Biodegradation of lignin in a compost environment: a review

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Abstract

Composting is nowadays a general treatment method for municipal solid waste. Compostable household waste contains, together with vegetable material, varying amounts of papers and boards. In the European Union composting is regarded as one recycling method for packages and this will probably favour compostable packages, like papers and boards, in the future. Paper is made up of lignocellulose and it may contain up to 20% of lignin. Efficient degradation of papers in composting plants means that biodegradation of lignin is also needed. However, very little is known about lignin degradation by mixed microbial compost populations, although lignin degradation by white-rot fungi has been extensively studied in recent years. Organic material is converted to carbon dioxide, humus, and heat by compost microorganisms. It is assumed that humus is formed mainly from lignin. Thus, lignin is not totally mineralized during composting. The elevated temperatures found during the thermophilic phase are essential for rapid degradation of lignocellulose. Complex organic compounds like lignin are mainly degraded by thermophilic microfungi and actinomycetes. The optimum temperature for thermophilic fungi is 40–50°C which is also the optimum temperature for lignin degradation in compost. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Paper and board are the best recycled materials in Europe. More than 40% of the total production is collected and recycled annually, and the proportion of recycling is constantly growing. Corrugated board forms the best recovered part of paper and board and, furthermore, almost half of all recycled fibres are used for manufacturing corrugated board while 12% is used for manufacturing newspaper (Goddard, 1997; Leppänen-Turkula, 1999).

According to the European Union Packaging and Packaging Waste Directive (1994) by the year 2001 25–45% of packaging waste must be recycled. This target can be met with paper and board, but in some cases, recycling as material is not environmentally sound. Then one option would be composting. 'Recycling' means 'reprocessing', including composting. Packaging, like paper products and newly developed biodegradable plastics, can be collected directly from consumers along with kitchen biowaste and composted in municipal composting plants, provided that they are compostable under such conditions (Itävaara et al., 1997). In recent years intensive research has been focused on the development of biodegradable plastics (Albertsson and Karlsson, 1995; Forssell et al., 1996; Itävaara et al., 1997), but relatively little attention has been paid to the biodegradability and compostability of paper products. More information is needed about the biodegradation of paper products in a compost environment, information about lignin degradation is especially important, because lignin is a recalcitrant polymer and paper may contain up to 20% of lignin (Biermann, 1993).

The European standardization organization, CEN, is developing test methods and recently finalized a test scheme for the evaluation of the compostability of various packaging materials (CEN TC261, SC4, WG2, draft, 1998). In the scheme the biodegradability of organic components is studied using mineralization and disintegration measurements. Wood fibres shall be

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accepted as being biodegradable, because of their natural origin, but must fulfil the criteria for disintegration and compost quality (CEN TC261,SC4,WG2, draft, 1998; Pagga, 1999).

What is currently known about the biodegradability and compostability of lignocellulosic materials – especially lignin – is reviewed in this literature survey. Microbial activity during composting is also briefly reviewed. The main emphasis is placed on thermophilic fungi because they occur frequently in compost and fungi, especially white-rot fungi, are also the most important group of lignin biodegraders in nature.

2. Lignocellulose

In nature, lignocellulose accounts for the major part of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle (Fig. 1). Lignocellulose, such as wood, is mainly composed of a mixture of cellulose (ca. 40%), hemicellulose (ca. 20–30%), and lignin (ca. 20–30%) (Sjöström, 1993). Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation (Argyropoulos and Menachem, 1997).

2.1. Lignin in biodegradation studies

The macromolecular properties and structural characteristics of lignin make biodegradation studies diffi-

cult. Suitable model compounds of lignin are difficult to obtain and there are only a few assays which are suitable for biodegradation studies (Buswell and Odier, 1987).

The ideal isolation method of lignin would allow the collection of chemically unmodified lignin with quantitative recovery and free of non-lignin contaminants (Lin and Dence, 1992). None of the existing methods fulfil all these requirements. Some of the methods are more suitable for quantitative lignin analysis, and others can be used to isolate lignin for purposes such as biodegradation studies (Table 1). Milled wood lignin (MWL) (= Björkman lignin) and cellulase enzyme lignin (CEL) are the best matrices for the structural analysis of lignin. However, they are not suitable for quantitative measurement because of their low yields of lignin (Sjöström, 1993; Argyropoulos and Menachem, 1997). The methods used for isolating lignin can be classified into methods in which lignin is selectively removed and recovered from the final solution, and methods in which lignin is left as an insoluble residue following dissolution of the carbohydrates. Determination of Kappa number and Klason lignin are the most common methods used to analyse lignin quantitatively. Kappa number is an important parameter in the pulp and paper industry and it is determined by oxidizing lignin selectively from pulp using a solution of potassium permanganate (Argyropoulos and Menachem, 1997). Klason lignin is determined gravimetrically after extracting the sample with sulphuric acid to dissolve out the other components (Dence, 1992).
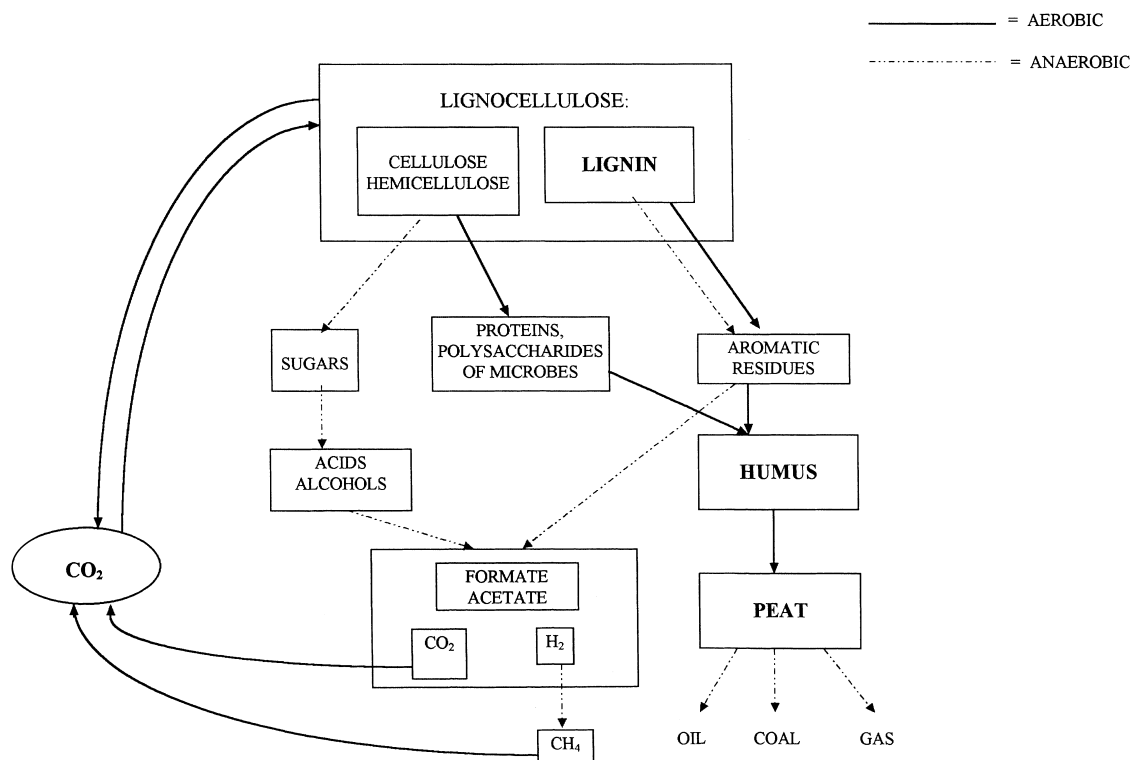

