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Waterlogged Archaeological Wood

Biodegradation and its implications for conservation

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SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

Although archaeological wood found in waterlogged environments is often described as well preserved, microbial degradation has taken place.

Microscopic investigations revealed that despite different types of soil, water, sediment, pH, wood species, and age, archaeological wood was mainly degraded by erosion bacteria, even though soft rot and tunnelling bacteria decay was also occasionally observed. Erosion bacteria seem to be the only wood degrading micro-organisms active in near anaerobic environments. A weak skeleton consisting of the lignin rich compound middle lamella remains after decay and maintains the form and integrity of the historical wood, as long as it is kept waterlogged. Erosion bacteria and their attack of wood cell walls are described and illustrated in detail. Presence of active erosion bacteria in 1200 year old Viking poles, suggests that degradation is generally a slow process, that proceeds until all cellulose rich parts of the wood cell wall are utilised.

The results indicate that depth of deposition in historic times as well as in reburial situations today, is an important factor for successful preservation in nature.

In laboratory experiments, it was found that both clay and sandy soils had an equal protective potential, and were significantly less aggressive to wood than top soil. However, 20 cm depth of burial in waterlogged soil did not prevent significant attack by soft rot fungi, but different cover sheets had some positive effect. Long term effects of soil and cover protections are still unknown.

From observations on aerobic decay patterns in archaeological materials, valuable information can be obtained about the history of these objects before waterlogging.

In active conservation situations, polyethylene glycol (PEG) in impregnation baths give rise to microbial growth at the liquid/air interface during immersion. However results indicate that the microbes present are not degrading the wood during treatment and therefore do not represent a threat for historical objects.

Knowledge on microbial degradation in wood, particularly waterlogged archaeological wood, is important for the development of passive and active conservation strategies in future in the interdisciplinary areas of conservation and archaeology.

Keywords: wood, archaeological wood, waterlogged, micro-organisms, degradation, erosion bacteria, soft rot, history, conservation, archaeology, reburial, *in situ* preservation, PEG.

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Preface

Care and respect for historical remains came to me at a young age, and seems to have stayed for good. Practising at the local museum at the age of 13, made my decision clear. Here my first meeting with waterlogged wood took place. However, at that time I was not particularly impressed by the old shipwreck or the special smell that arose from it. Later on, during my conservation education at The Royal Danish Academy of Fine Arts, conservation of waterlogged wood was interesting, but very different from the more spectacular and in most peoples eyes more valuable historical objects of textile, silver, gold, glass etc.

Sad to say, large rotten timbers did not have the same attraction. However, from my point of view, very old objects excavated by archaeologists from soil and water, seemed generally a lot more interesting to conserve, due to their very corroded and degraded state. These unique objects were *really* in desperate need of conservation and therefore also a greater professional challenge.

During several years as a conservator of mainly wet archaeological organic materials at the Central Board of National Antiquities in Stockholm, I increased my contact with waterlogged wood. Extremely large volumes of wooden artefacts from the ship Kronan were treated during the first years. Conservation treatments applied to this category of archaeological object as well as the routines involved became therefore well known. Occasional failures in treatment as well as problems of microbial growth during the impregnation period, arose my curiosity regarding the influence and importance of microbes in archaeological wood.

In 1988 I heard by coincidence about a "strange" Swedish professor who in front of an international forum had postulated that archaeological wood was decayed mainly by micro-organisms. At that time this was similar to swear in Church. Text books, papers and authorities within the field of conservation of waterlogged wood and wood chemistry, had all claimed for a couple of decades that decay of archaeological waterlogged wood was caused by chemical hydrolysis taking place during long term exposure in nature.

Shortly afterwards I contacted the professor in his department, where he briefly introduced me to wood degrading micro-organisms and their effect on wood in general and archaeological wood in particular. For him and his co-workers, this was just a well known fact, whereas for me it was to enter into a new world. When I recovered from the overwhelming shock and pressed "reset", I realised the need and also importance for conservators to increase their contacts with wood microbiologists and knowledge about micro-organisms.

In 1994, I finally convinced myself about my own role and responsibility for integrating microbiology into wood conservation. No one else seemed to care. However, would a professor in wood science support research in an area of this type where no economic support and interest from industry could be expected? What I did not know was that the professor had a special interest for history, especially archaeology. So by fortune and good luck, the professor accepted to be my supervisor; funds were kindly donated by the Central Board of National Antiquities, and I became a student once again. Just like the happy end of a fairy tale.

Looking back, my time as a Ph.D. student has been a great adventure. Interdisciplinary research is the name and applied knowledge is the result. However, to challenge such a wide area, is to accept that you will never be an expert in the individual disciplines. On the other hand, by scratching the surface, you know that a lot more is to come - if you just go on. In the safe hands of world leading experts I entered the most fascinating world of wood anatomy, and the wood degrading fungi and bacteria. Five years is just enough time to realise the width and complexity of the research field; inspiration is on top when it is time to finish.

But it is my wish that the national as well as international authorities in future will support and understand the need of this type of interdisciplinary work, so that unique historical remains can be saved for future generations with methods of conservation based on scientific work.

Contents

Appendix

Papers I - IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I Björdal, C. G.; Nilsson, T.; Daniel, G.; 1999. Microbial decay of waterlogged archaeological wood found in Sweden. *International Biodeterioration & Biodegradation*, *43* (1-2): 63-71.

II Björdal, C. G.; Nilsson, T.; 1998. Laboratory reburial experiments. *Proceedings from the 7th ICOM Group on Wet Organic Archaeological Materials Conference*, Grenoble, ICOM, pp 71-77

III Björdal, C. G.; Daniel, G.; Nilsson T.; 2000. Depth of burial, an important factor in controlling bacterial decay of waterlogged archaeological poles. *International Biodeterioration & Biodegradation*, (In press)

IV Björdal C. G.; Nilsson, T.; Observations on microbial growth during conservation treatment of waterlogged archaeological wood. *Studies in Conservation.* (Submitted)

Additional contributions, which are not included in the thesis due to their non-scientific form:

1. Björdal, G. C.; Nilsson, T.; 1996. Arkeologiskt trä- ett historiskt arkiv. Populär Arkeologi, *14* (2): 31-33

2. Björdal. C. G.; 1996. Konserveringsplan för Kronholmskoggen. Report. Länstyrelsen, Gotland.

3. Björdal. C. G.; 1999. Trämaterial - historiskt och arkeologiskt. In *Tidens Tand.* Ed. Monika Fjæstad, Stockholm, Riksantikvarieämbetet. pp 113-127.

1 Introduction

1.1 Archaeological wood, remains of human activities

Wooden remains from the past fascinate most people, especially when they are exposed in the form of magnificent shipwrecks like the Vasa, Mary Rose, Oseberg or the Viking ships in Roskilde. But only a few people think about the conditions in nature which led to the preservation of these large wooden objects and how unique these and other less glamorous findings are.

Throughout history, wood has served as an important raw material for larger constructions as well as for smaller delicate objects. Houses, trackways, bridges, and boats just as furniture, sculptures, weapons and tools were produced in many different styles; some with delicate artistic ornamentation. Wagons and cargo ships were used for travel and trade. Wood provided a basis for the development of early human cultures.

Archaeological wood can be defined as wood that has been used by humans for specific purposes for some length of time. From a preservation perspective, archaeological wood does not differ significantly from natural wood. The former wood may on rare occasions have received a superficial treatment in the form of paint or tar and the human influence on its final location is often obvious.

In theory, wooden objects should constitute the largest group of archaeological finds in our museums, but this is not the case. Like all organic materials, wood is decomposed by biological processes in nature if not already destroyed by fire (Levy, 1977). Biological degradation is rapid under warm and humid conditions, but slower in dry and cold climates (Feist, 1990; Nilsson and Daniel, 1990). Consequently, outdoor wooden monuments may, like the Buddha statue in northern India, survive 1200 years of exposure due to the very dry and cold climate (Malyon, 1986).

The fact that waterlogged wood decays much slower than wood exposed above water, must have been observed already when humans started to use timber for constructions. Is it even possible that they made use of this knowledge when constructing settlements in lakes and wetlands? In a historical report from dredging works in Stockholm, dated 1756, the extensive decay of parts of the poles exposed above water was compared with the much better condition of parts that had been under water (Knutberg 1756). The report also states that water alone will not cause considerable harm to the timber, it is the combination of air and water that contributes to the degradation!

In very special environments, archaeological wood is able to survive thousands of years (Blanchette, 1995). From the very dry and almost hermetically closed tomb of Tutankhamun, unique wooden finds, 3000 years old, were recovered in almost intact condition 1936 (Fig. 1) (Hepper, 1990). In Scandinavia, the 1200 year old Viking ship of Oseberg with its fantastic treasures of artefacts, was found well preserved in waterlogged clay 1904 (Fig. 2)

(Rosenquist, 1959; Marstrander, 1986). Today most archaeological wooden finds are recovered from waterlogged environments, and are often related to historical shipwrecks or prehistoric constructions in wetlands.

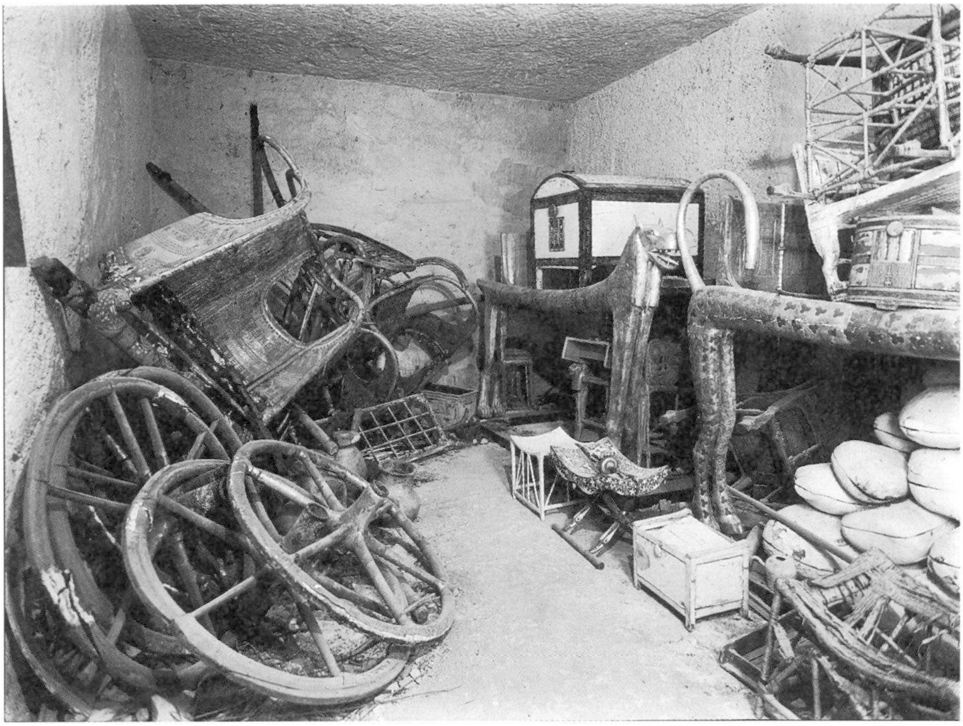


Fig. 1. In the tomb of Tutankhamun, magnificent wooden objects were found almost intact after 3000 years exposure in a very dry and capsulated environment. (Photo Burton 1923. Copyright: The Griffith Institute, Ashmolean Museum, Oxford).

1.2 Wood as an archive of past times

Today it is widely accepted that wooden objects are more than just physical remains from past. By further investigations of the wood tissue, additional information can be obtained (Hillam, 1990). Dendrochronology provides exciting possibilities to determine not only the age, but also the origin of living trees, important information for marine archaeologists discussing the origin of shipwreck timbers (Baillie, 1995). Dendrochronology and observations on insect attack or remains of eggs/larvae on the surface of wooden objects may provide information on previous fluctuations in climate of the area.

Microscopical examination of wood tissue may reveal evidence of decay types, which indirectly provides information on conditions of the wood during, and sometimes prior to waterlogging. An example is the mast of the warship Vasa, which showed extensive attack by the white rot fungus, *Phellinus pini*, a species only found in living trees (Nilsson and Daniel, 1992). Thus, the decay was present already when the tree was felled for mast timber. The poor

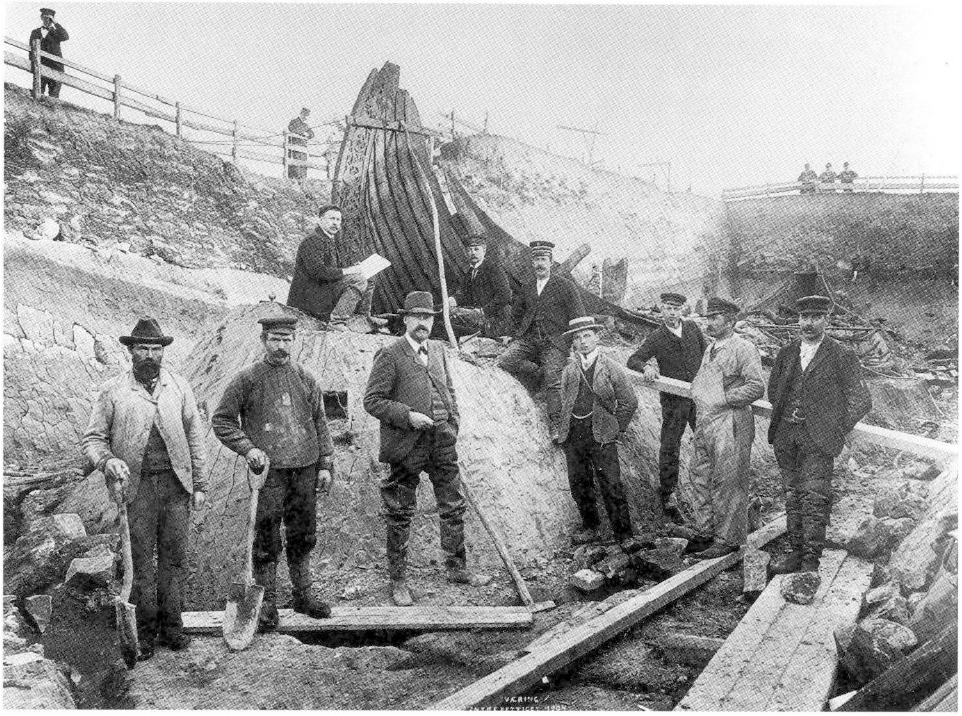


Fig. 2. Photo taken in 1904 at the excavation site of the Oseberg ship. Note the depth of burial as well as the compact clay layer that has protected the ship during 1200 years. (Photo Væring. Copyright Universitets Oldsaksamling, Oslo).

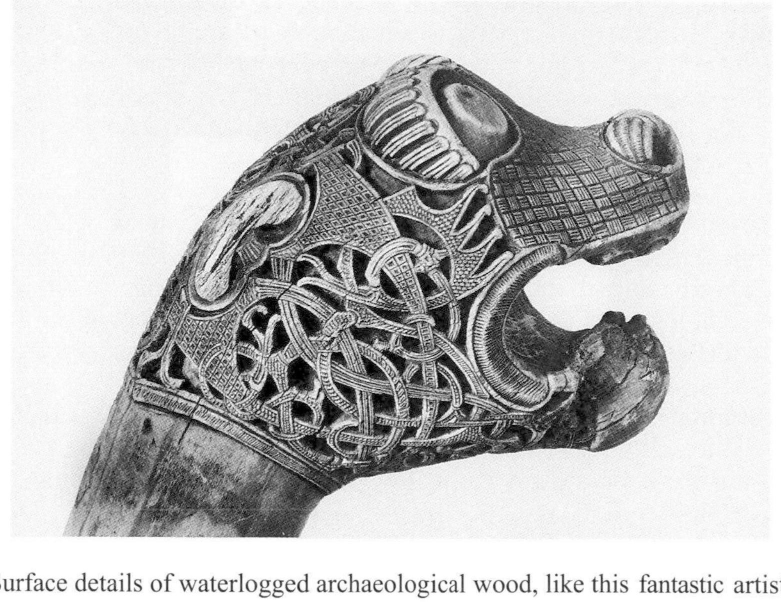


Fig. 3. Surface details of waterlogged archaeological wood, like this fantastic artistic work on an animal post from the Oseberg ship, may be extremely well preserved despite thousand of years of exposure in nature. (Copyright Universitets Oldsaksamling, Oslo).

condition of the mast escaped the attention of the carpenters, who are often thought of as very skilled. One may ask why the mast was used? A possible explanation is that this was the only mast timber available and the demands by the king to have the ship ready to sail made it impossible to find a sound log of the right dimensions. The same fungus was found in a log from the bulwark in Tingstäde Träsk, Sweden (Nilsson and Daniel, 1992), where the timber may have been used in a less critical situation.

