Sulfur-Related Conservation Concerns for Marine Archaeological Wood

The Origin, Speciation and Distribution of Accumulated Sulfur with Some Remedies for the Vasa

Yvonne Fors

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Department of Physical, Inorganic and Structural Chemistry
Stockholm University
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Department of Physical, Inorganic and Structural Chemistry
Arrhenius laboratory
Stockholm University
106 91 Stockholm
Sweden

Front cover:
Light microscopy picture of a transverse slice of an oak wood sample from the *Vasa's* orlop deck (core C9b at 0.5 cm depth).
Back cover:
The *Vasa* in Gustav V´s dry dock, Beckholmen, Stockholm 1961 (Photo: SMM)

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Faculty opponent:
Dr. Ian MacLeod, DSc
Executive Director, Western Australian Museum

Evaluation committee:
Docent Helena Lennholm, Wood chemistry, Royal Institute of Technology (KTH)
Dr. Poul Jensen, Department of Conservation, The National Museum of Denmark
Docent Jon Petter Gustafsson, Ecotechnology, Royal Institute of Technology (KTH)

Substitute:
Professor Gunnar Svensson, Physical, Inorganic and Structural Chemistry, Stockholm University

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To Krister and Tage

"I do not know whether it ought to be so, but certainly silly things do cease to be silly if they are done by sensible people in an impudent way."

Miss Emma Woodhouse

From Jane Austen's *Emma* (1816)

ABSTRACT

Synchrotron-based sulfur spectroscopy reveals that accumulation of several types of reduced sulfur compounds on the seabed is a common concern for marine archaeological wood. In the timbers of the Swedish warship *Vasa* (1628) the special seabed conditions restricted the sulfur accumulation to the surface layers (1-2 cm), while it is found to be rather uniform (around 1% S) throughout the hull for the *Mary Rose*, U.K. (1545). The distributions of reduced and oxidised sulfur species were separately mapped in the wood structure by means of scanning x-ray spectro-microscopy (SXM). Organically bound sulfur was found in unexpected amounts within lignin-rich parts, such as the middle lamella. Sulfur K-edge x-ray absorption near edge structure (XANES) spectroscopy identified mainly thiol (R-SH) and disulfide species. Inorganic sulfur compounds, consisting of particles of iron sulfides that may form in the presence of corroding iron, also appeared within the wood structure. Those two pathways for biogenic sulfur accumulation have been elucidated by laboratory experiments exposing fresh pine for two years to simulated seabed conditions. Organically bound sulfur formed in a reaction between the lignin in the wood, exposed by cellulose-degrading erosion bacteria, and hydrogen sulfide; H_2S, produced *in situ* by scavenging sulfate-reducing bacteria. In addition iron sulfide formed with bacteria inoculated from a shipwreck sample. Multi-elemental analyses by scanning electron microscopy (SEM) and x-ray photoelectron spectroscopy (ESCA), were combined with depth profiles of the sulfur and iron concentrations obtained by scanning x-ray fluorescence (XRF). Identification of crystalline compounds by x-ray powder diffraction (XRD) further contributed to the current insight in how sulfur and iron compounds penetrate the wood. Iron sulfides are known to oxidise under high humidity conditions and are probably the main cause of the numerous outbreaks of acidic sulfate salts occurring on the *Vasa'*s hull and on stored artefacts. The iron ions catalyse the production of acid, as well as other wood degrading oxidative processes. After the *Vasa's* conservation spray treatment was stopped in 1979 the continuing oxidation processes are estimated to have produced about 2 tonnes of sulfuric acid; H_2SO_4. The present amount of sulfur remaining in reduced forms within the hull timbers of each the *Vasa* and the *Mary Rose* is estimated to be at least 2 tonnes. A laboratory experiment to gently neutralise the acid in Vasa wood by ammonia gas has been conducted with promising results.

ABBREVIATIONS

DTPA	Diethylenetriamine-pentaacetic acid
EB	Erosion Bacteria
EDMA	Ethylenediiminobis (2-hydroxy-4-methyl-phenyl) acetic acid
EDS	Energy Dispersive Spectroscopy
ESCA	Electron Spectroscopy for Chemical Analysis
ESRF	European Synchrotron Radiation Facility
HPLC	High Performance Liquid Chromatography
MALDI-TOF	Matrix Assisted Laser Desorption Ionisation-Time of Flight
MFA	Microfibril Angle
NMR	Nuclear Magnetic Resonance (spectroscopy)
PEG	Polyethylene Glycol
RH	Relative Humidity
SEM	Scanning Electron Microscopy
SRB	Sulfate Reducing Bacteria
SSRL	Stanford Synchrotron Radiation Laboratory
SXM	Scanning x-ray Spectromicroscopy
XANES	X-ray Absorption Near-Edge Structure
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Powder Diffraction
XRF	X-ray Fluorescence

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by Roman numerals.

Paper I
The sulfur threat to marine archaeological artefacts: acid and iron removal from the *Vasa*.
M. Sandström, F. Jalilehvand, I. Persson, Y. Fors, E. Damian, U. Gelius, I. Hall-Roth, L. Dal, V. L. Richards and I. Godfrey. In *Conservation Science 2002*, Eds. J. H. Townsend, K. Eremin, A. Adriaens, Archetype Press, London 2003, Chapter 13, pp. 79-87.

Paper II
The *Vasa's* New Battle; Sulfur, Acid and Iron.
M. Sandström, Y. Fors and I. Persson.*Vasa studies 19*, The Vasa Museum, Stockholm, 2003**,** 80 p.

Paper III
Analyses of sulfur and iron in marine archaeological wood.
M. Sandström, Y. Fors, F. Jalilehvand, E. Damian and U. Gelius. *Proceedings of the 9th ICOM Group on Wet Organic Archaeological Materials Conference, Copenhagen 2004. Eds. P. Hoffmann, K. Strætkvern, J. A. Spriggs, D. Gregory, Bremerhaven, 2005 (ISBN 3-89757-308-3),* pp. 181-199 (available at http://icom.museum/publications/cc.html).

Paper IV
Sulfur Accumulation in the Timbers of King Henry VIII's Warship *Mary Rose*: A Pathway in the Sulfur Cycle of Conservation Concern.
M. Sandström, F. Jalilehvand, E. Damian, Y. Fors, U. Gelius, M. Jones and M. Salomé. *Proceedings of the National Academy of Sciences (U.S.A), PNAS* 2005, *102*, 14165-14170.

Paper V
Sulfur and Iron in Shipwrecks Create Conservation Concerns.
Y. Fors and M. Sandström. *Chemical Society Reviews 35* (2006) 399-415.

Paper VI
Ammonia Treatment of Acidic Vasa Wood.
Y. Fors, H. Egsgaard, K. Wickholm. In *Proceedings of the 10th ICOM Group on Wet Organic Archaeological Materials Conference,* Amsterdam 2007, Eds. Hoffmann, P. *et al*., ICOM, Committee for

Conservation, Working Group on Wet Organic Archaeological Materials, Bremerhaven, 2008, *In press.*

Paper VII
Sulfur Accumulation in Pine Wood (Pinus sylvestris) Induced by Bacteria in Simulated Seabed Environment: Implications for Marine Archaeological Wood and Fossil Fuels.
Y. Fors, T. Nilsson, E. Damian Risberg, M. Sandström and P. Torssander. *International Biodeterioration & Biodegradation,* 2008, *accepted for publication.*

Contributions by the author
This thesis summarises results obtained in fruitful multi-disciplinary cooperation with several experts in different fields. The author's involvement started with practical conservation work within the Vasa ship, with registration, mapping, pH-measurements and neutralisation of acidic sulfate precipitates. These experiences were later integrated into the research work led by Professor Magnus Sandström, where the author focused on elucidating the mechanism behind the sulfur accumulation processes in marine archaeological wood and the connection to the special environment at the *Vasa's* wreck site. This required considerable efforts to combine properties of wood from chemical, biological and microbial, mechanical, conservation and archaeological perspectives. The author has performed the laboratory experiments on wood supervised by Professor Thomas Nilsson, and the light microscopy studies with the help of Dr. Charlotte Björdal. The ammonia treatment of Vasa wood samples was planned and carried out by the author at Stockholm University. Another task was to select and prepare representative samples for the different elemental and spectroscopic analyses, and also to initiate the sulfur isotope study. During the synchrotron measurements the author's main contribution has been sample handling and preparation, while the data treatment has been performed by Professor Farideh Jalilehvand and Dr. Emiliana Damian Risberg. The SEM analyses were performed together with Emiliana, as also the ESCA measurements, which were supervised by Professor Ulrik Gelius.

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1 INTRODUCTION

1.1 Background

Sulfur* and iron in marine archaeological wood give rise to preservation challenges. Especially those of the Swedish 17th century warship *Vasa* are addressed in this thesis. The focus is on the origin and nature of the accumulation of sulfur in the wood, and on the future consequences and conservation actions that will be needed to prolong the preservation.

In February 2001, scientific investigations started which revealed that the *Vasa* had accumulated large amounts of different reduced sulfur and iron species during the 333 years on the seabed of the Stockholm harbour, and that the reduced sulfur compounds are being oxidised to sulfuric acid.[1,2,II] At an early stage the analyses showed that also other shipwrecks preserved in seawater around the world had accumulated reduced sulfur and iron compounds, making the *Vasa's* problems of general concern.[II-V] However, the analyses showed a large variety in amount, composition and distribution of the contaminants, which encouraged further studies of the accumulation mechanisms and conditions.[III,IV]

The iron compounds in the *Vasa's* wood mostly originate from the corrosion of the bolts on the seabed and from the corroding bolts inserted during and after the salvage. The conservation agent, polyethylene glycol (PEG), used to impregnate and dimensionally stabilise the degraded wood structure, has been found to increase the corrosion rate of metallic iron.[3] In the ambient conditions, moist wood exposed to oxygen, it seemed likely that iron ions catalyse oxidation not only of sulfur compounds but also of PEG and cellulose.[II] Acid hydrolysis and oxidative degradation could eventually reduce the mechanical stability of the wood structure.[V]

Core sampling of the ship show high total sulfur concentrations in the outermost layers (1-2 cm) of the *Vasa's* hull timbers. The variations are large with an average of ~1.0 mass% S.[II] This type of sulfur profile in the wood of the *Vasa´s* hull is special in our comparisons with other marine archaeological shipwrecks.[III-V] However, even though the sulfur contaminants are concentrated to the surface layers and the carrying parts still retain much of the original mechanical strength,[4] the surface carries important archaeological information, and any degrading process affecting the outer layers should be prevented.

* *Sulfur* is the approved spelling for use in a chemical context according to IUPAC´s *Nomenclature of Inorganic Chemistry, Recommendations 1990*, Ed. G.J. Leigh.

The source and distribution of the *Vasa's* accumulated sulfur content, as will be discussed in this thesis, has been connected to the former conditions at the wreck site, and the heavily polluted water of the Stockholm harbour. Sulfur reducing bacteria, as part of their metabolism of organic matter, transform the naturally occurring sulfate ions in the seawater to hydrogen sulfide, $H_2S(aq)$.[II,V]

The dissolved hydrogen sulfide reacted with wood components or iron ions, and accumulated as different reduced sulfur compounds in unexpected amounts, especially in bacterially degraded parts within the waterlogged wood.[V] When the ship was salvaged and the moist wood came into contact with oxygen, acid started to form in oxidation processes and sulfate salts could precipitate on the surface.[I] A database has been implemented at the Vasa Museum, registering and describing over 3000 (in April 2008) outbreaks of acidic sulfate salt (pH = 1-3.5) on the *Vasa's* surface hull and on loose objects. Information from the database has been used for statistics to locate areas with more frequent and severe outbreaks. The influence of high humidity on the rate of the production of acid will also be discussed.

Some of the steps in this sulfur cycle, including the products of the hydrogen sulfide reactions, will be discussed here with emphasis on the chemical reactions and microbial activity in seawater that affect waterlogged wood. Each wreck site has its unique environment and history, which will influence the amount and profile of contaminants, and thus the conservation procedures and the long-term stability of the marine archaeological artefacts.[5] A brief account will be provided of the special conditions that enabled the well preserved state of the *Vasa's* wood but also caused the present problems occurring as acidic salt precipitates. To illustrate the former conditions within the waters of the Stockholm harbour, results from water analyses from the 20th century, have been collected from the archives of the local waste treatment and water purification plant; *Stockholm Vatten*.

The composition and distribution of sulfur and iron compounds in the wood of the *Vasa* and other historical shipwrecks have been analysed by combining several different methods. Synchrotron-based x-ray absorption near edge structure (XANES) spectroscopy and scanning x-ray spectromicroscopy (SXM) have been very informative but are also exclusive techniques with restricted access. More basic information has been achieved from elemental analyses of total sulfur and iron, combined with x-ray powder diffraction (XRD), electron spectroscopy for chemical analysis (ESCA), scanning electron microscopy (SEM) with elemental analysis (EDS) and x-ray fluorescence (XRF) line scans. Such combined analyses have enabled new information to be achieved about the speciation of the sulfur compounds, and their location and reactivity within the wood structure. The scanning x-ray spectromicroscopy (SXM), XANES and SEM-EDS analyses indicate that a ma-

jor amount of reduced organosulfur compounds is concentrated in lignin-rich parts of the wood, i.e. the cell corners and the middle lamella (Chapter 1.5.5), and the inorganic reduced sulfur compounds to a large extent consist of different iron(II) sulfide particles distributed on the surfaces and in cavities in the wood structure.[IV,V]

The analyses indicates a variety of different reduced sulfur compounds in the *Vasa's* wood. The sulfur speciation provides necessary information for elucidating the mechanism of the reactions. Two different pathways are proposed for the accumulation processes in marine archaeological wood and their expected products were obtained in laboratory experiments where fresh pine wood was exposed to a simulated seabed environment, with conditions similar to those of the *Vasa's* wreck site. The test wood was analysed with both XANES and SXM, and the speciation and distribution of the accumulated sulfur compounds were compared to those of authentic marine archaeological wood.[VII]

A recurrent theme of this thesis is the sulfur distribution in timbers from marine archaeological shipwrecks from a wood morphology perspective. This is shown to be coupled to microbial wood degradation of certain wood components, and the access routes of bacteria, as also the transport of liquids are important for the chemical reactions in the wood. Therefore, basic knowledge of wood chemistry and terminology is relevant and will be presented in the first part (a ship terminology dictionary is found in Paper II) as well as some insights into biological wood degradation. Wood conservation ethics is also summarised, since such principles will strongly influence and restrict any future re-conservation work on the *Vasa*. Within that framework, the ultimate aim would be to find and device satisfactory procedures to remove or at least to reduce the detrimental effects of sulfur and iron compounds in marine archaeological wood. Here such modified conservation treatments are intended in the first place for the *Vasa*, but are also of interest for other historical shipwrecks, still on the seabed or presently under conservation treatment as the *Mary Rose* in Portsmouth, U.K.

To remove such potentially destructive contaminants from the wood will certainly be difficult. Any devised extraction method would be stressful to the weakened wood and the possible consequences must be carefully considered, especially to still retain the distinct features on the surfaces. Arresting the degrading processes in a more gentle way, e.g., by making the sulfur and iron compounds chemically inactive *in situ* seems more attractive.[V] Preliminary results of an experiment exposing Vasa wood to ammonia gas (as in a similar treatment of the *Batavia's* timbers) will be presented as a first step to evaluate the possibility to use the method on the *Vasa´s* hull in a larger scale.[VI] Further investigations are clearly needed and the treatments eventually chosen will certainly have to be adapted to each different object.

1.2 Archaeological wood

1.2.1 Definition and state of archaeological wood

Archaeological wood can be defined as wood that carries traces of cultural activity and has been preserved in a specific environment;[6] that is any wooden object that gives information about human development and culture (i.e. archaeological information). Historically, everyday articles were commonly made of wood, which often makes such artefacts representative for their time. Special conditions are required for long-term preservation of wooden artefacts, as an example the extremely dry environment in Tutankhamun´s tomb. Archaeological wood is also commonly excavated from anoxic marine or wet terrestrial (burial) sites and the waterlogged objects then often appear well preserved.[6,7]

The chemical and physical state of marine archaeological wood may vary from nearly sound to heavily degraded. Usually, the central portion is the best preserved, while the surfaces and the outer layers of wooden pieces can be degraded with high content of water and inorganic inclusions, and with loss of biopolymers (Chapter 1.5.4).[6] Generally, as the water content in the wood increases, the amount of cellulose is found to decrease relative to lignin.[8] This is a normal result of the microbial and chemical degradation (Chapter 1.6).[7,9] Non-uniform degradation of the wood within the same object is common, and can be related to inhomogeneous conditions of exposure on the seabed or in the sediments, but also to the species of wood, the history of usage, the time of burial, etc.[6,8]

1.2.2 Preservation of waterlogged wood

Several factors contribute to the relatively well preserved state often found for marine archaeological wood. The preservation in most cases requires near anoxic conditions, which normally occur in marine sediments at the seafloor. The cold, dark underwater environment with low oxygen level prevents many biological degraders.[10,11] Also, when buried by sand, silt and mud a protecting, relatively stable enclosing surrounding is created. Until recently, ancient remains under water have been relatively protected from human interference. This has partly changed with the new underwater technology and the rapid increase in the number of divers.[11] The actions of anyone in physical contact with a wreck site will alter the stable environment, especially if the sediment layers are disturbed. The deterioration of the object may then increase rapidly, and an eventual excavation and salvage operation then should be completed as soon as possible.[10]

Wood has been found to survive deep inside waterlogged anoxic sediments for considerable amounts of time (Chapter 5.1). In geological time spans

enclosed wood, deeply buried under sediments that prevent biological activity and oxygen access, and exposed to extreme temperatures and pressures wood is slowly mineralised forming brown coal.[6,9] Most buried marine archaeological wood is in the early process of diagenesis[*], which is the natural process in which sedimentary materials are slowly compacted and eventually form rock.[6]

Degraded waterlogged wood has higher density than water and is therefore not able to float. Its state can be very deceptive, with satisfactory appearance when wet and fully expanded with the internal water filling and supporting the weakened cells, and when cracks may have closed. However, the structure may be soft and the loss of cellulose usually correlates with the increasing water content in the wood.[8]

The fibre saturation point (FSP) is the moisture content at which all sites for adsorbed water in the cell wall are saturated (not to confuse with the moisture content in equilibrium at 100% RH). All water added above FSP is free water (in the wood cell cavites), which has no effect on the strength of the wood. Changes in moisture content of the wood cell wall below FSP has a major effect on many wood properties since the bound water interferes with secondary bonding (the hydrogen bonding) within polymers of the cell wall, which decreases the strength of the wood. The mechanical properties generally increase with decreasing moisture content (Chapter 1.5.4).[12,13] The FSP varies with the different chemical composition in different species of wood.[12] The maximum water content (of the cell wall) of archaeological wood is therefore related to the extent of degradation, i.e. the FSP is considered to increase as the cell wall degrades.[8]

1.2.2.1 The anisotropy of swelling and shrinking

Waterlogged wood, if allowed to dry without treatment, may go through drastic dimensional changes, In waterlogged wood all pore spaces are filled with water. If left to dry in air, the capillary tension when the water evaporates from the cell cavities (lumina) (Chapter 1.4.3) will reduce the internal gas pressure resulting in irreversible change or collapse of the weakened wood cell structure, with severe shrinkage, delamination and distortions.[8]

Anisotropic shrinkage occurs in the cell walls, with cracking of the sapwood and the outer layers when drying. The cut angle (from the annual rings) of the wood (Chapter 1.5.2) will indicate the overall shrinkage, and similarly individual planks swell differently depending on the direction from how it was sawed from the stem.[12] The wood will shrink most in the tangential direction (to the annual rings) followed by the radial (from the pith outwards),

[*] diagenetic = chemical and physical changes occurring in sediments during and after their deposition but before consolidation.

which is explained by the restraining strength of the wood rays (Chapter 1.5.1). Also the swelling is larger in the tangential direction. The rays also facilitate humidity release, which is faster than in the tangential direction.[12]

Differences in the transverse shrinkage are also connected to variations in the annual ring (Chapter 1.4.3) and the cell wall structure (Chapter 1.5.5). The shrinkage in the longitudinal direction along the grain is nearly insignificant,[8,12] but the longitudinal shrinkage of reaction wood (Chapter 1.5.5.1) is higher than in normal wood. Also, the microfibril angle (MFA) has a strong influence on both swelling and shrinkage. Increased fibril angle decreases the tangential shrinkage while the longitudinal shrinkage increases.[12] To prevent irreversible changes and retain the integrity of the object, waterlogged wood should always be kept wet until treatment (Chapter 1.3.1).[6,8]

1.3 The conservation process

1.3.1 Unique objects require special treatment

An important aspect on the conservation of waterlogged wood is the stabilisation of the object to maintain the dimensions.[6,8,10] The waterlogged objects should be kept wet (preferably fresh water) until conservation and under stable conditions before, as well as after, the treatment. A conservator's responsibility is to control the environment and establish conditions to minimise and arrest decay of the artefact. Recommended relative humidity (RH-value) is 45-55% with a maximum daily variation below 10%. Temperature should preferably be kept low, 15-22 ºC (to avoid biological growth), but more important is stability with small variations. The light level should not exceed 50 lux. The object must be kept well supported and handled gently to avoid mechanical stress or strain.[6,8,10]

Because of the variety of environments in which (marine) archaeological wood has been preserved, every artefact displays a unique problem profile. This situation creates a need for adapted conservation treatments, determined by the nature of the contaminants, the condition of the artefact and how it should be handled. Contaminants are usually disfiguring and/or chemically damaging, but may in rare cases have contributed to the preservation.[10]

1.3.2 Conservation ethics

The purpose of preserving archaeological wood is to save the artefacts for future exhibition, study and reference.[14] Beside the general agreement that "structural and decorative falsification" should be avoided, there are three commonly recognised ethical principles of conservation. The first principle is "the reversibility of processes". No process should be carried out which cannot at some later stage be altered; ideally it should be possible to return

the object to the initial (waterlogged) condition for retreatment.[8] The second states "that as far as possible decayed parts of an artefact should be conserved and not replaced". The third is that the "consequences of the ageing of the original materials (for example 'patina') should normally not be disguised or removed".[10] That means that any treatment should be based on minimum intervention.[8] Even if an effective method of treating an archaeological object is available; it is not always self-evident that it should be applied. The treatment could put extra stress on already weakened material, and make it less durable. The extraction of the chemical contaminations of an object could be an ethical problem since a general principle is to preserve the object as found, and in that respect the contaminants are considered as a part of its history.

Marine archaeological wood is often dark, and "black oak" is sometimes in popular view considered to possess special properties regarding strength and hardness. The dark colour may originate from iron(III)-tannin complexes or iron oxide particles, and the wood could be partly mineralised. Such wood may appear hard and resistant, but can be brittle and mechanically weakened.

1.3.3 Polyethylene glycol conservation

Polyethylene glycol (PEG) became commonly applied to stabilise the dimensions of waterlogged wood, especially for large objects, after the development of the PEG spray treatment for the *Vasa* (Chapter 2.5).[4,15] PEG is a polymer with oxygen (ether) bridges between CH_2CH_2 entities in a chain with terminal hydroxo groups; $HO\text{-}(CH_2CH_2O)_n\text{-}H$. It is used as a non-volatile replacement for water in the wood structure, able to form hydrogen bonds and provide mechanical support for the degraded wood. Polyethylene glycol polymers with a molecular mass higher than about 600 (PEG 600 has $n \approx 13$) has a waxy-like character at room temperature; however the large number of oxygen atoms in the chain makes even long chain PEG molecules water-soluble. With short length of the polymer chain the ability of PEG to penetrate into the wood structure increases, but also the hygroscopicity.[16]

Two different techniques to apply PEG are in use: the two-step (or twinned) method and the parallel method. In the two-step treatment aqueous solutions of low molecular PEG, which has better ability to penetrate the cell walls and bind to the fibrils, are applied first. Then solutions of high molecular PEG follow, with more bulking effect by filling out the lumen. The risk for osmotic collapse is lower when starting with low molecular PEG even though some may be expelled from the wood in the second step. The high molecular PEG penetrates slower but is less hygroscopic providing a less sticky character to the surface. In the parallel PEG approach where a treat-

ment with a mixture of low and high molecular PEG is used from the start the low molecular PEG may be hindered to reach the cell wall.[8]

The permeability of the wooden artefacts can vary highly even within the same object; because of differences in degradation, salt deposits or the presence of tyloses (Chapter 1.5.1).[8] Based on the U_{max} (maximum water content of wood, % of dry matter) guidelines are available for a recommended PEG mixture for the best anti-shrinkage efficiency (ASE).[8]

Despite its many advantages the use of PEG is under debate, especially since its chemical stability has been questioned;[17] it promotes corrosion of metallic iron,[3] and the treatment is not quite reversible. The terminal hydroxo groups form hydrogen bonds mainly to the carbohydrate polymers. In degraded archaeological wood the carbohydrates; cellulose and hemicellulose, may be absent or only present in reduced amounts (Chapter 1.6). Future non-reactive bulking agents should perhaps preferably be designed for lignin, the major remaining biopolymer.[6,18]

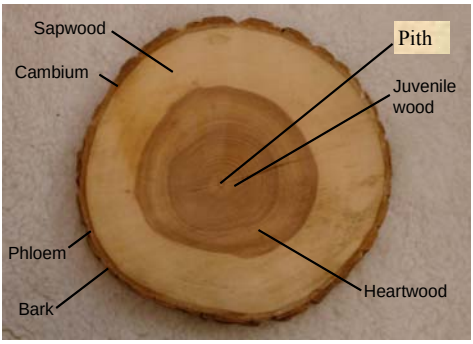
For the *Vasa*, a 1-4% boric acid-borax mixture was added to the PEG-solution as fungicide during the spray treatment.[18] Other measures to prevent biological activity in the solutions are the use of purified, frequently changed water, low temperature, dark storage, biocides, etc. Another option is gamma-ray sterilisation, tested by the Mary Rose Trust.[19]

1.4 Wood morphology

1.4.1 Softwood and hardwood

All plants have a common ancestor and can therefore be arranged in hierarchic levels into the botanic phylogenetic evolution tree of the eukaryotic kingdom *Plantae*. This thesis will focus on two species from two different phyla (or phylogenetic groups); pine and oak. The first comprises conifers, which is a phylum of gymnosperms (= naked seed). Conifers include species as pine and spruce, i.e. trees with needles. They are generally called softwoods.[20]

The second group is the phylum of agniosperms (= flower plants), where the class of eudicotyledones generally refers to trees with leafs with a central nerve fibre. Here we find species like oak, birch and aspen, which are called hardwoods. The term “hardwood” reflects that the wood mostly is harder than the wood of the often more porous coniferous trees; softwoods, even though this is not always the case.[20]



Picture (modified from): The Ljungberg Textbook

Figure 1. *Cross-section of a Scotch pine stem showing the layer structure. The darker heartwood is easily distinguished from the lighter sapwood.*

1.4.2 Stem layers

Wood is mainly composed of elongated cells forming wood fibres, running in longitudinal direction parallel to the stem. Bark is the outermost dead layer of the stem that provides protection against drying and physical, mechanical or biological damage (Figure 1). Underneath the living inner bark; the phloem is located, which transports nutrients. The vascular cambium is the following thin layer (about three cell rows) where the cell division takes place. Through repeated division the cambium cells are either developing to new phloem to the outside, or becoming xylem ("wood") to the inside. The outer part of the xylem is, except for the parenchyma cells, mainly dead sapwood (splintwood), where the water transport takes place. When the parenchyma cells (Chapter 1.5.1) die, the sapwood forms totally dead heartwood, which functions as support tissue. Products from the dying parenchyma are resin, phenols and pigment, and the colour of heartwood is usually darker because of the formation of these extractives, which function as natural protection against microorganisms, etc.[6] Finally, the most central part of the stem is the first years juvenile wood with the "herbal" growth pith in the middle with lower density and shorter cells.[6,20,21]

1.4.3 Annual rings

The xylem usually contains distinct oriented annual rings, each corresponding to the growth of one year (Figure 2). The earlywood (springwood) has thin cell walls and large lumen, which facilitate water transport. The latewood (summerwood) is characterised by small lumen and thick dense cell walls, which create physical support. The climate strongly influences the development of the annual rings, which are better defined in softwoods, as compared to hardwoods.[21] The annual rings allow dendrochronologic dating of archaeological wood, as a complement to the ^{14}C-technique. The unique pattern of the annual rings in the sample is compared to dated reference material.[20]

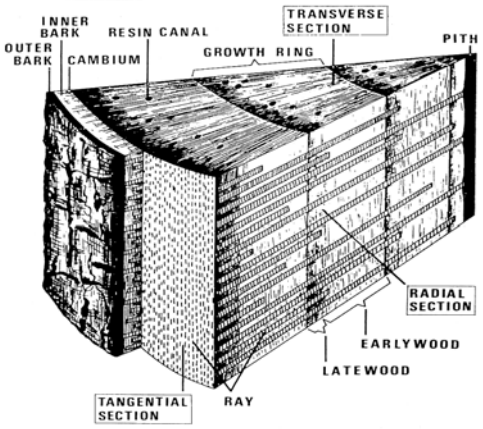


Figure: The Ljungberg Textbook

Figure 2. *The three (cutting) directions of wood, the transverse section and the longitudinal; tangential and radial sections.*

1.5 Wood anatomy

1.5.1 Transport of liquids

Softwoods are evolutionary more primitive and homogenous with a smaller number of cell types when compared to the more advanced set of (short) cell types with specialised functions in hardwoods. In softwood the wood cell fibres called tracheids are long and slender hollowed cells, providing both mechanical strength (esp. latewood) and water transport (esp. earlywood).[20]

In hardwoods the primary role of the corresponding fibres is to provide mechanical strength, while long, thin-walled open tubes; the vessels (Figure 3), provide the main transport channels for liquids.[20,21] The arrangement of vessels varies within the annual rings and is used to characterise hardwood types. Inside the vessels tyloses can be produced as outgrowths from adjacent parenchyma cells through pits. Tyloses are composed of cellulose, hemicelluloses and lignin and are impermeable for water. They are frequently formed in the vessels of certain hardwoods, such as oak (Figure 3).[21]

The resin canals of softwood (Figure 3) are intercellular spaces surrounded by living epithelial cells that form a network inside the sapwood structure, with the role of secreting resins into the lumen (from epithelial parenchyma cells) as defence against microbial attack. Both hardwoods and softwoods contain parenchyma cells that transport and store nutrients, and also rays to store and redistribute storage material, e.g. starch. Non-lignified pit pairs connect adjacent cells in both hardwoods and softwoods, and provide liquid

transport laterally and vertically through the cell walls.[21] The easily degraded pit membranes have often disappeared in archaeological wood. The tracheids in softwoods are end-closed, thus the pits are the major pathways for penetration of fluids, but may also serve as the entry of rot into the wood.[20] Also, natural openings in the wood structure, such as the vessels, may facilitate the penetration of conservation fluids.