Fig. 1. Global carbon cycle (Brown, 1985; Colberg, 1988).

Table 1
Commonly utilized methods for analyzing, isolating, and synthesizing lignin (Buswell and Odier, 1987; Dence, 1992; Argyropoulos and Menachem, 1997)

Name	Principle of the method	Advantages	Disadvantages
Quantitative measurement of lignin			
Klason lignin Acid-insoluble lignin	Residue after treatment of sample with 72% sulphuric acid	Suitable for a wide range of samples	Structure different from that of lignin
Acetyl bromide method	Lignin is dissolved in acetyl bromide-acetic acid, the amount of lignin measured from the absorbance at 280 nm	Rapid, simple, adaptable for small amounts of sample, minimal chemical modification of sample	Solubility of bleached pulps can be incomplete, oxidation during bleaching of the pulp can change the spectral absorption characteristics of the lignin
Kappa number	The amount of oxidant (potassium permanganate) consumed by sample corresponds to the amount of residual lignin	Rapid, simple	Used mainly in quality control in commercial pulping
Isolation of lignin			
Milled wood lignin (MWL = Björkman lignin)	Ultramilling of wood and extraction with neutral solvents followed by precipitation in water and ethyl ether	Best representation of native lignin, macromolecular	Low yield, preparation time-consuming and cumbersome, some contaminating sugars
Cellulase enzyme lignin (CEL)	Cellulase enzyme treatment	Good representation of native lignin, macromolecular	Low yield, contains protein impurities
Kraft lignin	Cooking in the presence of NaOH and Na_2SO_4	Easy availability	Structure far from native lignin, not entirely macromolecular, contaminating sugars
Brauns' lignin	Extraction with ethanol	Easier preparation compared to MWL	Low molecular weight
Synthetic lignin			
Dehydrogenation polymer (DHP)	Synthesized from its precursors, usually from coniferyl alcohol	Good representation of native lignin, macromolecular	Laborious synthesis

Lignin substructures and dimeric model compounds have been widely used in biodegradation studies because of the lack of good, or easily available, model compounds. Typical lignin models are the dimeric compounds which have the same linkages as found in lignin. The most important compounds are those with β-aryl ether linkages (Buswell and Odier, 1987; Kirk and Farrell, 1987). Aromatic acids such as veratric acid, vanillic acid and syringic acid have also been used as model compounds. The relevance of model compounds for understanding macromolecular lignin degradation has been criticized: the model compounds are water soluble and can be degraded by intracellular substrate-specific enzymes that are different from the enzymes which attack complex water-insoluble lignin (Crawford and Crawford, 1980).

Dehydrogenation polymers (DHPs) are synthetic lignins made of the primary lignin precursors; i.e. cinnamyl alcohols, usually coniferyl alcohol. They have been widely used as model compounds and their chemical and physical properties are well characterized. During synthesis the precursors can be labelled in different positions for radioisotopic studies (Crawford et al., 1980; Hatakka, 1994). The use of preparations of radioactive lignins has solved many problems in lignin degradation studies. ^{14}C-labelled lignins have made it possible to measure the fate of the ^{14}C-label, i.e. to mineralized

lignin, to solubilized lignin, etc., after microbial degradation. It is also possible to prepare specifically labelled lignins, e.g. the ^{14}C-label can replace special carbons on the propane side chains or in aromatic rings (Crawford et al., 1980). Nonisotopic methods are usually restricted to qualitative lignin degradation studies because they provide only a rough estimation of the quantity of degraded lignin compared to the radioactive methods (Crawford et al., 1980).

3. Microorganisms during composting

3.1. Compost environment

During composting microorganisms transform organic matter into CO_2, biomass, thermoenergy (heat) and humus-like end-product, (Figs. 1 and 2). The organic substrates, bulking agents and amendments used in composting are mostly derived from plant material. The main components of the organic matter are carbohydrates (e.g. cellulose), proteins, lipids and lignin. The capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate. The more complex the substrate, the more extensive and comprehensive is the enzyme system required. Through the synergistic action
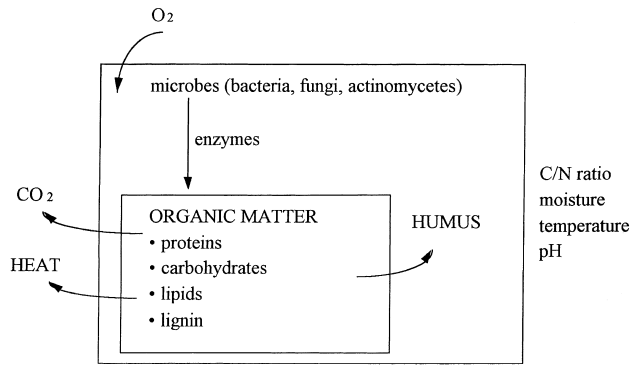
Fig. 2. The composting process (Itävaara et al., 1995).

of microorganisms complex organic compounds are degraded to smaller molecules which can then be utilized by the microbial cells (Golueke, 1991, 1992).

Microorganisms require a carbon source, macronutrients such as nitrogen, phosphorous and potassium, and certain trace elements for their growth. Carbon serves primarily as an energy source for the microorganisms, while a small fraction of the carbon is incorporated into their cells. Some of the energy formed is used for microbial metabolism, and the rest is released as heat. Nitrogen is a critical element for microorganisms because it is a component of the proteins, nucleic acids, amino acids, enzymes and co-enzymes necessary for cell growth and functioning. If nitrogen is a limiting factor during composting the degradation process will be slow. In contrast, if there is excess nitrogen, it is often lost from the system as ammonia gas or other nitrogen compounds. The optimum C/N ratio has been reported to be 25–40, but the value varies depending on the substrate (Golueke, 1991).

