Chapter 3
Decomposer Organisms

3.1 Introduction

The dominant primary decomposers in boreal and temperate forest soil systems are the microorganisms, encompassing both fungi and bacteria. Both these main groups of microorganisms can degrade cellulose, hemicellulose, and different lignins. This chapter will emphasize the functional roles of organisms (e.g., cellulolytic and lignolytic) rather than their taxonomy. The concepts white rot, brown rot, and soft rot and what they stand for functionally in terms of degradation processes will be presented.

Many microorganisms degrade cellulose and hemicellulose in nature. These organisms have in common the production of extracellular hydrolytic enzymes that are either bound onto the outside of the cell or released into the surrounding environment. Polymer carbohydrates may be degraded both aerobically and anaerobically, but a complete degradation of lignin (white-rot type) requires the action of aerobic organisms (fungi and/or aerobic bacteria). Partial lignin degradation (brown-rot type) may also be carried out by anaerobic bacteria, but is mainly found among fungi and aerobic bacteria.

We have used the functional concepts white rot, brown rot, and soft rot as a basis for the discussion of degradation of litter. Although the terms originally seem to refer to visually different types of lignin degradation, it now appears that the degradation of cellulose and hemicellulose is also different among the groups (Worrall et al. 1997). The terms refer to the type of rot rather than to a group of organisms, but we have adopted the common use of the terms and refer to fungi when using the terms white rot, brown rot, and soft rot. As regards degradation by bacteria, it is described and discussed as such.

The composition of the microbial population (e.g., cellulolytic versus lignolytic) may vary with general properties of the soil/litter ecosystem, such as nutrient status and pH. A specific functional property that may discriminate among systems and populations is their sensitivity to N concentrations in litter and humus, which may be either stimulating or suppressing. Such sensitivity is not universal, but common in species of both white-rot and brown-rot organisms.

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By tradition, soil animals, such as collembola, mites, and earthworms, have been considered important for litter decomposition. Such groups have different roles in decomposition, although those roles are not always clear. The decomposition by free-living microorganisms has also been considered important, but the relative influences of soil animals and soil microorganisms have not been apparent, thereby indirectly supporting studies of the more easily studied visible component, namely soil animals.

In recent decades, it has become increasingly clear that for some systems, at least boreal and temperate coniferous ones, the microbial component is of absolute dominance. For example, Persson et al. (1980) estimated that at least 95 % of the energy flow goes through the microbial population. The implications of this proportion are considerable, and we could express this in a somewhat simplified way by stating that the decomposition of litter in a given system is determined by conditions and limitations that are valid for the microbial community of that system, which may be quite different from those of the soil faunal community.

Considering that the focus of this book is directed toward boreal and temperate systems, which have a decomposition process dominated by microorganisms, we have described both the microbial population (Sect. 3.2) and the microbial enzymatic degradation mechanisms (Sect. 3.3). This chapter will describe those properties of the organisms that are important in the degradation of cellulose, hemicellulose, and lignin. The main combined effects on the decomposition of whole litter are given in Chaps. 2 and 6.

Mycorrhizae have been found to turn into aggressive decomposers under certain circumstances and may decompose humus that has been considered as stabilized. Such a degradation can take place at a high rate. This phenomenon may be related to nutrient stress in growing trees. The role of mycorrhizae in decomposition in general is still under dispute, and we present observations here without taking part in that dispute (Sect. 3.4).

The ecology of decomposer communities can influence the pattern of decay. The changes in the community and its function during the decay process and the cyclic nature of the successional process in the soil will be addressed. The effects of moisture and temperature on the activity of the microbiological decomposition process are presented later, in Chap. 7.

3.2 General Properties of a Given Microbial Population

The two main groups of litter decomposers are bacteria (including the filamentous bacteria that earlier were called actinomycetes) and fungi: two groups that appear to include some of the same basic physiological properties when it comes to degradation of fresh litter polymers. Still, the fungi are generally considered the most important group, and we know more about their litter-degrading properties and enzyme systems. Each of these two groups may be divided into functional subgroups with different properties, degrading different groups of chemical

Table 3.1 Some of the general properties of main groups of bacteria and fungi

Property	Bacteria	Fungi
Mobility	+	+
Spore-forming ability	+	+
Can degrade cellulose/hemicellulose	+	+
Can degrade lignin completely	+	+
Can degrade lignin anaerobically[a]	+	–
Can degrade intact fiber walls	+	+
Species with N repression of the ligninase system	?[b]	+
Species without N repression of the ligninase system	?[b]	+

[a] Incomplete degradation to be compared to the brown-rot type
[b] Not known

components. The taxonomy of both fungi and bacteria is complex and is beyond the scope of this book.

The bacteria include both aerobic and anaerobic organisms, which distinguishes them from the exclusively aerobic fungi. Both groups have organisms able to degrade all the main polymers: lignin, cellulose, and hemicelluloses. There are also organisms able to degrade woody tissue where all these components are combined into fibers. Complete degradation of lignin appears to be carried out by a small number of the fungi and aerobic bacteria. Some of the general properties of main groups of bacteria and fungi are listed in Table 3.1.

The biological diversity in the soil microbial community is high. The potential species diversity is evident just by comparing crude numbers of identifiable species. For just 1 m^2 of a given soil system, we may estimate that for bacteria, there may be 1,000–5,000 species and for fungi perhaps 100 dominant species.

The high density of bacteria in, for example, an organic soil creates a high potential for invading a new substrate, such as newly shed litter. Estimates of 10^9 bacteria g^{-1} organic soil in either an active or a resting stage are common when made by direct light microscopy counting. This figure is conservative since there are numerous bacteria that are simply too thin to be detected with light microscopy and have to be counted using electron microscopy. In the same soil, total mycelial lengths have been estimated to reach ca. 2,000 km L^{-1} of humus, of which perhaps 10 % would be live mycelium. Microorganisms will only be actively dividing and growing when environmental conditions are favorable. When the conditions cannot support growth, the microorganisms will be in some kind of resistant, resting stage, or spore form. Wind and animals easily transport fungal and bacterial spores. This means that spores may be transplanted among ecosystems and that a given ecosystem may have a passive gene bank able to quickly produce active microorganisms that can attack a particular litter type, possibly with new chemical components that are novel in a given environment.

