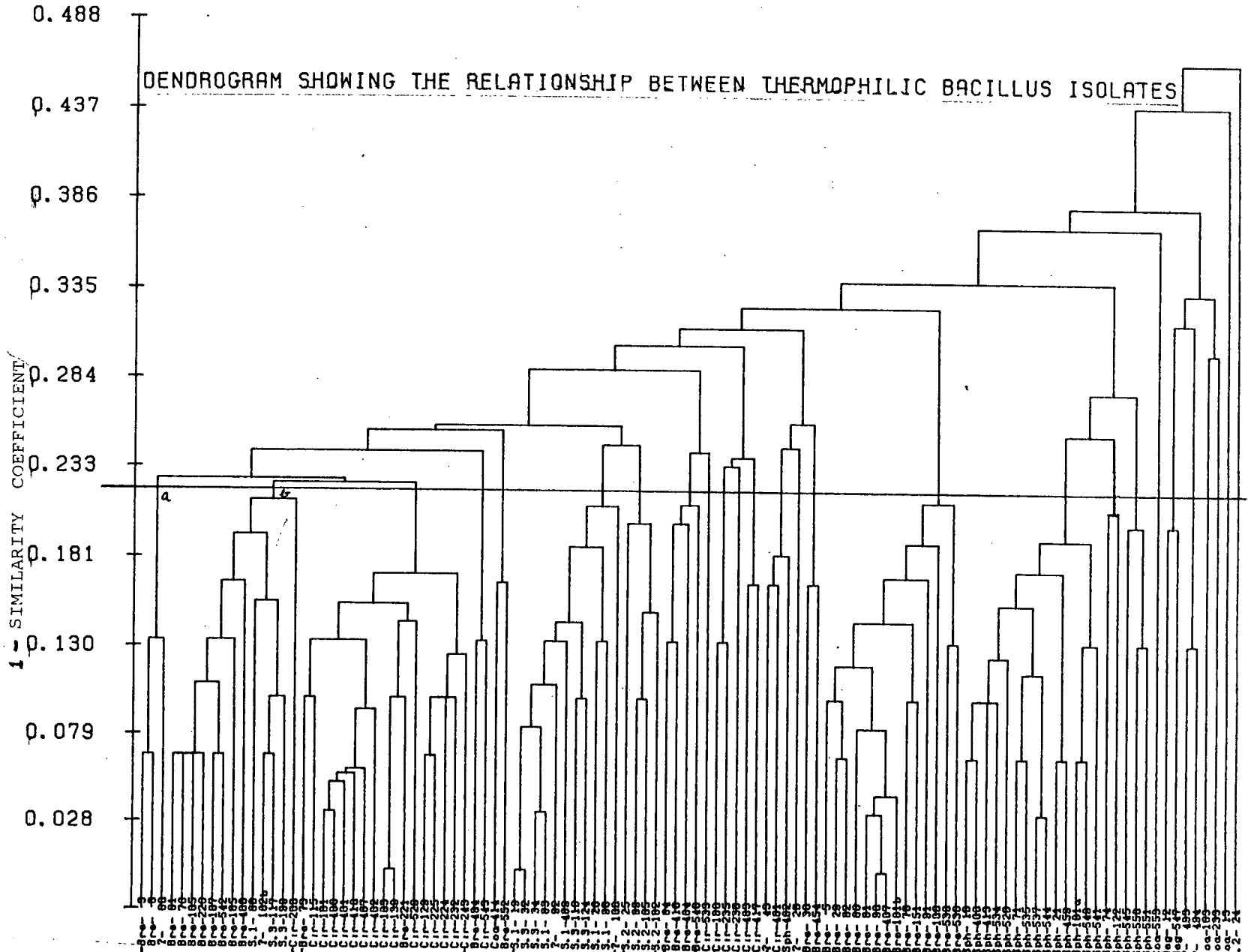

DENDROGRAM SHOWING THE RELATIONSHIP BETWEEN THERMOPHILIC BACILLUS ISOLATES
1 - SIMILARITY COEFFICIENT
0.488
0.437
0.386
0.335
0.284
0.233
0.181
0.130
0.079
0.028
a
b

TABLE - 17

Percentage Distribution of Thermophilic Isolates Among Taxa at Various Time Intervals R2 [1]

Initial C:N Ratio	45			65		
Week	1	2	4	1	2	4
Actinomycetes: (total)	0.0	1.2	7.5	0.7	0.6	5.2
Nocardia sp.	-	-	3.9 P	-	-	-
Streptomyces spp.	-	1.2 L	2.3 LP	0.7	0.6 P	0.3 P
Thermoactinomyces spp.	-	-	1.3 P	-	-	4.9 P
Bacillus (total)	100.0	58.4	51.3	99.3	72.7	15.5
B.brevis	53.6 L	+ L	6.4 L	4.1 L	7.9 L	1.2 L
B.circulans	3.0 L	32.3 L	28.5 L	+ L	50.1 L	13.2 LP
B.coagulans	1.6	+	-	+	-	1.1 P
B.megaterium	+ L	-	-	-	-	-
B.sphaericus	32.4 L	0.2 LX	+	33.1 P	11.6 L	+
B.stearothermophilus	8.4 L	25.9 LP	16.4 L	60.3 L	2.8 L	+ L
Bacillus spp.	-	-	-	1.8 L	0.3 L	-
Clostridium spp.	+ LP	-	-	-	-	-
Coryneforms	-	40.2 CL	41.1 CL	-	26.7 CP	79.3 CP
Lost	-	0.2	0.1	-	-	-

[1] Numbers refer to the percent of strains isolated on dilution plates with at least 10^6 CFU g^{-1} compost; + = present in low numbers; - = not isolated. Letter(s) indicate(s) the ability of representative isolates to hydrolyse C= NaCMC, L= Tween 20, P= pectin and X= xylan. See Appendix 6 for estimated numbers of bacteria and Appendix 9 for identification tables. Identifications were made using Bergey's Manual (1974) and the Key of Gordon, *et.al.* (1973) for *Bacillus spp.*

TABLE - 18

Percentage Distribution of Isolates Among Taxa at Various Time Intervals R4 [1]

Compost (initial C:N=45)	Fish-bark			Urea-bark		
Week	1	2	4	1	2	4
Thermophiles						
Actinomycetes:(total)	0.0	52.50	21.7	94.6	11.1	94.4
Micropolyspora spp.	-	0.4 PX	-	-	-	27.8
Streptomyces spp.	-	3.8 LX	21.7	4.1 CLX	9.8	66.6 CLX
Streptosporangium spp.	-	48.3 PX	-	22.2	1.3 PX	-
Thermoactinomyces spp.	-	-	+	68.3	+	-
Bacillus (total)	55.9	7.6	67.2	5.4	53.3	5.6
B.brevis	2.2 CLPX	+ L	4.2 LP	0.5 P	21.3	1.5 C
B.circulans	43.0 LP	6.3 L	17.7 LP	+ P	-	+
B.coagulans	-	-	+	-	-	+ L
B.megaterium	+	-	-	-	-	- P
B.sphaericus	8.6 L	-	44.8 LP	-	21.3 LP	4.1 CP
B.stearothermophilus	+ L	1.1 L	-	+	10.7 L	+ L
B.spp.	-	0.2 X	-	-	-	-
Clostridium sp.	+ PX	+ PX	-	-	-	-
Coryneforms	44.0CP	37.2CLP	11.1CP	-	1	-
Gram negative rods	-	2.6	-	-	35.6	-
Lost	0.1	0.1	+	-	-	-
Actinomycetes:		**Mesophiles**				
Streptomyces spp.	-	+ CPX	+ CPX	4.6 CPX	-	2.9 CPX
Bacillus (total)	93.4	99.9	24.9	58.0	70.9	10.5
B.brevis	38.1	+ CP	-	37.2	28.6	0.3
B.circulans	+	-	-	5.5 P	-	-
B.megaterium	-	3.7 CLP	0.5 P	0.3 CL	-	0.2 P
B.sphaericus	55.2 CP	56.4 CLP	24.4	15.0 P	42.3	10.0 CP
Clostridium spp.	-	-	-	+ PX	-	-
Coryneforms	-	-	16.8 LPX	-	+	42.6 LPX
Gram negative rods	6.7 CP	-	58.3 P	41.2 CP	+ P	8.1 P
Fungi:						
Aspergillus spp.	-	-	-	+ CPX	29.0 CPLX	-
Penicillium spp.	-	-	-	+ CPX	-	-
Trichoderma sp.	-	-	-	0.3 LP	-	-
Yeasts	-	-	-	-	-	73.3 LP

[1] Numbers refer to the percent of strains isolated on dilution plates with at least 10^6 CFU g^{-1} compost; + = present in low numbers; - = not isolated. Letter(s) indicate(s) the ability of representative isolates to hydrolyse C= NaCMC, L= Tween 20, P= pectin and X= xylan. See Appendix 6 for estimated numbers of microorganisms and Appendix 9 for identification tables. Identifications were made using Bergey's Manual (1974) and the key of Gordon, *et.al.* (1973) for *Bacillus spp.*

Table - 19

Estimated Numbers and % Distribution of Isolates Among Taxa During R7 & R8 [1]

Day, Temp Treat.	Total CFU isolated at compost temp.	% of Predominant Microorganisms Isolated at each Peak in Microbial Activity being: *Bacillus*	*Strepto-myces*	*Thermomono-spora*	G-ve Rods	Others[2]
R7) Fish-, Urea- & Sewage-bark, Initial C:N=35.						
2, 30°						
Fish-bark	178	100				
Urea-bark	1680	95	5			
Sewage-bark	43	100				
4, 40°						
Fish-bark	896	77	3		20	
Urea-bark	1460	91	1	8		
Sewage-bark	656	100				
6, 50°						
Fish-bark	199	62	23		5	
Urea-bark	1365	50	17	23	10	
Sewage-bark	89	77	3			20
8, 55°						
Fish-bark	159	56	34		10	
Urea-bark	987	4	1	95		
Sewage-bark	23	56			26	18
21, 55°						
Fish-bark	64	89	11			
Urea-bark	141	9	50	31		10
Sewage-bark	91	100				(yeasts)
R8) Urea-, Urea+p-benzoquinone- & IBDU-bark, Initial C:N=35. [3]						
Peak 1						
Urea-bark	1250	99	1	-	-	-
Urea-bark+q	1532	75	17	-	8	-
IBDU-bark	2163	68	22	-	-	10
Peak 2						
Urea-bark	1430	80	3	-	17	-
Urea-bark+q	1070	1	-	90	5	4
IBDU-bark	2036	5	8	71	1	5
Peak 3						
Urea-bark	128	70	15	-	15	-
Urea-bark+q	1510	5	17	55	8	15
IBDU-bark	1896	8	3	60	5	20
Peak 4						
Urea-bark	981	3	2	95	-	-
Urea-bark+q	1182	2	63	25	-	4
IBDU-bark	965	12	62	11	-	15
Day 28						
Urea-bark	89	28	11	43	-	8
Urea-bark+q	164	6	69	24	-	3
IBDU-bark	308	10	78	10	-	2

[1] Total numbers x 10^6 g^{-1} compost include counts on TSA (2.5.1.1) (bacteria) and on PDA (2.5.1.4) (yeasts and moulds). See Appendix 1 for composition of mixes.
[2] Isolates were identified as actinomycetes, coryneforms and fungi.
[3] Peaks in respiratory activity occurred at different times (Figure 19), consequently peaks 1 to 4 correspond to d 2, 3, 6 & 8 for the fish-bark compost, d6, 8, 14, 15 for the quinone (+q) amendment and d8, 12, 14 and 15 for the IBDU-bark compost.

3.3.1 Grouping of Thermophilic Bacilli Based on Isoenzyme Patterns

Isolates of *Bacillus spp.* capable of growth at 65° or above were examined for possible relationships based on their isoenzyme patterns. Of the seven extracellular enzymes examined (Appendix 9), RNase and lipase were most commonly produced (94% and 88% of isolates respectively). The isoenzyme patterns of these two enzyme complexes were therefore examined further.

3.3.1.1 Isoenzymes of RNase

Two isolates each of *B.stearothermophilus* of group 1 (26 & 68), group 2 (25 & 69) and group 3 (19 & 34) as well as isolates of *B.brevis* and *B.coagulans* were assayed for isoenzymes of RNase by polyacrylamide gel electrophoresis (Table 20). In general, groups 1 and 3 of *B.stearothermophilus* exhibited similar patterns of RNase activity and a greater degree of banding than found in group 2 isolates. Also, the numbers of intracellular isoenzymes decreased with the age of the culture while extracellular isoenzymes remained constant. There were however some isolates that showed no activity under the conditions used.

A number of parameters were varied in an attempt to improve strain differentiation by RNase isoenzyme patterns. Incubation of the gels at pH 6, 7 or 8 made no difference to the patterns observed, but fewer bands were obtained at pH 5. Temperature of incubation (25-70°) altered the speed of development of isoenzyme patterns (60° was optimal), but the final patterns were similar. Neither growth in a low P medium (TB of Sargeant, *et.al.*, 1971) nor the addition of gelatin (Arella and Sylvestre, 1979) improved the distinctions between, or similarities within, the three groups of *B.stearothermophilus*.

Table - 20

Electrophoretic Mobility of RNases in Extracts of Thermophilic *Bacillus* Cultures.

Isolate		R_f Values of Isoenzymes of RNase [1] Intracellular 18h	Intracellular 3d	Extracellular 18h	Extracellular 3d
B.stearothermophilus:					
group 1-	26	n.d.	n.d.	n.d	n.d.
	68	18 bands	0.06,<u>0.53</u>	0.17,<u>0.43</u>	0.17,<u>0.43</u>
group 2-	25	n.d.	0.11,0.29,<u>0.44</u>	0.10	0.10
	69	n.d.	n.d	n.d.	n.d.
group 3-	19	0.05,0.11 0.29,0.44	0.08,0.22 0.29,0.44	0.23,0.43	0.25,<u>0.43</u>
	34	21 bands	0.05,0.10 0.23,0.29 0.35,0.42	0.48	0.20,0.48
B.brevis	29	n.d.	n.d.	n.d.	n.d.
B.coagulans	13	n.d.	n.d.	n.d.	n.d.

[1] Bands of RNase activity were evident after incubating the gel at 60° for 2h, followed by staining with acridine orange for 2h then destaining in acetic acid for 2h. R_f values were relative to the bromophenol blue front and values underlined indicate strong activity.

n.d. - not detected.

3.3.1.2 Isoenzymes of Esterases

Various compost isolates and strains of *Bacillus brevis* and *B.coagulans* (B636 & B666 from the Australian thermophile collection) able to grow at 65° or above were examined for alpha-esterase activity (Table 21).

Identifications based on traditional methods generally matched the groupings based on esterase mobilities. All strains of compost isolates identified as *B.brevis* produced at least two bands, one of medium intensity at R_f 0.33 and another at about 0.49 and/or 0.73. The only other isolate (98) exhibiting

these isoenzymes also produced an intense band at about 0.68, an R_f band not seen in isolates of *B.brevis*, but common to group 1 *B.stearothermophilus*. *B.caldolyticus* (placed into group 1 *B.stearothermophilus* by Sharp, *et.al.* (1980)) lacked the intense band at about 0.68 common to other group 1 *B.stearothermophilus* isolates. This extreme thermophile showed greater similarity in esterase banding with *B.megaterium*, a species with members unable to grow at 65°. Group 1 *B.stearothermophilus* isolates had in common two intense bands at about R_f 0.53 and 0.68 and at least one other of lesser mobility and intensity. All group 2 isolates produced a single band at about R_f 0.50. Group 3 isolates, however, showed greater variability. Of the unidentified isolates, only one (isolate 100) showed isoenzymes in common with any group of *B.stearothermophilus* (group 2). Unfortunately neither of the known strains of *B.brevis* or *B.coagulans* (B636 & B666) demonstrated any esterase activity on alpha-naphthyl acetate.

3.3.1.3 Isoenzymes of Lipase

In developing an electrophoretic assay of lipase group of esterase isoenzymes various parameters of the assay were investigated. These included pH (during electrophoresis and subsequent incubation), temperature of incubation and substrate (tween 20, 40, 60, 80 or tributyrin) concentration. The best separation and greatest number of bands was achieved using 0.2% tributyrin in the gel buffered at pH 8.6. Following separation, gels were incubated in 0.1% $CaCl_2$ at pH 8.5 for 1h at 20°. However, staining showed cross reaction with esterases, as demonstrated by paired gels supplemented with 0.2% tributyrin, one stained for esterases the other for lipases.

Table - 21

Electrophoretic Mobility of Alpha-Naphthyl Esterases in Extracts of Thermophilic *Bacillus* Cultures. [1]

Identification	Strain	R_f [2] Value at : band 1	band 2	band 3	band 4	band 5	band 6
B.brevis	B636	n.d.					
" "	29			0.33(m)		0.50(m)	0.73(s)
	60			0.32(m)		0.48(w)	
	91			0.33(m)			0.73(m)
	151		0.19(m)	0.32(m)	0.49(s)	0.54(s)	0.72(s)
B.caldolyticus	B697	0.04(w)	0.11(w)	0.22(w)	0.33(s)	0.36(s)	0.58(s)
B.coagulans	B666	n.d.					
	13						0.68(m)
	163	0.04(w)	0.13(w)	0.33(w)	0.38(s)		0.95(s)
	233	n.d.					
	553						0.67(s)
B.licheniformis	B691			0.28(w)			
B.megaterium	12	0.17(m)	0.19(m)	0.33(w)	0.35(w)	0.37(s)	0.53(s)
B.stearothermophilus							
" "	B1518		0.18(w)		0.42(m)	0.55(m)	0.69(m)
" " group 1	26		0.11(w)			0.53(s)	0.60(m)
	68			0.22(w)		0.55(s)	0.68(s)
	83				0.42(m)	0.53(s)	0.68(s)
	98	0.06(w)		0.33(m)		0.52(s)	0.67(s)
	469	0.05(w)	0.13(w)			0.54(s)	0.68(s)
" " group 2	24					0.51(s)	
	25					0.51(w)	
	69					0.49(w)	
	165					0.47(m)	
	182					0.48(m)	
" " group 3	19				0.38(m)	0.55(s)	
	32				0.41(m)	0.52(s)	
	34					0.46(s)	0.69(w)
	116					0.50(s)	0.68(w)
	117				?	0.51(w)	
	124				0.36(m)	0.54(s)	
	199				0.40(w)	0.53(m)	
Bacillus.sp	28			0.42(w)		0.91(w)	
"	43				0.42(s)		
"	92				0.34(w)		
"	100					0.49(w)	
"	102b				0.36(w)		
"	493	n.d.					
"	494	n.d.					

[1] Bands indicating esterase activity were evident after incubating the gel at 20° for 1h in alpha-naphthyl acetate and naphthanil diazo at pH 7.0.

[2] R_f values were relative to the bromophenol blue front. Relative intensity of stained band:- s= strong, m= medium, w= weak. n.d.- not detected.

3.3.2 Production of Hydrolytic Enzymes by Compost Isolates

The 212 compost isolates were initially screened for hydrolytic enzyme production by agar plate clearing methods. The following results were obtained: 30% demonstrated amylase activity, 17% CMCase activity, 39% lipase activity, 35% pectic enzyme activity, 9% xylanase activity and 5% demonstrated activity towards lignin. The identifications of these isolates are given in Appendix 9, and their generic distributions at various composting intervals are indicated in Table 19. No isolate demonstrated laccase activity when assayed with the syringaldazine reagent, but six isolates (Appendix 9) showed laccase activity on LigA. Nevertheless most isolates (82%) could tolerate 0.05% ellagic or tannic acid and 6% caused a darkening (indication of laccase activity) or clearing reaction on tannic acid agar. Isolates possibly capable of degrading cellulose or lignin were studied in greater detail.

Most isolates which demonstrated CMCase activity on initial isolation could not degrade NaCMC after subculturing on TSA. The exceptions were the cellulolytic fungi, actinomycetes and *Cellulomonas spp.* which exhibited poor cellulolytic activity in pure culture (Table 22). Only the mesophilic *Aspergillus* and *Penicillium* isolates (490 & 437) were able to release dye from RBBR-cellulose, and only the *Aspergillus sp.* produced CMCase following growth on 0.1% microcrystalline cellulose. Unfiltered culture extracts were also assayed for the presence of cell-bound cellulases, but none were found.

During R8 samples of compost were examined for microorganisms capable of aerobic, microaerobic or anaerobic utilization of CMC in semi-solid agar deeps and in enrichment culture. Results from the agar deeps were inconclusive, but three cellulolytic strains of *Bacillus coagulans* were isolated from mesophilic enrichments in the medium of Teather and Wood (1982). These strains however, lost their CMCase activity on subculturing.

Possible lignoclastic isolates were examined for laccase and cellobiose:quinone oxidoreductase activities and for qualitative and quantitative biodegradation of Kraft lignin (Table 23). Only the mesophilic yeast (isolate 665) showed degradation of Kraft lignin in pure culture. All combinations of the possible lignoclastic isolates were also assayed for lignin degradation in co-culture. Degradation in excess of that in single-culture controls was found with co-cultures of isolates 440 and 441; 665 and 441; and with 665 and 600, with relative increased degradation of 3.4, 6.0 and 9.2% respectively.

Table - 22

Relative CMCase Activity Produced by Compost Isolates Grown on Various Cellulosic Substrates [1]

Isolate	Relative CMCase Activity in: following growth 0.1% micro-crystalline cellulose	0.5% NaCMC	Dye release from RBBR-cellulose
Thermophiles			
Cellulomonas sp. 203	-	19	-
Cellulomonas sp. 205	-	36	-
Cellulomonas sp. 492	-	40	-
Cellulomonas sp. 541	-	21	-
Streptomyces sp. 411	-	13	-
Streptomyces sp. 532	-	56	-
Mesophiles			
Aspergillus sp. 490	15	33	+
Penicillium sp. 437	-	26	+
Streptomyces sp. 436	-	21	-
Streptomyces sp. 599	-	15	-

[1] Relative CMCase assay values were means of duplicate determinations. Aliquots (1mL) of culture filtrate (10d growth at 28 or 55°) were incubated with 10mL 0.5% NaCMC in phosphate buffer (pH 6.0) for 1h at 55 or 60° for mesophiles or thermophiles respectively prior to relative assessment of CMCase activity by microviscometry. RBBR-cellulose dye release estimates were obtained from duplicate tubes. The incubation period was 10d at 28 or 55°. + = weak , - = no activity.

Table - 23

Lignoclastic Activity of Selected Compost Isolates [1]

Isolate	Bavendamm Test [2] in: 0.05% acid Ellagic	Tannic	Lignoclastic Activity: Qualitative [3] Kl+CMC	S&N	Quantitative [4]
Thermophiles					
B.brevis	404 g -	g +	L	-	-
B.brevis	530 g -	n.g.	C	-	-
B.brevis	542 g -	g -	C	-	-
B.brevis	548 g -	g -	C	B	-
B.circulans	543 g -	g -	C	-	-
B.coagulans	414 g -	g -	C	-	-
B.sphaericus	534 n.g.	n.g.	C	-	-
B.sphaericus	541 g -	g -	L	-	-
Brevibacterium sp.	416 n.g.	g -	C	-	-
Coryneform	240 g -	n.g.	C	-	-
Mesophiles					
Bacillus sp.	440 c	c	C	-	-
Bacillus sp.	441 c	r	C	C	-
Flavobacterium sp.	600 g -	g -	L	c	-
Klebsiella sp.	432 g -	g -	L	-	-
Klebsiella sp.	597 g -	g -	C	c	-
Aspergillus sp.	439 g -	d	L	-	-
Aspergillus sp.	490 c	c	C	c	-
Penicillium sp.	437 c	c	C	c	-
Trichoderma sp.	426 g -	g -	C	-	-
Yeast	665 g -	g -	C	c	3.9

[1] Isolates (Appendix 9) indicating activity towards Kraft lignin (Kl) on agar plates supplemented with NaCMC were selected in the course of R2 and R4. All tests were conducted in duplicate. Thermophiles were incubated at 55° and mesophiles at 28°.

[2] A positive test was indicated by c = clearing or d = darkening, while - = negative reaction. g = growth and n.g. = no growth on the agar in 10d.

[3] On the Kl+NaCMC agar C = cellobiose:quinone oxidoreductase activity (clearing of the agar) and L = laccase activity (darkening of the agar) (Westermask and Ericksson, 1974). On Sundman and Nase (1971) lignin agar (S&N) B = blue reaction and c = clearing reaction after flooding the plates with $FeCl_3$-$K_3(Fe(CN)_6)$ after 10d growth.

[4] After 1 month growth broths were purified and assayed against dioxane:water (1:1, v/v) at 281nm (Jansheker, *et.al.*, 1981). % reductions in absorbance ~~Readings of transmission~~ were corrected against the mean reading for three uninoculated controls (3.1%).

3.3.3 Isolation of Nitrifying Microorganisms During Composting

No autotrophic nitrifier was isolated in the course of R4 from fish- or urea-bark composts of initial C:N=45 using either of the described media for their isolation (2.5.1.4) following 7, 14, 21 or 28d composting. However, low numbers (< 10^4 CFU g^{-1}) of heterotrophic nitrifiers were isolated, but only from the urea-bark composts in the medium of Gunner (1963). These nitrifiers were identified as thermophilic strains of *Bacillus spp.* (two representative isolates were lost on subculture on TSA), and a *Streptomyces sp.*, all of which nitrified ammonia to nitrite.

Of the 212 isolates from R2 and R4 which were also screened for ability to nitrify on Gunner's (1963) medium, four were weakly positive. These were a *Streptomyces sp.* and the three pink coryneforms (isolates 94, 198 and 240). Considering the abundance of the coryneforms (estimated numbers 10^8-10^9 CFU g^{-1}) in fish-bark mixes during R2, thermophilic biological nitrification could be significant in such composts.

3.3.4 Estimated Numbers of Faecal Indicator Bacteria During Composting

Because of the potential health risk in handling sewage-bark compost, numbers of faecal coliform and faecal streptococci were estimated during R7 and R9. The decline in numbers of these faecal indicator bacteria from sewage-bark composts of initial C:N=35 and 25 are illustrated in Figure 37. From the AOV on the log transformed data (Appendix 6.7) and Figure 37 the significant ($p < 0.01$) decline in numbers and greater rate of decline at the lower C:N ratio, are apparent for both groups of bacteria. At d28, numbers of both groups of bacteria were at the lower limit of detection in both composts.

Figure 37

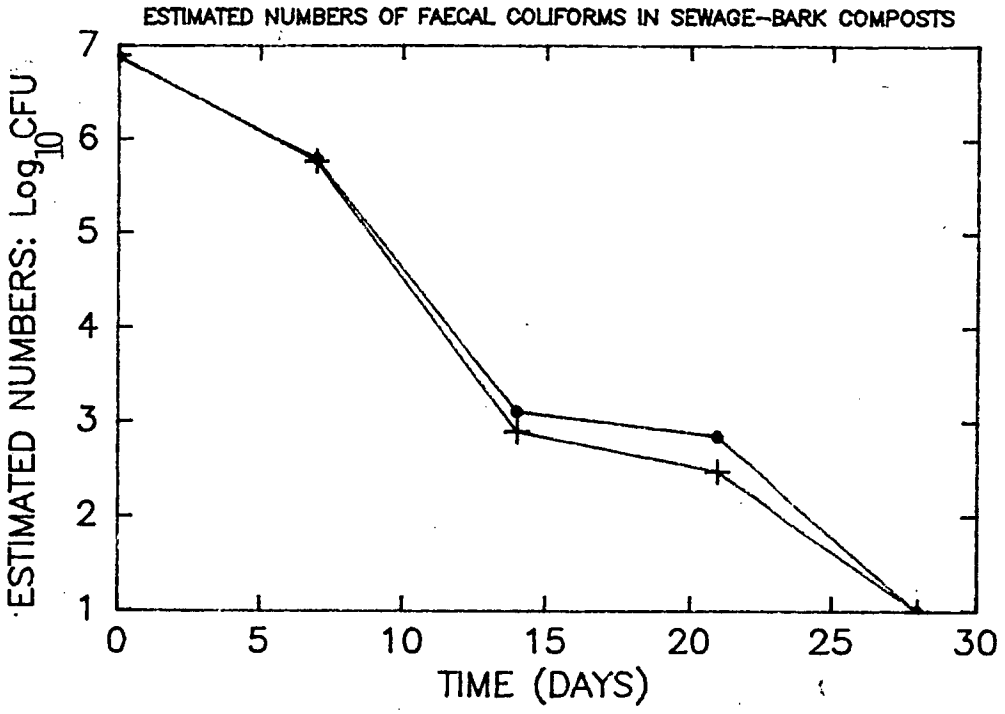
Estimated numbers of faecal coliforms and streptococci in sewage-bark composts.

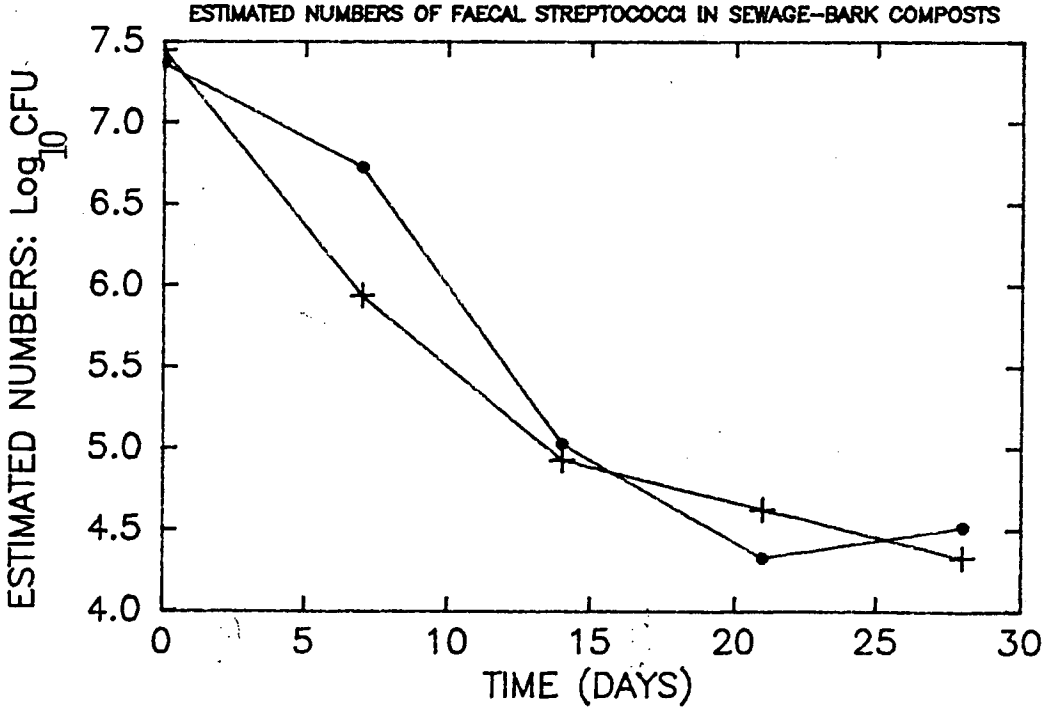
Faecal coliforms were enumerated on lactose teepol agar and confirmed by the IMViC biochemical tests (Mara, 1974). Faecal streptococci were enumerated on m-enterococcus agar and confirmed by their growth on MacConkey agar, morphology, and Gram and catalase reactions.

See Appendix 6.7 for data.

. C:N = 25

X C:N = 35


ESTIMATED NUMBERS OF FAECAL COLIFORMS IN SEWAGE–BARK COMPOSTS
ESTIMATED NUMBERS: Log10CFU
7
6
5
4
3
2
1
0
5
10
15
20
25
30
TIME (DAYS)


ESTIMATED NUMBERS OF FAECAL STREPTOCOCCI IN SEWAGE–BARK COMPOSTS
ESTIMATED NUMBERS: Log10CFU
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
5
10
15
20
25
30
TIME (DAYS)

3.3.5 Toxicity of Compost Components to the Predominant Compost Microflora

Compost isolates and samples were examined to determine whether detoxification of compost components was a significant factor controlling the microbial succession observed in bench-scale composts. During R7 and R8 the predominant microflora (Table 19) and the compost were sampled during peaks in respiratory activity for subsequent determination of their interactions. No effect on microbial succession was attributable to the initial changing temperature during R8, since all peaks in activity occurred at 55° (Figure 17).

Growing the three most numerous isolates obtained from each peak of activity during R8, on gradient plates containing compost obtained at various ages, clearly demonstrated that as compost aged, it progressively supported the growth of isolates obtained during the latter stages of composting (Figures 6 & 38).

3.4 Destruction of Phytotoxins by Composting

Tannins and phenolic acids responsible for the phytotoxicity of raw *E.delegatensis* bark were readily detected by a plant bioassay (Ashbolt, Hon. thesis, 1979). The non-phenolic phytotoxins were also determined by the use of a PVP pretreatment which removes most phenolics from the water extracts. Preliminary bioassays on composts of various ages (7, 14, 21 & 28d) revealed that composts less than 28d old completely inhibited lettuce seedling growth. Consequently, only the phytotoxicities of composts at 28d were subsequently assayed (Table 24). Volatile fatty acids were less than 50ppm in all 30d old composts.

Figure 38

Relative Tolerance to Composts (C:N=35) of the Predominant Flora Isolated at Each Peak of Respiratory Activity

Three predominant isolates present in compost at each peak in respiratory activity were streaked in duplicate across a gradient plate (the agar containing an increasing content of compost suspension from one side of the plate to the other). The suspensions were made from compost (fish-, or IBDU-bark composts R8) collected at each peak in respiratory activity, dried, milled (< 1mm) and then suspended in a mineral salts medium which was overlayed with TSA (Figure 4).

Each point is a mean obtained for three isolates. See Appendix 10 for data and AOV results.