Microorganisms are able to use organic molecules which dissolve in water. If the moisture content falls below a critical level, microbial activity will decrease and the microbes become dormant. On the other hand, too high a moisture content can cause a lack of aeration and the leaching of nutrients. In the subsequent anaerobic conditions the decomposition rate decreases and odour problems arise (Golueke, 1991).

Under optimal conditions, composting proceeds through three phases: (1) the mesophilic phase, (2) the thermophilic phase, which can last from a few days to several months, and (3) the cooling and maturation phase which lasts for several months (Fig. 3). The length of the composting phases depends on the nature of the organic matter being composted and the efficiency of the process, which is determined by the degree of aeration and agitation. At the start of composting the mass is at ambient temperature and usually slightly acidic. Soluble and easily degradable carbon sources, such as monosaccharides, starch and lipids, are utilized by microorganisms in the early stage of composting. The pH decreases because organic acids are formed from these compounds during degradation. In the next stage microorganisms start to degrade proteins, resulting in the liberation of ammonium and an increase in the pH. After the easily degradable carbon sources have been consumed, more resistant compounds such as cellulose, hemicellulose and lignin are degraded and partly transformed into humus (Crawford, 1983; Paatero et al., 1984).

Humus is the end product of the humification process, in which compounds of natural origin are partially transformed into relatively inert humic substances. Humic substances can be considered as a major reservoir of organic carbon in soils and aquatic environments (Aiken et al., 1985). The definitive structure of humic substances is not known, but they are usually divided into groups on the basis of chemical fractionation.
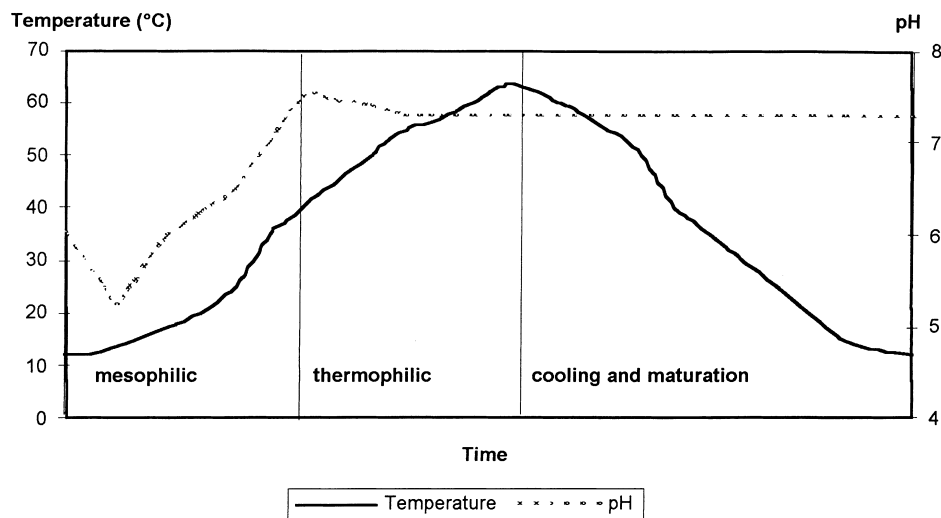

Fig. 3. Temperature and pH variation during natural composting process. The figure is redrawn from Golueke (1991).

Organic matter present in compost is usually chemically complex and difficult to fractionate. Extraction procedures may remove only a fraction of the organic matter, and, consequently, nondestructive methods such as ^{13}C-NMR (Almendros et al., 1992) and FTIR (Fourier transform IR) (Tseng et al., 1996) spectroscopy have been used for the analysis of humus. Aiken et al. (1985) divided humic substances into the following groups: humin (not soluble in water at any pH), humic acids (not soluble in water under acidic conditions) and fulvic acids (soluble in water under all pH conditions).

In general, immature compost contains high levels of fulvic acids and low levels of humic acids. As the decomposition proceeds, the fulvic acid fraction either decreases or remains unchanged while humic acids are produced. The degree of humification and compost maturity can be evaluated by means of the humification index. The humification index is the ratio between humic acids and fulvic acids (C_{HA}/C_{FA}) expressed as a percentage of the total organic carbon (Riffaldi et al., 1986; Inbar et al., 1990; Chen et al., 1996). Chen et al. (1996) studied the formation and properties of humic substances during composting. They found that the humification index increases during the process. The ^{13}C-NMR spectra of humic acids have been used to study the presence of aliphatic and aromatic structures. Both ^{13}C-NMR analysis and FTIR spectra indicate that the humic acid fraction extracted from a mature compost contains more aromatic structures and carboxyl groups and less carbohydrate components than that from an immature compost (Chen et al., 1996).

It has been claimed that humus is mainly formed from lignin, polysaccharides and nitrogenous compounds (Varadachari and Ghosh, 1984; Fustec et al., 1989; Inbar et al., 1989). The chemical pathway from organic matter to humus is very complex and involves a number of degradative and condensation reactions. Several schemes for the formation of humus have been proposed (e.g. Varadachari and Ghosh, 1984; Brown, 1985; Colberg, 1988). According to Varadachari and Ghosh (1984), lignin is first degraded by extracellular enzymes to smaller units, which are then absorbed into microbial cells where they are partly converted to phenols and quinones. The substances are discharged together with oxidizing enzymes into the environment, where they polymerized by a free-radical mechanism.

Composting is a dynamic process carried out by a rapid succession of mixed microbial populations. The main groups of microorganism involved are bacteria, including actinomycetes, and fungi (Golueke, 1991). Although the total number of microorganisms does not significantly change during composting, the microbial diversity can vary during the different phases of composting (Atkinson et al., 1996a). The precise nature of succession and the number of microorganims at each composting phase is dependent on the substrate and on the preceding microorganisms in the succession (Crawford, 1983).

At the beginning of composting mesophilic bacteria predominate, but after the temperature increases to over 40°C, thermophilic bacteria take over and thermophilic fungi also appear in the compost. When the temperature exceeds 60°C, microbial activity decreases dramatically, but after the compost has cooled mesophilic bacteria and actinomycetes again dominate (McKinley and Vestal, 1985; Strom, 1985a).

Composting is an aerobic process in general, but anaerobic microenvironments may develop. Atkinson et al. (1996b) estimated that almost 1% of all the bacteria found in municipal solid waste compost were anaerobic. All the anaerobic bacteria found were highly cellulolytic and thus may play a significant role in the degradation of macromolecules. The majority of the mesophilic anaerobic bacteria were facultative, while under thermophilic conditions more obligate anaerobic bacteria were found (Atkinson et al., 1996b).