Evidence of toolmarks in the intact surface layer of archaeological wood, increases information about the tools used for carving and provide in many finds superb evidence of advanced craftsmanship (Fig. 3). Identification of actual wood species gives important information on how carefully wood species were selected for their specific purposes and reveal that craftsmen in historic times were very skilled (Morris, 1990).

It is clear that archaeological wood contains a wealth of information that could be obtained if carefully examined by other disciplines. This possibility has, however, rarely been explored with the exception of dendrochronology. One can regard archaeological wood as an invaluable historical and environmental archive for information today and in the future.

1.3 Scope of this dissertation

Archaeologists realised very early that waterlogged archaeological wood does not behave as normal fresh wood upon drying (Herbst, 1861). In order to preserve wooden objects for the future, conservation methods have continuously been developed and improved over a period of *ca* 150 years (Jespersen, 1979; Florian, 1990). Despite, this progress in technical development in conservation, surprisingly little efforts has been made to understand the underlying fundamental processes that result in actual decomposition of the wood tissue. Only recently, attention and recognition of microbial decay in archaeological wood has been given and the knowledge obtained has replaced the previous view that the degradation of archaeological waterlogged wood was a result of chemical hydrolysis (Blanchette *et al.*, 1990).

This dissertation describes the microbial degradation processes of archaeological wood in waterlogged environments, and their implications for the closely related areas of reburial and *in situ* preservation and conservation of waterlogged archaeological wood i.e. topics that are of great importance for conservators and archaeologists as well as for museums worldwide.

The dissertation is based on four papers that are published or submitted for publication. Results from the papers support the statements given in the dissertation and are integrated in the thesis, which is based on a review of previous work within the field and on adequate background information. Therefore, each paper provides detailed information on materials, methods, and results. The wide research field has a interdisciplinary character, that includes archaeology, conservation, wood science, environmental aspects, and microbiology. Thus, the papers provide results of both applied as well as fundamental character.

2 Wood structure

2.1 Wood classification and general anatomical properties.

Archaeological finds show that man used wood from shrubs as well as from mature trees. Coppicing was often used to provide a renewable source of wood that was easy to cut.

Wood is produced by the cambium in trees, which is situated between the bark and the woody tissue. In temperate climates, annual rings are formed, often with two types of cells depending on the season. Earlywood cells, often thin-walled, are formed during the first part of the growing season, whereas late-wood cells, often thick-walled are formed during the second part of the growing season (Panshin and Zeeuw, 1964). The variation in density creates the well known annual ring patterns. The width of the annual rings is closely correlated with climate, a fact that provides the basis for dendrochronology (Baillie, 1995).

Trees are divided into two groups. Softwoods (Gymnosperms) like pine, spruce, and larch; and hardwoods (Angiosperms) like oak, elm, and beech. Softwoods have the simplest anatomy with only a few cell types. Tracheids, ray tracheids and parenchyma cells constitute the major cell types. In contrast, hardwoods have several cell types; fibre tracheids, libriform fibres, vessel elements and parenchyma cells (Panshin and Zeeuw, 1964). In softwoods 90-95 % of the volume is occupied by tracheids, making the structure of softwoods quite homogeneous, compared to the more complex and heterogeneous structure of hardwood tissue. The proportions of different cell types vary greatly between hardwoods. Water transport in the living tree is conducted through the tracheids of softwoods but mainly in the vessels in hardwoods.

The outer living part of a tree stem is called the sapwood. Here, almost all mature cell elements, with the exception of the parenchyma cells, are dead and devoid of protoplasm. The parenchyma cells die, as the heartwood forms near the centre of most hard- and softwoods (Panshin and Zeeuw, 1964) This leads to formation of extractives and cessation of water transport. In some wood species, heartwood can be identified by its darker colour, for example in pine and oak. The heartwood of some trees is definitely more resistant than sapwood to microbial attack, due to the presence of extractives.

2.2 Micro-structure

In all four papers, supporting the thesis, pine (*Pinus sylvestris L*) has been most frequently identified and also used for experimental work. Pine has like most softwoods, a fairly homogeneous and more easily understandable structure, in which microbial decay features become more easily recognisable. This is also due to the fact that softwood tracheids (fibres) generally are broader than hardwood fibres. The length of pine wood tracheids is *ca* 3-4 mm and the width *ca* 35µm (Siau, 1984). In the following chapters, examples focus on softwood.

Three different directions planes of the wood tissue are normally referred to when examining the wood structure: 1) Transverse or cross sections are cut across the fibre length; 2) radial sections are longitudinal sections cut parallel to the rays, and 3) tangential sections are longitudinal sections cut perpendicular to the annual ring (Grosser, 1977). In Fig. 4, the three dimensional microscopic structure of softwood is illustrated.

A wood fibre can be described as a long cell with an empty space (i.e. lumen) in the middle representing the space previously occupied by the living cell. The cell usually dies when the surrounding wall is completed. The empty space can then be used for water transport. In earlywood the cell wall is thin (*ca* 2.10 μm) whereas in latewood it is much thicker (*ca* 4.30 μm) (Fig. 4) (Fengel and Wegener, 1984).

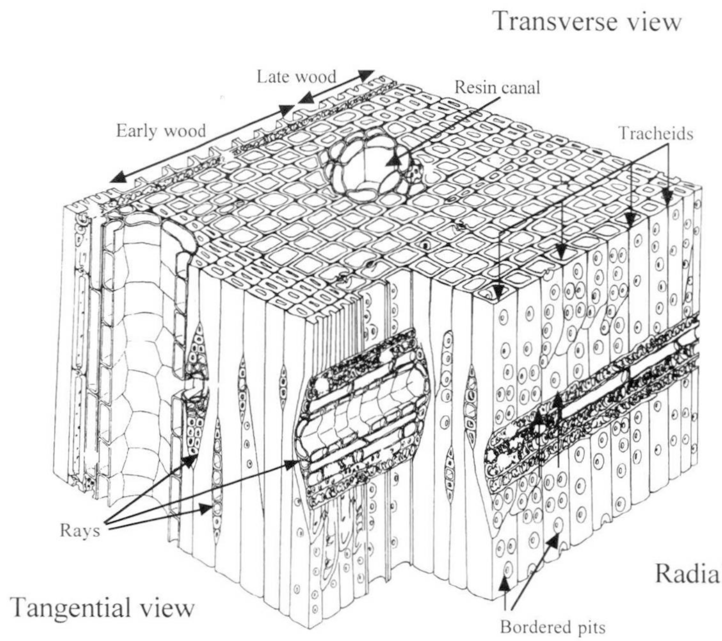


Fig. 4. Three dimensional view of softwood, illustrating the basic features observed in one growth ring. Note the thicker cell wall of latewood tracheids compared to earlywood tracheids, viewed in transverse section. A three dimensional network consisting of rays, tracheids, and pits ensures regulation of flow. (Modified from Siau (1984)).

To describe the wood cell wall in a three dimensional way, many studies and suggestions have been made. It is generally agreed that the wood cell wall can be divided into a primary cell wall and a secondary cell wall. The primary cell wall is very thin, whereas the thicker secondary cell wall can be sub-divided into 3 different layers, referred to as the S_1, S_2, and S_3 layers, where S_2 is far the thickest (Fig. 5) (Panshin and Zeeuw, 1964; Siau, 1984; Haygreen and Bowyer, 1989). In spruce fibres, the average thickness of S_1, S_2, and S_3 are respectively 0.38 µm, 3.69 µm, and 0.14 µm for latewood tracheids (Fengel and Wegener, 1984). Each layer consists of microfibrils, that, with the exception of the primary wall, are arranged parallel to one another and orientated in a characteristic angle to the longitudinal axis of the fibre. By the variable orientation of microfibrils, strength is built into the fibres, which consequently give strength to the living tree at the macro scale.

The inter-cellular layer (matrix) between the fibres, called the middle lamella, "glues" together the individual fibres forming the wood tissue. The middle lamella and the two adjoining primary walls are often referred to as the "compound middle lamella".

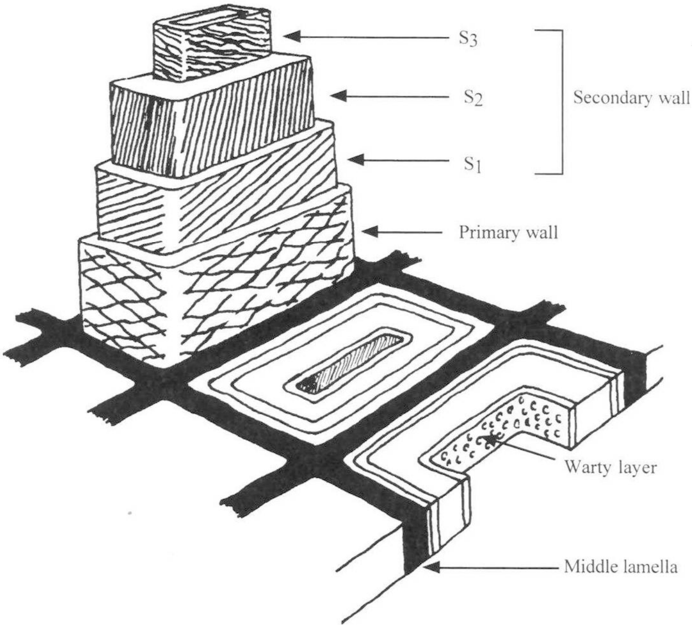


Fig. 5. Cell wall of tracheids consist of two layers; primary cell wall and secondary cell wall. The secondary cell walls are sub-divided into three layers; S_1, S_2 and S_3. Each layer is defined by their individual orientation of cellulose microfibrils; here illustrated by lines. Note the thickness of the S_2 layer. The lignin rich middle lamella is found between the individual cells. This example shows latewood cell walls of softwood. (Modified from Eaton and Hale (1993)).

For transporting water and nutrients, all cell types have pits to allow passage and regulation of flow (Fig. 4) (Haygreen and Bowyer, 1989). A three dimensional network ensures an even conduction around and along the stem axis.

Most fungi and bacteria infect the wood tissue through these pathways (Dickinson and Levy, 1992; Eaton and Hale, 1993). Furthermore, impregnation liquids, like polyethylene glycol (PEG) solutions are also transported through this network.

In leaning trees and branches, reaction wood is formed in response to gravity (Haygreen and Bowyer, 1989). The function of this is to bring the trunk back to its vertical position. The wood tissues formed are different from the normal wood with regard to chemical composition, microfibrillar angles and the presence of almost pure cellulose in a gelatinous layer in cell walls of tension wood (hardwoods) (Siau, 1984; Haygreen and Bowyer, 1989). Compression wood cells have a circular appearance when viewed in cross sections at a microscopic level (Core *et al.*, 1979).

2.2.1 Wood identification

Every wood species has its own specific anatomy, which is used for identification by wood anatomists. Fibre structure can be more easily observed using light microscopy and vessels, ray tracheids, ray parenchyma cells, epithelial cells around the resin canal, and tyloses are some features studied for identification purposes (Grosser, 1977; Schweingruber, 1978; Schweingruber, 1990). Vessels are only present in hardwoods whereas resin canals are significant structures for softwoods. The cross field pits are particularly important features for identifying softwoods (Grosser, 1977).

Problems frequently occur when identifying archaeological wood. Features used for identification are often missing or obscured due to decay, and occasionally complicated detective work involving observations on many sections is required before identification is finally achieved.

2.3 Chemistry

Wood tissue is mainly composed of cellulose, lignin and hemicelluloses. Between 40 - 50 % of the total cell wall mass is cellulose, 25 - 40 % hemicellulose and 18- 33 % lignin. Great variations exist in the chemical composition between the S_1, S_2 and S_3 layers and between wood species (Eaton and Hale, 1993).

Cellulose is a polymer of 8000 - 10000 units of glucose. These long chains are organised into larger units, called microfibrils, which are embedded in lignin and hemicelluloses (Fengel and Wegener, 1984). Highest cellulose concentrations are usually found in the S_2 layer of the secondary cell wall.

Lignin is considered as the "glue" between microfibrils and indeed between wood fibres, and is a complex three dimensional polymer composed mainly of phenylpropane units. Its exact structure is so far poorly understood. Lignin in softwoods consists mainly of guaiacyl propane units, whereas hardwoods in addition contain syringyl propane units. It is generally accepted that type and amount of lignin is important for the natural durability of wood species (Zabel and Morrell, 1992; Eaton and Hale, 1993). The distribution of lignin in wood cell walls is not uniform. The highest concentration (g/g) is found in the middle lamella, whereas the secondary cell walls contain less lignin (g/g). Hemicelluloses, also called polyoses are generally short chain branched polymers of xylose and other sugars. Like lignin, great variations also exist in the type and amounts of hemicelluloses between wood species (Fengel and Wegener, 1984).

2.4 Physics

Strength, elasticity and other mechanical properties of wood are strictly related to the structure and chemical arrangement at the micro- and ultrastructural levels. The thick-walled S_2 layer of latewood cells is often considered to be the most important layer regarding strength (Booker and Sell, 1998). As wood species differ chemically and anatomically, so do their physical properties. Furthermore, sapwood and heartwood have different properties. Throughout history craftsmen have shown their knowledge in chosing the "right" wood species for the "right" purpose.

2.5 Natural durability

The natural durablity of wood species varies widely. The resistance towards wood degrading micro-organisms is generally closely related to amount and type of lignin as well as type and amount of extractives. In heartwood the amount of extractives are generally higher than in sapwood (Eaton and Hale, 1993). This makes heartwood the most durable part of certain timbers. Durability of a certain wood exposed in nature depends on the interaction between the wood structure, mainly the chemical structure, and the environment. This is reflected in the great variability observed between wood exposed in ground and above ground contact. The durability of oak and pine heartwood is not greatly different from that of the sapwood, when exposed in soil and the degradation occurs quite rapidly (Militz *et al.*, 1996; Sierra-Alvarez *et al.*, 1998). In aquatic environments, however, where marine borers are excluded, oak heartwood appears to be much better preserved than pine sapwood. This was observed already in 1757 when oak and pine beams were recovered during dredging works for the Stockholm locks (Knutberg, 1756). Most of the timber used for ship building in Scandinavia in historic times was made from oak heartwood.

2.6 Archaeological wood

The above outlines the structure of sound wood, which is the origin of waterlogged archaeological wood. However, the properties and structure of sound wood are not identical to archaeological wood which has been modified during long term exposure to natural waterlogged environments. Structural changes due to microbial degradation are described in sections 3 and 4.

2.6.1 Chemical and physical properties

Chemical analyses of waterlogged archaeological wood generally show a significant decrease in cellulose and hemicellulose, whereas the lignin content remains more or less unchanged (Jong, 1977; Hoffmann and Parameswaran, 1982; Hedges, 1990; Sakai, 1991). However, great variations can exist. Larger timber dimensions are frequently found to have an inner core of seemingly sound wood. The greatest chemical changes have taken place in the outer layers (Squirrell and Clarke, 1987; Wilson *et al.*, 1993). Smaller and thinner objects are more homogeneously modified. A loss in cellulose is directly related to a decrease in strength and other physical properties of archaeological wood (Schniewind, 1990). Plank/boards from historical wrecks show significant loss in strength compared to sound wood (Barkman, 1967; Hoffmann *et al.*, 1986).

Umax (maximum moisture content) is frequently used as an indicator for the physical and chemical state of wood. Analyses show that the actual water content (i.e. when waterlogged), reflects the degree of chemical change within the decayed wood (Jong, 1979a; Jones and Rule, 1990; Kohdsuma *et al.*, 1996) (Wilson *et al.*, 1993). Density is also used to identify the state of the wood tissue, where low density indicates severely decayed wood with only little wood substance left (Barbour, 1984; Passialis, 1998). Both methods describe mean values for the selected sample and provide important information before a detailed conservation programs are selected.