The predominant flora at each peak in respiratory activity are identified as :-

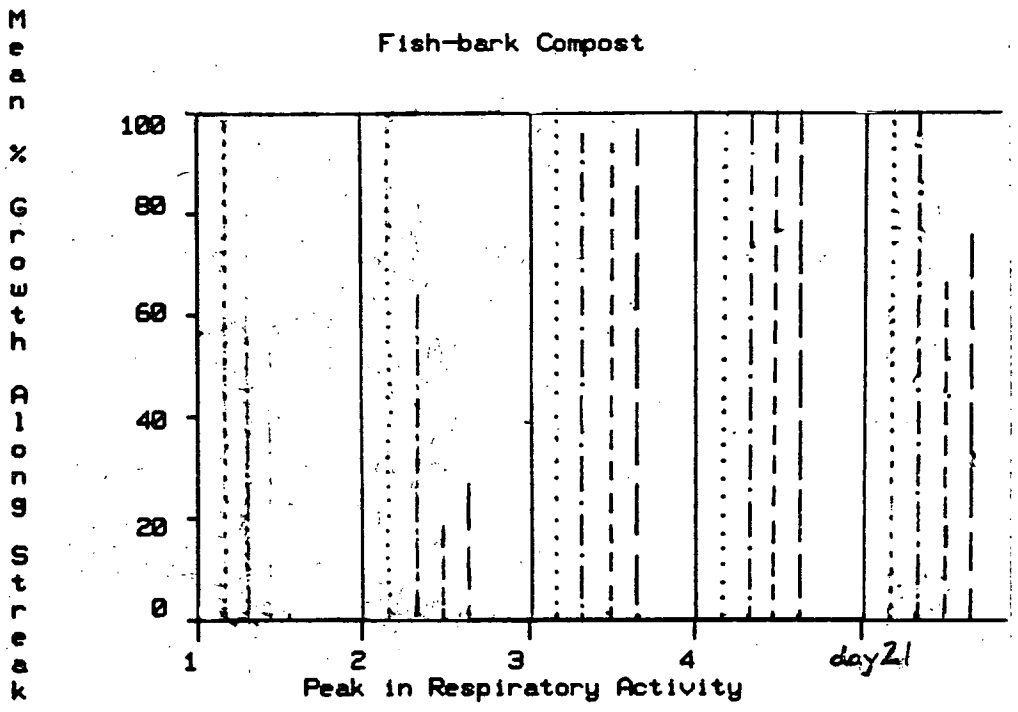
Peak 1 ————————

Peak 2 - - - - - - - - - -

Peak 3 —·—·—·—·—

Peak 4 — — — — — —

Flora at day 21. —— —— ——


Fish-bark Compost
Mean % Growth Along Streak
100
80
60
40
20
0
1
2
3
4
day 21
Peak in Respiratory Activity

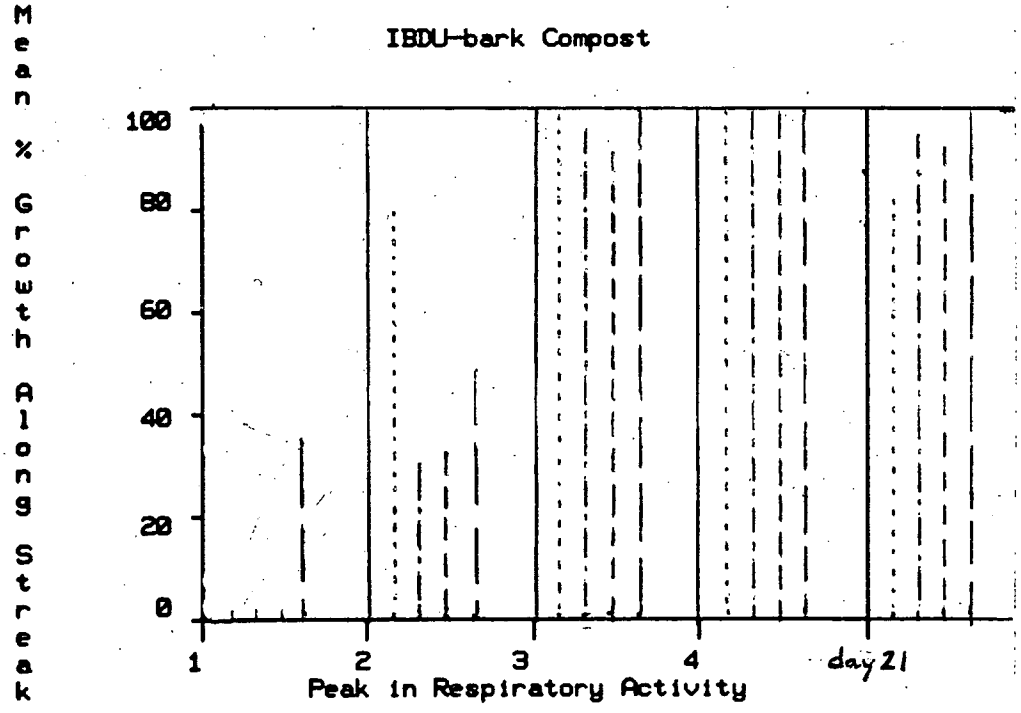

IBDU-bark Compost
Mean % Growth Along Streak
100
80
60
40
20
0
1
2
3
4
day 21
Peak in Respiratory Activity

Table - 24

Mean Lettuce Root Lengths Following Growth of Plants in Water Extracts of 28d Old Composts [1]

Urea-bark	C:N 25	+PVP [2]	0.0 a
Urea-bark	C:N 25	-PVP	0.0 a
Sewage-bark	C:N 25	+PVP	0.0 a
Sewage-bark	C:N 25	-PVP	0.0 a
Fish-bark	C:N 25	+PVP	0.4 a
Fish-bark	C:N 25	-PVP	0.4 a
Sewage-bark	C:N 35	-PVP	4.8 b
Sewage-bark	C:N 35	+PVP	5.8 b
Urea-bark	C:N 35	-PVP	8.6 c
Urea-bark	C:N 35	+PVP	9.3 c
Fish-bark	C:N 35	-PVP	14.3 d
Fish-bark	C:N 35	+PVP	21.1 e
Control		+PVP	23.7 f
Control		-PVP	24.3 f

[1] Comparisons were made using Duncan's new multiple-range test on transformed (square root (length mm + 0.5)) means. See Appendix 12 for data and AOV table. Any two means not followed by the same letter are significantly different ($p < 0.05$). Each assay is the mean of 20 roots. Controls were grown in phosphate buffered (pH 6.0) distilled water, tests also contained 20% (v/w) compost extract. Extracts were prepared by homogenizing 20g of milled (< 1mm) compost in 250 mL of water for three min. The extract was then filtered and centifuged before being concentrated under vacuum.

[2] Polyvinylpolypyrolidone (PVP) was shaken twice with the extracts, centrifuged, and the supernatant used in the bioassay.

3.4.1 Identification of Phenolics in Raw Bark & Composts

Liquid chromatography of methanolic extracts of raw bark and composts was carried out using HPLC techniques. Raw *E.delegatensis* bark was found to contain a considerable quantity of gallic acid with lesser quantities of catechol, p-hydroxybenzoic acid and p-coumaric acid. On the otherhand, mature composts (> 1 year old, from the large-scale study) contained some three times the quantity of p-coumaric acid, but undetectable levels of the other acids (Table 25). Hence, the ratio of peak heights of p-coumaric over gallic acid was used as an indicator of the maturity of fish-, urea- and sewage-bark composts (Table 25).

Table - 25

Predominant Phenolics Present in 28d Old Composts [1]

Compost	C:N Ratio	Catechol	Gallic acid	p-coumaric acid	p-Hydroxybenzoic acid	MI [2]
Fish-bark	25	1	5	16	6	3.2
Fish-bark	35	2	7	18	3	2.6
Urea-bark	25	2	12	16	9	1.3
Urea-bark	35	0	8	20	7	2.5
Sewage-bark	25	1	16	15	10	0.9
Sewage-bark	35	2	22	16	9	0.7
Raw Bark		4	65	13	16	0.2
1 year old Bark		n.d.	n.d.	35	n.d.	∞

[1] Extracts were prepared by shaking 0.5g compost in 5mL of methanol for 15 min, centrifugation (10,000g 10 min), filtration (0.45um), drying under vacuum and redissolving in 0.2 mL ethanol. Phenolics in the extracts were assayed by a HPLC fitted with a C_{18} Radpak column using a UV (280 nm) detector and Waters automated gradient controller as detailed in the Materials and Methods. Values are a mean of duplicates and reported as mm peak height. n.d. = not detected.

[2] Maturity Index (MI): ratio of p-coumaric/gallic acids.

3.5 Physical Properties of Bark Compost

The bulk density, particle size distribution and moisture characteristics of one year old urea- and sewage-bark composts (from the large-scale study) are given in Table 26.

Table - 26

Physical Properties of Large-scale Eucalypt Bark, Pine Bark Composts and of Peat Moss [1]

Material	Moisture Characteristic [2]					Bulk [3]	Particle Size Distribution [4]			
	AS	EAW	WBC	DAW	TP	Density	<0.5	0.5-1	1-2.5	> 2.5
Eucalypt:										
Urea-bark	35	18	3.5	6	72	470	20	15	43	22
Sewage-bark	28	15	3.2	8	65	510	28	8	44	20
Pine Bark:										
Normal	38	10	0.9	9	79	214	23	32	15	30
Fine	22	18	2.5	16	79	200	62	32	6	0
Peat Moss	24	30	4.9	16	89	114	23	32	14	30
Ideal [5]	20-30	20-30	4-10	0	85	?	35	--- 55 ---		10

[1] Data on Eucalypt bark were obtained after 1 years' composting while data on pine bark, Irish peat moss and the ideal substrate were from Prasad (1979).

[2] Air space (AS), Easily available water (EAW), water-buffering capacity (WBC), difficultly available water (DAW) and total porosity (TP) were calculated from the % of water at suctions of 10, 31, 50 and 100cm of water by the methods of Prasad (1979).

[3] Bulk density given as kg m^{-3}.

[4] Percentage of partical at various sizes in mm.

[5] According to Prasad (1979).

4 - DISCUSSION

4.1 The Bench-scale System

The bench-scale composter was designed to simulate most of the conditions found in the large-scale study and to allow for efficient sampling of gases and solids. Self-heating systems are unsuitable to study the prolonged thermophilic phase of bark composts (Figure 5), as they typically remain at thermophilic temperatures for only a week (Mote and Griffis, 1979). Total heat control was simply obtained by immersing the units (and air heat exchanger) in the one water bath rather than using separate heating water jackets for each unit (Cappaert, *et.al.*, 1976a) or separate air and unit temperature control systems (Bagstam, *et.al.*, 1974).

Composters providing recirculation of air (e.g. Mote and Griffis, 1979) do allow for sensitive detection of minor gas products such as N_2, N_2O and H_2S which were not detected in the present study. However, the flow-through system used here had the advantage of simple automatic analysis of gases unaffected by sampling of material. Also, improved detection of minor gases should be possible if N_2 in the carrier-gas mixture was replaced with He. (Cost considerations precluded this option in the present study).

Moisture content control was achieved by the use of chilled condensers as described by Cappaert, *et.al.* (1976a), rather than by the more demanding method of air humidification and drying used elsewhere (Jeris and Regan, 1968; Bagstam, *et.al.*, 1974). Using the conservative test of significance (Steel and Torrie, 1960) no significant ($p < 0.05$) deviation from the original moisture

content was found[1]. The mixing of compost provided better moisture and air distribution than would have been obtained in the static vessel described by Cappaert, *et.al* (1976a), or in rotating drums where balling of compost occurred (Jeris and Regan, 1968; Galler and Davey, 1971).

With metal bracing (Figure 2) the welded PVC units were strong enough to withstand the stress of prolonged use, and were inexpensive compared with equivalent units of stainless steel or glass. The reproducibility between R1 and R2 of the fish-bark composts was better than reported for other composters (Clark, *et.al.*, 1977). Coefficients of variation have only been reported for respiratory gas data, and the coefficient of variation for cumulative CO_2 production in this study was as good as the best reported (Clark, *et.al.*, 1977; Mote and Griffis, 1979).

4.2 Parameters Influencing the Composting of Bark

The principal parameters generally influencing composting include: temperature, aeration, particle size, moisture content, pH, nutrient availability and C:N ratio (Finstein and Morris, 1975; Cappaert, *et.al.*, 1976b). In addition to these factors the presence of tannins in bark (30 %, Table 4) may also be important, but this is discussed under factors controlling microbial successions in compost (4.4.1). Cappaert, *et.al.* (1976b) reported an optimal free air space for the composting of bark to be 35% (corresponding to 68% moisture on a wet weight basis), with a minimal O_2 level of 5%. The optimal C:N ratio range was reported by these workers to be 25-35 depending on the availability of C and N.

Assuming that bark has low availability of C (most being

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1. However, a significant ($p < 0.05$) deviation was indicated during R4, R8 and R9 using the more sensitive split-plot design (Steel and Torrie, 1969). Whenever such a conflict in the AOV occurred the conservative test has been used for the test of significance.

bound in the lignocellulose complex) an initial C:N ratio of 35 was chosen for the large-scale study. The heaps were compacted as little as possible to facilitate aeration and made up to a mean 68% moisture (214 % on a dry weight basis). The primary aim of the large-scale study was to provide a basis for comparison with the bench-scale system, both in physical and biological characteristics. The advantage of the latter system lay in its versatility in enabling the controlled assessment of a wide range of composting conditions.

4.2.1 Large-scale Studies

Estimated numbers of bacteria present in the two large-scale composts were similar (Figure 7), but temperature and CMCase activities (Figure 6) were highest in the urea-bark heap (Figure 6). A visibly greater degree of decomposition of the bark was also apparent in the urea-bark heap after a year's composting. As the C:N ratios (35), moisture contents (214%) and particle sizes were initially similar, other uncontrolled parameters such as aeration, temperature, nutrient availability or pH must have influenced the composting of these materials.

Levels of aeration would probably have been different from the onset of composting urea- and sewage-bark (despite the similar particle sizes of bark used) due to the inability to disperse the clay-like sewage cake as finely or as evenly as urea. Also, the lower temperatures produced in the sewage-bark heap combined with its lower permeability to air would have resulted in poorer air convection during composting. Despite these differences, there was an apparent greater moisture loss from the less active sewage-bark heap (Figure 6). Such a difference could however be accounted for by the increased production of water through microbial activity (Alexander, 1977) and greater permeability of the urea-bark heap to rain.

Regardless of the varying factors of aeration and moisture mentioned above, the different activities between the urea- and

sewage-bark heaps most likely resulted from the different nutrient availabilities of urea and sewage and from their influence on compost pH. As illustrated in the graph of compost pH against time (Figure 6), a rapid hydrolysis of urea was indicated by a sharp rise in compost pH coinciding with a rapid rise in microbial activity as indicated by the rapid rise in temperature. The result was a compost of near neutral pH and high CMCase activity. In contrast however the sewage, with a predominantly nitrate form of nitrogen (Table 4), showed a reduced influence on initial pH and temperature and produced a compost of about pH 5.4 with low CMCase activity. Similar effects of ammoniacal-N compared to that of nitrate-N amendments on composting activity and pH have been observed in the composting of other hardwood bark (Hoitink, 1980).

The relatively constant total numbers of CFU present in the large-scale composts is consistent with the results of other workers (Finstein and Morris, 1975). As expected the numbers of thermophiles were well correlated with temperature, however, the poorer negative correlation between mesophiles and temperature can be explained by the survival of large numbers of sporing mesophilic *Bacillus spp.* (e.g. Table 18) during thermophilic composting.

4.2.2 Bench-scale Studies

Previous workers on bench-scale studies have generally neither reported the variability between treatments nor identified the microorganisms involved in composting. The present study was conducted with these shortfalls in mind.

4.2.2.1 Temperature

Temperatures of compost in the bench-scale system were increased at 5° per day, to reflect the natural rise in temperature within large-scale heaps. A plateau temperature of 55° was however used for the bench-scale composts as compared with

60° in the large-scale heaps. The maximum temperature used for bench-scale composting was decreased for two reasons. Firstly, 55° was shown (R5) to support the highest respiratory activity in at least fish-bark composts of initial C:N=45 and 55. However, it must be pointed out that 60° was only held for 2d for fear that a leak may develop in one of the sterile units at this temperature [2]. Such a short time at the highest temperature may not have been sufficient for the adaptation of the microflora, particularly at the end of the composting period when readily available nutrients were low. The prime reason for the lower plateau temperature was that the optimum temperature for composting softwood or hardwood bark was reported as 40-50° (Cappaert, *et.al.*, 1976b, Bagstam, 1978; Hoitink, 1980). Nevertheless, respiratory activities in these studies were compared for only the first 10d of composting which is prior to the period of extensive degradation of the major bark components (Waksman, *et.al.*, 1939a) such as cellulose (Figures 21 & 22) (Poincelot, 1974). A period of 10d may also be insufficient time for the adaption of an obligate thermophilic flora (McKinley and Vestal, 1984). (Estimated numbers of microorganisms capable of producing wood-component hydrolases in the present study were also generally very low during the first week of composting (Tables 17 & 18)).

An understanding of the thermophilic nature of the degradation of bark in composts has not always been appreciated. Still, *et.al.* (1974) attempted to study the composting of bark by incubating small samples at 23°. Not surprisingly they only achieved up to 2.6% loss in organic-C as CO_2-C over 30d.

4.2.2.2 Aeration, Particle Size and Moisture Content

The interrelationship between aeration, particle size, moisture content and temperature during composting has been noted

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2. Subsequent runs were generally free of leaks following the use of silicon rubber sealant around the outside of the screw-cap assembly (Figure 2).

by Wiley and Pearce (1957). Reproducibility between composts in the present study was achieved by using a set moisture content, temperature programme, mixing period and a standard particle size distribution of bark (one batch of hammermilled bark of particle sizes 1-35 mm, with all fibres 1-3mm thick). Under such standardized conditions, the effect of altering single-variable factors (such as aeration) could be readily investigated.

The influence of aeration on the activity of fish-bark composts of initial C:N=45 was compared both by monitoring respiratory activity (R3 & R6) and by estimating numbers of thermophilic bacteria (R2 & R4). While the respiratory data indicated that greatest microbial activity occurred at the lowest rate of aeration used (Table 8) the reverse was indicated by data on bacterial numbers (Table 13). This apparent conflict is not unusual as estimated numbers often do not reflect activity (e.g. Schmidt, 1973). However, the situation here was probably confounded by the inability of the system to satisfactorily flush CO_2 out of a unit at an aeration rate of 10-15 mL min^{-1} and that the sample times (d7, 14 and 28) for bacterial counts in the treatment with the lowest rate of aeration did not coincide with peaks of respiratory activity (Figure 12). A considerable improvement in the correlation between respiratory activity and total estimated numbers was obtained by sampling during peak respiratory activities (Tables 12 & 14)). Another factor that may have reduced respiratory activity with increasing aeration is cool air entering the units and greater evaporative cooling with the higher flow rates. Thus a flow rate of gas through compost units of 20 mL min^{-1} was used for most experiments since no significant ($p < 0.01$) difference was apparent between compost aeration at 20 or 30 mL min^{-1}, and this rate would also ensure a residual O_2 level of > 5% in active compost. Such a situation would have been unlikely at any lower rate of aeration (Figures 8, 15, & 16). Higher rates of aeration were not investigated as they were outside the scope of this investigation to simulate as far as possible, passive composting conditions.

Generally workers on composting bark have not mentioned on

what basis the chosen rate of aeration of compost units was determined. Bagstam and Swensson (1976), however, used CO_2 output as an indicator of ideal aeration rate, suggesting 150 mL min^{-1} 150 g^{-1} Urea-spruce bark. However, only aeration rates of 50-170 mL min^{-1} were assessed and as a consequence of the decreased sensitivity with increased rate of aeration (Figure 13) only one major peak in respiratory activity was determined (Bagstam and Swensson, 1976), or three minor peaks when sewage replaced urea (Bagstam, 1977). Similarly the high aeration rate of 833 mL min^{-1} $100g^{-1}$ mixed-bark composts used by Deschamps, *et.al.* (1979) also resulted in a unimodial peak in respiratory activity.

Comparison of a eucalypt- and a similar (C:N=55) but highly aerated spruce-bark compost (Bagstam and Swensson, 1976) showed maximum CO_2 outputs (mg CO_2 g^{-1} compost h^{-1}) of three and 336 and total activities (as % CO_2-C loss) of six and ten respectively. These differences indicate two interesting points. Firstly, comparison of maximum respiratory activities gave little indication of the overall activity during batch composting [3]. And secondly, as softwood bark is generally more slowly degraded than hardwood bark (Cappaert, *et.al.*, 1976a) there is a possibility of achieving greater respiratory activity in eucalypt bark composts by using considerably higher rates of aeration. However, high rates of aeration through large-scale heaps would result in the necessity to add water during composting, and process control would also be improved by a feedback control of aeration based on compost temperature (Finstein, *et.al.*, 1983).

Microbial populations were found to be significantly ($p < 0.01$) different in composts under different aeration regimes (Table 13), but not too much weight can be placed on this finding considering the above discussion on the possible differences in microbial activities at the sampling times used. More frequent

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3. For continuous thermophilic composting however, maximum respiratory activity gives a most satisfactory measure for assessing different composting conditions (Suler and Finstein, 1977).

and consistent selection of sampling times would probably be necessary to show any real differences in microbial flora that may occur in different composts.

4.2.2.3 C:N Ratio, Nutrient Availability and pH

There are two basic problems in studying the nutrition of composts which are not generally recognized. Firstly, an unequivocal increase in the degradation of bark can only be assessed when using respiratory techniques by varying the concentration of bark and not a carbonaceous amendment as tried by Cappaert, *et.al.* (1976b) and Bagstam and Swensson (1976). The problem is that if the amendment is increased it is not possible to distinguish between differences in respiratory activity resulting from increased utilization of the amendment or the substrate under study(Mote and Griffis, 1980). This is exemplified in the present study by fish-bark composts during R2 and R5 as illustrated in Figures 8 and 11. This is particularly important with carbon containing amendments like sewage and fish wastes.

The second problem relates to the nitrogen economy of composting. While the increase in ammonia loss with decreasing C:N ratio is well understood (Finstein and Morris, 1975), there is no report on the losses of nitrogen oxides during composting. It was shown in the present study that there was a poor correlation between ammonia and N-oxide volatilization (Table 16). Consequently both respiratory activity (as CO_2-C loss) and N loss data are valuable in determining the economic as well as the microbiological optimal C:N ratio as illustrated in Figure 39. A comparison of initial and final C:N ratios will only show which compost gives the greatest C:N reduction. From Figure 39 it is clear that the fish-bark composts produced the highest respiratory activity and least loss of N, with the compost of initial C:N=35 producing the lowest N loss for a high loss of CO_2-C. Both the addition of quinone to urea or urea's replacement with IBDU resulted in a "poorer" performance. In the composting of straw Waksman, *et.al.* (1939b) also found that increased losses of N

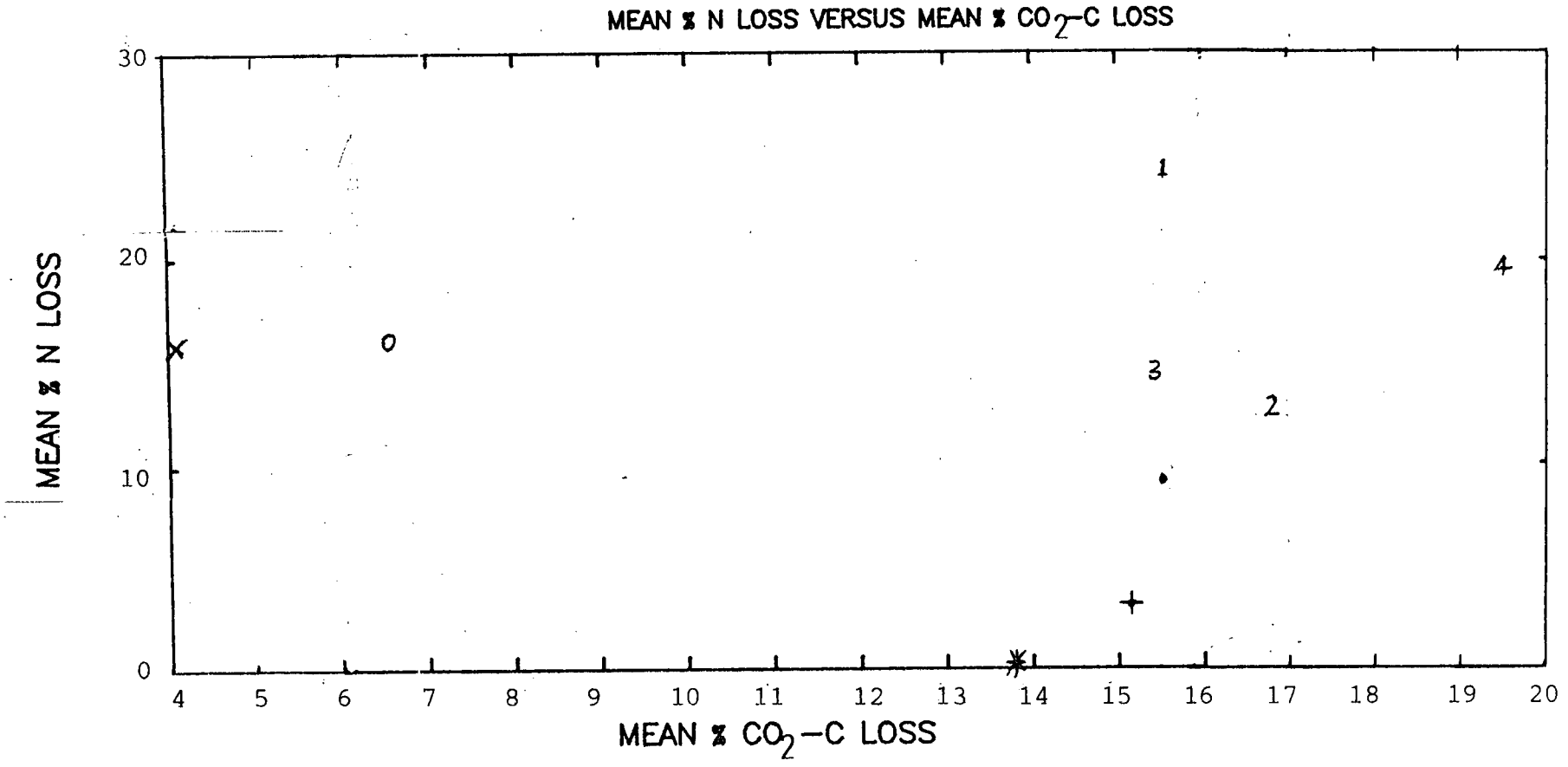
Figure 39

Mean percent losses of N versus CO_2 from various compost mixes. (28 days old)

Losses shown are relative to the initial total weight in each compost. Results are means from duplicate units in each run. CO_2 was automatically assayed every 5h by GC. Volatilized N was continuously collected in dilute acid (0.1 M H_2SO_4) and assayed by titration following steam distillation every 2d.

See Tables 9 and 16 and Appendix 4 for data.

	Compost	C:N Ratio
.	Fish-bark	25
+	Fish-bark	35
*	Fish-bark	45
O	Sewage-bark	25
X	Sewage-bark	35
1	Urea-bark	25
2	Urea-bark	35
3	Urea-bark+Quinone	35
4	IBDU-bark	35


MEAN % N LOSS VERSUS MEAN % CO2-C LOSS
MEAN % N LOSS
MEAN % CO2-C LOSS
0
10
20
30
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
1
2
3
4

occurred when decomposition was delayed. This increase N volatilization was probably a nonbiological response related to increased temperature and pH (Finstein and Morris, 1975). A slow release fertilizer with greater thermal resistance than IBDU may be more successful in conserving nitrogen. However, it is probably still preferable to have a relatively rapid initial release of N during the mesophilic stage. This would allow rapid microbial activity when conditions are less favourable to ammonia volatilization (low temperature and pH). Such an amendment could consist of urea plus a slow release N fertilizer.

The mean percentage weight loss data (Table 9) indicated that C availability rather than other nutrients was likely to be limiting respiratory activity in composts of initial C:N=25 or 35. It is also interesting to note that limiting high respiratory activity to the thermophilic compost microflora, by the use of IBDU, enhanced the loss of C over that detected from other mixes. At an initial C:N ratio of 45, however, other nutrient(s) were indicated to be limiting respiratory activity in fish-bark composts. Also, from the considerable excess of mineral N in composts of initial C:N=25-35 (Figures 24-26) it is evident that N was readily available. Considering the similar respiratory activities of urea and fish-bark composts of initial C:N=25-35 (Table 9), it is reasonable to assume that P and other amendments are unnecessary for urea-bark composts. The addition of P has been shown to be beneficial for the composting of softwood bark (Solbraa, 1979a), but was not found to be necessary for the composting of mixed softwood and hardwood species (Cappaert, *et.al.*, 1976a). The ability to compost eucalypt bark without addition of P is important considering the sensitivity of many native Australian plants to P (Nichols and Beardsell, 1981).

Considerable nutrient immobilization, however, was evident in the sewage-bark composts. Sewage-bark composts of initial C:N=25 resulted in similar weight losses to that found in fish-bark composts of initial C:N=55. This reduced activity was unlikely to be due to the presence of toxins in the sewage, as increasing the concentration of sewage increased respiratory activity. However,

composts of low pH such as the sewage-bark composts, are known to reduce respiratory activity (Cappaert, *et.al.*, 1976a). A low pH may directly reduce bacterial growth and/or indirectly reduce activity by reducing enzymic release of nutrients (Alexander, 1977). The low pH found with sewage-bark composts (about 5.5) was possibly due to the presence of nitrate N (Table 4) rather than ammoniacal N as produced from the other amendments (Hoitink, 1980).

4.3 Nutrient Transformations

4.3.1 Carbon Transformations

Transformations of the major components of fish-bark composts (R2) were examined after 35d composting. Most of the soluble carbohydrate and lipid was degraded at both C:N=45 and 65 (Table 6). However, apart from lignin degradation there was significantly ($p < 0.01$) less degradation of more complex components at the higher C:N ratio.

Comparing the weight loss data on the fish-bark compost of initial C:N=45 (Table 6) with that of a similar straw-manure compost (initial C:N=55, composted at 50° for 33d) (Waksman, *et.al.*, 1939b) showed that considerably greater degradation of cellulose, hemicelluloses and lignin (94, 85 & 42% respectively) occurred in the straw compost. Bagstam (1979) also demonstrated low levels of degradation of these components in a spruce-bark compost. Factors contributing to the degradation of these three components are discussed below.

Few workers have actually studied *in vivo* compost enzyme activities (Hankin, *et.al.*, 1976). In the present study, activities of hydrolytic compost microorganisms were generally confirmed by assay of the enzymes in the crude compost. These assays demonstrated that conditions during composting were suitable for enzyme induction and/or activity.

4.3.1.1 Lipid Biodegradation

Two esterase substrates, Tween-20 (a water soluble ester of lauric acid) and tributyrin (water insoluble, glyceryl tributyrate) were used to study potential lipase activity. Tween 20 was used in preference to insoluble lipids due to its ready hydrolysis and water solubility eliminating the need for an emulsifier with attendant problems of interference (Deployey, *et.al.*, 1981). Tributyrin, a lower triglyceride, was used in the enzyme mobility studies due to its water insolubility and low selectivity (Hugo and Beveridge, 1962). Other workers have shown however, that these substrates only indicate esterase and tributyrinase activity respectively and the presence of these enzymes was not always well correlated with the ability of a microorganism to hydrolyse higher triglycerides (lipase activity) (Hugo and Beveridge, 1962; Elwan, *et.al.*, 1977). It is generally accepted that there are two groups of lipolytic enzymes; one for triglycerides of short-chain saturated fatty acids (esterases) and the other primarily for the triglycerides of long-chain unsaturated fatty acids (lipases) (Khoo & Steinberg, 1975). With these shortfalls in mind esterase activity was used as an indication of lipolytic activity during composting.

Next to RNase, esterase was the most common hydrolase determined (39% of all fish-bark compost isolates), being detected from isolates at every sample interval (Tables 17 & 18). However, lipolytic activity in fish-bark composts was low during the mesophilic and later thermophilic stages of composting (Figure 23). These findings are consistent with the general concept of sequential microbial attack of increasingly complex carbon sources as the simpler components become limiting (Waksman, *et.al.*, 1939b). These results also highlight how misleading it is to only assay isolates' enzyme activities without concurrently studying crude compost samples. Few workers have reported studies on lipolytic activity in composts (Hankin, *et.al.*, 1979) (possibly due to its generally low occurrence).

4.3.1.2 Cellulose Biodegradation

The apparent higher rate of cellulose utilization at the lower fish-compost C:N ratio (45 compared to 65, Table 6) probably occurred after the first 8d (Figure 8) when the CMCase activity was greater at the lower C:N composition. Initial low cellulase activities could be due to the readily available simple C-compounds while the subsequently higher activity may be attributable to the greater N availability as degradation of the nitrogenous material progressed, as suggested by Freer and Detroy (1982) for lignocellulose biodegradation. No cellulolytic thermophilic bacterium was isolated at 7d from composts of either C:N composition, but during a different run with the same components (R4, C:N=45) cellulolytic mesophilic *Bacillus sphaericus* and Gram negative rods were isolated at 7d. Considering the temperature rise in the composts the CMCase activity initially measured during R2 may have been due to mesophilic microorganisms (which were not enumerated during R2). Nevertheless the compost cellulase assay temperature used (65°) was never significantly ($p < 0.01$) different from the optimum temperature for CMCase activity in fish-bark composts (Figure 20). It was also of interest to find that the estimated numbers of cellulolytic bacteria (Table 10) were always greater in the compost with the highest CMCase activity (Table 8).

From the above findings it appeared that CMCase activity correlated well with cellulose biodegradation. Further support for this was provided by determination of total CO_2-C loss (Table 9), with higher losses being associated with greater CMCase activity measured during composting, and in particular, the higher CMCase activity at 28d (Figures 21-22). This was also consistent with the large-scale study, where poorer degradation of sewage-bark corresponded with lower CMCase activity compared with that observed in the urea-bark compost (Figure 6).

All stable cellulolytic (CMCase) isolates obtained from dilution plates and from cellulose enrichments maintained this

activity in liquid culture. However, most isolates were poor degraders of crystalline cellulose as seen from the inability of all but the mesophilic fungi (*Aspergillus sp.* 490 & *Penicillium sp.* 437) to induce "C_1" cellulases (Table 22). Cellulolytic activity by microorganisms capable of anaerobic growth was only demonstrated for the facultative *Cellulomonas spp.* and *B.coagulans*. The inability to detect CMCase in all but *Aspergillus sp.* 490 culture broths containing micro-crystaline cellulose may be accounted for, in part, by adsorption of endogluconase to cellulose (Ryu, *et.al.*, 1984).

4.3.1.3 Hemicellulose Biodegradation

Degradation of xylan (the predominant hemicellulose in hardwood (Hillis, 1962)) was used as an indication of hemicellulase activity of isolates. Bacteria able to hydrolyse xylan were present at weeks 1, 2 and 4, but were predominant at week two in fish-bark and week four in urea-bark composts of initial C:N=45 (Table 18). Most of the xylanolytic isolates were species of *Sreptosporangium* and *Streptomyces*, but xylanolytic *Bacillus spp., Clostridium sp.*, coryneforms and *Micropolyspora spp.* were isolated. None of the *Bacillus spp.*, demonstrated by Williams and Withers (1983) to hydrolyse xylan anaerobically, were found to be active against xylan in the present study (i.e. *B.circulans* and *B.coagulans*).

4.3.1.4 Pectin Biodegradation

Pectic enzymes were detected from isolates taken at weeks one, two and four, but pectolytic bacteria generally predominated after the first week of composting. The exceptions were isolated from fish-bark composts of initial C:N=65. No direct assay of pectolytic activity within compost was undertaken. Consequently, actual pectolytic activity may well have been retarded by the presence of phenolics and tannins (Rexova-Benkova & Markovic, ~~et.al.~~, 1979; Obi and

Umezurite, 1981) until at least the second week of composting.

The predominant pectolytic bacteria isolated during the composting of fish-bark were initially *Streptosporangium spp.* and coryneforms, being followed by *Bacillus spp.* by the fourth week of composting. This situation was reversed during the composting of urea-bark, but with *Streptomyces spp.* replacing *Streptosporangium spp.* .

4.3.1.5 Lignin Biodegradation

The decrease in lignin degradation with the higher level of N in R2 was consistent with the concept of ammonium suppression of lignolytic activity and its biodegradation by secondary metabolism (Fenn and Kirk, 1981). This inability under reasonable levels of N availability for significant lignin degradation may explain the ability of mature eucalypt bark compost to hold its volume over a year in a plant pot under a normal fertilization programme (Clark, V.S. *pers. comm.*). Polyphenols and tannins were also included in the assay of lignin degradation in compost and may account for some of the loss of "lignin". Their bioconversion was evident from the change in composition of phenolic components of methanol extracts as determined by HPLC (Table 26). Also, partial reaction towards polyphenols was likely due to the presence of strains able to polymerize (darkening reaction) and depolymerize (clearing reaction) phenolic components in agar (Table 23).

The proposed mechanism of lignolytic enzyme activation (Fenn and Kirk, 1981) may explain why no laccase activity was detected in any compost. Nevertheless laccase-like activity was induced on agar plates (Table 23) and a mesophilic yeast appeared to degrade lignin. Greater lignolytic activity was observed in co-cultures with the yeast and either a *Flavobacterium sp.* (isolate 600) or *Bacillus sp* (isolate 441) and by two mesophilic *Bacillus spp.* (isolates 440 & 441) isolated from NaCMC enrichments. Although the assay of lignin biodegradation was not supported by ^{14}C-CO_2 assay, the method used does not detect

demethoxylation or metabolism of the side-chains in which the basic structural chromophores responsible for absorption at 281 nm are involved (Janshekar, *et.al.*, 1981). Pure cultures of the lignolytic yeasts *Geotrichum spp.* (was *Trichosporon spp.*, see Barnett, *et.al.*, 1983) have only been reported to degrade water soluble lignins (Glanser and Ban, 1983). Also, a mixed culture of these yeasts and bacteria significantly increased the rate of lignosulfonate degradation over that of pure cultures (Ban, *et.al.*, 1979).

4.3.2 Nitrogen Transformations

In general terms mineral N transformations depended on the net level of ammonification during composting, with net ammonification reflecting the level of available N from the amendment. Despite overall similar availabilities of urea and fish N (as discussed above) the order, in decreasing magnitude of ammonification was urea > fish > sewage. Ammonification was delayed by the addition of quinone to urea-bark and by urea's replacement with IBDU or sewage. With these relationships in mind the following discussion on urea-bark N transformations generally applied to other amendments.