3.2. Bacteria

Bacteria are typically unicellular with a size ranging from 0.5 to 3.0 μm. Because of their small size bacteria have a very high surface/volume ratio, which allows rapid transfer of soluble substrates into the cell. As a result, bacteria are usually far more dominant than larger microorganisms such as fungi. Some bacteria, e.g. *Bacillus* spp., are capable of producing thick-walled endospores which are very resistant to heat, radiation and chemical disinfection (Haug, 1993).

A wide range of bacteria have been isolated from different compost environments, including species of *Pseudomonas*, *Klebsiella* and *Bacillus* (Nakasaki et al., 1985; Strom, 1985a,b; Falcón et al., 1987). Typical bacteria of the thermophilic phase are species of *Bacillus,* e.g. *B. subtilis, B. licheniformis* and *B. circulans*. Strom (1985b) reports that as much as 87% of the randomly selected colonies during the thermophilic phase of composting belong to the genus *Bacillus*. Many thermophilic species of *Thermus* have been isolated from compost at temperatures as high as 65°C and even 82°C (Beffa et al., 1996).

Actinomycetes are bacteria which form multicellular filaments, thus they resemble fungi. They appear during the thermophilic phase as well as the cooling and maturation phase of composting, and can occasionally become so numerous that they are visible on the surface of the compost. Thermophilic actinomycetes have been isolated from a wide range of natural substrates, e.g. from desert sand and compost (Cross, 1968). The genera of the thermophilic actinomycetes isolated from compost include *Nocardia*, *Streptomyces*, *Thermoactinomyces* and *Micromonospora* (Waksman et al., 1939b; Strom, 1985a). Actinomycetes are able to degrade some

Table 2
Thermophilic fungi occurring in composts or which have a lignin-degrading capability

Fungus	Subdivision	Rot type	T_{opt} (°C)	T_{max} (°C)	Lignocellulose degradation	C/MC[a]	Ref.[b]
Aspergillus fumigatus	Deuteromycotina		35–43	52–55	Wood degradation, cellulose degradation, found in piles of hay and bark	C	3,5,7,8,9,10,11,16,19, 21,22,23,24
Chaetomium thermophilum var. *coprophile* var. *dissitum*	Ascomycotina		40–52.5	54–61	Very active cellulose degradation, found in piles of wood chips	C, MC	3,5,6,7,8,19,21,24
Coonemeria crustacea (= *Dactylomyces crustaceus*)	Ascomycotina	Soft-rot	40	<60	Found in self-heated material	C	9,11,14
Coprinus sp. *Coprinus cinereus* *Coprinus delicatulus*	Basidiomycotina	Brown-rot	45 35	55 45	Lignin modification	C, MC	3,6,15,25 4,7,21,22 6
Ganoderma colossum	Basidiomycotina	White-rot	40	>45	Effective lignin degradation		1,2
Malbranchea cinnamomea (= *Malbranchea sulfureum*) (= *Malbranchea pulchella*) (= *Thermoideum sulfureum*)	Deuteromycotina		45–47.5	53–57	Cellulose degradation, found in piles of hay	C, MC	3,4,5,6,14,19, 20,22,24
Melanocarpus albomyces (= *Myriococcum albomyces*)	Ascomycotina		37–50	55–57	Cellulose and hemicellulose degradation	C, MC	3,4,7,11,16,17
Myceliophthora thermophila (= *Sporotrichum thermophile*) (teleomorph: *Corynascus heterothallicus*)	Ascomycotina		36–50	52–65	Very active cellulose degradation, wood degradation, found in piles of wood chips		3,5,6,8,9,16,18,19
Paecilomyces spp. *Paecilomyces varioti*	Deuteromycotina	Soft-rot	45–50	55–60	Cellulose and some lignin degradation	C, MC	3,6,7,9 3,22,24
Phanerochaete chrysosporium (= *Sporotrichum pulverulentum*) (= *Chrysosporium pruinosum*)	Basidiomycotina	White-rot	36–45	46–55	Effective lignin degradation, newspaper degradation, in piles of wood chips		3,16,17,19,20
Rhizomucor pusillus (= *Mucor pusillus*)	Zygomycotina		35–55	55–60	Found in piles of hay	C, MC	3,5,6,7,9, 11,19,21,24
Scytalidium thermophilum (= *Torula thermophila*) (= *Humicola grisea*) (= *Humicola insolens*)	Deuteromycotina		35–47.5	55–58	Cellulose degradation	C, MC	3,5,6,9,14,22, 24
Stibella thermophila	Deuteromycotina		35–50	55	Cellulose degradation	MC	3,6,10,14,19

Table 2 (Continued)

Fungus	Subdivision	Rot type	T_{opt} (°C)	T_{max} (°C)	Lignocellulose degradation	C/MC[a]	Ref.[b]
Talaromyces emersonii	Ascomycotina		45–50	55–60	Wood degradation, found in piles of wood chips	C, MC	3,6,16,19,22
(anamorph: *Penicillium emersonii*) *Talaromyces thermophilus*	Ascomycotina		45–50	57–60	Weakly ligninolytic, found in piles of wood chips	C, MC	3,5,6,7,10,14,9,22,24
(= *Talaromyces dupontii*) (anamorph: *Penicillium dupontii*)							
Thermoascus aurantiacus	Ascomycotina	Soft-rot	45–52.5	55–62	Effective lignin degradation, found in piles of wood chips	C, MC	3,5,6,12,14,16,18,19, 21,22,24
Thermomyces lanuginosus (= *Humicola lanuginosa*)	Deuteromycotina		45–55	60	Found in piles of hay and wood chips, cellulose degradation (not for all isolates), some lignin degradation	C, MC	3,5,6,7,8,10,11,19,21, 22,24,25
Thermomyces ibadensis	Deuteromycotina		42–47	60–61	Palm kernel degradation		3,6,14
Thermomyces stellatus (= *Humicola stellata*)	Deuteromycotina		37–40	50	Found in piles of hay and peat		3,5,6,14,19,21,23
Thielavia terrestris (= *Allescheria terrestris*)	Ascomycotina	Soft-rot	40–47.5	55	Wood and some lignin degradation		3,6,7,16,19,22

[a] C = found in compost, MC = found in mushroom compost.