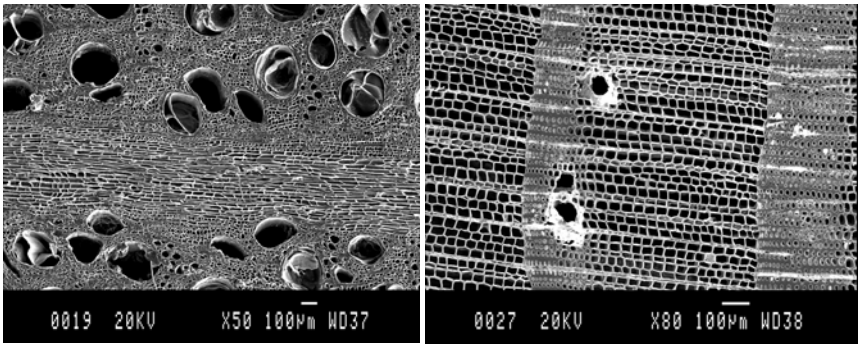
1.5.2 Cutting wood for microscopy

Light microscopy is an excellent and simple method for determining the species and the degree of deterioration of unknown wood samples. The wood slices must be very thin, and cut in certain directions (see below). Degraded archaeological wood is often soft and crumbles easily and cutting by hand is often the preferable method. A flexible razorblade that can be bent and adjusted to the shape of the wood sample is recommended. Because of large variations in the degree of deterioration even within the same sample, sometimes only a small area of the sample surface can be used for cuts, and the cut angle must be adjusted in every single case (Chapter 3.7).

Wood can be cut in three different directions, two of which are longitudinal and one horisontal relative to the direction of the longitudinal wood fibre (Figure 2). Three different types of cuts are needed in order to study different characteristic components in the wood structure. The transverse (horisontal) cut is made across the annual rings and the fibre direction, displaying the end side of the wood fibres as connecting rings (of lumen) in rows (Figure 3). This cross section gives information about transition from early to late wood cells, rays, resin canals and vessels. A tangential longitudinal cut is tangent to the annual ring, cutting the rays, which are seen as bundles of straws. The radial longitudinal cut is perpendicular to the tangential cut and runs parallel to the rays (Figure 2).

1.5.3 The main species of Vasa wood

Oak was the main construction material of the *Vasa* (about 90%), especially in load-carrying parts. Oak is hard, strong and the heartwood is very persistent against rot.[21] Earlier it was commonly used in shipbuilding and for other constructions in contact with water. Nowadays oak is mostly used in home decorations and furniture, where also so-called black oak (Chapter 1.3.2) is considered a decorative material. The species of oak in the *Vasa* has been identified as probably being *Quercus robur* (pendunculate oak) (Figure 3).



SEM photo: Yvonne Fors

Figure 3. *(left) SEM picture of a cross section of Vasa oak wood; site HS4a from the hold. The vessels, which are the main longitudinal conducting elements in hardwood, appear as large holes. The individual vessels are relatively short but can connect via plates throughout the wood. Tyloses are seen blocking some of the vessels.*[20] *(right) Transverse sections of fresh pine sample A208, stored in a bacterial culture of seawater,*[VII] *showing the annual rings (early and latewood cells), the position of rays, and epithelial cells surrounding the resin canals (note the folded edges). Trees under stress often develop large numbers of so-called traumatic resin canals.*

Pine, i.e. *Pinus sylvestris*, was the second most common species of wood used for the *Vasa's* construction, while alder and linden were used mostly for decoration and ornaments.[22] The annual rings between the light earlywood and the darker laterwood are sharp. Since the wood is relatively easy to work and impregnate, pine was chosen for the laboratory tests of sulfur accumulation in Paper VII (Chapter 2.4.1).

1.5.4 Chemical composition and wood mechanical properties

The microfibrils are formed by cellulose polymers held together by hydrogen bonds in bundles, which are surrounded by cross-linking hemicellulose and lignin.[23] The wood biopolymers are in order of decreasing stability: lignin > α-cellulose > hemicellulose.[6] The capacity to bind and store water is almost the opposite.[24] The pit membranes of cellulose (and pectin) therefore disappear by time in archaeological wood, while in a 100 million year old wood sample the lignin still remained.[6]

Table 1. *Cell component distribution in softwood and hardwood.*

	Cellulose	Hemicellulose	Lignin	Extractives
Softwood	40-45%	25-30%	15-35%	1-4%
Hardwood	40-50%	17-35%	20-30%	1-3%

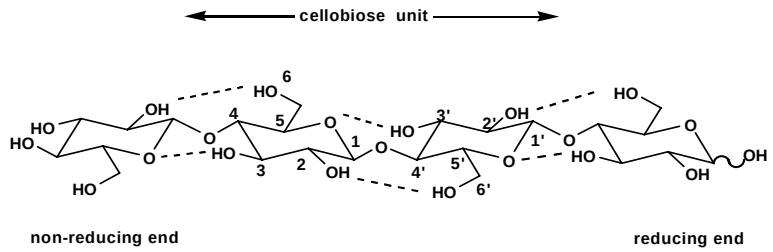


Figure: The Ljungberg textbook

Figure 4. *The structure of the cellulose chain. In acid hydrolysis the bond between oxygen and carbon atom 1 can be catalytically broken by a hydronium ion (H_3O^+) attaching to the oxygen atom linking to the next cellulose subunit.*

1.5.4.1 Cellulose

Cellulose (i.e. α-cellulose) in the skeletal matrix of wood is surrounded and encrusted by hemicellulose and lignin. Cellulose is a linear polysaccharide supported by hydrogen bonds within the chain (Figure 4). Hydrogen bonds also join adjacent cellulose chains into sheets, held together by van der Waals forces in a semi-crystalline structure. Bundles of cellulose chains are called microfibrils. The crystallinity varies within the microfibrils, and the less ordered cellulose on the surface provides contact surfaces between the fibrils.[23]

The strong covalent bonds within the cellulose chain are called the primary bonding. The secondary bonding, consisting of hydrogen bonds, van der Waals and dipole interactions between the cellulose chains, is weaker. Water weakens primarily the secondary bonding and decreases the stiffness of wood, which is reflected in a decreasing E-module*. High MFA (Chapter 1.5.5) of a wood sample increases its dependence of the secondary bonding and makes the weakening effect of water larger. The strength of the wood fibres, on the other hand, depends more on the primary bonding.[12]

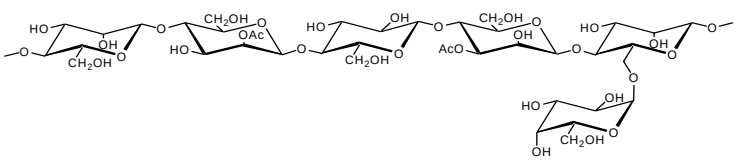


Figure: The Ljungberg Textbook

Figure 5*. The branched structure of hemicellulose (softwood galactoglucomannan).*

* E-module = Modulus of Elasticity or Young's Modulus used to determine stress-strain relationships of elasticity.

1.5.4.2 Hemicellulose

Hemicellulose is a group of branched and substituted polysaccharides with lower degree of polymerization (shorter chains) and crystallinity than the chemically related cellulose (Figure 5). The bulky and amorphous structure is more accessible and hemicelluloses are more reactive and hydrolyse more efficiently than cellulose. Wood usually contains 20-30% dry mass of hemicellulose, which together with cellulose forms the bulk of the cell wall (Table 1). The function is not fully understood but hemicellulose contributes to the mechanical properties of the cell wall, perhaps serving as an interface between cellulose and lignin.[24]

1.5.4.3 Lignin

Lignin basically makes the difference between cotton and wood by acting as a glue to keep the microfibrils together, thus giving stiffness to the cell wall. The cell wall has been described as a composite material with cellulose fibrils as the armouring fibres and lignin as a phenolic plastic.[25] Lignin is formed by polymerisation of three main monolignols (or phenylpropanol derivates) (Figure 6), which connect with three different types of ether bonds (C-O-C) and four types of carbon-carbon (C-C) bonds (or condensed bonds). The branched three-dimensional web structure (Figure 7) has no higher repeating units than the monomers. The aromatic rings and hydroxyl groups allow lignin to form non-covalent interactions and hydrogen bonds with cellulose and hemicellulose. The mixture of aromatic and aliphatic moieties makes it impossible to present a uniform "true" structure of lignin.[25]

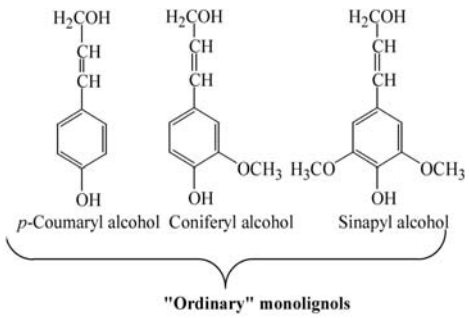


Figure (modified from): The Ljungberg Textbook

Figure 6. *The polymeric lignin is composed of monomeric monolignols*

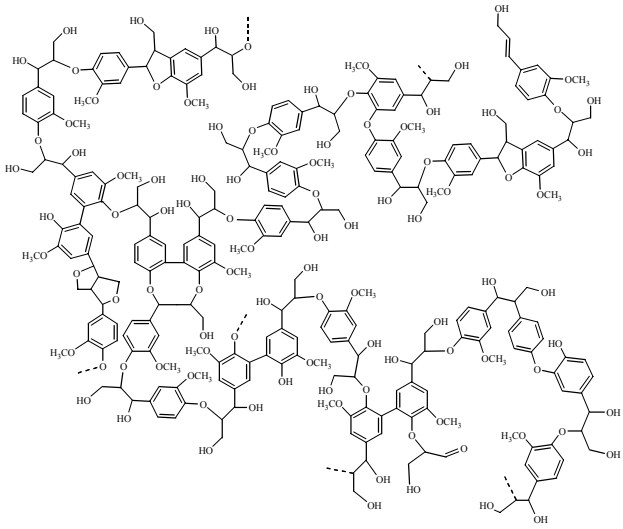


Figure: The Ljungberg Textbook

Figure 7. *A suggested structure of softwood lignin. There are a large number of accessible and reactive sites, e.g. ether bonds, keton and α-hydroxo groups, which can react with hydrogen sulfide to form thiols.*

The lignin content makes the cell wall hydrophobic and inhibits swelling, and is therefore an important component in water-transporting cell constructions. Also, the lignin functions as a defence against microbial degradation. The polysaccharide degrading enzymes from microorganisms usually cannot penetrate the cell wall (Chapter 1.6). Some specialised fungi and bacteria can degrade parts of the structure but the heterogeneity slows down the process.[25] Generally, the microbial resistance increases with higher lignin content.[9]

1.5.4.4 Hardwood and softwood lignin

In an evolutionary perspective softwoods were developed earlier than hardwoods (Chapter 1.5.1). That is also reflected in the less complex composition of the softwoods lignin, than that in hardwoods and other later developed species. Softwood lignin is dominated by coniferyl alcohol, with only small amounts of *p*-coumaryl alcohol (Figure 6).[25] Hardwood lignin consists of equal amounts of coniferyl and sinapyl alcohol together with smaller amounts of *p*-coumaryl alcohol, which introduces a higher content of methoxy groups. In hardwoods the high content of the three-substituted sinapyl alcohol residues also leads to less condensed structures, because they provide less networking opportunities during polymerisation, and an increased number of ether bonds. For steric reasons the sinapyl alcohol cannot couple

in 5-position (Figure 6), why hardwood lignin is believed to be more linear and less branched than softwood lignin. The softwood lignin is more cross-linked, but the lower amount of methoxy groups might facilitate flexibility.[25]

Both hardwoods and softwoods have high lignin concentration in the cell corners, the middle lamella and the S3-layer (Chapter. 1.5.5). Furthermore, residues of coniferyl alcohol may occur with high concentration in hardwood cell corners, whereas syringyl alcohol is found in larger amounts in the S2-layer. That means that lignin in the middle lamella could be more branched and condensed with higher content of carbon-carbon bonds, than lignin in the S2-layer. Still, the coniferyl alcohol is the dominating monolignol in both types of lignin. The lignin content is also higher in vessels (24-28%) and in ray parenchyma cells (27%), than in the libriform fibres (19-22%) with large amounts of coniferyl alcohol.[25] The differences in lignin content and composition of the monolignols may have consequences for the reactivity with hydrogen sulfide and thus the sulfur accumulation (Chapter 3.7).

1.5.5 Cell wall layers

The chemical composition varies in the layers of the cell wall in a wood fibre. Variations also occur between different species, in different parts in the growing tree and in wood grown under stress, so-called reaction wood (Chapter 1.5.5.1). The structure of the wood fibre is schematically shown in Figure 8. The altered orientation of the cellulose fibrils, or microfibril angle (MFA), in the lignified secondary cell wall layers; S1, S2, S3, together constitute a rigid and advanced construction.[21]

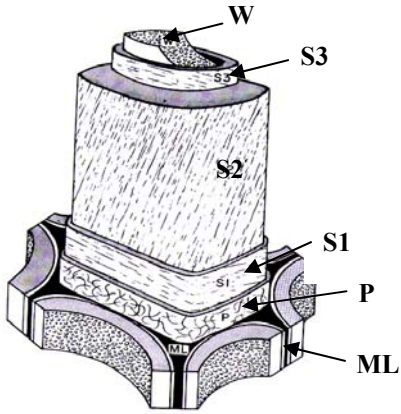


Figure (modified from): The Ljungberg Textbook

Figure 8. *The composition of the wood cell wall. From the outside the primary cell wall (P), the secondary cell wall; S1, S2, S3 and the warty layer (W). The lignin concentration is high in the S3-layer and the middle lamella (ML), which "glue" the wood cells together.*

The large volume of the S2-layer makes the S2-lignin the most abundant in the plant, totally 60-80%, or 0.2-0.3 g/g wood. Still, the cell corners, the thin middle lamella and primary wall (P) have the highest lignin concentration; 0.6-0.9 g/g, even though the content in the middle lamella is only 25-29% of the total lignin in the cell.[6,21] While the cellulose fibrils are randomly oriented in the primary cell wall, they are strictly ordered in the S1-layer, with a large angle to the longitudinal cell direction (high MFA). The lignin concentration is also higher (in g/g) than in the S2 and S3-layers. The cellulose fibrils of the S2-layer are also strictly ordered but with small MFA, and is the dominating layer in the cell wall (80-90% in latewood). The S3-layer is again arranged with a large angle between the cell direction and the cellulose direction. Finally, the warty layer (W) is lining the cell lumen.[21]

1.5.5.1 Reaction wood

Outer physical stress, e.g. hard wind or bending, causes wood to develop morphologically different cell compositions to keep the tree to upright growth. Softwood forms high-lignin compression wood on the lower parts of the tree and under branches, while hardwood develops tension wood in the upper regions. The tracheids in compression wood lack the S3-layer, are round in shape and disconnected from the cell corners. Tension wood develops a cellulose-rich gelatinous layer between the S2-layer and the lumen, and generally forms fewer and smaller vessels.[21] Reaction wood, as other natural occurring deviations in the wood structure, should not by mistake be considered degraded when analysing archaeological wood.

1.6 Biological degradation of wood

Degradation of waterlogged wood generally occurs in microbial rather than chemical processes, and is a part of the natural cycling of biomass. The rate and extent of such microbial degradation depend not only on the composition and chemical or morphological differences of the wood components; also the wood species, cell type and location in the cell wall layers are of importance.[26] The microbial attack breaks down or changes the structural components in the wood, resulting in decreasing density and mechanical strength.[9,10,20] Wood degrading organisms are found in different groups; insects, molluscs, fungi and bacteria, which can be specialised to degrade cellulose, hemicellulose or even lignin. The microorganisms causing wood decay are usually classified microscopically from their special ways of attacking wood. The appearance of the deterioration can also be used to identify cell wall layers or other features of the wood.[9]

Wood has developed defences against microbial degradation, with lignin acting as a barrier for enzymatic decomposition, and extractives that may function as toxins for microbes. The highly lignified middle lamella and S3-

layer (Chapter 1.5.5) are therefore the parts that are the most resistant to microbial degradation, and the polysaccharides generally degrade first. Only certain specialised wood degrading bacteria have been observed to be able to break down the lignin.[19,27] As for the pulp industry, the removal or modification of lignin to get access to the cellulose is usually the key for the wood degrading organisms.[9,25]

A most efficient degrader of marine archaeological wood is the mollusc *Teredo navalis*, or "shipworm", which rapidly can create tunnels lined with calcium carbonate in exposed wood in marine environments.[5,9] The physical damage may have impact on the later conservation, since the calcium carbonate may prevent conservation chemicals to penetrate the wood. Since the shipworms seem to require at least 12‰ salinity, the brackish waters of the Baltic Sea discouraged the *Teredo navalis* and other marine borers from degrading the Vasa wood. This was an important condition at the Vasa wreck site that enabled the relatively well preserved state of the exposed parts of the shipwreck (Chapter 2.2).[4,5]

Marine bacteria start coating a wooden object as soon as it enters the water, initiating the process of biodeterioration.[9] Still, degradation by marine bacteria is a slow process in comparison to deterioration by marine fungi or higher wood degrading organisms such as the *Teredo navalis*.[19,28] However, in near anoxic or anaerobic surroundings or in low salinity seawater the marine bacteria remain as the most active organisms.[29] The majority of marine bacteria seem to be non-culturable using standard procedures, and none of the wood degrading bacteria has as yet been isolated in a pure culture or identified. However, recent purified cultures of erosion bacteria show species belonging to the CFB (Cytophaga-Flavobacterium-Bacteroides) complex.[30]

Components of the wood structure, such as rays, resin canals, vessels and pits, often provide openings for the microorganisms.[9] Bacterial colonization and penetration of wood increase the permeability and predispose the wood structure to fungal and other microbiological attack.[29] Such bacteria that accompany other decay microorganisms as a part of the total microflora[26,31] are called scavenging bacteria or secondary wood degraders.[32,33,34] Scavenging bacteria are not involved in the primary degradation process but can be found in the residual material from erosion bacteria (Chapter 1.6.1), where they are thought to utilise simple sugars in the degraded cell wall materials as the carbohydrate source for their metabolism. Although some forms of scavenging bacteria are described as partly aerobic they can also thrive under anoxic conditions and exist in environments where organic compounds are being mineralised.[35]

1.6.1 Erosion bacteria (EB)

Erosion bacteria (EB) are the primary degraders of waterlogged archaeological wood,[30,34] and actively break down the lignocellulose structure of the cell walls throughout the wood tissue.[26,33] The EB function efficiently in near anaerobic conditions.[28,30] The bacterial invasion starts from the surface through rays and pits in the wood structure, and into the cell lumen from where the attack begins (Chapters 1.5.1 & 1.5.5). The EB progress outward, reaching the middle lamella by locally eroding and penetrating the S3-layer in grooves.[33,34] The attack continues through the cellulose-rich S2-layer, which is converted into an amorphous substance of granular residual cell wall material (Figure 9).[33,34] Even at very advanced stages of decay the lignin-rich middle lamella seems unaffected and often parts of the S3-layer remain. A characteristic feature is the non-homogeneous decay pattern, with heavily degraded wood cells distributed among sound ones.[31,36] Severe EB attacks has been observed deep into the timbers of the *Mary Rose*, with partial conversion of the cellulose components of the S2 and S3 layers of the wood cell wall into an amorphous mass, while the lignin-rich middle lamellae are not degraded.[19]

At the final stage, the remaining wood structure consists of a very fragile skeleton of middle lamellae, which will collapse irreversibly upon drying.[9] However, even if the cellulosic parts of the cell wall have been completely degraded, the waterlogged wooden object may retain its outer physical shape, and the ornaments and traces from tools, etc., which carry important archaeological information, could be preserved.[7,31,36]

1.6.2 Sulfate reducing bacteria (SRB)

Even in anoxic waters or in sediments, anaerobic bacteria may continue oxidation processes.[5] Sulfate reducing bacteria (SRB) are a distinctive group of anaerobic prokaryotes and have a long evolutionary history. SRB can reduce sulfate ions; SO_4^{2-}, to hydrogen sulfide; H_2S, when metabolising simple organic molecules (Chapter 2.3.2) The sulfate ion is used as terminal electron acceptor in the concomitant oxidation process in which the organic carbohydrate, denoted as (CH_2O) in reaction 1, acts as electron donor[37,38]:

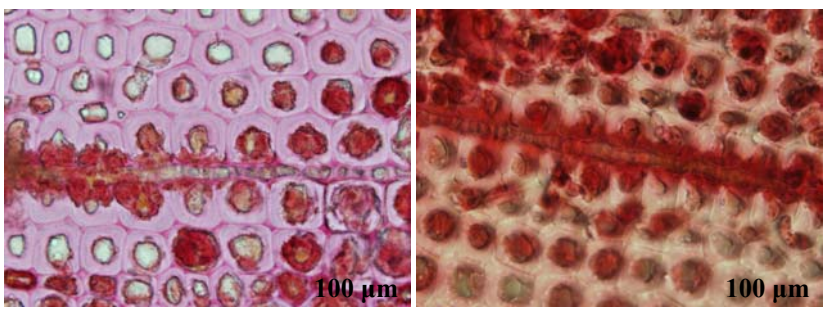
$$2(CH_2O) + SO_4^{2-} \rightarrow H_2S(aq) + 2HCO_3^- \qquad (1)$$

Reactions have been found to occur between the hydrogen sulfide and wood components, and also with iron ions from corroding iron metal forming iron sulfides. This is a concern for artefact deterioration and post-conservation stability (Chapter 2.6).[1,2,5,I-III]

The SRB are known to utilize a wide variety of compounds as electron donors. Suitable direct substrates are some inorganic compounds, hydrocar-

bons, monocarboxylic acids (aliphatic), dicarboxylic acids, alcohols, amino acids, sugars (glucose and microorganism-degraded cellulose), and also aromatic compounds including lignin and tannins, but without destruction of the ring.[39,40,41] The SRB are considered the penultimate organism in oxidation of organic matter but do not degrade natural biopolymers (starch, glycogen, protein or lipids). They then depend on the fermentation and degradation products from other organisms and are in that respect scavenging bacteria.[42,43]

The relationship between erosion (primary wood degraders) and scavenging bacteria (secondary wood degraders) is still largely unknown, but the absence of scavenging bacteria in areas of undecayed cell walls, suggests that they depend on the activity of wood degrading fungi and bacteria.[33,34,44] Consortia with closely related SRB and wood degraders cannot be ruled out.[19] The *Vasa* has, as other shipwrecks, e.g. the *Mary Rose* and the *Batavia*,[V] many areas of degraded wood with very soft, sometimes brittle surface, which may crumble at physical contact (Chapter 5.5). So far, it has not been demonstrated if the degradation, often accompanied with high acidity and precipitates of sulfate salts, is primarily a result of the acidity or caused by previous bacterial erosion. In any case, it seems likely that the EB facilitated the penetration of SRB, which produced hydrogen sulfide *in situ* in the areas with degraded, softened wood (Chapter 2.4).



Light microscope picture: Yvonne Fors

Figure 9. *Light microscopy images of transverse sections at the surface of pine wood sample 6 (Chapter 2.4.1) reveal the characteristic bacterial degradation of the wood cells (easily distinguished from so-called reaction wood, Chapter 1.5.5.1). Some intact wood cells are also visible. Erosion bacteria invade from the rays (elongated dark areas) and start degrading from within the lumen through the cell wall. The amorphous mass consisting of residual cell wall material and bacterial slime absorb the red safranin colour.*[31,36]

2 THE SULFUR RELATED PROBLEM

2.1 The history of the *Vasa*

In 1625 the Swedish King Gustav II Adolf ordered four warships for his navy, from the Dutch shipwrights Henrik and Arendt Hybertsson, who held the contract for the operation of the state shipyard at Skeppsgården (now Blasieholmen in Stockholm). The two larger of these ships were two-deckers, and the keel to the first was laid down early in 1626. This ship, later named the *Vasa*, was launched in 1627 and outfitted the following year with what was then the most powerful armament carried by any ship in northern Europe.[45,46] However, the ship lacked stability, and on its maiden voyage on the 10th of August 1628, heeled over in a gust and sank in Stockholm harbour after only a 1300 m cruise:

> "[Vasa] came level with Beckholmsudden, where she sustained a list, with the water coming in through the gun ports, until she slowly sank to the bottom, with all her flags flying..."[47]

The ship went down at 32 m depth about 100 m outside the island of Beckholmen in the middle of the entrance to the Stockholm harbour. Some early attempts to raise the ship failed, but brought the hull to an upright position. Most of the bronze cannons were recovered in 1664-1665 in a remarkable diving operation led by Hans Albrecht von Treileben. In 1956 the *Vasa* was relocated by Anders Franzén. Later, divers reported that the lower parts of the hull were partly covered by clay and sediments on the seafloor. On top of the clay a 3 m deep silt layer almost reached the portside gun ports, while the layer was thinner on the starboard side. The port side was facing land while the starboard side of the *Vasa* was more exposed to the circulating water of the fairway. The interior of the Vasa ship was at the salvage in 1961 after 333 years on the seabed partly filled with mud.[48]

2.2 Protection by water pollution with a sour aftertaste

Large marine archaeological wooden artefacts are relatively rare because of the efficient natural degradation of the wood in salty and/or oxygen-rich waters. The *Vasa's* relatively well preserved state after 333 years on the seabed is the result of a combination of several favourable circumstances. Important primary conditions were the brackish water of the Baltic Sea, which is an inhospitable environment for marine borers such as the *Teredo navalis*, and the low temperature that slows down natural deterioration processes.[4,49]

However, another quite important circumstance was that the *Vasa's* wreck site became heavily polluted.[50] The increasing amounts of organic waste

dumped into the waters of the Stockholm harbour had a most decisive effect on her fate.[II] The oxidation processes of that organic matter created sometimes almost anoxic conditions. The low oxygen concentration is inhospitable to wood-degrading microbes.[7] On the other hand it favours the SRB using sulfate ions; SO_4^{2} in the seawater as electron acceptor when metabolising the organic compounds (Chapter 1.6.2). The sulfate concentration is about 0.3 g/litre in the Baltic Sea. The end product of the SRB activity was hydrogen sulfide; H_2S in high concentrations in the water and in the wood structure.[II,V] Thus, the low oxygen levels saved the huge exposed hull from being attacked by many natural wood degrading organisms, but reactions of the dissolved hydrogen sulfide initiated the contaminations addressed in this thesis.[II] However, even in nearly anaerobic environments EB can slowly degrade waterlogged wood (Chapter 1.6.1). In the *Vasa's* timbers EB have affected mainly the outermost layer (down to 5-15 mm)[51] while the inner parts were spared from serious bacterial influence, and are in a quite well preserved condition in that respect.

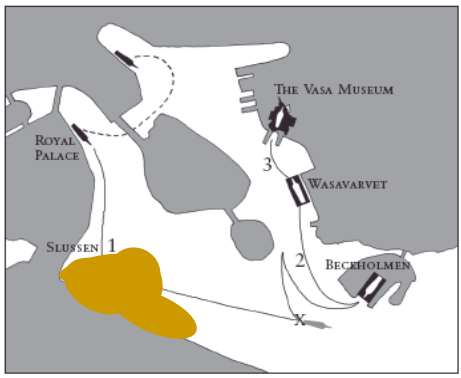


Figure (modified): SMM

Figure 10. *A map of the Stockholm harbour. The measuring site "Slussen" is located in the* Mälaren *west of the* Slussen*, which is a lock between sweet and brackish water (Site 1). The measuring place "Saltsjökvarn" is east of the* Vasa's *wreck site X, and is representative of the water of the* Saltsjön*. The yellow area demonstrates the water surface covered with elemental sulfur in December 1912.*

2.2.1 The preserving wreck site environment

The waters of Stockholm harbour have a long history of pollution. The growing city lacked a waste water system until the construction of sewers started late in the 19th century (see below). In attempts to keep the streets cleaner the surrounding waters became recipient of the waste of the growing city. The fishing used to be rich but organised fishing eventually ceased in the early 19th century.[50] In 1834 and 1835 the polluted water was suspected to be the cause of severe cholera epidemics.[52]

On a December morning in 1912 a dreadful stench of hydrogen sulfide was spreading from the Stockholm harbour, where dead fish were floating (close to *Slussen*; Figure 10).[53] The surface water close to the Vasa wreck site had turned yellow from floating elemental sulfur particles, and remarkable high concentrations of hydrogen sulfide; 0.27-2.05 mg H_2S/L were recorded.

> "Svavelfärgningen sträckte sig mitt i farleden ungefär så långt som mitt för Saltsjöbanans station [below Katarinavägen 19 (author's notes)[54]] Närmare södra landet sträckte den sig längre eller ungefär mitt för Schartaus handelsinstituts gamla byggnader [Stigbergsgatan 26 since 1865 (author's notes)]. Längre öster ut ägde vattnet normal färg".[53]

The unusual phenomenon, which also had been noticed in December 1906, was a joint effect of the seasonal recirculation of the water body when the surface density increases due to the cooling, and the stagnation in the surrounding sources (the *Mälaren* & the *Saltsjön*), which lead to an increased content of the sewer water. Winds from the west swept the surface water to the east and pushed the H_2S rich water at the seabed up to the surface where elemental sulfur precipitated.[53]

Thus, the *Vasa* was submerged in an increasingly polluted environment. The bacterial breakdown of the organic matter consumed most of the dissolved oxygen and infested the water with poisonous hydrogen sulfide, of which a lethal dose can be as low as 6 mg per kg body weight.[55] In 1943 concentrations of 4-8 mg H_2S/L in the entire water body were reported from the local sewage treatment work; Henriksdalsverket, where mechanical cleaning of the waste water had started in 1941.[18] Environmental legislation was non-existent and outlets from the surrounding industries continued until the 1950s. Chemical and biological purification were introduced in 1970, after which the water quality improved substantially.[50]

2.3 The source of the sulfur

In order to obtain a more comprehensive picture of the former conditions of the water in the Stockholm harbour some digging into the archives of the local water purification plant; *Stockholm Vatten,* became necessary. Results from analyses of the hydrogen sulfide concentration at various depths in the water, at locations close to the *Vasa's* wreck site (Figure 10), have been reported on a regular basis. However, coherent data were not available before the introduction of the sewage treatment works in the 1940s. The reported values, which display seasonal variations, show that on an annual basis the H_2S concentration decreased steadily from a high level after the water cleaning started of the sewage water. Also before that, the reported amounts of the organic waste drainage from the early 20th century indicate the potential of the H_2S to reach high levels (Figure 11).