Archaeological wood suffers from rapid and in most cases detrimental dimensional shrinkage upon drying (Fig. 6) (Christensen, 1951; Jong, 1979b). This physical change has sometimes been misinterpreted as an extremely rapid form of decay. This can be seen from an insert in an article published in New Scientist where the sentences read: "As soon as you remove a piece of ancient oak from a bog, decay will set in. The wood rots about 100 times as quickly as a piece from a modern oak tree and might fall apart after a few hours of exposure" (Coles, 1987). Shrinkage is most significant in the tangential direction, due to the anisotropic nature of wood, and sapwood is generally more affected than heartwood (Jong, 1977). It has been shown that the percent shrinkage is directly related to Umax (Hoffmann *et al.*, 1986; Imazu *et al.*, 1998).

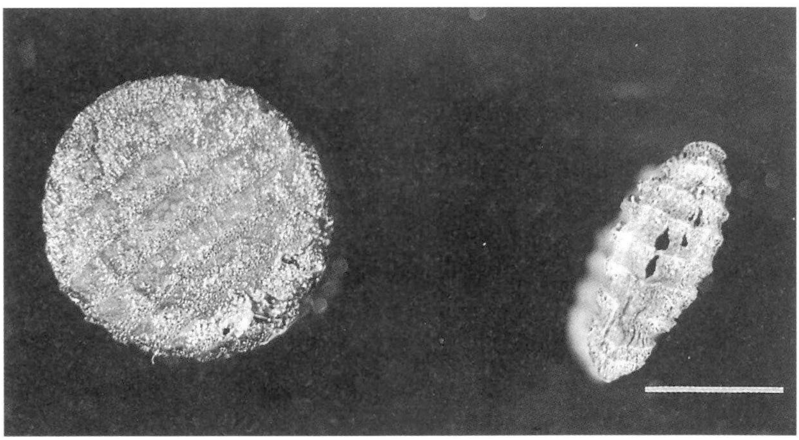


Fig. 6. Serious shrinkage and collapse of heavily degraded archaeological wood. Two discs of same dimensions, taken from a lance of ash wood (Iron age), observed in wet respective dry condition. Bar: 1.3 cm.

3 Biodegradation of wood

Biodegradation of wood is part of the cycling of carbon in nature, where lignin, cellulose and hemicellulose are decomposed by micro-organisms, liberating carbon dioxide, water and mineral elements (Paul, 1992). Decomposition in general, involves a large number of different organisms like insects, worms, nematodes, fungi, bacteria, and protozoa. Each has its role to play in this very complex system.

3.1 General about wood degrading fungi and bacteria.

Wood-degrading fungi and bacteria are specialised to degrade lignocellulosic materials. Fungi, attacking living trees are classified as parasites, whereas those that decay dead organic materials are called saprophytes. Fungi are eukaryotic organisms in contrast to the smaller bacteria (prokaryotes), which have a simpler cell structure. Wood exposed above ground is mainly decayed by fungi (Eaton and Hale, 1993).

Fungi are fascinating organisms. Long, thin threads (most ranging from 2 to 10 µm in diameter), called hyphae, grow continuously, forming mycelia which can expand to form a large "invisible" network, that can occupy areas of several square meters in soil. Within this network, nutrients from the surrounding environment are extracted and may be transported to other parts of the mycelia with less nutrient supply (Zabel and Morrell, 1992). For reproduction, fungi produce spores in sexual and/or asexual ways. This, in combination with the inner structure of the hyphae, is used for classification of fungi (Eaton and Hale, 1993). Under special conditions, especially in autumn, large fruiting bodies are produced by basidiomycetes for sexual reproduction. Such fruit bodies of certain species are collected from fields or forests and provide delicious meals world wide.

Most bacteria are single celled organisms that multiply by dividing. Bacteria appear to be present almost everywhere, tolerating quite extreme conditions. A large proportion of the bacteria cannot be isolated and cultured (Torsvik *et al.*, 1990).

3.1.1 Fungi

Wood rotting fungi can be divided into three main groups: white rot, brown rot, and soft rot. This classification is based on the appearance of wood after decay, chemical changes and micro-morphological characteristics of the attack (Blanchette *et al.*, 1990; Paul, 1992; Eaton and Hale, 1993; Blanchette, 1994). The following micrographs showing different decay types should be compared with the structure of sound wood shown in Fig. 7.

White rot gives the wood a bleached and fibrous appearance after decay, due to its ability to degrade both lignin and cellulose. This is the only group of fungi that have enzymatic tools for completely degrading the very resistant lignin component. Other enzymes are used for cellulose and hemicellulose decomposition, which provides white rot with tools able to degrade all the chemical components of wood. The aggressive effect of white rot on wood cell walls is shown in cross section in Fig. 8.

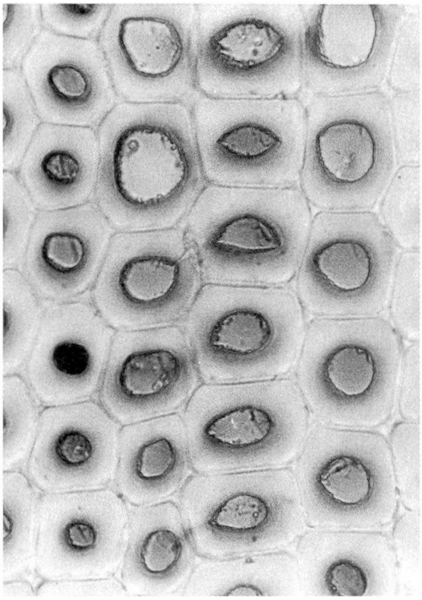


Fig. 7. Transverse section of sound softwood tracheids.

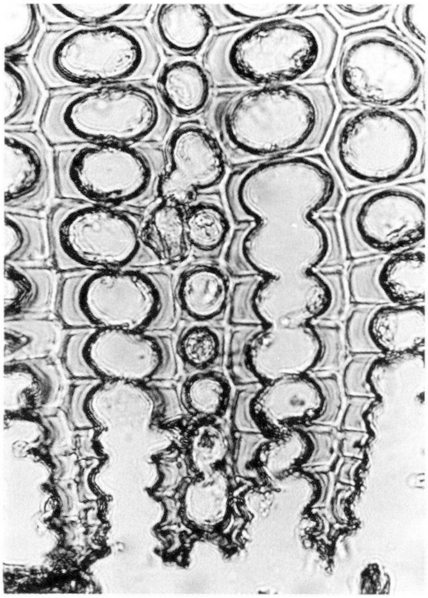


Fig. 8. Transverse section illustrating the ability of white rot to degrade all cell wall components including the middle lamella. By effective cell wall thinning the integrity of the wood tissue is successively lost.

Brown rot darkens the wood tissue, due to the incomplete degradation of lignin. When the wood is dry, deep and narrow brown cross-cracking of the wood into cubes occurs. These cubes are easily powdered, when the wood is pressed. Cellulose and hemicelluloses within the wood tissue are effectively degraded by enzymatic tools released by hyphae within the cell lumen. Using microscopy, wood cells attacked by brown rot show a change of cell wall integrity and a sligthly swollen appearance of the secondary cell walls (Fig. 9). In polarised light, the secondary cell walls show no birefringence, indicating great losses of crystalline cellulose. *Serpula lacrymans* is a well known brown rot fungus, often found in humid buildings, that can establish serious decay at relatively low moisture contents (Viitanen and Ritschkoff, 1991).

Soft rot is named by the soft surface of degraded wood, when wet; however in dry wood, brown appearance and cracks somewhat similar to brown rot arise. Soft rot fungi are common wood degraders in marine as well as wet environments on land. For further description, see 4.5.

Mould and blue stain fungi are other groups of fungi that affect wood tissue in a negative way. They are not regarded as true wood degraders, due to their minimal effect on wood cell walls and strength properties of wood. Staining fungi discolour the sapwood of wood by their dark coloured hyphae which utilise nutrients within the ray parenchyma cells and tracheid lumena (Fig. 10). Moulds grow superficially on wood from where coloured spores are liberated. This group together with bacteria are known as early colonisers of wet wood (Dickinson and Levy, 1992).

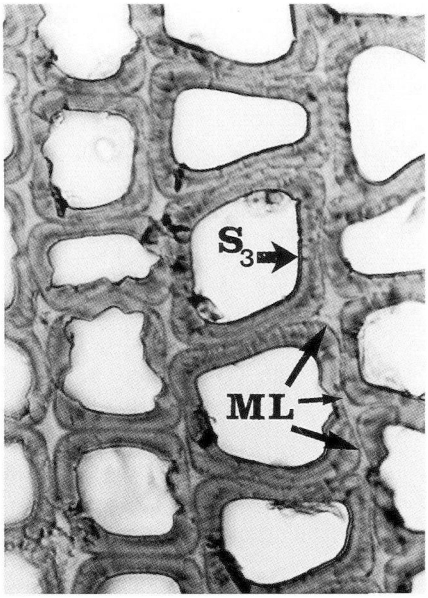


Fig. 9. Severe attack by brown rot transforms the secondary cell wall into a dark slightly swollen amorphous mass, which under wet conditions is held in place by the S_3 (arrow) and the more or less intact middle lamella (ML). The integrity and original form of the tracheids seems slightly changed.

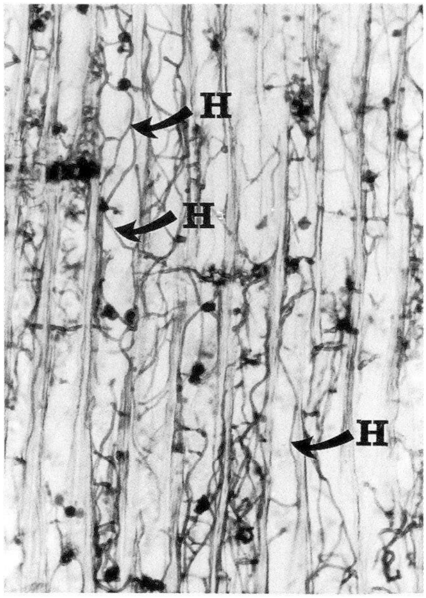


Fig. 10. Longitudinal section showing dark coloured hyphae (H) of blue stain fungi colonising the tracheids of pine where they utilise freely available carbohydrates in the lumena. No degradation of wood cell walls takes place.

Both white rot and brown rot fungi belong to the Basidiomycetes, whereas soft rot fungi belong to the Ascomycetes and Fungi Imperfecti (Jennings and Lysek, 1996). Within both groups, a wide variation in special requirements for growth exist. Also some species are found to act both as staining fungi and cause soft rot depending on conditions (Dickinson and Levy, 1992).

3.1.2 Bacteria

Wood degrading bacteria were recognised and described in detail only recently. The question whether bacteria could degrade the lignified wood cell walls or not was debated for a long time. The fact that most observations on what was supposed to be bacterial attack related to timbers exposed for a very long period of time, sometimes for centuries, made it difficult to find conclusive evidence. The possible presence and activity of wood degrading fungi was also difficult to exclude. Finally, the failure of demonstrating degradation when using pure cultures of bacteria, even cellulolytic species, suggested that bacteria for their attack required some form of preconditioning of the wood (Holt and Jones, 1978; Schmidt and Liese, 1982). Even today, a few recent textbooks state that bacteria can not degrade wood. The task to demonstrate bacterial attack was made simpler by the discovery that the attack observed in nature could be simulated in the laboratory within weeks (Boutelje and Kiessling, 1964; Courtois, 1966; Nilsson and Daniel, 1983). Using light and electron microscopy, active living bacteria could then be observed degrading the lignified wood cell walls. The finding that isolated bacterial consortia could degrade wood, without any form of preconditioning, finally proved that certain bacteria are able to degrade wood (Daniel and Nilsson, 1986). Cellulolytic wood colonising bacteria are able to degrade the non-lignified pit membranes and ray parenchyma cells effectively. The ability of these bacteria to open up pathways in the wood tissues, may cause problems related to increased absorption of water and other liquids (Daniel and Nilsson, 1997).

True wood degrading bacteria, are those which can degrade lignified cell walls. They are divided into three groups: Tunnelling bacteria, erosion bacteria and cavitation bacteria (Blanchette *et al.*, 1990; Singh and Butcher, 1991). These groups are recognised by their micro-morphological decay patterns as observed by light and electron microscopy, and are not groups based on taxonomic classification. Probably each group consists of many species, but unfortunately isolation and identification has so far not been possible despite extensive work (Daniel and Nilsson, 1986; Schmidt and Liese, 1994; Daniel and Nilsson, 1997).

The previous view that bacteria as a group were of minor importance for wood degradation changed, when tunnelling bacteria were found to be significant degraders of sound wood as well as preservative treated and naturally durable wood species (Nilsson and Daniel, 1983; Daniel and Nilsson, 1985; Drysdale *et al.*, 1986). Tunnelling bacteria can effectively degrade wood cell walls including the lignin rich parts such as the compound middle lamellae and the pit border regions (Singh, 1997b). However, decay is restricted to more aerobic environments.

In waterlogged near anaerobic environment, erosion bacteria are the principal degraders of wood (review by Singh and Kim (1997)). See 4.3.

3.2. Requirements for growth

Fundamental requirements for wood-degrading fungi and bacteria can be summarised as follows: water, oxygen, minerals, substrate, right temperature, and a suitable pH, and (Eaton and Hale, 1993).

In very dry or wet anoxic environments decay is absent or minimal (Nilsson and Daniel, 1990). There seems to be a need for the appropriate amount of both water and oxygen for active and effective decay (Dickinson and Levy, 1992).

3.2.1 Oxygen and water

In aerobic environments, for example in forests, white and brown rot fungi are the most common degraders of wood. Here fallen trees can be found on moist soil, half rotted with a white or brown appearance. Basidiomycete fungi require free access to oxygen for decay activities, and if wood becomes water saturated, air is excluded. In water saturated environments oxygen is only available by diffusion, which decreases with depth (Glinski and Stepniewski, 1983; Drever, 1997). Consequently, white and brown rot activity is drastically reduced or even completely suppressed under such conditions.

Absence of competition from the more aggressive basidiomycetes in wet environments, where oxygen concentrations have declined, favours soft rot and tunnelling bacteria (Eaton, 1976; Eaton and Hale, 1993). In wet soils and aerobic aquatic environments both groups show high activity. However, under very restricted oxygen concentrations, erosion bacteria seem to be the only active wood degrading microbes, judging by their dominance in waterlogged wood. There is no conclusive evidence for microbial degradation of lignified wood cell walls in the complete absence of oxygen.

3.3 Marine environment

In marine environments, wood deterioration is under strong influence of marine crustaceans and borers, organisms that are not present under terrestrial conditions. Marine crustaceans and borers are a great problem world wide due to their rapid destruction of wood and play a major role in degradation in aquatic environments, where marine fungi and bacteria are also active (Eaton and Hale, 1993; Cragg *et al.*, 1999). Marine fungi are mainly of the soft rot type.

Marine borers are divided into two groups; the molluscs and the crustaceans. In temperate waters *Teredo navalis* (shipworms) and *Limnoria* are regarded as the most destructive to wood. Shipwrecks situated on the seabed can be completely destroyed within a short period of time. Shipworms tunnel the interior of the wood tissue, whereas finer tunnels are formed by *Limnoria* in the outer parts (Eaton and Hale, 1993). Only within the sediment under the seabed is wood effectively protected from the aggressive borers. This explains why the historical warship ”Mary Rose” situated half buried in sediment, was only half preserved.

Furthermore, if salinity falls, the activity by marine borers is inhibited. Therefore, in Sweden, unique opportunities to find well preserved archaeological remains exist in the Baltic sea and within lakes such as Vänern and Vättern. Turbulence in water and sediments, due to storms and streams, is often extensive in marine environments, leading to great variation in the environment. Thus, oxygen concentrations may vary considerably from time to time.