[b] References: [1] Adaskaveg et al. (1990); [2] Adaskaveg et al. (1995); [3] Brock (1978); [4] Chang (1967); [5] Cooney and Emerson (1964); [6] Crisan (1973); [7] Dix and Webster (1995); [8] Eggins and Malik (1969); [9] El-Naghy et al. (1991); [10] Fergus (1964); [11] Kane and Mullins (1973); [12] Machuca and Durán (1996); [13] Maheshwari and Kamalam (1985); [14] Mouchacca (1997); [15] Nusbaumer et al. (1996); [16] Ofosu-Asiedu and Smith (1973); [17] Rayner and Boddy (1988); [18] Romanelli et al. (1975); [19] Rosenberg (1975); [20] Rosenberg (1978); [21] Sharma (1989); [22] Straatsma et al. (1994); [23] Stutzenberger et al. (1970); [24] von Klopotek (1962) and [25] Waksman et al. (1939a).

cellulose, and solubilize lignin, and they tolerate higher temperatures and pH than fungi. Thus, actinomycetes are important agents of lignocellulose degradation during peak heating, although their ability to degrade cellulose and lignin is not as high as that of fungi (Crawford, 1983; Godden et al., 1992). Under adverse conditions actinomycetes survive as spores (Cross, 1968).

3.3. Fungi

Temperature is one of the most important factors affecting fungal growth. Other important factors are sources of carbon and nitrogen, and the pH. A moderately high level of nitrogen is needed for fungal growth although some fungi, mainly wood-rotting fungi, grow at low nitrogen levels. Indeed, a low nutrient nitrogen level is often a prerequisite for lignin degradation (Eriksson et al., 1990; Dix and Webster, 1995). However, low nutrient nitrogen is a rate-limiting factor for the degradation of cellulose (Dix and Webster, 1995). Most fungi prefer an acidic environment but tolerate a wide range of pH, with the exception of the Basidiomycotina which do not grow well above pH 7.5. *Coprinus* species are the only Basidiomycotina which prefer an alkaline environment (Dix and Webster, 1995).

The majority of fungi are mesophiles which grow between 5°C and 37°C, with an optimum temperature of 25–30°C (Dix and Webster, 1995). However, in the compost environment the elevated temperature means that the small group of thermophilic fungi is an important biodegradation agent. In nature, thermophilic fungi grow in garden compost heaps, bird nests, coal tips, power plant cooling pipes and effluents, in the storage of many agricultural products (like hay, grain etc.) and in piles of wood chips and peat (Sharma, 1989; Dix and Webster, 1995). In mushroom compost, thermophilic fungi are responsible for the degradation of lignocellulose, which is a prerequisite for the growth of the edible fungus (Sharma, 1989).

Cooney and Emerson (1964) define thermophilic fungi as fungi with a maximum growth temperature of 50°C or higher and a minimum growth temperature of 20°C or higher. Thermotolerant species have a maximum growth temperature of about 50°C and a minimum well below 20°C (Cooney and Emerson, 1964). Crisan (1973), however, defines thermophilic fungi as fungi with a temperature optimum of 40°C or higher. Thermophilic and thermotolerant fungi which are known to have cellulolytic or ligninolytic activity, or which have been found growing in lignocellulose substrate or compost, are listed in Table 2.

Cooney and Emerson (1964), Crisan (1973), Rosenberg (1975), Brock (1978) and Mouchacca (1997) have enumerated several species and varieties of thermophilic fungi. The growth temperature optimum for most thermophilic fungi is between 40°C and 50°C and the maximum is 55°C (See Table 2). According to Cooney and Emerson (1964), 60°C is the temperature limit of fungi and, according to Brock (1978), 60–61.5°C is the temperature limit for all eukaryotes. Temperature tolerance varies within the genera and even between the isolates of fungal species. The substrate may also have some influence on the temperature tolerance (Ofosu-Asiedu and Smith, 1973). There are indeed some reports of fungal growth above 61.5°C. El-Naghy et al. (1991) reported saccharification activity of *Sporotrichum thermophile* (syn. *Myceliophthora thermophile*) at 65°C, and Ofosu-Asiedu and Smith (1973) found *Talaromyces emersonii* still to be very active after four weeks at 60°C indicating that the fungus might be active at even higher temperatures. However, the growth of the fungus near the temperature maximum is very slow compared to that at the optimum temperature (Rosenberg, 1975).

The ligninolytic capacity of all thermophilic fungi is not known (Table 2). However, most of them are known to be able to degrade wood or other lignocellulose, cellulose or hemicelluloses (e.g. Fergus, 1969; Ofosu-Asiedu and Smith, 1973; Sharma, 1989; Kuhad et al., 1997). The ability of fungi to hydrolyse hemicelluloses is probably more common than cellulose hydrolyzation (Dix and Webster, 1995).

The most effective lignin degraders are Basidiomycotina, but according to Cooney and Emerson (1964) and Mouchacca (1997) all Basidiomycotina are mesophilic. However, a few Basidiomycotina grow well at elevated temperatures. *Phanerochaete chrysosporium* (anamorph *Sporotrichum pulverulentum*) is a white-rot fungus with an optimum temperature of 36–40°C and maximum temperature 46–49°C (Mouchacca, 1997). *Ganoderma colossum* is another white-rot fungus which is still capable of growing at 45°C and has an optimum temperature of 40°C (Adaskaveg et al., 1990; 1995). In the genus *Coprinus* there are some species that have an optimum temperature of above 40°C (Crisan, 1973). Some of the wood-rotting *Coprinus* species are brown-rot fungi which modify rather than degrade lignin (Rayner and Boddy, 1988).

A thermophilic Ascomycotina, *Thermoascus aurantiacus*, has a high ligninolytic capacity (Machuca et al., 1995), and it has been isolated from compost (von Klopotek, 1962). Heat-tolerant soft-rot fungi, such as *Thielavia terrestris*, *Paecilomyces* sp. and *Talaromyces thermophilus*, are weakly ligninolytic (Dix and Webster, 1995).

Nusbaumer et al. (1996), Thambirajah and Kuthubutheen (1989), Thambirajah et al. (1995), von Klopotek (1962) and Waksman et al. (1939a,b) have studied the occurrence of fungi during composting. Waksman et al. (1939a,b) studied composting on a laboratory scale at temperatures of 28°C, 50°C, 65°C and 75°C. At 28°C the population was heterogeneous with bacteria being

dominant throughout the whole period, and fungi appearing later. Fungi, together with bacteria and actinomycetes, formed the microbial population in the compost at 50°C. At the beginning there were active thermophilic fungi, which were followed by bacteria and actinomycetes, some of them growing on the fungal mycelium. The composts used in the other studies mentioned above were full scale.