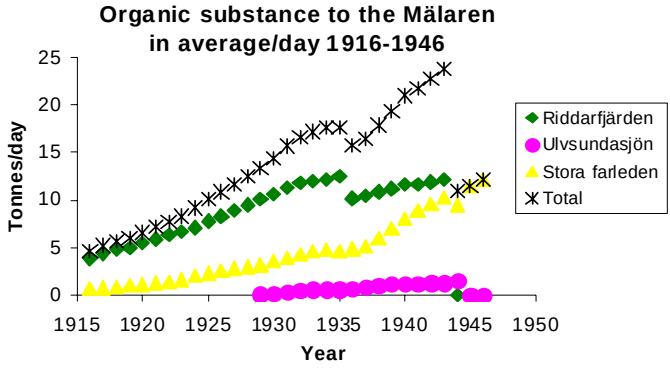


Figure 11. *Organic waste deposited into the lake* Mälaren *(west and upstream of the Vasa wreck site) from the sewage systems Riddarfjärden, Ulvsundasjön, and Stora farleden directly into the Stockholm harbour. The stepwise reductions in the amounts coincide with the relocation of the waste outlet in 1935, and the introduction of mechanical cleaning in 1943 in the Henriksdalsverket.*

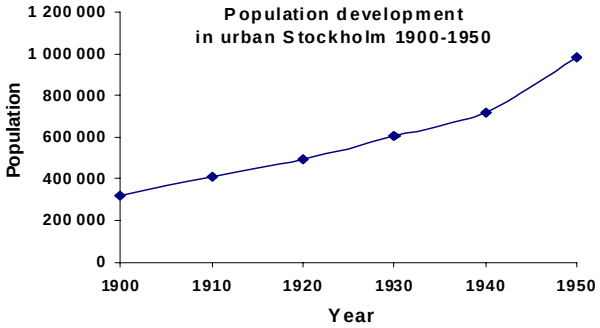


Figure 12. *The population development of urban Stockholm (StorStockholm) resembles the trend from the total amount of organic waste to the* Mälaren *for the same period (Figure 11).*

2.3.1 Drainage of organic waste to eastern *Mälaren*

The diagram in Figure 11 displays the daily amount of organic substances deposited into the lake *Mälaren*, based on the organisation of the drainage of the wastewater in urban Stockholm. Before 1935, 25% of the sewage from (central) Stockholm went to the *Riddarfjärden*, but in 1935 the amount decreased to 20%, until 1943 when the sewers were connected to the Henriksdal waste treatment works. The steps in the diagram correspond to those changes. Before 1929 lavatories were prohibited in Sundbyberg, northwest of urban Stockholm, and between 1929 and 1944 the wastewater from Sundbyberg was deposited in the *Ulvsundasjön*. After 1944 the sewers were connected to waste water treatment works in Åkeshov (northwest). Additional contaminating sources to the *Ulvsundasjön* were laundries and uncontrolled

outlets of about 100 people (not included in the diagram). The *Stora Farleden* was recipient of waste from Brännkyrka, Bromma, Lilla and Stora Essingen, and after 1944 also Sundbyberg, suburbs of Stockholm.[56]

The total estimated amounts above are based on the population, with an estimated percentage of the number of lavatories. For water, which has not passed a sewage treatment work the approximate amount of organic substance is 105 gram per person and day. For waste water, which had passed through the sewage treatment work in Åkeshov, a non-separated amount of 85-100 gram of organic substance per person and day was estimated from 1935 to 1944, depending on the degree of overload. After 1944 the amount was about 70 gram per person and day.[56] The distribution of organic substances to the *Mälaren* correlates well with the population development in urban Stockholm during the same period (Figure 12).[57,58]

2.3.2 Dissolved H_2S and O_2 fluctuations at the wreck site

After the waste water cleaning had commenced in *Henriksdalsverket*, the overall level of dissolved hydrogen sulfide ($HS^- + H_2S(aq)$) level decreased with time (Figure 13). The annual fluctuations of dissolved O_2 and H_2S are a natural result of the seasonal and biological cycles in the water. The O_2-levels fluctuate at all depths in the water body, but the levels are always lowest close to the seabed, as expected from the activity of the photosynthesis that needs sunlight. Generally, the O_2-level increases during spring and summer with max values up to 12 mg/L close to the surface, while at the seabed the levels may decline to 0-2 mg/L during the fall, when decomposing organic material depletes the oxygen. In contrast, dissolved H_2S often dominates in deeper water during late fall with maximum levels up to 7-14 mg/L. During the winter stagnation the oxygen levels usually decrease considerably due to the degradation processes. Oxygen levels below 4-5 mg/L harm most oxygen-dependent organisms. With decreasing amount of oxygen, prokaryotes can use other electron acceptors in their metabolism (in the order as follow):[59,60]

1. $NO_2^-/NO_3^- \rightarrow N_2O/N_2$
2. $Mn^{4+} \rightarrow Mn^{2+}$
3. $Fe^{3+} \rightarrow Fe^{2+}$ (critical limit of survival for many organisms)
4. $SO_4^{2-} \rightarrow S^{2-}$
5. $CO_2 \rightarrow CH_4$

Figure 13 gives an indication about the H_2S and O_2 fluctuations in the water around the *Vasa*, both west and east of the wreck site (Figure 10). The *Stockholm Vatten* data have been obtained from two sites where the waters of different sources, were separated by a lock. The water west of the wreck site, measured at “Slussen”, is at the outlet of the lake *Mälaren*, while the

brackish water to the east at “Saltsjön”, which is connected to the Baltic Sea, is more representative of the wreck site (Figure 13).

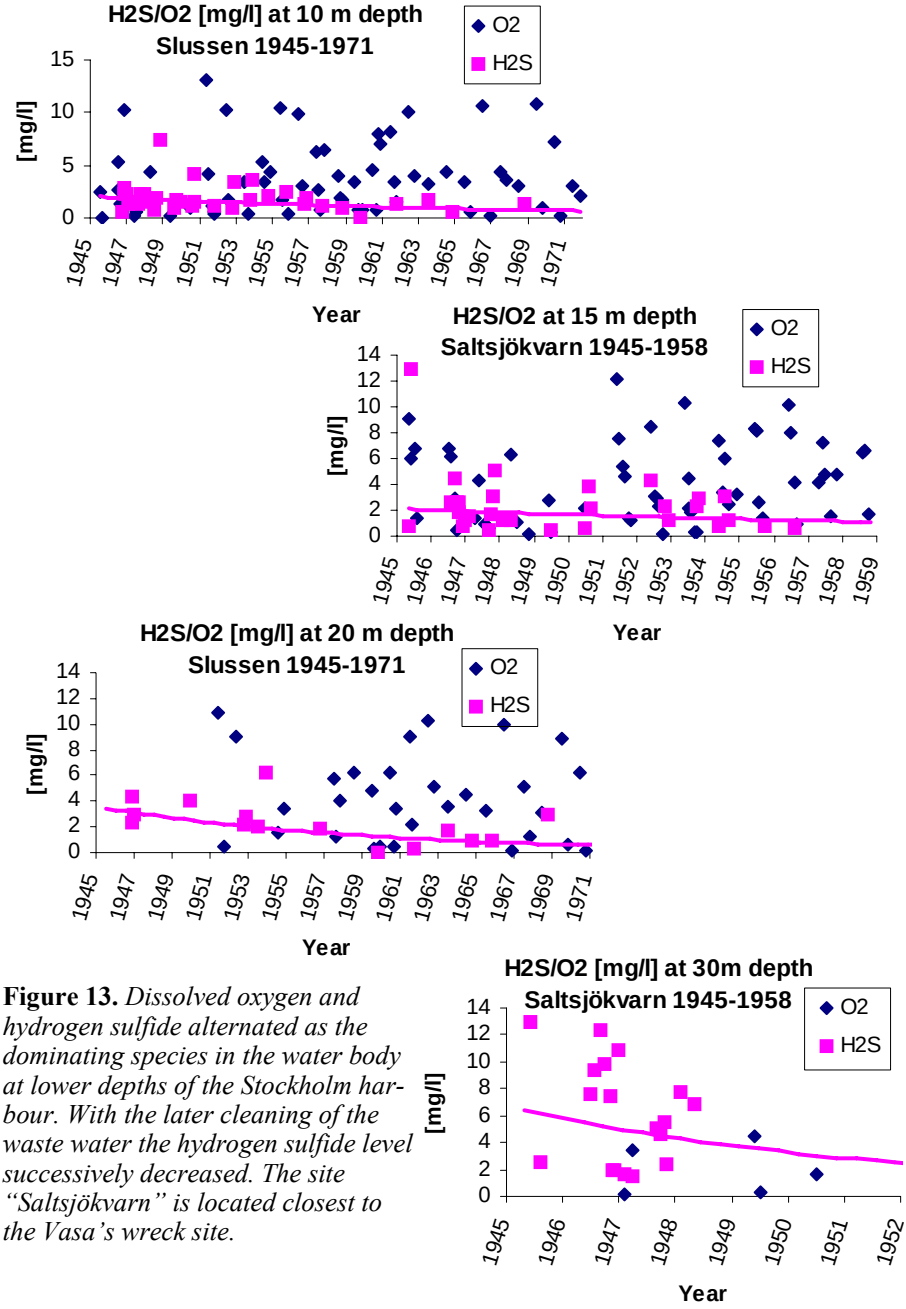


Figure 13. *Dissolved oxygen and hydrogen sulfide alternated as the dominating species in the water body at lower depths of the Stockholm harbour. With the later cleaning of the waste water the hydrogen sulfide level successively decreased. The site “Saltsjökvarn” is located closest to the Vasa’s wreck site.*

2.4 The mechanism of sulfur accumulation

The results of the water analyses from locations in the Stockholm harbour (*Strömmen* and *Saltsjön*) of interest for the *Vasa's* wreck site indicate that during the 1940-1970s dissolved O_2 and H_2S have alternately been dominating the lower water body (Figure 13). Evidently, the oxygen levels were occasionally high enough, especially at an early stage, to allow corrosion of the iron bolts and nails of mild steel in the hull. The penetration of iron(II) ions probably also allowed substantial amounts of iron sulfides to form in the wood.[IV,V] Possibly, these O_2/H_2S variations contributed to the accumulation of other forms of reduced sulfur in the waterlogged wood.[VII] The hydrogen sulfide dissolved in the penetrating water, or produced *in situ* of the wood by SRB, reacted with lignin components (Chapter 1.5.4.4) in the wood structure forming solid organosulfur compounds (Chapter 3.7). To verify the bacterial source behind the sulfur accumulation, and to achieve more information about the processes on the seabed, laboratory tests were carried out in simulated seabed environments (see below).[VII]

2.4.1 Reproducing the wreck site conditions

In order to follow how the bacterially reduced sulfur accumulates, series of experiments were conducted with wood in simulated seabed environments. Fresh pine wood blocks were submerged in media containing sulfate and iron(II) and inoculated with bacteria isolated from seawater. The aim was to simulate the conditions of the *Vasa's* wreck site. The experiments were conducted for over two years both in anaerobic and partly aerobic environments, before the wood was analysed and compared with authentic marine archaeological wood. Also fresh wood treated in a similar way with bacteria isolated from shipwreck samples in water were included in the study. The many different reduced sulfur compounds existing in the *Vasa's* and *Mary Rose's* wood could be identified also in the test wood of the experiment by synchrotron based sulfur spectroscopy (Chapters 3.2.3 & 3.7.3).[VII] The results of the successful experiments strongly supported the hypothesis that consortia of EB and scavenging SRB, from the inoculates, produced reduced sulfur compounds *in situ* in marine archaeological wood. Also, light microscopy images supported that following cellulose degradation by EB (Figure 9), scavenging bacteria, most likely including SRB, produced hydrogen sulfide within the wood (Chapter 3.7.3).[VII] A recent sulfur isotope study of a Vasa oak sample confirms that the accumulated sulfur has been reduced by bacteria (Chapter 2.4.2).[VII]

Earlier studies demonstrate cases where the EB disappear before the scavenging bacteria enter.[44] In the current case the SRB activity in the treated pine wood seems dependent on the EB, even though the degree of cooperation is unclear. Results for the partly aerobic series of samples indicate that

aerobic/anaerobic variations could stimulate the sulfur accumulation. The S2-layer that constitutes the major part of the cell wall,[61] is with its high cellulose content (Chapter 1.5.5) the primary target of the EB, which probably need some oxygen.[34] An EB degraded cell wall facilitates for the hydrogen sulfide from the SRB to reach and react with the lignin. However, increasing the sulfate concentration in the medium over a certain level had little effect on the sulfur accumulation (the SRB activity) in the wood.[VII]

2.4.2 Sulfur isotope study

The sulfur isotopic composition was analysed for different fractions of sulfur in an untreated (without PEG) Vasa sample. The result was consistent with the reactions deduced from the XANES analyses of the sulfur compounds in the wood.[VII] The ^{34}S content had decreased considerably in all fractions from the value $\delta^{34}S = 21‰$ for marine sulfate.[62] The result strongly suggests that the original marine sulfate had been largely transformed or washed out of the sample. The presently remaining sulfur originates from bacterially transformed sulfate in a reduction step.[63,64,VII]

2.5 Conservation of the *Vasa*

After the salvage in 1961 the *Vasa's* hull was kept wet by spraying with tap water. This is a necessary measure for waterlogged wood to prevent the degraded wood cells from collapse when drying, and thus the wooden object from shrinkage and cracking (Chapter 1.3.1). At that time no satisfactory conservation methods were available for such large archaeological wooden objects. The alum treatment previously applied, e.g. to the Norwegian Viking ships had been found unreliable.[65] After careful consideration an almost untested bulking agent was chosen for strengthening the waterlogged wood. The *Vasa* was the first major marine archaeological object for which polyethylene glycol, PEG, in aqueous solution was used.[4,15] Since then, PEG-treatment procedures have continuously been developed and are commonly used, also together with freeze-drying, as standard conservation procedures of most marine archaeological wooden artefacts (Chapter 1.3.3).[66] The museum shipwrecks of the *Bremen Cog*, the *Götavraket*, the *Batavia*, and the Skuldelev Viking ships have all been through PEG conservation treatment in tanks, and the hull timbers of the Mary Rose wreck, is currently being spray-treated in a similar way as the *Vasa* was.[I-IV]

The PEG molecules replace water in the wood cells, and for the *Vasa* it has been estimated that 580 tonnes of water were removed from the hull.[47] The mean moisture ratio (water weight/dry fibre weight) dropped from about 150%, measured in deck planks after the salvage, to about 12% in 1992.[18] The PEG-treatment of the *Vasa* was performed by spraying aqueous solu-

tions of PEG 4000 (n ≈ 90) and PEG 1500 (n ≈ 34) during the first years, 1962-1971, on the exposed surfaces of the hull, followed by PEG 600 solution after 1971. A 7:3 mixture of boric acid ($B(OH)_3$) and borax ($Na_2B_4O_7 \cdot 10H_2O$), 1-4%, was added to the PEG-solutions as fungicide. The PEG concentration was increased gradually, from an initial 10% to the final 45% before the spray treatment ceased in 1979. For 13 years, from 1965 to 1979, a closed-circuit automatic spray system was operating and during that time 240 tonnes of PEG was consumed. As a final surface treatment, 45% PEG 4000 solution was hand sprayed on the outside hull and the upper deck. The surplus PEG on the outside surface of the hull was melted away with a hot-air blower.[18] The distribution and penetration of the PEG through the wood was monitored by core sampling, in order to estimate the time span needed for the treatment. PEG analyses can be performed by weight measuring, but more advanced techniques include analysing with extractives (HPLC) and microscopy and in-situ determinations (print off techniques).[8] Also extraction methods combined with NMR spectroscopy have been used within the "Preserve the *Vasa*"-project.[51]

The *Vasa's* lower decks were only treated with hygroscopic PEG 600 and 1500. The wood on these decks has a damp and sticky feeling, and probably has relatively high water content. This could be a reason for the large number of salt precipitates in the hold (Chapter 2.6). In concentrated PEG solutions with high molecular mass the solubility of oxygen is low.[67] The PEG 4000 with high molecular weight, used on the outside hull and upper decks, might reduce the oxygen access to the wood, but not the ion-conductivity. PEG-treatments are time-consuming, and the long-term effects are not fully established, especially regarding oxidation processes in the presence of iron ions.[3,17] The complex chemical interaction between the PEG, wood, acid and iron is a subject that needs further investigation. However, PEG treatments are one of the most tested and remain as the primary choice for large waterlogged objects of marine archaeological wood.

2.6 Sulfuric acid and sulfate salts

During the rainy summer of 2000, the former climate system in the Vasa Museum could not prevent large variations in the relative humidity, which on several occasions reached level higher than 65%. During the fall, a large increase was reported in the number of acidic yellowish and white salt precipitates, both inside the ship's hull and on stored artefacts (Figure 14). X-ray powder diffraction analyses revealed these outbreaks to be sulfate salts.[1,2] The sampling of the sulfate salts showed that one of the most common crystalline precipitates inside the *Vasa* is the yellow-coloured natrojarosite; $NaFe_3(SO_4)_2(OH)_6$. Other commonly occurring iron sulfate salts are the white gypsum; $CaSO_4 \cdot 2H_2O$ and the bluish-white crystals of melanterite;

$FeSO_4 \cdot 7H_2O$. Also small crystals of elemental α-sulfur (with ring-shaped S_8 molecules) were found, mostly in the hold.[1,2]

Figure 14. *Sulfate and iron salt formation on the Vasa ship and on stored objects: (top, left) a piece of worked wood (sv. formstycke); No. 25624. (top, right) sculpture of a knight of the upper deck; 7896/ÖD3. (middle, left) knee on upper gundeck; ÖB5.2a. (middle, right) chest in the magazine, and (bottom, left) beam below the* Vasa's *galley in the hold.*

It was soon deduced that the high acidity and the sulfate precipitates on the Vasa wood were products of sulfuric acid, produced by oxidation of reduced sulfur compounds accumulated in the wood (Chapter 3.2).[1,2,I-III] However, salt precipitates were not a new phenomenon for the *Vasa*, even if the increase in RH during the summer of 2000 probably accelerated the problems. Already in 1963, Lars Barkman, the first chief conservator, sent some surface samples for x-ray powder diffraction (XRD) analysis. The salts, found on the gallion lion figure head and a sculpture, turned out to be the iron sulfates rozenite, $FeSO_4 \cdot 4H_2O$, and melanterite, $FeSO_4 \cdot 7H_2O$. The salt crusts could easily be removed by simple brushing and were not considered to be harmful. At that time the present advanced techniques for analysing sulfur species within wood were not available and further investigations were not performed.

Similar problems with high acidity and salt precipitates have also been reported from other shipwrecks with high iron content, e.g. the *Batavia* in the Western Australian Maritime Museum, where oxidation of pyrite; FeS_2, and other iron sulfides were found to be causing acidity problems.[68,69] After the reported outbreak of sulfate salts in 2001, the *Vasa* has been examined visually several times, and new acidic areas with salt precipitates were found regularly. However, the distinct wax-like white spots and stripes that can be seen all over the ship is excess of PEG from the conservation treatments, and the precipitated sulfate salts can sometimes be difficult to distinguish from other natural shades of the wood. The normal pH-value measured on a PEG treated Vasa wood surface without salt precipitates is around pH 4-5 (with pH-indicator paper), and pH-values below that (pH = 3.5 or lower) is considered as indicative of excess acid. The precipitates usually have pH ~ 3, but occasionally as low as pH 1 or 0. They can be found on all kinds of wood surfaces and can differ in size, shape and texture, with colour shades in yellow, white, bluish-white and red-brown. The yellow natrojarosite is considered to be the dominating sulfate salt on the timbers within the hull.[II]

The sulfate precipitates are often associated with heavily iron contaminated areas and where iron details as hinges, iron bolts, etc. formerly were present (e.g. the chest in Figure 14). The precipitates are often characteristic for the location. Inside the ship; e.g. in the hold, the orlop deck, the gundecks, the cabin floor and the poop deck the precipitates often display the yellowish colour typical of natrojarosite; these usually are the most acidic ones. Gypsum is a common precipitate on the outside of the hull, but also to some extent on the gundecks, upper decks and in the galleries. It often appears as a thin, dust-like white cover sometimes together with melanterite salts, in all cases (so far) on oak. The precipitates are more frequent on the starboard outside especially close to the bow, and often appear close to the oakum filled spaces between the planks.[II] Another special feature on the outside of the hull is some salt precipitates that are not acidic, at least not on the outer-

most PEG-layer. However, when surplus PEG is carefully removed a larger precipitate (often yellow) can often be revealed.[70] In such cases the acid underneath the PEG-layer has not penetrated the PEG up to the surface.

The special ion-conducting property of solid PEG (long-chain PEG has been tested as an ion-conducting medium for solid-state batteries),[71] is a probable reason why the salts often precipitate on the surfaces from the evaporating solutions during humidity variations. However, outbursts of expanding sulfate salts found underneath the surface coating of waxy PEG 4000 and upon stored objects, occasionally can be accompanied by detachment of the outer wood layer (Chapter 5.4.1).[V]

2.6.1 Statistics of sulfate salt outbreaks on the *Vasa*

As a complement to the visual mapping of the acidic salt precipitates on the *Vasa*, some statistics from a database (Market Store) at the Vasa Museum will be presented (Figure 15). The number of registered salt outbreaks with pH-values 3.5 or lower (measured with pH-indicator paper) is presently 3131 (April 2008), of which 2100 on the ship and the rest on loose objects in magazine storage. So far, most of the registered precipitates in the database (75%) show surface pH-values at 3-3.5 while 25% are lower (pH < 3). The acidic precipitates occur more frequently on the pine surfaces than the dominating wood species oak. New precipitates can be expected to be found on board, and the registration continues.

The results give some support to earlier observations, which indicated that the salt precipitates were more frequent on the starboard side; 54%, than the port side; 40% (centerline 6%), especially on the outside hull (the largest of the compared surface areas), where the number of precipitates is relatively large, but also on the upper gundeck and in the hold (Figure 15). This would be consistent with the ship's position on the seabed, with the starboard side facing the water fairway and thus more exposed to the circulating water. The port side was facing land (the Beckholmen island) and obtained a thicker cover of protecting mud and clay with time.[48]

The total number of precipitates, also those of high acidity (pH < 3) seems to be most frequent lower down inside the ship, i.e. in the hold. Further up in the ship the number decreases for every deck, with exception for the upper gundeck, which also includes the cabin and the steerage. One reason for the many acidic precipitates in the hold, even if the lower decks are more difficult to search, may be the more hygroscopic low molecular mass PEG (see above).

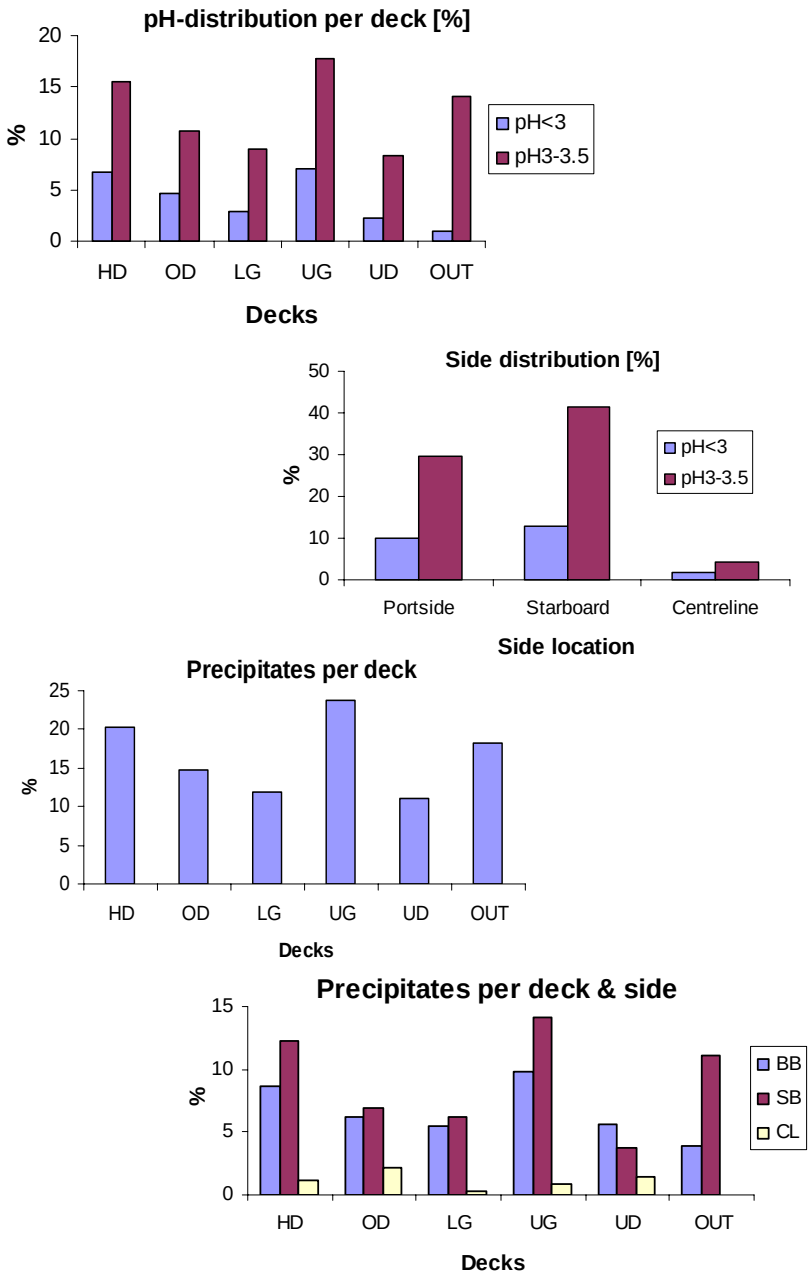


Figure 15. *Statistics on the* Vasa's *acidic sulfate salt outbreaks in and outside the ship, from the Vasa Museum database Market Store. Loose objects from the magazines not included in the diagrams. HD=hold, OD=orlop deck, GD=gundecks (LG+UG), LG=lower gundeck, UG=upper gundeck (the cabin and the steerage included), UD=upper deck (the head, the galleries, the poop deck, the top gallant poop and the coach included), OUT=outside hull.*

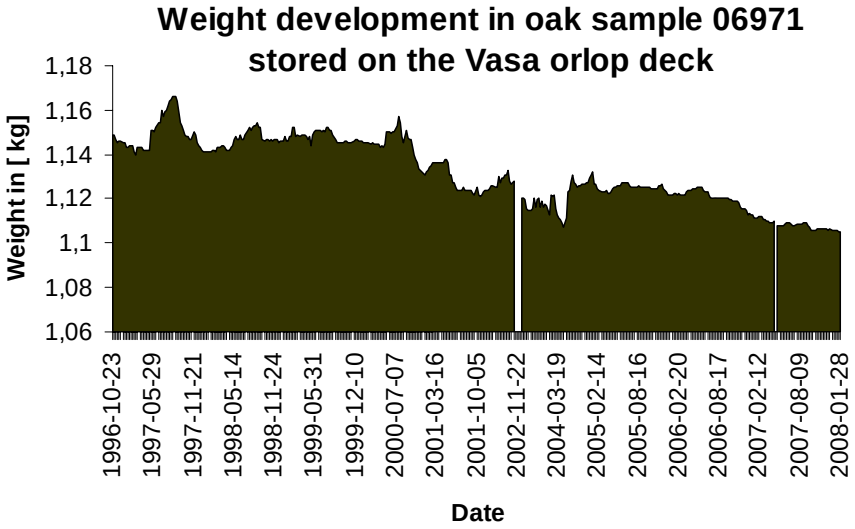


Figure 16. *Weight development of a piece of PEG-treated oak frame (No. 06971), monitored between October 1996 and January 2008. Peaks in the weight curve reflect increased relative humidity (RH) in the museum indoor climate, which causes water uptake in the treated wood. Note the high humidity peaks of 1997 and 2000. The break during November 2002 appeared when the ship was scanned for 3-dimensional imaging and the pieces were moved. During the break in May 2007 the scale was calibrated.*

2.7 Humidity effects on wood

The connection of salt formation to the seasonal variations in relative humidity (RH) in the ship hall strongly suggests that the water (and oxygen) transport in the moist wood plays an important role in the oxidation processes and the development of sulfuric acid.[III] The RH, which is directly correlated to the water activity in the moist wood at saturation, should therefore be kept at a stable level, and not exceed 55%. Previously, substantial RH-variations of 4-5% have been reported in the Vasa Museum, e.g. in 1993-1996.[72] The weight variations in a piece of oak wood on the *Vasa's* orlop deck monitor the absorption of water, which is enhanced by the hygroscopic PEG (Figure 16).[70] Some seasonal variations can be discerned with higher weight during summer periods, even though a long-term decrease has taken place since 1996. A new climate system was installed in the Vasa Museum in 2004, and between May 2004 and January 2005 the weight of the oak piece increased 2%, but has stabilised since then.