Nitrogen originating from urea was largely assimilated (99.3%) into the microbial biomass within the first four days of composting urea-bark of initial C:N=35 (Figures 26 & 29). After the largest peaks (mesophilic) of respiratory activity occurred, net ammonification steadily increased to reach a maximum some 4d after the thermophilic peaks in respiratory activity (d12). Many *Bacillus spp.* and in particular *B. brevis* isolated at this time produced detectable free ammonia on TSA plates. After a further 4d the level of net ammonification began to decline, reaching a level similar to that at d4 by the end of the composting period.

Net nitrification generally followed peak net ammonification (Figure 24) and appeared to be chemical rather than biological, since no nitrifying flora (neither autotrophic nor heterotrophic)

was isolated and similar levels of nitrification were apparent in sterile and non-sterile composts (Figure 30). It is possible, however, that the bulk of nitrate produced (by a heterotrophic population that did not grow on the isolation media used) is being immediately immobilized, to give apparent levels of nitrate similar to the sterile composts. This latter proposal is supported by the isolation from previous fish-bark composts (R2) of heterotrophs able to produce nitrite (4 coryneforms, 2 *Bacillus spp.* & 2 *Streptomyces spp.*) and that the addition of thiourea (inhibits autotrophic nitrification) increased net nitrification (and decreased net ammonification) (Figure 28). Only Golovacheva (1975) has isolated a thermophilic (autotrophic) nitrifier. All other heterotrophic nitrifiers isolated have been mesophiles and have included actinomycetes and fungi (Verstraete and Alexander, 1972a), *Alcaligenes sp.* (Castignetti and Gunner, 1981), *Arthrobacter sp.* (Focht and Verstraete, 1977) and Gram negative rods (Castignetti and Hollocher, 1984). While Waksman *et.al.* (1939b) has reported nitrification during thermophilic (up to 75°) composting, no nitrifier was isolated. He also found considerable nitrification only after ammonium- N was beginning to accumulate for the second time (after 47d when most carbohydrates had been reduced to a minimum).

Volatilization of ammonia was greatest during the period of greatest ammonification, temperature and pH (Figures 33 and 27). However, the later peaks in ammonia volatilization (Figure 33) occurred when net ammonification was at its lowest, but pH was rising. These peaks, although unique to the urea-bark composts of initial C:N=35, highlight the problem that net, not actual ammonium-N turnover was assayed. Delaying ammonification in the urea based composts (by the addition of quinone to urea or its replacement with IBDU) both delayed and increased ammonia volatilization. Waksman, *et.al.* (1939b) also noted an increased loss of ammonia when composting was delayed by too low or too high a temperature.

Data on volatilization of N oxides indicated that considerable nitrate may be produced before it was observed to

accumulate. The data showed net nitrification only began to increase after the decline in volatilization of N oxides (Figures 26 & 33). Also, whenever initial respiratory activity was delayed there was a significant increase in the volatilization of N oxides at 6d (Figures 32-34). While denitrification of the nitrate present in sewage was not unexpected there was no obvious source of nitrate in either the quinone+urea- or IBDU-bark composts for biological denitrification. However volatilization resulting from chemodenitrification (Smith and Chalk, 1980a, 1980b) was possible from accumulated nitrite (Figure 28) or the reaction of quinone or IBDU with urea via the formation of nitrosophenols and quinone oximes (Bremner and Nelson, 1968) and/or reaction with amide groups in organic matter via a van Slyke-like process (Reuss and Smith, 1965). Volatilization of nitrous oxide under aerobic conditions may also be explained, in part, by the activity of nondenitrifying (cannot reduce NO_3 to N_2) nitrate reducers such as *Bacillus* and *Citrobacter spp.*, whose activities are not inhibited by O_2 (Smith and Zimmerman, 1981). Whatever the source of the N oxides, their presence was thought to indicate undesirable nitrogen availability rather than conditions of low O_2 tension. Nevertheless Bagstam and Swensson (1976) reported an increase in loss of N with an increase in compost moisture content, but he did not assay for N oxides.

The presence of N oxides are also important with regard to the assay of ammonia. The acid produced from N oxides in solution may have caused severe underestimation of ammonia volatilization in systems where compost gases were collected in acid and ammonia assayed by back titration (e.g. Griffis and Mote, 1982).

4.4 The Microorganisms in Bark Compost

The spreading *B.brevis* and other spreading bacilli made counting most difficult when plates were incubated for over 4d even at an agar concentration of 2.0% . Interestingly no spreading

growth was noted on ellagic acid (0.05%) agar plates by the strains with a spreading habit, but lower counts were obtained after incubation for 4 or 7 days compared to those on TSA after 4d.

An apparent succession of bacteria occurred in fish-bark compost. Mesophilic *B.brevis* and *B.sphaericus* dominated during the initial temperature rise, with thermophilic *B.circulans* or *B.brevis* (at aeration rates of 10 or 30 mL min^{-1} respectively) taking over during the early thermophilic periods of composting (Tables 17-19) and finally *B.circulans, B.sphaericus* or *B.stearothermophilus* (Table 17-18) dominated during the later stages (after 2 to 4 weeks of composting). Thermophilic *Bacillus spp.* were strong ammonifiers (noted by the production of ammonia on TSA). Their tolerance of ammonia may have been important to their dominance in fish-bark compost, although actinomycetes were the predominant isolates from urea-bark compost at the lower rate of aeration. It was possible that the ammonia released from the fast growing bacilli suppressed the growth of sensitive microorganisms on TSA, but with parallel counts on LigA plates, from which no ammonia was noted, no significant difference in isolates was observations. The characteristic odour from one of the *B.brevis* isolates on agar plates was also evident from the composts during the first week.

Bacillus spp. continued to predominate throughout the composting of sewage-bark (initial C:N=35) and for the duration of most of the fish-bark composts. However, actinomycetes and coryneforms and in particular, *Streptomyces* and *Thermomonospora* predominated in urea based composts from the second or third peak in respiratory activity (after 8 days) until the last sampling (21 days) (Tables 17-19). It would appear that the actinomycetes and coryneforms play a dominant role in the degradation of bark. Since *Bacillus spp.* generally dominated when C sources other than bark were available, fewer *Bacillus spp.* were found to be capable of attacking bark (Tables 17 & 18).

Sampling for predominant microorganisms during peaks in respiratory activity and incubating the isolates at the

temperature of the compost was found to be necessary to reduce the variability in total CFU isolated from a particular compost and improve their correlation with respiratory activity (r^2=0.81). This correlation was relatively high considering the percentage of spores in a soil can range from 1-90% of the total bacterial population and from 5-100% of the *Bacillus* population (Mishustin and Mirsoeva, 1968). Estimated numbers of eubacteria in composts varied more than those in the urea-bark composts of Bagstam (1979), but were similar to those found in other composts (Waksman, *et.al.*, 1939b; Hankin, *et.al.*, 1976). Bagstam's (1979) total estimated numbers of 10^{10}-10^{11} bacteria per g compost seem rather high.

Estimated numbers of bacteria in the large-scale composts were generally of the same order of magnitude as those in the bench-scale composts. The high numbers of mesophiles isolated during thermophilic composting in both large- and bench-scale composts could be explained by the survival of spore-forming *Bacillus* strains from mesophilic temperatures. However, the apparent build up of mesophilic coryneforms and yeasts in the urea-bark compost of initial C:N=45 (Table 18), (and also observed in other composts (Von Klopotek, 1962)), may be due to the rapid growth of *Bacillus spp.* on agar media suppressing the growth of other isolates during the earlier isolation intervals. These bacteria, however, would rapidly decline in dominance as composting progressed enabling the isolation of yeasts and coryneforms at subsequent time intervals.

Very few strains of *Clostridium* were isolated (Appendix 9) despite conditions of high CO_2 production. Most isolates capable of anaerobic growth were identified as *B.coagulans*. As reported elsewhere (Bagstam, 1979), the microorganisms important in the composting of bark appear to be aerobes.

The decline in the estimated numbers of faecal indicator organisms in sewage-bark compost (Figure 37) would suggest that under the controlled conditions of the bench-scale system satisfactory die-off of pathogens was likely. Nevertheless in large-scale sewage-bark heaps, turning of the outer material into

the hotter interior would be necessary to ensure low survival of mesophilic pathogens.

4.4.1 Influence of Bark Components on Microbial Successions During Composting

In contrast to previous studies (Cappaert, *et.al.*, 1976a; Bagstam, 1979; Clark, *et.al.*, 1977) up to four clearly-separated peaks of respiratory activity were evident in the course of composting bark, using the bench-scale system. Possible reasons for these unusual results may lie with the low rate of aeration used and the presence of toxins in eucalypt bark. The low rate of aeration used may well have reduced the microbial activity possible at any one time and thus slowed down the normal succession of microorganisms to the degree that the activities of each major group were resolved. The second possibility was that eucalypt bark contains more potent toxins than other bark, and their slow decomposition restricted rapid microbial succession. The first theory was not tested by observing the number of peaks in activity at a very high rate of aeration, but data on the inhibition of compost on the growth of isolates (Table 24) supported the second theory. Microbial succession under isothermal conditions demonstrated that despite the predominance of different isolates, the four peaks in respiratory activity were influenced by toxins present in the compost. In contrast to the work of Clark, *et.al.* (1977) the availability of calcium did not influence the number of peaks in respiratory activity observed during composting.

These results imply that the toxin(s) must be reasonably thermostable and at least partly water soluble. Also, due to the likely requirement of a microbial succession for successful composting a continuous composting process is unlikely to speed-up the composting of eucalypt bark.

4.4.2 Taxonomy of Thermophilic Isolates

4.4.2.1 Nonsporeforming Genera

Three groups of thermophilic nonsporing bacteria were isolated from fish-bark composts. Firstly, some isolates keyed out to the genus *Thermus* being non-motile, yellow-pigmented, strictly aerobic Gram negative rods. However, *Thermus* is only reported to have been isolated from heated water (Buchanan and Gibbons, 1974). Strom (1978) also isolated non-motile, yellow-pigmented, strictly aerobic Gram negative rods, but identified them as thermophilic *Flavobacterium spp.* . The identification of these isolates as *Flavobacterium spp.* would appear to be inappropriate considering the present state of their taxonomy (Shewan and McMeekin, 1983) and that Brock (1978a) created the genus *Thermus* for such isolates.

The second group of isolates were placed with the coryneforms. These pink-pigmented, non-motile, strictly aerobic and generally coccoid (in pairs) Gram positive isolates were at first thought to belong to the *Nocardiacea* or *Mycobacteriaceae* (Buchanan and Gibbons, 1974). However, the lack of *meso*-DAP and arabinose in their cell wall and a %(G+C) = 79 do not fit the general description of these genera (comprising *meso*-DAP, arabinose & galactose in their cell wall & a %G+C range of 60-72).

The resurrection of the genus *Brevibacterium* (Skerman, *et.al.*, 1980) from *genera incertae sedis* (Buchanan and Gibbons, 1974) enabled the small Gram positive, strictly aerobic thermophilic rod shaped bacteria to be at least assigned to a genus. There is however, no thermophilic member mentioned in Bergey's Manual (Buchanan and Gibson, 1974).

4.4.2.2 Thermophilic *Bacillus*

Most of the 109 thermophilic *Bacillus* isolates could be identified to species using the key of Gordon, *et.al.* (1973), although to achieve this, 40% required a greater degree of variability in up to three characteristics than allowed for by Gordon, *et.al.* (1973). Most mismatches were in the fermentation of sugars. In most cases the species identifications which were made were supported by the numerical taxonomic study (Figure 36) and likely identifications of about half of the seven unassigned isolates were also indicated (Figure 36). The numerical taxonomy study was limited by the number of characteristics compared, but characteristics were selected from previous work to show differences between the species expected. Other workers have noted about a 15% mismatch on a few characteristics (Gordon, *et.al.*, 1973; Strom, 1978). Compared to Strom's (1978) 752 compost isolates most of the isolates in the present study fitted the description and identification given, however, no *B.licheniformis* was isolated and a greater percentage of *B.brevis* were identified in the present study. Also, the *B.coagulans* type A described by Strom (1978) were isolated. Type A strains differ from the more commonly observed type B in their negative V.P. reaction and ability to grow at 65°.

The separation of strains assigned to *B.stearothermophilus* into the three groups (Table 3) of Walker and Wolf (1971) was supported by the study on esterases produced by the isolates. However, generally fewer bands with different R_f positions were observed in polyacrylamide gels compared to those found in starch gels (Baillie and Walker, 1968). Sharp, *et.al.* (1980) also reported fewer bands in polyacrylamide gels and identified group 1 stains by the presence of two or more bands and groups 2 and 3 by the position of the single band of activity observed. In the present study group 1 strains were identified by at least three bands (two with similar R_f), one band from group 2 isolates and generally two bands from group 3 isolates. The group 3 isolates differed from those in group 1 by either giving a weak

band or none at all at R_f 0.68 and no bands at R_f's below 0.36. The differences in banding between the studies could not be accounted for by the culture medium as all used the medium of Sargeant, *et.al.* (1971), although TSB was shown to induce greater activity and was generally used in the present study. The differences probably reflected the differing enzyme concentrations and/or the age of the cultures. Such differences limit the versatility of this identification system.

The numerical taxonomic study also indicated greater similarity between group 1 and group 3 isolates than between either of these groups and group 2 isolates. Of the seven unidentified strains only isolate 102b fitted *B.stearothermophilus* (group 1) by both esterase mobility and the taxonomic study.

4.5 Suitability of Bark Compost as a Plant Growth Medium

4.5.1 Chemical Properties of Bark Compost

Successful eucalypt bark composting depends on the depletion of water soluble phytotoxins and a satisfactory N balance (Ashbolt, 1979, Hons. Thesis). The possible destruction of phytotoxic phenolics in most composts was difficult to assess due to the presence of non-PVP binding toxin(s) (probably ammonium-N) (Table 25). Nevertheless no additional growth was observed by removing phenolics from most compost water extracts. Only lettuce grown in water extracts of fish-bark compost of initial C:N=35 showed a significant ($p < 0.01$) increase in growth after the PVP treatment. However, all composts showed the presence of non-PVP binding phytotoxin(s). The levels of ammonium-N in these composts was sufficient to cause the phytotoxicity observed (Bennett and Adams, 1970; Imai, 1977) while levels of volatile fatty acids were insignificant in all 30d old composts. Nevertheless, phenolics which do not bind to PVP, such as gallic acid (Ashbolt, 1979, hons. thesis), may have contributed to the phytotoxicity observed in some composts. The other phenolics assayed by HPLC are less

likely to cause phytotoxicity due to their poorer water solubility (Patrick, 1971).

The maturity index (MI, ratio of p-coumaric to over gallic acid) correlated well with the results from the plant bioassay with regard to type of amendment, but did not reflect the highly phytotoxic nature of all composts with an initial C:N=25 due to the presence of non-phenolic phytotoxins. Both the MI data and the combined respiratory activity and N loss data indicated that the fish-bark composts were as good or better than urea-bark composts and that sewage-bark composts were considerable "poorer" products.

4.5.2 Physical Properties of Composts

The superior physical properties (Table 26) of the large-scale urea-bark compost over those of the sewage-bark (1 year old) probably resulted from its lower content of fine particles. These greasy, clay-like lumps of degraded sewage would necessitate considerable mixing of the compost mass to make it acceptable for sale to the general public.

Eucalypt bark composts generally had better physical properties than pine-bark composts and were as good as peat moss except for a four-fold increase in bulk density. These results are, however, limited in that the available water-holding capacities were not tested by plant performance (Beardsell, *et.al.*, 1979). The greater bulk density probably results from the well decomposed nature of one year-old eucalypt-bark compost and has the advantage over pine-bark composts of greatly reducing slumping in large pots (Clark, V.S., *pers. comm.*).

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APPENDIX - 1

Composition of Compost Mixtures & Conditions for Composting - Runs 1-9 [1]

Run Unit	Weight of: Bark	Fish	Urea	Initial C/N	Initial %N	Aeration mL/min	Other Conditions or Amendments
R1.							
1	148.0	16.01	-	45	1.0771	30	Unamended
4	148.0	16.01	-	"	"	"	"
5	148.0	16.01	-	"	"	"	"
2	148.0	8.34	-	65	0.7658	"	"
3	148.0	8.34	-	"	"	"	"
6	148.0	8.34	-	"	"	"	"
R2.	"As for R1 above"						
R3.							
3	148.0	16.01	-	45	1.0771	15	Unamended
4	148.0	16.01	-	"	"	"	"
2	148.0	16.01	-	"	"	"	Propylene oxide added
6	148.0	16.01	-	"	"	"	d0 (5mL) & d9 (10mL)
1	148.0	16.01	-	"	"	"	$CaCl_2$ (0.27g)
5	148.0	16.01	-	"	"	"	"
R4.							
1	148.0	16.01	-	45	1.0771	10	Unamended
2	148.0	16.01	-	"	"	"	"
3	148.0	16.01	-	"	"	"	Thiourea (0.01g)
5	148.0	16.01	-	"	"	"	"
4	148.0	-	2.81	"	1.1289	"	Unamended
6	148.0	-	2.81	"	"	"	"
R5.							
3	134.0	14.45	-	45	1.0730	30	Unamended. Temp. at 19d 50°, 24d 55°, 27d 60° & 29d 55°.
2	134.0	14.45	-	"	"	"	
1	184.6	14.45	-	55	0.9021	"	Unamended.
4	184.6	14.45	-	"	"	"	Temp. as above.
5	134.0	14.45	-	45	1.0730	"	Sodium azide (0.05%)
6	134.0	14.45	-	"	"	"	Temp. as above.

Run Unit:	Weight of: Bark	Fish	IBDU	Sewage	Urea	Initial C/N	Initial %N	Conditions or Amendments
R6.								
3	134.0	14.45	-	-	-	45	1.0730	Unamended. 10mL min^{-1}
5	134.0	14.45	-	-	-	"	"	" " "
6	134.0	14.45	-	-	-	"	"	" 20 "
1	134.0	14.45	-	-	-	"	"	" " "
4	134.0	14.45	-	-	-	"	"	" 30 "
2	134.0	14.45	-	-	-	"	"	" " "
R7.								
1	92.0	14.45	-	-	-	35	1.3230	Unamended.
2	92.0	14.45	-	-	-	"	"	"
6	105.2	-	-	-	2.81	"	1.4000	"
3	105.2	-	-	-	2.81	"	"	"
5	91.0	-	-	56.00	-	"	1.3241	"
4	91.0	-	-	56.00	-	"	"	"
R8.								
4	105.2	-	-	-	2.81	35	1.4000	Unamended.
6	105.2	-	-	-	2.81	"	"	"
3	105.2	-	-	-	2.81	"	"	p-benzoquinone (5mL)
5	105.2	-	-	-	2.81	"	"	"
1	91.0	-	3.75	-	-	"	1.4287	Unamended.
2	91.0	-	3.75	-	-	"	"	"
R9.								
3	56.3	14.45	-	-	-	25	1.7282	Unamended.
6	56.3	14.45	-	-	-	"	"	"
1	42.4	-	-	56.00	-	"	1.7419	"
4	42.4	-	-	56.00	-	"	"	"
2	65.2	-	-	-	2.81	"	1.9068	"
5	65.2	-	-	-	2.81	"	"	"

[1] All mixes were made with *Eucalyptus delegatensis* bark, inoculated with 15g of mature compost and made up to 214% m.c. See Table 1 for chemical compositions of raw materials. Mixes were aerated at 20mL/min unless noted otherwise. The temperature of all mixes was initially 20° & increased at 5° per d to 55° then modified as described in the table. Composts were mixed for 15 min every h at 15 rpm

APPENDIX - 2

Temperature in the Large-Scale Compost Heaps [1]

Day	Urea - Bark Heap					Sewage - Bark Heap				
	Probe 1	Probe 2	Probe 3	Mean	SE	Probe 1	Probe 2	Probe 3	Mean	SE
0.0	10	10	10	10	0	10	10	10	10	0
0.5	7	11	8	9	1	8	12	9	10	1
1.0	6	13	8	9	2	9	14	12	12	1
1.5	10	16	13	13	2	10	16	14	13	2
2.0	12	19	15	15	2	13	19	17	16	2
2.5	12	21	18	17	3	14	22	20	19	2
3.0	14	24	22	20	3	16	25	23	21	3
3.5	17	28	26	24	3	18	29	26	24	3
4.0	19	33	29	27	4	19	33	30	27	4
4.5	22	36	33	30	4	21	36	34	30	5
5.0	25	38	37	33	4	24	39	36	33	5
5.5	29	40	39	36	4	25	40	38	34	5
6.0	34	43	44	40	3	25	42	40	36	5
6.5	38	45	45	43	2	26	46	43	38	6
7.0	41	48	47	45	2	27	49	47	41	7
7.5	44	52	50	49	2	29	53	49	44	7
8.0	46	59	56	54	4	30	55	51	45	8
8.5	47	61	59	56	4	33	57	55	48	8
9.0	48	63	62	58	5	32	58	59	50	9
9.5	49	60	61	57	4	33	61	59	51	9
10.0	47	59	57	54	4	35	62	58	52	8
10.5	44	63	59	55	6	41	63	60	55	7
11.0	45	61	58	55	5	43	61	57	54	5
11.5	44	63	59	55	6	46	59	56	54	4
12.0	47	65	62	58	6	48	61	58	56	4
12.5	49	65	63	59	5	49	62	55	55	4
13.0	50	67	61	59	5	42	58	57	52	5
13.5	52	68	63	61	5	44	59	54	52	4
14.0	53	65	60	59	3	46	58	52	52	3
14.5	41	55	53	50	4	40	52	48	47	4
15.0	45	69	59	58	7	48	59	51	53	3
15.5	51	70	64	62	6	50	63	57	57	4
16.0	59	73	65	66	4	46	60	59	55	5
16.5	55	68	61	61	4	49	57	55	54	2
17.0	54	65	62	60	3	51	58	53	54	2
17.5	52	63	59	58	3	53	59	51	54	2
18.0	53	64	57	58	3	56	61	54	57	2
18.5	50	62	59	57	4	54	62	58	58	2
19.0	55	63	60	59	2	52	63	59	58	3
19.5	56	63	58	59	2	54	62	57	58	2
20.0	54	65	60	60	3	56	60	56	57	1
20.5	55	67	59	60	4	53	63	59	58	3
21.0	53	64	61	59	3	55	61	60	59	2
27.0	56	62	60	59	2	57	60	58	58	1
34.0	52	61	62	58	3	55	62	59	59	2
41.0	50	62	59	57	4	53	59	56	56	2
48.0	51	60	57	56	3	41	55	53	50	4
56.0	48	57	54	53	3	38	56	50	48	5
63.0	45	59	52	52	4	35	48	36	40	4
70.0	52	56	50	53	2	37	42	35	38	2
77.0	54	58	56	56	1	35	40	35	37	2
84.0	48	56	49	51	3	31	39	32	34	3
91.0	46	54	44	48	3	32	40	33	35	3
98.0	44	52	45	47	3	33	38	34	35	2
106.0	41	45	40	42	2	30	37	35	34	2
136.0	38	41	37	39	1	31	39	34	35	2
143.0	34	43	38	38	3	45	59	66	53	4
150.0	40	57	42	46	5	46	54	52	51	2
157.0	53	60	59	57	2	41	53	50	48	4
173.0	55	59	58	57	1	44	56	52	51	4
181.0	54	63	59	59	3	42	53	47	47	3
211.0	57	58	55	57	1	37	49	45	44	4
241.0	40	55	39	45	5	35	46	41	41	3
271.0	36	43	35	38	3	35	46	38	40	3
301.0	35	40	38	38	1	34	43	38	38	3

[1] Temperatures were monitored by three thermocouples placed at 0.3, 1.0 & 2.0m from the center top of each heap. **See Figure 5.**

CMCase Activity, Moisture, pH & Estimated Numbers of Bacteria in Large-Scale Compost Heaps [1]

Week	Relative CMCase Activity				Mean m.c.		Mean pH		Estimated No. Bacteria			
	Urea-Bark		Sewage-bark						Mesophiles		Thermophiles	
	Mean	SE	Mean	SE	U-B	S-B	U-B	S-B	U-B	S-B	U-B	S-B
0	5.22	2.33	39.15	2.25	215	218	4.4	4.6	5.2	7.6	3.7	4.4
1	10.55	3.48	23.11	1.26	203	220	8.4	5.2	8.1	7.8	4.0	4.6
2	13.27	5.47	38.81	5.65	196	203	7.2	5.9	5.3	6.0	9.3	8.6
3	45.22	7.66	42.61	3.18	223	214	6.9	6.0	5.5	5.7	8.1	7.9
4	36.95	6.04	45.58	1.06	191	202	6.7	6.5	5.3	5.4	8.0	7.8
5	40.12	5.97	46.74	2.97	220	209	6.5	6.3	5.0	4.8	8.3	7.9
6	43.58	6.37	44.38	3.18	209	211	6.5	5.8	4.6	4.5	8.4	8.0
7	46.17	5.87	46.84	5.48	174	196	6.3	5.6	4.7	4.3	8.1	8.0
8	47.07	4.17	43.77	4.24	185	189	5.8	5.6	4.9	5.2	7.8	7.6
9	53.71	3.05	45.99	3.62	187	184	6.2	5.5	7.3	6.5	7.5	7.1
10	62.41	4.66	36.42	4.96	225	215	6.5	6.2	6.4	7.6	8.0	6.3
11	43.82	3.12	26.83	2.03	215	220	7.0	6.0	6.4	7.8	7.8	6.4
12	48.66	3.28	27.51	3.16	214	209	6.3	6.3	6.3	7.7	7.9	6.3
13	37.75	6.17	29.44	3.66	225	211	6.2	6.4	7.8	7.9	7.6	6.5
14	42.07	7.76	31.47	7.81	207	200	6.7	6.1	7.6	7.8	7.7	6.4
15	41.62	5.83	33.47	5.78	221	186	7.1	5.9	n.t.	n.t.	n.t.	n.t.
16	46.74	6.77	26.32	3.44	237	196	7.3	6.1	n.t.	n.t.	n.t.	n.t.
17	49.26	5.88	30.41	2.19	239	220	7.6	5.8	n.t.	n.t.	n.t.	n.t.
18	44.58	3.09	24.44	6.77	245	226	7.8	5.9	7.4	7.8	6.1	5.8
19	56.43	2.45	23.47	5.31	240	214	7.3	5.7	n.t.	n.t.	n.t.	n.t.
20	58.64	3.66	36.18	7.11	243	203	6.8	5.7	n.t.	n.t.	n.t.	n.t.
24	66.30	4.42	46.66	6.28	236	189	5.4	5.4	n.t.	n.t.	n.t.	n.t.
28	51.55	6.74	34.62	4.08	223	180	5.9	5.2	6.2	6.3	8.0	7.9
32	75.44	7.05	30.74	3.11	241	193	6.8	5.3	n.t.	n.t.	n.t.	n.t.
36	65.41	5.44	24.08	4.00	250	187	6.9	5.4	n.t.	n.t.	n.t.	n.t.
40	69.47	6.27	22.05	5.73	255	192	6.7	5.2	n.t.	n.t.	n.t.	n.t.
44	62.33	5.32	26.14	4.12	260	199	6.8	5.2	7.3	7.2	7.1	7.0
48	50.64	4.68	24.91	5.33	261	184	6.6	5.3	n.t.	n.t.	n.t.	n.t.
52	51.66	5.02	22.06	4.55	263	189	6.7	5.4	n.t.	n.t.	n.t.	n.t.

[1] Assays were undertaken on subsamples from about 2kg wet compost (bulked from ten grab samples taken at random > 10cm from the compost surface). U-B, Urea-bark compost; S-B, Sewage-bark compost both of initial C:N = 35. Relative CMCase was assayed in triplicate on 1g samples, m.c. was assayed by oven drying (105°) about 20g wet compost, pH was determined by a glass electrode in a 1:5 suspention (20g compost: 100mL 2N KCl) & total numbers of bacteria (Log_{10} CFU g^{-1} compost) were estimated on TSA (2.5.1.1) incubated at 28° or 55°. **See Figures 6 & 7.**

APPENDIX - 3

Respiratory Activity During R1 & R2 [1]

Day		Initial C:N 45	45	45	65	65	65
				OXYGEN UPTAKE			
2	R1	7.77	7.54	8.03	4.75	4.75	3.20
	R2	7.09	7.32	7.16	3.33	2.43	3.23
4	R1	9.66	9.74	10.68	3.69	3.66	3.74
	R2	9.85	9.11	8.08	4.03	4.09	3.97
6	R1	5.53	4.54	5.45	2.56	2.76	2.90
	R2	4.98	5.67	5.92	2.36	2.91	2.20
8	R1	3.25	2.85	3.12	1.28	1.20	1.51
	R2	3.39	4.00	4.05	0.82	1.19	0.89
10	R1	4.09	3.71	4.02	0.43	0.48	0.78
	R2	3.57	3.90	4.03	0.76	0.95	0.96
14	R1	2.05	1.27	1.76	1.11	1.81	1.13
	R2	1.35	2.41	2.51	2.20	2.63	2.10
16	R1	2.68	2.03	3.15	1.13	1.82	1.20
	R2	2.20	2.63	2.10	1.36	1.93	1.26
18	R1	1.20	2.03	1.24	0.92	0.79	0.55
	R2	1.97	1.75	1.52	0.75	0.65	1.08
22	R1	2.64	2.53	2.20	2.53	1.88	1.81
	R2	1.90	2.11	1.97	1.11	1.27	1.58
24	R1	1.99	2.53	1.23	1.10	0.95	1.49
	R2	0.98	1.45	0.89	1.64	1.94	1.90
26	R1	0.88	0.89	0.96	0.42	0.36	0.53
	R2	0.99	0.79	0.84	0.55	0.63	0.59
28	R1	0.84	0.86	0.79	0.28	0.31	0.36
	R2	0.71	0.82	0.77	0.33	0.31	0.31
30	R1	0.89	0.87	0.69	0.38	0.46	0.33
	R2	0.67	0.48	0.56	0.40	0.50	0.48
Day				CARBON DIOXIDE OUTPUT			
2	R1	3.34	2.15	2.98	1.13	1.87	1.58
	R2	2.69	3.83	2.78	1.60	1.92	1.99
4	R1	4.40	4.58	4.30	2.66	2.69	2.74
	R2	4.73	4.56	4.58	2.24	2.73	2.46
6	R1	3.32	2.89	3.00	1.11	1.42	1.42
	R2	3.08	3.29	3.29	1.30	1.09	1.83
8	R1	3.56	2.62	3.13	0.88	0.66	0.74
	R2	3.01	2.95	2.38	0.57	0.69	0.55
10	R1	2.71	2.33	3.47	0.60	0.76	0.62
	R2	2.88	2.30	2.92	0.65	0.73	0.62
14	R1	1.25	0.87	0.95	0.90	0.57	0.45
	R2	0.97	1.25	1.24	0.48	0.50	0.53
16	R1	2.01	1.86	1.89	1.03	0.79	0.65
	R2	1.97	1.71	1.56	0.65	0.58	0.69
18	R1	1.15	1.56	1.10	0.79	0.45	0.43
	R2	1.54	1.38	1.23	0.51	0.39	0.74
22	R1	2.75	1.84	2.02	1.88	1.82	1.56
	R2	2.10	2.11	2.05	1.98	1.75	1.33
24	R1	1.73	1.52	1.43	0.69	0.43	0.55
	R2	0.95	1.35	0.86	0.45	0.72	0.42
26	R1	0.69	0.70	0.84	0.29	0.33	0.36
	R2	0.92	0.77	0.69	0.45	0.35	0.31
28	R1	0.74	0.68	1.00	0.33	0.28	0.25
	R2	1.03	0.80	0.78	0.36	0.33	0.37
30	R1	1.03	0.98	0.84	0.36	0.47	0.33
	R2	0.79	0.57	0.77	0.37	0.40	0.60

[1] Samples (1 mL) were injected manually into a dual column GC as described in Materials and Methods. Values of O_2 uptake or CO_2 production are given as a percentage of the effluent gas from each compost unit. Gas flow rate = 30 mL min^{-1}. **See Figure 8.**

Estimated Microbial Biomass in 28d Old Composts [1]

h	Net mg CO_2 10g^{-1}compost h^{-1} Fish [2]	Urea	IBDU	Sewage	Net mg CO_2-C 10g^{-1} Compost 5h^{-1} Fish	Urea	IBDU	Sewage
5	-0.811	-0.990	-1.295	-0.717				
10	-0.064	-0.360	-0.448	-0.175				
15	0.257	0.330	0.602	0.173	0.350	0.450	0.821	0.236
20	1.840	1.960	3.905	0.950	2.509	2.672	5.325	1.295
25	3.453	3.610	4.133	1.933	4.709	4.922	5.635	2.635
30	3.218	3.740	3.699	2.353	4.388	5.099	5.044	3.208
35	2.132	2.470	2.433	3.068	2.907	3.368	3.317	4.183
40	1.636	1.550	2.084	3.021	2.230	2.113	2.842	4.119
45	1.122	1.300	1.989	2.324	1.530	1.773	2.712	3.169
50	1.093	1.070	1.922	1.580	1.491	1.459	2.620	2.154
55	0.871	1.140	1.891	0.620	1.188	1.554	2.578	0.845
60	0.933	0.890	1.844	0.717	1.272	1.214	2.514	0.977
65	0.865	0.830	1.791	0.767	1.180	1.132	2.442	1.046
70	0.717	0.750	1.731	0.820	0.977	1.023	2.360	1.118
75	0.837	0.700	1.664	0.956	1.141	0.954	2.269	1.304
80	0.864	0.730	1.604	0.711	1.179	0.995	2.188	0.969
85	0.832	0.760	1.589	0.844	1.135	1.036	2.167	1.150
90	0.783	0.720	1.509	0.640	1.068	0.982	2.058	0.873
95	0.776	0.760	1.499	0.734	1.059	1.036	2.044	1.001
100	0.771	0.700	1.434	0.733	1.051	0.954	1.955	0.999
105	0.739	0.670	1.509	0.701	1.008	0.914	2.058	0.956
110	0.762	0.660	1.567	0.612	1.039	0.900	2.136	0.834
115	0.734	0.643	1.553	0.612	1.001	0.877	2.118	0.834
120	0.706	0.580	1.503	0.430	0.963	0.791	2.050	0.587
125	0.672	0.560	1.563	0.498	0.917	0.764	2.132	0.679
130	0.643	0.530	1.534	0.445	0.877	0.723	2.092	0.606
135	0.590	0.500	1.214	0.455	0.804	0.682	1.656	0.621
140	0.521	0.460	0.991	0.459	0.710	0.627	1.351	0.625
145	0.439	0.440	0.979	0.374	0.598	0.600	1.334	0.510
150	0.403	0.400	0.979	0.377	0.550	0.545	1.334	0.514
155	0.398	0.387	0.732	0.308	0.543	0.527	0.999	0.420
160	0.347	0.330	0.620	0.368	0.473	0.450	0.845	0.502
165	0.331	0.270	0.351	0.255	0.452	0.368	0.479	0.348
170	0.221	0.130	0.212	0.196	0.302	0.177	0.289	0.267
175	0.110	0.046	0.101	0.096	0.150	0.062	0.137	0.130
180	0.095	0.066	0.002	0.096	0.130	0.090	0.002	0.130
185	0.040	0.002	0.017	0.090	0.055	0.003	0.023	0.123
190	0.002	0.004	0.005	0.076	0.002	0.006	0.006	0.103
195				0.077				0.105
200				0.066				0.089
205				0.059				0.080
210				0.053				0.073
215				0.043				0.059
220				0.038				0.052
225				0.010				0.014
230				0.004				0.006
Biomass [3] (mg C g^{-1} Compost) =					15.99	15.97	22.66	15.68

[1] Biomass was determined from the flush in CO_2 output (assayed every 5h by GC) over that of a control after $CHCl_3$ fumigation and reinoculation during R7 & R8.

[2] All composts were made with an initial C:N=35 as described for R7 & R8 in Appendix 1.

[3] The k-factor of 0.45 (Jenkinson, *et.al.*, 1979) was used.