The size of most microorganisms gives them access to different parts of the fiber and tissues that make up litter (Fig. 4.2). For a main part of the bacteria, the diameters range largely from 0.1 to 2 μm and for filamentous fungi from ca. 1 to

less than 20 μm. The lengths of rod-shaped bacteria mainly range from ca. 1 to 10 μm, while those of the fungal mycelia are more undetermined.

Bacteria may be either immobile or mobile, with one or more flagella, a whip-like structure. Fungal mycelia demonstrate mobility in another way, in that they simply grow in one direction and thus move their protoplasm, leaving an empty cell wall structure behind.

The term 'decomposer' microorganism is sometimes used for those microbes that decompose plant litter structures, sometimes for the larger group that decomposes organic matter, thus including the whole group of free-living heterotrophic microorganisms. Free-living in this context simply means those microorganisms that do not live in obligate symbiosis. Here, we will focus on what may be called primary litter decomposers, namely those that attack and degrade (at least in part) the polymer structures to carbon dioxide and/or small, partly degraded molecules. We discuss below the hypothesis that not only free-living microorganisms play a role in the turnover of organic matter but that mycorrhizal fungi may also be important.

3.3 The Degradation of the Main Polymers in Litter

3.3.1 Degradation of Cellulose

Cellulose is degraded by numerous species of both bacteria and fungi. These organisms rely on extracellular enzymes that either are secreted into their immediate surroundings or are located on the cell surface. It is necessary that cellulose be degraded outside the microbial cell (Fig. 3.1), and that the insoluble macromolecules be degraded to monomers or oligomers of a few glucose units (Fig. 3.1), such as cellobiose, that can be taken into the cell and metabolized.

A common feature among all cellulose-degrading organisms is that they produce hydrolytic, extracellular enzymes that attack the cellulose polymer. Part of the cellulose in the plant fiber is arranged in a crystalline form that makes it harder to attack (see Chap. 4) and relatively few cellulolytic organisms have the necessary complete set of enzymes to degrade this structure. Many organisms are able to degrade the more amorphous kind of cellulose (see Eriksson et al. 1990).

The most studied group of cellulose-degrading organisms is the fungi, and no less than 74 species (Eriksson et al. 1990) have been studied in some detail. The traditional division of wood-degrading fungi into three main groups, white-rot, brown-rot, and soft-rot fungi, relates primarily to their mode of lignin degradation, but these groups also differ in the way they degrade polymer carbohydrates.

Perhaps the most studied wood decay fungus is the white-rot basidiomycete *Phanerochaete chrysosporium* Burdsall (previously called *Sporotrichum pulvurolentum* Novabranova). Much of what is known about the decay of lignocellulosic materials in nature is based on studies of this fungus (Ander and Eriksson 1977;

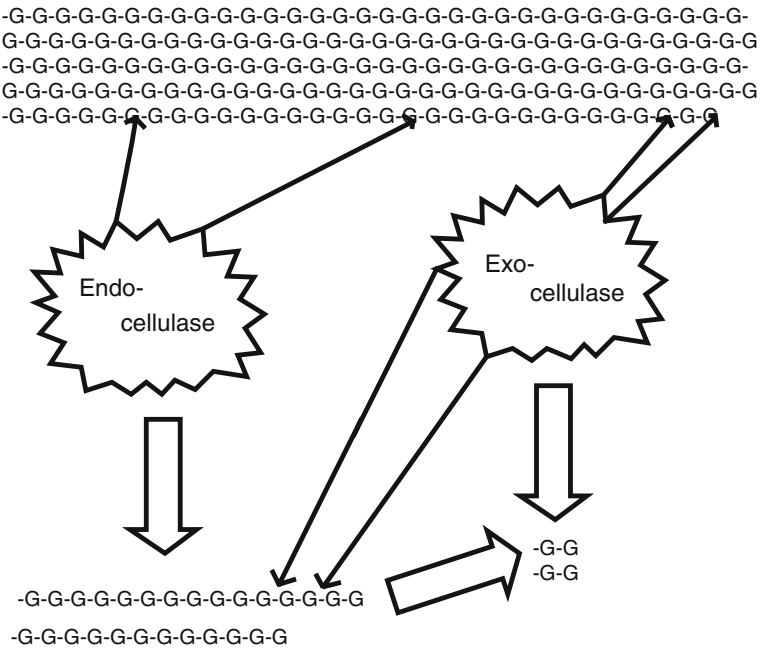

Fig. 3.1 Part of the cellulose fiber is attacked by an endo-1, 4-β-glucanase (endocellulase) breaking the chains and splitting off oligosaccharides in a random manner, including soluble shorter chains with, for example, 3–5 glucose units. An exo-1, 4-β-glucanase (exocellulase) splits off cellobiose units from the non-reducing end of the carbohydrate chains. *G* stands for a glucose unit

Higuchi 1993; Tien and Kirk 1984). Three main hydrolytic enzymes are involved in cellulose degradation; one type of enzyme (endo-1, 4-∃-glucanase) covers the cellulose chain and splits the glucosidic linkages in a random fashion (Fig. 3.1) producing oligosaccharide units of different lengths that may still be attached to the microfibril structure. Another enzyme, an exo-1, 4-∃-glucanase, splits off either cellobiose or glucose from the non-reducing end of the cellulose chain. Finally, 1,4-β-glucosidase hydrolyzes cellobiose and other water-soluble oligosaccharides, such as triose and tetraose, to glucose. One important aspect of this enzyme system is that the different enzymes with different specificities (the endo- and exoglucanases) exert a synergistic action that enables them to degrade both crystalline and amorphous cellulose.