The raw material of compost contains about 10^6 microbial counts of mesophilic fungi/g of raw material and thermophilic fungi 10^3–10^6/g (von Klopotek, 1962; Thambirajah and Kuthubutheen, 1989; Thambirajah et al., 1995). The predominant mesophilic fungus in the raw material has been *Geotrichum* sp. (von Klopotek, 1962; Nusbaumer et al., 1996) and the thermotolerant fungus *Aspergillus fumigatus* (von Klopotek, 1962). Counts of fungi decrease as the temperature rises, and at 64°C all the thermophilic fungi disappear. However, a mesophilic fungus, *Cladosporium cladosporioides*, grew well at 64–65°C, but no fungi were detected at 67°C (von Klopotek, 1962). In the studies of Thambirajah et al. (1995) and Waksman et al. (1939a,b), no fungi were detected when the temperature was over 60°C. In the study of Thambirajah and Kuthubutheen (1989) fungi survived at high temperature probably due to the short duration of peak heating. When the temperature decreases below 60°C mesophilic and thermophilic fungi reappear in compost (von Klopotek, 1962; Thambirajah et al., 1995). The dominating fungus after peak heating is *Aspergillus* sp. (Nusbaumer et al., 1996) or *Thermomyces lanuginosus* (von Klopotek, 1962), which was also found to dominate at 50°C. *T. lanuginosus* can decompose cellulose, hemicelluloses and even lignin, although to a much smaller extent than the other components (Waksman et al., 1939a,b). In the studies of Thambirajah and Kuthubutheen (1989) and Thambirajah et al. (1995) the number of mesophilic and thermophilic fungi (10^4–10^6/g) in mature compost were the same, but in the study of von Klopotek (1962) thermophilic fungi dominated especially in the drier parts of mature compost.

Coprinus sp. (von Klopotek, 1962; Nussbaumer et al., 1996), *Panaeolus* sp., *Corticium coronilla* and possibly *Mycena* sp. (von Klopotek, 1962) are Basidiomycotina occurring in compost. They were all isolated from compost during the cooling and maturation phase or from mature compost (von Klopotek, 1962; Nussbaumer et al., 1996).

4. Biodegradation of lignin

4.1. Lignin-degrading fungi

Lignin degraders in nature are mainly white-rot fungi (Table 3). There are several thousand species of white-rot fungi, most of them Basidiomycotina, in addition to a few Ascomycotina which can cause white-rot (Eriksson et al., 1990). White-rot fungi belonging to the subdivision Basidiomycotina attack either hardwood or softwood, while Ascomycotina probably degrade only hardwood (Kirk and Farrell, 1987). Lignin degradation by white-rot fungi is faster than that of any other organisms and they are responsible for most of the lignin decomposition in nature. However, the growth substrate is not only lignin, but also hemicelluloses and cellulose (Buswell and Odier, 1987; Kirk and Farrell, 1987; Blanchette, 1995). The growth of fungi decreases in nitrogen- or carbon-depleted conditions and ligninolytic activity appears as a form of secondary metabolism (Brown, 1985; Kirk and Farrell, 1987).

White-rot fungi can cause selective or nonselective delignification of wood. In selective delignification, lignin is removed without any marked loss of cellulose, and in nonselective delignification all the major cell wall components are degraded (Eriksson et al., 1990; Blanchette, 1995). Although some white-rot fungi always attack wood either in a selective or in nonselective way, there are fungi which are capable of both forms of degradation, e.g. *Heterobasidion annosum* (Eriksson et al., 1990; Blanchette, 1995). Among the best studied white-rot fungi are *Phanerochaete chrysosporium* and *Phlebia radiata*, which degrade lignin selectively, and *Trametes versicolor* which degrades lignin nonselectively (Hatakka, 1994).

Table 3
Lignin-degrading organisms (Buswell and Odier, 1987; Rayner and Boddy, 1988; Eriksson et al., 1990; Blanchette, 1995)

Organism	Subdivision	Lignin degradation	Environment	Genera (e.g.)
White-rot fungi	Basidiomycotina (Ascomycotina)	Lignin mineralization, selective or nonselective delignification	Mainly hardwood	*Phanerochaete*, *Phlebia*, *Trametes*
Brown-rot fungi	Basidiomycotina	Lignin modification	Mainly softwood	*Poria*, *Polyporus*
Soft-rot fungi	Ascomycotina or Deuteromycotina	Limited lignin degradation	Aquatic environment, wood with preserving chemicals, plant litter	*Chaetomium*, *Paecilomyces*, *Fusarium*
Bacteria	Actinomycetes or Myxobacteria	Limited lignin degradation	Sapwood, water-saturated wood, wood at late stage of decomposition, plant litter	*Streptomyces*, *Nocardia*, *Pseudomonas*

White-rot fungi degrade lignin by means of oxidative enzymes (Hatakka, 1994). Because of the nature and size of the lignin molecule, the enzymes responsible for the initial attack must be extracellular and nonspecific (Kirk and Farrell, 1987; Hatakka, 1994). The best studied extracellular enzymes of white-rot fungi are lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccase. The role of LiP and MnP in lignin degradation has been verified, while that of other enzymes is still uncertain (Hatakka, 1994). Different white-rot fungi produce different combinations of enzymes: there are fungi producing LiP and MnP, fungi producing MnP and laccase, fungi producing LiP and laccase and fungi which produce neither LiP nor MnP, but laccase and aryl alcohol oxidase (AAO) or some other enzyme (Hatakka 1994). *P. chrysosporium*, *P. radiata* and *T. versicolor* belong to the LiP–MnP group. Usually the members of this group also produce laccase, but *P. chrysosporium* is an exception, although very recently laccase production by this fungus has been reported under specific conditions (Srinivasan et al., 1995). Several fungi belonging to the LiP–MnP and the MnP-laccase groups are effective lignin degraders, while the lignin degradation capability of fungi belonging to the LiP-laccase group is much lower (Hatakka, 1994). This may be due to the essential role of MnP in lignin degradation. However, lignin degradation is a complex process and the enzymes have probably synergistic effects on each other. The mineralization of ^{14}C-lignin in optimal conditions by the most effective white-rot fungi can reach 50% or even 70%. In an experiment with several strains of *P. chrysosporium* 45–50% of β-^{14}C-DHP was mineralized and 69% of the ^{14}C-lignin from fir by one of the strains in 22 days (Hatakka et al., 1984).

Brown-rot fungi extensively degrade cellulose and hemicelluloses in wood, but lignin degradation is limited. Lignin is chemically modified by demethylation of its phenolic and nonphenolic units (Kirk and Farrell, 1987; Eriksson et al., 1990), and limited aromatic hydroxylation and ring cleavage of lignin also occurs (Kirk and Farrell, 1987). Brown-rot fungi are able to mineralize the methoxyl groups of lignin, but the mineralization of other parts is much lower (Buswell and Odier, 1987; Kirk and Farrell, 1987). Brown-rot fungi mainly colonize softwoods and only 6% of all wood-rotting Basidiomycotina are brown-rotters (Rayner and Boddy, 1988). Brown-rotted wood is brown in colour, consisting of a high proportion of modified lignin residue and little carbohydrates, and it persists in the forest for a long time (Blanchette, 1995).

