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Microbial Degradation of Wood from Aquatic and Terrestrial Environments

A better understanding of how wood is degraded by biotic (microorganisms) and abiotic (chemical and environmental) agents has emerged during the past few decades, and this information is valuable to conservators to preserve historic and archaeological wooden cultural properties. Wood exposed to any environment will degrade over time. If conditions are conducive for decomposition, the attack may be swift and extensive, but if the factors governing decay and deterioration are limited, degradation processes can be exceedingly slow and wood can survive for long periods. Wood is more recalcitrant than many other organic materials because of its multilayered cell structure and the presence of complex structural components such as lignin. Extracellular extractives deposited in the heartwood of trees can make the wood obtained from this region of the tree more durable than others. The ability of some woods to resist degradation is a desirable characteristic, and this knowledge has been known since ancient times. Cedar, cypress, juniper, chestnut, oaks, and other woods rich in extractives were selected for use in buildings, especially when wood was used in contact with the ground, and for ship construction. In structures like the wooden tomb chamber found within Tumulus Midas Mound at Gordion, Turkey, dating to the 8th century BC, the floor timbers were made of very resistant cedar (*Cedrus libani*), while less-resistant pine timbers were used for the walls and ceiling (16). Additional protection, however, was provided around the chamber made of pine wood by surrounding it with huge juniper logs and separating it from the mountain of soil and rubble placed on the tomb (54). Studies of wood from ancient ships show that cedar, oak, and other resistant woods were also used for thousands of years in ship building because of their excellent durability (53).

All wood, even if the cells are filled with resistant heartwood extractives, can be degraded over time, and there are few environments where decomposition can be held in check and normal ecosystem processing and nutrient recycling delayed. One example, however, of an environment where wood has been preserved for millions of years can be found in the Canadian high Arctic. Surprisingly, this region supported forests when warm climatic conditions were present during the Tertiary period (1.8 to 65 million years ago), and wood from some of these forests was buried by huge deposits of sediment during past catastrophic events (21, 30). Wood has recently been found from these locations, and studies have shown that it is in remarkably good condition with little to no change taking place during its long entombment (34).

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This ancient wood is now being released from receding glaciers and geological events and subjected to the current environmental conditions, which include colonization by modern day fungi that have begun to attack and degrade the wood. Other ancient woods buried in the Arctic under conditions that have excluded microorganisms have also been found, but these woods have been severely altered by abiotic processes and appear to be transformed by slow acid hydrolysis, pressure, and other factors, resulting in characteristics that are similar to the early stages of coal formation (6, 40). These studies demonstrate that only the most limiting environmental conditions prevent microbial and chemical degradation from taking place, and it is exceedingly rare to find both microbial decay and chemical deterioration being inhibited.

Investigations of wooden cultural properties recovered from terrestrial and aquatic sites have shown them to be changed in different ways and to consist of varied physical and chemical properties (4, 5). These altered wood characteristics impact how they will respond to conservation treatment and need to be considered to select procedures that will ensure successful preservation. In general, environmental and substrate conditions dictate the type of organisms that can grow and the extent of decay that will take place. Water, oxygen, temperature, nitrogen, and other nutrients are a few of the factors that can influence microbial growth. If these essential components for biological activity are not available, decay is inhibited, as seen in the mummified Arctic woods previously discussed (34). However, even in the most extreme environments, there are some microbes that have adapted to these conditions and can attack wood and other organic materials. Although there are many thousands of fungal and bacterial species that can colonize wood, the decay that results can be categorized into a few groups based on how the wood is altered. This categorization is not complete and more groups and subgroups will undoubtedly be identified as additional research is completed, but substantial information is currently available to help differentiate the types of decay. The review of microbial decay found in archaeological wood from different environments included in this section (4) presents a summary of the most crucial information.

WOOD CELL WALL STRUCTURE

For conservators who work with wood, some knowledge of the woody cell wall structure is important. Not all wood is the same, and significant differences in the anatomical structure exist among different genera of trees, allowing identification by observing microscopic characteristics. Differences in anatomy between species within a genus are usually not great and only in a few cases can microscopic analyses be used. The xylem cell structure of hardwoods (angiosperms) and softwoods (gymnosperms) are very different, but their purpose is the same: to provide support and water transport to the tree. Since wood consists of a network of tubes (some up to a meter long), it can be relatively easy for microorganisms to grow in these porous cells. If these tubes are filled with extractives or other substances that are produced by the tree, they can block the passage of microorganisms by physical as well as chemical barriers. Since the anatomical structure of woods can vary greatly, identifying the type of wood is important. This often requires detailed anatomical study and confirmation by an expert, but new resources are available that make working with wood and identification by conservators easier. Recently, a database of information about the anatomical nature of wood has been assembled, and an interactive web site is available that provides information and search capabilities to view descriptions of thousands of woods and over 34,000 images. This massive collection is an excellent tool for obtaining information and micrographs on the characteristics of any wood world wide (InsideWood database at the University of North Carolina, http://insidewood.lib.ncsu.edu). Texts such as *Identifying Wood* (29) and books of wood technology (26, 41, 47) are also valuable resources.