In addition to hydrolytic enzymes, some cellulolytic organisms produce cellobiose dehydrogenase. This enzyme is found in a variety of fungi and appears to have roles in both lignin and cellulose degradation. There was much confusion in the early literature about this enzyme, but recent work has resulted in a renaming of both cellobiose oxidase and cellobiose:quinone oxidoreductase to cellobiose dehydrogenase (Cameron and Aust 2001).

The soft-rot fungi as a group appear to have a cellulose-degrading system similar to that of the white rots. However, in contrast to white-rot fungi, brown rots have not been found to have the synergistic enzymes that are found in white rots and they appear not to have the exoglucanase mentioned above. However, Highley (1988) found several species of brown-rotters that were able to solubilize microcrystalline cellulose. Thus, the generally held conclusion that these fungi merely seem to depolymerize cellulose without producing soluble monomers or dimers may not be entirely correct. Still, no other enzyme has been found to substitute for the missing exoglucanase that splits off soluble units. This has led Eriksson et al. (1990) to conclude that there may be a non-enzymatic mechanism involved. An observation that hemicellulose is virtually absent in wood decayed by brown rots suggests that brown-rot fungi may degrade hemicelluloses. Although the mechanisms for degradation of cellulose are far from clear, work on a basidiomycete (Wolter et al. 1980) suggested that at least for some species, a less specific or multifunctional enzyme that could degrade several different polysaccharides was active, an observation that suggests that this enzyme also has an effect on cellulose.

The ability to degrade crystalline cellulose is also found in many bacteria. Detailed studies on *Clostridium cellulolyticum* show that the organism produces at least six different cellulases, each with slightly different structural and catalytic properties. The cellulases, along with xylanases, are held together in a large structure, the cellulosome, by a scaffolding protein (Bélaich et al. 1997), much as was envisioned by Eriksson et al. (1990). In the anaerobic bacterium *Clostridium thermocellum*, a multicomponent complex of cellulolytic enzymes was named 'cellulosome' in the very early work of Viljoen et al. (1926). A close contact between the cellulose substrate and the organism often appears to be necessary.

The degradation of cellulose by bacteria has been suggested to be hydrolytic, although the mechanisms seem to be different from those found in fungi. For bacteria, the cellulolytic enzymes are arranged in clusters and act in a combined way as described above. There are a few other groups of cellulolytic bacteria that have been studied, including *Cytophaga*, *Cellulomonas*, *Pseudomonas*, and *Cellvibrio*. It appears that these have their cellulolytic enzymes bound to the cell wall, and therefore, a close contact is needed between the cell and the substrate (Berg et al. 1972; Eriksson et al. 1990). This property seems today to be widely recognized (Wiegel and Dykstra 1984).

Actinomycetes, in contrast to some other bacterial groups, appear to degrade cellulose in a manner similar to that of the fungi and can also degrade the crystalline form. Several strains have the ability to degrade the lignocellulose complex. The fungal model for enzymatic attack on the cellulose molecule, namely that an endo- and an exocellulase act synergistically, appears to be valid for actinomycetes, supporting their similarity to white-rot and soft-rot fungi.

The synthesis of cellulases is induced by cellulose, cellobiose, sophorose, and lactose. The presence of cellulose appears to be the best induction agent. On the other hand, the presence of glucose seems to repress the synthesis of the cellulase system. As cellulose is a large and non-soluble molecule, it cannot be absorbed

into the microbial cells and exert an inducing effect. Today, the accepted theory is that the organisms have a constant, basic level of cellulase on their surface. Upon contact with cellulose, low amounts of inducing substances are released from the cellulose, enter the microbial cell, and induce cellulase formation. It is likely that both the type of compounds, for example cellobiose or cellotriose, and a low intracellular concentration of these compounds influence the synthesis of cellulase. There are also theories that metabolic transfer products of glucosyl are active as inducing agents, one of these being sophorose (Eriksson et al. 1990).

3.3.2 Degradation of Hemicelluloses

In wood, the total concentration of hemicelluloses usually ranges from 20 to 30 % (Chap. 4). There are clear differences in the composition and structure of hemicelluloses in softwood as compared to hardwood litters. The composition of hemicelluloses is clearly different between hardwoods and softwoods (Table 4.1). The hemicelluloses are composed of both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, and D-glucuronic acid. These individual sugars may be methylated or acetylated, and most hemicellulose chains contain between two and six different kinds of sugars. Hemicelluloses from hardwoods have average degrees of polymerization in the range of 150–200 units, and most hemicelluloses are based on the 1,4-ß-linkage of their main sugars.

Degradation of hemicelluloses requires more complex enzyme systems than are needed for the hydrolysis of cellulose. For example, xylan-based hemicellulose contains both 1,4-ß-linkages and branched heteropolysaccharides, which require a complex set of enzymes for degradation (Dekker 1985). Figure 3.2 shows the possible structure of a xylan-dominated molecule. The xylan backbone is made up of both acetylated and non-acetylated sugar units. On the branches, there are units of glucose and arabinose. The degradation of such a molecule requires the concerted action of several different hydrolytic enzymes (Eriksson et al. 1990).

3.3.3 Degradation of Lignin

Lignin degradation is regarded as a process that differs between the three general groups of decomposers: white-rot, soft-rot, and brown-rot fungi. Although the names are old and refer to characteristics easily seen by the eye, there are also functional differences in the degradation mechanisms, motivating the continued use of the terminology. The names are used in connection with fungi although bacteria are also lignin degraders.