3.4 Biodiversity

For micro-organisms, archaeological wood is "just" a substrate to live on. Microbial invasion of wood is highly dependent on environmental conditions, especially with respect to moisture and oxygen. Absence of erosion bacteria in aerobic environments, does not necessarily imply that physical conditions restrict the activity. Their slow activity may be repressed by the very fast and aggressive basidiomycetes. Within the wood tissue, local microcosms are formed where antagonistic and symbiotic behaviour of micro-organisms towards each other controls their growth, development, and wood degradation (Zabel and Morrell, 1992). Within archaeological waterlogged wood, soft rot and erosion bacteria are occasionally found within the same part of the wood tissue, suggesting no strong antagonistic behaviour to be present (**Paper I, III**). However, sometimes single wood cells are found decayed by only soft rot, despite that the surrounding cells all are degraded by erosion bacteria (Fig. 11). Defence mechanisms may be involved.

Fig. 11. Despite severe decay by erosion bacteria in this 1200 year old softwood, single tracheids are sometimes found attacked by only soft rot (arrows). The antagonistic or symbiotic behaviour between soft rot fungi and erosion bacteria is unknown.

In aquatic environments, marine borers prefer to infect areas previously infected by soft rot and bacteria (Jones and Byrne, 1976; Eaton and Hale, 1993).

Only recently scientists are beginning to understand the complexity of ecological systems within terrestrial and aquatic systems in nature. Biodiversity as a result of metabiotic activity is a new way of understanding this complexity, where one group of organisms will modify an ecosystem for the next group of organisms (Waid, 1999). The ability of cordgrass (*Spartinia*) and fungi to translocate oxygen into waterlogged strata (Padgett and Celio, 1990) are examples of metabiotic activity that may be of importance for waterlogged wood. Burrowing by animals may change the structure of marine sediments, thereby increasing local oxygenation (Ferrari and Adams, 1990). Archaeological wood is a substrate for a wide range of micro-organisms, which exist in very complex ecosystems, where interactions are possible, not only between the micro-organisms, but also with other organisms.

4 Microbial degradation of waterlogged wood

4.1 Waterlogged archaeological wood

Waterlogged wood may be described as wet tissue, where all capillary systems are filled with water, and from which air is consequently excluded. To be waterlogged, wood has to be situated in a water rich environment, which can be terrestial or aquatic. Sea water, rivers and lakes are typical examples of aquatic environments, whereas peat bogs, other wetlands, and soils with high water table are typical land site environments.

In waterlogged environments, archaeological wood is often found well preserved in contrast to drier sites, where the only remaining evidence of historical wood may be a black contour of decomposed wood in contact with the soil. The ship of Sutton Hoo remained only as a ghost in the sand (Evans, 1977), just as the Ladby ship in Denmark. The remains of wooden posts can be seen as round areas that are darker in colour compared with the surrounding soil. This makes it possible to determine the approximate diameter of the posts even after thousands of years.

In the wetlands of Switzerland and France, unique settlements on poles were found in lakes in the late 19th century (Coles, 1987; Schlichtherle, 1997). In wet clay soils, magnificent ships were found in Norway (Marstrander, 1986). Here, the extreme ability of clay to maintain and even raise the waterlevel by capillary forces, established favourable preservation conditions within the mud. During the 20th century the volume of well preserved archaeological wood recovered from waterlogged conditions has increased enormously (Jong, 1979a; Heide, 1979b; Coles, 1989; Nayling, 1990).

While still wet, well preserved archaeological wooden objects can retain their dimensions and integrity. This helps archaeologists to make useful recordings in the field. Despite the well preserved appearance at excavation, archaeological wood does not normally dry as sound wood. The swamp-like condition, when pressed, reveals a very fragile inner structure, described by many archaeologists and conservators. When dried, waterlogged wood undergoes destructive shrinkage and warping, resulting in samples which are useless for historical collections (See 2.7).

4.2 Research history of decay in waterlogged environments

As far back as 1861, Herbst described archaeological wood to be swamp-like in nature, but no further investigations on the wood tissue were made and the reasons for the condition were not discussed. Chemical analysis of archaeological wood samples carried out through the 20th-century, have all shown an increase in lignin content and a decrease in hemicellulose and cellulose (Sen and Basak, 1957; Wazny, 1976; Hoffmann *et al.*, 1986; Hedges, 1990). Chemical hydrolysis was suggested to be the cause or leaching of cellulose by several authors in the 1970s and 1980s (Stamm, 1970; Ambrose, 1971; Cutler, 1975; Barkman *et al.*, 1976; Hoffmann and Parameswaran, 1982). Only a few authors paid attention to microbial degradation (Florian *et al.*, 1977). Sen and Basak (1957) proposed two prinicipal processes for deterioration of ancient buried wood; one by biological agents and the other in the form of chemical hydrolysis. Chemical alteration, i.e. hydrolysis of wood tissue, was generally the most accepted hypothesis. Different arguments, like absence of fungi and the supposedly anaerobic conditions (Sakai, 1991) have been used to support the idea of abiotic degradation processes. Bacterial degradation of wood was generally not recognised. Few, if any measurements were carried out on oxygen concentrations.

Barghoorn (1949) used microscopy to study degradation of plant tissues, including wood, in sediments. He observed that degradation followed a characteristic sequence leading to a stage where the main part of the secondary wall was transformed into a granular amorphous structure. The primary wall and in many cases the outer part of the secondary walls remained. Unchanged secondary walls were also observed among severely degraded cells. It is clear from his illustrations that the middle lamella was preserved. It is interesting to note that he observed similar degradation in silicified fossil wood from the cretaceous period. He assumed that the degradation observed was caused by chemical hydrolysis.

Degradation of waterlogged wood was later observed by a number microbiologists and wood anatomists. Microscopically, the degradation was quite similar to that described earlier by Barghoorn in 1949 and bacteria were now suggested to cause the attack (Varossieau, 1953; Harmsen and Nissen, 1965; Courtois, 1966; Boutelje and Bravery, 1968; Greaves and Levy, 1968). However, this could first be verified in the 1980s, with help of high resolution electron microscopy. Now, wood degrading bacteria and their decay patterns could be described in detail (Holt and Jones, 1983; Nilsson and Daniel, 1983; Singh *et al.*, 1990). This knowledge was more or less unknown to archaeologists and conservators until 1990, when the book "Archaeological Wood" was published by the American Chemical Society as a result of an interdisciplinary international conference held in Los Angeles 1988. Due to the long delay in fully understanding the cause of decay of archaeological woods, a lot of confusion and alternatives to explain degradation of waterlogged wood existed and still exists within the disciplines of marine archaeology and wood conservation. This will be discussed in the next paragraphs.

4.2.1 Anaerobic / aerobic environment

Conclusions have carelessly been drawn on the environment from where archaeological wood is found. The waterlogged condition found in sediments or soils is often interpreted as being anaerobic or anoxic (Caple, 1994). The absence of aerobic fungi have possibly played some role in this misunderstanding. But it should be stated that absence of aerobic fungi does not necessarily indicate that the environment is completely anaerobic. Anaerobic or anoxic means a total absence of oxygen. Such conditions may be rare in nature. It is more likely that different concentrations of oxygen exist throughout a waterlogged environment, possibly creating aerobic and anaerobic microenvironments close to each other. Instruments have difficulties in measuring very minute levels of oxygen and are of little use in environments where every arriving oxygen molecule would be consumed immediately. As an alternative, it would be interesting to use a biologically inert substrate that would react as a more efficient scavenger of oxygen than the substrates and micro-organisms present in the sediments. Most waterlogged environments may rather be regarded as near anaerobic environments, e.g. very low in oxygen.

4.2.2 Degrading activities of anaerobic bacteria

As a consequence of the above mentioned belief that wood degradation occurred under anaerobic conditions, a lot of interest has been given to anaerobic bacteria and their ability to degrade wood (Schmidt and Liese, 1994; Pointing *et al.*, 1996). Sulfate-reducing bacteria are able to use sulfate as an electron acceptor instead of oxygen (Paul, 1992), and they are known to participate in the decomposition of organic matter under anaerobic conditions. There is, however, so far no evidence for wood degradation by sulfate-reducing bacteria. However, they are indeed present in large numbers in environments surrounding waterlogged archaeological wood (Florian *et al.*, 1977), and may use wood surfaces as shelter. Often, a distinct smell of H_2S, resulting from the reduction of sulfate, is associated with marine sediment finds.

To understand what was believed to be anaerobic bacterial degradation of wood, cellulose fibres and textiles have often been used as test materials in laboratory experiments or *in situ* (Gregory, 1998). Results show that a number of anaerobic cellulose degrading bacteria exist (Leschine, 1995), but the implication that they degrade wood is unfounded. They may, however, degrade non-lignified cellulosic materials in wood like ray parenchyma cells and pit membranes. It should be stressed that the ability of bacteria to degrade pure cellulose does not indicate that they also are able to decay ligno-cellulosic materials. Cellulose in wood is embedded in lignin and is therefore not easily accessible unless the right tools for also ”dealing” with the lignin exist. So far no anaerobic cellulose degrading bacteria have accomplished conclusive degradation of wood cell walls.

4.3 Decaying micro-organisms in waterlogged archaeological wood

For the last twenty years, there has been an increasing interest in archaeological wood among wood biologists. In archaeological wood, unique possibilities exist to study long term degradation processes in near anaerobic environments.

Erosion bacteria are now regarded as the most common degraders of wood found under waterlogged near anaerobic conditions (Singh and Kim, 1997). These observations reflect results from experimental laboratory work on the decay of sound wood samples as well as investigations on degraded archaeological materials. However, most of the archaeological material and more recent wood is mainly recovered from aquatic environments such as lakes, sea water and sediments (Blanchette and Hoffmann, 1993; Singh and Kim, 1997). Only a few samples from terrestrial environments have been investigated (Blanchette, 1995). Consequently, in **PAPER I**, ninety two archaeological samples from seven archaeological waterlogged sites of mainly terrestrial character were investigated regarding the type and extent of microbial attack. It was verified that erosion bacteria were also the main degrading microbes in various waterlogged terrestrial environments. This suggests that these bacteria appear to be omnipresent in wood exposed under wet and oxygen poor conditions and judging form the reports published, these bacteria are cosmopolitan organisms. Decay patterns produced by soft rot fungi and tunnelling bacteria were also observed in wood degraded by erosion bacteria, but were less frequent. Presence of basidiomycete (i.e. white rot and brown rot) decay was regarded as reflecting attack taking place before waterlogging (See section 1.2).

Erosion bacteria decay progresses slowly inwards, until all cellulose rich areas are utilised. According to **PAPER III** this process may go on for at least 1300 years. Both soft rot and tunnelling bacteria are often concentrated to the outer parts of wood samples, probably as a consequence of the relatively higher access to oxygen (Nilsson and Daniel, 1992; Daniel and Nilsson, 1997).

It was apparent that the degree of decay is not just a result of time of burial, which was also suggested by Hoffman (1982) and Borgin *et al* (1979). In fact, the local environment and type of wood species seems to play just as important role for the development of decay (**Paper I**). Thus, wooden remains from the iron age were occasionally better preserved than 1000 years younger Viking objects.

In the following section, typical decay features of erosion bacteria and soft rot will be described in more detail.

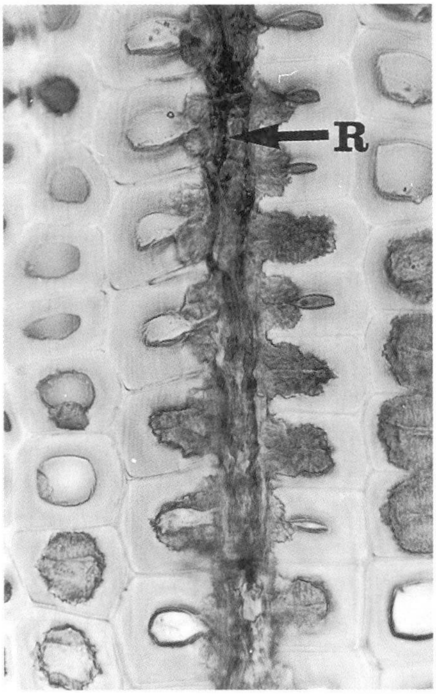
4.4 Erosion bacteria

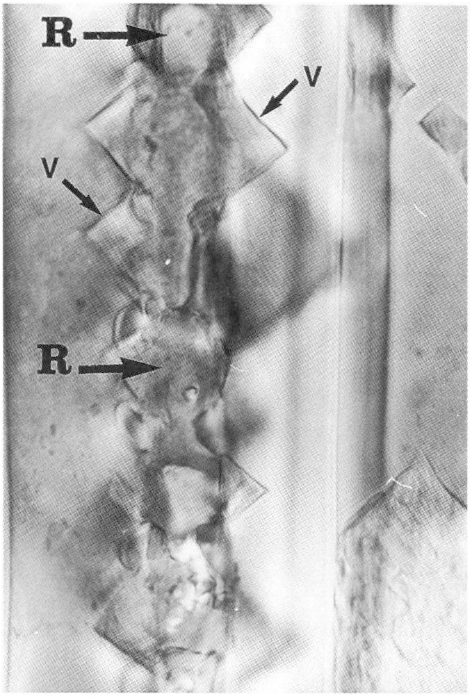
From light microscopy (LM), as well as TEM (transmission electron microscopy) and SEM (scanning electron microscopy), the decay patterns and morphology of erosion bacteria are now well known. The majority of micrographs illustrating the decay patterns are a result of LM, TEM and SEM observations made for **PAPERS I** and **III**.

4.4.1 Decay pattern

Erosion bacteria were named by their way of eroding the wood cell walls.
Invasion starts from the wood surface through rays and pits, from where erosion bacteria enter the cell lumen of tracheids (Figs 12 a, b). Here the S_3 is locally eroded and penetrated. Erosion bacteria continue their attack by progressing into the S_2 layer, the most cellulose rich part of the cell wall leaving behind a granular residual material after decay (Fig. 13). In moderately degraded wood, a special pattern of apparently sound tracheids adjacent to heavily degraded cells are observed (Fig. 14).

However, cross sections only provide documentation of the condition at one plane of the fibre length. Degradation can vary along the length of the same fibres. The diamond- or V-shaped pattern, viewed in longitudinal sections, provides evidence of such variation within a fibre, and consequently represents a typical decay pattern of erosion bacteria (Fig. 15).





a) b)

Fig. 12 a, b. LM micrographs showing the initial invasion of erosion bacteria into soft wood tracheid along the rays (R). a) From rays, colonisation disperse into adjacent tracheids, here viewed in cross section. b) In tangential sections, decay can be seen to proceed from individual horizontal ray cells, (R) into adjacent longitudinal tracheids forming characteristic V-shaped patterns (V).

The compound middle lamella seems however, to be almost unaffected even at very advanced stages of decay (Daniel and Nilsson, 1986; Kim and Singh, 1994; Singh, 1997a). Polarised light reveals birefringence in middle lamella regions, indicating that some cellulose remains (Fig.16). Despite this fragile state, surface details from previous manufacturing and the structure of the wood remain as long as it is kept waterlogged. The integrity of heavily decayed archaeological waterlogged wood will depend on this very fragile network of the compound middle lamellae, which in contrast to sound wood, are not supported by a cellulose rich strong secondary cell wall.

It is interesting to note that the attack observed being caused by erosion bacteria is very similar to the degradation described by Barghoorn already in 1949 (Barghoorn, 1949). His observation that similar patterns can be observed in petrified wood, fits the idea that petrification is preceeded by waterlogging, during which erosion bacteria have an opportunity to attack the wood, until the mineralisation is completed.