As microorganisms colonize wood, they produce extracellular enzymes and other substances that break down the complex polymers in cell walls (15). These low-molecular-weight fragments from the wall are then utilized by the organism. Some cells, such as the ray parenchyma that can store starch and simple sugars produced by the tree, are thin walled and easily degraded by many different organisms. These cells are usually the first to be colonized, and since the rays connect outer parts of the xylem with inner regions, they provide access for the organism into the wood. Other cells, such as the vessels in hardwoods and tracheids in conifers, can also be avenues for relatively rapid colonization of the woody substrate. The cell walls of tracheids, vessels, and fibers have an interwoven matrix of lignin and hemicellulose that surrounds cellulose, resulting in a more resistant structure. In addition, the cell wall has different layers, and each layer has a different composition and orientation of microfibrils. When attacking these cells, microorganisms must not only degrade a substrate impregnated with lignin but they also confront wall layers of varying compositions. A complex multifaceted degradation system is essential. Most fungi and bacteria do not have such a system or capacity to degrade woody cell walls, but many can. In fact, different degradation systems

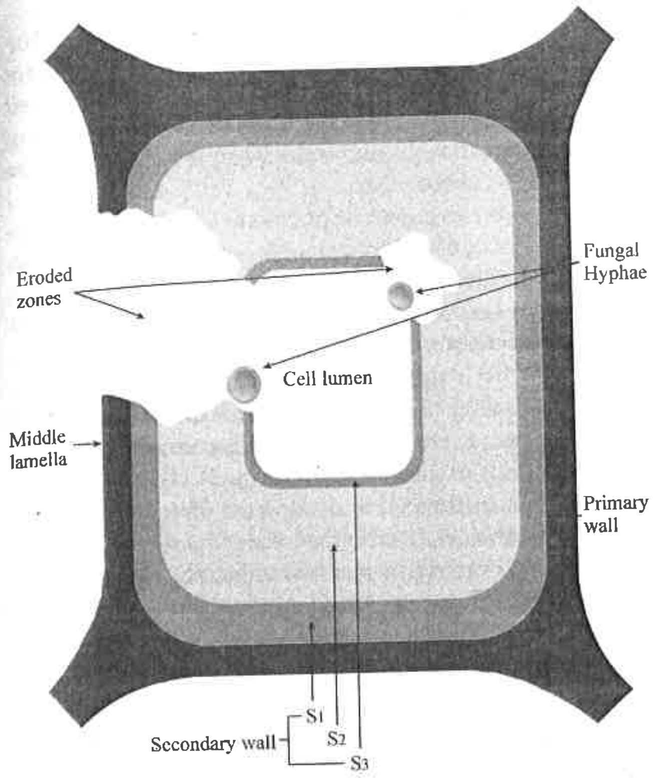

Figure 1 Illustration showing a transverse section of wood cell wall degraded by a white rot fungus causing a simultaneous attack of all cell wall components. Erosion of the cell wall occurs adjacent to the fungal hyphae. A progressive attack takes place that degrades the secondary walls layers and middle lamella. Illustrations courtesy of Joel Jurgens, University of Minnesota.

have evolved among the thousands of microorganisms that live on wood. The degradation processes result in different types of attack and a degraded residue with characteristics that can reveal how it was degraded. Not only can there be visual color changes taking place in the decayed wood but the patterns of degradation can be elucidated by microscopic, ultrastructural, and chemical analyses. Investigations on the altered wood can provide a great deal of knowledge about its current condition as well as past events, such as environmental conditions, that influenced the degradation processes. This information can assist conservators in the selection of an effective preservation strategy for this valuable resource.

DECAY FUNGI

As wood decays, the color of the substrate changes and fungi have been separated into broad groups based on what the resulting wood looks like. Although this seems to be a simplistic way to separate the decay fungi, it reflects the type of enzymes that are produced and their mode of action. White rot fungi, for example, have the ability to degrade all cell wall components, including lignin. They also have the ability to degrade a wide variety of other aromatic compounds, such as polycyclic aromatic hydrocarbons and even coal. The attack on wood is facilitated by nonspecific enzymes such as laccase, lignin peroxidase, and manganese peroxidase (14, 35). As lignin or other dark-colored aromatic substances are altered, they are bleached, and decayed wood may turn very white in advanced stages. Since different groups of enzymes are needed to attack cellulose, hemicellulose, or lignin, fungi have an arsenal of enzymes. The type, quantity, and mix of enzymes produced by different species of white rot fungi can cause different patterns of decay. They all have the potential to degrade lignin, but they may also remove varying amounts of hemicellulose and cellulose in the process. White rot fungi may cause a simultaneous rot where all cell wall components are degraded (Fig. 1), or they can be very selective and attack lignin without degrading cellulose (Fig. 2). It is also possible to