The number of different enzymatic mechanisms of lignin degradation with which organisms operate appears to be large, and only a few are well described. In

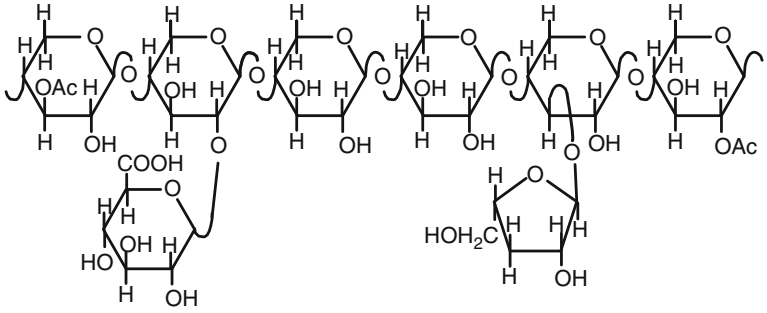

Fig. 3.2 An example of a fragment of a xylan molecule. The backbone of the molecule is made up of xylan units of which part are acetylated (Ac) and part not. The branches in this case are composed of glucose (*left*) and arabinose (*right*) units. The main enzyme attacking the unbranched part of the chain would be an endo-1, 4-β-xylanase, producing oligomers of different lengths. β-xylosidases split the oligomers into simple xylose units. Other enzymes are necessary to split off the side chains as well as, for example, the acetyl substituent. (Eriksson et al. 1990)

fact, today it appears that only one mechanism of lignin degradation is well described, namely that for *P. chrysosporium*, a white-rot fungus. Some characteristics for each of the groups are given below, starting with white rots since these are not only the most studied ones, but also probably the strongest lignin degraders known.

3.3.3.1 Lignin Degradation by White-Rot Fungi

White-rot fungi possess the ability to completely mineralize lignin to CO_2 and H_2O. The result, for wood, is that the entire lignocellulosic complex is degraded more or less simultaneously. A large group of the white rots may even degrade lignin preferentially to cellulose (Hatakka 2001).

The attack on lignin structure has long been considered to start with a removal of the methoxyl group (Figs. 3.3 and 3.4). Newer research has shown that a combination of hydroxylation and demethylation is followed by an oxidative attack on the aromatic ring (Eriksson et al. 1990). The cleavage of the aromatic ring (Fig. 3.4) is an oxygen-demanding step, and the data in Table 3.2 illustrate the importance of the presence of O_2.

The lignolytic enzyme system of our example fungus (*P. chrysosporium*) is synthesized as part of several physiological events that appear to be triggered by N starvation. As described by Kirk (1980), a whole set of enzymes are synthesized under conditions of N starvation (see below). Almost all white-rot fungi produce Mn peroxidase, a fact that may create an ecological niche, based on Mn as a limiting nutrient.

Although we may know more about the lignolytic system of *P. chrysosporium* than those of other white rots, it appears that the lignolytic systems are species

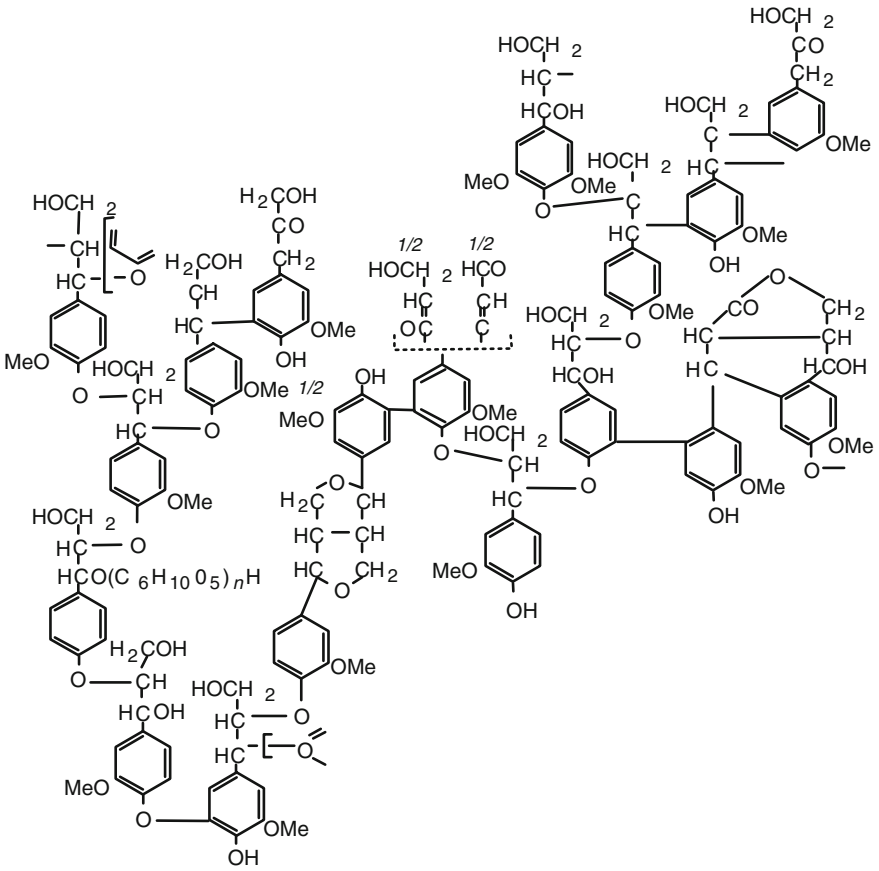

Fig. 3.3 Part of a lignin molecule from spruce

specific and it has been suggested that they depend on the ecological niche of the fungus in question (Hatakka 2001). For example, the white-rot *Ganoderma lucidum* produces Mn peroxidase in a medium with poplar wood but not in one with pine wood (D'Souza et al. 1999). An observation like this latter one may support the finding that white-rot fungi are more commonly found on angiosperm than on gymnosperm wood (Gilbertson 1980).

3.3.3.2 Lignin Degradation by Brown-Rot Fungi

Brown-rot fungi decompose mainly the cellulose and hemicellulose components in wood and have the ability to significantly modify the lignin molecule, but are not able to completely mineralize the compound (Eriksson et al. 1990). They allow for the degradation of cellulose with a relatively small loss of lignin mass.