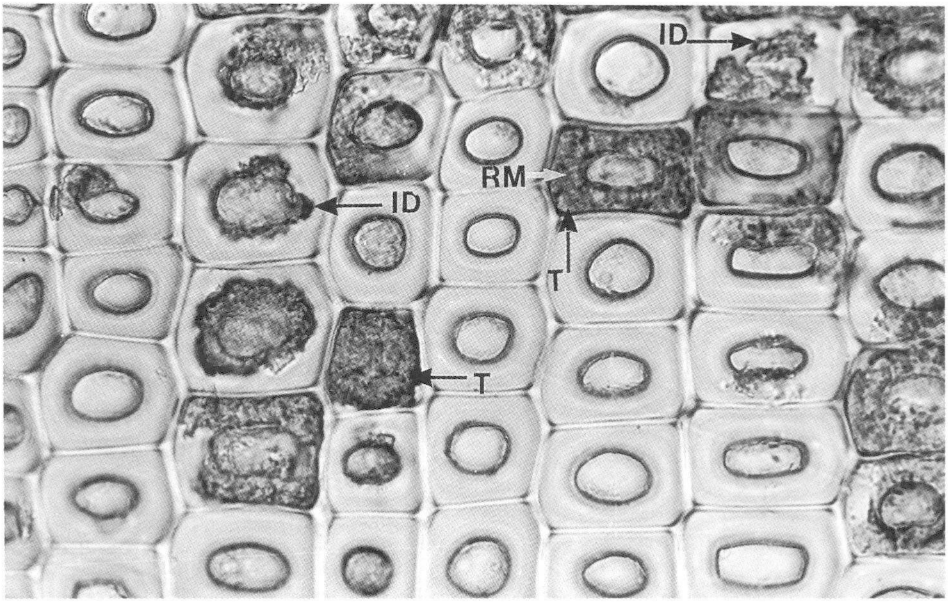


Fig. 13. LM micrographs showing erosion bacteria decay of the secondary cell wall in pine tracheids viewed in cross section. Most tracheids are still sound (the light tracheids) whereas adjacent cells represent varying stages of decay; from initial (ID) to totally (T) degraded. During decay the secondary cell wall is transformed into a granular residual material (RM).

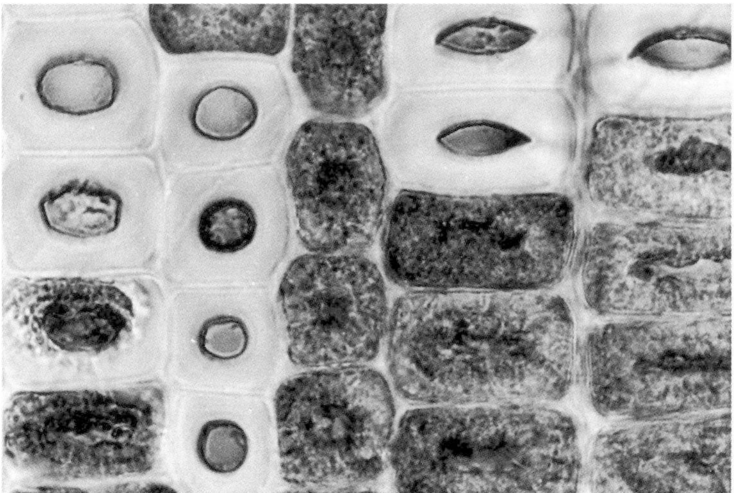


Fig. 14. A common decay feature of erosion bacteria in moderately degraded wood observed in LM. In cross section a heterogeneous pattern of totally degraded cells are recognised among undegraded ones, forming a distinct black / white chequered pattern.

Fig. 15. The heterogeneous decay of individual tracheids by erosion bacteria, may even be observed in longitudinal LM-sections of moderately decayed wood. The typical diamond or V-shaped pattern (V) of degraded areas can be observed in polarised light.

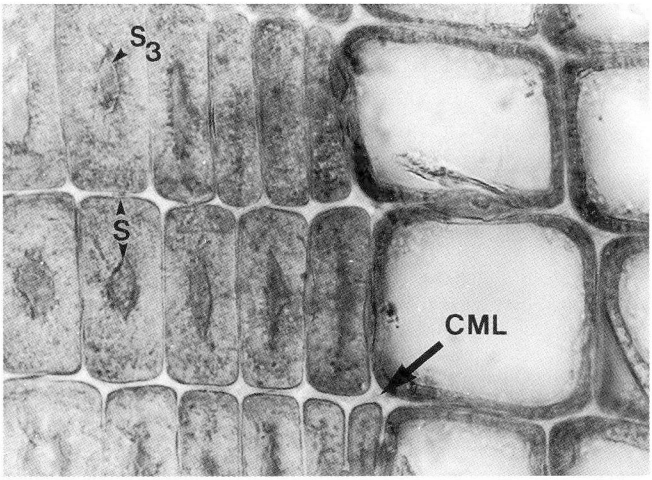


Fig. 16. The compound middle lamellae (CLM) remains undegraded despite total decay by erosion bacteria. This very fragile network retains the integrity of each cell, so that dimensions and ornamentation of wooden objects are kept intact as long as the wood is waterlogged. Note the amorphous appearance of the secondary cell wall (S) and the traces of undegraded S_3. The LM micrograph is a cross section of pine, observed using half polarised light.

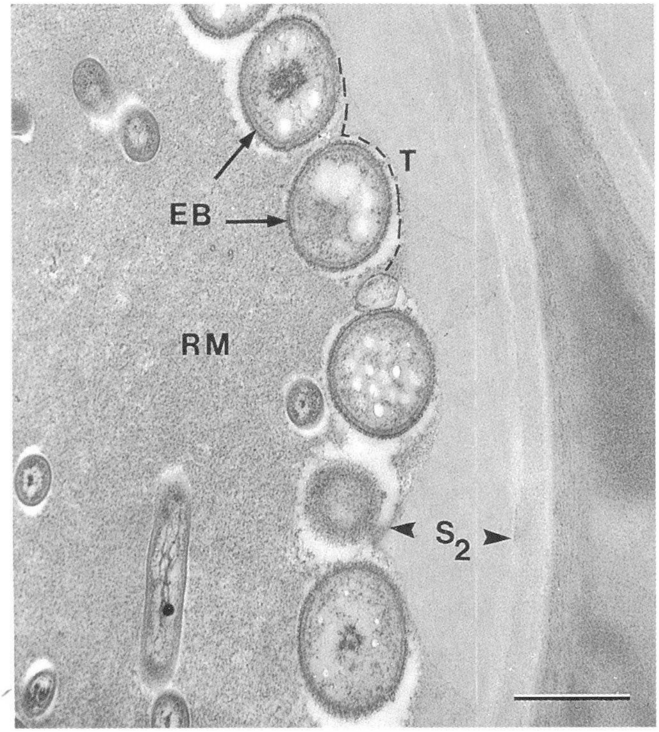


Fig. 17. Using TEM on 1200 year old pine, active erosion bacteria (EB) were found aligned close to the undegraded part of S2, leaving behind a homogeneous granular amorphous residual material (RM) after decay. Characteristic troughs (T) are formed during decay. Bar: 0.5 µm.

In most archaeological wood examined by TEM or SEM, erosion bacteria have been absent, and identification has been based on their distinct decay patterns left behind like fingerprints. The characteristic troughs that can be seen in the eroded cell walls is a particular distinguishing feature. **PAPER III** describes active erosion bacteria found in the 1200 year old wood, and in cross sections their decay patterns were studied in detail at high magnification. During decay of wood cell walls, groups of bacteria are aligned along the microfibrils close to each other forming individual erosion troughs (Fig. 17). A granular amorphous material is left behind. Apparently decay is not always limited to the S_2 layer and erosion bacteria were occasionally observed degrading the S_3 and even parts of S_1 in softwoods (Figs 18 a,b). This was also observed by Singh and Butcher (1985), Kim *et al* (1996), Daniel and Nilsson (1986). From SEM observations, erosion bacteria were found to be rod shaped organisms (Fig. 19), 1 - 5 µm long and 0.5 - 0.9 µm in diameter (Daniel and Nilsson, 1986; Singh *et al.*, 1990; Daniel and Nilsson, 1997).

4.4.2 Slime

At advanced levels of decay, lumen and degraded cell wall regions are filled with amorphous residual material (Figs 16, 17), probably representing residual lignin, bacterial slime and bacteria (Singh and Butcher, 1991; Kim *et al.*, 1996). In **PAPER III**, two types of slime were distinguished using TEM. One homogeneous layer close to the active bacteria, and the other more heterogeneous in areas characterised by total decay and absence of active erosion bacteria (Figs 17, 20). Some tracheids contained both types of slime material (Fig. 21). Scavenging bacteria were observed primarily in the heterogeneous slime and these secondary degraders are suggested to utilise simple sugars associated with the slime (Singh and Butcher, 1991). Due to their absence in areas of undecayed cell walls, it is assumed that they are not true wood degraders (Kim and Singh, 1994). The relation between erosion bacteria and scavenging bacteria are unknown.

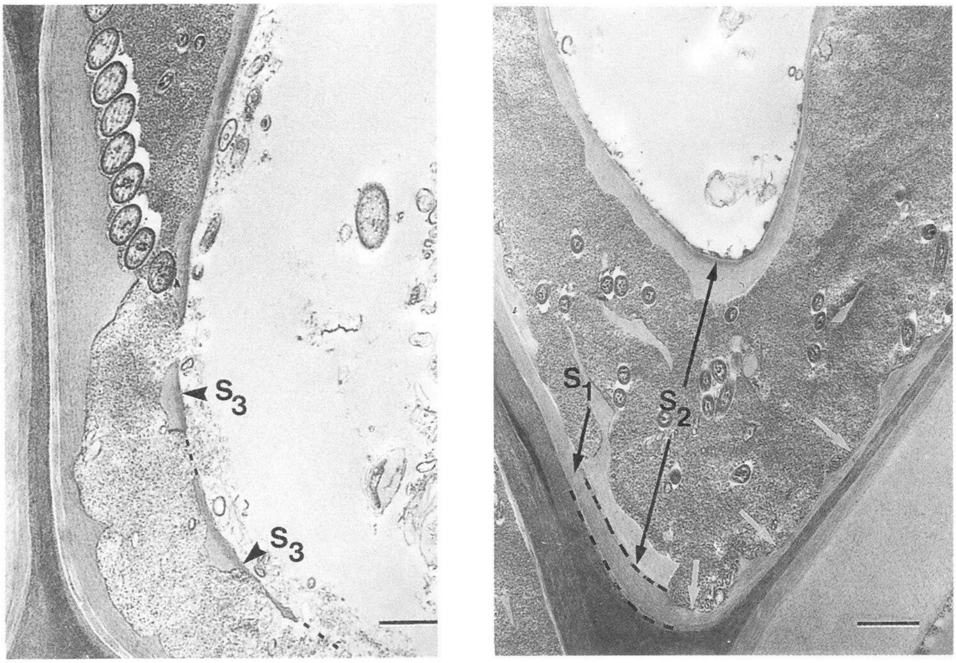
The frequent presence of slime associated with erosion bacterial degraded wood cell walls, may slow down the penetration rate of PEG solutions, used world wide as conservation liquid for archaeological wood. This could explain the occasionally observed failure of impregnation treatments. Extraction of slime may improve the penetration rate. No information on the chemical structure of the slime is currently available.

During almost two decades, efforts have been made to isolate erosion- and tunnelling bacteria. So far it has not been possible to isolate them from wood and establish pure cultures in artificial media under laboratory conditions (Schmidt and Liese, 1994; Daniel and Nilsson, 1997). Modern molecular biology techniques may help us identify species of wood degrading bacteria in the future, so that further details about their specific requirements for growth can be obtained.

4.5 Soft rot

In aquatic as well as terrestrial environments, soft rot fungi are present in large numbers (Eaton, 1976; Eaton and Hale, 1993). Soft rot fungi cause two different types of attack. Soft rot observed in archaeological wood seems to be exclusively Type 1, characterised by cavities produced along the cellulose microfibrils in the S_2 layer and holes in the secondary cell wall when viewed in cross sections (Fig. 22 a,b). Cavities are absent from the S_3 layers and are quite rare in the S_1 layer (Khalili, 2000).

It has been shown that soft rot fungi are able to attack wood exposed in soil with very high moisture contents, and also in fresh and sea water. Marine soft rot fungi are known for their fascinating spores (Ingold, 1971; Ingold, 1976; Eaton and Hale, 1993). Both marine and terrestrial soft rot fungi are considered to have lower requirements for oxygen than basidomycetes, which are rare in aquatic environments.



a) b)

Fig. 18 a,b. TEM micrographs showing cell wall degradation by erosion bacteria, including parts of the S_1 and S_3. a) S_3 is partially degraded (arrows). Bar: 1 μm. b) In addition to the preferential decay of the S_2 layer, the S_1 has also been degraded (white arrows). Bar: 1 μm.

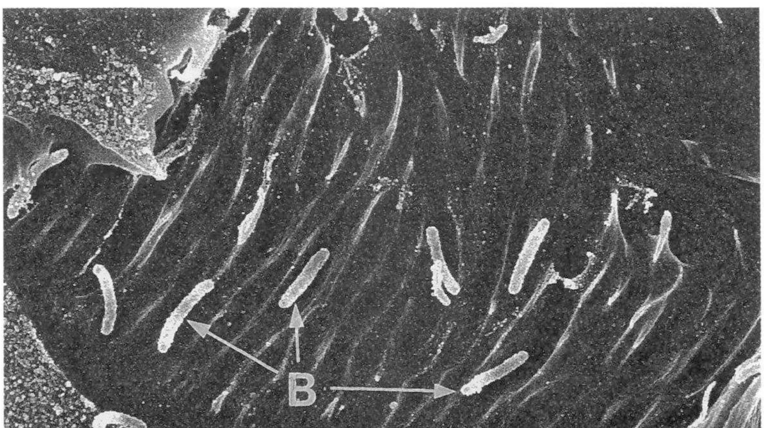


Fig. 19. Using SEM, rod shaped erosion bacteria (B) were found aligned in their individual troughs degrading the cell wall.

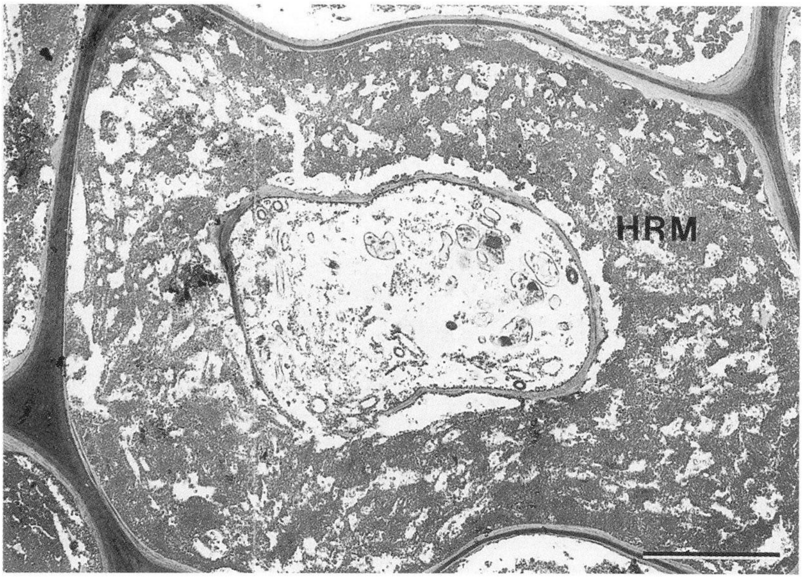


Fig. 20. Heterogeneous residual material (HRM) observed in totally degraded tracheids where erosion bacteria no longer were present. This differs from the more homogeneous residual material that is produced immediately after decay, next to the active bacteria (Fig. 17). TEM. Bar: 4 µm.