Soft-rot fungi are Ascomycotina or Deuteromycotina which degrade both hardwood and softwood, although hardwoods are degraded to a greater extent than softwoods (Kuhad et al., 1997). All wood components are degraded, but the rate of degradation is minimal compared to that of white-rot or brown-rot fungi (Eriksson

et al., 1990). Soft-rot fungi degrade wood in environments that are too severe for white- or brown-rot fungi, generally in wet environments (Blanchette, 1995). They also decompose plant litter in soils. Haider and Trojanowski (1980) and Rodriguez et al. (1996) studied the lignin mineralization capacity of soft-rot fungi. In 35 days, *Chaetomium piluliferum* mineralized 17% of ring-^{14}C-labelled corn stalk lignin, but the amount mineralized by other soft-rot fungi was much less (Haider and Trojanowski, 1980). Mineralization of side chain-and methoxy-labelled lignins by all fungi was slightly higher (Haider and Trojanowski, 1980). The soil fungi *Penicillium chrysogenum*, *Fusarium solani* and *Fusarium oxysporum* mineralized 20–27% of ^{14}C-MWL from wheat straw in 28 days. *P. chrysogenum* can also attack kraft and organosolv lignins (Rodriguez et al., 1996). Little is known about the enzyme system of soft-rot fungi or their lignin degradation capacity as litter decomposing organisms (Haider and Trojanowski, 1980; Kirk and Farrell, 1987). Lignin-related compounds, like vanillic acid and phenols, are rapidly degraded by soft-rot fungi (Haider and Trojanowski, 1980). This could mean that soft-rot fungi are an important group of lignin degraders in mixed populations, although their lignin degradation capacity alone is limited.

4.2. Lignin-degrading bacteria

There are many genera of actinomycetes and eubacteria which can degrade extracted lignin and DHP (Buswell and Odier, 1987). Many bacterial strains, especially actinomycetes, can solubilize and modify the lignin structure extensively, but their ability to mineralize lignin is limited (Buswell and Odier, 1987; Ball et al., 1989; Eriksson et al., 1990; Godden et al., 1992). Actinomycetes degrade lignin as their primary metabolic activity and at high nitrogen levels compared to white-rot fungi, most of which degrade lignin via their secondary metabolism. Some species of *Streptomyces* and *Nocardia* have been shown to degrade some lignin in soil (Haider and Trojanowski, 1980). *Streptomyces badius* mineralizes 3.2% of ^{14}C-MWL in 30 days, and mineralization was further enhanced to 11% when cellulose and yeast extract were added to the medium (Eriksson et al., 1990). There is some evidence that the grass lignins are attacked by actinomycetes more efficiently than the wood lignins (Buswell and Odier, 1987).

The lignin-degrading eubacteria can be divided into erosion, cavitation and tunnelling bacteria (Eriksson et al., 1990; Blanchette, 1995). Wood is degraded by bacteria under certain extreme environmental conditions, e.g. wood saturated with water, almost anaerobic conditions or wood with a high extractive content. However, the rate of degradation is very slow (Eriksson et al., 1990; Blanchette, 1995). Erosion bacteria grow towards the middle lamella of the wood cells and cause

erosion of the fibre wall, while tunnelling bacteria grow within the cell wall. The species of the tunnelling bacteria have not been identified (Eriksson et al., 1990). Bacteria of several genera such as *Pseudomonas*, *Alcaligenes*, *Arthrobacter*, *Nocardia* and *Streptomyces* can degrade single-ring aromatic compounds. The role of bacteria may be significant in consuming the small molecular weight intermediate compounds produced by fungi (Vicuña, 1988; Rüttimann et al., 1991).

Although aerobic microorganisms are primarily lignin degraders in most environments, it has been shown that anaerobic rumen microorganisms are cabable of degrading plant fibre cell walls (Kuhad et al., 1997). Colberg (1988) has reviewed the anaerobic microbial degradation of lignin compounds and concluded that the intermediate metabolic products called oligolignols, released during aerobic degradation, may be partially degraded to CO_2 and CH_4 by anaerobic microorganisms. Also, polymeric lignin is mineralized in anoxic sediments at a slow rate. In many studies, however, no anaerobic degradation of lignin has been observed (e.g. Hackett et al., 1977; Micales and Skog, 1997). Benner and Hodson (1985) reported that an elevated temperature of 55°C enhances the anaerobic degradation of lignin, nevertheless mineralization of DHP stays low. Chandler et al. (1980) have formulated an equation for the anaerobic degradation of different organic materials. Degradation is directly proportional to the lignin content of the material:

$$B = 0.830 - 0.028X, \tag{1}$$

where B is biodegradable fraction and X is the lignin content, % of the volatile solids. The data were collected from the anaerobic fermentation process using a wide range of lignocellulosic materials (Chandler et al., 1980).

4.3. Studies in the compost environment

Stutzenberger et al. (1970) studied the composting of municipal solid waste containing paper products for 49 days. Lignin may have inhibited the degradation of cellulose, because 40% of the cellulose remained undegraded. The cellulose content of the waste was 46–56% but degradation of lignin was not determined (Stutzenberger et al., 1970). Micales and Skog (1997) reported that lignin also inhibits the anaerobic degradation of cellulose in landfills.

Lignin degradation of plant residues has been studied in the composting experiments summarized in Tables 4 and 5. Mineralization of lignin in the compost environment was not studied in any of the experiments. However, Hackett et al. (1977), Haider et al. (1977) and Martin and Haider (1980) studied lignin mineralization by a mixed population of microorganisms in soil. According to Haider et al. (1977), soil microbes could mineralize 27% of the ring-^{14}C-corn stalk lignin and 21% of the ring-^{14}C-DHP, while 54% and 61% of the ^{14}C-label, respectively, was found in humic substances after 28 weeks of incubation. Hackett et al. (1977) studied the lignin mineralization capacity of 30 different soil samples from five different sites. Mineralization of ring-^{14}C-DHP varied from 3.1% to 42.4% in 41 days. The highest mineralization was achieved in soil from a geyser basin at its natural temperature of 35°C (Hackett et al., 1977).

The effect of temperature on lignin degradation has been studied in a few composting experiments (Table 5). Horwath and Elliott (1996) composted ryegrass for 45 days at a mesophilic temperature of 25°C, as well as in a temperature profile in which the temperature was either 25°C or 50°C. The proportion of lignin degraded, as determined by the Klason method, was 7% and 27%, respectively. At both temperatures the elemental ratio of the residual Klason lignin changed, and it was estimated that only 6% of the residual Klason lignin was unaltered after composting (Horwath and Elliott, 1996).