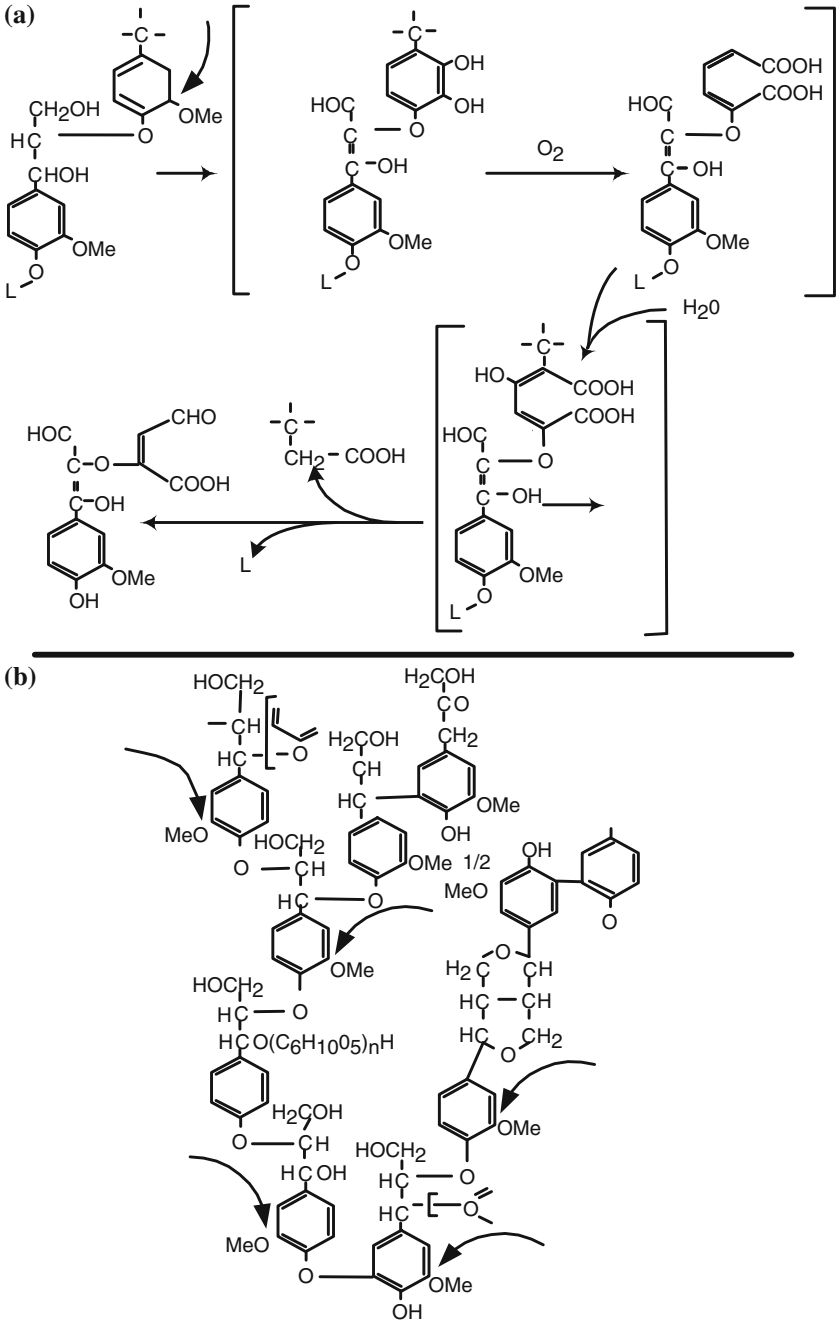

Fig. 3.4 Part of a lignin molecule of spruce during degradation. **a** Under degradation by white rot, demethoxylation and hydroxylation are followed by an oxidative step leading to ring cleavage (from Kirk 1984). **b** The same molecule under attack by brown-rot fungi, resulting in just a demethylation where the methoxy groups (MeO) are replaced by OH groups

Table 3.2 Degradation of aspen wood lignin by different white-rot fungi in the presence of air or pure oxygen

Fungal species	$^{14}CO_2$ evolution (%)		Klason lignin loss (%)	
	Air	O_2	Air	O_2
Phanaerochaete chrysosporium	10.8	35.2	13	40
Coriolus versicolor	14.6	35.5	24	46
Gloeoporus dichrou	9.7	18.1	22	24
Polyporus brumalis	16.6	33.0	19	33
Merulius tremellosus	14.0	22.3	30	40
Pychnoporus cinnabarinus	13.6	22.6	18	37
Lentinus edodes	9.7	18.0	18	41
Bondarzewia berkeleyi	9.0	13.8	25	27
Pleorotus ostreatus	11.7	11.6	17	17
Grifola frondoza	9.2	10.6	8	15

Determinations were made as $^{14}CO_2$ evolution and as Klason lignin. (Reid and Seifert 1982)

Brown-rot fungi are considered to have similarities in degradation mechanisms to white-rot fungi. In both cases, the formation of hydroxyl radicals that attack wood components is important and high oxygen tensions support the degradation (Hatakka 2001). It has been assumed that all brown-rot fungi use the same mechanism for wood decay. However, newer research has indicated that in parallel with white rots, brown-rot fungi appear to have different mechanisms. The initiation of the degradation of both lignin and cellulose appears to be by diffusible small molecules that can penetrate the cell wall. In contrast to white rots, only one brown rot has been found to produce Mn peroxidase.

The radicals formed by brown-rot fungi can remove methoxyl groups from lignin and produce methanol, leaving residues of mainly modified lignin (Eriksson et al. 1990). Relative to native lignin, brown-rotted lignins are structurally modified and have a decreased number of methoxyl groups (Fig. 3.4) and an increase in phenolic hydroxyl groups (Crawford 1981). Brown-rotted lignin is more reactive than native lignin due to the increased content of phenolic hydroxyl groups. Carbonyl and carboxyl groups are also formed (Jin et al. 1990).