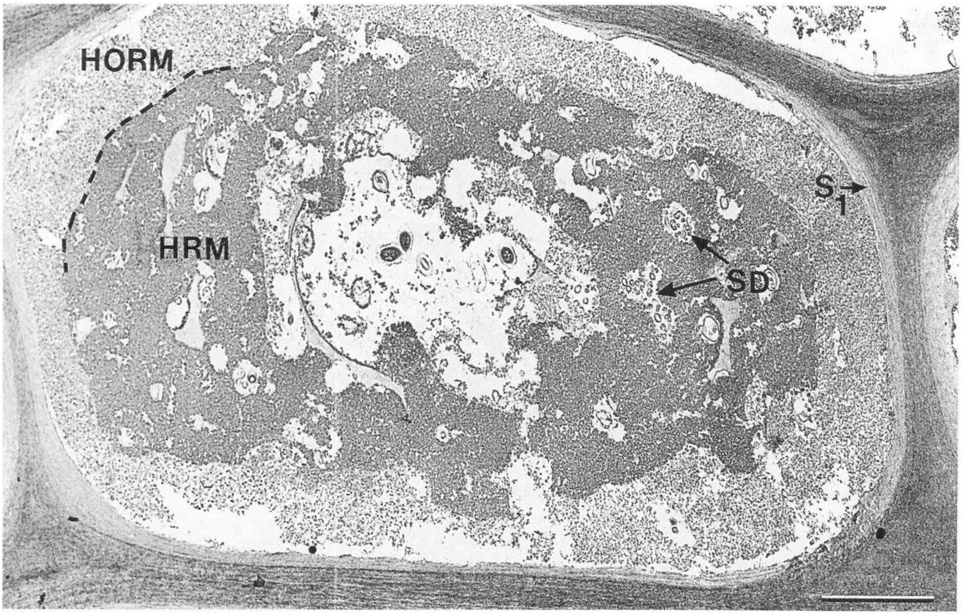
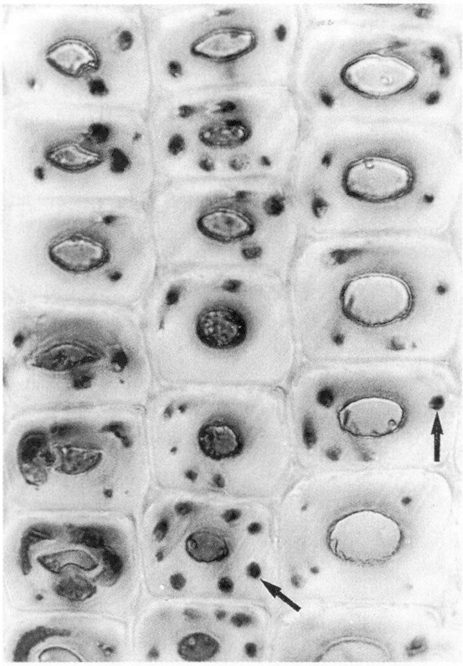


Fig. 21. TEM micrograph showing the occurrence of both types of slime in one individual tracheid. The slime materials are distributed in almost concentric circles within the former secondary cell wall (dotted). Within the heterogeneous residual material (HRM) secondary degraders (SD) are found. These bacteria are almost absent in the more dense, but homogeneous residual material (HoRM) close to the S_1. Bar: 2 µm.



a) b)

Fig. 22 a,b. LM micrographs showing typical decay features of soft rot viewed in longitudinal, respectively transverse sections of pine. a) Typical soft rot cavities (arrows) produced when hyphae cause decay along the microfibrils within S_2 (polarised light). b) cross section of the hyphae and their cavities, producing holes (arrows), preferentially within the S_2 layer.

4.6 Environmental factors

4.6.1 Oxygen and depth

Oxygen plays a very important role for micro-organisms in general. The rare occurrence of basidiomycetes in aquatic environments is thought to be related to restrictions in oxygen whereas soft rot fungi are found active under reduced oxygen concentrations (Eaton, 1976). In soils and water, oxygen is repressed, compared to above ground situations. The more frequent occurrence of soft rot attack in wood from marine environments relates probably to the more aerated state of seawater, compared to waterlogged clay soils on land sites (Glinski and Stepniewski, 1983; Drever, 1997).

It has been shown that oxygen concentrations in marine sediments are highly dependent on the aeration of the surrounding water. However within sediments, oxygen concentrations decrease rapidly in the surface layers to reach zero levels at a depth of a few mm (Rasmussen and Jørgensen, 1992). Preliminary reports from experimental work show that decay in wood samples decreases significantly between intervals of 10 cm in sediments (Björdal and Gregory D, 1999; unpublished). The ships and artefacts from the Vasa, Kronan, and the Mary Rose also imply the protective effect of sediments.

However, how much the effect depth of burial in water or sediment has on the state of preservation has been poorly investigated. Some variations in decay along poles inserted in rivers or waterlogged soil have been recorded (Eslyn and Clark, 1976; Eslyn and Moore, 1984; Grinda, 1997) But for long-term preservation no information has so far been published. However observations by conservators and archaeologists show that vertically inserted boards in wells, vary significantly in preservation along their axis. The lower parts are always better preserved (Fig. 23). From the excavation of the Oseberg ship in 1904, photo documentation of the intricately ornamented outer stern post, also suggest different preservation conditions between the upper and lower parts (Fig. 24).

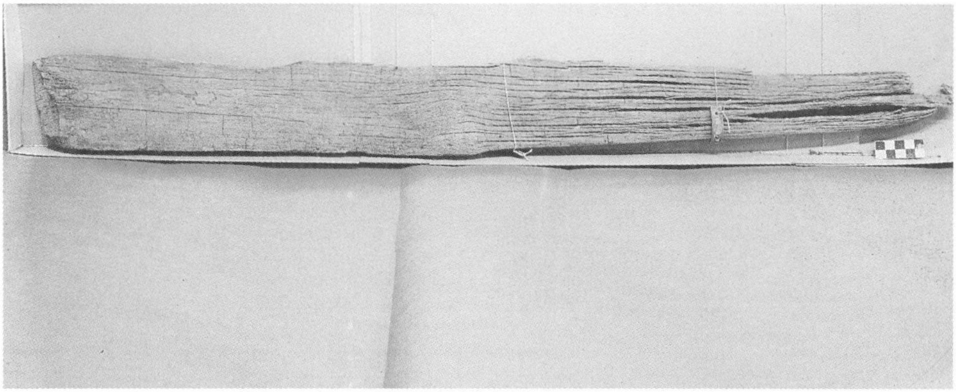


Fig. 23. From a prehistoric well found at a depth of 3 meters in heavy clay, where one meter long boards were inserted vertically. After conservation, the upper and lower parts of the boards responded differently to the treatment, indicating different states of preservation. The integrity of the lower parts were maintained, whereas the upper parts showed serious distortion.

To investigate the effect of depth on state of preservation, two Viking age poles, vertically inserted into the seabed sediment, were excavated from a maritime Viking blockade and investigated to obtain new information (**PAPER III**). Despite a short distance of 40 cm between the upper and lower discs, taken from each pole, significant differences were found in degree of decay. The upper parts, close to the air / water interface had more severe decay by erosion bacteria, compared to the lower parts. Within the lower parts of the poles, erosion bacteria were found to be still active. This indicates that a distance of only 40 cm is important enough for obtaining differences in available oxygen and decay rate. In waterlogged soils and sediments diffusion of oxygen is very low, and decreases with depth. It is therefore suggested that there is a significant increase of preservation with depth of burial, due to the slower decay rate of erosion bacteria when less oxygen is available. It also shows the ability of erosion bacteria to be active at different oxygen levels.

Circumstantial evidence suggests that erosion bacteria require oxygen to be able to degrade wood. Such evidence is based on observations on the degree of preservation of archaeological wood in certain environments and from laboratory experiments that indicate that a complete exclusion of oxygen will inhibit the activity of erosion bacteria (Björdal and Nilsson, not published). The fact that the attack starts in the outer layers of waterlogged wood also supports the view that the bacteria are oxygen dependent. This is, however, not based on actual measurements of prevailing oxygen concentrations.

Fig. 24. A detail photo taken of the stern, at the excavation site of the 1200 year old Oseberg ship, suggest better preservation conditions at lower levels, where animal ornaments seem more distinct. (Copyright: Oldsaksamlingen, Oslo).

4.6.2 pH
In **PAPER I** , the pH of seven archaeological sites was shown to vary between 5.5 and 7.5, levels which are a commonly found at other archaeological sites (Caple *et al.*, 1996). Within this range, erosion bacteria as well as soft rot had successfully decayed wood. Unfortunately very little is known about microbial degradation under acid conditions that may occur at peat sites.

4.6.3 Nitrogen and other elements
The influence of nitrogen and other elements on the degradation of water-logged wood is not known. Wood is poor in nitrogen and this element is known to greatly stimulate the activity of soft rot fungi (Worrall and Wang, 1991), but the effect on attack by erosion bacteria seems not to have been specifically investigated. However, Boutelje and Göransson (1975) reported on a possible correlation between high nitrogen and phosphate concentrations and the extent of damage observed in waterlogged wooden pilings supporting buildings in Swedish cities. The damage was caused by soft rot and bacteria. The fact that the timber of Vasa was relatively well preserved may be due to the fact that the harbour waters, where she sunk, were used for disposing all sorts of refuse. This would have led to increased nitrogen levels, but large parts of the refuse was probably easily degraded, thereby consuming most of the oxygen. This would have resulted in very reduced oxygen levels around the ship.

4.7 Environmental history of the objects
Knowledge about the different environmental requirements of wood degrading micro-organisms, mainly their oxygen requirements, allows us to draw conclusions when typical terrestrial decay types are found in waterlogged environments. In archaeological samples from Vadstena (**PAPER I**), brown rot was present in more than half of the samples. Brown rot is regarded as one of the most aggressive types of decay found in wooden buildings (Viitanen and Ritschkoff, 1991). The Vadstena samples indicate a widespread attack by brown rot within the actual house foundations before waterlogging had occurred. From this we can conclude that house rot was also a serious problem in the middle ages.

Brown/black poles from the bulwark of Tingsträde träsk was interpreted by archaeologists as evidence of fire, which supported the legend that the construction, built in the lake, had burned down. But observations using microscopy revealed, however, that heavy soft rot attack had discoloured the wood and that there was no evidence of coal (Nilsson and Daniel, 1992). Furthermore, the occurrence of white and brown rot attack, in the waterlogged timbers, which could be identified as having once been part of house constructions, suggested that decay, rather than fire was the cause of destruction.

It is also suggested in **PAPER I**, that soft rot attack in the planks of the ship wreck ”Kraveln”, may be concentrated to certain areas, reflecting the waterline of the ship, which in turn may indicate the weight of cargo transported.

Other examples of how microbial analysis can reveal important information for archaeologists are described in section 1.2. For archaeologists, microbial observations can provide new unexpected information, enriching their understanding of the previous history of samples and sometimes also of historical events.

5 Conservation

5.1 Archaeology and conservation

An increased interest for history especially historical remains, escalated in Scandinavia during the 1800-century when systematic records and preservation efforts were initiated. Archaeological unique finds like the ships Nydam in Denmark and Oseberg in Norway led to the need for conservation strategies, and in Denmark wood conservation started very early (Christensen, 1970). As far back as in 1859, during excavation of wooden artefacts from peat soil (Viemose in Denmark), archaeologists were aware of the need for special care for the objects recovered to avoid deformation and loss of integrity (Herbst, 1861). Bows, lances, and ship- parts were transported in very wet peat soil to avoid drying out during transport from the excavation area to the conservation facilities at the National museum in Copenhagen. Even at that time, it was well known that archaeological wood should not be allowed to dry out before treatment.

5.1.1 Well preserved wood?

At excavation sites, archaeological wooden objects often seem to be well preserved despite several hundred and even thousand years of burial. As long as the wood is kept waterlogged, the dimensions of the cell wall are maintained due to the bulking effect of water. Integrity, dimensions and colour remain and information on the surface (like runes, tool marks etc.) can still frequently be observed (Figs 3, 25). This false image of sound wood has led many archaeologists to think of such samples as ”well preserved” and no precautions against drying have been made. However, if the wooden objects are allowed to dry without conservation treatment, serious shrinkage and distortion will take place and the integrity of the historical object would be lost (See Fig. 6) (Speerschneider, 1861; Ambrose, 1971). This is an irreversible process, where capillary forces cause cellular collapse. The effect can be more or less extreme depending on the extent of decay (Jong, 1977).

This very drastic change in dimensions, and total distortion of an object showed very clearly the need for some kind of preservation treatment (Christensen, 1951).

5.2 Historical review on conservation methods

Initially, linseed oil, creosote and waxes were used, probably recommended by boat builders and furniture craftsmen. Some ships found during the 19th and 20th century were treated this way (Heide, 1979b). Later on, it was clear that these methods were only successful when the wood was in an extremely good condition, as for the Oseberg hull (Rosenquist, 1959), and many vessels were lost, due to the lack of knowledge and adequate preservation methods (Heide, 1979a).

It was obvious that degraded wood did not respond successfully to methods used for sound wood, and therefore future methods were based on an understanding for bulking and stabilisation of individual cell walls.

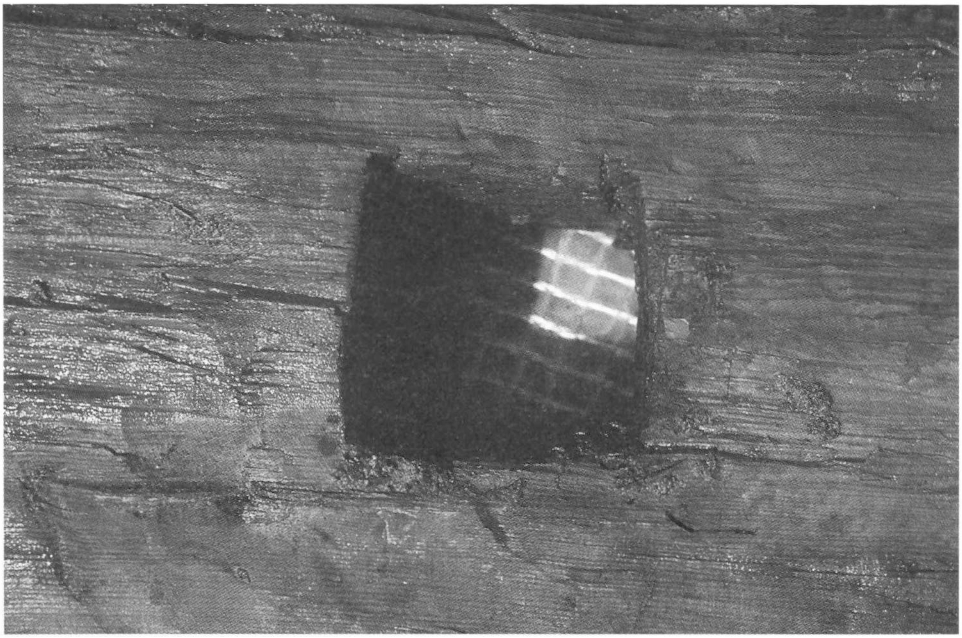


Fig. 25. Structural timber from a 1900 year old well. Detail showing the excellent preserved condition of the wood when wet. The hole for joining looks like it was manufactured yesterday and can successfully be recorded by the archaeologists. (Photo Anna Onsten)

5.2.1 Alum method

In 1861 Herbst described a novel method with successful results, which was termed the alum method. This method became the most frequently used during the next 100 years in Scandinavia. Detailed information on improvements and experimental work carried out until the middle of the 20th century at the National museum in Copenhagen is exciting reading (Christensen, 1970). Due to the hygroscopicity of alum, especially when glycerin was added, long-term storage experiences showed a high instability and self destructive tendency of treated wood. The alum method was consequently abandoned when alternative methods were found (Christensen, 1951; Rosenquist, 1959; Barkman *et al.*, 1976). Recently, costly re-conservation of important finds like the Hjortespring boat has been carried out in Denmark (Christensen, 1966). However it is important to know that there is still a lot of alum treated historical objects in museums that are in excellent condition, due to a very stabile storage climate.

5.2.2 Conventional PEG method

In the late 1950s, the first experiments on impregnation of archaeological wood with polyethylene glycol (PEG) were carried out (Håfors, 1990). PEG is a synthetic straight chained polymer of ethylene glycol units, which is produced in both low and high molecular weights. The general formula is HO--$(CH_2CH_2O)_n$--H. PEG solutions penetrate the wood tissue, and physically as well as chemically stabilises degraded wood cell walls during drying, to avoid collapse. In conservation the most frequently used PEGs are probably PEG 400, 600, 1500 and 4000. They are diluted with water in concentrations of normally 10 to 60 %, depending on the conservation method used, size of the object, state of decay, and wood species (Pearson, 1979).

A lot of initial experimental work, testing molecular sizes versus penetration rates, were carried out around 1960 in both Sweden and Denmark, where the sensational raising of the Vasa and the Viking ships of Roskilde, were in desperate need of appropriate conservation treatment (Christensen, 1970; Håfors, 1990). Also the failure of previous methods (alum, creosote, methylcellulose) was a reason for this interest, and thus a new conservation area, based on modern scientific analysis was initiated. Improvements of PEG treatments have continuously been made through the years (Grattan, 1982; Hoffmann, 1984; Hoffmann, 1988; Dean *et al.*, 1996; Hoffmann, 1996; Jensen, 1996)

5.2.3 PEG and freeze-drying

Since the 1970s, PEG impregnation has been combined with freeze-drying (Ambrose, 1971; Cook and Grattan, 1984; Watson, 1996). During freeze-drying water is evaporated by sublimation, e.g. from ice directly to the gas phase, and capillary forces caused by evaporating liquid water are avoided (Ambrose, 1990). This method has a lot of advantages, but its use is often limited to smaller objects for technical reasons (Fig. 26).