Carbon Dioxide Output During Runs 3 & 4, Initial C:N=45 [1]

RUN 3

Day	Unamended		Fish-bark Compost Sterilized		Plus CaCl [2]	
1	0.933	0.875	0.003	0.002	1.043	0.979
2	1.750	1.699	0.003	0.003	1.790	1.697
3	1.709	1.648	0.007	0.004	1.593	1.633
4	1.738	1.759	0.093	0.007	1.850	1.957
5	1.783	1.800	0.143	0.097	1.739	1.783
6	1.323	1.578	1.198	1.377	1.205	1.308
7	1.284	1.136	1.450	1.531	1.229	1.321
8	1.473	1.400	1.845	1.637	1.205	1.215
9	1.153	1.241	1.437	1.759	0.892	1.003
10	0.981	1.022	0.001	0.001	0.523	0.638
11	0.780	0.825	0.003	0.003	0.277	0.215
12	0.583	0.476	0.003	0.002	0.242	0.208
13	0.339	0.313	0.005	0.005	0.370	0.327
14	0.450	0.534	0.005	0.005	0.485	0.490
15	0.499	0.503	0.003	0.004	0.431	0.444
16	0.573	0.560	0.004	0.004	0.523	0.503
17	0.833	0.794	0.005	0.004	0.559	0.548
18	0.775	0.644	0.006	0.007	0.514	0.523
19	0.697	0.687	0.006	0.007	0.489	0.473
20	0.530	0.491	0.005	0.008	0.422	0.438
21	0.201	0.357	0.006	0.006	0.479	0.499
22	0.238	0.258	0.023	0.010	0.466	0.487
23	0.309	0.343	0.066	0.049	0.481	0.473
24	0.290	0.291	0.095	0.095	0.421	0.431
25	0.384	0.344	0.089	0.143	0.333	0.392
26	0.404	0.562	0.094	0.155	0.489	0.400
27	0.560	0.538	0.108	0.141	0.520	0.497
28	0.593	0.579	0.117	0.131	0.559	0.529
29	0.600	0.641	0.144	0.184	0.563	0.579
30	0.621	0.597	0.329	0.259	0.548	0.540
31	0.633	0.589	0.809	0.699	0.407	0.432
32	0.537	0.511	0.990	0.891	0.392	0.410
33	0.306	0.433	0.878	0.798	0.335	0.361
34	0.433	0.377	0.974	0.894	0.385	0.328
35	0.466	0.306	1.048	1.231	0.276	0.280

RUN 4

Day	Unamended		Fish-bark Composts plus Thiourea [2]		Urea-bark Compost	
1	0.237	0.264	0.180	0.178	0.186	0.171
2	2.397	2.487	1.079	1.175	1.711	1.794
3	2.065	2.187	0.894	0.859	1.502	1.354
4	2.365	2.302	2.580	2.438	2.023	1.914
5	2.421	2.252	2.802	2.314	2.156	2.161
6	2.120	2.131	1.973	1.458	1.570	1.422
7	2.494	2.605	2.342	2.216	1.991	1.897
8	2.761	2.662	2.519	2.692	2.226	2.031
9	2.366	2.447	2.165	2.403	1.782	1.893
10	2.035	2.088	2.293	2.267	1.520	1.434
11	2.088	1.868	2.122	1.979	1.742	1.674
12	1.915	1.754	1.861	1.977	1.573	1.497
13	1.862	2.017	1.983	1.790	1.369	1.416
14	1.963	2.064	2.054	1.855	1.661	1.563
15	2.265	2.477	2.396	2.332	1.535	1.644
16	2.340	2.415	2.442	2.456	1.457	1.510
17	2.197	2.258	2.326	2.191	1.784	1.696
18	2.121	2.130	2.201	2.266	1.891	1.742
19	2.034	2.062	2.050	2.542	1.462	1.599
20	1.462	1.351	1.478	1.373	0.953	0.825
21	0.966	0.811	1.073	0.818	0.454	0.378
22	0.692	0.728	0.755	0.642	0.468	0.454
23	0.853	0.798	0.815	0.766	0.530	0.472
24	0.800	0.872	0.953	0.871	0.664	0.573
25	1.094	1.016	1.048	1.157	0.809	0.858
26	1.218	1.191	0.966	1.093	0.894	0.903
27	1.350	1.117	1.122	1.171	0.921	0.889
28	1.402	0.908	1.221	1.285	0.696	0.819
29	0.724	0.784	0.572	0.653	0.402	0.648
30	0.274	0.289	0.228	0.227	0.299	0.321
31	0.157	0.219	0.229	0.168	0.361	0.271
32	0.143	0.191	0.182	0.128	0.242	0.276
33	0.217	0.154	0.190	0.156	0.206	0.250
34	0.106	0.159	0.172	0.156	0.206	0.249

[1] Samples (1 mL) were injected manually into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit. Gas flow rate = 15 and 10 mL min^{-1} for R3 and R4 respectively. See Figures 13 and 14.

[2] Amendments: R3, 0.27g $CaCl_2$; R4, 0.01g thiourea.

Carbon Dioxide Output During R5, Fish-bark Initial C:N=45 & 55 [1]

h	Sterile		C:N=45		C:N=55	
20	.0025	.0015	.0023	.0033	.0019	.0019
25	.0018	.0021	.0028	.0030	.0023	.0018
30	.0035	.0031	.0045	.0039	.0039	.0034
35	.0028	.0030	.0295	.0255	.0122	.0124
40	.0029	.0024	.0674	.0610	.0341	.0303
45	.0026	.0040	.0696	.0635	.0321	.0361
50	.0043	.0016	.4832	.4440	.2196	.2684
55	.0054	.0028	1.007	1.057	.4992	.5521
60	.0046	.0029	.9553	.9338	.2988	.3091
65	.0031	.0025	.3378	.4100	.1900	.2332
70	.0028	.0021	.1869	.1919	.1060	.1302
75	.0030	.0040	.1907	.2534	.1145	.1210
80	.0029	.0013	.3186	.3261	.1852	.2098
85	.0690	.0289	.5218	.5419	.0661	.1149
90	.0041	.0025	.4428	.3926	.0572	.0905
95	.0050	.0040	.3550	.3600	.0479	.0793
100	.0056	.0049	.2609	.2634	.0497	.0498
105	.0052	.0049	.2800	.2644	.0470	.0487
110	.0053	.0044	.2985	.2609	.0394	.0447
115	.0055	.0051	.1367	.1183	.0830	.0894
120	.0053	.0041	.2772	.2622	.1379	.1332
125	.0026	.0041	.3274	.3462	.1558	.1779
130	.0040	.0041	1.109	1.052	.2449	.2054
135	.0238	.0138	1.026	1.017	.2102	.2024
140	.0138	.0019	1.031	1.006	.2102	.1820
145	.0025	.0038	1.144	1.159	.2168	.2105
150	.0013	.0013	1.377	1.303	.3397	.3396
155	.0025	.0025	1.095	1.232	.1520	.2217
160	.0025	.0025	.9433	1.029	.1426	.1993
165	.0028	.0038	.8454	.8693	.1332	.1820
170	.0054	.0041	.7551	.7940	.1229	.1657
175	.0041	.0054	.7589	.8128	.1530	.2003
180	.0055	.0054	.7601	.7664	.1398	.1962
185	.0038	.0025	.7626	.7890	.1332	.2064
190	.0040	.0036	.7476	.7614	.1361	.0146
195	.0060	.0045	.5105	.5293	.1229	.1302
200	.0031	.0028	.4453	.4792	.0957	.0986
105	.0068	.0090	.1443	.1907	.0967	.1119
210	.0046	.0029	.1167	.1242	.0610	.0834
215	.0079	.0083	.1204	.1179	.0638	.0732
220	.0059	.0090	.1242	.1279	.0769	.0803
225	.0104	.0110	.1217	.1154	.0826	.0722
230	.0103	.0127	.1154	.1217	.0723	.0671
235	.0103	.0127	.1217	.1141	.0676	.0651
240	.0120	.0128	.1116	.1029	.0535	.0691
245	.0146	.0142	.0934	.0924	.0480	.0750
250	.0142	.0217	.0765	.0890	.0409	.0494
255	.0140	.0148	.0729	.0817	.0465	.0524
260	.0142	.0149	.0861	.0765	.0363	.0541
265	.0140	.0146	.0966	.0951	.0437	.0546
270	.0130	.0140	.0694	.0730	.0323	.0582
275	.0139	.0140	.0718	.0717	.0356	.0562
280	.0109	.0120	.0698	.0639	.0333	.0486
285	.0115	.0124	.0595	.0626	.0411	.0428
290	.0066	.0112	.0542	.0569	.0400	.0471
295	.0102	.0124	.1658	.1523	.1012	.1006
300	.0098	.0140	.2341	.2257	.1427	.1351
305	.0090	.0120	.3063	.3066	.1477	.1638
310	.0082	.0124	.2783	.2909	.1581	.1367
315	.0056	.0114	.2181	.2289	.1256	.1194
325	.0060	.0120	.3510	.3189	.1780	.2031
330	.0082	.0114	.3998	.3143	.1799	.2119
335	.0094	.0104	.4000	.2554	.1853	.1928
340	.0059	.0108	.4693	.3693	.2138	.2017
345	.0056	.0098	.3120	.2987	.1903	.2215
350	.0039	.0074	.3377	.2936	.1653	.2045
355	.0077	.0108	.5000	.5291	.2334	.2642
360	.0071	.0102	.6657	.6562	.4425	.4729
365	.0085	.0097	.6342	.6176	.4481	.4507
370	.0078	.0073	.6357	.6092	.3952	.4451
375	.0080	.0097	.9125	.9277	.5469	.5592
380	.0090	.0110	.9790	.9637	.4482	.4394
385	.0085	.0092	.8008	.8750	.4399	.4390
390	.0044	.0074	.9122	.9197	.4529	.4731
395	.0051	.0069	1.063	1.045	.3733	.4082
400	.0041	.0045	1.117	.9889	.4689	.5871
405	.0041	.0056	.7670	.7389	.3026	.3170
410	.0018	.0041	.8208	.7731	.3698	.4579
415	.0065	.0030	.7793	.7516	.3596	.4288
420	.0071	.0119	.7269	.7547	.3446	.4126
425	.0060	.0104	.4329	.4872	.3374	.3289
430	.0058	.0103	.4144	.4473	.3230	.3879
435	.0137	.0124	.4832	.4839	.3135	.3805
440	.0128	.0114	.3911	.4364	.2490	.3273
445	.0082	.0094	.4271	.4710	.2839	.3299
450	.0066	.0094	.3497	.4642	.2294	.2012
455	.0071	.0109	.3840	.4248	.2586	.2829
460	.0030	.0040	.4764	.4631	.2991	.3248
465	.0094	.0109	.4450	.4430	.3900	.3503
470	.0078	.0117	.4681	.4368	.2298	.2075
475	.0051	.0124	.4963	.4640	.2225	.2444
480	.0054	.0115	.4908	.4331	.2193	.2047
485	.0020	.0066	.2699	.3648	.2347	.2090
490	.0052	.0049	.2800	.2644	.0470	.0487
500	.0011	.0045	.1871	.2423	.0946	.0990
505	.0015	.0045	.2050	.2510	.0928	.1046
510	.0014	.0040	.1790	.2535	.0912	.1010
515	.0019	.0040	.1914	.2638	.0940	.1006
520	.0015	.0031	.1929	.2581	.1031	.1165
525	.0010	.0026	.2354	.1890	.1414	.1048
530	.0026	.0045	.2576	.3107	.1739	.2066
535	.0028	.0036	.3280	.3320	.1797	.2090
540	.0028	.0045	.3421	.3313	.1825	.2142
545	.0030	.0035	.3059	.3210	.1271	.1931
550	.0019	.0028	.2786	.3046	.2150	.1884
555	.0028	.0033	.2654	.2821	.1719	.1988
560	.0031	.0033	.2378	.2581	.1545	.1469
565	.0029	.0034	.2300	.2443	.1499	.1659
570	.0031	.0039	.2445	.2640	.1664	.1463
575	.0029	.0033	.2374	.2535	.1561	.1563
580	.0034	.0039	.2390	.2501	.1458	.1763
585	.0029	.0034	.2448	.2431	.1297	.1242
590	.0030	.0040	.3720	.3896	.3783	.4957
595	.0045	.0055	.4833	.4454	.2973	.3665
600	.0058	.0069	.4094	.4254	.2774	.2505
605	.0040	.0049	.4034	.4205	.2612	.2424
610	.0035	.0040	.3709	.3831	.2204	.2427
615	.0030	.0045	.3730	.3655	.1956	.2114
620	.0031	.0043	.3698	.3891	.2220	.2189
625	.0031	.0053	.3814	.3767	.2069	.2394
630	.0041	.0046	.3717	.3817	.2080	.2523
635	.0034	.0041	.3787	.3803	.2206	.2733
640	.0028	.0040	.3530	.3698	.2160	.2153
645	.0034	.0039	.3777	.3631	.2776	.2483
650	.0029	.0044	.4028	.3751	.2716	.2707
655	.0028	.0039	.3692	.3645	.2574	.2595

...

h	Sterile		C:N=45		C:N=55	
660	.0030	.0035	.3819	.4028	.2608	.2799
665	.0033	.0039	.4028	.4034	.2273	.2726
670	.0142	.0149	.0861	.0765	.0363	.0541
675	.0140	.0146	.0966	.0951	.0437	.0546
680	.0130	.0140	.0694	.0730	.0323	.0582
685	.0139	.0140	.0718	.0717	.0356	.0562
690	.0109	.0120	.0698	.0639	.0333	.0486
695	.0115	.0124	.0595	.0626	.0411	.0428
700	.0066	.0112	.0542	.0569	.0400	.0471
705	.0102	.0124	.1658	.1523	.1012	.1006
710	.0098	.0140	.2341	.2257	.1427	.1351
715	.0090	.0120	.3063	.3066	.1477	.1638
720	.0082	.0124	.2783	.2909	.1581	.1367
725	.0056	.0114	.2181	.2289	.1256	.1194
730	.0117	.0124	.2505	.2650	.1466	.1456
735	.0060	.0120	.3510	.3189	.1780	.2031
740	.0082	.0114	.3998	.3143	.1799	.2119
745	.0094	.0104	.4000	.2554	.1853	.1928
750	.0059	.0108	.4693	.3693	.2138	.2017
755	.0056	.0098	.3120	.2987	.1903	.2215
760	.0039	.0074	.3377	.2936	.1653	.2045
765	.0077	.0108	.5000	.5291	.2334	.2642
770	.0071	.0102	.6657	.6562	.4425	.4729
775	.0085	.0097	.6342	.6176	.4481	.4507
780	.0078	.0073	.6357	.6092	.3952	.4451
785	.0080	.0097	.9125	.9277	.5469	.5592
790	.0090	.0110	.9790	.9637	.4482	.4394
795	.0085	.0092	.8008	.8750	.4399	.4390
800	.0044	.0074	.9122	.9197	.4529	.4731
805	.0051	.0069	1.063	1.045	.3733	.4082
810	.0041	.0045	1.117	.9889	.4689	.5871
820	.0035	.0040	.3072	.3333	.2287	.2600
825	.0036	.0039	.3177	.2990	.2039	.2276
830	.0033	.0035	.2505	.2490	.1777	.1875
835	.0031	.0036	.2454	.2477	.1572	.1730
840	.0031	.0035	.2062	.1878	.1246	.1295
845	.0031	.0033	.2392	.2576	.1468	.1467

[1] Samples (1 mL) were injected automatically into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit.
Gas flow rate = 30 mL min^{-1}. The temperature of all mixes was initially 20° & increased at 5° per d to 55° then modified to 50° at 19d, 55° at 24d, 60° at 27d and 55° at 29d. Composts were mixed for 15 min every h at 15 rpm. See Appendix 1 for composition of compost mixtures. See Figure 11.

Carbon Dioxide Output During R6, Fish-bark Initial C:N=45 [1]

h	10		20		30	
20	.0938	.1094	.0893	.0740	.0024	.0023
25	.2866	.2784	.2074	.1948	.0029	.0030
30	.4329	.3645	.2975	.2794	.0058	.0065
35	.8947	.7493	.6430	.6740	.0313	.0034
40	1.494	1.584	.8910	.9019	.0642	.0598
45	1.846	2.048	1.264	1.109	.0727	.0808
50	2.494	2.409	1.438	1.389	.4832	.4440
55	2.030	2.113	1.163	1.202	1.007	1.057
60	2.144	2.235	1.211	1.133	.9553	.9338
65	2.035	2.109	1.246	1.158	.3378	.4100
70	2.088	2.024	1.327	1.294	.1869	.1919
75	1.915	1.004	1.393	1.303	.1907	.2534
80	1.862	1.987	1.348	1.424	.3186	.3261
85	1.963	2.008	1.442	1.499	.5218	.5419
90	2.265	2.198	1.509	1.639	.4428	.3926
95	2.340	2.386	1.790	1.746	.3550	.3600
100	2.422	2.343	1.837	1.849	.2609	.2634
105	2.121	2.124	1.953	2.011	.2800	.2644
110	2.034	2.231	1.815	1.783	.2985	.2609
115	2.330	2.402	1.491	1.510	.1367	.1183
120	2.504	2.478	1.470	1.458	.2772	.2622
125	2.387	2.466	1.438	1.412	.3274	.3462
130	2.124	2.233	1.348	1.377	1.109	1.052
135	2.100	2.121	1.144	1.209	1.026	1.017
140	2.093	1.983	1.404	1.174	1.031	1.006
145	1.989	2.090	1.564	1.498	1.144	1.159
150	2.043	2.112	1.466	1.660	1.377	1.303
155	2.240	2.199	1.397	1.403	1.095	1.232
160	2.326	2.323	1.355	1.284	.9433	1.029
165	2.399	2.443	1.273	1.244	.8454	.8693
170	2.487	2.404	1.233	1.118	.7551	.7940
175	2.502	2.553	1.141	1.099	.7589	.8128
180	2.349	2.421	1.156	1.110	.7601	.7664
185	2.460	2.491	1.178	1.241	.7626	.7890
190	2.561	2.645	1.230	1.179	.7476	.7614
195	2.434	2.476	1.098	1.114	.5105	.5293
200	2.215	2.330	.6624	.8039	.4453	.4792
105	2.354	2.129	.6510	.7635	.1443	.1907
210	2.224	2.321	.6043	.6302	.1167	.1242
215	2.322	2.410	.5893	.5908	.1204	.1179
220	2.109	2.238	.5241	.5103	.1242	.1279
225	2.098	2.124	.5326	.5002	.1217	.1154
230	1.956	2.208	.5087	.5114	.1154	.1217
235	2.030	2.121	.6248	.6340	.1217	.1141
240	2.124	2.201	.6615	.6793	.1116	.1029
245	2.033	2.022	.6697	.6329	.0934	.0924
250	1.894	2.001	.6364	.5789	.0765	.0890
255	1.904	1.956	.5991	.5330	.0729	.0817
260	1.876	1.882	.4526	.4810	.0861	.0765
265	1.873	1.823	.5033	.4966	.0966	.0951
270	1.798	1.774	.5087	.5108	.0694	.0730
275	1.691	1.890	.5015	.5048	.0718	.0717
280	1.743	1.743	.4977	.4799	.0698	.0639
285	1.770	1.774	.4542	.4483	.0595	.0626
290	1.839	1.844	.4218	.4390	.0542	.0569
295	1.744	1.823	.4212	.4110	.1658	.1523
300	1.695	1.848	.4093	.5239	.2341	.2257
305	1.732	1.774	.5185	.5124	.3063	.3066
310	1.567	1.589	.3771	.4093	.2783	.2909
315	1.598	1.610	.3650	.3510	.2181	.2289
320	1.604	1.633	.3512	.3347	.2505	.2650
325	1.657	1.599	.3022	.3245	.3510	.3189
330	1.898	1.599	.2790	.2900	.3998	.3143
335	1.904	1.821	.2352	.2416	.4000	.2554
340	1.890	1.843	.2576	.2311	.4693	.3693
345	1.798	1.908	.2417	.2250	.3120	.2987
350	1.910	1.920	.2015	.1739	.3377	.2936
355	1.980	1.930	.1193	.1430	.5000	.5291
360	2.002	2.110	.1112	.0899	.6657	.6562
365	2.210	2.223	.0994	.1440	.6342	.6176
370	2.014	2.101	.2399	.5028	.6357	.6092
375	2.155	1.189	.5877	.6238	.9125	.9277

h	10		20		30	
385	2.130	2.067	.8909	.7900	.8008	.8750
390	2.048	2.120	.9077	.9987	.9122	.9197
395	1.985	2.304	1.444	1.388	1.063	1.045
400	1.967	2.231	1.510	1.491	1.117	.9889
405	1.947	2.121	1.208	1.321	.7670	.7389
410	1.920	1.994	1.055	1.139	.8208	.7731
415	2.012	1.937	.9987	1.000	.7793	.7516
420	1.969	2.000	.8767	.9302	.7269	.7547
425	1.989	2.090	.3877	.5688	.4329	.4872
430	1.880	1.935	.4789	.5201	.4144	.4473
435	1.844	1.821	.5088	.5504	.4832	.4839
440	1.789	1.774	.4809	.4877	.3911	.4364
445	1.833	1.750	.4767	.4909	.4271	.4710
450	1.785	1.900	.4590	.5091	.3497	.4642
455	1.732	1.821	.4909	.5210	.3840	.4248
460	1.685	1.751	.5110	.4901	.4764	.4631
465	1.643	1.732	.4886	.4901	.4450	.4430
470	1.677	1.689	.4789	.4782	.4310	.4221
475	1.633	1.650	.5104	.5000	.3919	.4012
480	1.584	1.564	.5034	.5012	.3877	.3987
485	1.599	1.640	.4330	.4629	.3545	.3648
490	1.575	1.544	.3299	.4012	.2800	.2014
500	1.532	1.439	.3100	.3727	.1871	.1788
505	1.438	1.377	.3745	.3284	.2050	.1893
510	1.399	1.327	.3379	.3218	.1790	.1974
515	1.343	1.210	.3109	.3013	.1914	.2078
520	1.278	1.035	.2901	.2838	.1929	.2217
525	1.181	.9340	.3018	.2908	.2354	.1890
530	.9845	.8994	.3478	.3174	.2576	.3107
535	.9034	.8557	.3900	.3794	.3280	.3320
540	.8434	.8089	.3762	.3641	.3421	.3313
545	.7984	.7444	.3319	.3299	.3059	.3210
550	.7321	.6335	.3787	.3936	.2786	.3046
555	.6439	.5910	.3476	.3287	.2654	.2821
560	.5897	.5688	.3319	.3128	.2378	.2581
565	.5211	.5548	.2965	.3019	.2300	.2443
570	.5891	.5474	.3078	.2874	.2445	.2640
575	.6948	.6312	.2899	.2798	.2374	.2535
580	.6894	.7867	.2788	.2639	.2390	.2501
585	.7209	.7444	.2577	.2310	.2448	.2431
590	.7895	.7432	.2909	.2740	.2284	.3089
595	.8098	.7937	.2875	.2612	.2433	.2238
600	.7648	.7809	.2666	.2350	.2199	.2019
605	.7744	.8055	.2349	.2173	.2040	.2237
610	.8124	.8239	.2119	.2089	.2138	.2389
615	.8330	.8644	.2338	.2238	.2541	.2554
620	.8841	.9081	.2373	.2188	.2108	.1988
625	.9122	.9433	.2018	.2129	.1908	.1830
630	.9310	.9804	.1928	.1988	.1789	.1610
635	.9797	.9844	.1829	.1900	.1389	.1438
640	.9803	.9644	.1635	.1789	.1409	.1397
645	1.054	1.085	.1348	.1365	.1355	.1365
650	1.099	1.129	.1362	.1210	.1231	.1193
655	1.155	1.179	.1237	.1124	.0939	.1098
660	1.095	1.132	.1038	.1094	.0819	.0994
665	1.101	1.281	.0894	.0938	.0790	.0983
670	1.178	1.190	.0939	.0994	.0861	.0765
675	1.224	1.204	.0989	.0876	.0966	.0951
680	1.341	1.260	.1188	.1074	.0694	.0730
685	1.458	1.289	.0722	.0889	.0718	.0717
690	1.357	1.325	.0733	.0889	.0698	.0639
695	1.324	1.380	.0753	.0684	.0595	.0626
700	1.249	1.433	.0471	.0684	.0542	.0569
705	1.039	1.231	.0565	.0938	.1658	.1022
710	.9784	.9877	.0697	.1039	.1288	.0994
715	.8664	.9099	.0686	.0898	.0978	.0790
720	.7432	.8761	.0659	.0998	.0683	.0539
725	.6188	.6589	.0867	.0783	.0592	.0489
730	.4400	.5838	.0858	.0994	.0438	.0432
735	.3729	.4574	.0771	.0783	.0227	.0320
740	.3330	.3211	.0554	.0682	.0207	.0231
745	2978	.2650	.0503	.0276	.0112	.0121

[1] Samples (1 mL) were injected automatically into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit. Gas flow rate = 10, 20 or 30 mL min^{-1}. See Appendix 1 for composition of compost mixtures.

Carbon Dioxide Output During R7, Fish-, Urea- and Sewage-bark, Initial C:N=35 [1]

h	Fish-b		Urea-b		Sewage-b		h	Fish-b		Urea-b		Sewage-b	
20	.0398	.0427	.0559	.0601	.0363	.0347	340	.2153	.2576	.4944	.5534	.2069	.1450
25	.2433	.2810	.0813	.0922	.0961	.1174	345	.2665	.2417	.5791	.5640	.1991	.1374
30	.5493	.5230	.2977	.3329	.1224	.1555	350	.2287	.2015	.6768	.5635	.1919	.1327
35	.7002	.6297	.4774	.6343	.1523	.1719	355	.1984	.1193	1.116	.5042	.1867	.1199
40	.9133	.8974	1.190	1.909	.5878	.4847	360	.1433	.1112	1.257	.3750	.1852	.1202
45	1.203	1.136	2.863	2.709	.8111	.6807	365	.1129	.0994	1.432	.4633	.1864	.1262
50	1.398	1.322	2.968	2.778	.7831	.6884	370	.1004	.0927	1.553	.3932	.1762	.1304
55	1.064	1.163	2.697	2.591	.7461	.6610	375	.0932	.0827	1.562	.4782	.1852	.1506
60	1.199	1.211	2.554	2.550	.7540	.6555	380	.0965	.0811	1.384	.5360	.1886	.1345
65	1.254	1.246	2.287	2.405	.3941	.3343	385	.0896	.0697	.8042	.6145	.1980	.1390
70	1.372	1.327	2.003	1.949	.4389	.3700	390	.0753	.0637	.8307	.6937	.2321	.1615
75	1.412	1.393	2.210	2.479	.7468	.6046	395	.0443	.0494	.7575	.6518	.2177	.1606
80	1.377	1.348	2.369	2.322	.4929	.4904	400	.0408	.0386	.7391	.6301	.2096	.1604
85	1.430	1.442	2.325	2.262	.5057	.4459	405	.0391	.0206	.5236	.4396	.1590	.1210
90	1.491	1.509	2.881	2.956	.9987	.8776	410	.0253	.0239	.4878	.5297	.1770	.1340
95	1.774	1.790	2.797	2.887	1.321	1.300	415	.0298	.0248	.4848	.5388	.1855	.1323
100	1.923	1.837	2.433	2.250	1.135	1.157	420	.0334	.0228	.4396	.5261	.1786	.1312
105	1.934	1.953	2.456	2.405	.8684	.8496	425	.0308	.0212	.4494	.5181	.1813	.1385
110	1.831	1.815	2.229	2.142	.8156	.8034	430	.0539	.0255	.4515	.4792	.1859	.1382
115	1.533	1.491	2.079	2.013	.8329	.8039	435	.1093	.0824	.4817	.5431	.1748	.1391
120	1.457	1.470	2.014	1.987	.7563	.7782	440	.1132	.0965	.4633	.5607	.1758	.1263
125	1.422	1.438	1.852	1.787	.6720	.6939	445	.0905	.0882	.4540	.5675	.1730	.1376
130	1.388	1.348	1.616	1.540	.5561	.5715	450	.0863	.0722	.4575	.5834	.1719	.1204
135	1.231	1.144	1.497	1.347	.5054	.4733	455	.0786	.0668	.4386	.6236	.1723	.1238
140	1.330	1.404	1.470	1.270	.5247	.5006	460	.0553	.0485	.4444	.6352	.1723	.1215
145	1.391	1.472	1.549	1.391	.7214	.7657	465	.0432	.0378	.4083	.6278	.1623	.1171
150	1.511	1.466	2.135	2.004	.7657	.8031	470	.0408	.0351	.4159	.6180	.1678	.1147
155	1.400	1.397	2.410	1.543	.7681	.7599	475	.0322	.0270	.3561	.5711	.1512	.1035
160	1.365	1.355	2.505	1.511	.4328	.4768	480	.0174	.0165	.3026	.4666	.1266	.0844
165	1.336	1.273	1.898	1.298	.3805	.4250	485	.0218	.0139	.2478	.3606	.0960	.0609
170	1.221	1.233	1.617	1.511	.4040	.3285	490	.0103	.0094	.2092	.2771	.0722	.0506
175	1.002	1.141	1.241	.9115	.3226	.3778	495	.0091	.0076	.1905	.2481	.0540	.0434
180	1.094	1.156	1.176	.8481	.2863	.3351	500	.0083	.0074	.1602	.2100	.0521	.0452
185	1.132	1.178	1.436	1.324	.2800	.3273	505	.0192	.0098	.1272	.1517	.0357	.0322
190	1.199	1.230	1.590	1.468	.3213	.3370	510	.0144	.0132	.1194	.1501	.0352	.0324
195	1.100	1.098	1.539	1.561	.2991	.3054	515	.0124	.0118	.1378	.1557	.0465	.0390
200	1.009	.6624	1.268	1.245	.2719	.1936	520	.0093	.0027	.2491	.3397	.1093	.1171
205	.8873	.6510	1.196	.9445	.2446	.2484	525	.0053	.0049	.2652	.3392	.1262	.1427
210	.7021	.6043	.9703	.8961	.2287	.2340	530	.0068	.0056	.2153	.3144	.1089	.0883
215	.6343	.5893	.8078	.6367	.2550	.2188	535	.0054	.0069	.1983	.2728	.0933	.0856
220	.5211	.5241	.7626	.5746	.2561	.2187	540	.0031	.0049	.1671	.2291	.0808	.0740
225	.5038	.5326	.6387	.4865	.2857	.2360	545	.0027	.0025	.1491	.2039	.0769	.0853
230	.4896	.5087	.6135	.4227	.2227	.1971	550	.0154	.0105	.1491	.1787	.0717	.0823
235	.5889	.6248	.6634	.5100	.2173	.1814	555	.0205	.0118	.1671	.2112	.0919	.1168
240	.6387	.6615	.6975	.5759	.2462	.2130	560	.0194	.0123	.1509	.2122	.1016	.1016
245	.6449	.6697	.6829	.5796	.2498	.2075	565	.0187	.0139	.1663	.2334	.1186	.1363
250	.6872	.6364	.6652	.5574	.2428	.1974	570	.0153	.0145	.2087	.2092	.1360	.1839
255	.6223	.5991	.6937	.4668	.2368	.1712	575	.0155	.0139	.2101	.1990	.1443	.1776
260	.5301	.4526	.5706	.4368	.1813	.1507	580	.0142	.0139	.1772	.1983	.1377	.1738
265	.5223	.5033	.6281	.5645	.1977	.1664	585	.0144	.0141	.1803	.1899	.1322	.1669
270	.4996	.5087	.6379	.5844	.2029	.1798	590	.0141	.0133	.1773	.1903	.1201	.1665
275	.4732	.5015	.6306	.5890	.2036	.1617	595	.0139	.0138	.1802	.1873	.1044	.1430
280	.4223	.4977	.6220	.5771	.2025	.1720	600	.0133	.0133	.1799	.1882	.1000	.1340
285	.4103	.4542	.6465	.5877	.2198	.1892	605	.0142	.0127	.1783	.1830	.0993	.1223
290	.3998	.4218	.6422	.5998	.2213	.2018	610	.0144	.0132	.1562	.1747	.0944	.1203
295	.4091	.4212	.5898	.5987	.2198	.1957	615	.0135	.0133	.1776	.1733	.0921	.1221
300	.3936	.4093	.5130	.5935	.2179	.1874	620	.0133	.0134	.1734	.1704	.0884	.0994
305	.4773	.5185	.5832	.5569	.2151	.1711	625	.0132	.0132	.1732	.1772	.0882	.0931
310	.4423	.3771	.5524	.5900	.2213	.1615	630	.0129	.0129	.1448	.1559	.0832	.0859
315	.4022	.3650	.5355	.5776	.1820	.1459	635	.0136	.0125	.1586	.1534	.0778	.0912
320	.3884	.3512	.4656	.5466	.2068	.1468	640	.0133	.0122	.1562	.1432	.0733	.0883
330	.3008	.2790	.4371	.4557	.1814	.1465	645	.0128	.0125	.1490	.1500	.0772	.0883
335	.2543	.2352	.4734	.5181	.2005	.1453	325	.3201	.3022	.4305	.4964	.1982	.1730
							650	.0122	.0126	.1445	.1463	.0667	.0800

[1] Samples (1 mL) were injected automatically into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit. Gas flow rate = 20 mL min^{-1}. The temperature of all mixes was initially 20° and increased at 5° per d to 55°. Composts were mixed for 15 min every h at 15 rpm. See Appendix 1 for composition of compost mixtures. See Figure 15.