A test was performed by storing pieces of an acidic Vasa pine plank for four years in desiccators at different constant relative humidities (from 44 to 74%) maintained by saturated salt solutions. The relative moisture content of

the wood pieces was compared with a wood moisture conductivity meter, and was found to range from about 6 to 50% (no independent calibration was performed). The study indicated significantly higher moisture values and softer wood in the salt affected side of the pieces, with increasing relative humidity in the desiccators.[VI,73]

2.7.1 Changes in sorption behaviour

Sorption is influenced by such wood properties as its water affinity and the size of the internal surface. The hemicelluloses are with their hydrophilic side groups considered to have the highest sorption capacity followed by cellulose and lignin (Chapter 1.5.4). In wood fibres the cellulose contribution is estimated to be about 47% of the total sorption of wood (hemicelluloses 37%, lignin 16%). Drying and rewetting the same fibres in several cycles cause degradation of the cell wall structure, as well as extraction of hemicelluloses and extractives, and will shift the equilibrium attained at 100% RH. This can be demonstrated with sorption curves in a plot of moisture content vs. relative vapour pressure, where the difference between the curves is referred to as sorption hysteresis of wood.[12,13] Drying and rewetting of wood increase both the rate and the extent of swelling, with higher water access to the cell wall with each cycle.[13] Different explanations for the hysteresis phenomenon are presented in the literature, and some modification of the wood structure components seems to occur. Studies of the exothermic water adsorption indicate breaking of hydrogen bonding.[12]

2.8 Sulfur in other shipwrecks

Alas, sulfur accumulation is not restricted to the *Vasa*, even though the *Vasa's* sulfur concentrations reach the highest values found so far (Chapter 3.4 & 3.5). From analyses of core samples from a number of shipwrecks the conclusion can be drawn that the sulfur accumulation problem is omnipresent for waterlogged marine archaeological wood, which has been preserved in seawater.[V]

Analyses of a few samples from the Swedish warship *Kronan,* sunk in battle 1676 and presently resting on the seafloor outside Öland in the Baltic Sea, vary from low values up to 4 mass% total sulfur, almost all in reduced form.[74] This indicates future conservation concerns if the *Kronan* would be salvaged. Other shipwrecks still on the seabed, for which samples with significant amount of sulfur have been obtained and analysed, are: the *Stora Sofia* outside Göteborg, *Riksnyckeln* (at Viksten outside Järflotta), *Tattran* (Nynäshamn), Sweden,[74] the *James Matthews* outside Fremantle, Western Australia, and the *Pandora* on the outer Great Barrier Reef off Australia's north-east coast, and *USS Monitor*, John Ericsson pioneering iron-clad warship sunk outside Cape Hatteras in 1862.[75] In museums, the hull timbers of

the Viking ships of Skuldelev, Denmark,[74] the *Batavia* in the Western Australian Maritime Museum[75], and the *Mary Rose* salvaged in 1982 and now under conservation in Portsmouth, U.K. (Chapter 3.2.2), also show accumulation of reduced sulfur compounds.[II,V] So far, the only exception is the *Bremen Cog* from 1380, recently PEG treated and on display in Bremerhaven, Germany. The *Bremen Cog* was discovered in 1962 during dredging operations of the river Weser downstream Bremerhaven, and is the best-preserved example of the cog, which was the dominating type of merchant ship during the time of the Hanseatic League.[16] The highest sulfur concentration obtained for core samples of the *Bremen Cog* is less than 0.2% on the surface and even lower inside. The sulfur speciation, as displayed by the XANES technique, is similar as for the other ships, even though the total sulfur amount is much lower,[IV,V] probably because the wreck was preserved in river water with low sulfate concentration.[III, IV]

3 MAPPING THE SULFUR

3.1 Core sampling for analysis

Core samples were collected from the Vasa ship on several occasions. The cores, diameter 4.2 mm and length 50 to 160 mm, were sampled manually with an increment bore by conservator Bo Lundvall and Ove Olsen. After sampling the cores were kept in sealed nitrogen-filled glass tubes. The sampling was distributed over both visibly and non-visibly salt affected areas for a representative mapping of the amount and distribution of sulfur with as few core samples as possible (Figure 17).[II] Detailed analyses will here be reported for some of the cores (Figures 18-22). A brief summary of the total sulfur amount of the cores is provided in Figure 30.

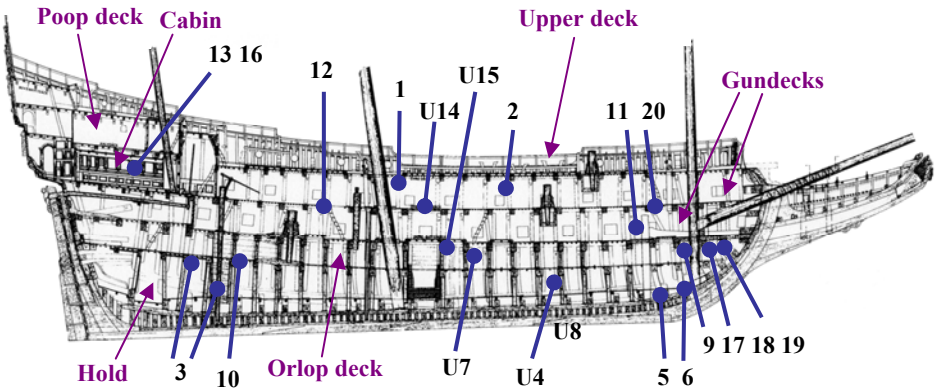


Figure 17. *Cross-section of the* Vasa's *hull from the starboard side, with the positions of the collected core samples 1-20 (U for outside hull).*

3.2 Sulfur speciation by XANES-analyses

Sulfur has been considered a spectroscopically "silent element" before the advent of synchrotron-based sulfur absorption spectroscopy.[76] With ordinary chemical techniques the many different species occurring in natural samples are difficult to separate and analyse, but the absorption features of the bright x-ray radiation from synchrotrons allow characteristic sulfur groups to be identified. For that purpose we used the dedicated beamline 6-2 at Stanford Synchrotron Radiation Laboratory (SSRL) in California, where we explored the sulfur K-edge x-ray absorption near-edge structure (XANES) to characterise sulfur species in wooden samples at atmospheric helium pressure.

The sulfur XANES spectra of core samples from the *Vasa* at different depths from the surface and inwards, generally display two major peaks at 2473 and

2483 eV (Figure 18), corresponding to reduced and oxidised sulfur species, respectively. The peaks originate from absorption of x-rays exciting the most tightly bound (1s) electrons of the sulfur atoms in the different compounds present in the wood.[76] Later we could employ beamline ID21 at the ESRF, which is equipped with a scanning x-ray spectro-microscope. Its energy resolution of 0.5 eV and spatial resolution of 0.5 μm enabled detailed studies of the distribution of the reduced sulfur compounds in the cell components of the wood structure (Chapter 3.7).[IV,V,VII]

3.2.1 Sulfur speciation in Vasa wood

The sulfur 1s electrons become excited to unoccupied molecular orbitals with p character, and the energy (the position in the spectrum) of the transitions is sensitive to the chemical oxidation state of the sulfur atom. The shape of the composite peaks depends on the intensity and energy of their inherent overlapping transition features. Deconvolution of the absorption features allow different types of sulfur compounds in the wood to be identified by fitting with standard spectra from known compounds with sulfur groups in a similar chemical surrounding as in the samples. For the speciation it is helpful to use Principal Component Analysis to determine the number of components in the overlapping peaks.[76,V]

The relative amount of the reduced sulfur species contributing to the 2473 peak, containing thiol (-SH) groups, disulfides; R-S-S-R, elemental sulfur and also pyrite; FeS_2 could be obtained in this way (Figures 18-25).[II,III] Occasionally a peak appears at 2470-2471 eV, which corresponds to sulfur in contact with iron(III), as in pyrrhotite; $Fe_{1-x}S$.[VII] A minor peak at 2476 eV occurs in most samples and corresponds to a few percent of the sulfur in sulfoxide species; $R_2S{=}O$.[I] The peak at 2482-3 eV originates from sulfate ions, often with a shoulder on the low energy side from sulfonates; RSO_3^-.[1,2,I,II] The sulfonate ions, with the functional group $-SO_3^-$, are conjugate bases of sulfonic acids with formula RSO_2OH.

Analyses of samples at different depths along cores from the *Vasa*, indicate that oxidised sulfate (oxidation number +VI) occurs primarily in the surface layer, but also deep inside the wood where the total sulfur content is low, approaching the natural level in fresh wood. The amounts of reduced sulfur species are substantial in the outer layers of the cores. Note that the sulfate peak is about three times larger than for the same concentration of reduced sulfur (Figure 18 *right*). The XANES spectra are normalised, which means that the total sulfur concentration must be determined at each depth by elemental or XRF analysis.

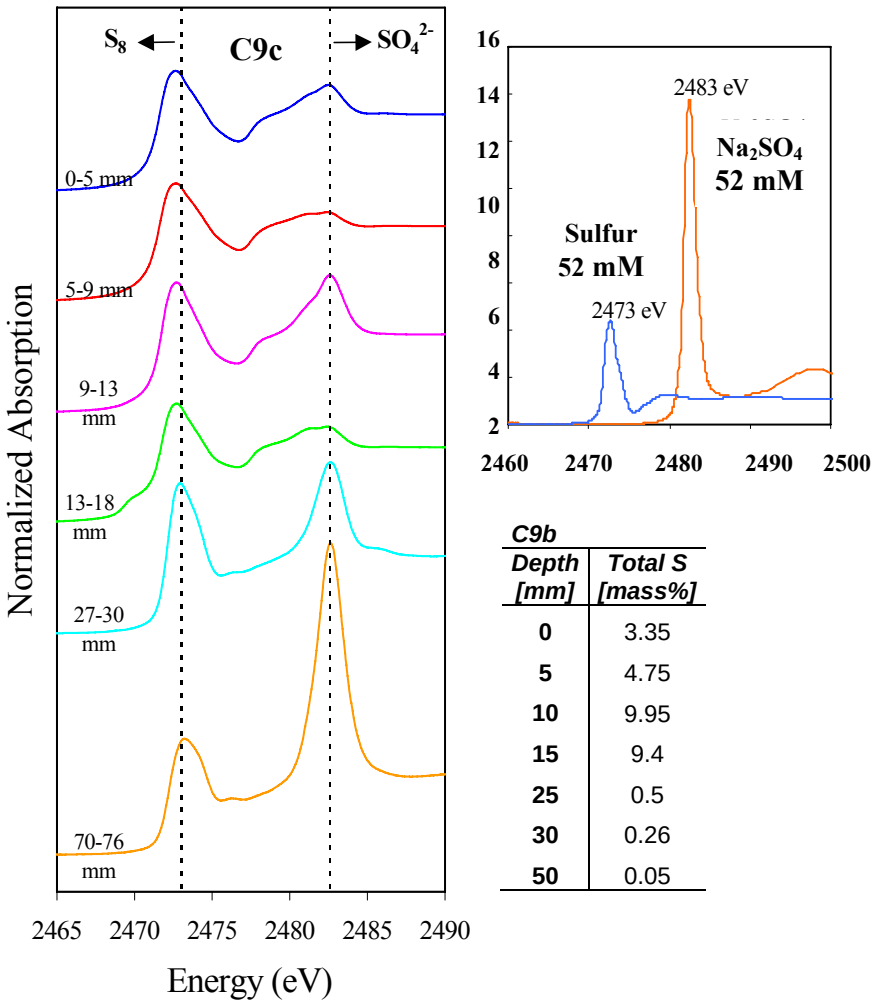
Included below are all XANES spectra analysed of cores sampled from the *Vasa* ship (Figure 17), and from sediments and wood from the wreck site;

the Vasa pit (Figures 18-23). The XANES profiles differ somewhat for the cores but all indicate both reduced and oxidised sulfur from the surface down into the wood.

The occurrence of both thiols and iron sulfides, and their respective oxidation products in the spectra shows two pathways of sulfur accumulation and oxidation (Chapter 3.2.4). There are two main groups or reservoirs of reduced sulfur compounds, namely organosulfur and inorganic iron sulfides. The oxidation pathway of the first group starts with the thiols; R-SH, goes through the intermediates disulfides, sulfoxides and occasionally sulfones, and ends as sulfonates; $R\text{-}SO_3^-$. This stepwise oxidation to the end product, which is found especially in the surface layers of the wood, seems to require prolonged exposure to oxygen. The second group starts with the iron sulfides, such as pyrite; FeS_2 or pyrrhotite; $Fe_{1-x}S$, where the byproduct from the decomposition and oxidation is elemental sulfur.[69] Other intermediates in that pathway are not evident in the XANES spectra and the oxidation to sulfates (and sulfuric acid) is relatively rapid.[74]

It is therefore important to distinguish between the relative amounts of the inorganic and organically bound reduced sulfur. One way to discriminate thiols and disulfides from elemental sulfur and pyrite would be further careful analysis of the broad peak at 2472-2473 eV in the XANES spectra, as for the *Mary Rose*.[V] The relative amounts of S:Fe could also give an indication of the original amount of iron sulfides in the wood (Chapter 3.8), especially when there is a clear correlation between the Fe and S concentration profiles, as for some Vasa cores (Chapter 3.5). In such a case it is probably in the form of pyrite and/or iron sulfides that most of the iron has accumulated the wood. However, by XRD only traces of pyrite have been found in the *Vasa's* wood, and just in one XANES spectrum, at the depth 13-18 mm of core 9 (Figure 18) a minor peak at 2471 eV indicates the presence of pyrrhotite, $Fe_{1-x}S$.[II] Based on those indications, it seems that most of the iron sulfides could already have been oxidised to sulfates during and after the conservation procedures of the *Vasa*.

However, a considerable amount of acid, estimated as equivalent to two tonnes of H_2SO_4 (Chapter 5.4), would then be present in the wood, together with appreciable amounts of iron ions. There are recent indications that the iron ions in the interior of the wood may continue to catalyse oxidation processes primarily of the PEG molecules to organic acids, e.g. formic acid.[17]



C9b

Depth [mm]	*Total S [mass%]*
0	3.35
5	4.75
10	9.95
15	9.4
25	0.5
30	0.26
50	0.05

Figure 18. *Sulfur K-edge XANES spectra for Vasa core C9c (oak bilge stringer, orlop deck, bow) at increasing depth from the surface. The peaks originate from different sulfur compounds with the highest total concentration close to the surface. The shoulder at 15 mm depth (2469 eV) corresponds to inorganic sulfide (S^{2-}), probably $Fe_{1-x}S$ (pyrrhotite). The sulfate peak at 2483 eV is about three times larger than the peak at 2473 eV for the same sulfur concentration. The total sulfur analyses come from the nearby sampled core C9b.*

C4c

Depth [mm]	0	10	20	25	30	45	75	105	115
Total S [mass%]	0.11	0	0.5	0.02	0.35	0.02	0.23	0.53	0.31

C6b

Depth [mm]	0	15	30	45	60	70	80
Total S [mass%]	0.32	0.22	0.35	0.33	0.41	0.37	0.36

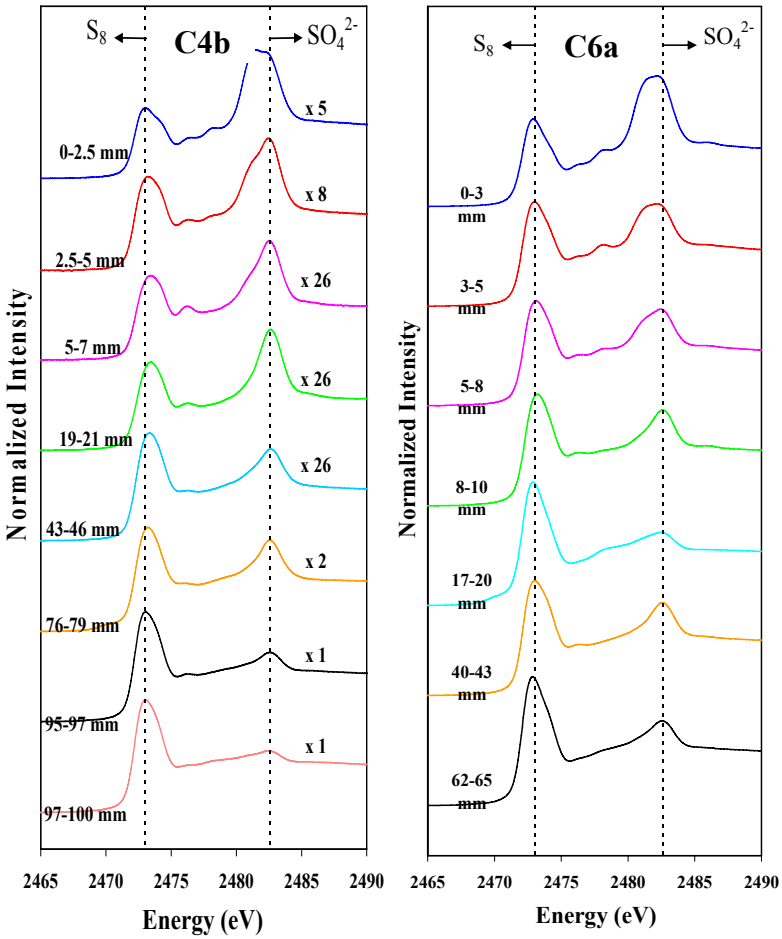


Figure 19. *The XANES spectra for Vasa U4 from the outside planking (oak, starboard, bow) and C6a (oak deck plank, hold, port, bow) are quite similar. Total S analyses were performed for adjacent cores close to the XANES cores. C4b: Disulfides, sulfoxides (2476 eV) and sulfonates (2481 eV) at the surface. The relative amount is high of thiols, disulfides, elemental sulfur and sulfates (2482.5 eV) at the first 2 cm. At the first 10 mm there are indications of oxidation products of the organically bound sulfur. At larger depths the relative amount of sulfate decreases generally. C6a: The surface spectra indicate oxidation of thiols through disulfides, sulfoxides, sulfones (at 2478 eV) to sulfonates.*[74]

C5a

Depth [mm]	0	5	10	15	25	65	125
Total S [mass%]	0.43	0.18	0.21	0.25	0.24	0.02	0.02

C13d

Depth [mm]	0-3	3-4	8-11	49	60	63-68	68	69-72	74	106
Total S [mass%]	2.8	3.8	0.32	0.28	0.3	0.37	0.31	1.57	0.3	0.34

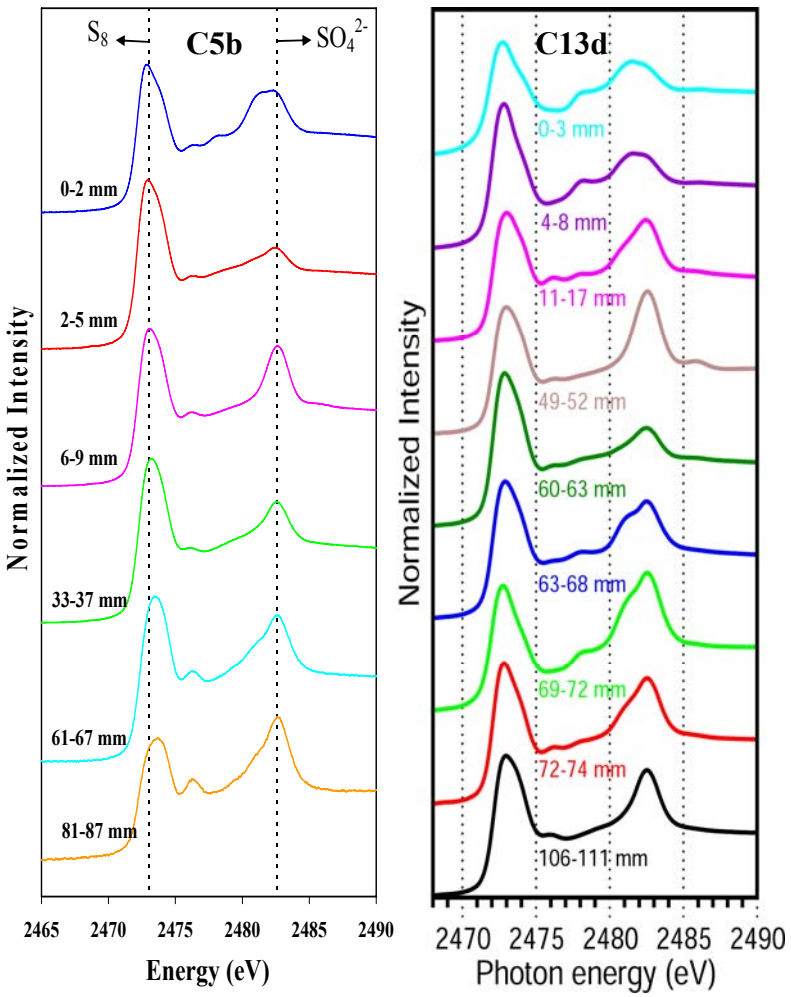


Figure 20. *Vasa sample C5b (oak deck beam, hold, star board, hull). The sulfoxides (2476 eV) at 81-87 mm indicate a crack in the wood; otherwise oxidised sulfur intermediates usually do not show up at that depth. Total sufur analyses of C5a, taken next to XANES sample C5b. C13d (oak ceiling, cabin, upper gundeck, port, stern). Sulfones in the surface layers.*[74] *Core drilled through several layers of the hull and when reaching the new "surface" at the frame (Chapter 3.6.1) just after ~60 mm the amounts of sulfates increases (60-63 and 63-68 mm).*[II]

C17a

Depth [mm]	**2-5**	**11**	**18**	**22-25**	**50**	**70**
Total S [mass%]	0.3	0.15	0.1	0.1	0.1	0.1

C20a

Depth [mm]	**3-7**	**7-18**	**18**	**30**
Total S [mass%]	0.1	0.1	0.1	0.1

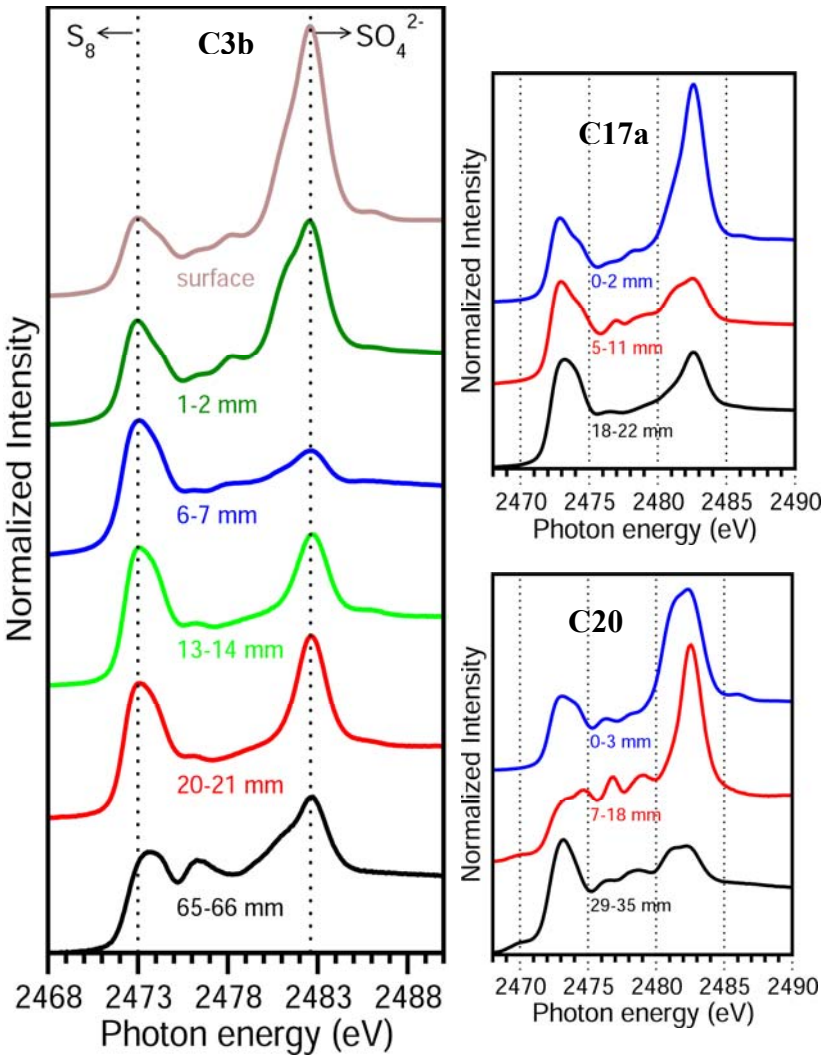


Figure 21. *Vasa sample C3 (rider under oak beam 23, hold, portside, stern) and C17a (oak knee 2, orlop deck, port, bow). Indicate breakdown of iron sulfides and thiols. C20a (oak deck plank between gun port 4 & 6, upper gundeck, port, bow). Plank treated in PEG bath at 60° C. The oxidation has gone further with more oxidation products clearly indicated (sulfonate).*[74]

C1c

Depth [mm]	**0**	**2,5**	**5**	**10**	**15**	**20**	**35**	**45**	**55**	**70**	**85**
Total S [mass%]	5.6	6.0	5.7	2.95	1.92	0.40	0.05	0.05	0.03	0	0.03

C18a

Depth [mm]	**3-4**	**8-9**	**14**	**28**	**30**	**63**
Total S [mass%]	0.34	0.1	<0.1	<0.1	<0.1	<0.1

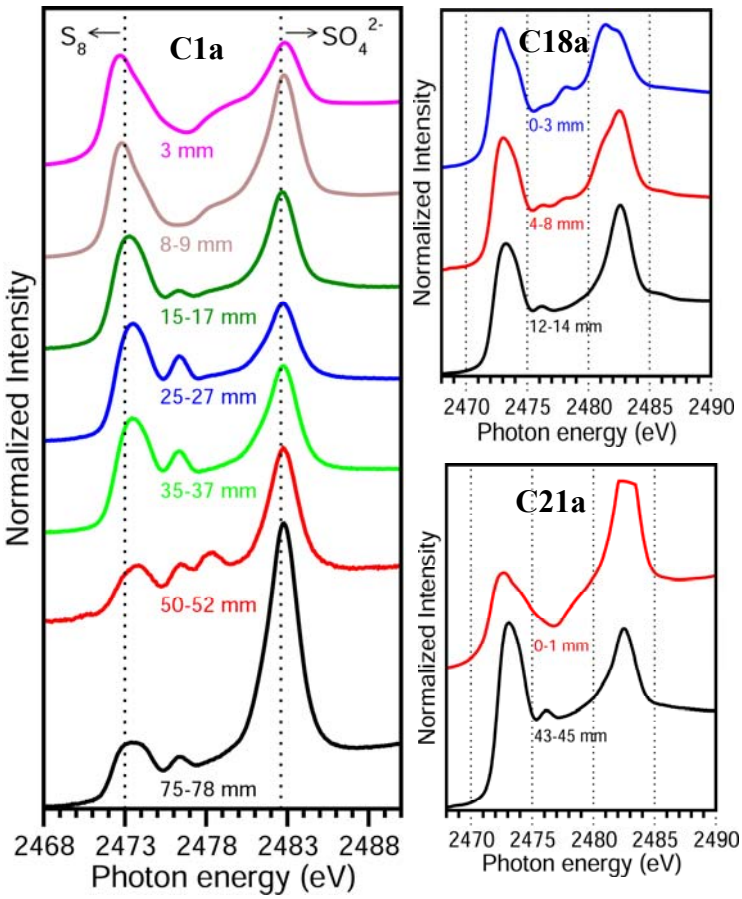


Figure 22. *Vasa sample C1a (oak knee, beam 15, upper gundeck, port, amidships). C18 (oak knee 3, orlop deck, starboard, bow): sulfonate as a sign of thiol oxidation at the surface. Decomposing of sulfides at lower depths. C21a (outside planking, starboard, 25.21).*[74]

In Figure 23 XANES spectra of some samples of different origin and sulfur content have been brought together. So far only qualitative analyses have been performed, based on the peak positions and shapes, with the following tentative conclusions.

The two top spectra (i and h) are from two different sediment samples from the Vasa pit. Spectrum (i) contains organically bound sulfur (possibly in lignin) including thiols and sulfoxides. The second spectrum (h) seems to contain also some elemental sulfur S(s) and sulfates. Samples (f) and (g) were taken from an oak plank found at the Vasa wreck site in 2003, which has been dendrochronologically dated to the 17th century. Sample (f) corresponds to elemental sulfur in the 2473.1 eV peak, while (g) also indicate disulfides and sulfoxides. Sample (g) was taken from the surface and is as expected more oxidised than the inner sample (f) with sulfonates and probably sulfates in the 2482 eV peak. Samples (d) and (e) are from "fresh wood" also found in the sediments at the wreck site. The peak corresponding to oxidised sulfur, S(VI), is larger in sample (e). Otherwise their appearance is rather similar to that of sample (g) with thiols and sulfonate. Sample (b) is taken from the space between the outer planking (hull outside), which used to be filled with oakum and sealed with tar. Its large peak at 2483 eV with a minor feature at 2486 eV indicates that gypsum; $CaSO_4 \cdot ½H_2O$, is the major sulfur compound. The XANES spectrum for the old Vasa core (a), sampled in the 70's and stored in a test tube, indicates thiols but has relatively low concentration of oxidised sulfur.