Recently, the wood conservation centre at the Danish National museum increased the volume of their already large freeze-dryer, so now even smaller boats can be treated by this successful method (Meyer, 1996). However, improvement of the methods are still in progress (Hoffmann and Fortuin, 1990b; Jensen *et al.*, 1993; Koefoed *et al.*, 1998).

Today both PEG, and a combination of PEG and freeze-drying, are the most commonly used methods worldwide.

5.3 Microbial growth during impregnation

Wood for freeze-drying is usually impregnated for months rather that years, whereas oak timber from wrecks treated by conventional PEG methods needs several years. In large baths or by spraying, archaeological wood is penetrated by PEG solutions of different concentrations. Since the use of PEG as a conservation chemical for archaeological waterlogged wood, microbial growth at the liquid/air interfaces of the impregnation baths has regularly been observed

(Fig. 27). This has been regarded as one of the drawbacks of PEG, and fungicides are frequently used (Christensen, 1970; Barkman, 1975; Meyer, 1996).

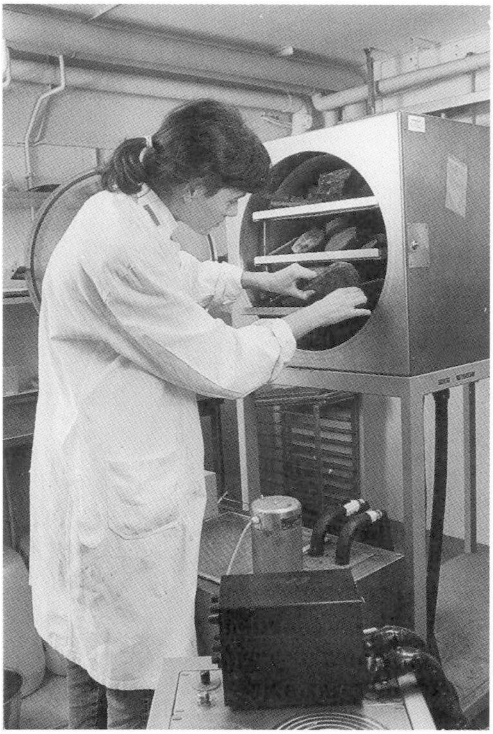


Fig. 26. Freeze-drying of archaeological wood samples at the Institute of Conservation, RAÄ, Sweden. In this type of conventional vacuum freeze-dryer, only smaller artefacts can be treated. (Photo Bengt Lundberg, RAÄ)

The reason for adding biocides (i.e. why microbial growth should be eliminated during treatment) was and still is, not very clear. Sodium pentachlorophenate was first tried "to control the rot problem", later borax and boric acid was added to the PEG solutions used for the conservation of Vasa (Barkman, 1965). In the literature the fundamental problems regarding micro-organisms during impregnation is poorly described.

What effect microbial growth at the liquid/air interface has on the degradation of the actual archaeological objects within the bath, or in what way microbial growth at the interface is related to the actual microbial decay forms within the archaeological objects that are being conserved is not known. Nor is it known if the decay present within the archaeological samples will continue during the conservation process. Experiments, in the laboratory simulating attack by erosion bacteria, suggest, however, that erosion bacteria may still be active if the samples are stored in only water (Singh *et al.*, 1990).

To highlight these problems, and increase our understanding of microbial processes during impregnation in PEG solutions, experiments were carried out as described in **PAPER IV**. In the following paragraphs the results are summarised.

5.3.1 Observations from impregnation baths

During PEG-impregnation several different microbial related events take place: 1) microbial colonisation at the liquid/air interface; 2) slime development in the upper part of the liquid; 3) foam development; 4) partial discoloration of wood samples; and 5) development of an unpleasant smell. In Figs 27 and 28, microbial colonisation of the impregnation bath, as well as discoloration of test samples are shown.

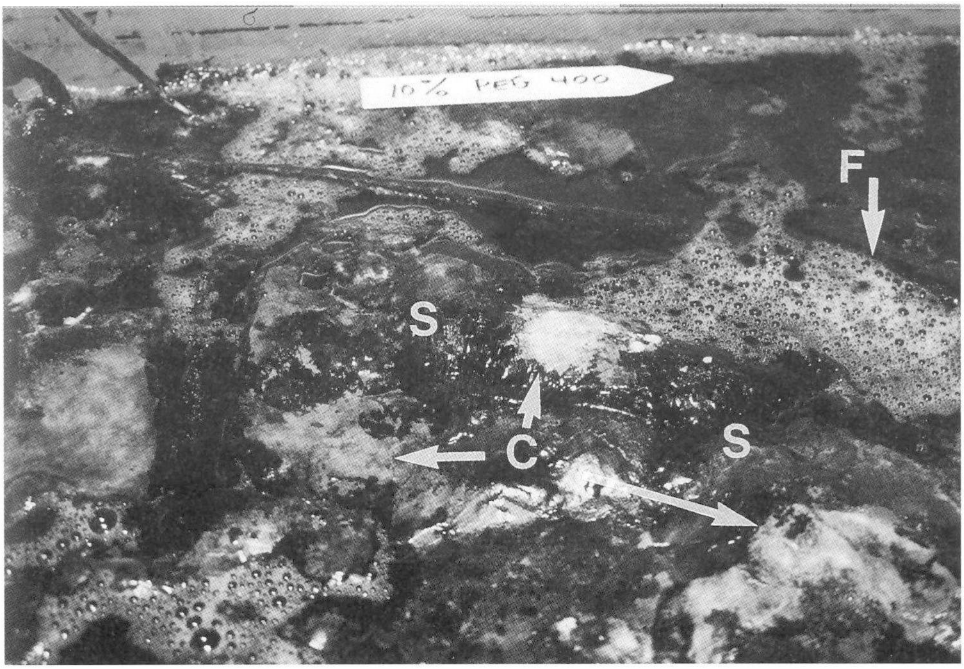


Fig. 27. Microbial colonisation at the interface during impregnation in PEG solutions. Colonies (C) are formed upon the biofilm/slime (S). Foam (F) are also present.

5.3.2 Micro-organisms

Most interesting is the absence of wood degrading activity within the baths. No degradation of the wood cell walls in test samples, made from recent sound wood was present after 7 months of impregnation, even though extensive microbial growth was present at the liquid/air interface. The microbial growth at the interface, consisting of multicoloured colonies, was apparently not growth of wood degrading organisms, but rather different types of mould and staining fungi, that are common in wet environments. In contrast to fresh water environments, wood decaying organisms appears to be inhibited in PEG solutions. Here, increased concentrations of PEG will reduce the amount of free water available for micro-organisms within the liquid (Steuter *et al.*, 1981; Avari and Allsopp, 1983). At higher PEG concentrations even microbial growth at the liquid/air interface is excluded (Stamm and Baechler, 1960). Generally, most fungi and bacteria require high humidity and free access to oxygen for growth, and their metabolism also requires a stabile osmotic pressure within the cells. Only a few specialised organisms are able to live at low water potentials, e.g. high osmotic pressure (Park, 1968; Sparrow, 1968; Eaton and Hale, 1993).

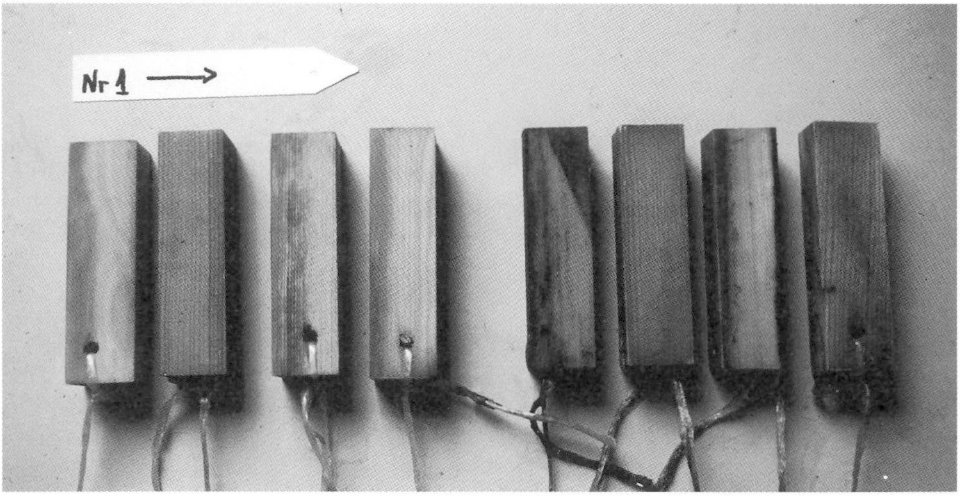


Fig. 28. Partial discoloration of wood samples by blue stain fungi. See also Fig. 10.

5.3.3 Slime formation and PEG penetration

Development of biofilm and slime, is a result of bacterial growth, usually observed in water solutions. Within slime layers, which can be several cm thick, microcosms are created where both more aerobic as well as less aerobic regions exist (Hobbie and Fletcher, 1988; Characklis and Marshall, 1990). Consequently, the biodiversity is high, and includes also other organisms in addition to bacteria, like fungi, algae and protozoa (Gilbert *et al.*, 1993).

In solutions low in nutrients, archaeological wood will represent a potential food source for bacteria forming biofilms, and the wood will be covered with slime, which also happens when wood is situated close to the liquid/air interface. From the results in **PAPER IV**, wood samples covered with slime had no evidence of decay, and therefore the slime producing bacteria or any other associated bacteria present, were not directly "harmful" for the archaeological wooden objects. However, slime may decrease the penetration rate of PEG, which is also suggested by Grattan (1982) and Dean (1990). Biofilm development may represent a possible explanation, for the occasional failure of PEG impregnation, where freeze-dried wood after treatment appears unimpregnated, but further research is needed.

5.3.4 Discoloration

Staining fungi are known to colonise wood under humid conditions (Dickinson and Levy, 1992). In impregnation baths, these fungi are able to colonise wood situated close to the liquid/air interface, a feature recognised in **PAPER IV**. Fortunately, most staining fungi are not real wood degraders. Their main negative effect is the development of dark coloured hyphae, which stain the wood, during their utilisation of easily available nutrients within cell lumena and non-lignified cell walls (Eaton and Hale, 1993).

5.3.5 Health aspects

From a health perspective, presence of microbial growth at the liquid/air interface should not be accepted by conservation staff. Volatile compounds are liberated as a result of microbial metabolism. Moulds, which are probably the most common colonisers in this environment, are known to release noxious compounds which can give health problems (Mouzouras *et al.*, 1990). Recently it was also found that PEG during its long-term degradation liberates formic acid and formaldehyde, both known to have ill effects on humans (Glastrup, 1996). Consequently, there are good reasons for looking over safety precautions in conservation laboratories, and research on methods for minimising microbial growth should be encouraged.

5.3.6 Guidelines

Some of the described microbial-related negative effects, namely slime development and discoloration, can be easily minimised in the baths, by placing the objects at some distance from the liquid/air interface, i.e. deeper, where less oxygen is available for bacterial activity. However, to combat the microbial colonisation at the interface and the distinct smell that arises, use of biocides may be the only way. To decrease temperature during impregnation, is not enough to avoid microbial growth, and furthermore impregnation processes are prolonged.

6 Reburial and *in situ* preservation

6.1 History of reburial and *in situ* preservation

During almost two centuries of archaeological excavations, significant amounts of archaeological wood have been abandoned or even burnt after recording, as a result of economic and academic priorities (Heide, 1979a). The quantities excavated are simply too large to conserve. In the light of very costly conservation treatments and storage facilities (especially of shipwrecks and construction wood) these drastic decisions are made by archaeologists even today. Need for conservation has too often come as a "bad" surprise" for enthusiastic archaeologists, which lead Pearson (1979) to state that "Excavation without conservation is vandalism".

A shipwreck represents a relatively minor preservation problem compared with constructions like trackways, the Sweet track, or settlements (such as Biskupin and Flag Fen) that are spread over large areas.

Only recently, awareness of archaeological wood as an archive of historical information, has increased the interest for passive, less costly conservation processes (Caple, 1994). Reburial and *in situ* preservation are two techniques for long term storage in nature, suitable for shipwrecks and wooden constructions found in terrestrial or aquatic environments. Reburial involves excavation and examination of the archaeological material and sometimes transport to a different location. The material is then re-buried in an environment that has been designed to provide the best possible preserving conditions. *In situ* preservation is carried out at the same location where the material was found, with as little disturbance as possible.

At the Biskupin site, efforts were done on several occasions to protect the area from drying out (Piotrowski, 1990). In 1932 hot wax was dispersed over an area of 1000 square meters, which must be regarded as one of the first examples of methods for *in situ* preservation. A lack of methods and understanding of the degrading processes in nature, has been frequently mentioned during the last decades. The International UNESCO symposium 1979, the WARP conference 1990 and the PARIS meeting on Preservation *in situ* 1996 and several WOAM conferences, represent some efforts to highlight the problems, but very little has so far developed in terms of recommendations and methods of reburial and *in situ* preservation.

6.1.1 Marine environments

Marine archaeologists have, with a few exceptions, showed little interest in the degradation processes in the most abundant archaeological material, wood. Degradation by marine borers has usually been recognised, but the microbial processes appear to have been largely unknown. This may be a result of the earlier widespread assumption that chemical hydrolysis was the main cause of the degradation observed (see section 4.2). The often spectacular discoveries of shipwrecks seem to have led to an excitement, that made seeking for an understanding of degradation processes a lesser priority. It is significant that a modern encyclopedia of underwater and maritime archaeology (Delgado, 1997) contains very little information on degradation of wood in the aquatic environments. Under the entry ”conservation” it is stated that: ”In time, through hydrolysis, cellulose in the cell walls disintegrates, leaving a lignin network to support the wood”. The encyclopedia contains no entries for ”marine borers” or ”shipworm”.

Large historical ships found in marine environments are often preserved *in situ* if possible, to avoid the great cost of excavation, transport and later on reburial. However, *in situ* preservation in sea waters and sediments can be a real challenge when fighting against marine borers, streams and erosion of the seabed. Only a few cases have so far been reported, but at present several *in situ* projects are going on in Canada, Denmark, Sweden and England. So hopefully useful information will soon follow.

In marine environments, mainly above the seabed, shipworms and *Limnoria* are effective wood degraders, which during a short period of time can transform invaluable historical ships into skeletons. However, they are highly dependent on salinity, and are therefore normally absent in brackish waters (Eaton and Hale, 1993). The Vasa found in brackish water was in excellent condition, whereas the Mary Rose had suffered from serious attack by the marine invaders and was only well preserved in parts that were found protected by the sediments. Activity by wood borers decline dramatically with depth in sediments, probably reflecting the fast decline in the diffusion of oxygen.

Experience of reburial in marine environments has been rarely reported although some experimental work describing the wood decay potential of marine sediments has recently been carried out (Pointing *et al.*, 1996; Gregory, 1998). Failure to include control wood samples e.g. sound wood, at the time of reburial makes it very difficult to assess if deterioration slowed down or was prevented (Riess and Daniel, 1997).

In Sweden however, a marine archaeological investigation of wrecks in Marstrand harbour, has recently involved both reburial and *in situ* preservation, within the same area. One wreck was left *in situ*, with a covering of clay, plastic and sand bags, whereas another wreck was removed and dismantled due to exploitation. At present new trenches within the harbour area are planned for reburial of the dismantled wreck.

6.1.2 Land site environments

On land, observations and records are made under more easily controlled conditions (Fig. 29).

Archaeological log boats and ships are also frequently and surprisingly found at land sites. This could be due to raising of natural land areas, drying out of peats, and lakes or drainage. Many historical remains are today found on land. However, a small exclusive group of ships used in funeral ceremonies, were already in historical times placed on land. Such examples include the three

Norwegian ships Oseberg, Tuna ship and the Gokstad boat as well as Sutton Hoo in England (Evans, 1977; Marstrander, 1986).