Carbon Dioxide Output During R8, IBDU- and Urea-bark

Initial C:N=35 ¹

h	Urea-b		Urea+Q-b		IBDU-b	
20	.0635	.0644	.0197	.0145	.0078	.0074
25	.0965	.0934	.0108	.0042	.0110	.0041
30	.4182	.3981	.0222	.0056	.0080	.0048
35	.5382	.5047	.0208	.0183	.0035	.0117
40	1.184	1.162	.1697	.1378	.0193	.0104
45	1.839	1.920	.2488	.1933	.0234	.0163
50	2.943	3.091	.0745	.0483	.0076	.0357
55	2.576	2.644	.0842	.0743	.0169	.0507
60	2.355	2.422	.0962	.0948	.0098	.0517
65	2.329	2.385	.1037	.1206	.0115	.0495
70	1.577	2.218	.1567	.1601	.0166	.0496
75	2.973	3.077	.1773	.1938	.0140	.0436
80	3.020	3.211	.1987	.2488	.0220	.0423
85	2.985	3.248	.2890	.2929	.0650	.0827
90	3.045	2.802	.2933	.2633	.1065	.1230
95	3.151	2.903	.5863	.8322	.7188	.6596
100	2.683	2.613	.9877	1.062	.6536	.6645
105	2.227	2.166	1.998	2.098	.3703	.5344
110	1.716	2.474	2.183	2.221	.3120	.3299
115	2.034	1.833	2.384	2.518	.3328	.3988
120	2.046	1.847	2.606	2.885	.4088	.4389
125	1.933	1.783	2.483	2.708	.3895	.4234
130	1.797	1.703	2.861	2.940	.6697	.5889
135	1.684	1.502	3.158	2.764	.8213	.8999
140	1.900	1.962	2.963	2.967	.8633	.9034
145	1.959	2.074	3.046	2.745	.8652	.9634
150	2.189	2.413	3.123	2.943	.8925	.8634
155	2.699	2.192	2.741	2.725	.8478	.8036
160	2.167	2.101	2.775	2.507	.8282	.8674
165	1.744	1.540	2.626	2.467	.7431	.8001
170	1.627	1.440	2.522	2.158	2.112	1.938
175	1.042	1.175	2.262	2.039	2.273	2.440
180	1.033	1.175	1.113	2.416	2.476	2.549
185	1.393	1.287	2.219	2.166	2.682	2.986
190	1.673	1.497	2.057	2.139	2.810	3.092
195	1.642	1.592	1.902	1.929	2.792	2.644
200	1.080	1.186	.5074	.8466	2.874	2.783
205	1.001	1.096	.4165	.5294	2.924	2.895
210	.8044	.7462	.4090	.8467	2.758	2.567
215	.7182	.6317	.3284	.7221	2.420	2.198
220	.6668	.5963	.2624	.6641	2.095	1.897
225	.7834	.6032	.3086	.3198	.9374	.7652
230	.6696	.7831	.3173	.4664	.7901	.5439
235	.5679	.5401	.2553	.2867	.6208	.4490
240	.5358	.6373	.1921	.1640	.4534	.8837
245	.5724	.6432	.1661	.2500	.5822	.9907
250	.5979	.6697	.1462	.2528	.4353	1.744
255	.8455	.9395	.1334	.4903	1.872	2.077
260	.8194	.7712	.1614	.2690	1.606	2.245
265	.7172	.6712	.1583	.2388	2.335	1.983
270	.6564	.6532	.4164	.5294	2.631	2.584
275	.7044	.7723	.7365	.8467	2.758	2.400
280	.7182	.7312	.8478	.7221	2.420	2.239
285	.6684	.7113	.6848	.6641	2.095	1.183
290	.7089	.6296	.3086	.3198	.9374	.6649
295	.6927	.5488	.3173	.4664	.7901	.5598
300	.5679	.5412	.2551	.2445	.6208	.4893
305	.5355	.5372	.1921	.2247	.4534	.5043
310	.4997	.5321	.1998	.2010	.4889	.4981
315	.5097	.6032	.2024	.1974	.5487	.5299
320	.3997	.4267	.0975	.1661	.5822	.5910
325	.4239	.4966	.1462	.1737	.4247	1.447
330	.4558	.3957	.9983	1.420	1.872	1.976
325	.5119	.7743	1.544	1.985	2.664	2.572
330	.7172	.6794	2.324	2.549	2.549	2.667
335	.6786	.6954	1.984	2.172	2.391	2.103
340	.8861	.8654	1.500	1.698	1.838	1.745
345	.9732	.8999	1.104	1.169	.8577	.9533
350	1.095	1.159	.8373	.8626	.7424	.8013
355	1.696	1.470	.7839	.6929	.6307	.9949
360	1.158	1.781	1.339	1.698	1.841	1.848
365	1.091	1.144	1.938	1.897	1.617	1.535
370	1.086	1.149	1.646	1.816	1.623	1.490
375	.9079	.9155	.8773	.7128	.8194	.7784
380	.8655	.9568	.6643	.5587	.7749	.6676
385	.5468	.8955	.5438	.4750	.5080	.5211
390	.5756	.6874	.4339	.4674	.5312	.5335
395	.6125	.6533	.4873	.4456	.5222	.5225
400	.5862	.5708	.4483	.4196	.5184	.5008
405	.6365	.4854	.5464	.4623	.5665	.4459
410	.6092	.5178	.4983	.4327	.5308	.4433
415	.5799	.4337	.3875	.3978	.4938	.4560
420	.5322	.4456	.4340	.4093	.4555	.4435
425	.5709	.4963	.4022	.3988	.4428	.4505
430	.5233	.4614	.4807	.3419	.4383	.4486
435	.4744	.3259	.3398	.2940	.3937	.4225
440	.6012	.7083	.2154	.3964	.4698	.4310
445	.5867	.5591	.2735	.3516	.4138	.4249
450	.6007	.5801	.2750	.3407	.4089	.4224
455	.6696	.6004	.2510	.3632	.4506	.4489
460	.7007	.5322	.2803	.3821	.4573	.4233
465	.5598	.5683	.3097	.3544	.4107	.4059
470	.4919	.5882	.2988	.3123	.3719	.3895
475	.4882	.4985	.2919	.2991	.3718	.3700
480	.4849	.4703	.3350	.2949	.3660	.3496
485	.4704	.3754	.2864	.2884	.3617	.3501
490	.4338	.4496	.2736	.2721	.3549	.3346
495	.4288	.3554	.2766	.2656	.3276	.3443
500	.4396	.3979	.2816	.2842	.3519	.3355
505	.3321	.3086	.2827	.2792	.3296	.3560
510	.3932	.2942	.2771	.2859	.3354	.3225
515	.2096	.2023	.2662	.2437	.2954	.3010
520	.2092	.1952	.2532	.2294	.2978	.2900
525	.2014	.2117	.2540	.2258	.3094	.2448
530	.1952	.2474	.2439	.2729	.3533	.3224
535	.1884	.2041	.2650	.2681	.3296	.3113
540	.1394	.2009	.2463	.2293	.3018	.2992
545	.1373	.2021	.2374	.2283	.3083	.3113
550	.1405	.1745	.2298	.2174	.3316	.3199
555	.1354	.1967	.2149	.2278	.2844	.2997
560	.1334	.1808	.2146	.2187	.2754	.2887
565	.1314	.1769	.2087	.2093	.2555	.2998
570	.1381	.1198	.2344	.2340	.2887	.2855
575	.1433	.1543	.2240	.2239	.2766	.2688
580	.1643	.1321	.2019	.2480	.2598	.2560
585	.1295	.1406	.1882	.2236	.2473	.2533
590	.1314	.1219	.1746	.2174	.3004	.2798
595	.1875	.1621	.1609	.2099	.2969	.2665
600	.1809	.2056	.1858	.1847	.3510	.2877
605	.1807	.2335	.1773	.1856	.2971	.2670
610	.1986	.2333	.1896	.1993	.2704	.2444
615	.1792	.2483	.1755	.1852	.2481	.2554
620	.1757	.2774	.2046	.1870	.2441	.2400
625	.1208	.2143	.1968	.1870	.2419	.2344
630	.1909	.2132	.1950	.1845	.2336	.2409
635	.1418	.2176	.1747	.1773	.2661	.2415
640	.1609	.2165	.1636	.1775	.2567	.2332

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h	Urea-b		Urea+Q-b		IBDU-b	
645	.1907	.1854	.1051	.1800	.2399	.2450
650	.1611	.1648	.2077	.1978	.2866	.2444
655	.1644	.1543	.2012	.1926	.2719	.2510
660	.2091	.1632	.1989	.1836	.2571	.2333
665	.2591	.1757	.2000	.1510	.2036	.2348
670	.2398	.1759	.2371	.2502	.2308	.2230
675	.2336	.1763	.2212	.2258	.2436	.2338
680	.2724	.1903	.1998	.2246	.2970	.2890
685	.2093	.1914	.1989	.2197	.2710	.2898
690	.2381	.1762	.2045	.1636	.2693	.2755
695	.2433	.1783	.2400	.2695	.2421	.2385
700	.2341	.1775	.2458	.2450	.2120	.2388
705	.2153	.1514	.2690	.2754	.2465	.2119
710	.1901	.1265	.2646	.2698	.1755	.2668
715	.2118	.1336	.2434	.2590	.2146	.2012
720	.2301	.1524	.2877	.2710	.2003	.2276
725	.2062	.1566	.2789	.2654	.2517	.2187
730	.2102	.1592	.2885	.2621	.2376	.2485
735	.2083	.1593	.2755	.2671	.2439	.2583
740	.2238	.1653	.2337	.2755	.2572	.2342
745	.2184	.1666	.2656	.2461	.2555	.2279
750	.2537	.1648	.2483	.2305	.2415	.2534
755	.1988	.1473	.2600	.2450	.2444	.2337
760	.2077	.1563	.4988	.2436	.2665	.2444
765	.2005	.1382	.9887	.5544	.4440	.3977
770	.1986	.1338	1.004	.9983	.8864	.5995
775	.1893	.1327	2.284	1.555	1.117	.6755
780	.1919	.1227	2.599	1.900	1.890	1.221
785	.1873	.1206	1.900	2.433	2.009	1.801
790	.1967	.2416	1.399	2.172	2.391	2.211
795	.1847	.2185	1.125	1.698	1.838	1.779
800	.1908	.1538	.8847	1.169	.8577	.5584
805	.1736	.1593	.9846	.8626	.7424	.4398
810	.1693	.1037	1.139	.6929	.6307	.9986
815	.1917	.1635	1.444	1.698	1.841	1.434
820	.1906	.1444	1.548	1.466	1.617	1.229
825	.1854	.1493	1.009	1.816	1.623	1.113
830	.1709	.1553	.8777	.7128	.8194	.9909
835	.1645	.1956	.6635	.5587	.7749	.8328
840	.1543	.2327	.5595	.4750	.5080	.5537
845	.2088	.2341	.7078	.4674	.5312	.4904
850	.1613	.2465	.5544	.4456	.5222	.5099

[1] Samples (1 mL) were injected automatically into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit. Gas flow rate = 20 mL min^{-1}. The temperature of all mixes was initially 20° and increased at 5° per d to 55°. Composts were mixed for 15 min every h at 15 rpm. See Appendix 1 for composition of compost mixtures. IBDU - isobutylidene diurea. Q - p-benzoquinone. See Figure 17.

Carbon Dioxide Output During R9, Fish-, Urea- and Sewage-bark, Initial C:N=25 [1]

h	Fish-b		Urea-b		Sewage-b	
20	.0478	.0512	.0632	.0679	.0690	.0660
25	.6638	.5973	.0918	.1042	.1826	.2230
30	1.116	1.072	.3365	.3762	.2325	.2954
35	2.163	2.398	.5395	.7167	.2894	.3265
40	2.797	2.525	1.344	2.157	1.117	1.051
45	3.067	3.198	3.235	3.061	1.541	1.755
50	2.524	2.677	3.354	3.139	2.071	2.136
55	1.572	1.848	3.048	2.927	1.870	1.882
60	1.439	1.453	2.886	2.881	1.857	1.815
65	1.504	1.496	2.584	2.718	1.592	1.520
70	1.647	1.593	2.263	2.202	1.406	1.465
75	1.695	1.672	2.497	2.801	1.698	1.577
80	1.652	1.617	2.676	2.624	1.713	1.750
85	1.716	1.730	2.628	2.556	1.895	1.855
90	1.789	1.811	2.373	2.475	2.146	1.963
95	2.129	2.147	2.638	2.630	2.510	2.470
100	2.308	2.204	2.750	2.542	2.156	2.198
105	2.320	2.344	2.775	2.718	1.650	1.614
110	2.197	2.178	2.519	2.421	1.550	1.527
115	1.839	1.790	2.349	2.274	1.582	1.527
120	1.749	1.764	2.276	2.245	1.437	1.479
125	1.707	1.725	2.093	2.020	1.277	1.318
130	1.665	1.617	2.383	2.261	1.057	1.086
135	1.477	1.373	2.016	2.143	.9603	.8993
140	1.596	1.685	2.109	1.948	.9969	.9511
145	1.669	1.766	2.117	2.258	1.435	1.455
150	1.814	1.759	2.412	2.265	1.455	1.526
155	1.680	1.676	2.723	1.743	1.459	1.444
160	1.638	1.626	2.830	1.707	.8223	.9059
165	1.604	1.527	2.145	1.467	.7230	.8074
170	1.465	1.480	1.827	1.708	.7676	.6242
175	1.203	1.369	1.402	1.030	.6129	.7179
180	1.313	1.388	1.329	.9584	.5439	.6367
185	1.359	1.413	1.623	1.496	.5320	.6219
190	1.439	1.476	1.796	1.659	.6105	.6403
195	1.320	1.318	1.739	1.764	.5683	.5802
200	1.211	.7948	1.433	1.406	.5166	.3679
205	1.065	.7812	1.351	1.067	.4648	.4720
210	.8425	.7251	1.096	1.013	.4345	.4446
215	.7612	.7072	.9128	.7194	.4845	.4158
220	.6253	.6289	.8617	.6493	.4865	.4155
225	.6046	.6391	.7217	.5498	.5427	.4485
230	.5875	.6104	.6932	.4776	.4232	.3744
235	.7067	.7498	.7497	.5763	.4128	.3447
240	.7664	.7938	.7882	.6507	.4678	.4048
245	.7739	.8037	.7716	.6550	.4746	.3943
250	.8246	.7637	.7517	.6299	.4613	.3750
255	.7468	.7190	.7839	.5275	.4500	.3253
260	.6361	.5431	.6447	.4936	.3444	.2864
265	.6268	.6040	.7097	.6379	.3756	.3161
270	.5995	.6104	.7209	.6604	.3854	.3417
275	.5678	.6018	.7126	.6655	.3869	.3072
280	.5068	.5973	.7029	.6521	.3848	.3268
285	.4924	.5450	.7306	.6641	.4175	.3595
290	.4798	.5061	.7257	.6778	.4205	.3833
295	.4909	.5054	.6664	.6765	.4177	.3718
300	.4723	.4911	.5797	.6707	.4140	.3560
305	.5728	.6222	.6590	.6293	.4086	.3251
310	.5308	.4525	.6242	.6667	.4205	.3069
315	.4826	.4380	.6051	.6527	.3459	.2772
320	.4661	.4214	.5261	.6176	.3929	.2790
325	.5762	.5440	.4865	.5609	.3765	.3286
330	.5414	.5022	.4939	.5150	.3447	.2784
335	.4577	.4234	.5349	.5854	.3810	.2760
340	.3875	.4636	.5586	.6253	.3932	.2756
345	.4797	.4351	.6544	.6373	.3783	.2611
350	.4117	.3627	.7648	.6367	.3646	.2522
355	.3571	.2147	1.261	.5697	.3548	.2278
360	.2579	.2002	1.421	.4237	.3518	.2284
365	.2032	.1789	1.618	.5235	.3542	.2397
370	.1807	.1669	1.755	.4443	.3349	.2477
375	.1678	.1488	1.765	.5404	.3518	.2861
380	.1737	.1460	1.564	.6057	.3584	.2555
385	.1613	.1255	.9088	.6943	.3762	.2641
390	.1355	.1146	.9387	.7839	.4410	.3069
395	.0797	.0889	.8560	.7366	.4137	.3051
400	.0734	.0696	.8352	.7120	.3982	.3048
405	.0704	.0370	.5917	.4967	.3022	.2299
410	.0455	.0430	.5512	.5985	.3364	.2546
415	.0536	.0446	.5478	.6088	.3524	.2513
420	.0601	.0410	.4967	.5945	.3393	.2492
425	.0554	.0382	.5079	.5854	.3444	.2632
430	.0970	.0458	.5101	.5415	.3533	.2626
435	.1967	.1484	.5444	.6137	.3322	.2644

[1] Samples (1 mL) were injected automatically into a dual column GC as described in Materials and Methods. Values of CO_2 production are given as mg CO_2 g^{-1} compost h^{-1} from each compost unit.
Gas flow rate = 20 mL min^{-1}. The temperature of all mixes was initially 20° and increased at 5° per d to 55°. Composts were mixed for 15 min every h at 15 rpm See Appendix 1 for composition of compost mixtures. See Figure 16

APPENDIX - 4

MOISTURE, pH, NITROGEN LEVELS, CMCase ACTIVITY, HUMIFICATION INDICES AND WEIGHT LOSSES DURING COMPOSTING:

R1 & R2.) Fish-bark of Initial C:N 45 and 65, Unamended. [1]

Treatment C:N	45	45	45	65	65	65
% N (Initial)	1.0771	1.0771	1.0771	0.7658	0.7658	0.7658
Initial Weight	179.01	179.01	179.01	171.34	171.34	171.34
Day 2						
R1 m.c.[2]	227.45	189.64	194.67	210.78	216.31	200.47
R2	216.34	189.37	200.01	218.34	187.49	220.72
pH [3]	4.3	4.4	4.4	4.0	4.0	4.1
	4.6	4.4	4.5	4.1	4.0	4.0
Compost NH_4^+-N [4]	0.10	0.09	0.09	0.13	0.15	0.14
	0.10	0.08	0.09	0.13	0.13	0.14
Compost NOx^--N [4]	0.25	0.35	0.30	0.26	0.24	0.28
	0.32	0.28	0.31	0.23	0.30	0.25
CMCase Activity [5]	26.44	29.35	29.66	59.41	58.56	61.86
	26.71	29.55	22.75	58.85	59.47	60.59
P_2O_4 Index [6]	2.78	2.85	3.30	3.46	4.25	3.55
	2.62	2.99	3.20	3.94	3.65	4.75
$E_{440/660}$ [6]	4.01	3.66	3.84	1.84	2.33	2.00
	3.79	3.64	3.56	1.66	2.07	2.00
Day 4						
m.c.	226.17	190.11	203.16	210.47	216.67	213.44
	216.54	184.61	215.32	189.04	195.46	206.58
pH	6.8	6.5	6.5	5.8	6.3	6.0
	6.9	6.6	6.8	5.7	5.6	6.0
Compost NH_4^+-N	0.28	0.42	0.31	0.27	0.22	0.27
	0.40	0.37	0.43	0.30	0.30	0.31
Compost NOx^--N	0.25	0.27	0.27	1.26	1.21	1.27
	0.27	0.28	0.29	1.20	1.29	1.21
CMCase Activity	50.80	57.83	55.90	69.11	64.32	67.57
	54.83	51.22	50.45	69.32	66.21	69.18
P_2O_4 Index	4.86	4.79	5.63	4.09	4.93	4.10
	5.14	5.41	5.47	4.71	4.57	4.40
$E_{440/660}$	3.81	3.94	3.69	3.78	3.56	3.67
	3.59	3.66	4.01	3.42	4.04	4.03
Day 6						
m.c.	227.98	200.42	187.69	209.77	210.47	207.55
	178.65	186.34	200.34	178.36	172.89	195.06
pH	6.5	6.9	7.1	5.9	6.2	6.0
	6.9	6.7	7.1	6.1	5.8	6.2
Compost NH_4^+-N	0.37	0.32	0.38	0.26	0.32	0.27
	0.40	0.37	0.46	0.29	0.30	0.26
Compost NOx^--N	1.98	1.86	2.02	1.02	1.39	1.26
	2.09	2.00	1.93	2.00	1.18	1.26
CMCase Activity	45.22	50.10	48.91	74.31	78.10	70.20
	48.65	46.50	47.72	69.04	69.31	67.66
P_2O_4 Index	4.22	4.99	4.08	6.22	5.91	5.22
	4.78	4.71	4.52	4.28	5.59	5.67
$E_{440/660}$	2.22	2.41	2.99	3.78	3.56	3.00
	2.68	2.49	3.31	3.62	2.74	2.80
Day 8						
m.c.	216.32	200.44	212.51	198.93	204.68	200.33
	200.45	189.30	207.61	200.14	200.33	220.17
pH	7.8	7.6	7.6	6.4	6.2	6.4
	7.8	7.6	7.3	6.4	6.3	6.4
Compost NH_4^+-N	0.32	0.30	0.35	0.29	0.35	0.25
	0.39	0.30	0.30	0.30	0.32	0.35
Compost NOx^--N	2.80	2.76	2.40	2.00	0.99	0.97
	2.41	2.36	2.43	1.92	1.98	1.98
CMCase Activity	57.20	61.57	59.50	55.80	51.57	57.20
	61.57	55.73	58.94	52.55	55.21	59.47
P_2O_4 Index	4.63	4.23	4.59	6.00	5.98	6.50
	4.47	4.37	4.21	6.20	6.62	6.90
E440/E660	3.61	3.84	3.58	3.22	3.17	3.26
	3.59	3.66	3.72	3.08	3.83	3.04
Day 10						
m.c.	204.38	196.78	200.47	196.53	214.36	204.08
	200.48	204.36	217.09	204.06	210.47	230.78
pH	7.5	7.4	7.4	6.0	5.8	6.0
	7.3	7.5	7.5	6.4	6.1	6.3
Compost NH_4^+-N	0.34	0.29	0.37	0.21	0.21	0.26
	0.29	0.32	0.31	0.23	0.21	0.24
Compost NOx^--N	1.72	1.89	1.83	1.04	1.03	1.26
	1.86	1.79	1.79	1.03	1.06	1.05
CMCase Activity	60.30	62.33	59.70	44.50	45.00	43.50
	65.51	62.31	60.98	40.32	39.11	41.40
P_2O_4 Index	3.07	3.33	3.75	5.61	5.12	5.44
	3.63	3.47	2.85	5.19	6.18	5.36
$E_{440/660}$	3.61	3.84	3.58	3.22	3.17	3.26
	3.59	3.66	3.72	3.08	3.83	3.04

Continued....

Treatment C:N	45	45	45	65	65	65
% N (Initial)	1.0771	1.0771	1.0771	0.7658	0.7658	0.7658
Initial Weight	179.01	179.01	179.01	171.34	171.34	171.34
Day 12						
m.c.	187.33	226.47	220.34	179.68	196.07	230.47
	188.47	215.04	230.47	213.89	214.67	231.03
pH	7.3	6.9	7.1	6.8	6.6	6.6
	6.9	7.2	6.9	6.5	6.8	6.6
Compost NH_4^+-N	0.30	0.28	0.31	0.28	0.27	0.27
	0.30	0.30	0.28	0.29	0.28	0.27
Compost NOx^--N	1.62	1.66	1.63	0.89	0.94	0.92
	1.62	1.70	1.71	0.93	0.91	0.92
CMCase Activity	63.60	66.66	65.11	38.91	48.97	40.22
	62.56	62.60	66.41	46.22	40.50	40.51
P_2O_4 Index	5.66	5.98	5.36	4.06	3.55	3.67
	3.44	5.72	5.94	3.74	3.85	3.33
$E_{440/660}$	2.17	2.07	2.69	2.00	1.36	1.78
	1.93	2.53	2.41	1.30	1.44	1.22
Day 14						
m.c.	212.55	213.69	220.04	215.34	206.31	228.47
	177.64	224.67	236.07	216.47	226.85	235.44
pH	7.2	6.8	6.8	6.4	5.7	6.3
	6.6	7.0	6.8	6.0	6.0	6.5
Compost NH_4^+-N	0.26	0.25	0.25	0.19	0.21	0.19
	0.24	0.25	0.25	0.19	0.19	0.18
Compost NOx^--N	1.20	1.22	1.20	0.86	0.89	0.88
	1.18	1.11	1.40	0.88	0.89	0.89
CMCase Activity	63.40	61.53	68.60	41.22	45.40	42.90
	66.66	65.21	65.64	46.81	46.50	44.20
P_2O_4 Index	4.62	4.36	3.99	3.12	3.53	2.89
	4.48	4.54	3.21	3.98	3.37	3.21
$E_{440/660}$	2.60	3.18	2.55	6.12	6.54	7.00
	2.30	3.32	2.55	7.48	7.36	7.50
Day 16						
m.c.	188.44	219.86	224.26	231.47	204.16	215.07
	205.65	223.01	213.48	206.35	214.70	223.45
pH	7.2	6.9	7.0	6.8	6.5	6.4
	7.1	7.1	7.5	6.1	6.2	6.7
Compost NH_4^+-N	0.21	0.20	0.20	0.19	0.19	0.20
	0.20	0.22	0.20	0.21	0.16	0.21
Compost NOx^--N	2.01	1.98	1.99	0.46	0.47	0.48
	1.85	1.90	1.83	0.51	0.49	0.52
CMCase Activity	69.72	79.81	70.33	46.61	56.52	48.97
	72.15	73.14	74.78	51.32	50.22	57.23
P_2O_4 Index	3.99	3.98	3.78	3.61	3.55	3.06
	4.11	3.42	4.12	3.49	3.35	3.54
$E_{440/660}$	3.99	3.98	3.78	3.61	3.55	3.06
	0.71	0.72	1.22	1.09	1.15	1.64
Day 18						
m.c.	200.53	218.46	216.07	223.46	208.96	214.07
	198.45	200.46	216.89	226.30	236.14	233.89
pH	7.1	6.8	7.0	6.9	6.6	6.8
	6.7	6.8	7.2	6.6	6.7	7.0
Compost NH_4^+-N	0.22	0.24	0.23	0.15	0.16	0.16
	0.24	0.23	0.24	0.16	0.15	0.14
Compost NOx^--N	0.83	0.81	0.83	0.42	0.41	0.44
	0.86	0.86	0.82	0.42	0.41	0.42
CMCase Activity	79.32	80.32	77.63	61.22	59.33	60.18
	83.15	70.20	82.36	60.21	59.46	61.36
P_2O_4 Index	3.78	3.46	4.32	3.46	3.55	3.61
	3.42	3.84	3.68	3.64	3.05	3.59
$E_{440/660}$	1.95	2.03	1.97	1.00	1.09	1.05
	1.75	1.57	1.83	1.20	0.91	0.85
Day 20						
m.c.	204.38	230.14	223.78	189.64	201.04	224.78
	178.96	204.15	233.55	230.14	225.37	214.68
pH	6.9	6.4	6.7	6.5	6.1	6.4
	6.5	6.8	6.8	6.5	6.4	6.8
Compost NH_4^+-N	0.14	0.24	0.51	0.19	0.16	0.17
	0.26	0.26	0.26	0.16	0.18	0.18
Compost NOx^--N	0.84	0.83	0.84	0.41	0.42	0.42
	0.85	0.83	0.84	0.41	0.42	0.40
CMCase Activity	65.20	70.00	69.30	56.55	59.44	57.91
	72.77	72.52	69.44	58.54	57.91	58.54
P_2O_4 Index	3.99	4.15	3.22	3.45	3.56	3.00
	3.81	4.55	3.08	3.35	3.34	3.20
$E_{440/660}$	1.07	1.88	1.46	0.76	1.00	0.85
	1.53	3.32	1.44	0.94	1.10	0.45
Day 22						
m.c.	186.37	185.66	223.14	221.77	235.69	230.14
	221.56	236.38	224.67	193.87	207.89	218.32
pH	6.4	6.0	6.7	6.2	5.9	6.6
	6.2	6.9	6.8	6.4	6.4	6.5
Compost NH_4^+-N	0.21	0.20	0.20	0.18	0.20	0.19
	0.14	0.12	0.13	0.14	0.16	0.17
Compost NOx^--N	2.69	2.76	2.74	0.61	0.62	0.61
	2.81	2.77	2.67	0.63	0.63	0.61
CMCase Activity	88.43	89.07	86.73	60.47	61.89	62.05
	86.33	87.42	84.64	59.04	59.44	59.73
P_2O_4 Index	4.18	4.75	4.92	6.01	5.64	5.04
	4.37	4.88	4.99	5.45	5.89	5.64
$E_{440/660}$	1.92	2.04	2.30	1.66	1.46	1.39
	1.28	1.66	2.10	2.14	1.64	1.41

Continued....

Treatment C:N	45	45	45	65	65	65
% N (Initial)	1.0771	1.0771	1.0771	0.7658	0.7658	0.7658
Initial Weight	179.01	179.01	179.01	171.34	171.34	171.34
Day 26						
m.c.	215.66	217.94	216.31	223.16	230.04	227.31
	216.45	216.87	236.54	216.78	223.65	204.72
pH	6.6	6.5	6.7	6.6	6.3	6.5
	6.8	6.9	7.0	6.6	6.7	6.5
Compost NH_4^+-N	0.20	0.19	0.22	0.14	0.13	0.15
	0.19	0.22	0.18	0.16	0.14	0.13
Compost NOx^--N	2.95	2.94	2.94	0.89	0.80	0.86
	2.95	2.94	2.91	0.89	0.89	0.84
CMCase Activity	86.73	85.71	84.74	53.86	52.84	56.47
	85.71	87.79	83.51	51.48	58.94	57.99
P_2O_4 Index	5.00	5.06	5.50	4.66	4.06	4.57
	4.50	4.74	4.70	4.04	3.84	4.33
$E_{440/660}$	1.44	1.68	1.88	1.22	1.36	1.42
	1.75	1.64	2.06	1.41	1.34	1.30
Day 30						
m.c.	210.09	215.31	216.21	217.88	216.19	225.34
	224.09	210.47	223.95	203.04	195.64	207.65
pH	6.2	6.1.	6.4	6.0	5.7	6.3
	6.0	6.5	6.6	6.0	6.0	6.2
Compost NH_4^+-N	0.30	0.28	0.28	0.22	0.22	0.24
	0.26	0.28	0.27	0.25	0.23	0.22
Compost NOx^--N	2.90	2.96	3.00	0.85	0.89	0.84
	2.81	3.00	2.91	0.87	0.85	0.86
CMCase Activity	82.85	84.73	86.48	56.47	58.57	49.58
	87.35	57.44	81.42	49.88	50.36	51.07
P_2O_4 Index	3.62	3.02	3.00	6.37	6.71	6.00
	3.02	3.37	2.42	6.97	6.49	6.20
$E_{440/660}$	1.31	1.23	1.09	2.06	1.64	2.20
	1.76	1.01	1.38	1.84	1.94	2.33

[1] See Appendix 1 for compostition of compost mixes & composting conditions. See Table 4 for the chemical composition of raw materials.

[2] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[3] The pH was determined on a 1:5 suspension in 2N KCl.

[4] Determined by steam distillation on the 2N KCl extracts (mg g^{-1} compost). NO_x-N = NO_2^- + NO_3^-.

[5] Percent reduction in viscosity of NaCMC g^{-1} compost h^{-1}.

[6] Humification was assayed by absorbance in 0.025M $Na_4P_2O_4$ extracts at 550nm and the ratio of absorbance at 440 and 660 nm.

R2.) Percentage Weight Losses of Compost Components.[1]

Component	C:N=45 Rep 1	Rep 2	Rep 3 (SE)	C:N=65 Rep 1	Rep 2	Rep 3 (SE)
Ash	3.17	4.90	4.32 (0.51)	2.72	2.45	2.99 (0.16)
Cellulose	26.71	25.51	26.92 (0.44)	7.00	6.26	6.76 (0.22)
Hemicellulose	31.28	32.89	30.07 (0.82)	18.04	19.29	16.49 (0.81)
Lignin	0.54	0.64	0.34 (0.10)	2.98	3.27	4.55 (0.48)
Lipid	86.66	85.11	84.10 (0.74)	87.37	83.74	86.87 (1.47)
Protein	5.34	10.34	11.90 (1.98)	0.14	0.65	0.52 (0.15)
Soluble Carbohydrate	94.69	94.75	94.13 (0.20)	90.94	86.40	91.77 (1.67)
Total Weight	24.24	24.68	24.33 (0.13)	14.37	14.30	14.18 (0.06)
CO_2-C [2]	13.19	12.52	12.64 (0.21)	6.16	6.28	6.51 (0.10)

[1] Components were determined by proximate analysis (Allen, 1974) and losses shown are percentages of the initial dry weight of each component. Standard errors (SE) are given in parentheses. Detailed compositions of raw materials are given in Table 6.

[2] Results were obtained from bidaily GC analysis of compost gases.

MOISTURE, pH, NITROGEN LEVELS & WEIGHT LOSSES DURING COMPOSTING:

R3.) Fish-bark (C:N 45) Unamended, Fumigated & Calcium amended. [1]

Treatment	Unamended		Fumigated		Ca-amended	
% N (Initial) [2]	1.0771		1.0771		1.0771	
Initial Weight	179.01		179.01		179.01	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Day 4						
m.c. [3]	198.80	195.72	210.39	198.41	209.96	187.41
pH [4]	4.8	4.9	4.2	4.3	4.8	5.0
Compost NH_4^+-N[5]	2.34	3.95	5.68	4.53	1.59	3.21
Compost NOx^--N[5]	10.64	12.18	61.44	56.98	46.58	42.71
Day 8						
m.c.	209.52	207.97	197.37	181.43	200.00	230.00
pH	6.8	6.7	6.0	5.7	6.6	6.8
Compost NH_4^+-N	4.56	6.25	4.47	3.65	9.68	6.84
Compost NOx^--N	14.77	17.94	21.43	20.95	17.33	16.18
Day 12						
m.c.	197.39	200.11	208.45	188.27	220.82	213.75
pH	6.9	6.8	5.9	6.3	6.7	6.7
Compost NH_4^+-N	5.24	4.95	7.24	6.28	7.13	8.60
Compost NOx^--N	25.87	30.05	27.22	25.74	30.12	28.98
Day 16						
m.c.	186.88	199.22	213.72	198.76	217.33	221.07
pH	7.0	6.9	6.4	6.7	6.9	7.2
Compost NH_4^+-N	4.89	6.06	5.21	7.28	6.04	7.69
Compost NOx^--N	29.65	30.73	29.63	27.88	32.02	30.28
Day 20						
m.c.	198.04	206.73	213.64	167.35	210.81	208.89
pH	6.8	7.1	6.5	6.6	6.7	6.9
Compost NH_4^+-N	5.45	8.31	4.45	3.81	9.77	9.49
Compost NOx^--N	36.82	40.00	40.23	38.84	41.52	37.66
Day 24						
m.c.	210.33	220.81	201.77	186.34	219.36	210.64
pH	6.9	6.9	6.7	6.6	6.5	6.6
Compost NH_4^+-N	9.06	9.34	4.33	2.98	9.32	10.07
Compost NOx^--N	20.64	25.68	23.42	20.07	29.27	25.98
Day 28						
m.c.	198.54	210.44	210.46	188.69	206.77	207.25
pH	6.5	6.4	6.6	6.3	6.4	6.5
Compost NH_4^+-N	6.86	6.46	4.47	3.99	7.03	7.12
Compost NOx^--N	19.03	21.27	14.25	15.30	22.00	28.54
% Weight Loss (CO_2-C)[6]	20.56	20.46	10.27	10.64	18.53	18.74

[1] See Appendix 1 for composition of compost mixes & composting conditions.

[2] See Table 4 for chemical composition of raw materials.

[3] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[4] The pH was determined on 1:5 suspension in 2N KCl.

[5] mg g^{-1}, assayed by steam distillation. NOx^--N = NO_2^-+NO_3^-.

[6] Results were obtained from daily GC analysis of compost gases.

MOISTURE, pH, NITROGEN LEVELS AND WEIGHT LOSSES DURING COMPOSTING:

R4.) Fish-bark and Urea-bark, Initial C:N=45. [1]

Treatment	F-B+thiourea		Fish-Bark		Urea-Bark	
% N (Initial)[2]	1.0771		1.0771		1.1289	
Initial Weight	179.01	179.01	179.01	179.01	165.81	165.81
Day 4						
m.c. [3]	221.97	217.61	206.45	217.33	213.14	220.14
pH [4]	5.0	4.8	5.1	5.2	7.3	7.4
Compost NH_4^+-N [5]	2.69	2.67	2.13	3.19	10.08	7.48
Compost NO_2^--N [5]	0.00	0.00	0.00	0.00	4.48	3.74
Compost NO_3^--N [5]	2.29	2.47	2.19	2.20	1.53	3.67
Day 8						
m.c.	225.36	216.77	216.09	209.88	221.03	215.67
pH	6.7	6.3	6.1	6.4	7.9	7.8
Compost NH_4^+-N	2.41	2.94	4.20	3.80	12.54	10.89
Compost NO_2^--N	0.00	0.00	0.00	0.00	1.04	1.42
Compost NO_3^--N	2.81	2.90	2.38	2.47	7.17	6.03
Day 12						
m.c.	216.34	222.86	219.34	215.79	206.99	223.82
pH	6.8	6.5	6.7	6.8	7.1	7.3
Compost NH_4^+-N	4.42	4.64	3.82	4.38	16.55	17.75
Compost NO_2^--N	0.83	0.49	0.36	0.49	13.57	12.29
Compost NO_3^--N	3.26	3.10	3.36	3.54	50.51	55.55
Day 16						
m.c.	219.33	230.05	211.69	223.37	208.73	225.50
pH	6.8	6.7	6.9	6.8	7.0	6.9
Compost NH_4^+-N	1.42	1.64	3.82	4.38	16.55	17.75
Compost NO_2^--N	0.83	0.62	0.36	0.49	13.57	12.29
Compost NO_3^--N	6.82	8.21	7.36	8.78	44.09	46.47
Day 20						
m.c.	206.78	226.91	217.30	217.66	214.32	220.81
pH	6.6	6.3	7.1	7.1	6.8	6.9
Compost NH_4^+-N	1.47	1.51	1.27	1.36	10.55	12.29
Compost NO_2^--N	0.09	0.13	0.22	0.10	0.00	0.00
Compost NO_3^--N	8.53	6.74	6.29	5.24	12.30	15.55
Day 24						
m.c.	198.89	219.37	209.64	214.81	208.09	221.34
pH	6.4	6.3	6.5	6.6	6.7	6.8
Compost NH_4^+-N	0.70	0.60	0.53	0.69	9.47	10.37
Compost NO_2^--N	0.06	0.04	0.14	0.21	0.76	0.86
Compost NO_3^--N	2.63	3.34	2.29	4.78	9.89	11.60
Day 28						
m.c.	206.55	207.35	218.77	201.04	189.79	215.36
pH	6.3	6.4	6.6	6.7	6.4	6.5
Compost NH_4^+-N	0.51	0.63	0.63	0.93	13.47	18.82
Compost NO_2^--N	0.03	0.02	0.00	0.00	0.47	0.34
Compost NO_3^--N	3.21	4.26	2.82	5.85	6.57	4.10
% Weight Loss (CO_2-C)[6]	36.77	35.80	35.19	38.26	28.95	28.47

[1] See Appendix 1 for composition of compost mixes & composting conditions.

[2] See Table 4 for chemical composition of raw materials.

[3] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[4] The pH was determined on 1:5 suspension in 2N KCl.