Table 2. *Total sulfur analysis and details of the samples in Figure 23.*

No.	***Sample name***	***Sample details***	***Total S [mass%]***
a	Vasa Old core 151	Old Vasa core from the 70s (0mm)	1.76
b	Vasa Tar	From oakum filled spaces between hull planks, sealed with tar)	0.61
c	Vasa oak untreated 24248 (21675)	Plank (no PEG) stored in tap-water at ~5º C, since salvage	0.54
d	Vasa pit 65379	"Fresh" wood in sediment (65380) from Vasa pit Schaft 1 (2005)	-
e	Vasa pit 65381	"Fresh" wood in sediment (65382) from Vasa pit Schaft 2 (2005)	-
f	Old oak Vasa pit 46-48mm	1^{7th} century oak found close to Vasa wreck site	0.36
g	Old oak Vasa pit 0-2mm	17th century oak found close to Vasa wreck site	0.54
h	Sediment Vasa pit B2 wet	Sediment from Vasa pit (2003)	2.8
i	Sediment Vasa pit C1 dry	Sediment from Vasa pit (2003)	2.7

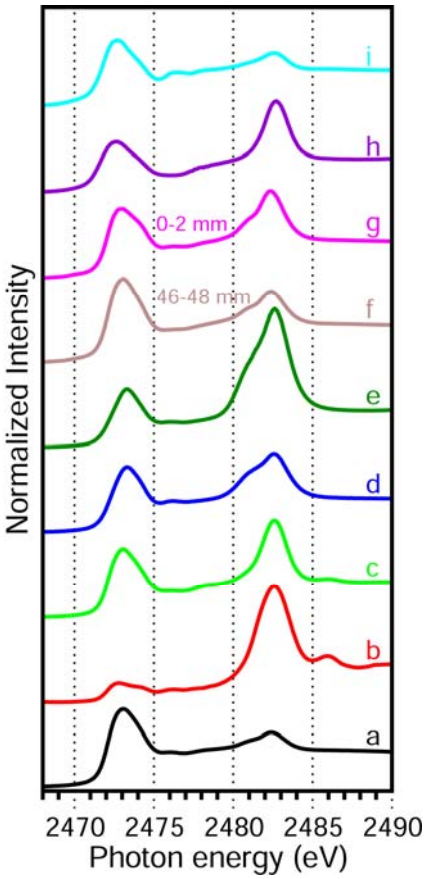


Figure 23. XANES spectra of w*ood and sediment samples from the* Vasa *or the wreck site (see details Table 2). Tentative assignments are proposed for the dominant sulfur groups with peak values (within brackets) reported for the maximum of the reduced sulfur peak. The corresponding peak maxima for standard compounds are: cysteine (thiols) 2473.3 eV; cystine (disulfide) 2472.7 and 2474.4 eV; elemental sulfur in xylene solution 2473.0 eV; methionine sulfoxide 2476.4 eV; sulfonate 2481.2 eV, sulfate 2482.6 eV.*[IV]

a. Thiols; -SH (2473.1 eV); sulfate.
b. Elemental S, thiols, disulfides S-S (2472.8 eV); sulfate in the form of gypsum.
c. Elemental S, thiols (2473.1 eV); sulfate; SO_4^{2-}.
d. Thiols(2473.3 eV); sulfonate R-SO_3^- and sulfate (Sediment analyses Figure 25).
e. Thiols (2473.3 eV); sulfonate. More sulfate than in (d).
f. Thiols, disulfides (2473.1 eV); sulfonates and possibly sulfate.
g. Thiols, disulfides (2473.0 eV); sulfonates and some sulfate.
h. Elemental S (2472.6 eV); sulfate (see also SXM Figure 35).
i. Organosulfur in sediment; elemental S, disulfides (2472.7 eV); sulfoxides and sulfates.[74]

MR80T108 Core 1

Depth [mm]	**0-5**	**7,5**	**15**	**34**	**90-95**	**99**	**107**	**112**	**145**
Total S [mass%]	1.24	0.81	1.04	1.02	1.13	0.85	1.04	1	1.02

MR2A Core 2

Depth [mm]	**0-3**	**8**	**24**	**79**	**118**	**162**	**193**
Total S [mass%]	0.45	0.25	0.4	0.34	0.5	0.89	0.73

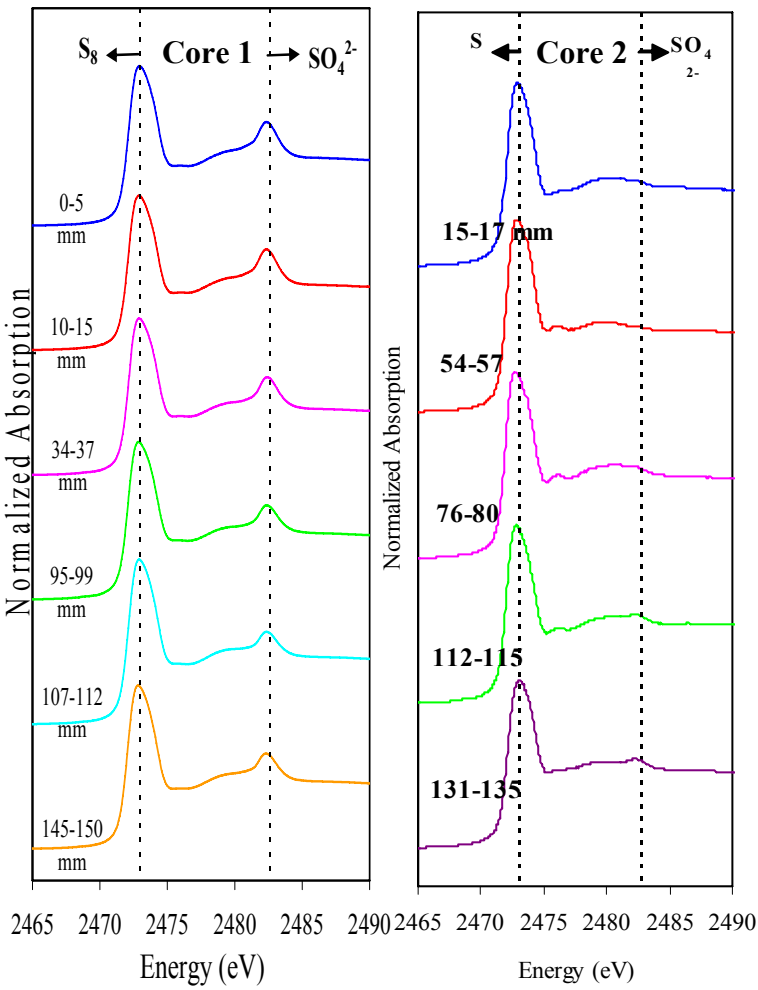


Figure 24. *Normalized XANES spectra from slices along oak wood cores of the* Mary Rose *from the surface and inwards. (left) Core 1(MR80T108); beam stored in magazine. (right) Core 2 (MR2A); Hull rider under PEG treatment. The major peak in the spectra at 2473 eV originates from reduced sulfur species (thiols; R-SH, disulfides; R-SS-R´, elemental sulfur; S_8, and possibly pyrite; FeS_2) and the minor peak at 2482.4 eV (Core 1) to sulfate.*[IV]

3.2.2 Sulfur speciation in Mary Rose wood

The warship *Mary Rose* served in Henry VIII's navy for 35 years until she in 1545, when maneuvering at the start of a battle, went down at a depth of 14 m outside Portsmouth, U.K. After microbial actions and tidal currents had eroded away the exposed port side, the starboard hull timbers and some remaining deck planks became protected by a layer of compact clay.[19] The normalized XANES spectra for sections along oak cores from hull timbers show a main absorption peak at 2473 eV that corresponds to reduced sulfur and consists of several components (Figure 24). Curve fitting with XANES standard spectra has been performed to quantitatively reveal thiols (R-SH), disulfides (R-SS-R´, with a characteristic shoulder at 2474.4 eV) and elemental sulfur (S_8) in all spectra. The analysis indicated that also pyrite (FeS_2) sometimes occurs in particles. The minor peak, visible at 2482.4 eV, corresponds to sulfate (SO_4^{2-}) in about 5 atom% of the total sulfur. Sulfoxides (R_2SO), with a characteristic feature at about 2476 eV, can be discerned as a minor component in all XANES spectra for the *Mary Rose*. Note that for cores sampled from hull timbers being washed by the conservation liquid (Figure 24 *right*), oxidised sulfur(VI) compounds in significant amount are only present at the surface.[IV]

3.2.3 Sulfur speciation in wood from wreck site simulation

The laboratory experiments, exposing pine wood to bacterially produced hydrogen sulfide in a simulated seabed environment (Chapter 2.4.1), reveal sulfur accumulation processes consistent with the previous observations for marine archaeological wood. The peak at about 2472-2473 eV in the XANES spectra showed that organic sulfur, mainly thiols (R-SH) and some organic disulfides, had accumulated in EB-degraded parts of the wood of the treated samples (exemplified by the analyses of sample 3 and 6 (Figure 25)). The sulfur content in the wood increased more than 10 times in two years.[VII] However, elemental sulfur; S_8, often found in marine archaeological wood and especially in the *Vasa*, seems to be absent.[VII] Since elemental sulfur is a common intermediate product in the oxidation of iron sulfides,[69] its absence is expected if no iron sulfides had formed. A characteristic peak at about 2476 eV indicates the presence of a small amount of sulfoxides, which is an oxidation product of the organosulfur compounds.

In another series with fresh wood stored together with active inoculums from marine archaeological wood (samples A20 and A21), the sulfur XANES spectra showed after four years anaerobic treatment considerable amounts also of inorganic iron sulfides, corresponding to $Fe_{1-x}S$. The amount of elemental sulfur also increased considerably when the iron sulfide ($Fe_{1-x}S$, peak energy 2470.4 eV) in sample A21 oxidised at atmospheric exposure. A sediment sample from the *Vasa's* seabed (SED 65380) was also rich in iron sulfides, including pyrite; FeS_2 (Figure 25d *right*).[VII]

Table 3. *Total sulfur analyses of pine wood from simulated seabed environments and of sediment from the Vasa pit (see also Figure 25).*

Sample Id	*Sample details*	*Total S [mass%]*
3 surface	Pine wood; simulated wreck site. Anaerob	0.08
3 mid~1cm	Pine wood; simulated wreck site. Anaerob	0.07
6 surface	Pine wood; simulated wreck site. Partly aerob	0.12
6 mid~1cm	Pine wood; simulated wreck site. Partly aerob	0.075
A20 & 21	Pine wood; ~4 years with inoculums from iron-rich marine archaeological wood (de Rob).	0.18 & 0.58
Fresh pine	Fresh *Pinus sylvestris*	0.006
SED 65380	Vasa wreck site sediment, 1 m below seafloor	1.46

The other major peak at ~2482 eV in the XANES spectra corresponds to oxidised S(VI) species, sulfate and sulfonates. In the samples from the ship-wrecks those oxidised sulfur compounds are mainly oxidation products formed after the salvage, as for the *Vasa*. However, for the pine samples in the current study the sulfate mostly originates from that added to the media, with higher amount at the surface. Also fresh pine contains some sulfate and sulfonate, even though in much lower concentration.[VII]

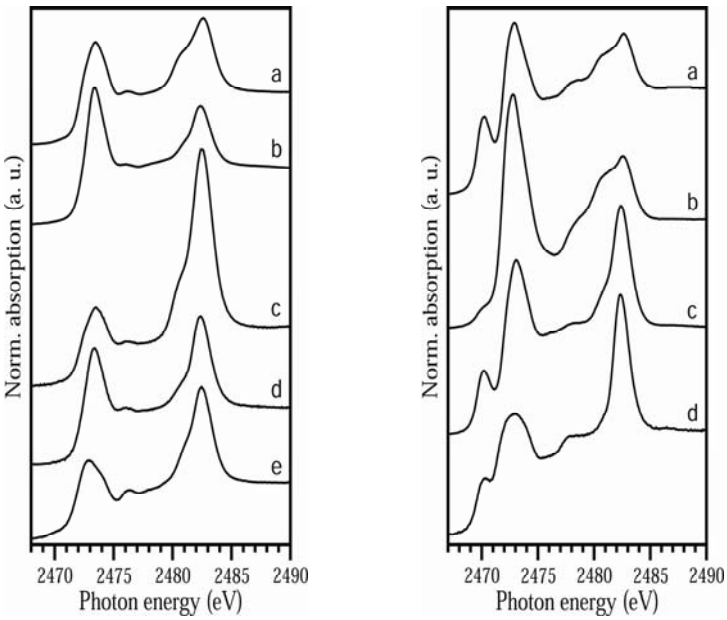


Figure 25. *(left) Normalized XANES-spectra of pine wood after treatment in simulated seabed conditions, see Table 3. a) 3 (surface); b) 3 (mid) ~1 cm depth; c) 6(surface); d) 6 (mid) ~1 cm depth; e) Fresh pine. (right) a) A21 (newly prepared); b) A21 (1-day atmospheric exposure); c) A20; d) SED 65380, sediment 1 m below seabed at the* Vasa's *wreck site. Sample details Table 3.*

3.2.4 Two pathways for sulfur accumulation

The two series of fresh pine wood treated with different inoculums of bacteria clearly illustrate the two different pathways[IV,V] for accumulation of reduced sulfur in wood via bacterial formation of hydrogen sulfide: 1) reaction with lignin to form thiols, and 2) formation of iron(II) sulfides, pyrite and probably pyrrhotite. Especially the pyrrhotite ($Fe_{1-x}S$) fraction is expected to be instable in high humidity;[69] consistent with the repeated XANES measurement for A21, which shows that the corresponding peak at 2470.4 eV has almost disappeared after one day of atmospheric exposure (Figure 25). The increase in the fraction of elemental sulfur corresponding to the oxidation of the $Fe_{1-x}S$ particles of filed wood is consistent with the reaction scheme provided by MacLeod and Kenna.[69] There are no significant changes in the other components, including pyrite.[VII] The sediment sample SED 65380 from the *Vasa´s* wreck site with high total sulfur and iron concentration displays a somewhat different peak shape for reduced sulfur than the wood samples, because of the high amount of pyrite.[VII] Another sediment sample from the seabed beside the *Vasa's* wreck site showed small particles (< 1 μm), which were identified as elemental sulfur by focused micro-XANES, and certainly result from bacterial activity (Figure 35). There is also support for bacterial involvement in the reduction step (of sulfate) in the sulfur accumulation in a recent sulfur isotope study of a *Vasa* oak sample (Chapter 2.4.2).

Organic substances and reactive iron compounds compete to react with the hydrogen sulfide and trap the reduced sulfur in sediments. Generally, reactive iron ions are considered to be a more successful sink for reduced sulfur than organic matter. In an excess of corrosion products like iron(III) hydroxides; FeOOH, which readily react with H_2S if moisture is present, the primary reaction products $Fe_{1-x}S$, FeS_2 and elemental sulfur are favoured. The aqueous oxidation of H_2S is orders of magnitudes slower than if the reactions are catalysed by iron oxides.[77] When reactive iron is limited, the disulfide concentrations in the waters rise and solid organosulfur compounds may form by direct reaction with active sites in organic compounds in lignin, carbohydrates, etc.[77,78]

3.3 ESCA for multi-elemental analyses

X-ray photoelectron spectroscopy (XPS) was used to complement the XANES results, providing a quantitative survey of most elements in the cores. The Scienta 300 ESCA (Electron Spectroscopy for Chemical Analyses) instrument at the Ångström Laboratory, Uppsala University, was used for the multi-element analyses. Reduced and oxidised sulfur species can be distinguished as for the core sample C9a, for which two broad peaks are produced at 164 eV and 169 eV, proportional to the concentration of reduced

and oxidised sulfur forms, respectively (Figure 26). The cores usually display the highest sulfate concentration closest to the surface, while reduced sulfur compounds dominate deeper inside the wood (Figure 26, Table 4). The increase in the oxidised sulfate is consistent with increased oxygen access. With a core that runs through more than one layer of the hull the sulfate concentration is enhanced close to all surfaces of the different layers (Chapter 3.6.1).[2,II]

ESCA measurements allow quantitative analyses of light elements (except hydrogen). The content of borate was found to be high and uniform throughout the Vasa cores and indicates that boric acid from the fungicide mixture added in the conservation treatment easily penetrated the wood (Chapter 2.5) Silicon is found especially on the surface and in cracks of the wood, probably originating from silicates in clay and sediments. Also iron was found in varying concentration throughout the core (Table 4).

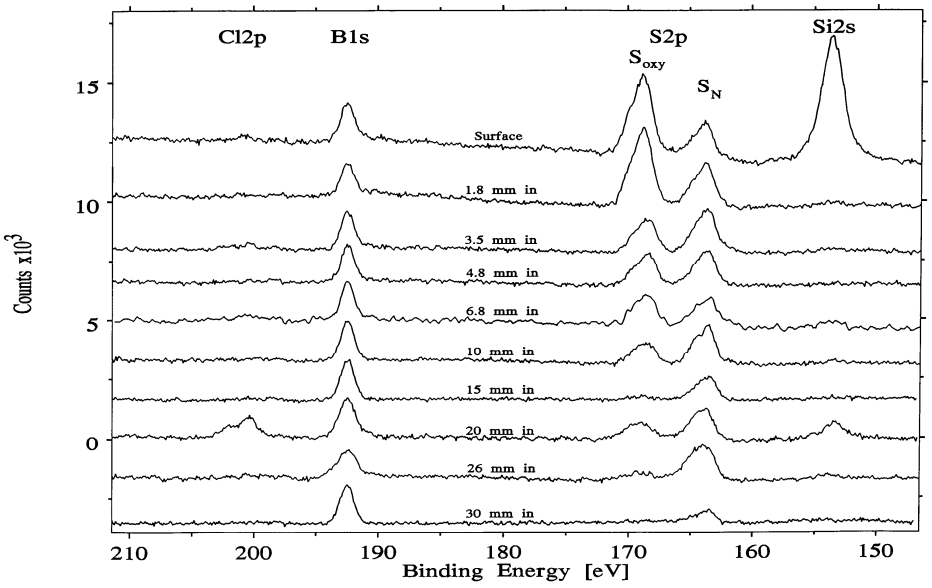


Figure 26. *ESCA analyses of core sample C9a from the* Vasa *at different depths. Sulfate or other oxidised sulfur forms (S_{oxy}) are found mostly in the surface. The amounts of reduced sulfur compounds (S_N) are almost constant down to 27 mm. The peak B_{1S} indicated that boric acid, $B(OH)_3$ has penetrated the whole core in large concentrations. Chlorides and silicon from silicates in sand and clay particles are found mostly close to the surface and in cracks (at 20 mm).*

Table 4. *ESCA analyses of oak core C9a from an oak bilge stringer at port side bow at the orlop deck. The concentrations are given in atom% (excluding H). A crack was noticed at 27mm, which might have influenced the amount of contaminants.*

Depth mm	Fe atom%	S atom%	B atom%	N atom%	Si atom%	Cl atom%	Na atom%	Ca atom%	O atom%
0	0.37	0.82	0.58	0.23	2.22	0.01	0.23	0.04	22.97
1.7	0.27	0.93	0.44	0.47	0.12	0.01	0.13	0.03	26.05
3.5	0.12	0.54	0.49	0.31	0	0.03	0.07	0	25.27
4.8	0.16	0.49	0.54	0.28	0	0.01	0.09	0.01	25.24
6.5	0.12	0.6	0.71	0.18	0.12	0.06	0.12	0	29.16
10	0.16	0.52	0.72	0.17	0.05	0.02	0.11	0	25.92
15	0	0.21	0.65	0.04	0	0.03	0.1	0.02	23.49
20	0.06	0.3	0.55	0.18	0.18	0.09	0.23	0.02	27.74
27	0.12	0.38	0.6	0.38	0.09	0.04	0.28	0.06	23.49
30	0.06	0.22	0.5	0.21	0.07	0.03	0.19	0.03	31.23
40	0.08	0.23	0.38	0.24	0	0.09	0.23	0	28.57
50	0.14	0.17	0.48	0.25	0	0.03	0.24	0.02	30.84
60	0.12	0.15	0.4	0.17	0.04	0.04	0.17	0.03	29.93
70	0.16	0.16	0.46	0.24	0.07	0.05	0.26	0.07	27.37
80	0.16	0.08	0.4	0.22	0.05	0.05	0.22	0.03	28.41
90	0.21	0.12	0.5	0.28	0.11	0.14	0.23	0.01	25.24

3.3.1 ESCA analyses for the Mary Rose wood

The ESCA spectra indicated large variations in the carbon and oxygen concentrations along the core with an inverse correlation (high C corresponds to low O), and also in the relative sizes of the two C_{1s} peaks at 285.0 and 286.5 eV, which mainly correspond to CH_2 (lignin) and C-O (cellulose) groups, respectively (Chapter 1.5.4). The lignin to cellulose ratio increases when the wood is degraded and the variations indicate that pockets or zones of degraded wood extend throughout the core, consistent with the relatively uniform concentration profiles of total sulfur. This again indicates that degradation of the wood structure by EB facilitates $H_2S(aq)$ and $Fe^{2+}(aq)$ accumulation (Chapter 2.4.1).[IV]

3.4 Total sulfur concentration

The results above were complemented with elemental analyses of the total sulfur concentration by Mikrokemi AB in Uppsala.[79] The elemental analyses confirmed that all core samples from the *Vasa* contain sulfur, taken both from areas with and without visible salt precipitates, even though the concentrations vary considerably.[III] In the cores from the *Vasa*, the total sulfur concentrations are usually high within 1-2 cm from the surface and decrease sharply further inside. For the outermost layer the highest sulfur concentration is mostly found a few mm below the wood surface (Figure 27).[II] The somewhat lower sulfur concentration closest to the surface could be due to the washing out of the sulfate during the prolonged spraying during the PEG conservation treatment.

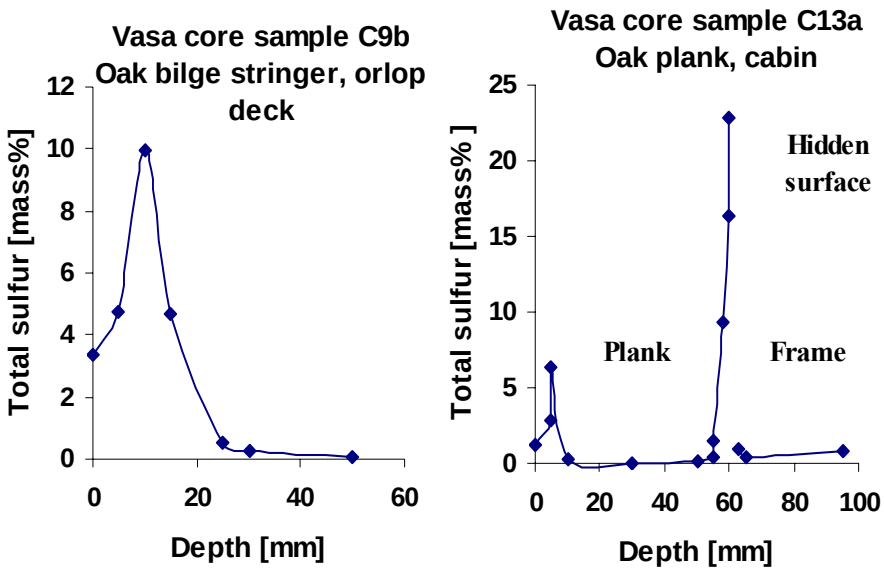


Figure 27. *Penetration profile of total sulfur (mass%) within core sample C9b into an oak beam from the orlop deck, and in C13a through an oak dunnage in the cabin, with very high sulfur concentrations close to the surfaces. In C13a the highest level, 23 mass% S, was found in a hidden surface between a 60 mm dunnage and an oak beam of the frame behind, while the maximum level close to the exposed outer surface was 6.4 mass% S.*

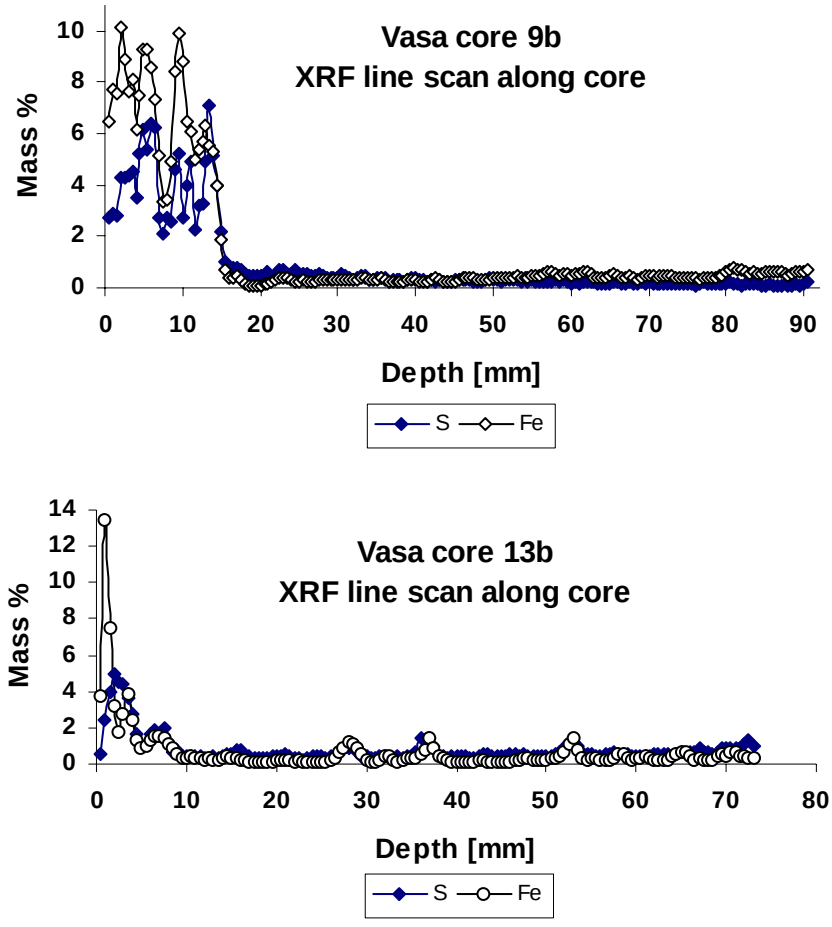


Figure 28. *X-ray fluorescence (XRF) line scan along core. Total iron and sulfur profiles in mass% along the Vasa cores C9b and C13b. Parts of the cores were consumed in the destructive elemental analyses and the complete cores could therefore not be scanned.*

3.5 Sulfur and iron profiles by XRF line scan

Cox Analytical Systems has developed a method for automatic x-ray fluorescence (XRF) line scan along cores of wood and sediments.[80] The method could be adapted for use on cores of archaeological wood to obtain concentration profiles with fairly high resolution (< 0.5 mm) of total sulfur and iron, and also other elements, for example calcium; Ca. The sensitivity covers the range from about 0.02 mass% to 20 mass% (Figures 28, 29). Repeated line

scans and calibration with pressed wood pellets containing known amounts of iron sulfate and sulfur indicated the accuracy to be within ±10% of the reported values. The results from scanning some of the *Vasa's* wood cores confirm with high resolution that the sulfur mainly has accumulated in the outermost surface, the first 1-2 cm. Deeper into the wood the sulfur levels are much lower, even approaching those (< 0.1%) in fresh wood.[I-III,4] From the diagram it is also clear that the sulfur and iron profiles often follow each other closely. The concentration of total sulfur corresponding to the XRF-profiles in Figures 28 and 29 sometimes differs somewhat from the total sulfur analyses (Mikrokemi). Parts of the wooden cores, including the surfaces, were consumed in the destructive elemental analyses of sulfur, and the complete sulfur (and iron) profiles could therefore not be obtained for the same cores.[IV]

For the *Mary Rose* the total sulfur and iron concentrations were also determined by several methods (ESCA, XRF and elemental analyses). The distribution is much more uniform than for the *Vasa,*[IV] and normally varies between about 0.5 and 1.5 mass% S throughout the cores (Figure 29).

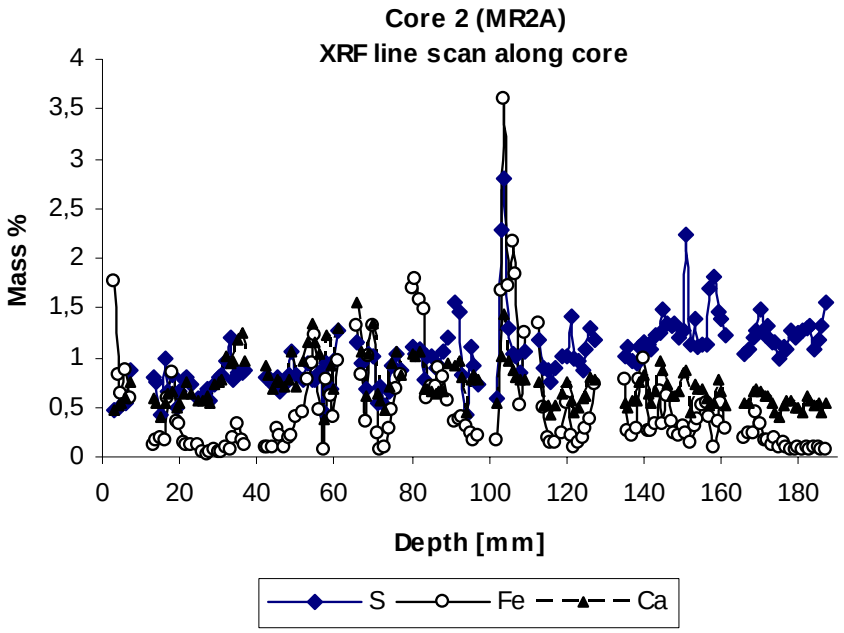


Figure 29. *X-ray fluorescence (XRF) line scan along Mary Rose oak wood core 2 (MR2A), from hull rider under PEG treatment showing total sulfur, iron and calcium in mass%. A crack at 105 mm corresponds to high iron and sulfur concentrations. The gaps are due to missing pieces of the core lost in destructive analyses.*

3.5.1 Different wreck sites – different sulfur profiles

The different accumulation profiles of sulfur and iron in the samples from the *Vasa* and the *Mary Rose* are connected to the special conditions that prevailed at the wreck sites. Around the *Vasa's* exposed hull the dissolved oxygen was largely consumed by decomposing organic matter in the increasingly polluted water of Stockholm's harbour. The records occasionally show very high concentrations of hydrogen sulfide in the entire surrounding water volume, before the salvage in 1961 (Chapter 2.3).[V,VII]

At the wreck site of the *Mary Rose,* the seawater has higher salinity but the remaining timbers were covered by compacted clay, restricting the flow of anoxic seawater.[19] In the experiment with the simulated seabed samples there were no obvious indications that the SRB activity was stimulated by higher sulfate concentration (Chapter 2.4.1).[VII] However, a certain level of sulfate is required for the sulfur accumulation processes to take place. This becomes obvious with the low total sulfur concentrations in the wood of the *Bremen Cog*, which was preserved in river water (Chapter 2.8).[III] For the *Mary Rose* the EB has degraded the wood throughout the hull, consistent with the sulfur profile, and in contrast to the *Vasa* where only the surface wood has been degraded and is sulfur contaminated. The iron and sulfur concentration profiles are often correlated for the *Vasa* and to some extent also for the *Mary Rose*, indicating that initially iron sulfides were formed from hydrogen sulfide and iron(II) ions in the waterlogged wood (Chapter 3.5).[IV,V] Possibly, the EB degradation is coupled also to the penetration of the iron ions into the wood.

3.6 The amount of sulfur in the *Vasa* & *Mary Rose*

The average total sulfur concentration in the top 2 cm surface layer is 1.0 mass% in 20 analysed *Vasa* core samples distributed over the ship (Figure 17), in locations selected to represent different affected and visibly non-affected areas (Figure 30). The sulfur concentration further inside the wood is usually negligibly low (< 0.1%). Since the cores were collected from different parts of the ship, this mean value has been assumed to be fairly representative for the entire surface area of the *Vasa*. With the approximate total area of 14 000 m^2 of the *Vasa's* surfaces, and the density of 0.8-0.9 g/cm^3 in the PEG-impregnated parts of the wood, the total amount of sulfur would be about 2.5 tonnes, parts of which have already been oxidised to sulfates (corresponding to about 2 tonnes sulfuric acid) and sulfonates (Chapter 3.2.1).[II]

Even though much less remains of the *Mary Rose* hull, the total amount of sulfur is substantial, especially since the distribution is much more uniform than in the *Vasa*. The estimated amount of totally 2 to 3 tons sulfur in the

hull of about 280 tons oak wood is not less than the total amount estimated for the more than 1000 tons oak timbers of the *Vasa's* hull.[IV]

3.6.1 Sulfur variation between Vasa cores

The clay and silt, which coated the keel and the lower part of the *Vasa's* hull at the seafloor (Chapter 2.1), may to some extent have prevented the formation or penetration of hydrogen sulfide. Core sample U8 collected from the *Vasa's* keel (Figure 17), which was buried about 2.5 m down in compact clay, shows relatively low total sulfur content, max 0.4 mass%. However, also other cores taken higher up in the ship sometimes have rather low sulfur concentration (Figure 30).