3.3.3.3 Lignin Degradation by Soft-Rot Fungi

The traditional view has been that soft-rot fungi do not degrade lignin, but act to soften wood by breaking down the middle lamella of the cell wall (Fig. 4.2). Most soft-rot fungi are ascomycetes and deuteromycetes and are most active in moist wood. Crawford (1981) reviews a number of studies in which purported soft-rot fungi were able to decrease the lignin content of decaying wood.

Today, it has been well confirmed that soft-rot fungi do degrade lignin: In laboratory experiments, up to 44 % was degraded at a wood mass loss of 77 % (Nilsson et al. 1989). In general, they are considered to degrade lignin to some extent.

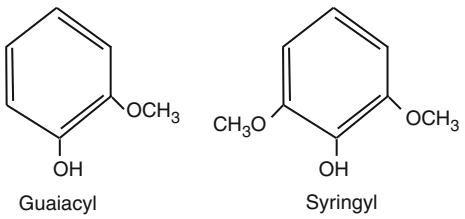

Fig. 3.5 Structures of guaiacyl and syringyl components of lignin

Evidence from a study on the fungus *Daldinia concentrica* may explain why these fungi preferentially degrade hardwoods. This fungus degraded birch wood efficiently but not that of pine (Nilsson et al. 1989). The lignolytic peroxidases of soft-rot fungi do not have the potential to oxidize the softwood lignin which has a high level of guaiacyl units (Fig. 3.5). In contrast, soft-rot fungi readily oxidize the syringyl lignins in hardwoods (Nilsson et al. 1989).

3.3.3.4 Enzymes Directly Affected by Mn Concentration in the Substrate

Manganese peroxidase belongs in a group of enzymes that are classified as phenoloxidases. Manganese is essential for the activity of the lignin-degrading Mn peroxidase (Perez and Jeffries 1992). Although not much was published on this enzyme before 1983, Lindeberg (1944) discovered in the 1930s that *Marasmius sp*. was dependent on Mn for their growth and that a low level of Mn in a substrate hampered the degradation of lignin. This finding was not pursued, and it was not until the 1980s that additional detailed studies followed.

Manganese is also involved in the regulation of other lignolytic enzymes, including laccase (Archibald and Roy 1992) and lignin peroxidase (Perez and Jeffries 1992). The role of Mn peroxidase in lignin degradation is not clear although one of its roles may be to form H_2O_2. The enzyme itself shows no affinity for non-phenolic compounds, which on the other hand are readily attacked by ligninase. Blanchette (1984) found that Mn often accumulates as MnO_2 in wood attacked by white rots, which suggests that Mn peroxidases are important for the degradation of lignin. It has also been found that MnO_2 stabilizes lignin peroxidase.

3.3.3.5 Effect of N Starvation on Lignin Metabolism

Lignin degradation may be repressed by high N levels in the substrate, an effect seen mainly in white-rot fungi but also in brown rots and soft rots. As mentioned above, Kirk (1980) described a set of effects for *P. chrysosporium* that were regulated by N starvation. A drastic effect on lignin degradation was seen when the

Table 3.3 Some fungal species for which raised N concentrations have or, alternatively, have not elicited a repressing effect on lignin degradation

Species	Comments	Reference
Sensitive to N		
Phanerochaete chrysosporium	Isolated from wood	Keyser et al. (1978) Eriksson et al. (1990)
Phlebia brevispora		Leatham and Kirk (1983)
Coriolus versicolor		Leatham and Kirk (1983)
Heterobasidion annosum	Some repression	Bono et al. (1984)
Not sensitive to N		
Pleurotus ostreatus		Freer and Detroy (1982)
Lentinus edodes		Leatham and Kirk (1983)
NRRL 6464 not identified	Isolated from cattle dung	Freer and Detroy (1982)

N concentration in the culture medium was increased from 2.6 to 5.6 mM (Keyser et al. 1978), namely that the lignolytic activity (measured as transformation of ^{14}C-lignin to $^{14}CO_2$) was repressed by 83 %. The same property has since been described for several fungal species in laboratory experiments with pure cultures, although the levels of N and the magnitude of the effect vary. For three species (*Phlebia brevispora*, *Coriolus versicolor*, and *Pholiota mutabilis*), there were effects at 7.8 and 34 mM N in the culture, but not at 2.6 mM N. The magnitude of the effect varied from an almost complete repression in *P. chrysosporium,* to about approximately a 50 % repression in *P. mutabilis*. When using ^{14}C-labeled lignin from red maple wood, there was a clear effect of 20 mM N. There are also several fungi that are not sensitive to N. For example, a white-rot strain isolated from an N-rich environment (cattle dung) showed no sensitivity to raised N concentrations. Table 3.3 lists a number of species investigated for this property.

The results suggest that repression of lignin degradation by N is common but not always the rule. The addition of N to fungal cultures may in certain cases even increase their ability to utilize lignin. We would expect that such fungi, and tolerant fungi in general, would be found in environments with high N concentrations, as in the example given above with cattle dung, whereas most white-rot fungi that grow in and on wood are adapted to low N concentrations. Many of the fungi that have been studied were isolated from wood, and the low N content in wood (with C-to-N ratios in the range from 350 to 500) may explain the generally strong influence of increased N levels.

3.3.3.6 Effect of the C Source on Lignin Degradation

It appears that the presence of a carbon source other than lignin stimulates lignin degradation in several white-rot species including *P. chrysosporium, C. versicolor, Coriolus hirsutus*, *Polyporus sp*., and *Lentinus edodes*. One observation was that

cellulose had a stronger stimulating effect on lignin degradation than glucose, an observation that was ascribed to its *lower* availability; thus, an influence of catabolite repression could be expected (cf. Sect. 3.3.1). The major organic components in litter are normally the insoluble ones such as lignin, cellulose, and hemicelluloses. The latter two normally supply the lignin-degrading organisms with alternative carbon sources.