[5] mg g^{-1} compost, assayed by steam distillation.

[6] Results were obtained from daily GC analysis of compost gases (Appendix 3).

MOISTURE, pH, NITROGEN LEVELS, CMCase ACTIVITY AND WEIGHT LOSSES DURING COMPOSTING

R5.) Fish-bark, Initial C:N=45 or 55. [1]

Treatment (C:N)	Fish-Bark Sterile (45)		Fish-Bark (45)		Fish-Bark (55)	
% N (Initial) [2]	1.0730		1.0730		0.9021	
Initial Weight	163.45	163.45	163.45	163.45	214.05	214.05
Day 2						
m.c. [3]	229.38	230.77	184.73	190.22	205.34	220.33
pH [4]	4.4	4.4	4.3	4.2	4.5	4.4
Day 4						
m.c.	233.10	220.36	200.71	230.82	204.33	179.39
pH	4.5	4.5	4.9	5.0	4.6	4.7
Loss NH_3-N [5]	0.0	0.0	0.6	0.4	0.0	0.0
Loss NOx-N [5]	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N [6]	0.70	0.60	0.39	0.22	0.09	0.07
Compost NOx^--N [6]	0.01	0.00	0.12	0.09	0.03	0.02
CMCase Activity [7]	0.37	0.61	31.05	32.19	83.06	82.85
Lipase Activity [8]	0.00	0.00	0.00	0.00	0.05	0.07
Day 6						
Loss NH_3-N	0.1	0.1	0.1	0.1	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 8						
m.c.	221.56	234.88	219.32	221.08	223.31	201.23
pH	5.0	5.5	6.3	6.5	5.9	6.1
Loss NH_3-N	0.0	0.0	0.0	0.0	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	0.29	0.56	1.41	1.23	0.51	0.96
Compost NOx^--N	0.04	0.00	0.76	0.86	0.57	0.76
CMCase Activity	0.00	0.09	46.15	45.31	56.67	62.16
Lipase Activity	0.00	0.00	0.95	0.90	1.60	1.72
Day 10						
Loss NH_3-N	0.1	0.1	0.2	0.2	0.1	0.1
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 12						
m.c.	230.21	200.90	221.43	225.62	219.77	214.38
pH	5.0	4.7	6.8	6.7	6.2	6.1
Loss NH_3-N	0.1	0.1	0.2	0.2	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	0.70	0.76	2.53	2.48	1.76	1.84
Compost NOx^--N	1.17	1.29	1.01	1.29	0.87	0.94
CMCase Activity	0.00	0.01	64.00	62.36	52.54	50.86
Lipase Activity	0.00	0.00	1.53	1.59	1.80	1.83
Day 14						
Loss NH_3-N	0.0	0.0	0.2	0.2	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 16						
m.c.	225.31	201.08	218.73	219.77	223.27	219.05
pH	5.0	4.9	6.9	7.0	6.4	6.5
Loss NH_3-N	0.0	0.0	0.2	0.2	0.1	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	2.29	2.05	0.89	0.93	0.83	0.90
Compost NOx^--N	8.54	8.79	9.11	9.28	8.68	9.01
CMCase Activity	0.0	0.01	63.24	62.79	40.37	41.56
Lipase Activity	0.0	0.0	4.00	3.91	2.75	2.67
Day 18						
Loss NH_3-N	0.0	0.0	0.2	0.3	0.2	0.2
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 20						
m.c.	208.43	194.98	220.34	217.39	220.32	216.88
pH	5.2	5.3	7.3	7.0	6.8	6.8
Loss NH_3-N	0.1	0.1	0.4	0.6	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	0.70	0.85	1.73	1.69	1.15	1.09
Compost NOx^--N	8.56	9.00	9.20	9.46	6.20	6.37
CMCase Activity	0.0	0.0	73.44	75.09	42.39	44.43
Lipase Activity	0.0	0.0	2.75	2.81	1.00	1.06

Continued....

Treatment (C:N)	Fish-Bark Sterile (45)		Fish-Bark (45)		Fish-Bark (55)	
% N (Initial) [2]	1.0730		1.0730		0.9021	
Initial Weight	163.45	163.45	163.45	163.45	214.05	214.05
Day 22						
Loss NH_3-N	0.1	0.1	0.6	0.6	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 24						
m.c.	199.37	201.23	210.84	215.25	218.94	221.22
pH	4.8	5.0	6.8	6.6	6.3	6.4
Loss NH_3-N	0.0	0.0	0.6	0.7	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	0.45	0.33	0.99	0.98	0.53	0.44
Compost NOx^--N	0.30	0.37	0.58	0.62	0.51	0.49
CMCase Activity	0.00	0.00	72.31	71.09	40.04	39.12
Lipase Activity	0.00	0.00	1.38	1.40	0.90	0.96
Day 26						
Loss NH_3-N	0.0	0.1	0.4	0.6	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Day 28						
m.c.	184.99	196.08	216.33	220.40	203.22	216.21
pH	5.0	5.2	6.7	6.7	6.3	6.2
Loss NH_3-N	0.0	0.0	0.7	0.7	0.0	0.0
Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N	0.44	0.50	1.06	1.11	0.40	0.45
Compost NOx^--N	0.10	0.22	1.49	1.60	1.10	1.06
CMCase Activity	0.00	0.00	73.65	74.08	33.77	29.79
Lipase Activity	0.00	0.00	1.25	1.33	0.94	0.87
Total Loss NH_3-N	0.6	0.8	4.2	4.6	0.4	0.3
Total Loss NOx-N	0.0	0.0	0.0	0.0	0.0	0.0
% Weight Loss (CO_2-C) [8]	0.16	0.19	13.82	13.90	6.00	6.02

[1] See Appendix 1 for compostition of mixes & composting conditions.

[2] See Table 4 for chemical compostition of raw materials.

[3] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[4] Determined on 1:5 compost: 2N KCl suspension.

[5] Nitrogen (mg) collected in 0.1 M H_2SO_4 trap, assayed by steam distilation.

[6] mg g^{-1} compost, assayed by steam distillation.

[7] % reduction in viscosity of NaCMC g^{-1} compost h^{-1}.

[8] Results were obtained from 5 hourly GC analysis of compost gases (Appendix 3).

R6.) Fish-bark, Initial C:N=45, Aerated at 10, 20 or 30 mL min^{-1}. [1]

Treatment (Aeration)	10		20		30	
% N (Initial)	1.0730		1.0730		1.0730	
Initial Weight	168.45	168.45	168.45	168.45	168.45	168.45
% Weight Loss (CO_2-C)	37.44	36.24	13.62	13.98	10.19	10.52

[1] See Appendix 1 for compostition of mixes and composting conditions and Table 4 for chemical composition of raw materials. CO_2-C loss was determined from 5 hourly analysis of the effluent gas (Appendix 3).

MOISTURE, pH, NITROGEN LEVELS, CMCase ACTIVITY AND WEIGHT LOSSES DURING COMPOSTING:

R7.) Fish-bark, Urea-bark and Sewage-bark, Inital C:N=35. [1]

Treatment	Fish-Bark		Urea-Bark		Sewage-Bark	
% N (Initial)	1.3241		1.4000		1.3241	
Initial Weight	121.45		123.01		162.00	
Day 2						
m.c. [2]	223.98	217.31	213.84	218.47	222.52	214.37
pH [3]	4.9	4.8	6.3	6.2	5.2	5.2
Day 4						
m.c.	221.33	220.07	221.65	220.74	219.88	217.37
pH	5.3	5.2	7.3	6.9	5.3	5.4
Loss NH_3-N [4]	1.1	1.4	9.1	10.2	0.0	0.0
Loss NOx-N [4]	0.0	0.0	0.0	0.0	0.0	0.0
Compost NH_4^+-N [5]	0.44	0.52	12.52	11.26	0.21	0.30
Compost NOx^--N [5]	0.60	0.52	2.61	2.26	1.96	1.90
CMCase Activity [6]	9.33	9.94	9.37	10.05	15.79	17.66
Day 6						
Loss NH_3-N	1.1	0.8	4.9	5.9	0.3	0.3
Loss NOx-N	7.8	10.9	8.0	10.8	19.6	18.4
Day 8						
m.c.	219.98	215.31	183.84	216.47	222.52	220.37
pH	6.2	6.3	7.7	7.8	5.6	5.7
Loss NH_3-N	0.8	1.1	12.1	13.3	0.6	0.3
Loss NOx-N	0.1	0.3	1.7	1.3	31.4	36.7
Compost NH_4^+-N	1.83	1.77	16.39	18.01	1.46	1.57
Compost NOx^--N	0.89	0.95	4.53	5.71	6.81	8.04
CMCase Activity	37.61	36.05	32.91	33.41	24.66	22.34
Day 10						
Loss NH_3-N	1.0	1.5	10.5	8.5	1.5	1.3
Loss NOx-N	0.3	0.4	0.7	0.6	10.5	13.7
Day 12						
m.c.	189.98	207.31	210.84	218.47	212.52	223.37
pH	6.9	7.0	6.8	6.8	5.8	5.9
Loss NH_3-N	1.0	0.7	20.0	26.0	4.6	4.0
Loss NOx-N	0.0	0.1	0.8	0.7	4.2	3.2
Compost NH_4^+-N	4.42	5.02	29.22	28.28	4.21	3.68
Compost NOx^--N	2.20	2.15	16.98	20.05	52.32	59.02
CMCase Activity	56.42	57.11	56.51	53.64	28.12	29.09
DAY 14						
Loss NH_3-N	0.8	0.7	25.8	30.7	0.6	0.7
Loss NOx-N	0.1	0.3	1.0	1.1	11.7	10.6
Day 16						
m.c.	217.07	231.37	200.96	186.90	221.46	214.50
pH	5.4	5.3	6.6	6.5	5.6	5.7
Loss NH_3-N	0.3	0.4	7.7	13.7	1.0	0.8
Loss NOx-N	0.4	0.3	4.7	3.2	7.3	6.7
Compost NH_4^+-N	2.46	2.51	28.96	30.18	5.33	4.38
Compost NOx^--N	10.65	9.98	15.32	16.87	35.48	36.33
CMCase Activity	66.85	67.11	78.93	77.69	23.41	22.08
Day 18						
Loss NH_3-N	1.3	0.8	8.4	8.9	0.3	0.4
Loss NOx-N	3.5	4.9	4.5	4.6	1.9	4.0
Day 20						
m.c.	221.07	216.37	181.96	195.90	211.21	222.97
pH	6.4	6.3	6.8	6.9	5.7	5.9
Loss NH_3-N	0.6	0.4	18.2	18.3	0.4	0.6
Loss NOx-N	7.5	9.2	3.6	3.2	3.2	2.9
Compost NH_4^+-N	3.48	4.02	22.23	21.58	10.21	11.08
Compost NOx^--N	8.30	8.89	3.66	4.03	13.22	12.57
CMCase Activity	80.33	81.67	80.32	79.21	36.78	38.07

Continued,

Treatment	Fish-Bark		Urea-Bark		Sewage-Bark	
% N (Initial)	1.3241		1.4000		1.3241	
Initial Weight	121.45		123.01		162.00	
Day 22						
Loss NH_3-N	1.0	0.7	12.0	13.7	0.0	0.0
Loss NOx-N	5.9	5.3	2.1	1.8	3.1	2.4
Day 24						
m.c.	189.88	237.22	241.44	215.15	233.46	244.20
pH	6.8	6.9	7.0	7.1	6.1	6.0
Loss NH_3-N	1.3	1.5	26.4	24.7	0.4	0.3
Loss NOx-N	3.6	2.8	3.6	4.6	3.6	7.5
Compost NH_4^+-N	1.28	1.02	11.33	10.37	5.41	6.01
Compost NOx^--N	0.90	0.89	1.06	1.03	5.82	4.57
CMCase Activity	63.51	62.78	70.33	69.09	15.38	16.84
Day 26						
Loss NH_3-N	0.8	1.1	18.7	27.9	0.4	0.3
Loss NOx-N	3.5	2.8	4.0	4.5	5.2	3.9
Day 28						
m.c.	177.07	216.37	201.96	185.23	221.16	209.84
pH	6.5	6.4	6.8	7.0	6.1	5.8
Loss NH_3-N	0.1	0.5	6.5	3.4	0.0	0.1
Loss NOx-N	1.2	0.7	0.9	1.2	1.2	1.4
Compost NH_4^+-N	1.55	1.67	11.99	11.00	3.11	4.01
Compost NOx^--N	1.10	1.39	1.36	1.59	5.98	5.14
CMCase Activity	52.99	50.44	55.26	52.94	10.44	9.84
Total Loss NH_3-N	11.2	11.6	180.3	205.2	10.1	9.1
Total Loss NOx-N	33.9	38.0	35.6	37.6	105.9	111.4
% Weight Loss (CO_2-C)[7]	15.14	14.93	17.49	16.06	4.43	4.02

[1] See Appendix 1 for composition of compost mixes & composting conditions. See Table 4 for chemical composition of raw materials.

[2] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[3] The pH was determined on 1:5 compost:2N KCl suspension.

[4] mg Nitrogen collected in 0.1 M H_2SO_4 acid traps, assayed by steam distilation

[5] mg g^{-1} compost, assayed by steam distillation.

[6] % reduction in viscosity of NaCMC g^{-1} compost h^{-1}.

[7] CO_2-C loss was determined from 5 hourly analysis of the effluent gas (Appendix 3).

MOISTURE, pH, NITROGEN LEVELS, CMCase ACTIVITY AND WEIGHT LOSSES DURING COMPOSTING:

R8.)Urea-bark, Urea-bark + p-Benzoquinone & IBDU-bark, Inital C:N=35. [1]

Treatment	Urea-Bark		Urea-Bark+Quinone		IBDU-Bark	
% N (Initial)	1.4000		1.4000		1.4287	
Initial Weight	123.01		123.01		109.75	
Day 2						
m.c. [2]	200.34	205.56	198.98	191.71	180.72	193.75
pH [3]	6.3	6.3	6.4	6.3	4.6	4.8
Day 4						
m.c.	192.27	194.22	188.76	181.99	189.32	193.44
pH	6.8	6.7	6.6	6.7	5.2	5.1
Loss NH_3-N [4]	9.7	11.8	0.1	0.3	0.1	0.03
Loss NOx-N [4]	0.0	0.0	2.0	2.4	25.7	19.7
Compost NH_4^+-N [5]	8.65	13.43	4.38	4.43	1.96	1.64
Compost NOx^--N [5]	4.07	3.55	17.51	17.59	19.41	14.92
Compost Urea-N [6]	5	9	90	120	145	153
CMCase Activity [7]	12.04	10.88	4.67	6.35	7.89	5.33
Day 6						
Loss NH_3-N	8.1	8.7	6.3	7.5	0.1	0.3
Loss NOx-N	15.2	13.0	8.0	6.4	22.3	18.2
Day 8						
m.c.	195.23	199.98	195.51	200.48	198.50	195.07
pH	7.9	7.7	6.8	7.0	6.0	5.9
Loss NH_3-N	11.4	17.6	27.7	35.1	0.3	0.8
Loss NOx-N	0.013	0.017	0.00	0.00	0.00	0.00
Compost NH_4^+-N	19.51	17.97	6.05	7.15	3.54	1.57
Compost NOx^--N	4.21	6.05	9.33	11.95	15.22	15.09
Compost Urea-N	1	2	14	9	102	98
CMCase Activity	42.64	38.69	20.26	25.41	26.76	27.22
Day 10						
Loss NH_3-N	13.5	12.1	20.9	11.6	3.2	3.5
Loss NOx-N	0.1	0.7	2.1	1.1	0.0	0.0
Day 12						
m.c.	191.72	200.57	211.73	199.62	223.25	203.41
pH	6.8	6.9	6.4	6.6	7.1	7.0
Loss NH_3-N	27.2	26.3	20.7	17.0	2.7	1.5
Loss NOx-N	0.1	0.7	2.1	1.1	0.0	0.0
Compost NH_4^+-N	26.82	22.19	13.68	15.04	16.21	18.77
Compost NOx^--N	20.47	17.96	6.33	5.01	0.02	0.02
Compost Urea-N	1	1	14	10	22	24
CMCase Activity	52.37	56.07	32.49	37.93	26.44	25.31
Day 14						
Loss NH_3-N	11.0	13.8	6.1	7.3	0.7	0.6
Loss NOx-N	0.1	0.4	0.0	0.0	0.0	0.0
Day 16						
m.c.	189.21	209.45	217.15	212.63	213.46	206.54
pH	6.4	6.5	6.7	6.5	7.6	7.6
Loss NH_3-N	12.1	8.9	7.3	7.5	46.6	43.6
Loss NOx-N	2.6	2.3	1.0	0.2	4.6	3.5
Compost NH_4^+-N	23.65	26.71	24.62	26.46	20.58	18.97
Compost NOx^--N	0.23	0.20	0.33	0.27	0.01	0.01
Compost Urea-N	0.0	0.0	0.2	0.1	0.8	0.6
CMCase Activity	76.88	74.89	62.38	61.00	53.64	54.17
Day 18						
Loss NH_3-N	15.1	16.8	6.6	7.3	33.0	34.1
Loss NOx-N	0.6	0.4	2.2	3.1	2.9	12.6
Day 20						
m.c.	188.28	208.84	211.34	215.23	223.65	210.87
pH	6.7	6.7	6.8	6.6	7.0	7.1
Loss NH_3-N	14.9	17.0	29.0	28.5	46.5	43.4
Loss NOx-N	0.8	0.4	2.9	4.3	0.7	0.1
Compost NH_4^+-N	22.00	20.86	18.34	21.93	30.14	28.67
Compost NOx^--N	3.66	1.97	1.27	1.62	0.55	0.89
Compost Urea-N	0	0	0	0	2	2
CMCase Activity	83.36	80.12	86.77	87.64	68.99	71.45
Day 22						
Loss NH_3-N	14.1	18.4	14.7	17.5	26.1	31.4
Loss NOx-N	0.7	0.3	2.0	3.2	0.6	0.3

Treatment	Urea-Bark		Urea-Bark+Quinone		IBDU-Bark	
% N (Initial)	1.4000		1.4000		1.4287	
Initial Weight	123.01		123.01		109.75	
Day 24						
m.c.	179.28	238.84	227.15	225.63	221.25	206.54
pH	6.8	6.9	7.0	6.8	6.7	6.7
Loss NH_3-N	17.0	18.6	41.6	43.0	45.2	42.5
Loss NOx-N	1.8	1.5	2.7	2.0	0.7	0.3
Compost NH_4^+-N	13.14	10.22	10.33	9.63	23.54	27.10
Compost NOx^--N	0.96	0.97	0.27	0.29	0.33	0.59
Compost Urea-N	0	0	0	0	0	0
CMCase Activity	60.58	65.44	68.32	70.47	79.38	80.77
Day 26						
Loss NH_3-N	15.4	16.1	41.8	38.7	45.0	43.9
Loss NOx-N	1.5	1.3	0.6	0.3	0.1	0.1
Day 28						
m.c.	182.77	224.87	220.75	222.49	213.22	209.98
pH	6.5	6.6	6.7	6.7	6.9	6.8
Loss NH_3-N	7.0	7.3	5.9	6.1	4.6	4.3
Loss NOx-N	0.5	0.6	0.2	0.3	0.4	0.3
Compost NH_4^+-N	12.10	9.98	8.45	7.63	14.31	12.98
Compost NOx^--N	0.69	0.78	0.18	0.12	0.20	0.13
Compost Urea-N	0	0	0	0	0	0
CMCase Activity	51.89	49.90	60.32	61.78	73.68	77.01
Total Loss NH_3-N	175.6	193.4	228.7	227.4	254.1	250.2
Total Loss NOx-N	24.5	23.3	25.8	24.4	58.0	55.1
% Weight Loss (CO_2-C)[8]	16.68	16.69	15.10	15.78	19.62	19.35

[1] See Appendix 1 for composition of compost mixes & composting conditions. See Table 4 for chemical composition of raw materials.

[2] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[3] The pH was determined on 1:5 suspension in 2N KCl.

[4] N (mg) collected in 0.1 M H_2SO_4 traps and assayed by steam distillation.

[5] mg g^{-1} compost, assayed by steam distillation.

[6] ppm, Spectrophotometric assay.

[7] % reduction in viscosity of NaCMC g^{-1} compost h^{-1}.

[8] CO_2-C was determined from compost gasses assayed every 5 h by GC (Appendix 3).

R9.) Fish-bark, Urea-bark and Sewage-bark, Inital C:N=25 [1].

Treatment	Fish-Bark		Urea-Bark		Sewage-Bark	
% N (Initial)	1.7282		1.7419		1.9068	
Initial Weight	85.75		113.40		83.01	
Day 2						
m.c. [2]	217.33	220.67	211.84	219.07	220.77	217.37
pH [3]	5.5	5.4	6.7	6.8	5.4	5.3
Day 4						
m.c.	223.65	216.33	220.07	214.00	220.33	222.08
pH	6.2	6.2	7.9	7.8	6.0	5.9
Loss NH_3-N [4]	3.1	3.4	29.1	30.2	0.9	0.10
Loss NOx-N [4]	0.0	0.0	0.4	0.5	15.0	13.4
Compost NH_4^+-N [5]	0.68	0.72	18.52	17.21	0.43	0.47
Compost NOx^--N [5]	0.67	0.63	2.69	2.55	2.30	2.22
CMCase Activity [6]	9.00	9.21	8.33	8.95	12.79	14.76
Day 6						
Loss NH_3-N	2.2	1.8	54.9	55.9	0.5	0.4
Loss NOx-N	12.0	13.3	3.7	5.5	30.8	36.6
Day 8						
m.c.	221.63	215.71	199.74	217.55	222.52	221.37
pH	7.2	6.9	8.4	8.3	6.6	6.7
Loss NH_3-N	8.8	9.1	97.8	103.3	1.8	2.3
Loss NOx-N	8.1	9.5	1.8	2.2	70.0	67.7
Compost NH_4^+-N	2.09	1.98	27.11	28.34	1.72	1.66
Compost NOx^--N	1.68	1.75	8.39	9.01	8.32	8.66
CMCase Activity	33.47	31.36	9.81	10.33	25.77	24.67
Day 10						
Loss NH_3-N	3.2	3.5	34.4	38.5	6.2	7.2
Loss NOx-N	3.1	3.6	1.0	0.9	29.5	31.1

Continued....

Treatment	Fish-Bark		Urea-Bark		Sewage-Bark	
% N (Initial)	1.7282		1.7419		1.9068	
Initial Weight	85.75		113.40		83.01	
Day 12						
m.c.	189.98	207.31	187.66	201.47	219.30	223.79
pH	7.6	7.6	8.0	7.9	5.8	5.9
Loss NH_3-N	8.7	7.9	86.1	93.0	9.6	10.6
Loss NOx-N	0.9	1.1	0.8	0.7	9.2	10.6
Compost NH_4^+-N	31.45	32.47	96.78	108.28	44.17	43.84
Compost NOx^--N	30.99	32.47	20.78	21.44	59.99	63.78
CMCase Activity	42.78	44.91	19.51	20.64	36.12	39.09
Day 14						
Loss NH_3-N	2.3	2.7	49.5	50.1	4.4	3.1
Loss NOx-N	7.8	7.0	1.0	1.1	18.7	16.5
Day 16						
m.c.	206.47	213.70	200.96	196.30	223.46	224.52
pH	7.4	7.3	7.6	7.5	6.3	6.7
Loss NH_3-N	2.3	2.2	29.5	31.4	7.6	7.2
Loss NOx-N	12.4	13.3	6.6	6.0	10.6	11.0
Compost NH_4^+-N	21.36	20.14	76.11	75.64	32.89	34.15
Compost NOx^--N	15.26	16.00	18.41	17.29	37.89	38.76
CMCase Activity	72.18	74.22	53.66	53.09	41.33	43.19
Day 18						
Loss NH_3-N	1.0	1.3	16.2	15.8	14.8	5.3
Loss NOx-N	20.9	21.9	5.5	6.2	9.2	8.8
Day 20						
m.c.	221.07	216.37	181.96	195.90	221.47	230.17
pH	7.4	7.5	7.8	7.9	6.7	6.8
Loss NH_3-N	0.6	0.8	13.7	13.3	1.4	1.2
Loss NOx-N	10.0	9.2	6.6	6.2	9.2	9.9
Compost NH_4^+-N	12.53	13.67	20.45	28.03	29.66	31.07
Compost NOx^--N	12.44	13.58	10.55	12.63	18.41	19.60
CMCase Activity	75.29	74.36	75.91	76.47	42.31	43.07
Day 22						
Loss NH_3-N	1.2	1.4	23.4	22.7	1.1	0.9
Loss NOx-N	8.6	8.3	4.2	3.8	5.3	4.8
Day 24						
m.c.	200.70	212.66	204.14	205.31	226.67	230.02
pH	7.0	6.9	7.5	7.5	6.5	6.7
Loss NH_3-N	2.6	2.2	29.4	28.8	0.6	0.4
Loss NOx-N	9.2	9.8	5.3	5.6	4.5	4.1
Compost NH_4^+-N	6.99	7.65	13.46	16.07	9.55	7.89
Compost NOx^--N	4.11	4.06	3.81	3.00	9.16	10.88
CMCase Activity	82.77	84.01	79.20	78.55	36.28	30.11
Day 26						
Loss NH_3-N	1.8	1.6	17.0	17.8	2.0	1.5
Loss NOx-N	2.2	2.6	4.8	4.5	4.2	4.9
Day 28						
m.c.	199.37	217.92	215.96	207.39	219.47	224.09
pH	6.8	6.9	7.0	7.2	6.7	6.8
Loss NH_3-N	1.7	2.2	8.0	8.8	0.8	1.1
Loss NOx-N	1.7	1.4	2.0	1.8	3.2	3.3
Compost NH_4^+-N	7.55	8.02	15.87	14.33	7.34	9.11
Compost NOx^--N	2.69	2.78	4.18	4.12	6.20	6.13
CMCase Activity	86.26	85.12	84.92	86.01	32.14	33.76
Total Loss NH_3-N	39.5	40.1	489.0	509.6	41.7	42.2
Total Loss NOx-N	96.9	101.0	43.1	44.1	201.5	222.7
% Weight Loss (CO_2-C)[7]	15.61	15.49	16.46	14.81	6.69	6.35

[1] See Appendix 1 for composition of compost mixes & composting conditions. See Table 4 for chemical composition of raw materials.

[2] The % moisture (m.c.) was determined on 5g samples after drying at 105° 24h.

[3] The pH was determined on 1:5 compost:2N KCl suspension.

[4] mg Nitrogen collected in 0.1 M H_2SO_4 acid traps, assayed by steam distillation

[5] mg g^{-1} compost, assayed by steam distillation.

[6] % reduction in viscosity of NaCMC g^{-1} compost h^{-1}.

[7] CO_2-C was determined from 5 hourly GC assay of compost gasses (Appendix 3).

APPENDIX - 5

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

R1 & R2.) Fish-bark, Initial C:N=45 or 65. [1]

		Oxygen Uptake		Carbon Dioxide Output	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	1	109.6875	1009.350 ***	47.1130	628.413 ***
Run	1	0.4175	3.842 ns	0.2601	3.469 ns
Error (unit)	7	0.10867		0.07497	
SAMPLE (subplot)					
Time	12	43.4882	256.555 ***	9.4834	175.392 ***
Time.Treat	12	8.8432	52.779 ***	1.4525	26.864 ***(**)
Error(Sample)	122	0.16755		0.05407	
% Coefficient of Variation		14.5		14.9	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.87		0.50	
any time or treatment:		1.15		0.48	

		M.C.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	1	59.0	0.113 ns	9.2564	18.151 **
Run	1	360.5	0.692 ns	0.2625	0.515 ns
Error (unit)	7	520.87		0.50998	
SAMPLE (subplot)					
Time	12	528.59	3.097 ns	2.3053	10.364 ***(**)
Time.Treat	12	204.16	1.196 ns	0.4359	1.959 *(ns)
Error(Sample)	122	170.69		0.22244	
% Coefficient of Variation		3.4		2.9	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		27.90		1.01	
any time or treatment:		28.40		1.41	

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	1	95857.0	228.460 ***	36019241	0.001 ns
Run	1	1114.7	1.718 ns	1869	0.000 ns
Error (unit)	7	648.636		35284	
SAMPLE (subplot)					
Time	12	53804.3	78.073 ***	4233008	554.194 ***
Time.Treat	12	5359.7	7.777 ***(*)	1581182	207.012 ***
Error(Sample)	122	689.157		7638.131	
% Coefficient of Variation		8.7		3.9	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		56.1		71.4	
any time or treatment:		74.8		101.7	

Continued...

Humification Indices

	df	Abs 550 nm MS	F	Abs 440/660 nm MS	F
UNIT (plot)					
Treatment	1	6.5641	58.268 ***	0.05026	0.847 ns
Run	1	0.05769	0.512 ns	0.01641	0.126 ns
Error (unit)	7	0.11265		0.13004	
SAMPLE (subplot)					
Time	12	5.48264	47.426 ***	14.49192	131.614 ***
Time.Treat	12	6.38341	55.218 ***	5.59803	50.841 ***
Error(Sample)	122	0.11560		0.11011	
% Coefficient of Variation		7.1			9.7
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.73			0.27
any time or treatment:		0.97			0.95

CMCase Activity

	df	MS	F
UNIT (plot)			
Treatment	1	888.688	30.252 ***
Run	1	73.281	2.558 ns
Error (unit)	7	28.649	
SAMPLE (subplot)			
Time	12	1750.426	230.767 ***
Time.Treat	12	526.576	69.421 ***
Error(Sample)	122	7.585	
% Coefficient of Variation		4.0	
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:		5.88	
any time or treatment:		8.67	

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$. Significance indicators in parentheses were for the conservative test using 1 & 7 df.

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

R3.) Fish-bark, Initial C:N=45. [1]

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	2.3421	1301.537 ***
Error (unit)	3	0.0018	
SAMPLE (subplot)			
Time	37	0.8187	272.567 ***
Time.Treat	74	0.3209	106.858 ***(**)
Error(Sample)	111	0.0030	

% Coefficient of Variation 9.2

$LSD_{(0.01)}$ between two means at -
different times in one treatment: 0.14
any time or treatment: 0.32

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	721.01	1.303 ns	0.8931	30.008 *
Error (unit)	3	553.23		0.0298	
SAMPLE (subplot)					
Time	6	47.90	0.659 ns	3.4365	189.912 ***
Time.Treat	12	126.58	1.743 ns	0.0803	4.439 **(*)
Error(Sample)	18	72.64		0.0181	
% Coefficient of Variation		4.2		2.1	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		24.53		0.39	
any time or treatment:		69.43		0.82	

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	22.356	14.557 *	197.787	13.176 *
Error (unit)	3	1.536		15.011	
SAMPLE (subplot)					
Time	6	9.467	13.713 ***(*)	412.732	134.735 ***(**)
Time.Treat	12	6.304	7.134 ***(ns)	183.260	59.824 ***(**)
Error(Sample)	18	0.884		3.063	
% Coefficient of Variation		15.4		6.2	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		2.71		5.04	
any time or treatment:		5.77		12.76	

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$. Significance indicators in parentheses were for the conservative test using 2 & 3 df.

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

R4.) Fish-bark & Urea-bark, Initial C:N=45. [1]

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	2.5668	151.904 ***
Error (unit)	3	0.0169	
SAMPLE (subplot)			
Time	31	3.1255	212.343 ***
Time.Treat	62	0.0983	6.681 ***(ns)
Error(Sample)	93	0.0147	
% Coefficient of Variation			8.5
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:			0.32
any time or treatment:			0.71

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	16.53	0.048 ns	2.3016	43.742 **
Error (unit)	3	212.53		0.0526	
SAMPLE (subplot)					
Time	6	221.55	2.335 *(ns)	0.9007	97.009 ***(*)
Time.Treat	12	25.26	0.266 ns	0.5581	60.098 ***(*)
Error(Sample)	18	94.88		0.0093	
% Coefficient of Variation		4.5		1.5	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		28.04		0.28	
any time or treatment:		61.73		0.73	

		Ammonification		Nitrification (Nitrate)	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	557.188	531.187 ***	1100.410	290.653 ***
Error (unit)	3	1.049		3.786	
SAMPLE (subplot)					
Time	6	17.679	15.544 ***(*)	344.965	209.907 ***
Time.Treat	12	8.256	7.258 ***(ns)	261.732	159.261 ***
Error(Sample)	18	2.504		1.643	
% Coefficient of Variation		18.1		13.6	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		3.07		3.69	
any time or treatment:		6.20		8.15	

		Nitrification (Nitrite) [2]		CMCase Activity	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	1.0127	1409.856 ***	888.688	30.252 ***
Error (unit)	3	0.0007813		28.649	
SAMPLE (subplot)					
Time	6	0.24932	326.589 ***	1750.426	230.767 ***
Time.Treat	12	0.11084	145.193 ***(**)	526.576	69.421 ***(**)
Error(Sample)	18	0.0007634		7.585	
% Coefficient of Variation		12.3		4.0	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.08		3.57	
any time or treatment:		0.16		6.89	

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$. Significance indicators in parentheses were for the conservative test using 2 & 3 df.

[2] Data was transformed by $\log_{10} + 1$.

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

R5.) Fish-bark, Initial C:N=45 or 55[1].

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	13.2655	1825.863 ***
Error (unit)	3	0.0073	
SAMPLE (subplot)			
Time	163	0.1090	248.971 ***
Time.Treat	326	0.5135	117.317 ***(**)
Error(Sample)	489	0.000438	
% Coefficient of Variation		10.2	
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:		0.056	
any time or treatment:		0.122	

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	76.27	1.691 ns	8.9031	763.122 ***
Error (unit)	3	45.11		0.0117	
SAMPLE (subplot)					
Time	6	160.79	2.141 ns	1.7466	95.268 ***(**)
Time.Treat	12	306.63	4.083 **(ns)	0.1942	10.593 ***(*)
Error(Sample)	18	166.56		0.0183	
% Coefficient of Variation		4.1		2.3	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		24.23		0.38	
any time or treatment:		50.64		0.79	

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	1.0129	116.104 **	1.569	29.372 *
Error (unit)	3	0.0087		0.053	
SAMPLE (subplot)					
Time	6	1.3013	108.878 ***(**)	90.31	9252.257 ***
Time.Treat	12	0.5306	44.396 ***(**)	0.881	90.306 ***(**)
Error(Sample)	18	0.01195		0.0097	
% Coefficient of Variation		15.4		6.2	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.31		0.36	
any time or treatment:		0.64		0.58	

		CMCase Activity		Ammonia Volatilization [2]	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	14580.014	129949.410 ***	0.00862	405.912 ***
Error (unit)	3	0.112		0.00002	
SAMPLE (subplot)					
Time	6	20.707	11.655 ***(*)	0.00031	16.600 ***(*)
Time.Treat	12	540.083	304.001 ***	0.00039	20.733 ***(*)
Error(Sample)	18	1.777		0.000019	
% Coefficient of Variation		3.6		31.9	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		3.57		0.01	
any time or treatment:		7.78		0.03	

Continued...

Lipase Activity [2]

	df	MS	F
UNIT (plot)			
Treatment	2	0.01868	374.420 ***
Error (unit)	3	0.00004989	
SAMPLE (subplot)			
Time	6	0.13914	2159.202 ***
Time.Treat	6	0.01785	276.988 ***
Error(Sample)	12	0.00006444	
% Coefficient of Variation			2.2
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:			0.024
any time or treatment:			0.078

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$. Significance indicators in parentheses were for the conservative test using 2 & 3 df.

[2] Data was transformed by $\log_{10} + 1$.

R6.) Fish-bark, Initial C:N=45[1].

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	124.722	76486.833 ***
Error (unit)	3	0.0016	
SAMPLE (subplot)			
Time	145	1.0215	235.764 ***
Time.Treat	290	0.2116	48.834 ***(*)
Error(Sample)	453	0.0043	
% Coefficient of Variation			7.8
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:			0.17
any time or treatment:			0.38

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$. Significance indicators in parentheses were for the conservative test using 2 & 3 df.

R7.) Fish-, Urea-, & Sewage=bark, Initial C:N=35. [1]

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	23.8306	171.791 ***
Error (unit)	3	0.1387	
SAMPLE (subplot)			
Time	110	1.5610	112.536 ***(**)
Time.Treat	220	0.1857	13.385 ***(*)
Error(Sample)	330	0.01387	
% Coefficient of Variation			19.8
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:			0.32
any time or treatment:			0.69

Continued...