The Netherlands has an outstanding tradition of reburial and *in situ* preservation. Due to the large amount of wrecks found during building of the Polders, storage methods in nature were required. Experience showed that wrecks left unattended over a long period until full excavation was made, suffered from severe decay (Jong, 1979a). In 1979, de Jong described initial work on *in situ* preservation, based on a local rise of the water level. Unfortunately, very little has been published on the results. However, today reburial of dismantled wrecks, takes place at a special selected site where the water table is high. Water levels are checked occasionally using vertically inserted pipe lines (L. Van Dijk, 1999, personal communication).

Fig. 29. Excavation of the Kronholms cog found in wet sand. Recordings and observations are easily obtained at land site excavations compared to marine environments.

In Denmark, reburial became a matter of scientific interest in the late 1970s, when large historical shipwrecks were excavated. A sandy waterlogged soil with minimum microbial activity was selected for reburial of dismantled ships (Jespersen, 1984). Just now, at the beginning of the new millennium, this area has received new attention due to the rescue process of Nydam mose (peat bog). This unique and historically well known site, has during last 100 years been occasionally excavated due to the incredibly large amounts of well preserved unique wooden and metallic artefacts from the iron age. Questions have been raised by archaeologists if the preservation condition in the peat has changed for the worse, since objects recently excavated seem to be in a poorer condition compared to previous excavations. Consequently, greater attention has now been given, and a full investigation of the conditions within the peat and the artefacts, involving a lot of different experts, is being carried out (Gregory, D., personal communication)

In Great Britain, reburial and *in situ* preservation in urban as well as wetland areas, has led to similar projects, where monitoring and covering-techniques have been discussed and tested (Foley, 1990; Caple, 1994; Corfield, 1996; Brunning, 1996a; Brunning, 1996b).
About 10 years ago, environmental monitoring at different archaeological sites was started where pH, redox potential, and oxygen concentrations were related to the state of preservation (Caple, 1993; Caple *et al.*, 1996). The interpretations were, however, more complicated than expected and so far no recommendations or conclusions have been published. From *in situ* preserved sites in London, the present monitoring may give interesting results in future (Corfield, 1996).

In Sweden two wrecks were recently discussed regarding *in situ* preservation. The Kronholm cog was found on the isle of Gotland in wet sand, one metre above the water table, 30 cm under ground. A Viking ship (Äskekärr II) was buried in heavy clay, some decimetres under the present water table. Both wrecks were left *in situ*, due to the lack of economic support for complete excavation and conservation. In Sweden, like in many other countries, no strategies for *in situ* preservation on land sites (as well for marine environments) exist. Consequently, no special attention has been given for the routine covering and protection of historical remains after recording. The condition and preservation prognoses for the future of ships or constructions left *in situ* has so far only been given very little attention.

6.2 Factors affecting environmental preservation

Some of the individual parameters that affect preservation are discussed in the following paragraphs. In **PAPER I** it was shown that erosion bacteria, the ultimate degraders in waterlogged environments low in oxygen, are capable of degrading wood in aquatic and terrestrial environments, despite varying pH, soil type, salinity and depth of burial. **PAPER III** shows that even small differences in depth probably affect the rate of decay by erosion bacteria, which is suggested to relate to the decrease in oxygen with depth of burial.

All factors discussed in the following paragraphs are in some way related to microbial degradation. Microbiology has from my point of view been the missing link for understanding the often excellent preserving conditions in nature. However, the research area as a whole, including microbial ecology, is very complex, and today understanding of microbial systems is still embryonic both in description and prediction (Klug and Odelson, 1992; Caple *et al.*, 1996).

6.2.1 Oxygen

As stated earlier, oxygen appears to be the most important factor to control. If erosion bacteria are dependent on oxygen, all measures that can exclude oxygen, should lead to a long term preservation of waterlogged archaeological wood.

6.2.2 The wood itself

Wood species and whether it is sapwood or heartwood has a strong influence on the rate of attack. There is little information on the durability of different wood species exposed in aquatic environments. Some timbers have a reputation for being very durable when submerged in water. Scientific evidence is, however, mostly lacking. The size of the wooden objects is also likely to be of importance, since the degradation starts from the surface and progresses inwards. Thus it is obvious that thinner objects will be degraded throughout sooner than more massive objects. In large size beams and logs, penetration rates of oxygen can be assumed to decrease with the distance from the outermost part of the object. This will, if oxygen is required for the activity of erosion bacteria, result in a slower progression of attack.

6.2.3 Water

A waterlogged environment is essential for long term preservation in nature. Two important functions are recognised; 1) to fill up the degraded cell wall with water to physically retain the dimensions of the cell; 2) in water and particularly in sediments under water, oxygen concentrations are reduced. This will repress or eliminate the activity of aggressive aerobic fungi.

If water is enriched with nutrients, like polluted waters, microbial degradation of wood may accelerate, depending on the presence or absence of more easily degradable substrates. Shipwrecks found in such environments should therefore be subjected to reburial in a more protective environment.

In waters with high salinity, like most oceans and seas, shipworms and *Limnoria* cause the most rapid decay of wood. Only under the seabed, within the sediment is wood protected. Therefore most ships sunk at sea during history have disappeared quickly, without leaving a trace. In brackish waters, like the Baltic sea, the preservation potential is much higher.

6.2.4 Soil types

In sandy soils, the larger grain size porosity is greater than for clay and silty soils (Fetter, 1994). This has a negative effect on the water holding capacity for sand compared to clay in situations where an area is occasionally drying out. However, in a stabilised waterlogged environment, these differences may have no practical influence in terms of preservation potential. From experience with the Kronholm cog and the Äskekärr Viking ship, two very different burial conditions were present; wet sand and waterlogged clay. Both ships were situated about 20 cm from the surface. What kind of wood degrading microbes will occur and how fast is the decay rate under such conditions? To investigate the effect of soil type used in reburial situations on land sites, the experiments in **PAPER II** were carried out.

From the results it was concluded that waterlogged clay and wet sand provided similar protection, whereas wood samples covered with top soil from the fields had an extremely high aggressive biological activity. However, the long term effects may be different. Observations by Boutelje and Göranson (1975) suggest that old submerged piles were better preserved in clay soils than other types of soil. More experimental work is needed to verify this statement.

Another way to measure the preservation potential of soils is to measure redox potential. Low mV values indicate little reduction and oxidation processes within the soil, which is a more protective environment that soils with high redox potential (Paul, 1992; Caple *et al.*, 1996). However, with this method, all reducing/oxidising processes in soil are measured, including those not directly related to wood degradation.

6.2.5 Cover

Reburial failures are seldom published, but are indeed extremely important for the development of methods. During excavation, it is not unusual that wooden remains are temporarily reburied for full excavations later on. It is also known that wood during periods of months or years can decay dramatically and turn into useless fragments. Such an unexpected event was described by Goodburn-Brown and Hughes (1996). When the "protective" layers consisting of polyethylene sheeting, dried up peat, and backfillings were removed, they found fungal multi-coloured growth and wood lice feeding on the historical remains, and "it was obvious that further surface decay had occurred during the period of temporary reburial". Despite the good intentions, a locally dry environment had been created which favoured aerobic wood degrading fungi.

Plastic sheets, sand, sand bags and geotextile have been widely used for protection of wrecks in marine environments. Lately, archaeologists have started questioning the effect of such methods, and *in situ* preservation is now a hot topic (Riess and Daniel, 1997; Gregory, 1998; Pournou *et al.*, 1998). There is a distinct lack of knowledge regarding the effect of cover techniques used on land as well as in marine environments in terms of protection from wood degrading micro-organisms.

In **PAPER II**, two different cover techniques were tested under laboratory conditions. Both sawdust and geotextiles were found to decrease microbial activity, compared to reburial without cover, but their effect as a measure for long term use as well their use under other conditions is unknown. Probably the initial effect is just a physical barrier between the wood and soil; the substrate and the wood degrading organisms. The idea of using sawdust was that it should represent a sacrificial oxygen consuming substrate. Further observations on their effects in both marine and terrestrial situations during long term exposure should provide useful information.

6.2.6 Depth

One may suspect depth of burial in waterlogged environments to affect the state of preservation (Wazny, 1976), but here a lack of research exists. Decrease of diffusion of oxygen into sediments is clearly correlated with depth

(Rasmussen and Jørgensen, 1992). Contribution from **PAPER III** gives new information on the decrease of decay rate with depth. In poles submerged for 1300 years in brackish water at 5 metres depth, significant differences in degree of decay were observed between samples taken at two different levels. A short distance of 40 cm had an important effect in terms of microbial activity. Depth of burial in the past as well as reburial of archaeological wood today plays an important role for further success of preservation.

In the reburial experiment (**PAPER II**), a depth of 10 - 20 cm from the surface was too small to avoid serious decay by soft rot in all soil types. Diffusion of oxygen into the soil, even though it was waterlogged, created an environment too "anaerobic" for basidiomycetes but perfect for soft rot decay. Therefore, both the Kronholm cog and the Äskekärr II, two examples of land site reburial, are presumably not in safe hands.

Recent work on marine sediments by Gregory (1998), showed that microbial activity decreased significantly at a depth of 50 cm.

Further research is needed, where other levels of depths are investigated regarding type and rate of microbial activity, before final recommendations can be given.

6.3 Environmental monitoring

Recently, interest has been focused on the chemical, electro-chemical characterisation of anoxic waterlogged environments. Redox potential, pH, soil moisture, hydrology, analysis of soil nutrient content, and oxygen concentrations are the most commonly methods so far used (Corfield, 1993; Brunning, 1996b). Most of these measurements are however not easily achieved (Caple, 1993). Results from redox potential measurements are highly dependent on the electrodes used, and there are discussions by experts that suggest that great variation in data can be obtained due to the use of different electrodes (Gregory, D.; 1999, personal communication).

When oxygen concentrations are measured, the result is often zero, and suggestions of an anaerobic environment are given (Pointing *et al.*, 1996). But for a microbiologist this rather tells something about the limitations of the instrument than the actual oxygen concentration. For small organisms, like erosion bacteria, even low concentrations seem to be of great importance according to **PAPER III**. Within the scope of this thesis, development of oxygen measurements in soil environments is of great interest for future research.

Looking back in the literature based on monitoring work, low redox potentials have usually been found in environments where wood is regarded as "well preserved" (Caple *et al.*, 1996). However, no further description of the state of the wood or actual wood species has been given, and comparisons as well as the scientific use are questionable. Hopefully, results from monitoring of Nydam, where many factors are systematically recorded, combined with investigations on metal corrosion and decay of wood, will provide useful information in the future (Fig. 30).

Fig. 30. Different electrodes inserted in the peat bog at different levels are continuously recorded obtaining environmental information. The photo is taken at the Nydam excavation in 1999.

7 Conclusions and strategies for future work

It is said that research produces more questions to be answered, and unfortunately I have to agree. A lot of new ideas have been discussed during my Ph.D. period. Some have led to more experimental work, others have been rejected, but most are saved for future work. In the following sections conclusions drawn from my own studies and from the literature have been used to outline strategies for further work.

7.1 Isolation and identification of erosion bacteria

Even though erosion bacteria are slow degraders, their degradation of archaeological waterlogged wood has been proven to be the most important factor to consider for future decisions for archaeological wood that still remains *in situ*. The fact that these bacteria cannot be isolated, cultured and identified makes it almost impossible to study their growth characteristics and their physiological requirements. Here, temperature and pH ranges are of interest, but most important of all is to find out if they require oxygen for their wood degrading activity or if they can be seen as truly anaerobic degraders. The answer, whatever it may be, will have a very significant influence on design of measures for preservation *in situ* or reburial and for selecting the best possible storage conditions for objects waiting to be conserved. Collaboration should be established with experts in molecular biology to initiate projects leading to identification and isolation of these bacteria. Then, extensive experimental work in the laboratory could be carried out, and information on other interesting aspects of their physiology could be obtained. The effect of polluted waters on the speed of degradation should be analysed under carefully controlled conditions.

7.2 Combating erosion bacteria

Knowing that bacteria represent a problem, the question arises: How can they be prevented from degrading wood? The advice to keep the wood dry to avoid decay is not applicable to situations where erosion bacteria are active. If oxygen can be proven to be an essential requirement, all measures to prevent oxygen coming in contact with the bacteria would slow down or prevent degradation. This means that increasing depths in the waterlogged situation and covering with clay and/or geotextiles should help to preserve the timber. Sawdust and other more easily degradable substrates could also be supplied to provide a nutrient that, when consumed, would use up most of the available oxygen.

Biocides have occasionally been used, although the reasons for using them have not been clear. Such chemicals could play a role in the future for water storage and impregnation baths, but it is very unlikely that biocides will be accepted for use in nature, even if the archaeological objects are unique.

7.3 The environment

Certain parameters, like oxygen, redox potential, pH etc. have been measured and are still being measured at several sites. Such measurements should be encouraged, as well as the attempts to develop better probes. The information obtained will be invaluable for a more complete understanding of on-going processes. Little is known, however, about the micro-organisms present in, or around the wooden objects and their aggressiveness. The presence of micro-organisms other than erosion bacteria, like soft rot and tunnelling bacteria, is well confirmed in timber from certain environments. Understanding the degradation processes in different environments usually require deeper insights in the ecosystem. Terrestrial microcosms have successfully been used in the laboratory for wood preservation research to assess the effects of different soils and their varying micro-flora on the degradation of treated and untreated wood (Edlund, 1998). A similar technique was used in this work for laboratory reburial experiments (**Paper II**). Microcosm studies could be initiated to study the inherent aggressiveness of the micro-flora residing in the soil, water or sediment next to the archaeological wooden objects. Experiments can then be carried out, using sound wood samples, in a convenient way in the laboratory under relatively controlled conditions. Information could then be obtained on presence and activity of various types of wood-degrading organisms. The microcosms will represent an easily accessible source of organisms, for those interested in specific studies of the micro-organisms.

7.4 Conservation

The microscopical observations reported in **Paper I** clearly demonstrated that the deterioration of archaeological wood may be due to different types of decay leading to distinct differences in the structure of the degraded wood. It is also obvious that interior parts of massive objects may appear completely sound when analysed using light microscopy. This does not, however exclude that significant chemical changes may have occurred in the interior parts. It is clear that chemical analyses alone, can not provide enough information on the cause for the changes observed during biological deterioration of wood. Microscopy is in most cases required for identifying the responsible organisms, but microscopy is less well suited for detecting subtle abiotic chemical changes. Thus, the two techniques should be used in combination for future studies aiming at a more complete understanding of the actual wood structure and the deteriorating processes.

All the information on actual structure of the archaeological wood is not readily available for conservators. Drilling or needle techniques and Umax measurements are often used to determine the degree of deterioration, but information on type of decay is usually not obtained. Therefore, timbers in a similar state of preservation, are treated in a similar way. Terrestrial attack by brown and white rot fungi, that occurred before waterlogging, may in combination with the decay during waterlogging have resulted in a weakened residual structure. Archaeological wood suffering from a multitude of decay forms, may thus require special care during conservation. Slime and residual products arising during attack by erosion bacteria, might decrease the penetration rate of PEG, whereas bore holes, pit membrane degradation and other structural changes probably increase the penetration rates.

7.5 Reburial and *in situ* preservation

More research is required before optimal designs and measures can be recommended for the best possible environment. As stated earlier, the question whether or not oxygen is required for the degradation of waterlogged wood is the most important. Another important question is if chemical degradation has significant effects on the structure during extended waterlogging. If so, the influence on chemical degradation by various environmental factors should be studied.

Methods should be developed that will make it possible to assess the success of reburial or *in situ* preservation techniques. One very simple method, that was adopted during the *in situ* preservation of the Kronholm cog, is to install sacrificial wood samples next to the archaeological wood. Such samples can then be easily recovered and investigated after a certain length of time to provide valuable information. For the Kronholm cog, sound pine wood stakes were used. Thus, any degradation observed will have occurred in the environment where the archaeological wood is situated. One objection against this method is that sound wood will be affected in a different way from true archaeological wood, which already suffers from deterioration. It would, however, be difficult to use samples of archaeological wood for monitoring, due to the heterogeneous attack in most samples.

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