Waksman et al. (1939b) studied the degradation of lignin in composts at different temperatures (Fig. 4). The highest degradation of lignin occurred at 50°C, while degradation was to some extent lower at 28° and 65°C. At 75°C no biodegradation of lignin occurred, but 12% of the lignin was solubilized as a result of the high

Table 4
Studies in which lignin degradation during composting or in soil incubation has not been reported

Waste	Time/d	Compost/Soil	Original lignin-content/%	Note	Lignin analysis method	Ref.
Grape pulp	12	Compost, 70 l	39.4	Thermophilic phase 3 d	Acid detergent?	Faure and Deschamps (1990)
Grass, leaf, corn cob	14	Compost, bench scale	12.4	Fixed temperature of 50°C	Acetyl bromide (and FTIR)	Tseng et al. (1996)
Cotton	90	Compost, 150 l	14.2	Thermophilic phase about 7 d	Acid detergent?	Baca et al. (1992)
Wheat internodes	224	Soil (sample in bags)	9.4	Buried	H_2SO_4	Robinson et al. (1994)
Market waste, hay, fresh wood	295	Compost, 785 l	25.1	Thermophilic phase 8d, frozen for 54 days	Klason lignin	Nusbaumer et al. (1996)

Table 5
Studies in which lignin degradation during composting or in soil has been reported

| Waste	Time/d	Compost/soil	Original lignin-content/%	Degraded lignin/%	Note	Analyzig method of lignin	Ref.
Ryegrass straw	45	Compost, 140 ml	12.15	7	25°C	Modified Klason lignin	Horwath and Elliott (1996)
				27	30 d 50°C 15 d 25°C		
Horse manure, wheat straw	47	Compost, 600 g	20.5	26	28°C	–	Waksman et al. (1939b)
				43	50°C		
				33	65°C		
				12	75°C		
Olive mill waste water, wheat straw	140	Compost, pile	–	70	Prolonged thermophilic phase of 35 d	Modified Klason lignin	Tomati et al. (1995)
Canola residue (*Brassica campestris*)	154	Soil (sample in bags)	11.5	17	Buried	Acid detergent	Franzluebbers et al. (1996)
				17	Not buried		
Wheat leaves	224	Soil (sample in bags)	5.92	99.2	Buried	H_2SO_4	Robinson et al. (1994)
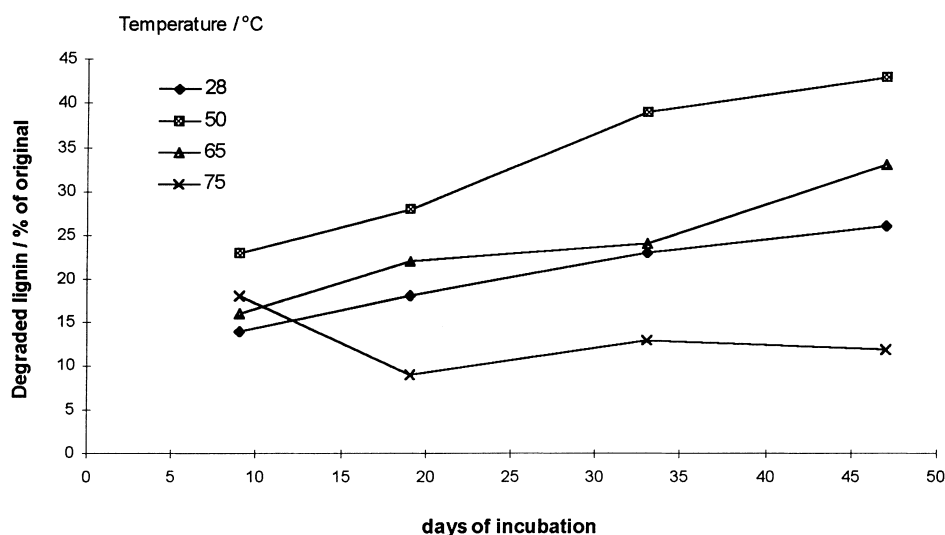
Fig. 4. Lignin degradation in horse manure and straw compost. Original lignin content 20.5%. The data of the figure are derived from Waksman et al. (1939b).

temperature and alkaline reaction of the compost (Waksman et al., 1939b).

In experiments where the thermophilic phase lasted for a short time, between 7 and 14 days, lignin was not degraded (Table 4), not even when the total composting time was as long as 295 days (Nusbaumer et al., 1996). According to a study by Tomati et al. (1995), 70% of lignin was degraded during 35 days when the temperature of the compost was kept at 50°C, whereas only negligible degradation occurred later during compost maturation. According to these studies, it seems probable that sufficient duration of the thermophilic phase is an important factor for lignin degradation in the compost environment. However, the degradation of lignin in agricultural residues in soil has been reported in some studies (Robinson et al., 1994; Franzluebbers et al., 1996). The original lignin content and thickness of the material has an influence on lignin degradation. When buried in soil for 224 days the lignin of wheat leaves was almost totally degraded, while the lignin of wheat internodes did not degrade at all. The leaves contained 5.92% lignin and the internodes 9.2% lignin (Robinson et al., 1994).

Waksman et al. (1939a) studied the degradation capacity of some microorganisms isolated from compost. Two thermophilic actinomycete isolates degraded

0.7–2.5% of the lignin in 42 days at 50°C, the thermophilic fungus *Thermomyces lanuginosus* 4.2%, but neither of the two bacteria studied were capable of lignin degradation alone. However, the natural microbial population in manure degraded 11.5% of the lignin. The natural population also decomposed 62% of the total dry material as compared to 40% for the fungus (Waksman et al., 1939a).

5. Conclusions

This literature survey shows that very little is known about the degradation of lignin in compost. Although it would appear that lignin can be degraded or transformed extensively during composting, the lignin loss in a compost environment is hardly due to extensive mineralization of lignin. The organisms most efficient at mineralizing lignin, white-rot fungi, do not survive the thermophilic phase of composting, and thus cannot play any significant role in lignin degradation. The basidiomycetous fungi that have been found in composts appear during the cooling and maturation phase or in mature compost (von Klopotek, 1962; Nusbaumer et al., 1996). The other microorganisms in compost, mainly thermophilic microfungi, are probably the most important lignin degraders (Waksman et al., 1939a,b) and synergistic effects with other organisms enhance the degradation significantly (Waksman et al., 1939a). The mineralization of lignin by compost microorganisms is probably of the same order of magnitude as that by a mixed population of soil microorganisms, and the degraded lignin fragments are building units for humic substances both in soil (Hackett et al., 1977; Haider et al., 1977; Martin and Haider, 1980) and in compost (Horwath and Elliott, 1996). Lignin degradation in composts is regulated by temperature, the original lignin content and the thickness of the material.

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　　　　M. Tuomela et al. / Bioresource Technology 72 (2000) 169–183

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