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	888.50	3.562 ns	5.542	4656.600 ***
Error (unit)	3	249.41		0.001	
SAMPLE (subplot)					
Time	6	374.90	2.246 ns	0.6176	46.047 ***(**)
Time.Treat	12	211.42	1.267 ns	0.3361	25.056 ***(*)
Error(Sample)	18	166.9		0.0134	
% Coefficient of Variation		6.1		1.8	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		30.48		0.31	
any time or treatment:		75.46		0.68	

		Ammonification		Nitrification (Nitrate)	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	1134.511	7717.013 ***	946.902	552.277 ***
Error (unit)	5	0.147		1.715	
SAMPLE (subplot)					
Time	6	79.793	241.335 ***	523.386	349.298 ***
Time.Treat	12	38.802	177.356 ***(**)	201.004	134.145 ***(**)
Error(Sample)	18	0.331		1.498	
% Coefficient of Variation		6.8		13.9	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		1.59		3.56	
any time or treatment:		3.36		7.15	

		Nitrogen Volatilization [2]			
		Ammonia		Nitrogen Oxides	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	5.3884	609.486 ***	0.75189	1009.620 ***
Error (unit)	3	0.0884		0.00075	
SAMPLE (subplot)					
Time	12	0.0865	24.738 ***(*)	0.23576	77.458 ***(**)
Time.Treat	24	0.0667	19.081 ***(*)	0.13895	45.653 ***(**)
Error(Sample)	36	0.0035		0.00304	
% Coefficient of Variation		17.7		17.1	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.18		0.15	
any time or treatment:		0.35		0.32	

		CMCase Activity	
	df	MS	F
UNIT (plot)			
Treatment	2	4527.171	3002.899 ***
Error (unit)	3	1.508	
SAMPLE (subplot)			
Time	6	1878.578	1802.541 ***
Time.Treat	12	331.084	317.683 ***
Error(Sample)	18	1.042	
% Coefficient of Variation		3.6	
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:		3.03	
any time or treatment:		5.96	

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$, () significance level with 2 & 3 df.

[2] Data was transformed by $\log_{10}+1$.

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

R8.) Urea-bark, Initial C:N=35. [1]

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	0.1211	3.107 ns
Error (unit)	3	0.03896	
SAMPLE (subplot)			
Time	164	2.0196	109.438 ***(**)
Time.Treat	328	0.8211	44.493 ***(**)
Error(Sample)	492	0.01845	
% Coefficient of Variation		18.3	
$LSD_{(0.01)}$ between two means at -			
different times in one treatment:		0.35	
any time or treatment:		0.79	

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	329.69	0.498 ns	0.1866	71.273 **
Error (unit)	3	661.83		0.0026	
SAMPLE (subplot)					
Time	6	350.08	3.659 *(ns)	0.3666	39.479 ***(**)
Time.Treat	12	88.46	0.925 ns	0.7028	75.684 ***(**)
Error(Sample)	18	95.67		0.0093	
% Coefficient of Variation		4.7		1.4	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		38.25		0.26	
any time or treatment:		57.13		0.56	

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	86.514	58.537 **	1.934	2.876 ns
Error (unit)	5	1.478		0.672	
SAMPLE (subplot)					
Time	6	295.863	95.278 ***(**)	163.569	153.422 ***
Time.Treat	12	63.862	20.566 ***(*)	62.111	58.258 ***(**)
Error(Sample)	18	3.105		1.066	
% Coefficient of Variation		11.5		20.2	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		4.88		2.89	
any time or treatment:		10.29		6.03	

		Nitrogen Volatilization [2]			
		Ammonia		Nitrogen Oxides	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	6.953	62.998 **	0.0267	1.822 ns
Error (unit)	3	0.110		0.0146	
SAMPLE (subplot)					
Time	12	7.642	159.416 ***	0.3497	17.818 ***(ns)
Time.Treat	24	3.251	43.298 ***(**)	0.1312	6.685 ***(ns)
Error(Sample)	36	0.0751		0.1118	
% Coefficient of Variation		12.1		63.8	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.80		0.40	
any time or treatment:		1.60		0.82	

		CMCase Activity		Residual Urea [2]	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	130.673	20.319 *	2.887	293.845 ***
Error (unit)	3	6.441		0.0098	
SAMPLE (subplot)					
Time	6	3964.310	1099.676 ***	2.2056	404.108 ***
Time.Treat	12	221.626	61.478 ***(**)	0.1503	27.588 ***(*)
Error(Sample)	18	3.605		0.00545	
% Coefficient of Variation		3.8		8.4	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		5.76		0.22	
any time or treatment:		11.09		0.46	

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$, () significance level with 2 & 3 df.

[2] Data was transformed by $\log_{10}$ (ammonia) & $\log_{10}+1$ (N oxides & urea)

R9.) Fish-, Urea-, & Sewage-bark, Initial C:N=25. [1]

Carbon Dioxide Output

	df	MS	F
UNIT (plot)			
Treatment	2	0.63498	18.574 *
Error (unit)	3	0.034186	
SAMPLE (subplot)			
Time	114	2.94492	480.626 ***
Time.Treat	228	0.28237	46.085 ***(*)
Error(Sample)	342	0.006127	

% Coefficient of Variation 9.9

$LSD_{(0.01)}$ between two means at -
different times in one treatment: 0.21
any time or treatment: 0.46

---

		m.c.		pH	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	1420.82	13.758 *	5.9172	273.099 ***
Error (unit)	3	103.27		0.0217	
SAMPLE (subplot)					
Time	6	107.27	2.929 *(ns)	0.3587	37.983 ***(**)
Time.Treat	12	106.97	2.921 *(ns)	0.3407	36.080 ***(**)
Error(Sample)	18	36.62		0.0094	
% Coefficient of Variation		2.8		1.4	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		19.55		0.30	
any time or treatment:		35.35		0.57	

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	1107.524	588.215 ***	559.692	319.844 ***
Error (unit)	3	1.883		1.750	
SAMPLE (subplot)					
Time	6	264.937	126.076***(**)	1052.212	1869.873 ***
Time.Treat	12	100.405	47.780 ***(*)	124.244	220.793 ***
Error(Sample)	18	2.101		0.5627	
% Coefficient of Variation		10.0		5.5	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		4.17		2.16	
any time or treatment:		8.41		5.00	

		Nitrogen Volatilization			
		Ammonia		Nitrogen Oxides	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	2	107.796	1997.841 ***	12.588	3248.991 ***
Error (unit)	3	0.05396		0.003874	
SAMPLE (subplot)					
Time	12	6.939	642.940 ***	2.595	297.421 ***
Time.Treat	24	4.883	452.398 ***	2.457	281.639 ***
Error(Sample)	36	0.01079		0.008724	
% Coefficient of Variation		7.0		10.0	
$LSD_{(0.01)}$ between two means at -					
different times in one treatment:		0.37		0.34	
any time or treatment:		0.69		0.53	

CMCase Activity

	df	MS	F
UNIT (plot)			
Treatment	2	2438.818	739.972 ***
Error (unit)	3	3.296	
SAMPLE (subplot)			
Time	6	2813.554	2076.209 ***
Time.Treat	12	392.305	289.494 ***
Error(Sample)	18	1.355	

% Coefficient of Variation 2.7

$LSD_{(0.01)}$ between two means at -
different times in one treatment: 3.68
any time or treatment: 6.80

---

[1] Calculated from the data given in Appendices 3 & 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$,

***- significant at $p < 0.001$, () significance level with 2 & 3 df.

SPLIT-PLOT AOV OF COMPOST CHARACTERISTICS:

Percentage Weight Loss as CO_2-C in Composts of Various C:N Ratios after 28d Composting. [1]

	CO_2-C Loss		
	df	MS	F
UNIT (plot)			
Treatment	19	185.617	439.247 ***
Error (unit)	20	0.423	

% Coefficient of Variation 4.1

$LSD_{(0.05)}$ between any two means : 1.64
$LSD_{(0.01)}$ between any two means : 1.85

[1] Calculated from the data given in Appendix 4.

***- significant at $p < 0.001$.

Appendix - 6

ESTIMATED NUMBERS OF MICROORGANISMS DURING COMPOSTING:

R2) - Millions CFU g^{-1} Fish-Bark Compost, Initial C:N 45 or 65 [1]

DAY, TEMP TREAT	Total Count Thermophiles				Nos. of Thermophiles being:			
	Aero	Anero	Actino-mycetes	Fungi	Cellulo-lytic	Ligno-lytic	Pectino-lytic	Lipo-lytic
2, 30°								
C:N 45	0.54	0.15	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.54
"	0.50	0.10	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.50
C:N 65	0.50	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.50
"	0.48	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.48
7, 50°								
C:N 45	12500.00	7.00	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	697.00
"	9870.00	7.10	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	841.00
C:N 65	190.00	5.00	1.19	< 0.01	< 0.01	< 0.01	< 0.01	168.00
"	143.00	4.70	1.14	< 0.01	< 0.01	< 0.01	< 0.01	143.00
14, 55°								
C:N 45	14500.00	51.00	179.00	< 0.01	6020.00	< 0.01	2530.00	14020.0
"	16200.00	59.00	189.40	< 0.01	6160.00	< 0.01	2840.00	16090.0
C:N 65	153.00	0.56	1.00	< 0.01	23.00	< 0.01	335.00	106.0
"	178.00	0.66	0.99	< 0.01	20.30	< 0.01	341.00	113.0
28, 55°								
C:N 45	752.00	< 0.01	56.40	< 0.01	312.00	< 0.01	335.00	702.00
"	769.00	< 0.01	57.65	< 0.01	289.00	< 0.01	341.00	719.00
C:N 65	453.00	< 0.01	2.36	< 0.01	2.60	< 0.01	436.00	65.00
"	476.00	< 0.01	2.47	< 0.01	3.50	< 0.01	440.00	67.00

[1] Bacteria were enumerated on TSA(2.5.1.1.) (total aerobes) or TSA plus cysteine hydrochloride (total anaerobes), on mineral salts agar plus 0.5% Na carboxymethylcellulose (NaCMC), 0.3% Indulin AT + 0.1% NaCMC, or 0.5% pectin; or on Tween 20 agar (2.5.5.3) after incubation at 55° for 4d.

Split-plot AOV of Bacterial Counts During R2. [1]

		Total Count Anerobic		
	df	MS	F	
Unit (plot)				
Treatment	1	3.1185	2356.134	***
Error (units)	2	0.00132		
Sample (subplot)				
Time	2	6.1433	1021.503	***
Time.Treat	1	1.6162	268.744	***(**)
Error(sample)	3	0.00601		

Means & LSD after Log Transformations [2]:

	Treatment	
Days	C:N=45	C:N=65
2	-0,912 a1	-2.153[3] b1
7	0.848 a2	0.686 a2
14	1.739 a3	-0.216 b3

$LSD_{(0.01)}$
Comparison of two treatments:
(a) at the same time 0.66
Comparison within one treatment:
(1) at different times 0.45

		Total Count Aerobic			% Lipolytic	
	df	MS	F		MS	F
Unit (plot)						
Treatment	1	4.0728	7228.226	***	3.7806	3735.9 ***
Error (units)	2	0.00056			0.001012	
Sample (subplot)						
Time	3	11.2471	4200.587	***	9.1736	8117.1 ***
Time.Treat	3	1.0633	397.130	***(**)	0.7783	688.7 ***(**)
Error(sample)	6	0.00267			0.00113	

Means & LSD after Log_{10} Transformations [2]:

	Treatments				
Days	C:N=45	C:N=65		C:N=45	C:N=65
2	-0.284 a1	-0.310 a1		100.0 a1	100.0 a1
7	4.046 a2	2.217 b2		6.9 a2	92.7 b2
14	4.185 a3	2.218 b2		96.7 a1	65.8 b3
28	2.881 a4	2.667 b3		86.9 a3	14.1 b4

$LSD_{(0.01)}$
Comparison between two treatments (designated by a letter) at the same time: 0.46 0.13

Comparison within one treatment (designated by a number) at different times: 0.19 0.33

[1] The data from the above table was transformed (log_{10}) prior to the analysis. *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$. Significance levels in parentheses are for the conserative test (using plot d.f.).

[2] Means were significantly different (LSD $p < 0.01$ or $p < 0.05$ if underlined) if followed by a different letter (6 df) in the one row or by a different number (conservative test 2 df) in the same column.

ESTIMATED NUMBERS OF MICROORGANISMS DURING COMPOSTING

R4) - Millions CFU g-1 Fish- & Urea-bark Compost, Initial C:N=45. [1]

Day, Temp Meso Treat Thermo	Total CFU g-1 Compost Eubacteria Aero.	Eubacteria Anaero.	Actino-mycetes	Fungi	Nos. of Total Flora being: Cellulo-lytic	Pectino-lytic	Xylano-lytic	Lipo-lytic
7, 50°								
Fish-b MESO	82.6	< 0.1	< 0.1	< 0.1	32.4	48.9	0.1	< 0.1
" "	83.1	< 0.1	< 0.1	< 0.1	31.5	48.8	0.1	< 0.1
" THERMO	226.0	3.0	< 0.1	< 0.1	158.0	158.0	2.5	80.9
" "	234.0	9.0	< 0.1	< 0.1	149.3	149.3	2.3	76.1
Urea-b MESO	364.0	0.5	20.0	1.1	63.5	63.5	7.7	1.5
" "	347.0	0.5	14.0	0.8	49.3	49.3	11.6	0.7
" THERMO	15.6	0.1	262.0	< 0.1	1.9	0.3	1.9	6.4
" "	12.1	0.1	251.0	< 0.1	1.6	0.3	1.6	5.3
14, 55°								
Fish-b MESO	88.0	< 0.1	< 0.1	< 0.1	81.9	81.9	< 0.1	20.0
" "	99.0	< 0.1	< 0.1	< 0.1	76.0	79.0	< 0.1	22.0
" THERMO	1077.0	69.0	1482.0	< 0.1	678.1	1774.4	784.6	937.9
" "	1620.0	76.0	1498.9	< 0.1	674.1	811.5	1945.7	1028.8
Urea-b MESO	33.2	< 0.1	< 0.1	27.0	12.0	7.0	12.0	12.0
" "	35.1	< 0.1	< 0.1	31.0	17.8	7.8	17.8	17.8
" THERMO	928.0	< 0.1	112.0	< 0.1	< 0.1	3.1	3.1	190.3
" "	1210.0	< 0.1	120.0	< 0.1	< 0.1	8.0	8.0	282.0
28, 55°								
Fish-b MESO	16.0	< 0.1	< 0.1	< 0.1	< 0.1	10.2	2.0	2.3
" "	20.0	< 0.1	< 0.1	< 0.1	< 0.1	13.2	2.3	3.0
" THERMO	94.0	< 0.1	20.8	< 0.1	9.2	79.9	< 0.1	69.2
" "	113.0	< 0.1	29.4	< 0.1	11.1	100.5	< 0.1	82.7
Urea-b MESO	22.0	< 0.1	< 0.1	60 y	1.9	74.0	< 0.1	60.8
" "	20.0	< 0.1	< 0.1	55 y	0.8	70.5	< 0.1	54.3
" THERMO	69.0	< 0.1	1040.2	< 0.1	276.2	29.9	531.6	479.2
" "	55.0	< 0.1	1050.1	< 0.1	240.9	30.9	520.5	503.9

[1] Bacteria were enumerated on TSA(2.5.1.1.) (total aerobes), TSA plus cysteine hydrochloride (total anaerobes), on mineral salts agar plus 0.5% Na carboxymethylcellulose(NaCMC), 0.3% Indulin AT plus 0.1% NaCMC, or 0.5% pectin; or on Tween 20 agar (2.5.5.3) after incubation at 55° (thermo) or 28° (meso) for 4d. CFU of fungi were determined using PDA (2.5.5.6) at 55° or 28°.

y - yeast.

Split-plot AOV of Microbial Counts During R4. [1]

	df	Total Count Aerobic MS	F	% Lipolytic MS	F
Isolate (plot)					
Treatment	1	0.51569	102.324 **	0.770	0.053 n.s.
Error (units)	2	0.00504		14.485	
Sample (subplot)					
Time	3	0.49675	186.041 ***(**)	3053.220	411.254 ***(**)
Time.Treat	3	0.57494	215.326 ***	756.559	101.905 ***(**)
Error(sample)	6	0.00267		7.424	
Incubation (Sample Subplot)					
Temperature	1	4.27966	14107 ***	527.325	80.004 ***(*)
Temp.Time	3	0.78112	2574 ***	126.840	19.244 ***(*)
Temp.Treat	1	0.03996	131.721 ***(**)	2310.805	350.587 ***(**)
Temp.Treat.Time	3	0.09555	314.955 ***	490.130	74.361 ***(*)
Error	8	0.000303		6.591	

Mean Counts & LSD [2]:

Total Aerobic Count with Log Transformations

Days	Mesophiles Fish-bark	Mesophiles Urea-bark	Thermophiles Fish-bark	Thermophiles Urea-bark
7	1.918 a1	2.570 b1	2.362 c1	2.440 c1
14	1.970 a1	1.800 b2	3.451 c1	3.070 d2
28	1.253 a2	1.894 b2	2.107 c2	3.044 d2

% Lipolytic

Days	Mesophiles Fish-bark	Mesophiles Urea-bark	Thermophiles Fish-bark	Thermophiles Urea-bark
7	0.00 a1	0.30 a1	34.15 c1	2.15 a1
14	22.45 a2	23.45 a2	30.75 c1	19.75 d2
28	14.65 a3	73.30 b3	59.20 c2	44.40 d3

$LSD_{(0.01)}$

Comparison of two treatments at the:	Total Count	Lipolytic
(a) same time and temperature	0.122	1.68
(b) same time, different temperature	0.096	5.24
Comparisons within one treatment and temperature: at different times	0.123	6.47

	df	% Cellulolytic MS	F	% Pectinolytic MS	F
Unit (plot)					
Treatment	1	5262.935	151.187 *	14970.015	621.635 **
Error (units)	2	34.811		24.082	
Sample (subplot)					
Time	3	2167.536	133.345 ***(**)	1161.551	59.175 ***(*)
Time.Treat	3	1192.657	62.367 ***(*)	1518.939	77.382 ***(*)
Error(sample)	6	19.123		19.629	
Incubation (Sample Subplot)					
Temperature	1	626.300	71.762 ***(*)	2181.227	581.660 ***(**)
Temp.Time	3	1555.943	178.281 ***(**)	828.203	220.854 ***(**)
Temp.Treat	1	20.163	2.310 ***(ns)	1561.707	416.455 ***(**)
Temp.Treat.Time	3	814.452	93.320 ***(*)	1687.815	450.084 ***(**)
Error	8	8.728		3.750	

Means & LSD after Log Transformations [2]:

% Cellulolytic

Days	Mesophilic Fish-bark	Mesophilic Urea-bark	Thermophilic Fish-bark	Thermophilic Urea-bark
7	38.55 a1	15.05 b1	66.85 c1	0.65 d1
14	84.95 a2	23.45 b2	23.60 b2	0.00 d1
28	0.00 a3	1.65 a3	7.90 a3	3.35 a1

% Pectinolytic

Days	Mesophilic Fish-bark	Mesophilic Urea-bark	Thermophilic Fish-bark	Thermophilic Urea-bark
7	58.95 a1	15.05 b1	66.85 c1	0.10 d1
14	86.45 a2	1.75 b2	64.60 c1	0.45 d1
28	64.95 a1	92.10 b3	70.10 a1	2.75 c1

$LSD_{(0.01)}$

Comparison of two treatments at the:	% Cellulolytic	% Pectinolytic
(a) same time and temperature	20.74	13.59
(b) same time, different temperature	8.12	7.43
Comparison within one treatment and temperature: (1) at different times	10.38	10.51

Split-plot AOV of Microbial Counts During R4. [1]

% Total Flora being *Bacillus spp.*

	df	MS	F
Unit (plot)			
Treatment	1	1375.620	331.975 **
Error (units)	2	4.144	
Sample (subplot)			
Time	3	663.889	293.269 ***(**)
Time.Treat	3	1308.650	578.090 ***(**)
Error(sample)	6	2.264	
Incubation (Sample Subplot)			
Temperature	1	1505.750	1578.768 ***
Temp.Time	3	7153.650	7500.551 ***
Temp.Treat	1	1068.000	1119.791 ***
Temp.Treat.Time	3	1044.455	1095.104 ***
Error	8	0.954	

Means & LSD for % of the Total Flora being[2]: *Bacillus spp.*

	Mesophilic		Thermophilic	
Days	Fish-bark	Urea-bark	Fish-bark	Urea-bark
7	93.35 a1	59.00 b1	55.90 c1	5.40 d1
14	100.00 a2	70.90 b2	7.60 c2	53.30 d2
28	24.90 a3	2.90 b3	67.20 c3	66.60 c3

$LSD_{(0.01)}$

Comparison of two treatments at the:	
(a) same time and temperature	6.86
(b) same time, different temperature	3.08
Comparison within one treatment and temperature:	
(1) at different times	3.57

[1] The data is expressed as a percentage of the total aerobic count except for total aerobic counts which were transformed (log_{10}) prior to the analysis. Day(s) when both treatments gave counts < 0.01 were not used in the anova (n.u.). *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$. Significance levels in parentheses are for the conserative test (2df).

[2] Means were significantly different (LSD $p < 0.01$) if followed by a different letter (4 df) in the one row or by a different number (2df) in the same column.

Estimated Numbers of Microorganisms During Composting:

Comparison of Numbers of Thermophiles in Fish-bark Mixes Composted with Aeration at 10 or 30 mL min^{-1}. [1]

	df	MS	F
Unit (plot)			
Treatment	1	3.522	762.272 **
Error (units)	2	0.004621	
Sample (subplot)			
Time	2	1.750	738.246 ***(**)
Time.Treat	2	0.2723	114.686 **(**)
Error(sample)	4	0.002370	

Means & LSD after Log Transformations [2]:

Days	Treatments: 10 mL min^{-1}	30 mL min^{-1}
7	2.362 a1	4.049 b1
14	3.451 a2	4.191 b1
28	2.085 a3	2.912 b2

$LSD_{(0.01)}$

Comparison of two treatments:	
(a) at the same time	0.483
Comparison within one treatment:	
(1) at different times	0.257

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[1] The data from the above table was transformed (log_{10}) prior to the analysis. *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$. Significance levels in parentheses are for the conserative test (using plot d.f.).

[2] Means were significantly different (LSD $p < 0.01$) if followed by a different letter (4 df) in the one row or by a different number (conservative test 2df) in the same column.

Estimated Numbers of Faecal Coliforms & Faecal Streptococci in Sewage-bark Composts of Initial C:N=25 & 35 [1]

Initial C:N	Estimated Numbers x 1000 g^{-1} Compost: Faecal Coliforms 25	Faecal Coliforms 35	Faecal Streptococci 25	Faecal Streptococci 35
Day				
0	7980.0 7890.0	7620.0 7440.0	28190.0 23660.0	23660.0 23710.0
7	612.0 588.0	636.0 598.0	863.0 892.0	5381.0 5440.0
14	0.9 0.8	1.2 1.4	80.2 89.1	120.0 95.8
21	0.2 0.3	0.6 0.7	45.1 40.6	22.6 20.0
28	< 0.1 < 0.1	< 0.1 < 0.1	22.8 20.1	36.7 30.1

[1] Counts of faecal coliforms were estimated form the number of yellow colonies on lactose teepol agar which gave the appropriate IMVC reactions (Mara, 1974). Counts of faecal streptococci were estimated from the number of red colonies on m-enterococcus agar and shown to be minute red colonies on MacConkey agar and Gram positive catalase-negative cocci in chains.

Split-plot AOV of Faecal Indicator Bacteria. [1]

	df	Faecal Coliforms MS	Faecal Coliforms F	Faecal Streptococci MS	Faecal Streptococci F
Unit (plot)					
Treatment	1	0.07341	60.901 **	0.09908	36.046 **
Error (units)	2	0.00121		0.00275	
Sample (subplot)					
Time	4	23.3425	9768.3 ***	6.8244	6737.6 ***
Time.Treat	4	0.0350	14.6 ***(*)	0.1676	165.5 ***
Error(sample)	8	0.0024		0.00101	

Means & LSD [2]:

Days	Treatments C:N=25	C:N=35	C:N=25	C:N=35
0	3.900 a1	3.877 a1	4.451 a1	4.375 a1
7	2.773 a2	2.795 a2	2.943 a2	3.733 b2
14	-0.071 a3	0.113 b3	1.927 a3	2.030 a3
21	-0.611 a4	-0.188 b4	1.631 a4	1.328 b4
28	-2.000 a5	-2.000 a5	1.331 a4	1.522 b5

$LSD_{(0.01)}$

Comparison between two treatments (designated by a letter) at the same time: 0.16 0.11

Comparison within one treatment (designated by a number) at different times: 0.27 0.22

[1] The data from the above table was transformed (log_{10}) prior to the analysis. *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$. Significance levels in parentheses are for the conserative test (using plot d.f.).

[2] Means were significantly different (LSD $p < 0.01$ or $p < 0.05$ if underlined) if followed by a different letter (6 df) in the one row or by a different number (conservative test 2 df) in the same column.

APPENDIX - 7

ENZYME ACTIVITIES DURING COMPOSTING.

1. RELATIVE CARBOXYMETHYL CELLULASE ACTIVITY DURING COMPOSTING:

A.) Effect of pH on the CMCase Activity of Fish-bark Compost. [1]

	pH								
	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
Rep 1	59.11	65.32	72.59	77.21	75.11	68.42	63.86	60.19	58.35
Rep 2	60.36	63.98	72.02	78.10	75.45	68.03	64.83	61.22	57.60
Mean	59.74	64.65	72.31	77.66	75.28	68.23	64.35	60.71	57.98

[1]Fish-bark compost (initial C:N=45) was composted for 20d prior to assay (0.5g in 10mL NaCMC for 1h at 65°). The pH was controlled by a citrate-phosphate buffer (0.15mM). See Figure 19.

B.) Effect of Temperature on the CMCase Activity of Fish-bark Compost. [1]

Split-plot AOV

Source of Variation	df	MS	F-test
Units (Plots) Stratum			
Temperature	6	16998.23	7.10 * [2]
Error(temp.)	6	2394.66	
Sample (Subplots) Stratum			
Time	5	4131.82	15.92 ***(**)
Treat.time	30	92.54	0.35 ns
Error(subplots)	35	259.47	

Day	Temperature 50°	55°	60°	65°	70°	75°
4	79.48 a1[3]	85.29 a1	84.43 a1	81.83 a1	79.61 a1	49.44 b1
8	70.35 a2	72.97 a2	69.36 a2	68.68 a2	64.38 a2	41.49 b1
12	50.30 a3	54.79 a3	53.18 a3	57.03 a3	42.61 b3	28.51 c2
16	48.21 a3	50.54 a3	51.71 a3	51.04 b3	40.43 c3	26.83 d2
20	40.83 a3	41.26 a3	42.53 a3	48.06 a4	39.13 b3	19.99 c3
24	31.54 a4	32.89 a4	39.50 b3	42.16 b4	38.78 b3	19.94 c3
28	20.57 a5	22.36 a5	33.54 b3	38.46 b4	35.33 b3	17.44 c3

[1] Fish-bark compost (initial C:N=55) was sampled at the days specified and assayed (0.5g in 10mL 0.4% NaCMC pH 6.0) at temperatures designated for 1h. Reults are a mean of duplicate assays. See Figure 20.

[2] Significant at: *- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$.

[3] Means followed by the same letter in the same row or the same number in the same column are not significantly ($LSD_{(0.01)}$ = 6.44 & 9.24 respectively) different.

C.) Mean Relative CMCase Activity in Fish-bark Composts of Various C:N Ratios. [1]

Split-plot AOV

Source of Variation	df	MS	F-test
Units (Plots) Stratum			
Treatment	5	272.275	332.407 *** [2]
Error(treat)	6	0.819	
Sample (Subplots) Stratum			
Time	6	669.280	124.547 ***
Treat.time	30	1048.652	195.145 ***
Error(subplots)	36	5.374	

Day	Fish-bark of Initial C:N Ratio: 25	35	45	55	65
4	9.10 a[3]	9.55 a	31.62 b	82.96 c	83.26 c
8	32.41 a	38.92b	45.73 c	59.42 d	71.01 e
12	43.84 a	56.77 b	62.95 b	51.70 c	48.23 c
16	73.20 a	67.50 ab	64.10 b	40.96 c	52.06 d
20	74.83 a	80.99 a	76.83 a	44.41 b	49.77 b
24	83.39 a	78.63 a	73.96 a	40.42 b	46.23 b
28	85.69 a	77.10 a	75.57 a	29.20 b	31.84 b

[1] Fish-bark mixes were composted as described in Appendix 1. Samples (0.5g) were assayed in 10mL 0.4% NaCMC at pH 6.0 65° for 1h. Results are a mean of duplicates reported in Appendix 4.

[2] Significant at $p < 0.001$

[3] Means followed by the same letter in the same row or the same number in the same column are not significantly ($LSD_{(0.01)}$ = 6.38 & 8.76 respectively) different. See Figure 21.

D.) Mean Relative CMCase Activity in Fish-, Urea- & Sewage-bark Mixes of Initial C:N=25 or 35. [1]

Split-plot AOV

Source of Variation	df	MS	F-test
Units (Plots) Stratum			
Treatment	5	2324.144	2565.489 *** [2]
Error(treat)	6	5.436	
Sample (Subplots) Stratum			
Time	6	5077.519	3172.169 ***
Treat.time	30	423.519	264.593 ***
Error(subplots)	36	1.601	

Day	C:N=25 Fish	C:N=25 Urea	C:N=25 Sewage	C:N=35 Fish	C:N=35 Urea	C:N=35 Sewage
4	9.10 a1[3]	8.64 a1	13.78 b1	9.63 a1	9.71 a1	16.73 b1
8	32.42 a2	10.07 b1	25.22 c2	36.83 d2	33.16 a2	23.50 c2
12	43.85 a3	20.07 b2	37.60 c3	56.77 d3	55.08 d3	28.56 e3
16	73.20 a4	53.38 b3	42.26 c4	66.98 d4	78.31 e4	22.75 f2
20	74.83 a4	76.19 a4	42.69 b5	81.00 c5	79.76 c4	37.43 d4
24	83.39 a5	78.88 b4	33.20 c6	63.15 d3	69.71 e5	16.11 f1
28	85.69 a5	85.46 a5	32.95 b6	51.72 c6	54.10 c3	10.14 d4

[1] Fish-bark mixes were composted as described in Appendix 1. Results are a mean of duplicates reported in Appendix 4. See Figure 22. Samples (0.5g) were assayed in 10mL 0.4% NaCMC at pH 6.0 65° for 1h.

[2] Significant difference at $p < 0.01$.

[3] Means followed by the same letter in a row or same number in a column were not significantly different ($LSD_{(0.01)}$=3.444 & 4.541 respectively).

E.) Mean Relative CMCase Activity in Urea-, Urea+p-benzoquinone- and IBDU-bark Mixes of Initial C:N=35. [1]

Day	Urea-bark	Urea-bark + p-benzoquinone	IBDU-bark
4	11.46 a1[2]	5.51 b1	6.61 b1
8	40.67 a2	22.84 b2	26.88 b2
12	54.22 a3	35.21 b3	25.88 c2
16	75.89 a4	61.69 b4	53.91 c3
20	81.74 a4	87.21 b5	70.22 c4
24	63.01 a3	69.40 b4	80.08 c4
28	50.90 a23	61.06 b4	75.35 c4

[1] Fish-bark mixes were composted as described in Appendix 1. Results are a mean of duplicates reported in Appendix 4. Samples (0.5g) were assayed in 10mL 0.4% NaCMC at pH 6.0 65° for 1h.

[2] Means followed by the same letter in the one row or same letter in the same column were not significantly different ($LSD_{(0.01)}$ =5.77 & 11.09 respectively, see Appendix 5 for AOV).

2.) LIPASE ACTIVIY DURING COMPOSTING:

R5) Lipase activity During the Composting of Fish-bark, Initial C:N=45 & 55

Split-plot AOV [1]

Source of Variation	df	MS	F-test
Units (Plots) Stratum			
Treatment	1	0.01868	374.420 **
Error(treat)	2	0.00004989	
Sample (Subplots) Stratum			
Time	6	0.13914	2159.202 ***
Treat.time	6	0.01785	276.988 *** (**)
Error(subplots)	12	0.00006444	
% Coefficient of Variation		2.2	
$LSD_{(0.01)}$ between two means at - different times in one treatment:		0.025	
any time or treatment:		0.078	

[1] Calculated from the data given in Appendix 4 after transformation (log_{10} + 1). See Figure 23.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$, ***- significant at $p < 0.001$, () significance levels with 1 & 2 df. Means after Log_{10} + 1 Transformation.

APPENDIX - 8

NITROGEN TRANSFORMATIONS and pH DURING COMPOSTING:

A.) Fish-bark Composts, Initial C:N=25 to 55. [1]

Split-plot AOV

		Ammonification.		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	4	142.143	5604.402 ***	61.331	504.810 ***
Error (unit)	5	63.189		27.326	
SAMPLE (subplot)					
Time	6	25.167	151.246 ***	116.842	3857.324 ***
Time.Treat	24	10.341	62.148 ***	11.210	370.084 ***
Error(Sample)	30	6.736		0.030	
% Coefficient of Variation		16.2		5.3	
LSD(0.01) between two means at -					
different times in one treatment:		1.12		0.48	
any time between treatment:		1.54		0.84	

		Nitrogen Volatilization [2]					
		Ammonia			Nitrogen Oxides		
	df	MS	F	df	MS	F	
UNIT (plot)							
Treatment	3	0.0589	11821.769 ***	1	0.2158	758.614 ***	
Error (unit)	4	0.00000498		2	0.0002844		
SAMPLE (subplot)							
Time	12	0.00288	70.815***	12	0.0477	175.935	***(**)
Time.Treat	36	0.00318	78.223***	12	0.0167	61.545	***(*)
Error(Sample)	48	0.00004069		24	0.0002714		
% Coefficient of Variation		15.9			10.4		
LSD(0.01) between two means at -							
different times in one treatment:		0.171			0.461		
any time or treatment:		0.284			1.638		

[1] Calculated from the data given in Appendix 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$,

***- significant at $p < 0.001$, () significance levels with 3 & 4 df.

[2] Data was transformed by $\log^1 + 1$ & reported transformed.

B.) Fish-, Urea- and Sewage-bark Composts, Initial C:N=25 and 35. [1]

Split-plot AOV

		Ammonification		Nitrification	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	5	1049.174	1018.424 ***	644.559	342.565 ***
Error (unit)	6	1.030		1.882	
SAMPLE (subplot)					
Time	6	315.057	257.173 ***	1563.569	1518.078 ***
Time.Treat	30	61.431	50.145 ***	136.100	132.140 ***
Error(Sample)	36	1.225		1.030	
% Coefficient of Variation		9.6		8.9	
LSD(0.01) between two means at -					
different times in one treatment:		3.01		2.76	
any time between treatment:		4.06		3.99	

		Nitrogen Volatilization			
		Ammonia		Nitrogen Oxides [2]	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	5	5461.694	375.338 ***	0.28855	2133.515 ***
Error (unit)	6	14.551		0.0001352	
SAMPLE (subplot)					
Time	12	420.544	117.025 ***	0.09711	247.005 ***
Time.Treat	60	284.912	79.282 ***	0.03156	80.285 ***
Error(Sample)	72	3.594		0.02144	
% Coefficient of Variation		18.8		10.5	
LSD(0.01) between two means at -					
different times in one treatment:		5.027		0.0526	
any time or treatment:		7.807		0.0716	

[1] Calculated from the data given in Appendix 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$,

***- significant at $p < 0.001$,

[2] Data was transformed by $\log_{10} + 1$ & reported transformed.

pH DURING COMPOSTING:

pH in Fish-, Urea- and Sewage-bark Composts, of Various Initial C:N Ratios. [1]

Split-plot AOV

		Fish-, Urea- & Sewage-bark Composts, C:N=25 & 35.		Fish-bark Composts, C:N=25 to 65.	
	df	MS	F	MS	F
UNIT (plot)					
Treatment	5	7.746	835.535 ***	2.596	183.551 ***
Error (unit)	6	0.00927		0.0142	
SAMPLE (subplot)					
Time	7	2.257	210.903 ***	3.8156	314.227 ***
Time.Treat	35	0.3217	30.065 ***	0.2548	20.987 ***
Error(Sample)	42	0.3217		0.0124	
% Coefficient of Variation		1.6		1.7	
LSD(0.01) between two means at -					
different times in one treatment:		0.38		0.51	
any time between treatment:		0.28		0.30	

[1] Calculated from the data given in Appendix 4.

ns- not significant ($p < 0.05$), **- significant at $p < 0.01$,

***- significant at $p < 0.001$,

APPENDIX - 9

IDENTIFICATION & BIOCHEMICAL CHARACTERISTICS OF COMPOST ISOLATES

All *Bacillus spp.* were identified by the methods of Gordon, *et.al.* (1973) while other bacteria were identified using Bergey's Manual (1974) and fungi were identified morphologically (Barrow, 1968).