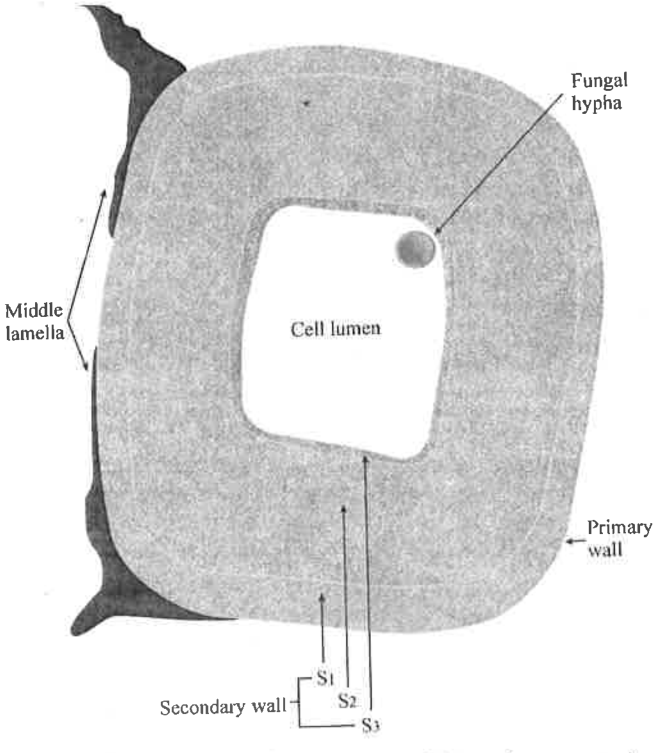

Figure 2 Cell wall degradation by a white rot fungus causing a selective attack on lignin. A diffuse type of attack originates from the hypha in the cell lumen and removes lignin from the secondary wall layers and middle lamella. The cellulose-rich secondary wall remains while the middle lamella can be completely degraded, or as illustrated here, some remnant parts of the middle lamella may be left in less-advanced stages of decay.

find many intermediate types of attack. Some species can even cause a combination of decay patterns when environmental, stress, or other factors influence the production of enzymes and nonenzymatic processes produced by these fungi (3, 15, 46). Simultaneous rot, white pocket rot, white stringy rot, white mottled rot, and many other descriptive terms can be used to describe the visual patterns observed in the decayed wood.

White rot fungi can be very aggressive and are common in terrestrial environments of temperate regions. Many of the most important wood decay pathogens that attack living trees are white rot fungi. The fossil record also shows that some of the oldest types of decay fungi found in ancient trees (such as *Araucarioxylon*, a gymnosperm from the Triassic age that likely had high levels of aromatic compounds in its wood) were white rot fungi (51). Cultural artifacts, such as wooden statues that have been placed outside or wooden structures, can be affected by white rot fungi, since they are common in soil. In waterlogged archaeological wood from wetlands and aquatic environments, however, white rot fungi are not found unless drainage has occurred and an increase in oxygen takes place. As moisture is reduced in the wood, it can become a substrate for these organisms (2). These studies show that changing environments, such as the draining of wetlands or leaving excavated wooden objects under different burial conditions, could lead to severe decay by modern white rot fungi before any detrimental changes may occur due to desiccation. Investigations of site characteristics are therefore exceedingly important, since changes in conditions can have a significant impact on biodegradation (32, 42). This information becomes even more crucial if preservation of wood in situ is selected to maintain the wood for long periods of time.

Another broad category of decay fungi contains those that cause brown rot. These fungi are common wood destroyers and are a major cause of decay problems in buildings. They can also be frequently found in forest ecosystems, especially in conifer forests where they decompose woody forest debris. Brown rot fungi selectively degrade hemicellulose and cellulose from wood, leaving lignin-rich residues after decay that result in a brown coloration (15). The mechanisms causing the brown rot attack have not been investigated as much as those of white rot fungi. Current research suggests that demethylation of lignin occurs, creating small pores in the lignocellulose matrix (17). Depolymerization of cellulose and hemicellulose then takes place by compounds smaller than enzymes (31). This nonenzymatic depolymerization is achieved oxidatively using an iron-peroxide reaction known as the Fenton reaction (23, 52). Early depolymerization occurs randomly along cellulose chains and throughout the cell wall, and the result is rapid strength loss. Cellulases and other enzymes follow, causing extensive loss of the wood polysaccharides.

An important aspect of brown-rotted wood is the large reduction of wood strength properties that occurs very early in the decay process even after limited biomass loss. Often, before evidence of appreciable decay is visible, significant strength losses have taken place. This is due to the early depolymerization of the cellulose, which provides binding power to the cell walls. In advanced stages of decay, the resulting residue consists of high concentrations of altered lignin (Fig. 3). The lignin skeletal remains of the cell wall are exceedingly weak and can fragment and disintegrate into fine particles when subjected to even slight pressure. Since brown rot fungi cause serious decay in wood used in service, it is not unexpected to find them as major decay-causing organisms in historic buildings. Strategies for eradication and

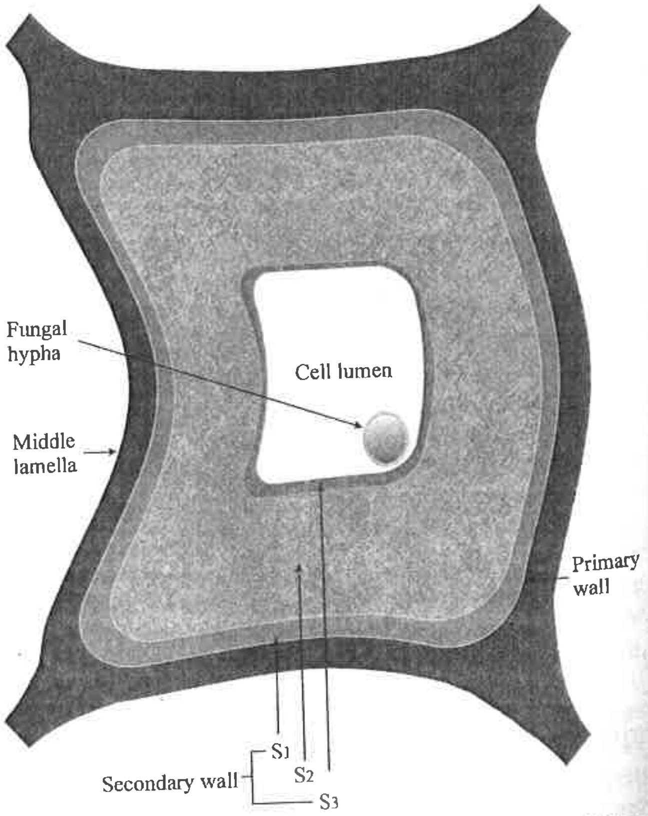

Figure 3 Decay caused by a brown rot fungus. A diffuse attack on cellulose and hemicellulose has taken place, leaving lignin. Depolymerization and degradation of cellulose reduce wood strength and weaken the cell wall.