Those sculptures that were detached early and buried in the mud of the seabed are reported to retain sharper contours than the exposed ones.[22] The clay prevented mechanical abrasion of the wooden surfaces, which probably is the main reason why also the exterior of the quarter galleries generally is in good condition, since they fell off from the stern and were covered with mud and clay. The amount of sulfur in the sculptures has not yet been determined, but many display acidic salt precipitates.[II]

The sulfur accumulation in the *Vasa's* wood is inhomogeneous and differs even between samples taken next to each other (compare mass% S in Figure 18, 27, 28). The variation in sulfur content, as also the distribution of the sulfate salt precipitates, to some extent depends on the location of the sample and the side of the ship (Chapter 2.6.1), but also the species of wood, and the state of degradation.[II] Two oak cores; 9b and 13a from the orlop deck and the cabin, revealed the locally highest total sulfur concentration measured so far at the *Vasa* (Figures 27, 28). The highest concentration of 23 mass% of sample 13a was found in the hidden inner surface (see below) between the ceiling and the underlying frame on the port side of the cabin, connected to the upper gundeck (Core 16 was taken on the opposite starboard side).[II]

From the inside and out the outside the *Vasa's* hull is constructed of three layers: ceiling, frame (ribs) and planking, with wooden nails and iron bolts running through (Figure 38). Concealed spaces and surfaces were formed in between, of which the spaces between the ribs of the hull, were filled with tightly assembled balks of varying quality. Sapwood has sometimes been used,[81] which usually is more hygroscopic and less resistant to biological degradation than heartwood (Chapter 1.4.2). This may explain why the sulfur concentration often differs considerably even between adjacent surfaces in a core sample drilled through more than one layer, and why it can be very high in the hidden surfaces between the layers of the hull (Figure 27).[II] No documentation or registration of the acidity and the sulfate salt outbreaks on the hidden surfaces has yet been possible.

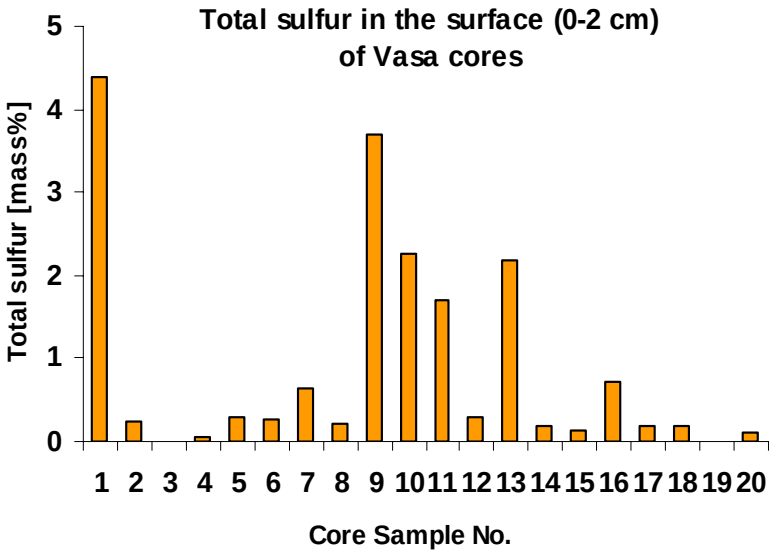


Figure 30. *Sulfur concentration (mean value in surface layers; 0-2 cm) in core samples 1-20 (except 3 & 19) from the* Vasa*. Locations on the ship of the individual samples are indicated in Figure 17.*

3.7 Sulfur distribution in the wood structure

In order to map the distribution of sulfur within the wood structure, microscopic information was necessary. Because scanning electron microscopy (SEM) with x-ray fluorescence (EDS) detection (Chapter 3.8) cannot differentiate between oxidation states of the elements, and thus does not provide sulfur speciation, the scanning x-ray spectromicroscope (SXM) at beamline ID21, ESRF in Grenoble, France was utilised. The reduced sulfur compounds could be mapped in oak wood and sediment samples from the wreck sites of the *Vasa,* the *Mary Rose* and the pine wood from the simulated wreck site tests with revealing results. The samples were transversely cut by hand with razorblades (Chapter 1.5.2).

3.7.1 Sulfur distribution in Vasa timbers

The SXM images obtained for a sample from the *Vasa's* orlop deck (C9b) at the x-ray energy 2473 eV clearly indicate that reduced organically bound sulfur, mainly as thiols and disulfides, is enriched in lignin-rich parts of the wood structure, especially in the middle lamella and the cell corners (Figure 31, Chapter 1.5.5). For the oak wood from the *Mary Rose* and also pine wood from the simulated wreck site experiment low energy peaks in their XANES spectra also indicate the presence of sulfide particles. Sulfate parti-

cles seem to be randomly distributed in the cavities in the wood structure. The combined results from the XANES and SXM studies indicate that the acidity in the Vasa wood probably originates mainly from reactive iron sulfides, while the organosulfur compounds may be more protected in lignin-rich parts of the wood. The results clearly support the hypothesis that the accumulation and reactions of sulfur compounds in marine archaeological wood follow two separate pathways (Chapter 3.2.4).[V,VII]

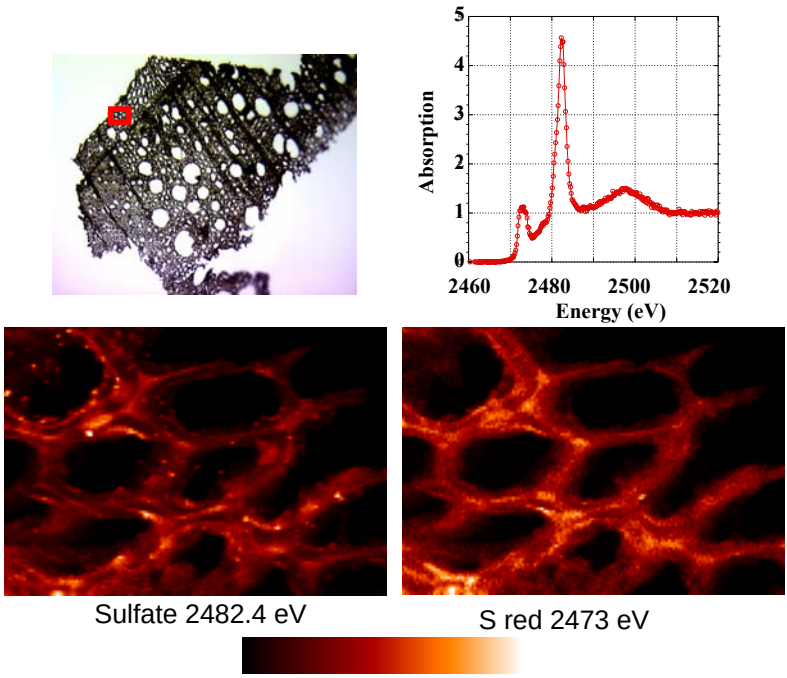


Figure 31. *Vasa sample C9b at 6.5-11 mm depth. Focused XANES-spectra of points in the images of core 9b at 6.5-11 mm depth, show reduced sulfur of energy 2473.6 eV in the middle lamella and the cell wall (Chapter 1.5.5); the energy is close to that of cysteine thiol groups (2473.3 eV). The large peak at 2483 eV is due to sulfate, which indicates that acid has been formed in an oxidation process. Higher sulfur concentration → brighter color.*

3.7.2 Sulfur distribution in Mary Rose timbers

Also the SXM images from freshly salvaged Mary Rose oak consistently reveal reduced sulfur, mostly as thiols and disulfides, in high concentration, especially in the middle lamella and cell corners (Figure 32). Iron sulfides occur in separate particles. Complementary studies by means of scanning electron microscopy and elemental analysis by x-ray fluorescence (SEM-EDS), showed sulfur but no iron in the middle lamella.[IV]

The Vasa samples show higher amounts of sulfate than the Mary Rose samples and indicate that sulfuric acid has been released in oxidation processes. The fact that the Mary Rose samples only reveal small amounts of sulfate is probably due to the constant washing of the timbers, which still are under spray conservation treatment, or freshly salvaged as in Figure 32.[IV]

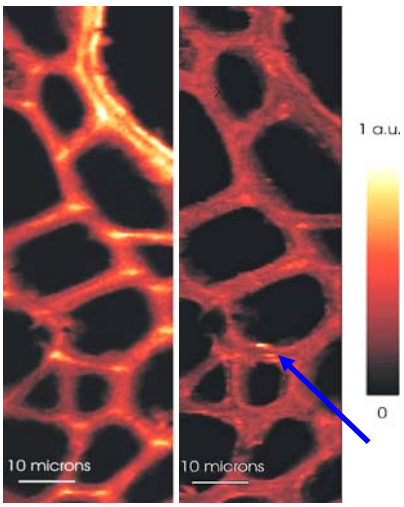


Figure 32. *Freshly salvaged oak wood from the* Mary Rose *after 459 years below the seafloor (pixel size: 0.5 µm, integration time / pixel: 600 ms). The left image, at 2473 eV, shows thiols in high concentration in the lignin-rich middle lamella between the cells (Chapter 1.5.5) and in the lignin-reinforced vessel wall (Chapter 1.5.1). In the image to the right at 2483 eV a bright spot shows a sulfate particle (blue arrow). Higher sulfur concentration → brighter color.*

3.7.3 Sulfur distribution in wood from wreck site simulation

Scanning x-ray spectro-microscopy (SXM) images of the pine wood samples exposed for 2 years to simulated wreck site environments in a laboratory experiment (Chapter 2.4.1) again showed that sulfur, mainly as thiols (R-SH), had accumulated in the middle lamella and the cell corners in EB-degraded parts of the wood in both samples; number 12 (partly aerobic) and 2 (anaerobic) (Figure 33, 34). SXM-images could simultaneously be obtained for total phosphorus, the distribution of which resembles that of the reduced sulfur (Figure 34b,c). Phosphates are present in all bacteria[82,83] and the phosphorous content has been found to increase substantially with higher degree of bacterial degradation of wood.[84] Thus, the correlation of the phosphorus and sulfur distributions indicates bacterial activity both of the EB degrading the wood, and the scavenging SRB producing hydrogen sulfide *in situ*. The silicate distributions differ (Figure 34d), and probably reflect the presence of sand grains from the inoculum.[VII]

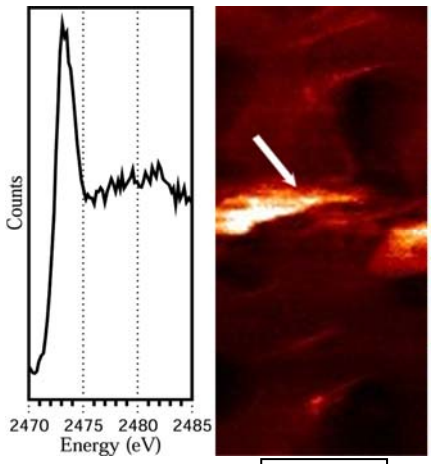


Figure 33. *Pine wood sample 2 (anaerobic). (a) Micro-XANES at arrow; the peak energy (2473 eV) corresponds to thiol (-SH) groups. (b) SXM-image of reduced sulfur (for the energy 2473± 0.5 eV) shows high concentrations, especially in the ray; one of the entrances of the EB into the wood structure (Chapter 1.6.1).*

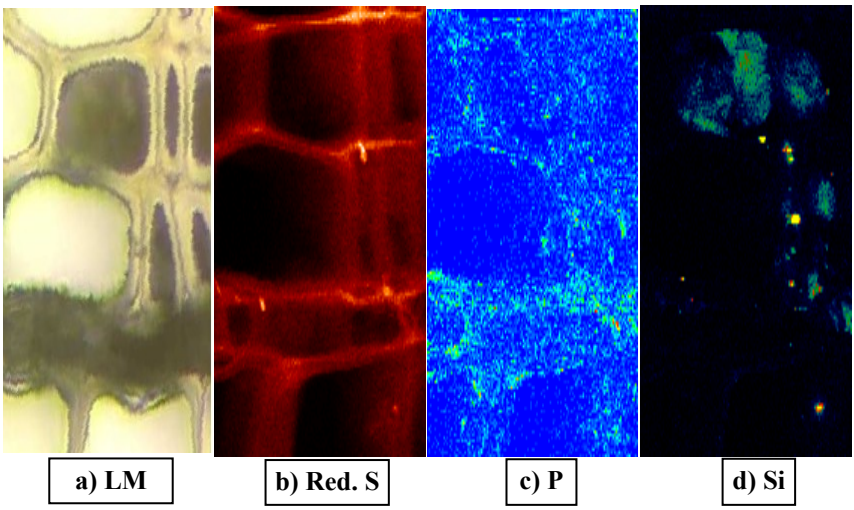


Figure 34a-d. *Pine wood sample 12 (partly aerobic): (a) LM-image of EB degraded area (not red safranin coloured as in Figure 9) at the border between early (large lumen) and late wood cells. (b) SXM image at 2473 eV ± 0.5 eV showing accumulation of reduced sulfur in lignin-rich parts of cell walls, such as the middle lamella and the cell corners. (c) Total phosphorous, indicating bacteria. (d) Silicon distribution, probably small silicate particles.*

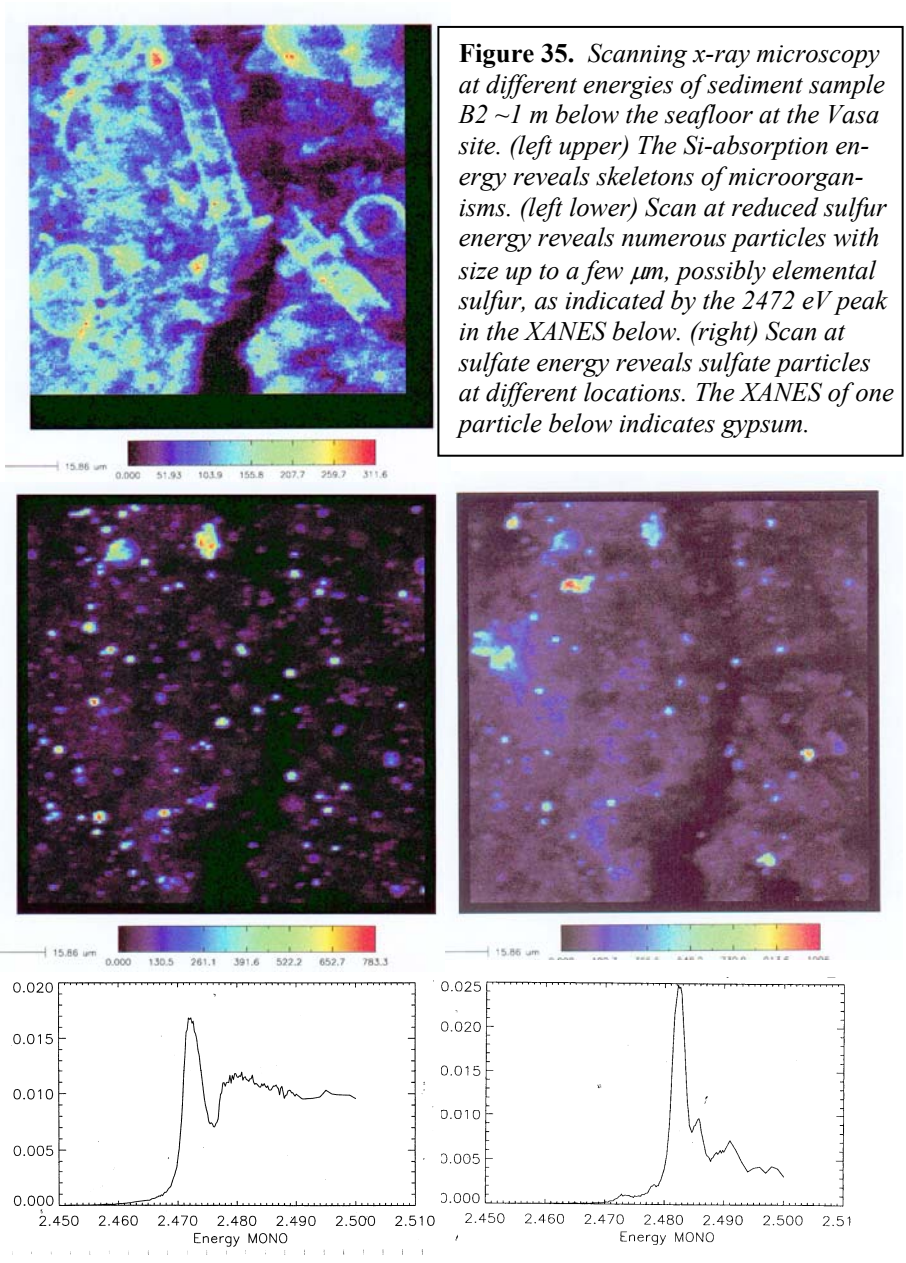


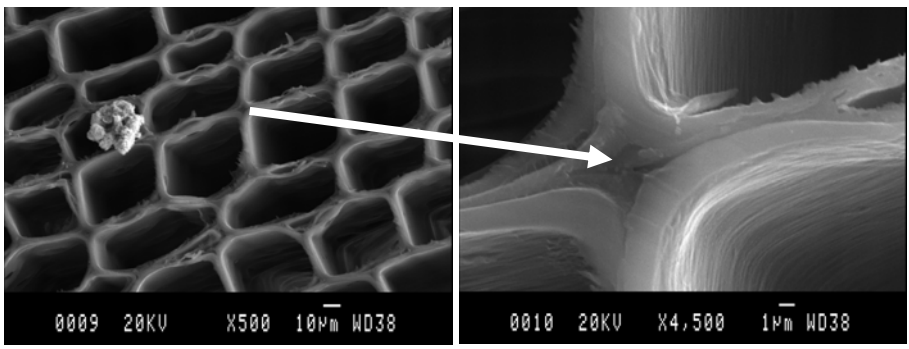
Figure 35. *Scanning x-ray microscopy at different energies of sediment sample B2 ~1 m below the seafloor at the Vasa site. (left upper) The Si-absorption energy reveals skeletons of microorganisms. (left lower) Scan at reduced sulfur energy reveals numerous particles with size up to a few μm, possibly elemental sulfur, as indicated by the 2472 eV peak in the XANES below. (right) Scan at sulfate energy reveals sulfate particles at different locations. The XANES of one particle below indicates gypsum.*

3.8 SEM-analyses

The information from scanning electron microscopy analyses (SEM with EDS) can be used for judging if the total sulfur and iron distribution on a microscopic scale is primarily in particles of iron-sulfur compounds or in organically bound sulfur compounds in lignin-rich parts. The SEM-EDS technique does not have the energy resolution as the highly exclusive synchrotron-based XANES and SXM measurements, but is more available and can be quite informative. The samples in the study were as for the SXM measurements, transversely cut by hand with razorblades (Chapter 1.5.2).

The results from the SXM analyses (Chapter 3.7) are consistent with SEM analyses subsequently performed on Vasa wood at Stockholm University. Figure 36 and 37 indicate enrichment of sulfur in the lignin-rich parts of the wood structure, especially in the middle lamella, the cell walls and the cell corners. Particles containing iron (possibly iron sulfides sulfides or iron sulfates) were also present in the transverse sample cut from the *Vasa's* hold (Figure 37). The high sodium content is most likely a result from the bicarbonate-soda treatment and the zinc content originates from the corroding galvanised bolts. Furthermore, the SEM image showed a particle of framboidal appearance (Chapter 5.2.5) for which the EDS gave the sulfur to iron ratio 1:2. in a sample from a Dutch shipwreck (Figure 36).[V]

The ratio and total amounts of S and Fe in the wood could provide important information about the relative amounts of inorganic/organic sulfur in marine archaeological wood, which is important for predicting the reactivity of the reduced sulfur compounds in the different oxidative pathways leading to sulfuric and sulfonic acids, respectively (Chapter 3.2).



SEM photo: Yvonne Fors

Figure 36. *(lower) SEM-picture of transverse cut from the Burgzand Noord (BZN) wreck site 3, in Waddensea, the Netherlands. The EDS revealed 1:2 ratio of iron and sulfur for the particle, probably pyrite; FeS_2 with framboidal appearance. (upper) The EDS showed high sulfur content but very little iron in the lignin-rich middle lamella and the cell corner (sample prepared by Professor Thomas Nilsson).*

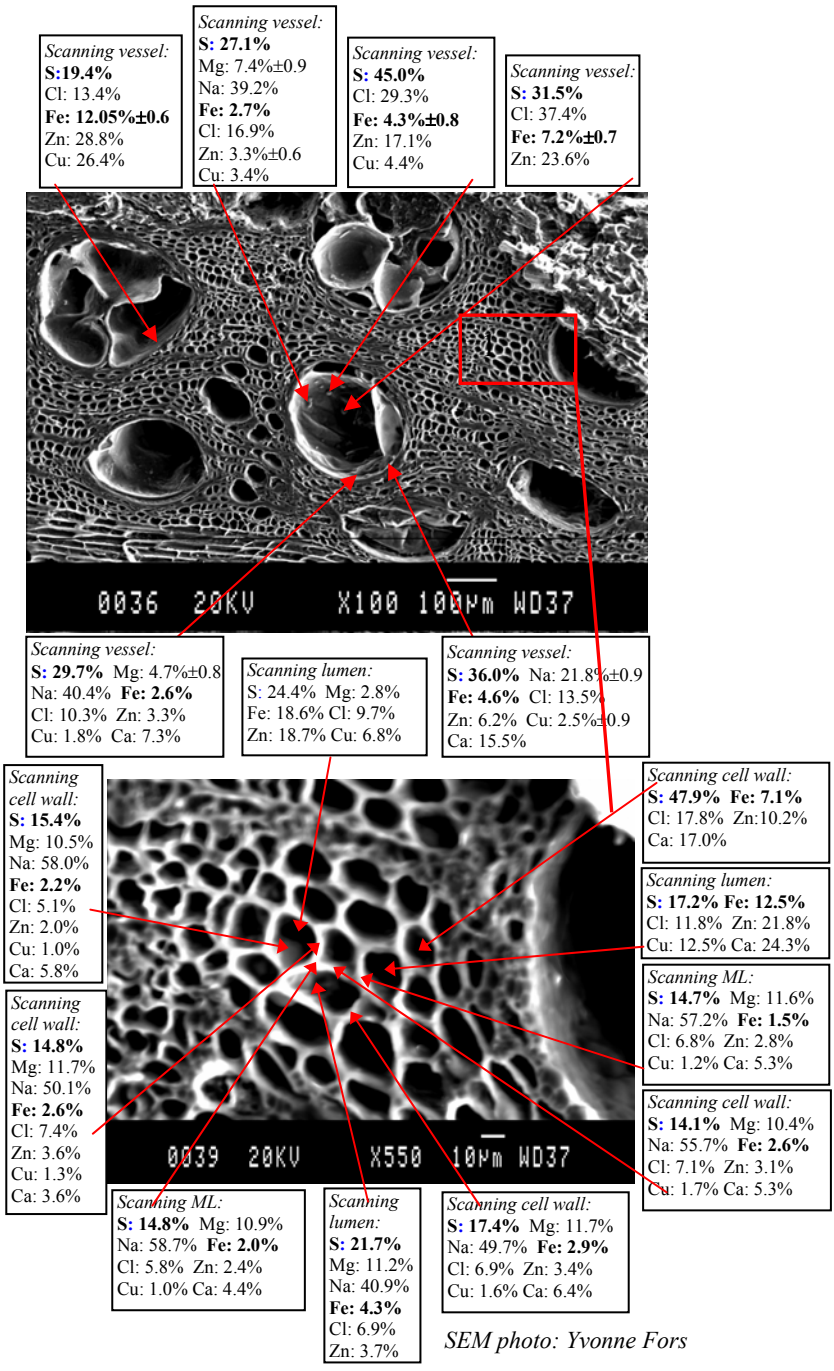


Figure 37. *Cross section of Vasa HS4A (oak knee, pH = 4), in the hold. The SEM-analyses support the SXM-studies, which indicated enrichment of sulfur in lignin-rich parts as the middle lamella and cell walls.*

4 IRON

4.1 Iron contamination of Vasa wood

Elemental analyses of Vasa oak samples after the salvage revealed iron contents up to 2 mass%.[4,85] The ESCA analyses confirm high iron concentration, about 1.5 mass% in the surface layer of dry (black) oak, decreasing to 0.1% or lower deeper inside the core (Table 4). However, the x-ray fluorescence line scans (Figure 28) reveal the concentration of iron and sulfur along the cores with much higher resolution, sometimes with similar values and with related profiles.

The iron contamination originates from iron(II) ions released on the seabed from the corroding iron bolts in the hull. Especially during the first time on the seabed the oxygen level in the water must have been high enough for rapid corrosion of all articles of mild steel, bolts, nails, etc.[4] The iron(II) species primarily formed could by time oxidise further to rust particles or form iron(II) sulfides with bacterially produced hydrogen sulfide.

During and after the *Vasa's* salvage in 1961 about 5500 new zinc and epoxy coated iron bolts were inserted into the holes of the old corroded ones (Figure 38). The recommendation of the Conservation Council to use stainless steel was unfortunately not followed.[18] Now, over forty years later those iron bolts display severe corrosion damage providing a new source of iron ions. The increased acidity has dissolved the zinc coating, and which was unforeseen, the corrosion of the metallic iron accelerates in contact with PEG in humid wood.[3] Furthermore, the released iron ions catalyse oxidative processes in humid environments with detrimental effects on the wood.[2,II]

XANES spectra show that also large amounts of iron sulfides; $Fe_{1-x}S$ and FeS_2, form when pine wood is exposed to active bacterial cultures containing both EB and SRB from inoculums of iron-rich marine archaeological wood in the presence of iron(II) ions (Chapter 3.2.3).[VII] It is not clear, however, if it is the increase in the amount of available iron (from the inoculums) or a strain of bacteria better adapted to iron-rich surroundings that triggers such a clear difference both in the amount and chemical form of the accumulated sulfur and iron in the wood. Previous laboratory experiments of pyritisation of plants, both with microbially and chemically mediated reactions, showed rapid mineralisation driven by an anaerobic bacteria-mediated degradation process. However, also in a chemical model system, with Fe^{2+} ions and Na_2S/H_2S added, but without bacterial inoculum, some mineralization took place (Chapter 5.1).[86] Iron sulfides, probably mostly pyrrhotite and pyrite, are also abundant in sediments from the Vasa wreck site (Figure 35, Chapter 3.2.3). Especially the iron sulfides of pyrrhotite type, which were found to oxidise rather rapidly with oxygen access to sulfates and also form elemental

sulfur, could in a humid aerobic environment cause long-term preservation problems and possible degradation of marine archaeological wood. The oxidation of pyrite gives rise to increased acidity that may hydrolyse cellulose,[III] while Fenton types of oxidative reactions catalysed by iron ions are capable of direct oxidative degradation of cellulose and also of PEG.[59] The iron activity and its influence on the mechanical stability of the wood is another important field for which the information is limited.[VII]

Generally, in humid environments iron ions could in photochemical reactions catalyse oxidative processes,[59] such as the oxidative degradation of cellulose.[87] Formation of organic radicals occurs with oxygen. In the Fenton reaction, hydrogen peroxide; H_2O_2 forms as an intermediate, which then reacts with iron(II) to reactive hydroxyl radicals. The hydroxyl radicals are very strong non-selective oxidising agents, capable of oxidising organic matter in general.[59] To reduce the generation of such radicals, catalysed by iron(II) ions, the light level is kept low (max. 50 lux) in the main exhibition hall of the Vasa Museum.[18]

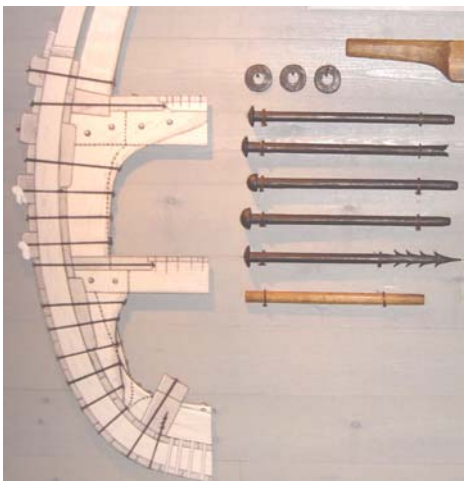


Photo: Magnus Sandström

Figure 38. *Cross section of the* Vasa's *hull. The epoxy and zinc coated iron bolts, inserted in the hull after the salvage to replace the old corroded ones, show corrosion damage. Subsidence when the timbers dried will complicate the removal and replacement of the bolts without damage to the hull.*

Considerable loss of tensile strength of wood has been found over time in contact with iron.[88] Moreover, the iron ions probably degrade the PEG molecules.[3,17] In a recent study the PEG-profiles from Vasa wood and PEG solutions stored since the conservation period were analysed and compared with results from a statistical simulation of a possible PEG degradation process.[17]

Mass spectrometric analyses of aqueous extracts from Vasa wood below the surface region (3-10 cm) indicate degradation processes resulting in random cleavage of PEG molecules and the formation of low-molecular hemicellulose, while the surface wood was reported to be less affected. This condition was accompanied by low pH, high iron content, but relatively low content of sulfur. The detrimental effects of iron provide strong reasons to remove or deactivate iron compounds in the wood of the *Vasa*.[II,17]

4.2 Iron extraction by the chelates EDMA and DTPA

Tests have been carried out where iron compounds are dissolved and removed from samples of Vasa wood at the same time as the acid is neutralised.[II,17] Such an action is in a way a chemical contradiction, since iron(III) oxyhydroxides (rust) precipitate already at low pH-values from aqueous solutions. However, special chelates can be made that form very strong and soluble complexes with iron(III) ions. The bonding with six surrounding donor atoms in the well-known chelate EDTA; ethylenediaminetetraacetic acid, can be enhanced by adjusting the size of the cage enclosing the iron(III) ion.[89] Such chelating agents derived from EDTA are EDMA; ethylenediiminobis(2-hydroxy-4-methyl-phenyl) acetic acid and DTPA; diethylenetriamine-pentaacetic acid.[II] Stability constants indicate that EDMA can dissolve rust and keep iron(III) ions in alkaline solution up to pH = 11, before goethite; FeOOH(s), would precipitate. DTPA lacks the phenol rings that enhance the donor ability of EDMA (but also give a dark-red colour to the Fe(III)-EDMA complex), and DTPA therefore forms somewhat weaker complexes (goethite precipitates at pH > 8).[II]

The iron extraction by means of the EDMA and DTPA chelates in alkaline solution has been monitored for years in experiments on Vasa wood. The efficiency was found to be enhanced by stirring and by higher chelator concentrations, but not obviously by elevated temperature.[90] Most of the surface iron was extracted during the first three months, but longer time, even years, could be needed for more complete extractions. Pyrite is not soluble in aqueous EDMA, but the visible precipitates, including jarosite, were dissolved together with most of the surface PEG and other water-soluble compounds. The degree of iron extraction was found to be correlated to the remaining amount of sulfur in the wood,[90] indicating that the lignin-bound reduced sulfur compounds (thiols) remain (Chapter 3.7.1).