Thermophilic *Bacillus spp.* were also analysed using the Clustan 1C program (Wishart, 1968). Most strains were placed into one of 12 phenons (Figure 36) grouped, as indicated in the tables, by the UPGMA algorithm (Sneath and Sokal, 1973) using the simple matching coeffifient (Sokal and Michener, 1958). Strains of *B. stearothermophilis* were also placed into the three groups (g1, g2 or g3) of Walker and Wolf (1971).

KEY

Gram: Gram stain reaction (+/-).

Morphology: morphology under high power (d- dipteroid, f- filamentous, F- fungal, p- pleomorphic, pr- palisade rods, r- rod, y- budding yeast).

Mycelium: aerial mycelium (+/-).

Spore
Swollen: swelling of the sporangium (+/-).
Location: (c- central, p- paracentral, s- subterminal, t- terminal c1- chain of > 50 spores, cs- chain of < 20 spores, mf- mycelium fragments into spores, a- aerial spores only, as- aerial & substrate borne).

Spreader: spreading growth on TSA (2.0% agar) (+/-).

Metabolism: oxidative or respiratory metabolism in Huge & Leifsons medium.

Flaglla: presence of flagella (s-single polar, p- peritricous, or -).

Pigment: colony colour (B- blue, b- brown, G- green, g- grey, r- red-pink, v- violet, w- white, y- yellow or - nil).

Acid fast: acid fast cell wall (+/-).

Cell Wall: cell wall type (1, 2, 3, 4).

M.R.: methyl red test for acid (+/-).

V.P.: Voges-Proskauer test for acetoin production (+/-).

V.P. pH: pH in unbuffered V.P. broth after 7d incubation; uninoculated control = 6.8 (pH).

Catalase: prduction of O_2 from cells in a drop of 0.3% peroxide (+/-).

30°, 55°, 65°, 70°: growth on TSA or at 70°, TS medium (+/-).

7% salt: growth in nutrient broth with 7% NaCl added.

Citrate: utilization as sole C source (+/-).

Azide: growth in dextrose broth plus 0.02% Na azide (+/-).

Anaerobic: growth on TSA slant under anaerobic conditions (S- strict anaerobe,+/-).

pH 7.5: growth at pH 5.7 on Sabouraud dextrose slant (+/-).

Arabinose, Xylose, Mannose, Glucose: growth and acid production on slant of basal medium (inorganic N) with 0.5% sugar added (+/-).

Starch: hydrolysis after 5d.

NO_2: reduction of nitrate to nitrite within 14d.

N_2: reduction of nitrate to dinitrogen within 14d.

DNase: hydrolysis on DNase agar (Oxoid) within 5d (+/-).

RNase: hydrolysis, as for DNase except bovine RNA replaced DNA (+/-).

Cellulase, Lignase, Pectinase, Xylanase: hydrolysis of NaCMC, Indulin AT lignin, pectin or xylan in mineral salts agar with 0.5% C source added to the top layer (s- strong, m- medium, w- weak hydrolysis or - nil).

Lipase: hydrolysis of Tween 20 (+/-).

Thermophilic *Bacillus spp.* Isolates, R2

Isolate	Identification	Phenon	Swollen	Position	Spreader	Pigment	V.P.	pH V.P.	Catalase	30°	55°	65°	70°	7% Salt	Citrate	Azide	Anaerobic	pH 5.7	Arabinose	Xylose	Mannose	Glucose	Starch	NO_3^-	N_2	DNase	RNase	Cellulase	Lipase	Pectinase	Xylanase
TSA-3	*B.brevis*	1a	+	s	+	b	-	8.4	+	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	+	+	-	m	-	-
TSA-8	*B.brevis*	1a	+	s	+	b	-	8.3	+	-	+	-	-	-	+	-	-	-	+	+	-	+	-	-	+	+	-	m	-	-
TSA-61	*B.brevis*	1a	+	s	-	-	-	8.2	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	w	-	-
CMC-76	*B.brevis*	1a	+	s	-	-	-	8.0	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	m	-
LIG-105	*B.brevis*	1a	+	s	-	-	-	8.5	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-
LIG-107	*B.brevis*	1a	+	s	-	-	-	8.4	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
TSA-185	*B.brevis*	1a	-	s	-	-	-	8.6	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-
TSA-226	*B.brevis*	1a	+	s	-	-	-	8.1	+	-	+	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	+	-	-	-
CMC-73	*B.brevis*	2	+	s	-	-	-	8.6	+	-	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	+	w	w	-
TSA-221	*B.brevis*	2	+	s	-	-	-	8.3	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	-	w	-
TSA-64	*B.brevis*	5	+	s	-	-	-	8.5	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	m	-
TSA-66	*B.brevis*	5	+	p	+	b	-	8.3	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	m	-
LIG-30	*B.brevis*	7	+	c	+	b	+	8.6	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	m	-
TSA-5	*B.brevis*	8	+	c	+	b	-	8.5	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	m	-
LIG-29	*B.brevis*	8	+	c	+	b	-	8.4	+	-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	m	-
PA -78	*B.brevis*	8	+	c	+	-	-	8.2	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
LIG-81	*B.brevis*	8	+	c	+	b	-	8.2	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-
LIG-82	*B.brevis*	8	+	p	+	b	-	8.6	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	w	-
TSA-90	*B.brevis*	8	+	c	+	b	-	8.1	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-
TSA-91	*B.brevis*	8	+	c	+	b	-	8.0	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	w	-
PA -101b	*B.brevis*	8	+	p	+	-	-	8.4	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-
LIG-106	*B.brevis*	8	+	s	+	+	-	8.6	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-
TSA-151	*B.brevis*	8	+	c	+	-	-	8.0	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-
PA -103	*B.circulans*	2	+	t	-	-	-	4.6	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	-	-
TSA-115	*B.circulans*	2	+	s	-	-	-	4.9	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	w	-	-
PA -129	*B.circulans*	2	+	s	-	-	-	6.2	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	s	-	-
TSA-139	*B.circulans*	2	+	s	-	-	-	5.1	+	+	+	-	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+	-	-	-
TSA-181	*B.circulans*	2	+	t	-	-	-	4.7	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	-	-	-
TSA-224	*B.circulans*	2	+	t	-	-	-	5.4	+	-	+	-	-	+	-	-	-	-	+	+	-	+	+	+	-	+	+	-	m	-
TSA-225	*B.circulans*	2	+	t	-	-	-	4.8	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	w	-
PA -232	*B.circulans*	2	+	s	-	-	-	5.0	-	-	+	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	m	-
LIG-243	*B.circulans*	2	+	s	-	-	-	5.5	+	+	+	-	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	m	-
TSA-180	*B.circulans*	6	+	c	-	-	-	6.0	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-	s	-
LA -235	*B.circulans*	6	+	c	-	-	-	5.8	+	-	+	-	-	+	+	-	-	-	-	+	-	+	+	+	-	+	+	-	m	-
LA -238	*B.circulans*	6	+	c	-	+	-	5.5	-	-	+	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	+	-	s	-
LIG-200	*B.circulans*	-	+	t	-	-	-	5.7	+	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	w
CMC-13	*B.coagulans*	-	-	s	-	+	-	4.4	+	-	+	+	-	+	-	+	+	+	-	+	+	+	-	-	-	+	+	-	-	-
CMC-163	*B.coagulans*	-	+	s	+	-	-	4.8	-	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-
PA -233	*B.coagulans*	-	+	t	-	-	-	4.3	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	-	+	+	-	m	-
PA -12	*B.megaterium*	12	+	s	-	-	-	4.8	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	s	-
TSA-18	*B.sphaericus*	9	+	s	+	-	-	7.6	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
TSA-21	*B.sphaericus*	9	+	s	+	-	-	7.4	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
LA -71	*B.sphaericus*	9	+	t	-	-	-	8.2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	m	-
CMC-74	*B.sphaericus*	9	+	s	-	-	-	7.3	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
PA -101a	*B.sphaericus*	9	-	s	-	-	-	8.0	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	m
TSA-122	*B.sphaericus*	10	-	s	-	-	-	8.3	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	m	-	w
LA -68	*B.stearo. g1*	1b	+	s	-	-	-	5.8	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	s	-	-
CMC-26	*B.stearo. g1*	3	+	t	-	+	-	4.9	+	+	+	+	-	+	-	-	-	-	+	+	-	+	+	+	+	-	+	-	s	-	-
LIG-83	*B.stearo. g1*	3	+	t	-	-	-	5.2	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-	m	-	-
CMC-98	*B.stearo. g1*	3	+	t	-	-	-	5.0	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	m	-	-
PA -24	*B.stearo. g2*	-	+	s	+	-	-	5.8	-	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	s	-	-
PA -25	*B.stearo. g2*	4	+	t	-	+	-	5.9	+	-	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	s	-	-
LA -69	*B.stearo. g2*	4	+	t	-	-	-	5.2	+	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	s	-	-
PA -165	*B.stearo. g2*	4	+	s	+	-	-	5.5	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	s	-	-
TSA-182	*B.stearo. g2*	4	+	c	-	-	-	5.6	+	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	s	-	-
TSA-117	*B.stearo. g3*	1b	+	t	-	-	-	5.7	+	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	s	-	-
LIG-199	*B.stearo. g3*	1b	+	s	-	-	-	5.0	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	m	m	-	-
TSA-19	*B.stearo. g3*	3	+	s	-	-	-	4.7	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	s	-	-
lig-32	*B.stearo. g3*	3	+	t	-	-	-	4.8	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	s	-	-
TSA-34	*B.stearo. g3*	3	+	t	-	-	-	4.9	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	m	-	-
TSA-116	*B.stearo. g3*	3	+	t	-	-	-	5.7	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
CMC-124	*B.stearo. g3*	3	+	t	-	-	-	5.8	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	s	-	-
CMC-28	*B. sp.*	-	+	c	-	-	-	5.2	+	+	+	+	-	+	-	-	-	-	+	+	-	+	-	+	-	-	-	-	m	-	-
LIP-43	*B. sp.*	6	+	t	-	-	-	4.9	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	m	-	-
TSA-60	*B. sp.*	1a	+	s	+	-	-	8.6	+	-	+	+	-	-	-	-	-	-	+	+	-	+	-	+	-	+	+	-	s	-	-
TSA-92	*B. sp.*	3	+	s	-	-	-	8.2	+	-	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+	-	s	-	-
CMC-100	*B. sp.*	3	-	s	-	-	-	6.4	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	w	m	-	-
PA -102b	*B. sp.*	1b	+	s	-	-	-	5.6	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	w	m	-

Other Thermophiles, R2

Isolate Identification	Gram Stain	Morphology	Mycelium	Spore	Metabolism	Flagella	Pigment	Acid fast	Cell wall	M.R.	V.P.	Catalase	30°	55°	65°	70°	7% Salt	Citrate	Azide	Anaerobic	pH 5.7	Arabinose	Xylose	Mannose	Glucose	Starch	NO_2^-	N_2	DNase	RNase	Cellulase	Lignase	Lipase	Pectinase	Xylanase
CMC-203 *Cellulomonas sp.*	+	p	-	-	o	-	y	-		-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	+	m	-	w	w	+
CMC-205 *Cellulomonas sp.*	+	p	-	-	f	-	y	-		-	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	m	-	w	w	+
TSA-184 *Corynebacterium sp.*	+	r	-	-	f	-	-	-		-	-	+	-	+	+	-				+		-	-	+	+	+	+	-	+	+	-	-	w	a	-
CMC-123 Coryneform	+	c	-		o	-	r	-	1	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-
LIG-198 Coryneform	+	d	-		o	-	r	-	1	-	-	+	-	+	-	-	-	-	-	-	-	-		-	+	-	+	-	-	-	w	-	-	-	-
LIG-240 Coryneform	+	d	-		o	-	r	-	1	-	-	+	-	+	+	-	+	-	-	-	-	+	+	-	-	-	+	-	+	+	c	w	-	-	-
TSA-166 *Clostridium sp.*	+	r	-	t	f	-	-	-		+	-	-	+	+	-	-	-	+	+	s	+	+	+	+	+	+	+	-	+	+	-	-	-	a	w
TSA-167 *Clostridium sp.*	+	r	-	st	f	-	-	-		+	-	-	+	+	-	-	-	-	+	s	+	-	+	+	+	+	+	-	+	+	-	-	-	a	-
LIG-201 *Nocardia sp.* T3	+	f	-	mf	o	-	y	+	4	-	-	+	-	+	-	-								+	+	-	+	+			-	-	-	w	-
LIP-94 *Streptomyces sp.*	+	f	+	cl	o	-	y	-	1	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-
TSA-118 *Streptomyces sp.*	+	f	+	cl	o	-	p	-	1	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	m	-
LIP-191 *Streptomyces sp.*	+	f	+	cl	o	-	y	-	1	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	s	m
LIP-192 *Streptomyces sp.*	+	f	+	cl	o	-	y	-	1	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	-	-	s	-
TSA-188 *Thermomonospora sp.*	+	f	+	as	o	-	w		4			+	-	+	-	-				-		-	-	+	+		+	-			-	-	-	w	-

Thermophilic *Bacillus spp.*, R4

Isolate	Identification	Phenon	Swollen	Position	Spreader	Pigment	V.P.	pH V.P.	Catalase	30°	55°	65°	70°	7% Salt	Citrate	Azide	Anaerobic	pH 5.7	Arabinose	Xylose	Mannose	Glucose	Starch	NO_2^-	N_2	DNase	RNase	Cellulase	Lipase	Pectinase	Xylanase
TSA-408	*B.brevis*	1a	+	s	+	-	-	8.3	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	s
CMC-542	*B.brevis*	1a	+	s	-	-	-	8.0	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-
TSA-404	*B.brevis*	2	+	s	+	-	-	8.6	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	w	-	-
CMC-464	*B.brevis*	5	+	s	-	-	+	8.4	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-
TSA-418	*B.brevis*	5	+	s	-	-	+	8.3	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
GAA-548	*B.brevis*	5	+	c	-	-	-	8.0	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-
TSA-454	*B.brevis*	7	+	c	-	-	-	8.0	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	-	+	+	-	s	-	-
TSA-407	*B.brevis*	8	+	c	+	b	-	8.5	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-
TSA-530	*B.brevis*	8	+	c	+	b	-	8.4	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	w	-	-
CMC-536	*B.brevis*	8	+	c	+	-	-	8.1	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
TSA-401	*B.circulans*	2	+	s	-	-	-	5.8	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-
TSA-402	*B.circulans*	2	+	t	-	-	-	6.0	+	-	+	-	-	-	+	-	-	-	+	+	-	+	+	-	-	-	+	-	s	-	-
TSA-406	*B.circulans*	2	+	s	-	-	-	5.9	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-
TSA-410	*B.circulans*	2	+	s	-	-	-	5.5	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	w	-
LIG-467	*B.circulans*	2	+	t	+	-	-	5.0	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-
TSA-529	*B.circulans*	2	+	s	-	-	-	6.0	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-
CMC-543	*B.circulans*	2	+	s	-	-	-	5.7	+	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	s	s	-
TSA-552	*B.circulans*	2	+	s	-	-	+	6.0	-	-	+	-	-	-	-	-	+	-	+	-	-	+	-	+	-	-	+	-	s	-	-
TSA-403	*B.circulans*	6	+	c	-	-	-	4.9	+	-	+	-	-	+	+	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-
TSA-417	*B.circulans*	6	+	p	-	-	-	5.6	+	-	+	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-
LA -461	*B.circulans*	6	+	s	-	-	+	5.0	+	+	+	-	-	+	-	-	-	-	+	+	-	+	-	-	-	+	-	-	s	-	-
LA -533	*B.circulans*	6	+	s	-	p	-	6.2	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	s	s	-
LA -414	*B.coagulans*	2	+	t	-	-	+	4.8	+	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-
TSA-553	*B.coagulans*	-	+	c	-	-	-	4.3	-	-	+	+	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	s	-	-
LIG-547	*B.megaterium*	12	-	s	-	-	-	6.3	+	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	s	-
LA -462	*B.sphaericus*	6	+	s	-	y	-	7.6	-	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	s	-	-
TSA-400	*B.sphaericus*	9	+	s	+	-	-	8.0	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
LA -413	*B.sphaericus*	9	+	s	+	-	+	8.4	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	s	-	-
TSA-459	*B.sphaericus*	9	+	t	-	-	-	8.4	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
TSA-528	*B.sphaericus*	9	+	s	-	-	-	8.0	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
LA -534	*B.sphaericus*	9	-	s	+	w	-	8.6	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	w	-	-
LA -535	*B.sphaericus*	9	-	s	-	-	-	8.3	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-
CMC-537	*B.sphaericus*	9	-	s	-	-	-	8.6	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMC-541	*B.sphaericus*	9	-	s	-	-	-	8.3	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	s	s	w	-
CMC-544	*B.sphaericus*	9	-	s	-	-	-	7.6	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIG-545	*B.sphaericus*	11	-	s	-	-	-	7.4	+	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
GAA-549	*B.sphaericus*	11	-	s	-	-	-	7.6	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	w	-
TSA-550	*B.sphaericus*	11	-	t	-	-	+	7.4	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	w	-
TSA-551	*B.sphaericus*	11	-	t	-	-	+	7.9	-	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
LIG-469	*B.stearo.* g1	3	+	t	-	-	-	5.8	+	-	+	+	+	-	-	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-
TSA-493	*B. sp.*	-	+	s	-	-	+	4.9	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	-	-	-
TSA-494	*B. sp.*	-	+	t	-	-	+	5.3	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	-	-	-	s

Mesophilic *Bacillus spp.*, R4

TSA-421	*B.brevis*		+	c	-	-	-	8.2	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
TSA-481	*B.brevis*		+	s	-	-	-	8.4	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	w	-	w	-
CMC-488	*B.brevis*		+	c	-	-	-	8.6	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	w	-	w	-
CMC-489	*B.brevis*		+	s	-	-	-	8.3	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
TSA-673	*B.brevis*		+	c	-	-	-	8.4	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-	-
TSA-424	*B.circulans*		+	c	-	-	-	5.0	+	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-
TSA-427	*B.circulans*		+	c	-	-	-	5.2	+	+	+	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-
TSA-594	*B.circulans*		+	s	-	-	-	5.7	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	w	-
TSA-429	*B.megaterium*		+	c	-	-	-	6.0	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	w	w	s	-
TSA-483	*B.megaterium*		+	s	-	-	-	4.8	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	+	w	w	-	-
LIP-487	*B.megaterium*		+	c	-	-	-	5.2	+	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	-	w	w	-	-
GAA-601	*B.megaterium*		-	c	-	-	-	4.6	+	+	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	w	-	-
LIG-669	*B.megaterium*		+	s	-	-	-	5.0	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	w	-	-
TSA-422	*B.sphaericus*		+	t	-	-	-	7.6	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-
TSA-425	*B.sphaericus*		+	c	-	-	-	8.1	+	+	+	-	-	+	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
CMC-433	*B.sphaericus*		+	s	-	-	-	7.6	+	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	+	w	-	-	-	-
TSA-480	*B.sphaericus*		+	s	-	-	-	7.8	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	w	-	-	-	-
TSA-482	*B.sphaericus*		+	s	-	-	-	7.9	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	w	-	-	-	-	-
TSA-499	*B.sphaericus*		-	s	-	-	-	7.7	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSA-580	*B.sphaericus*		+	s	-	-	-	7.8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-
TSA-582	*B.sphaericus*		+	s	-	-	-	7.5	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	w	-
CMC-668	*B.sphaericus*		-	c	-	-	-	7.5	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	w	-
TSA-672	*B.sphaericus*		+	s	-	-	-	8.3	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
TSA-660	*B.subtilis*		-	s	-	-	+	5.2	+	+	+	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	w	-	s	-

Other Microorganisms. Isolated at 55°, R4

Isolate	Identification	Gram Stain	Morphology	Mycelium	Spore	Metabolism	Flagella	Pigment	Acid fast.	Cell wall	Catalase	30°	55°	65°	70°	7% Salt	Citrate	Azide	Anaerobic	pH 5.7	Arabinose	Xylose	Mannose	Glucose	Starch	NO_2^-	N_2	DNase	RNase	Cellulase	Lignase	Lipase	Pectinase	Xylanase
LIG-416a	*Brevibacterium sp.*	+	r	-	-	o		-	-		-	+	+	-	+	-	-	-	-	-	-	-					-	-	-	w	c	-	w	-
TSA-492	*Cellulomonas sp.*	+	r	-	-	f	-	-	-	1			+	-	+	-	-	-		-	+	-			+	+	-	-	-	m	-	m	w	-
CMC-541	*Cellulomonas sp.*	+	r	-	-	f	-	-	-		-	+	-	+	-	-	+	-	+	-	-			-	-	-	-	-	-	w	b	-	w	-
TSA-495	*Clostridium sp.*	+	r	-	st	f	-	-	-		+	-	-	+	+	-	-	-	-	-	s	+	-	+	+	+	+	+	-	-	-	-	m	w
LIG-468	Coryneform	+	r	-	-	o		-	-		-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	w	-	-	-	-
TSA-452	*Flavobacterium sp.*	-	r	-	-	o	-	y	-		-	-	-	+	-	-	-	-	-	-	-				-	-	-	-	-	-	-	-	-	-
LIP-460	*Flavobacterium sp.*	-	r	-	-	o	-	y	-		-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSA-521	*Flavobacterium sp.*	-	r	-	-	o	-	y	-		-	-	-	-	+	-	-	+	-	-	-	-			-	-	-	+	+	-	-	-	-	-
CMC-540	*Klebsella sp.*	-	r	-	-	f	-	-	-		-	+	+	+	-	-	-	-	-	-	-	+			-	+	-	-	-	-	-	-	-	-
CMC-465	*Micropolyspora sp.*	+	f	+	cs	o		g	-	4	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	m	m
TSA-522	*Micropolyspora sp.*	+	f	+	as	o	-	b	-	4	-	-	+	-	+	-	-	-	-	-	-	-				-	-	+	+	-	-	-	-	-
TSA-401	*Pseudomonas sp.*	-	r	-	-	o	s	-	-		-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSA-451	*Pseudomonas sp.*	-	r	-	-	o	s	-	-		-	-	+	-	+	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-
TSA-520	*Pseudomonas sp.*	-	r	-	-	o	-	-			-	-	+	+	+	-	-	-	-	-	-	-				-	+	-	-	-	-	-	-	-
CMC-538	*Pseudomonas sp.*	-	r	+	-	o	s	-	-		-	-	+	-	+	-	-	-	-	-	-	-				-	-	-	-	-	-	-	-	-
TSA-405	*Streptomyces sp.*	+	f	+	cl	o	-	w	-		-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
TSA-411	*Streptomyces sp.*	+	f	+	cl	o	-	y	-	1	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	w	-	-	-	k
CMC-415	*Streptomyces sp.*	+	f	+	cl	o	-	v	-	1	-	-	+	-	+	-	-	-	-	-	-	-					+	-	-	-	-	m	-	-
CMC-420	*Streptomyces sp.*	+	f	+	cl	o	-	v	-		-	-	+	+	+	-	-	-	-	-	-	-					+	-	-	-	-	-	-	-
TSA-450	*Streptomyces sp.*	+	f	+	cl	o	-	w	-		-	-	+	-	+	-	-	-	-	-	-	-					-	-	-	-	-	m	-	-
TSA-455	*Streptomyces sp.*	+	f	+	cl	o		w	-				+	+	+	-	-	+	-	-	-	-					-	-	+	-	-	m	-	-
TSA-456	*Streptomyces sp.*	+	f	+	cl	o		g	-				+	-	+	-	-	+	-	-	-	-					-	+	-	-	-	-	-	s
TSA-458	*Streptomyces sp.*	+	f	+	cl	o		y	-	1	-	-	+	+	+	-	-	+	-	-	-	-					-	+	+	+	-	-	-	-
LIP-463	*Streptomyces sp.*	+	f	+	cl	o		w	-		-	-	-	+	+	+	-	-	-	-	-	-					-	+	-	-	-	-	-	-
CMC-466	*Streptomyces sp.*	+	f	+	sb	o		w			-	-	-	-	+	-	-	-	-	-	-	-					-	-	+	-	-	-	-	-
TSA-523	*Streptomyces sp.*	+	f	+	cl	o	-	B	-		-	-	+	-	+	-	-	-	-	-	-	-					-	-	-	-	-	-	-	-
TSA-524	*Streptomyces sp.*	+	f	+	cl	o	-	w	-		-	-	+	-	+	-	-	-	-	-	-	-					-	+	-	-	-	-	-	-
LIP-532	*Streptomyces sp.*	+	f	+	cl	o	-	g	-		-	-	+	-	+	-	-	-	-	-	-	-					-	-	-	-	s	-	m	-	s
CMC-539	*Streptomyces sp.*	+	f	+	cl	o	-	g	-		-	-	+	+	+	-	-	-	+	+	-	-					-	-	-	-	-	-	-	-	-
TSA-412	*Streptosporangium sp.*	+	f	+	sb	o	-	w	-	3	-	-	+	-	+	-	-	-	-	-	-	-					-	+	+	-	-	-	-	w	w
TSA-453	*Streptosporangium sp.*	+	f	+	s	o	-	w	-	3	-	-	+	-	+	-	-	-	-	-	-	-					-	+	-	-	-	-	-	-	-
CMC-466	*Streptosporangium sp.*	+	f	+	sb	o		w			-	-	-	-	+	-	-	-	-	-	-	-					-	-	+	-	-	-	-	-	-
TSA-409	*Thermoactinomyces sp.*	+	f	+	as	o	-	w	-	3	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	m	-	-
TSA-457	*Thermoactinomyces sp.*	+	f	+	as	o		w		3	-	-	+	-	+	-	-	-	-	-	-	-					-	+	-	-	-	-	-	-	-
LIP-531	*Thermomonospora sp.*	+	f	+	a	o	-	y	-	4	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-

Other Microorganisms. Isolated at 28°, R4

Isolate Identification	Gram Stain	Morphology	Mycelium	Spore	Metabolism	Flagella	Pigment	Acid fast.	Cell Wall	Catalase	30°	55°	65°	7% Salt	Citrate	Azide	Anaerobic	pH 5.7	Arabinose	Xylose	Mannose	Glucose	Starch	NO_3^-	N_2	DNase	RNase	Cellulase	Lignase	Lipase	Pectinase	Xylanase
TSA-585 *Brevibacterium sp.*	+	r	-	-	o	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-
TSA-496 *Clostridium sp.*	+	r	-	t	f	-	-	-	+	-	+	+	-	-	-	-	-	+	s	-	-	-	-	-	-	-	-	-	-	-	w	-
TSA-497 *Clostridium sp.*	+	r	-	t	f	-	-	-	+	-	-	+	-	-	-	-	-	+	s	-	-	-	-	-	-	-	-	-	-	-	w	-
TSA-501 *Corynebacterium sp.*	+	r	-	-	o	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	w	-	-	-	-
TSA-502 *Corynebacterium sp.*	+	r	-	-	o	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	w	-
TSA-581 *Corynebacterium sp.*	+	r	-	-	o	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	w	-
TSA-583 *Corynebacterium sp.*	+	r	-	-	o	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	w	-
CMC-592 *Corynebacterium sp.*	+	r	-	-	o	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	s	-
LIG-598 *Corynebacterium sp.*	+	r	-	-	o	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	s	-
TSA-662 *Corynebacterium sp.*	+	r	-	-	o	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	s	-	-	w	-
CMC-435 *Cytophaga sp.*	-	r	-	-	o	-	y	-	-	-	+	+	-	-	-	-	-	+	-	-			-	-	-	-	-	-	s	-	g	-
TSA-661 *Enterobacter sp.*	-	r	-	-	f	p	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	g	-
TSA-420 *Flavobacterium sp.*	-	r	-	-	o	s	y	-	-	-	+	+	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-
CMC-596 *Flavobacterium sp.*	-	r	-	-	o	-	y	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIG-600 *Flavobacterium sp.*	-	r	-	-	o	-	y	-	-	-	+	+	-	-	-	-	+	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-
TSA-432 *Klebsiella sp.*	-	r	-	-	f	-	-	-	+	-	+	+	-	-	-	+	+	+	+	+			-	+	-	+	-	-	-	-	w	-
TSA-500 *Klebsiella sp.*	-	r	-	-	f	-	-	-	-	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
CMC-597 *Klebsiella sp.*	-	r	-	-	f	-	-	-	+	-	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	+	+	-	c	-	w	-
CMC-434 *Pseudomonas sp.*	-	r	-	-	o	s	-	-	-	-	+	+	-	-	-	-	+	+	-	-			-	-	-	-	-	s	-	-	w	-
TSA-586 *Pseudomonas sp.*	-	r	-	-	o	s	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMC-591 *Pseudomonas sp.*	-	r	-	-	o	s	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	s	-
CMC-595 *Pseudomonas sp.*	-	r	-	-	o	s	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	w	-
TSA-423 *Streptomyces sp.*	+	f	+	cl	o	-	w	-	-	-	+	+	-	-	-	+	-	+	-	-			-	-	+	-	-	w	-	-	s	m
CMC-436 *Streptomyces sp.*	+	f	+	cl	o	-	g	-	-	-	-	+	-	-	-	+	+	+	-	-			-	-	+	-	-	w	-	-	s	s
TSA-498 *Streptomyces sp.*	+	f	+	cs	o	-	w	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	s	s
CMC-593 *Streptomyces sp.*	+	f	+	cl	o	-	w	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+	-	w	-	-	w	-
LIG-599 *Streptomyces sp.*	+	f	+	cl	o	-	w	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	w	-	-	s	-
TAA-439 *Aspergillus sp.*	+	F	+		o	-	G	-				+	-	-	-	-	-	+	-	+			-	-	-	-	-	-	c	-	s	s
LIG-490 *Aspergillus sp.*	+	F	+		o	-	w	-				+	-	-	-	-	+	+	-	+			-	-	-	-	-	w	d	-	s	s
TAA-437 *Penicillium sp.*	+	F	+		o	-	G	-				+	-	-	-	+	-	+	-	+			-	-	+	-	-	w	c	-	s	s
TSA-426 *Trichoderma sp.*	+	F	+		o	-	w	-	-	-	+	+	+	-	-	+	+						-	-	+	-	-	-	c	w	s	-
CMC-665 Yeast	+	y	-		f	-					+	+	-	-	-											-	-	g	-	-	-	-

Appendix - 10

Relative Tolerance to Composts (C:N=35) of the Predominant Flora Isolated at each Peak of Respiratory Activity [1]

Genus	Isolate-Peak [2]	% Growth Along Streak on Compost Sampled at Peak: 1		2		3		4		& Climax	
Urea-bark Compost											
Bacillus sp.	B01-1	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B02-1	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B03-1	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B17-2	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B18-2	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B19-2	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B29-3	90	82	95	98	100	100	100	100	100	100
Bacillus sp.	B30-3	56	57	61	74	96	100	100	100	100	100
Streptomyces sp.	B31-3	0	0	5	7	88	100	100	100	100	100
Thermomonospora sp.	B50-4	0	0	12	16	100	100	100	100	59	68
Thermomonospora sp.	B51-4	0	0	6	10	100	100	100	100	36	23
Streptomyces sp.	B54-4	0	0	33	39	100	100	100	100	100	100
Thermomonospora sp.	B65-C	0	0	0	0	100	100	100	100	86	90
Thermomonospora sp.	B66-C	0	0	8	5	93	88	100	100	40	31
Streptomyces sp.	B67-C	0	0	69	66	100	100	100	100	100	100
IBDU-bark Compost											
Bacillus sp.	B12-1	100	100	100	100	100	100	100	100	100	100
Bacillus sp.	B13-1	100	100	100	100	100	100	100	100	100	100
Streptomyces sp.	B14-1	88	100	100	100	100	100	100	100	100	100
Thermomonospora sp.	B24-2	0	0	55	84	100	100	100	100	44	60
Thermomonospora sp.	B25-2	0	0	66	45	89	100	100	100	83	70
Pseudomonas sp.	B26-2	12	20	100	100	100	100	100	100	100	100
Thermomonospora sp.	B45-3	0	0	12	0	100	100	100	100	89	33
Thermomonospora sp.	B46-3	0	0	22	15	93	81	100	100	100	100
Streptomyces sp.	B47-3	0	0	79	66	100	100	100	100	100	100
Streptomyces sp.	B59-4	0	0	55	30	100	100	100	100	100	100
Streptomyces sp.	B60-4	0	0	55	67	100	100	100	100	100	100
Thermomonospora sp.	B61-4	0	0	0	0	69	73	100	100	73	57
Streptomyces sp.	B73-C	0	0	14	22	100	100	100	100	100	100
Streptomyces sp.	B74-C	0	0	39	24	100	100	100	100	100	100
Bacillus sp.	B77-C	90	100	100	100	100	100	100	100	100	100

[1] The three most numerous isolates obtained from R8 at each peak in respiratory activity (Figure 19) and at d28 (climax flora) were streaked across a sterile compost suspention of varing concentration in solid agar. The precentage growth along the streak is given for duplicate runs.

[2] Isolates were named by culture number and associated peak in respiratory activity (-1, -2, -3, -4 or C).

Anaylsis of Variance of Bacterial Growth in Agar Containing Compost of Various Ages. [1]

Source of Variation	df	MS	F-Test
Peak of Respiration	4	20.586	2377.027 ***
Isolate	21	0.7627	88.070 ***
Peak.Isolate	84	0.3726	43.023 ***
Residual	110	0.00866	

Coefficient of Variation 6.1 %

$LSD_{(0.01)}$ between any two means : 0.244

[1] Calculated on $\log_{10} + 1$ transformed data given in the above table. *** - significant differance at $p < 0.01$.

Appendix - 11

PHYTOTOXINS OF BARK AND COMPOST

A.) Plant Bioassay of Phytotoxins in 28 Day Old Composts [1]

Compost	C:N	PVP[2]	Length of Lettuce Radical (mm) for Rep: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Fish-bark	25	+	0	0	0	0	1	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0
		-	0	2	2	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	0
	35	+	12	20	15	16	10	13	11	15	17	15	13	18	12	12	15	14	18	13	16	16
		-	15	10	13	17	15	12	13	14	16	10	9	14	17	22	18	13	14	16	14	13
Urea-bark	25	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	35	+	16	13	8	7	12	13	8	6	7	7	8	4	12	9	10	7	9	10	11	9
		-	7	9	8	9	13	12	12	11	7	6	8	9	10	5	6	7	8	12	11	10
Sewage-bark	25	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	35	+	7	12	5	6	6	6	5	4	2	3	3	3	2	2	6	4	9	8	10	6
		-	3	3	9	8	10	13	5	4	6	3	4	4	3	7	3	5	2	2	1	3
Water Control		+	22	25	29	20	23	23	24	25	21	21	23	28	25	22	24	26	27	22	20	23
		-	26	26	25	28	24	22	28	23	23	20	20	24	25	22	27	26	23	25	24	25

[1] Lettuce seedling were grown in buffered (pH 6.0) water extracts (20%) of composts for 72h at 28° in the dark.

[2] Polyvinylpolypyrrolidone (PVP) was shacken twice with the extracts, centrifuged, and the supernatant used in the bioassay.

Analysis of Variance of Lettuce Root Lengths Following Growth of Plants in Water Extracts of 28d Old Compost [1]

Source of Variation	df	MS	F-Test
Compost	7	5.491	244.129 ***
Residual	152	0.0225	

Coefficient of Variation 18.1 %

$LSD_{(0.01)}$ between any two means : 0.122

[1] Calculated on $\log_{10} + 1$ transformed data given in the above Table. *** = significant difference $p < 0.01$. See Table 24.