control are required (11). Often, construction inadequacies and moisture accumulation are factors in establishment of the decay. Although some brown rot fungi can grow over brick, stone, and plaster surfaces, they need an organic carbon source and adequate moisture. The common term "dry rot" is often used to describe this type of decay, but as with all wood-destroying fungi, an adequate amount of moisture is needed for decay to take place. Brown rot has been found affecting some of the oldest wooden objects known, such as wooden statues and other cultural artifacts excavated from ancient tombs in Egypt dating to more than 4,000 years ago (7). These studies and others (5, 9, 15) demonstrate the wide range of terrestrial sites where brown rot can cause wood decay.

Soft rot fungi represent another category of decay organisms that attack wood. They can be found in terrestrial as well as aquatic environments. This group of fungi has had the least amount of research carried out on their biology and physiology, despite increased awareness in recent years of their importance in degrading historic and archaeological woods throughout the world (4, 5, 10). Soft rot fungi can be found anywhere, but they dominate in places with extreme environmental conditions. With the exclusion of aggressive white and brown rot fungi, the more tolerant soft rot fungi appear to have control of the substrate and can utilize it over long periods without competition from other types of decay fungi. When soft rot is found in wood under wet conditions, the decay appears brown and soft. It proceeds progressively from the edges of the substrate to the center. Soft rot fungi can also be found at the opposite environmental extreme under relatively dry conditions or in cold environments. They are often the only fungi attacking wood in arid lands, and recent investigations have found soft rot fungi to be the primary decomposers of wood in the polar regions where historic woods were brought to the area by early explorers during expeditions to the North and South Poles (1, 10, 27). Historic huts built by Scott and Shackleton from 1901 to 1911 in the Ross Sea region of Antarctica, Northumberland House built on Beechey Island in 1852 in the Arctic by the search and rescue ships trying to find Franklin's lost expedition, and Fort Conger built in 1881 and the Peary huts built in 1900 on northern Ellesmere Island in the Canadian high Arctic are all attacked only by soft rot fungi (8). High salt concentrations or heavy metals that effectively inhibit growth of most white and brown rot fungi have little effect on many soft rot fungi. There are many different genera and species, and more are being discovered and described as investigations of wood decay take place in historic woods from extreme environments.

Soft rot from terrestrial sites also is brown and, in advanced stages, can look very similar to the decay caused by brown rot fungi. Microscopic examination needs to be used for diagnostic purposes, since most of these fungi produce distinct decay patterns consisting of cavities formed within the cell walls (type 1 form of soft rot) (Fig. 4). The hyphae colonize wood and penetrate the secondary cell wall layers (15). As they grow within the wall, the hyphae erode channels which develop into cavities. The cavities run in alignment with the microfibrillar orientation of the secondary wall, causing long chains of cavities with conical ends to appear in spiral patterns (Fig. 5). In transverse section, the cavities appear as distinct holes in the secondary wall layer of the wood cells (Fig. 4), but in longitudinal view, they appear as chains of cavities much like a string of sausage links. Some soft rot fungi do not produce cavities (39). Instead, they erode the entire secondary wall but do not degrade the middle lamella (type 2 soft rot). In advanced stages, the remaining cells may have all secondary walls completed eroded and only middle lamella

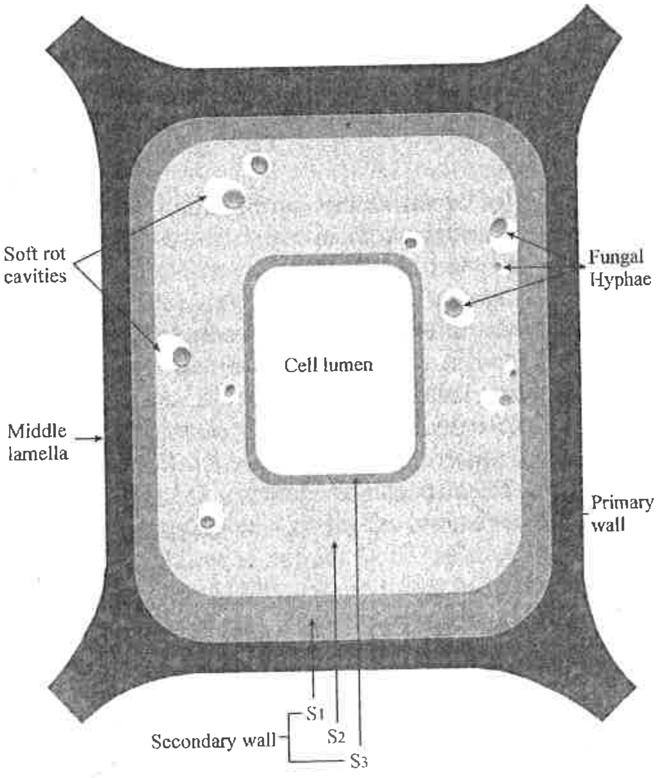

Figure 4 Decay caused by a soft rot fungus. Fungal hyphae penetrate the secondary wall and produce cavities. As decay progresses, the cavities enlarge and may coalesce, resulting in large holes in the wall. See Fig. 5 for a longitudinal view of the cell wall attack.