3.4 Degradation of Fibers

3.4.1 Bacteria

Though bacteria have long been known to be involved in litter decomposition, they have received far less study than fungi. In most cases, bacteria coexist with fungi, particularly basidiomycetes and yeasts, and their presence has been shown to double the rate of fungal growth on wood and increase the overall rate of decay (Blanchette and Shaw 1978). Although it was once thought that bacteria were not capable of degrading lignified cell walls without some type of pretreatment, a variety of fiber-degrading bacteria have now been identified. Three types of bacterial degradation are recognized, based on the manner in which they degrade the cell walls of the substrate: tunneling, erosion, and cavitation (Blanchette 1995). Bacterial decomposition seems to be more common in situations where fungi are under stress. Bacteria have also been found to degrade substrates, especially wood, that are resistant to fungal decay (Singh et al. 1987).

3.4.2 Soft Rot

Soft rots generally occur under conditions that are not favorable for basidiomycetes. However, a key for good growth of soft rots is a high availability of nutrients. It is also generally held that soft rots require moist conditions, though this requirement may not be different from that of basidiomycetes (Worrall et al. 1991). Two forms of soft rots are identified based on the morphology of the degradation they cause (Blanchette 1995). Type I causes the formation of cavities in the secondary wall and is most commonly found in conifers, where lignin-like materials accumulate on the edge of the cavities. Type II causes cell wall erosion, but unlike white rot, it does not degrade the middle lamella (Fig. 4.2). It is possible that the middle lamella is resistant because it contains more guaiacyl propane units. Type II is more common in angiosperms.

3.4.3 Brown Rot

Brown-rot fungi have the ability to degrade holocellulose in plant cell walls without first removing lignin. Brown rots apparently begin their attack on fibers by degrading the hemicellulose matrix because xylans begin disappearing before cellulose (Highley 1987). They do this by first causing a rapid decrease in the degree of polymerization of the holocellulose polymers. The decomposition occurs in a diffuse manner and, in wood, with a rapid loss of strength. These two factors suggest that agents smaller than enzymes are involved (Green and Highley 1997). This initial degradation is generally accompanied by relatively little mass loss.

When attacking fibers, brown-rot fungi appear to attack the S2 layer first, leaving the S3 layer until later (Fig. 4.2; Highley et al. 1985). The reason for this is not known, but Hirano et al. (1997) offer a proposed mechanism that agrees with the observations. They suggest that the brown-rot fungus grows into the cell lumen and releases a low molecular weight substance (1–5 kDa) that diffuses into the S2 layer. Fe(III) is then reduced to Fe(II) and chelates it. The newly formed complex with the Fe(II) catalyzes a redox reaction that produces hydroxyl radicals. These hydroxyl radicals are able to cut canals through the S3 layer large enough for cellulases to penetrate. Clearly, more work is needed to validate this mechanism and to identify the unknown substances required for its operation.

3.4.4 White Rot

White-rot fungi carry out two different types of fiber degradation: simultaneous rot and selective lignin degradation. Some species can carry out both types (Blanchette 1991). In simultaneous rot, the fungi are able to either erode the cell wall adjacent to the hyphae, creating erosion channels, or they generally erode the lumen surface, resulting in an overall thinning of the cell wall. In addition, the hyphae move from cell to cell through pits or by boring through the wall. The other type, selective delignification, often results in cell separation as well as overall thinning of the cell walls. Anagnost (1998) provides numerous photomicrographs that illustrate the various types of decay.

White-rots sometimes seem to have a delay or a lag time of relatively slow mass loss before a period of more rapid mass loss (Fig. 8.1). Blanchette et al. (1997) used a novel biotechnological approach to demonstrate why this might occur. They incubated loblolly pine wood with a white-rot fungus, *Ceriporiopsis subvermispora*. They then placed the wood, in various stages of decay, in solutions containing proteins of known size. Using immunocytochemical techniques, they were able to show that proteins of the size of cellulases and lignin-degrading enzymes could not freely pass through the wood until later stages of decay. After cell walls had been thinned enough to increase their porosity, it was possible for extracellular enzymes to move freely from lumen to lumen, thus initiating the stage characterized by a higher rate of mass loss.

3.5 Mycorrhizae

In undisturbed soil systems, there also appear to be mechanisms that can change the composition of the microflora in ways that enhance its ability to degrade the otherwise stable humus. Hintikka and Näykki (1967) gave a good description of the mycorrhizal basidiomycete *Hednellum ferrugineum* and its effects on the humus layer. The development of thick mycelial mats under the mor layers was described, as well as bursts of soil respiratory activity, followed by a large decrease in the amount of humus in the FH layer. The effect was observed on dry, sandy, nutrient-poor sediment and till soils and could be attributed to plant growth. It appears to be a powerful mechanism driving humus decomposition. Unestam (1991) discussed this effect for certain other mycorrhizal fungi. Further, Griffiths et al. (1990) studied the effects of the ectomycorrhizal fungus *Hysterangium setchelli* on respiration in humus under Douglas-fir and identified patches with very high respiratory activity.

3.6 Ecological Aspects

The composition of the microbial community that invades litter depends on the properties of the litter that falls onto the soil system and the changes in those properties over time. The community of decomposers undergoes many of the same ecological processes that act on communities of primary producers. These processes include succession and competition, while the pathway of decay may be influenced by modifications in these processes.

Microbial succession, the change in community composition over time, occurs as the quality of the decomposing substrate changes, but it also occurs because different organisms invade substrates at different rates. Griffith and Boddy (1990) followed the development of the fungal community on common ash, common oak, and European beech twigs. The primary colonizers included endophytes that were present on the twigs while they were still alive. Secondary invaders were not endophytic and did not show up in appreciable numbers until about 11 months after twig death. They identified a third type of colonizer, the superficial, that appeared on the surface rather early into decay but was not present on the living twig. This pattern is probably similar for all litter types, though of course the species and timing may differ. For example, spruce needles can persist on the twigs for some time after death and decomposition can begin then. However, when the needles ultimately fall to the forest floor, the changing environmental conditions and the availability of a rich variety of inocula result in a change in the microbial community.