The experiments demonstrate the potential of these iron(III) chelates as iron extraction agents for marine archaeological wood, but all aspects of the method must be evaluated before any large scale treatment can be initiated. The treatment in alkaline solutions could be developed into an efficient iron removal method, where the acid is neutralised and the iron compounds re-

moved by EDMA or DTPA extraction.[II] The efficiency of the chelates is higher in alkaline solutions, but the high pH values (> 9) may during prolonged exposure affect especially the lignin in degraded wood.[87] As a final step, the wood has to be reconserved with new PEG, preferably restoring a surface layer of PEG 4000.[II] Even though DTPA is not as effective as chelating agent for iron(III) as EDMA, discoloration is avoided. The rinsing with water that is required to remove the dark-red EDMA complex from the surface,[90] would be stressful for the weakened wood.[V]

Before any such treatment of the *Vasa's* hull, the removable iron bolts should be replaced with bolts of inert material, probably epoxy reinforced carbon fibre bolts. However, it is likely that some of the iron bolts now wedged in the hull can not be removed without serious damage to the hull. Some of the bolts are inaccessible, others jammed when the hull has dried and subsided (Figure 38).[II]

5 THE SULFUR CYCLE

5.1 Sulfur in wood and other cellulose-rich material

The studies of the Vasa and Mary Rose wood show unexpected build-up of reduced sulfur compounds from bacterially produced hydrogen sulfide in the lignin-rich parts of the wood structure (Chapter 3.7). However, there are previous reports of accumulation of reduced organosulfur compounds in humic material and in marine sediments, serving as additional examples of the natural cycling of sulfur in the environment.

SEM-EDS analyses indicated an organic form of sulfur, assumed to be lignin sulfonate, in the middle lamella in 6000 year-old subfossil Totara heartwood from marine environments in New Zealand.[91] The sulfur concentration was reported to be large compared to that in the secondary wall (Chapter 1.5.5) and was considered to have contributed to the preservation. Since the middle lamella were chemically modified and the distribution of sulfur seems to follow that of lignin in the cell wall, it is likely that also this reported accumulation is a result of hydrogen sulfide reacting with the cell wall components, as in the present cases. Among other inorganic elements, minerals such as silica, calcite and hematite were reported in the cell lumen, while salt deposits of $CaSO_4$, NaCl and FeS or FeS_2 were present mainly in ray and axial parenchyma cells and within tracheid cell walls (Chapter 1.5.1). The presence of inorganic deposits was traced approximately to the position of the S1 or S2-layer, while framboidal pyrite was found separated from the cell walls and distributed along the vessels.[91,92]

Sulfurisation has also literally taken place in the literature, such as within antique books. The Archivo Histórico Nacionale in Madrid has reported precipitates in the form of framboidal pyrite in 16th and 17th century books stored in archives for centuries. During the Middle Ages and the Renaissance, tannin was commonly mixed with iron sulfates (melanterite; $FeSO_4 \cdot 7H_2O$) to produce ink. The anoxic environment inside the books evidently allowed reduction of the sulfate in the ink and the formation of pyrite crystals. The cellulose in the paper and the gum Arabic used in the ink provided the carbon and nitrogen sources needed to sustain the activity of iron- and sulfate reducing bacteria.[93]

Pyritisation seems to be a part of a fossilisation process of cellulose-rich material. Deposits within the cell walls of 32000 year-old pine, recovered from a quarry in Italy, turned out to be submicroscopic polysulfides (pyrite).[86] Microcrystalline pyrite has also been found in fossils of plants in Eo-

cene* London clay; in the inner surface of the cell walls, within the middle lamella and the secondary lignified cell wall.[93] Pyrite has also been reported in silicified herbaceous plants, in trees from Messinian marine sediments, and in buried gymnosperms (Chapter 1.4.1).[92]

However, there is also laboratory evidence for very rapid (80 days) pyritisation or mineralisation where the wood anatomy was preserved, in an environment driven anoxic by bacterial activity. At reaction with H_2S, pyrite formed mainly in the parenchyma cells along the inner cell walls. The fossil wood cells (from the Eocene London clay) differ by the greater amount of pyrite in the cell interiors and lignified areas such as the middle lamella.[93]

5.2 Sulfur in nature

5.2.1 Organosulfur in geochemistry

The transformation and accumulation of certain reduced sulfur compounds in organic material constitute significant parts of the natural sulfur cycle, in particular the formation of organosulfur compounds in wet humic matter and in anoxic marine sediments (of low iron content), which eventually may end up in fossil fuels, coal and oil. Sulfate reduction is the main terminal process in anaerobic mineralisation of organic matter in marine sediments; for which up to half of the deposited organic matter has been found to undergo anaerobic mineralization via sulfate reduction.[77,94,95] Sulfur present in that organic fraction of anoxic marine sediments mainly originates from the (abiotic) uptake of reduced inorganic sulfur during early diagenetic processes, while most organic sulfur species in soil and rivers are considered to originate from biological sources.[77,78,95,96,97,98,99] The incorporation of reduced sulfur into organic matter is believed to be the second most significant sink for sulfur in nature after the formation of pyrite.[100]

In marine sediments and in petroleum the major classes of organosulfur compounds are thiols, organic sulfides, disulfides, sulfate esters, sulfur amino acids and thiophene derivates. The large concentration of organosulfur in some sediments contrasts with the relatively low organic-bound sulfur content in the biomass. Unlike the inorganic forms, organic sulfur compounds show little variation with depth in natural waters.[98,101]

Direct reaction between reduced inorganic sulfur species; e.g. H_2S and/or polysulfides, with active sites in organic compounds in humic matter; carbohydrates, functionalised lipids, etc., can occur to form organosulfur com-

* Eocene = geologic time above Auversian, below Ludian. Also known as Marinesian.

pounds. Those reactions occur without the need of bacterial involvement, even if the H_2S originated from bacterial sulfate reduction.[77,78] However, small particles (globules) of elemental sulfur (as in the sediments in Figure 35), indicate bacterial sulfide-oxidising processes as the origin.

Reduced sulfur produced by the dissimilatory bacterial reduction of dissolved sulfate has an isotopic composition significantly depleted in ^{34}S relative to sulfate in seawater. In contrast, biosynthetic sulfur incorporated in living tissue is isotopically close to the sulfate in seawater. Therefore, an analysis of the composition of the stable sulfur isotopes is helpful in revealing the relative contributions in organic matter from different sulfur sources.[77,98] A sulfur isotope analysis has also been conducted to elucidate and confirm the bacterial involvement in the sulfur accumulation in the *Vasa's* wood (Chapter 2.4.2).[VII]

5.2.2 Sulfur nucleophilic reactions

Clay minerals coated with humic substances may function as reactive surfaces that offer a variety of functional groups. By reaction with sulfur nucleophiles as hydrogen sulfide (HS^-) and polysulfide ions, sulfur may be incorporated as thiols and polysulfides into humic substances.[95,99] In sedimentary humic matter the major forms of sulfur have been found to be organic sulfides, di- and polysulfides, sulfonates and organic (ester-bonded) sulfates. Those reduced sulfide structures are essentially intramolecular, while the highly oxidised sulfonates and ester-bonded sulfates only can be present as end groups.[99]

Vairavamurthy have suggested an interesting mechanism for the formation of thiols in sediments.[95] The Michael addition mechanism, where the sulfur nucleophiles react with organic molecules containing activated unsaturated bonds, could be a major pathway for organosulfur formation in marine sediments (incorporation of sulfur into sedimentary organic matter). Hydrogen sulfide (H_2S) and hydrogen sulfide ions (HS^-) would be the primary sulfur nucleophiles in reducing sediments, and the abiotic reaction probably occurs by a nucleophilic attack of hydrogen sulfide ions (HS^-) to the activated double bond (at the β-carbon) in the α, β-unsaturated carbonyl system of acrylic acid, during early diagenesis in marine sediments.[95,98] A disulfide bond would be formed from the oxidation of two thiol molecules. Macromolecular structures, held together by disulfide cross-linking, are subsequently converted to sedimentary deposits.[99]

Another possibility may be an electrophilic reaction between hydrogen sulfide and acrylic acid, where the initial protonation would be followed by a HS^- attack on the double bond. On the other hand, the reactivity of carbon-carbon double bonds (e.g. in acrylic acid) towards electrophiles is restricted

by strong electron-withdrawing groups, such as -C=O, -COOH, -COOR and -CN. Lower reactivity would favour the nucleophilic mechanism proposed above.[95,98]

5.2.3 Sulfur accumulation mechanisms in wood

Our discovery of organosulfur accumulation within waterlogged wood in anoxic seawater directly connects to the environmental implications described above. The high concentration of organosulfur in the form of thiols and disulfides in the lignin-rich middle lamella (Chapter 3.7) of the wood indicates a nucleophilic reaction between the hydrogen sulfide (or HS^- ions) with active sites in lignin. However, not only activated double bonds,[18,20] but probably also ether, carbonyl and α-hydroxy groups must be reactive in lignin to explain the observed effects.

Probable precursors to the organosulfur compounds found in oil and coal bearing sediments are compounds such as thiols or larger aggregates with disulfide bonds. Lignin is an important constituent in humic matter and the specific reaction with hydrogen sulfide could be a major pathway for such organo-sulfur compounds to enter marine sediments via waterlogged wood and eventually end up in kerogens and finally fossil fuels.[IV,VII]

When corrosion products of iron are present in sufficient amount the hydrogen sulfide will also form iron(II) sulfides (e.g. pyrite, pyrrhotite) in reactions competing with those of the organic substances.[14,21]

5.2.4 Iron sulfides

A number of different iron sulfides are known as minerals, e.g. greigite (Fe_3S_4), mackinawite ($Fe_{1-x}S(am)$), marcasite (FeS_2), pyrite (FeS_2), pyrrhotite ($Fe_{1+x}S$) and smythite (Fe_9S_{11}). Each iron sulfide is formed by a distinct mechanism, and all six above can be synthesised from aqueous solutions at low temperatures. Of the ten reactions involving iron sulfides, only three are reversible. The equilibration reactions and the mackinawite – greigite, -marcasite and – pyrite transformations are all irreversible (Figure 39). The major controlling factor is the oxidation state of the sulfur bearing reactant, since the oxidation state of the iron is ferrous in all these minerals. In particular, the presence of aqueous sulfide or polysulfide ion, or elemental sulfur, can decide the nature of the final product. The rates of the reactions vary widely. The direct precipitation reaction, including the reactions between dissolved ferrous iron and sulfide or polysulfide giving mackinawite, marcasite or pyrite, are extremely rapid.[102]

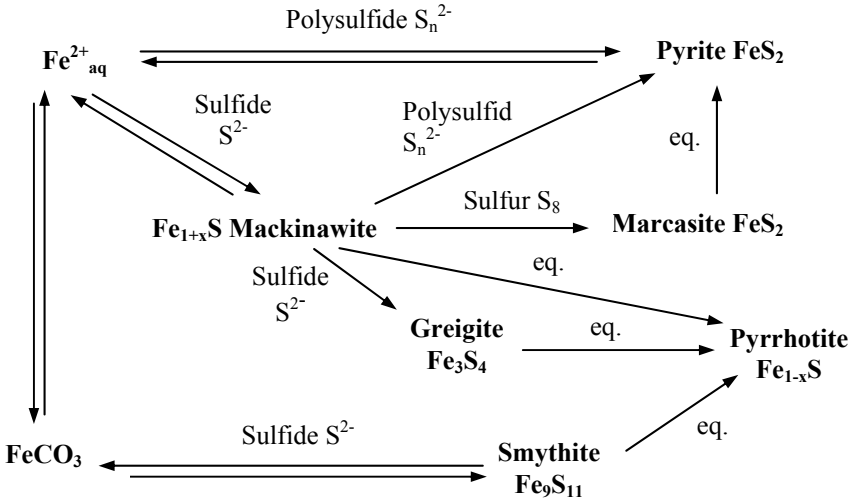


Figure 39. *Iron sulfide reaction schemes.*[102]

5.2.5 Pyrite and framboid formation

Microbial reduction of seawater sulfate under partly open system conditions led to the formation of high concentrations of pyrite; FeS_2 (Chapter 3.2.3), which occurs in a wide set of geological environments, e.g. marine sediments with organic matter, such a coal, slate and limestones.[92,103] Pyrite has been found in the wood of several archaeological shipwrecks,[IV,V] and oxidises in humid conditions relatively rapidly at room temperature to sulfuric acid. Possibly marcasite, also of composition FeS_2, could be formed initially (Figure 39). Marcasite forms from acid solutions, while pyrite, the more stable form of FeS_2, forms under conditions of higher temperatures and lower acidity.[102]

In order for either pyrite formation or organic matter sulfurisation reactions to occur, certain conditions of the sedimentary environment must be fulfilled. A sufficient amount of inorganic sulfide (i.e. H_2S) must be present, which implies anoxic conditions and the occurrence of microbial sulfate reduction, schematically written as in reaction 1 (Chapter 1.6.2). Sulfate occurs with unlimited supply in seawater. The resulting dissolved hydrogen sulfide (H_2S(aq) or HS^- above pH = 7) is then utilized both in the formation of pyrite and organic sulfur compounds. Finally, there must be sufficient initial availability of reactive iron species (e.g. iron oxides and oxyhydroxides) and reactive organic matter.[100]

Densely packed, often spherical “raspberry-like” aggregates of submicron-sized pyrite crystals generally termed framboids,[104,105,106] is the dominant form of pyrite in modern anoxic and ancient sedimentary rocks, where the morphology often is preserved in shales, carbonates and coals (Figure 36 *left*).[106,107,108] The formation of framboids seems to be a common phenomenon occurring specifically in the zone between oxic and anoxic/redox interfaces (separating waters containing dissolved oxygen and sulfide, respectively), and probably takes place during the earliest stages of anoxic diagenesis.[105,106,108] Whether framboidal pyrite is formed anaerobically via the oxidation of amorphous iron(II) monosulfide, $Fe_{1-x}S_{(am)}$ (mackinawite) by H_2S in aqueous solutions without biological intervention[92,101,104,106,108] or is produced from a biotic source,[109] is still under debate.

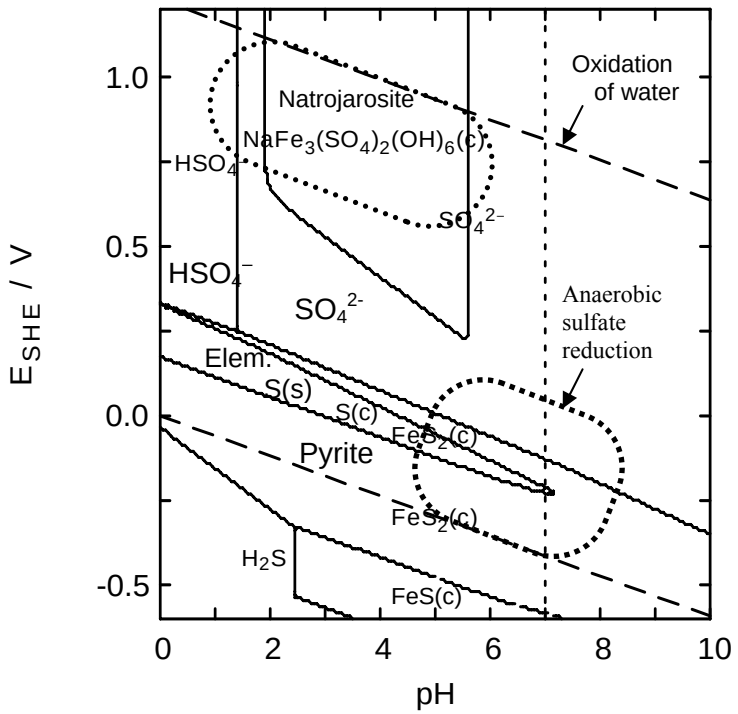


Figure 40. *Pourbaix diagram showing stable sulfur compounds in aqueous solution at different redox potential. pH calculated for the sulfate, iron and sodium ion concentrations $[SO_4^{2-}]_{tot}$ = 350 mM, $[Fe^{3+}]_{tot}$ = 50 mM , $[Na^+]_{tot}$ = 400 mM, and t = 25 °C. The slowly forming iron(III) oxides have been omitted.*

5.3 The sulfur cycle in marine archaeological wood

As discussed above, SRB are producing hydrogen sulfide from sulfate in anaerobic marine environments, e.g. seabeds. The hydrogen sulfide reacts with wood components or iron(II) and accumulate in waterlogged wood. A Pourbaix diagram of sulfur for conditions similar to those at the seabed of the Stockholm harbour (Figure 40) indicates that hydrogen sulfide cannot be the thermodynamically stable sulfur form at the approximate pH and redox potential of the wreck site. At the oxygen deficient seabed (with redox potentials close to the lower dashed, sloping line) elemental sulfur could be the stable form, and with sufficient amount of iron present pyrite may be coexisting. The upper dashed sloping line in the diagram represents the oxidising conditions in contact with air, where sulfate is the stable sulfur form. When the slowly forming iron(III) oxyhydroxides and oxides are omitted, the natrojarosite salt can form.[II] The sulfur cycle is completed.

5.3.1 Sulfuric acid production in shipwrecks

When the reduced sulfur compounds in the moist wood of the shipwreck came into contact with oxygen in the museum environment, oxidation processes started and sulfuric acid could be produced. According to the Pourbaix diagram the sulfate ions and the solid natrojarosite are the most stable sulfur species in the current conditions, when the slowly forming iron oxides are excluded (Figure 40). The oxidation of elemental sulfur may occur according to the overall redox reaction:

$$S(s) + \tfrac{3}{2}O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-} \qquad (2)$$

Normally the oxidation of elemental sulfur to sulfuric acid is quite slow and must be catalysed.[110] Also, organosulfur compounds in lignin, such as thiols and disulfides, might be fairly stable. However, the small particles of iron sulfides, which were found to be present, e.g. in the Mary Rose wood, are not stable in humid conditions and will oxidise fairly rapidly with oxygen access. The oxidation of such iron sulfide particles might be responsible for most of the acid present in the wood.[IV,V]

Bacterial enzymes can be very efficient and selective catalysts, but the investigations for the *Vasa* have so far not been able to prove any significant microbial bacterial activity at present in the timbers.[51] On the other hand, iron ions are well known for catalysing oxidative reactions in humid environment.[2,II] It seems likely that the large amount of iron (Chapter 4.1) distributed in the *Vasa's* timbers could catalyse several types of oxidation processes in the humid wood.

5.3.2 Pyrite oxidation

In the 1960s, the essential role of bacteria in the oxidation of pyrite, especially in coal, had been well established. It has been proposed that pyrite oxidation is catalysed primarily by bacteria of the genus *Thiobacillus*.[111]

$$FeS_2 + {}^{15}/_4\, O_2 + {}^{5}/_2\, H_2O \rightarrow FeOOH + 2H_2SO_4(aq) \qquad (3)$$

Pyrite oxidation, as also oxidation of other sulfides, gives rise to formation of sulfuric acid, which is completely dissociated in aqueous solution and forms sulfates with available metal ions. The solid end products of the pyrite oxidation could in the case of the *Vasa* be natrojarosite; $NaFe_3(SO_4)_2(OH)_6$, or goethite; α-FeOOH,[111] but most likely without bacterial catalysation.[51] Such non-bacterial pyrite oxidation reactions have been described by Lowson.[112]

5.4 The sulfuric acid formation

The total amount of sulfuric acid being formed within the timbers of the *Vasa* can be estimated in different ways. A 3:7 mixture of borax ($Na_2B_4O_7{\cdot}10H_2O$) and boric acid ($B(OH)_3$) was used as fungicide in the recirculating PEG spray-solution during the conservation treatment (Chapter 2.5). The concentration (calculated as boric acid) varied between 1 and 4 mass%. The pH of the recirculated PEG solution was monitored and it was found that the acidity increased steadily by time. The Conservation Council of the Vasa Museum discussed the reason for this continuous decrease in pH and it was suggested: "This might indicate oxidation and possible decomposition of the PEG molecule or that acid products have been dissolved from the wood of the hull". It was decided to add more borax to neutralise the acid, and 5 tonnes of borax were added during the period 1965-1977 to maintain the pH at about 7.5 in the recirculating PEG-liquid.[18] One probable source for this pH decrease would be the oxidation of iron sulfides or reduced sulfur compounds to sulfuric acid, which started as soon as the wood came in contact with oxygen after the salvage. The alkaline borax neutralised the acid, through this huge pH-titration, to boric acid and dissolved sodium sulfate; $Na_2SO_4(aq)$, which was washed away. The 5 tonnes of borax would be sufficient to neutralise 1.3 tonnes of sulfuric acid, which in turn makes it possible to approximate the rate of the sulfuric acid production during 1965-1977 to about 100 kg $H_2SO_4(aq)$ annually.[II]

From this estimate follows that if the sulfuric acid production did continue at the same rate after the conservation spray treatment ceased in 1979, then about 2.5 tonnes of acid would now be present in the wood of the *Vasa*.[II] Due to lower moisture ratio in the wood and possibly lower access of oxygen after the final PEG 4000 treatment of the surface, the rate of sulfuric acid

production probably has decreased.[V] In addition, there is a possibility that e.g. formic and acetic acid also may have formed, due to PEG-oxidation.[17] However, the estimated amount of acid that could have accumulated in the wood after the washing by the spray treatment was stopped in 1979, seems nearly consistent with the estimated amount of sulfate in the wood from the XANES-analyses of the core samples (Chapter 3.2.1).[II]

The measured XANES spectra indicate that about 20-25% of the total remaining amount of sulfur (estimated to 2.5 tonnes S(s) from the elemental analyses (Chapter 3.4), is present as sulfate in the surface layer of the Vasa wood. If about 0.7 tonnes of (elemental) sulfur S(s) would be transformed to sulfate, this is equivalent to a formation of 2 tonnes of sulfuric acid. The large amount of reduced sulfur still remaining unoxidised in the Vasa wood could theoretically produce an additional 5-6 tonnes of acid if fully oxidised.[II] However, the different types of reduced sulfur should have different rates of oxidation, and possibly those (iron sulfides) oxidising most rapidly are already largely consumed (Chapter 3.7.1).[V]

5.4.1 Volume expansion at crystallisation

The transformation to hydrated sulfate salts (Chapter 2.6) from reduced sulfur compounds such as iron sulfides or pyrite often involves a large volume expansion of about 5-10 times per sulfur atom, which theoretically could cause severe mechanical damage of the fragile wood. Still, the volume expansion has not so far caused any major problems since the soluble salts generally have been transported through the PEG-layer to crystallise at the wood surface when the water evaporates at reduced relative humidity. One exception with low solubility is gypsum, which could crystallise inside the wood in the presence of calcium and build up expanding crystalline aggregates.[II] Sulfate salts has sometimes been found underneath the PEG 4000 on the outside of the Vasa hull and upon objects in the magazines, and there are some examples where the surface wood has detached.[V] The volume per S-atom in gypsum, $CaSO_4(H_2O)_2$, is 124 $Å^3$, compared to the volume 52 $Å^3$ per S-atom of orthorhombic α-sulfur; S_8. The volume per S-atom of other sulfate salts is for pyrite; FeS_2: 20 $Å^3$; rozenite; $FeSO_4 \cdot 4H_2O$: 163 $Å^3$; melanterite $FeSO_4 \cdot 7H_2O$: 244 $Å^3$, jarosite; $NaFe_3(SO_4)_2(OH)_6$: 133 $Å^3$.[VI]

5.5 Acidic hydrolysis of cellulose & wood degradation

Large accumulation of sulfuric acid in the wood would eventually become a serious problem since high acidity results in acid hydrolysis of the cellulose chain, which gradually might reduce the mechanical strength of the wood.[113] The catalytic degradation starts with a proton (H_3O^+) attack at the glycosidic linkage between the oxygen and carbon atom number 1. When a water mole-

cule binds to the carbon atom 1 in a nucleophilic reaction, another proton is released (Figure 4). The rate of cellulose hydrolysis depends on pH, temperature, humidity, iron presence, the species of wood, etc.[87] On the other hand, in laboratory experiments, where milled ancient soft- and hardwood were exposed to 3% sulfuric acid at 15-25°C for 3600 hours, only hemicellulose and not cellulose were solubilised, while cotton linters suffered severe loss in DP (degree in polymerisation).[113] Still, the situation may be different in the iron rich matrix of humid, PEG treated marine archaeological wood.[V]

It is still not known how fast the *Vasa's* wood is deteriorating in ambient conditions. There are soft, degraded areas in the wood, and generally the salt affected areas feel softer, sometimes with a brittle surface layer when pin-pricking the wood.[II,V] EB decay extends only to the outer 5 to 15 mm of the wood,[51] but so far the above described degradations cannot clearly be distinguished as being either the result of bacterial actions or acid hydrolysis (or both). Further experimental investigations are needed to predict how rapidly the destabilisation of marine archaeological wood would continue under ambient conditions.[V] Size exclusion chromatography (SEC) analyses on Vasa samples showed the presence of organic acids, which was interpreted as signs of hydrolytic or oxidative degradation of the holo-cellulose in inner parts of the wood. Still, some preliminary mechanical tests using High Energy Mechanical Impact (HEMI) did not indicate any substantial loss of mechanical strength in the wood.[51]

For the *Vasa* the sulfur accumulation is confined to the outermost 1-2 cm surface layer of the hull (Chapter 3.5). The inner wood of the *Vasa's* hull timbers is not severely sulfur contaminated and in that respect not at any immediate risk.[V] Within the surface layer of the Vasa wood, a reduction of the local mechanical stability of the wood structure has already occurred. The S2-layer, which constitutes the major part of the cell wall,[114] has with its high cellulose content been the primary target of the EB, probably followed by the SRB (Chapter 1.6).[34] Further degradation may occur chemically by iron catalysed oxidation processes,[3,17] and by acid hydrolysis due to the accumulated contaminants, but may not be of immediate concern for the stability. However, the ion-conducting property of the bulking PEG agent in the wood, together with humidity fluctuations, would certainly distribute an increasing acidity also to parts of the wood without bacterial degradation and sulfur contamination.[VII]

5.6 Remedies; acid neutralisation in wood

The *Vasa*, with its present atmospheric exposure and obvious signs of chemical processes, will most likely need renewed conservation treatment at some stage. However, any (re)conservation treatment should be well moti-

vated and thoroughly tested. The methods should be adapted to the species of wood and the state for each object and applied with great care to minimise the strain on the weakened wood. The principles of conservation ethics must also be considered (Chapter 1.3.2). The most desirable conservation procedure would have been to leave the sulfur and iron compounds as found, and preserve the archaeological artefact in an indoor environment that slows down the deterioration reactions sufficiently. However, in the present case the amounts of contaminants with possible long-term detrimental effects constitute a risk for irreversible damage, even though the rate of deterioration is as yet not known under ambient conditions. The acid already formed, now present in the *Vasa's* wood, should be neutralised prior to other consolidation treatments. Renewal of the spray treatment for the *Vasa* would be stressful, especially for the fragile surface layer, and is also technically a major undertaking.

5.6.1 Bicarbonate & soda treatments

The accessible wooden surfaces inside the *Vasa's* hull displaying acidic sulfate salts, were during 2001-2004 treated with an alkaline solution (pH ≈ 9) containing 5% bicarbonate ($NaHCO_3$) and soda ($Na_2CO_3 \cdot 10H_2O$) in the weight ratio 7:3. The surface pH increased temporarily, but many of the treated wood surfaces reverted to pH ≤ 3 within a few weeks or months.[II] The troublesome and time-consuming treatment, carried out by applying wet poultices or by hand spraying, also removed some of the PEG, which left the wood surface with a dry and fragile appearance. In retrospective, it would probably have been beneficial to add PEG to the bicarbonate-soda solution. The rather inefficient treatment, which beside adding mechanical stress to the sensitive surface layers also increased the humidity inside the ship, was therefore abandoned in 2004.[V]

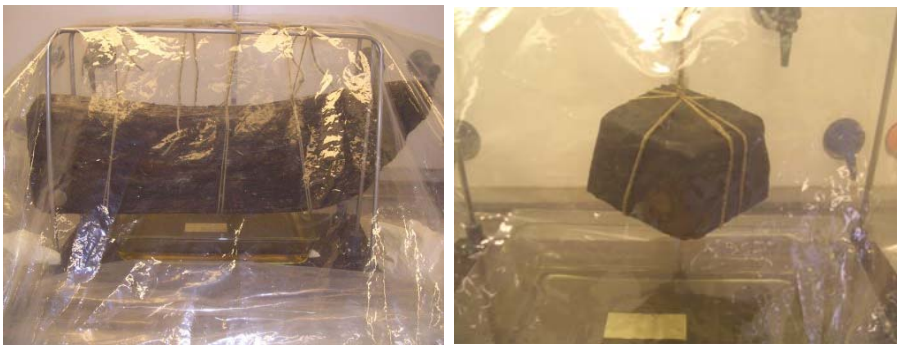


Photo: Yvonne Fors

Figure 41. *Glove bag treatment of Vasa oak with concentrated ammonia. The wood is suspended in strings over the evaporating ammonia for full exposure during 24 hours. To avoid marks on the wood surface soft and flexible hemp strings were used.*

5.6.2 Ammonia treatments of Vasa wood

Ammonia gas treatments have been shown to efficiently neutralise acid,[115] and would mechanically be a more gentle method to treat PEG-conserved archaeological wood than by applying wet poultices. Moreover, all wood surfaces would be easily accessed, including the hidden surfaces within the hull (Chapter 3.6.1). Both gaseous ammonia and saturated ammonia solutions proved to be effective in changing the surface pH of some acidic Batavia timbers at the Western Australian Maritime Museum. The acidity of the Batavia wood decreased dramatically after 24 hours of ammonia exposure. The pH-value, which was about 6 after the treatment, reached within about 25 days a plateau at around pH 4, and was after several years reported to be stable. The impact on the wood was described to be limited to a slight loss of hemicellulose.[69,116] This result is consistent with earlier studies, which mostly show degradation and possibly reorientation of partially solubilised hemicelluloses within the cell wall upon ammonia treatment.[117]

Guided by the positive experiences from the Batavia experiment,[116] a series of Vasa samples was set up to expose the wood to ammonia vapour from a concentrated aqueous solution (28 w/w, 31.8 g/L NH_3 puriss, VWR) at ambient temperature and pressure. The purpose was to study the post-treatment long-term stability of the pH, and the possible effects on both the wood structure and the PEG.