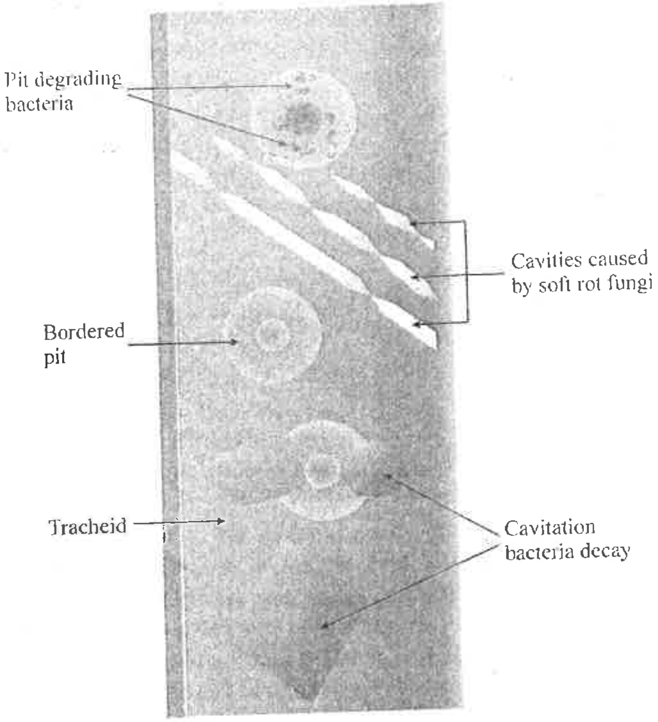

Figure 5 Illustration of a longitudinal section from a wood cell wall showing cavity-forming bacteria degrading pit membranes on a bordered pit as well as chains of cavities formed inside the cell wall caused by soft rot fungi. Reprinted from reference 33 with permission.

remains. A lot of variability can be found among these fungi, and some can produce both type 1 and type 2 soft rot when growing in different types of wood (conifer versus angiosperm). These unusual fungi are very tough and can persist in extreme environments, causing decay very slowly when conditions are suitable for degradation. The historic huts in Antarctica are examples where decay takes place for a very limited amount of time each year when temperatures are conducive to fungal activity (1, 10, 27). The fungus goes dormant for many months and becomes reactivated the following year as temperatures once again rise. Degradation of wood is slow in this environment. The huts are now 90 to 100 years old, and sufficient time has elapsed for these fungi to cause significant decay in areas where wood is in ground contact. Other artifacts at the huts, such as paper, textiles, and leather, are also being degraded by these organisms.

Many studies of biodeterioration in archaeological wood have revealed soft rot fungi as the major source of decay. This is likely due to the environments where this wood is found. Fast decomposition has often been inhibited due to the physical and chemical conditions of the site. But the persistent and tolerant soft rot fungi slowly take hold and may cause slow decomposition over hundreds and even thousands of years. A good example of the unique biology of soft rot fungi was elucidated in a study of the decay found within the 8th-century wooden tomb structure, Tumulus Midas Mound in Gordion, Turkey. As mentioned previously, decay-resistant cedar was used for the floor and juniper logs surrounded the inner tomb structure. A huge cedar coffin containing a body, as well as a rich array of magnificent furniture and bronzes, were also found in the funeral chamber, which had been covered by 53 m of limestone-rich earth (48). Microbiological and stable nitrogen isotope analyses showed that significant decay was present throughout the tomb, and all of this decay was caused by a soft rot fungus (16). The fungus had apparently started its attack at the coffin and mobilized the nitrogen in the body to degrade wood throughout the tomb. Although moisture and nutrients were undoubtedly limited, the fungus in this closed system tolerated the limited moisture availability and high pH conditions from the surrounding mountain of limestone-rich earth and attacked some of the most resistant wood species (cedar of Lebanon and juniper). Even under the most suitable conditions, degradation of the enormous cedar and juniper logs would take very long periods of time. Within the tomb, the fungus was able to recycle nitrogen from the body and utilize moisture to cause extensive decay. The advanced stages of decay and distribution of the fungus in the tomb (in association with the nitrogen) suggests that the soft rot fungus was active for many hundreds of years or possibly for millennia within the tomb. The physical characteristics of how soft rot affected the wood were important to understand, as conservators planned treatments to conserve the wooden furniture and coffin (49, 50). Knowledge of the degraded wood properties and testing of consolidants has led to the successful conservation of the furniture and has allowed many of the objects with decay to be displayed in the Ankara Museum of Anatolian Civilizations (48).

Soft rot fungi can also occur in aquatic environments. However, as sediments accumulate and cover wooden artifacts, the availability of oxygen becomes limited and the soft rot stops. Although fungal attack may be inhibited by low-oxygen conditions, other forms of decay caused by bacteria continue. Bacteria are major decomposers of wood in wet environments. They can also occur in terrestrial systems, but wet conditions are needed, and in some cases, waterlogged wood is the main target for wood-degrading bacteria.