In addition to the change in microbial community that occurs along with decay, there are seasonal changes in the microbial community reflecting temperature and moisture. For example, Kayang (2001) followed fungi, bacteria, and selected

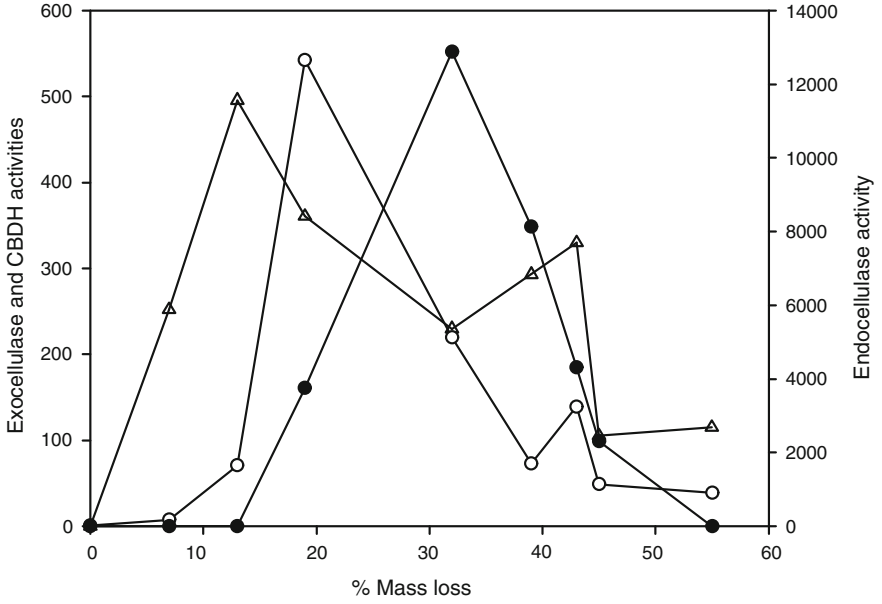

Fig. 3.6 Activities of exocellulase (*triangle*), endocellulase (*circle*), and cellobiose dehydrogenase (CBDH) (*filled circle*) during chestnut oak leaf litter decay in microcosms (Data from Linkins et al. 1990)

enzyme activities in freshly fallen leaves of Nepalese alder in India. The climate was described as subtropical monsoon. The dry season occurred from November through March, with frosts during December and January. The fungal and bacterial propagule numbers varied by a factor of nearly 5 between winter and summer. Enzyme activities (invertase, cellulase, and amylase) reached their peaks before the peak of microbial numbers, between April and June, and then fell slowly. The sequence of peaks suggests a succession of enzyme activities, with invertase, an enzyme involved in sucrose metabolism, peaking first. Amylase, which catalyzes the hydrolysis of starch, and cellulase appear later.

Examining activities of cellulases and cellobiose dehydrogenase on leaf litter in laboratory microcosms, Linkins et al. (1990) observed similar patterns for three different litter species. The species, flowering dogwood, red maple, and chestnut oak, differed in lignin contents and decay rates. However, all three species exhibited an increase in cellulase activity that reached a peak at the same time that cellulose disappearance rate was at its maximum. Cellulase activity then began to decline, and cellobiose dehydrogenase activity began to increase (Fig. 3.6).

As enzyme activities are changing, so are the fungal communities. Osono and Takeda (2001) studied the fungal populations on Japanese beech leaves as they decomposed in a cool temperate deciduous forest. Total and living fungal biomass, estimated using a modified Jones and Mollison (1948) technique (Ono 1998), increased during the first year of decay and then fluctuated for the remainder of the

study period. The percentage of fungi that were basidiomycetes increased for the first 21 months of the study, reaching a maximum of 25–35 % of the total living fungal biomass. They noted that the relative abundance of basidiomycetes was linearly and negatively related to the lignocellulose index (Chap. 2), an index of litter quality equal to the fraction of holocellulose in the lignocellulose. They identified over 100 fungal taxa on the beech leaves during their study and distinguished three groups: an early-appearing group, a late-appearing group, and a constantly appearing group. The early-appearing fungi were present during the period of net nutrient immobilization and the late-appearing fungi increased in number as the litter moved into the phase of net mineralization.

Decomposer populations may work synergistically or in competition. Competition is visible in decaying logs where discrete zones of decay caused by different organisms can be easily discerned. In some cases, the organisms define their boundaries with black zone lines. The interspecies dynamic can change as decomposition proceeds. For example, Bengtsson (1992) found a synergism with no evidence of competition between fungi and bacteria on common beech leaves during their first year of decay in stream microcosms. In comparison, Møller et al. (1999) found clear evidence of competition between fungi and bacteria on 1-year-old beech leaf litter, also in a microcosm study. This difference probably relates to the age, and hence the state of decomposition and the quality of the litter. Though there are not many studies on this phenomenon, it is possible that as litter quality decreases, the competition for the remaining resource becomes more intense.

As decomposition proceeds, the microorganisms themselves can become important substrates for the microbial community. Some fungi, including wood decay fungi, are able to use the cell walls of other fungi or bacteria, presumably as an N source. Some bacteria are able to degrade hyphal walls (Tsuneda and Thorn 1995).

There are many interactions among the organisms involved in decomposition and these interactions change over time. These complex, dynamic systems are not easily described. However, this natural complexity does have implications for the interpretation of pure culture and microcosm studies. Such studies are often the only way to control variability enough to ask a precise question. On the other hand, the behavior of a single, isolated species or of a simple community in a mesocosm may not reflect its behavior in the more complex natural environment.

Molecular microbial ecology promises to be a powerful tool for the study of decomposition and nutrient cycling (Zak et al. 2006). As molecular analytical tools become more available, molecular databases more accessible, and computer systems to analyze them become more powerful, molecular tools will be able to provide information on microbial community structure and function at a level previously not possible. For example, Blackwood and Buyer (2007) have demonstrated the potential of terminal restriction fragment length polymorphism (T-RFLP) to identify microorganisms from a variety of soils.