BACTERIAL DECAY

A few decades ago, bacterial degradation of wood had not been demonstrated and the lignified cells walls of wood were assumed resistant to the actions of bacteria. Many bacteria found in trees or wood from temperate sites were considered scavengers without the capacity to degrade wood cell walls. When decay was observed in wood obtained from sunken ships or in buried waterlogged environments, it was thought to be caused solely from chemical hydrolysis, especially when it was from sites appearing to be in anaerobic or near-anaerobic conditions. With diagnostic tools such as electron microscopy, it was shown that bacteria can degrade wood and that there were many different types of bacterial attack. Some may only degrade the membranes on pit apertures between cells (Fig. 5) or remove extractives from wood (12), but others can directly degrade the woody cell wall. Distinct micromorphological features resulting from bacterial degradation are used to distinguish and categorize the different types of cell-wall-degrading bacteria that exist. Three broad categories are recognized based on how they degrade the cell wall. Some bacteria cause broad cavities, while others erode the wood or tunnel into the cell wall. These decay patterns can be identified using scanning and transmission electron microscopy. Frequently, bacterial attack is accompanied by scavenging bacteria, and physical changes from wave action, invertebrates, or other factors can mask the primary cause of the decay (33).

Erosion bacteria are the major degraders of waterlogged woods and actively break down cell walls by eroding the wall from the lumen toward the middle lamella. The attack will start on wood surfaces, but bacteria will move through rays and pit apertures into the lumens of woody cells (vessels, fibers, or tracheids). The bacteria then penetrate the outermost secondary wall layer (S1), and a progressive attack and erosion on the inner secondary wall (S2 and S1) takes place (Fig. 6). The bacteria utilize cellulose and hemicellulose from this layer of the cell wall, leaving a residue of amorphous granular material after the attack. These altered and partially degraded wall substances are colonized by many different types of scavenging bacteria (Fig. 6). The attack by erosion bacteria continues until it reaches the middle lamella. This highly lignified layer is not degraded and is left unaltered. In advanced stages of bacterial decay, the wood cells consist only of a framework of middle lamella and a matrix of degraded wall residue resulting from the destroyed secondary walls. The erosion bacteria can tolerate low-oxygen conditions and function in near-anaerobic conditions, so they can cause significant decay in waterlogged woods even if covered in deep sediments.

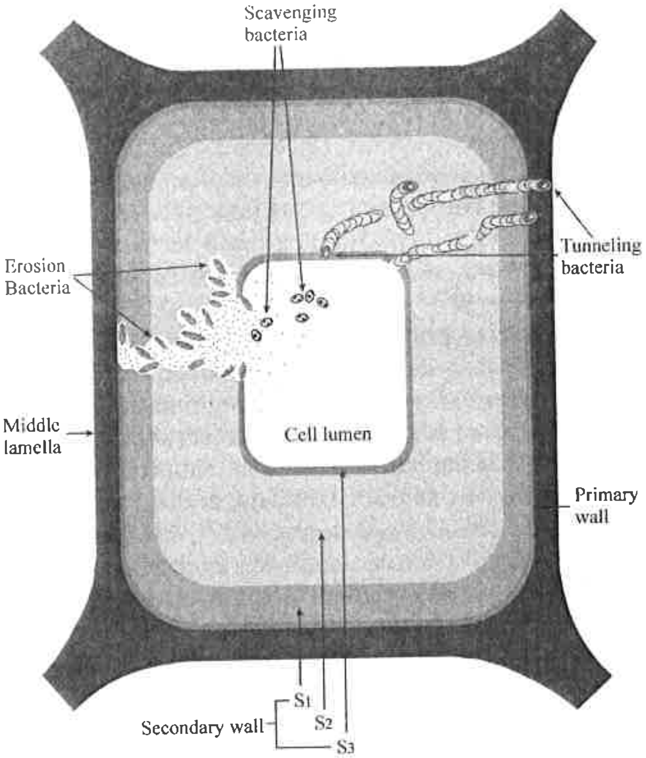

Figure 6 Decay patterns caused by erosion and tunneling bacteria. Scavenger bacteria are also commonly found associated with erosion and tunneling bacteria in the cell lumen and degraded cell wall matrix. Reprinted from reference 33 with permission.

In waterlogged woods where oxygen concentrations are not as limited, tunneling and cavitation bacteria are found, and they may share this niche with soft rot fungi (Fig. 5). Tunneling bacteria erode minute holes in the cell wall and do not cause degradation at large distances away from the bacterial cells. They can cause extensive degradation of the secondary wall as they ramify through this cellulose-rich region. They can also penetrate the middle lamella, producing holes, but as with other types of bacterial attack, much of the cell wall lignin remains in the residual decayed substrate.

An enormous problem resulting from bacterial degradation of waterlogged wood is the loss of wood strength. Although much of the residual cell wall substances may remain and the wood structure has some integrity while still waterlogged, physical changes from drying can destroy the wood structure. Collapse occurs due to the capillary forces associated with removal of water in the lumen, and shrinkage takes place from the water loss inside the cell wall. Proper treatment is essential if the wood is to be preserved in a dry state. Many conservation treatments have been used on degraded waterlogged woods. Some of these treatments have been successful, but others have failed. The failures can be attributed to an incomplete understanding of the nature of the degraded wood and the processes that have caused its alterations.

In the past few decades, many archaeological sites with buried waterlogged wood and wood from sunken ships have been found, and material recovered from these sites has given rise to increased problems with storage and conservation (13, 25, 32). The size of many wooden objects, large numbers being found, and conservation treatment failures has led to new archaeological management options which often now include preservation of the materials in situ, usually by reburial. It was assumed that if reburial conditions could be properly implemented to provide an environment similar to that of preexcavation, decay processes and future damage to the archaeological wood would be minimized. Unfortunately, studies of reburied artifacts at wet sites have demonstrated that the burial conditions reestablished often favored renewed microbial activity, and the wood was aggressively attacked and degraded (32, 43). Incomplete understanding of burial conditions and what prevents or stimulates wood decomposition in wet archaeological sites has prompted the need to obtain more information and monitor environmental conditions at reburial sites. More baseline data from undisturbed waterlogged sites that have the least decay hazard are needed, so similar conditions can be mimicked when preserving wood in situ (24, 32, 37, 42).

The isolation of bacteria responsible for decaying wood has not been possible using conventional methods. Success using anaerobic conditions and detailed culturing procedures have provided cultures for molecular characterization (28, 36, 38). The nucleotide sequences of 16S rRNA gene regions have been determined for some of the isolates and phylogenetic analyses completed (28). Many of the bacteria are similar to those found at sites where biodegradation of humic substances is taking place. To date, laboratory studies to produce in vitro decay with individual organisms isolated from waterlogged woods have failed, suggesting that unique environmental conditions are needed and, possibly, a consortium of bacterial species may be needed.

ROLE OF SULFUR BACTERIA

Scavenging bacteria are not involved in the primary degradation process of wood, but they appear to have a significant influence on the wood and their activities affect subsequent conservation treatment. Sulfur-reducing bacteria are a distinct group of anaerobic prokaryotes that reduce sulfate ions to hydrogen sulfide as they metabolize simple organic substances. Reactions have been found to occur between hydrogen sulfide and wood components and with iron from corroding metals forming iron sulfides. Recently, this has raised serious concerns for postconservation stability and deterioration of conserved woods. This sulfur problem has received the greatest attention after reports that the *Vasa*, a 17th-century Swedish ship that was excavated and conserved by a polyethylene glycol consolidation process taking 17 years to complete and then many additional years of slow drying, was deteriorating (45). Problems with acidic sulfur salt outbreaks were noticed soon after conservation treatment ended, and investigation showed that tons of sulfur had accumulated in the *Vasa* and that deterioration from sulfuric acid and other chemical processes were taking place. This magnificent ship displayed in the *Vasa* Museum had become one of Sweden's major tourist attractions, and its deterioration after conservation treatment was unexpected and alarming to researchers and the public. As a result, a large number of investigations were completed, and a recent publication from this work is presented in this volume (paper 15) describing how sulfur accumulates in waterlogged woods and the important implications for improving conservation methods (19).

During the several hundred years that the *Vasa* had been on the bottom of the Stockholm harbor, bacterial degradation had taken place in the timbers. Degradation was significant but restricted to the outer regions of the ship's timbers. After excavation, conservation procedures used polyethylene glycol as a bulking material to replace water and give the degraded wood stability during drying. Once the long and tedious process of consolidation was complete and the ship was displayed, acidic sulfate salts started to appear on the surface of hull timbers (20). In addition, the surfaces of some wood layers that had received a coating of high-molecular-weight polyethylene glycol (polyethylene glycol 4000) were being lifted off by an underlying precipitation of sulfate salts. Elemental analyses revealed that high levels of iron were also present in the ship's wood, apparently originating from iron ions during corrosion of iron bolts and other iron in the ship. Reactions with sulfur produced iron sulfides in the wood. With both sulfur and iron contamination in large amounts within the wood, several different deterioration processes can be expected to occur. The acid that forms in oxidation processes can degrade cellulose by hydrolysis, and iron ions can catalyze oxidation processes such as Fenton type reactions to degrade cellulose and oxidize polyethylene glycol. Both degradation processes can destroy the wood. Also of importance is the ion-conductivity of the polyethylene glycol that can allow transport of acidity from the degraded sites (outer regions of the timbers) into nondegraded parts of the ship's wood. This movement of ions takes place with fluctuating humidity, which has been documented in the *Vasa* Museum (18).

To study the sulfur problem, sound wood blocks were submerged in sulfate- and iron(II)-containing media and inoculated with a consortium of microorganisms previously obtained from seawater that contained wood-degrading erosion bacteria and scavenging sulfate-reducing bacteria (38). To simulate partly aerobic conditions, some inoculated flasks were aerated by bubbling air for 10 minutes once a month, while other flasks were closed and developed anaerobic conditions during the 2 years of incubation. Additional experiments were set up where pieces of archaeological wood with bacterial decay were placed in seawater under near-anaerobic conditions and sound wood was added to them. These chambers were incubated for 4 years.

Wood blocks examined after 2 years showed evidence of erosion bacteria degrading the ray parenchyma cells and the adjacent wood cell walls. The decay observed was similar to what would be expected during incipient stages of bacterial attack where the erosion bacteria moved into the wood via the rays and affected the tracheids that contact the ray cells. Total sulfur concentrations increased in the experimental woods to more than 10 times the reference control woods. This increase was found to be primarily in the outermost 1 cm of the wood where most of the bacterial degradation and activity were observed. Iron did not accumulate in most of the samples, but in wood exposed to archaeological wood with high iron content for 4 years, the experimental wood blocks had elevated iron concentrations.

Examination of the wood cell walls with scanning X-ray spectromicroscopy at the X-ray energy for reduced sulfur showed that the greatest concentrations were in the lignin-rich middle lamella and cell corners. Sulfides produced reacted with lignin-producing thiols and complex organosulfur compounds within the residual lignified cell wall material. The distribution of the bacteria in wood could also be identified using X-ray spectromicroscopy images of phosphorus, since phosphates are in all bacteria and phosphorus concentrations increase with the degree of bacterial degradation of wood (22). The distribution of phosphorus was observed within the degraded cell walls in association with areas that sulfides were accumulating. It appears that erosion bacteria open up the cell wall by degrading cellulose and hemicellulose, allowing the secondary scavenging bacteria and sulfate-reducing bacteria to move into the degraded matrix. They can easily come in contact with the residual middle lamella lignin located inside the cell wall as the secondary wall is altered and destroyed. As they reduce sulfates to sulfide within the degraded substrate, the sulfides react and combine with the altered lignin to form the thiols. As the process continues over time, high concentrations of sulfur accumulate in the wood. Cells without erosion bacteria and wall degradation have no free carbon sources for the sulfate-reducing scavenging bacteria, and access to inner regions of the cell wall are not available. In these cells, no sulfur accumulates.

Additional spectral analysis using XANES (X-ray adsorption near edge structure) spectroscopy showed the reduced sulfur in thiols and disulfides in organic sulfur compounds. Large amounts of iron sulfides were found in the wood experiments exposed to the bacterial cultures from the iron-rich archaeological wood. The iron sulfides can oxidize rapidly in the presence of oxygen, forming elemental sulfur. These results show that there are two pathways for accumulation of reduced sulfur in wood by bacteria, the reaction with lignin to form thiols and the formation of iron(II) sulfides with pyrite and likely pyrrhotite also being produced (18).

These processes, shown in vitro, would be common in marine environments, and the *Vasa* would not be expected to be the only excavated ship with these problems. Indeed, studies have shown that the accumulation of reduced sulfur is common in wooden shipwrecks preserved in seawater (44). One ship, the *Mary Rose*, an English war ship that sank in 1545, is currently under conservation, and a polyethylene glycol spray treatment is being used. Timbers of this ship have extensive bacterial degradation by erosion bacteria that occurs deep within the timbers. Reduced sulfur compounds were concentrated in the degraded wood cells with thiols present in the lignin-rich middle lamella and disulfides and elemental sulfur in particulate form in the wood. Analyses indicate that substantial amounts of sulfur, at least 2 tons, exist in the ship's hull (the hull is approximately 280 tons in total weight). As learned from studies of sulfur accumulation in the *Vasa*, this large amount of reduced sulfur will undoubtedly have severe consequences in the polyethylene glycol-conserved timbers of the *Mary Rose* due to the oxidation of sulfides and other reduced sulfur compounds producing sulfuric acid and the possibility of direct oxidative degradation of cellulose by iron ions. There is also the possibility of degradation of the polyethylene polymers used as a consolidant through formation of hydroxyl radicals that could affect the stability of the degraded wood. Not all sunken ships are affected, however. The Bremen Cog, a ship that sank in freshwater with low sulfate concentrations, has been conserved using polyethylene glycol and has very low sulfur and apparently no potential long-term problems from acidic sulfate salts (20).

With information about the possible damaging future effects of acidic sulfate salts now available, conservators can focus on how to alleviate the problem during conservation treatment. Methods to dissolve and remove iron(III) compounds and neutralize acid are being tested (18). Derivatives of the chelating agent EDTA, such as EDMA, are possible treatments to remove and make reactive iron species inert and to prevent the lignin-bonded organosulfur from forming. Other methods, such as a mild oxidation treatment by singlet oxygen generated by UV radiation in conservation liquid, are also being proposed to exhaust the strongly acid-forming iron sulfides (44). For the *Vasa*, some emergency procedures to neutralize acidic areas have been under way using poultices of baking soda applied to affected timbers. These treatments have worked for short-term control measures to eliminate the acidity problem in localized areas, but more efficient measures are needed for neutralizing the large quantities of acid being produced (18).

Of the utmost importance is a stable museum climate where humidity is controlled, preferably held at approximately 55% relative humidity. For the *Vasa* and other ships and artifacts that have been already treated, controlling the humidity, although not easy in a large open museum where ships are on display, is very important. In Denmark, where the Skuldelev Viking ships are displayed, sulfur is present in these ships. Stable environmental conditions in the museum have been achieved, and outbreaks of sulfate salt have not been detected (18).

The problems associated with the conservation of the *Vasa* and other ships and artifacts are unfortunate and can be attributed to an incomplete understanding of degradation processes taking place within waterlogged environments. It is essential that conservators work closely with microbiologists, geochemists, and other researchers to get more information on these important processes that affect the physical and chemical nature of the wood. Additional research is needed not only for woods coming from wet environments but for archaeological wood being recovered from a multitude of various terrestrial sites as well. In many situations, the identity of the organisms involved with the decay is just being determined, and knowledge of how they function and what effects their degradation processes have on wood and other organic materials is often not known. The results presented in the following papers demonstrate that significant advances have been made to better understand microbial decay processes in wood, and conservators can use this information to help plan effective conservation strategies. However, there is still much to be learned, and the efforts of interdisciplinary research teams, such as those now investigating the *Vasa*, are essential if we are to advance our knowledge so that we can be assured of success when conserving wood.

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