Oak and pine wood, both with and without acidic sulfate salt outbreaks, were used in the experiment as well as an untreated (no PEG) Vasa oak sample, together with fresh pine and oak wood as references. The wood was sampled prior to the ammonia treatment and the pH measured on the surface but also further down to the middle of the wood samples through stepwise drilling.[VI] The experiments were carried out in glove bags with the wood suspended in hemp strings over vessels with concentrated ammonia (Figure 41). The ammonia vapour exposure of the Vasa wood was, as in the treatment of the Batavia timbers, carried out for 24 hours. Concerns were raised that ammonia treatments would seriously decrease the crystallinity of the cellulose. Therefore, wood pulp was exposed to ammonia vapour in two additional experiments, for 24 hours and 14 days, respectively, with the same procedure as for the wood and then analysed with solid state ^{13}C-NMR. Also samples of PEG solutions were exposed and later analysed by MALDI-TOF/GC-MS for signs of degradation.[VI]

The surface pH of the acidic Vasa wood samples increased upon the 24 hour ammonia exposure from initial pH-levels around 3 to pH ~ 8, but had not after 103 days stabilised at a final plateau value. No significant difference could be noticed between oak and pine samples in the test. The pH-profiles throughout the wood of the samples were monitored digitally at different

depths before and after the treatment, but the data were inconsistent. The differences in the results can partly be related to the inhomogeneous sulfur and acidity distribution in the wood (Chapter 3.6.1), but also to the limited accuracy in the pH-monitoring methods. The pH-profiles for the reference fresh pine, fresh oak and untreated Vasa oak (no PEG) are generally more uniform throughout the wood than those of the PEG-treated Vasa samples (Figure 42).[VI]

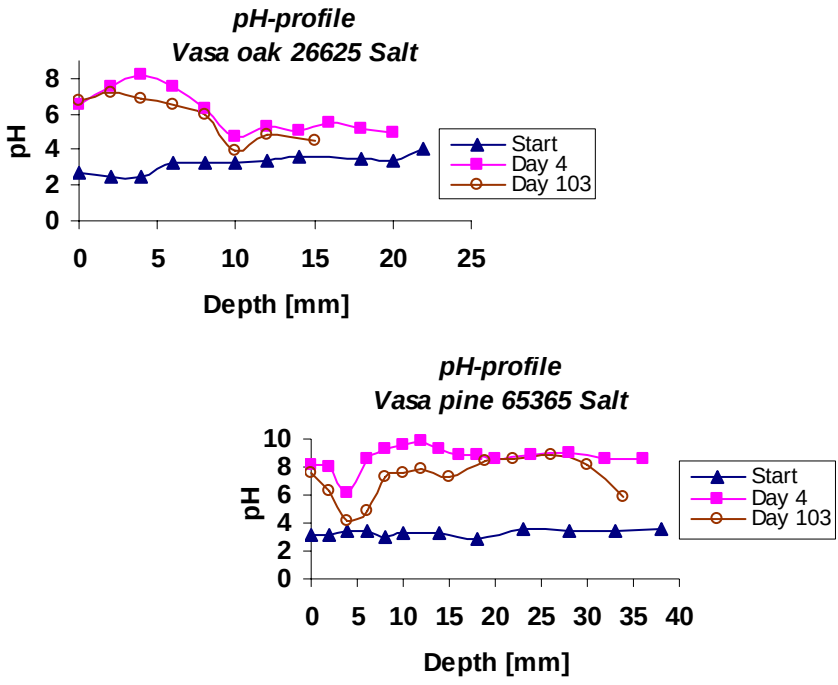


Figure 42. *pH-profiles through the wood before and after the 24 hours ammonia exposure.*

The colour of the sulfate salts on some of the wood surfaces changed after the ammonia exposure from yellow to characteristic red-brown. XRD-analyses could identify iron hydroxy oxides and also some ammonium salts, e.g. the Fe(III)-salt ammoniumjarosite; $(NH_4)Fe_3(SO_4)_2(OH)_6$, probably re-crystallised from natrojarosite. Also, mohrite and mascagnite; $(NH_4)_2SO_4$, were found most probably formed from hydrated Fe(II)-sulfates. Some of the already known sulfate salts, e.g. natrojarosite and melanterite, could still be identified after the ammonia treatment. Damaging volume expansion from the new salts is not to be expected considering that the volume factor is small compared to when pyrite oxidises to rozenite and melanterite. No significant cracking related to the ammonia exposure was noticed in the samples.[VI] According to Schuerch and coworkers swelling and warping during

ammonia treatments may be controlled by a rapid and uniform penetration, and shrinkage and cracking during drying by slow evaporation.[118]

For a thorough evaluation of the effect of ammonia exposure on the Vasa wood samples several chemical and mechanical tests are required, of which some still are in progress. However, the preliminary results from the ammonia treatment of Vasa wood are promising. According to the MALDI-TOF/GC-MS-analysis, no major degradation of the PEG was found even after 4 weeks of exposure. One remaining issue is if the ammonia treatment reduces the crystallinity of the cellulose, as proposed in the literature,[VI] and thus the stability of the wood. The results so far indicate that this is not the case for the present treatment. No major degrading effect was observed for pulp cellulose by means of solid state ^{13}C-NMR analysis.

There are a number of literature reports of tests performed at elevated temperatures and pressure and thus not for conditions relevant for the *Vasa*.[VI] According to literature references ammonia treatments may result in plasticisation and changes in sorption behaviour related to structural changes within the cell wall,[119,120] but the degree of plasticisation strongly depends on the ammonia content in the wood.[121]

Further chemical and mechanical testing should be performed to assess the effect on the stability and mechanical strength of the wood as a function of the degree of exposure. The current experiment can only be considered a first step toward finding a proper treatment. A large scale ammonia treatment of the entire hull would require enclosing the *Vasa*, e.g. with gastight canvas in a “tent”. The practical and technical feasibility of such a project needs to be evaluated.[VII]

6 EXPERIMENTAL METHODS

Most of the instrumental methods used in this work are based on various applications of x-rays for analytical purposes (Figure 43). X-ray powder diffraction (XRD) can be used to identify crystalline compounds, such as the salts precipitating on the surfaces, while x-ray fluorescence techniques (XRF) and also x-ray photoelectron spectroscopy (XPS) or Electron Spectroscopy for Chemical Analyses (ESCA) can provide multielement distributions of the total amount of an element. Laboratory sources of x-rays are convenient and sufficient in most cases for such analyses, but the extremely bright x-rays from a synchrotron source allow more sensitive analyses of small amounts of samples, which often are advantageous for archaeological wood samples. However, the sulfur spectroscopic analyses (XANES), to obtain specific information on the chemical state and bonding of the sulfur functional groups in natural samples, can only be performed with special purpose-built instruments at the brightest synchrotron sources. That instrumental development has made possible the speciation of sulfur compounds, even on microscopic level (SXM) in wood in the current study, and opened up a new level of understanding of the processes in the natural sulfur cycle.

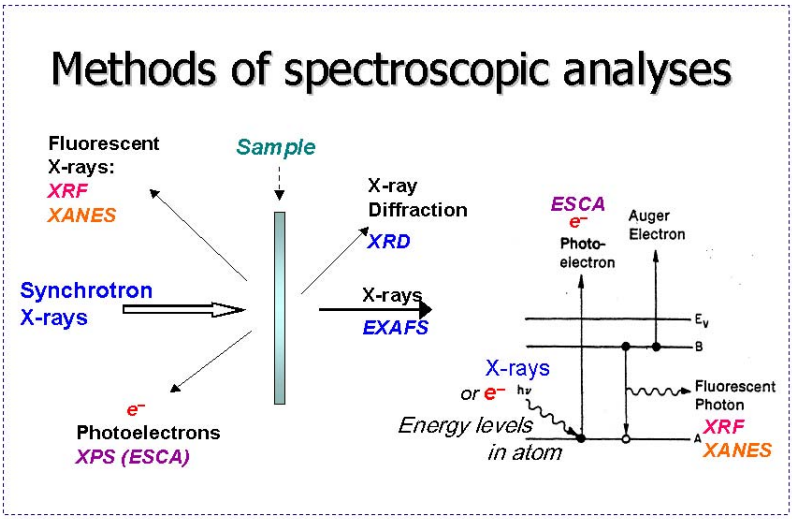


Figure 43. *Scheme of principles for some x-ray analytical methods used.*

6.1 X-ray powder diffraction (XRD)

Crystalline compounds, such as the precipitated salts, were identified by means of x-ray diffraction. For this purpose a Guiner-Hägg camera with focused monochromatic Cu Kα_1 x-ray radiation, 1.5406 Å (or in a few cases Cr Kα_1, 2.2897 Å, in order to reduce fluorescence from iron) was used. The film data were digitalised and the background eliminated by means of a spe-

cially constructed scanner. Spectra were obtained in the 2θ range 10 to 87 degrees. This method is suitable for small amounts of sample, and provides high resolution with sufficient accuracy to allow identification of crystalline components by computer search in the powder diffraction (PDF) database. No internal calibration compound (such as pure silicon Si) was necessary, which would further complicate the already line-rich spectra. XRD measurements were also tested on powdered wood samples at beamline I711 at MAX-lab in Lund, in search for iron(II) sulfides within the wood, but without success.

6.2 X-ray photoelectron spectroscopy (ESCA or XPS)

For quantitative multi-element analyses (except hydrogen) including sulfur analyses of fair accuracy, x-ray photoelectron spectroscopy (XPS) was applied. Thin slices of about 0.3 to 1.5 mm were cut from the wooden cores at various depths, mounted on tape and transferred in vacuum into the sample chamber of a Scienta ESCA 300 instrument. Monochromated Al K_α x-ray radiation (1487 eV) excited core photoelectrons from all elements in the sample. A sample area of approximately 4x2 mm was irradiated and a mask of aluminium foil was used to prevent contributions from the sample holder. To reduce the vapour pressure (mostly water) from the samples down to levels for which measurements were possible, at least one hour pumping is required. The time depends on how much water and, in this case, PEG (prevents evaporation of the water) the sample contains. The pressure was 10^{-8} mbar when starting the measurements, and often at $4 \cdot 10^{-9}$ mbar at the end of the analyses.

The bonding energy of the core electrons was obtained by measuring the kinetic energy of the photoelectrons from atoms of all elements in the samples, ejected by the x-ray radiation of known energy. The extreme surface sensitivity of electron spectroscopy requires careful sample handling to avoid contamination, and for PEG rich samples smearing when cutting may affect the quantitative results. To prevent uncontrolled charging of the electrically insulating samples the surface potential of the sample is kept constant by an excess current of low energy electrons (1 eV) supplied by a Scienta flood-gun. Only after an extended long exposure the signal for the oxidised sulfur showed a small reduction; this indicated that radiation damage was negligible during the normal XPS measurements.

The energy resolution of the S 2p photoelectrons allowed a quantitative distinction between reduced and oxidised sulfur compounds in the samples. The amounts were determined by considering three different sulfur components in a least squares fitting procedure, representing each sulfur component by two slightly asymmetric Gaussian peaks with relative intensity 1:2, spin-orbit split by 1.18 eV, and with the same full width at half maximum.[IV] In a similar way the C_{1s} lines (of which the well-resolved $C_{1s}(CH_2)$ line, assumed

to be at 285.0 eV, was used for charge-calibration) could be used to estimate the relative amounts of lignin and cellulose components in the wood slices (Chapter 3.3.1).

6.3 X-ray absorption near edge structure (XANES) spectroscopy

The speciation of sulfur compounds in some surface and core samples was investigated by means of sulfur K-edge x-ray absorption near-edge spectroscopy XANES at atmospheric pressure (1 atm He) with a specially designed instrument at the wiggler beam line 6-2 in Stanford Synchrotron Radiation Laboratory (SSRL). The SPEAR synchrotron of SSRL operated at 3.0 GeV and a maximum current of 100 mA. The energy of the very intense x-rays from the wiggler beam line was varied using a Si (111) double-crystal monochromator and a nickel-coated mirror to reject higher order harmonics of the x-rays passing through the monochromator. The x-ray intensity incident on the sample was monitored using a helium-filled ion chamber (I_0). All the beam-path is through vacuum or helium, and the sample was held in helium at atmospheric pressure. At the sulfur K-edge (~2.47 keV) a core electron (1s) is exited, and the "empty hole" then created in the energy level is filled very rapidly and fluorescence radiation is emitted, which is detected in the XANES spectra (see below). Also secondary electrons, so-called Auger electrons, are emitted. The x-ray fluorescence, which occurs after the emission of a photoelectron, is proportional to the x-ray absorption in the sample, and was used for the detection. The fluorescence was measured by means of a Stern-Heald-Lytle fluorescent ion chamber detector without filter or Soller slit. The energy scale was calibrated before every measurement against the lowest energy peak of a sodium thiosulfate standard, $Na_2S_2O_2{\cdot}5H_2O$, set to 2472.0 eV at the peak position. The relative amounts of the different types of sulfur compounds in a sample can be obtained by fitting the normalised spectra, but overlap and absorption problems can make quantitative analysis difficult. The position and shape of the sulfur K-edge, i.e. the energy needed for releasing a photoelectron, is sensitive to the oxidation state and can be used to identify the type of chemical surrounding of the sulfur atoms down to low concentrations (~0.01 percent) in wooden samples.

The energy resolution of the spectra is 0.5 eV, and the precision of the relative peak positions better than ± 0.05 eV. All data were collected at room temperature. Core samples were handled in an inert atmosphere. A nitrogen-filled glove box was used for dry samples, and an argon-filled glove bag for wet samples. Each 4.2 mm diameter core was sectioned at various depths into 2 mm sub-samples, which were filed or ground in an inert atmosphere, and the powder was mounted in a thin layer on sulfur-free tape Mylar tape on an Al-frame and covered by a polypropylene film.

6.4 X-ray fluorescence spectroscopy (XRF)

An Itrax wood scanner from Cox Analytical Systems was used to obtain concentration profiles of total sulfur and iron by automatic line scans along cores.[80] Focused Cu K_α x-rays (1.5420 Å) from a conventional x-ray aggregate was used to excite x-ray fluorescence by means of a special collimator utilizing total reflection to obtain a condensed high intensity parallel beam with a diameter of ca 300 μm, and an analytical depth of about 0.1 mm into the woodAn energy dispersive solid-state x-ray detector was used to monitor the element specific x-ray fluorescence, with 0.5 mm between the scan points, with 1 mm resolution and an analytical depth into the wood of about 0.1 mm. Quantitative intensity calibration was performed by measuring standard sample pellets of FeO and $Fe_2(SO_4)_3 \cdot 5H_2O$ with known concentrations mixed into a matrix of milled oak wood and PEG.

6.5 Scanning electron microscopy (SEM / EDS)

Scanning electron microscopy (SEM), equipped with energy dispersive x-ray fluorescence detection (EDS), was used to map sulfur and iron ratios and distributions in cell walls and particles, with a resolution of about 1 μm. We used a JEOL 829 SEM instrument, equipped with a LINK AN10000 EDS microanalysis system. The samples were coated with a thin graphite layer by sputtering and mounted on sample holder with sulfur free tape. A low-grade vacuum is maintained during measurements. This method is capable of distinguishing different elements, but not oxidation states. Quantitative measurements relative to other elements are possible from sodium to heavier elements.

6.6 Scanning x-ray spectromicroscopy (SXM)

Scanning x-ray spectro-Microscopy at the beamline ID21 at the European Synchrotron Radiation Facility (ESRF), Grenoble provides the high energy and spatial resolution, needed for detailed information of the sulfur speciation and distribution in the wood structure. It would also be possible to distinguish between iron compounds in oxidation states +2 and +3, but that was outside the scope of the present investigations. In this specially constructed instrument the x-ray beam from the storage ring first passes through a double crystal monochromator and is focused on the sample by a zone plate objective and a pinhole aperture. A small area, max. 100x100 μm^2, of the sample is raster scanned with a beam size of approximately 0.5 μm. A photodiode is used to detect transmitted x-rays, and a Ge-detector registers the emitted x-ray fluorescence. A low-grade vacuum, $\sim 10^{-4}$ mbar, is maintained in the instrument, while the sample is enclosed between 4 μm sulfur-free Ultralene films, and is not exposed to vacuum. Very thin transverse slices from the archaeological wood were required for transmission (Chapter 1.5.2).

6.7 Elemental analyses

Analyses were performed of the total sulfur content with depth in core samples from different locations in the *Vasa's* hull. Mikrokemi AB in Uppsala carried out the elemental (sulfur) analyses, with the following method.[79] The sample (a few mg) is weighed in a tin-capsule. When oxygen is injected the temperature momentarily rises to about 1800 ^{o}C when the tin metal oxidises. The combustion gases CO_2, H_2O, NO_x, SO_2 and SO_3 are led into a reduction chamber where SO_3 is reduced to SO_2. The amount of SO_2 is then determined by gas chromatography. This type of analysis requires total sulfur concentrations over about 0.1 mass% for good accuracy; it is destructive and provides total sulfur concentration profiles with fairly low resolution.

7 CONCLUSIONS

7.1 Sulfur, iron & acid in marine archaeological wood

The discovery in 2001 that large amounts of reduced sulfur had accumulated in the Swedish warship *Vasa* (1628), and also in other marine archaeological artefacts, opened the door for new possibilities of cooperation and exchange of knowledge between conservators and chemists, as also an exciting opportunity to new multi-disciplinary research. The analyses of the accumulation mechanisms and the nature of the sulfur and iron compounds in wood, made possible by the development and application of new scientific methods, shed new light and were able to explain the previous and present observations of some conservation problems of marine archaeological wood.

The source of the sulfur and iron contaminants has been traced to the conditions on the seabed where bacterially *in situ* produced hydrogen sulfide; H_2S, reacts with lignin or with iron ions; Fe^{2+}, and accumulates in bacterially degraded wood. Speciation of the different reduced sulfur compounds could be performed by means of synchrotron-based spectroscopic sulfur analyses (XANES). Scanning x-ray spectromicroscopy (SXM) studies were used to map the location of reduced and oxidised sulfur compounds in the wood structure, complemented by micro-XANES studies of sub-micron particles and areas. From those studies two pathways of accumulation and oxidation processes involving the reduced sulfur compounds could be proposed and elucidated:

1. The main parts of the *Vasa's* and the *Mary Rose's* (U.K.) total amount of sulfur are strongly bound to lignin components, and located mainly in the middle lamella and the cell corners in the wood structure. This type of organically bound sulfur starts out as thiols; R-SH, and is rather inaccessible for treatments but also not readily oxidised unless the wood is strongly degraded. The thiols seem to easily connect to disulfides, then sulfoxides and finally sulfonates may form, mostly at exposed surfaces.[74]

2. The inorganic iron(II) sulfides, including pyrite; FeS_2 are distributed as small particles in wood cavities, are unstable in high humidity and oxidise to sulfuric acid. The iron sulfides form elemental sulfur as a byproduct in a fairly rapid oxidising process and are the most immediate concern towards formation of sulfuric acid in the wood.[74] The acid and sulfates are being washed out of the *Mary Rose's* timbers in the ongoing spray treatment, while the amount of iron sulfides remaining in the *Vasa's* wood is not yet known.

The released iron ions are efficient non-selective oxidation catalysts in humid conditions in so-called Fenton reactions,[59] which together with acid hydrolysis of the cellulose, may degrade the wood and reduce its mechanical stability.[87,88] Further investigations of the relative quantities of these sulfur and iron compounds are needed, as well as more information about their reactivity in the present conditions.

The former conditions of the *Vasa's* wreck site were assessed with the help of data from *Stockholm Vatten*, which indicate that the water at least at lower depths were occasionally nearly depleted in oxygen and contained high amounts of hydrogen sulfide. To some extent this foul environment preserved the wood from microbial degrading attacks but also initiated the challenges addressed in this thesis. The studies of the wreck site conditions gave inspiration to laboratory experiments, where samples of fresh pine wood were exposed to bacterially produced hydrogen sulfide in solution under simulated seabed conditions (shipwreck environment). The results from the experiments revealed and confirmed some steps of the proposed mechanisms behind the sulfur accumulation. Analyses by XANES and SXM of the exposed pine wood showed the same accumulation pattern in the wood structure as in the previous observations for the authentic marine archaeological wood, with high concentrations of reduced sulfur primarily as thiols (R-SH) in lignin-rich parts. The distribution of phosphorous, indicating the presence of bacteria in the wood structure, is similar to that of reduced sulfur and supports the hypothesis that the hydrogen sulfide is produced *in situ* in the waterlogged wood by scavenging sulfate reducing bacteria (SRB). Also the fractionation of the sulfur isotopes in a Vasa oak sample supports bacterially mediated reduction of the accumulated sulfur. The relationship between the erosion bacteria (EB) and SRB is not yet fully understood but since the SRB cannot degrade natural biopolymers,[42,43] they would depend on the remains of the wood degradation by the EB.

The biogenic accumulation of organically bound sulfur specifically in lignin-rich parts of waterlogged wood may have geochemical significance as a pathway for sulfur compounds to enter fossil fuels of marine origin, since lignin-rich humic matter is an important constituent in the diagenetic transformation to kerogens from anoxic marine sediments.[98,101] It would be of interest to perform separate studies of the reactions between hydrogen sulfide (HS^-) and different lignin components to evaluate the stability (and/or reactivity) of the organo-sulfur compounds.

From XRF line scanning of sulfur and iron concentration profiles it was concluded that the sulfur had accumulated almost entirely within the outer surface layers of the *Vasa's* wood (1-2 cm). This seems consistent with the depth of the EB penetration, which managed to degrade the first 5-15 mm[51] of the Vasa wood surface layer during the 333 years on the seabed of the

Stockholm harbour. In the case of the *Mary Rose* the EB have degraded the timbers all through and also the sulfur concentration profile is fairly uniform throughout the core samples. Despite the large differences in weight and size between the remaining shipwrecks of the *Vasa* and the *Mary Rose* the average total amount of sulfur is nearly the same, about 1 mass% in the degraded parts, or totally 2 tonnes sulfur. Comparisons of the various sulfur compounds in wood from shipwrecks preserved under different conditions show large variations in distribution and amounts, which can be related to special conditions at the wreck sites, and depend on the state of wood degradation.

The iron ions are expected to catalyse the oxidation process not only of the reduced sulfur compounds, but also of the wood components,[88] and there are also indications of PEG decomposition.[3,17] High iron concentrations often follow the sulfur levels rather closely, which was demonstrated by XRF line scanning along cores especially from the *Vasa*. This indicates that the origin would be iron(II) sulfides formed in the wood of the shipwrecks, even though a similar concentration profile may remain as e.g. iron sulfates after oxidation. It is not known how much of the reduced sulfur that presently remains as iron(II) sulfides, and by XRD (powder diffraction) only traces of pyrite has been found for the *Vasa*. The total remaining amount of iron(II) sulfides in the *Vasa's* wood is therefore a subject of interest for further analyses. Fe K-edge XANES spectroscopy should be able to distinguish between iron(II) and iron(III) compounds, and also between iron(II) sulfides and oxides.[122]

However, the amount of oxidised sulfur(VI) corresponding to the 2482-3 eV peak (mostly sulfates) is consistent with nearly the same continued oxidation rate of reduced sulfur to acid, after the conservation spray treatment was stopped in 1979. Comparable XANES-spectra from core samples of the *Mary Rose* indicate that sulfates are washed away efficiently during the spray treatment. Thus, a reasonable estimate of the current amount of sulfuric acid, formed after 1979 and now present in the *Vasa's* wood, seems to be about 2 tonnes. The oxidation of primarily the iron sulfides to sulfates (sulfuric acid) can clearly be followed in the development of acidic sulfate salt outbreaks on the surface of the Vasa wood, as well as on other famous shipwrecks such as the *Batavia* (Australia).[69] At the moment over 3000 precipitates with low pH (≤ 3.5) have been registered on the ship hull and on stored objects from the *Vasa*. X-ray powder diffraction (XRD) at an early stage showed the presence of natrojarosite; $NaFe_3(SO_4)_2(OH)_6$. High acidity might with time have detrimental effects on the wood by acid hydrolysis, and there are many areas of softened wood within the *Vasa*, especially at sulfate salt infested surfaces. Still, there is no clear evidence at present for acid hydrolysis in the Vasa wood. It is difficult to distinguish between deterioration from acid hydrolysis and the earlier bacterial degradation, especially since the sulfur contaminants largely follow the degradation pattern of the EB. Since

the distribution of the sulfur in the Vasa wood is limited to mainly the EB degraded surface layer the mechanical stability of the carrying inner parts of the timbers are not in that respect at any immediate risk. However, the acid is mobile due to the ion-conducting PEG, and acid may also be forming in the wood by iron-catalysed oxidation of the PEG.[17] Thus, further chemical degradation will certainly occur, although the rate with which the mechanical stability deteriorates is not known.

Laboratory tests have been carried out on how the acid can be neutralised with ammonia vapour, as earlier performed on Batavia timbers. The effects of the ammonia treatment on both the Vasa wood and the PEG have been studied. A MALDI-TOF/GC-MS-analysis did not show any major degradation effect on the PEG even after 4 weeks of ammonia exposure. Also, a solid state ^{13}C-NMR study showed no obvious degrading effect on pulp cellulose treated with ammonia. Further investigations are needed to check, as indicated in the literature, if the ammonia treatment could significantly reduce the crystallinity of the cellulose, and thus the stability of the wood. However, many of the literature reports concern tests performed at elevated temperatures and pressure and not for conditions relevant for the *Vasa*.

So far, the results from the ammonia treatment seem promising. The ammonia vapour would access the hidden surfaces within the hull and probably also deactivate the iron catalysis. However, the size of the ship will make an ammonia treatment a technically advanced task.

7.2 New conservation challenges

The unexpected amounts of reduced sulfur compounds in marine archaeological wood, and the connected acidity development in a museum environment, call for development, modification and perhaps extension of the present conservation methods. The preservation of such a complex material as in the *Vasa* will eventually make renewed treatments of some kind necessary. Future tasks must be, in collaboration with the foremost expertise available, to find, test and devise the most appropriate methods for continuing the conservation, and to recommend when and how the treatments should be applied. For devising specially adapted and lasting conservation treatments, it will be necessary to monitor the distribution, location and removal/deactivation of the acid-producing reduced sulfur and iron compounds within the wood. The conservation spray treatment of the *Mary Rose* that will continue for some years offers a timely possibility to deal with and learn about the behaviour of the sulfur and iron contaminants. There is no doubt that the historical, cultural and scientific values of the *Vasa* and the *Mary Rose*, as well as many other marine archaeological artefacts are so great that every reasonable effort should be made.

For the *Vasa* the factors or substances that accelerate the oxidation and degradation processes need to be investigated. The role of the humidity should be further looked into since it is evidently connected to the development of new sulfate salt outbreaks. The transport of ions, water and oxygen in the PEG impregnated timbers is limiting for the reactions occurring in the wood.

It is known that iron ions catalyse oxidative degradation of cellulose in a chain reaction involving free radicals and molecular oxygen.[59] Thus, cellulose in contact with rusting iron will oxidise to oxycellulose, and over time the wood may loose a considerable part of its tensile strength.[88] It seems therefore important not only to neutralise the acid, but also to remove the iron compounds from the wood. It will probably not be possible to prevent the remaining iron(II) sulfides from oxidising and eventually form acid in the moist PEG impregnated wood. Therefore, iron(II) sulfides should be removed or deactivated as far as possible. The chelates EDMA and DTPA have in tests been able to dissolve most iron containing precipitates, including jarosite.[90] Since EDMA will remove most iron(III) compounds, oxidation of pyrite will be facilitated. Therefore, an EDMA-extraction treatment would also speed up the removal of remaining iron(II) sulfides. However, the high pH and the rather long time needed for an efficient treatment required are serious concerns.[III,90] For the *Vasa's* hull a renewed spray treatment of the hull, apart from being technically a major undertaking, would dissolve the stabilising PEG and be stressful especially for the fragile surface layer of the wood.

It does not seem possible so far to extract or remove the lignin-bound organosulfur. The thiols and disulfides seem to be firmly bound in organosulfur aggregates, from which removal would not be possible without serious degradation of the wood structure. After neutralisation of the existing acid and removal and/or oxidation of non-stable sulfur compounds, it may be possible to stabilise the lignin-bound reduced sulfur in the wood, e.g. by creating a stable micro-climate. Further investigations would help to decide what stabilising conditions that are required to keep the organosulfur in the wood from reacting further, e.g. coating with high molecular PEG could possibly reduce oxygen access.

Even though PEG treatments are not completely reversible, they have become the preferred method for stabilising waterlogged wood after the *Vasa's* conservation, and the techniques have developed with time.[66] Concerning the long-term stability of the PEG, little research has yet been done. However, there is an inherent risk for decomposition due to iron-PEG interactions.[3,17]

Among alternative treatment methods, the use of sugars has sometimes provided better dimensional control than PEG, but not much is known about the long-term stability in waterlogged wood. Sugars give the wood a "natural"

appearance and are relatively inexpensive, but there is a risk of post-treatment microbial attacks.[8] Impregnating the wood with non-hygroscopic silicones, has after polymerisation given results with satisfactory visual appearance of the objects,[123] but the treatment is unfortunately irreversible. Most conservation treatments intend to bind the bulking chemicals, such as the PEG to the polysaccharides, i.e. the cellulose, in the wood. Since the degradation of marine archaeological wood primary concerns the hemicellulose and cellulose, it would seem more appropriate that future conservation research should focus on finding a method where the chemicals primarily bind to the lignin.[6]

Still, any retreatment and reconservation must be considered stressful, and any proposed method must be carefully evaluated before being applied in a large scale treatment. One of many tasks for a conservator is to "arrest deterioration" of the object, but also to "repair damages, whether biological, chemical or mechanical".[124] The contaminants of the objects can be considered as a part of their history. Thus, their removal would also remove some part of the history and characteristics of the object. On the other hand, that loss should be balanced against the probable long-term detrimental effects of the contaminants.

In the present case, the most desirable conservation action would be to leave the sulfur and iron compounds in place and try to find a way to preserve the objects as found. For example, oxidation processes may be arrested by an inert oxygen free atmosphere or environment. Could this be done in practice? There is no general answer to that. It will always be necessary to device a method for every unique archaeological object, with regard to its state, history and purpose of the preservation, and how the object should be handled and exhibited.[10]

With this in mind, there are still many questions that need to be answered before any continued treatment of the *Vasa:*
- What are the relative quantities of the reduced sulfur and iron compounds in the wood and how reactive are they in the present atmosphere?
- How rapidly and in what way do acid and iron compounds cause decomposition of the wood?
- What suitable methods can be devised to remove acid and to prevent or delay new acid to form?
- How can the reduced sulfur and iron compounds in the wood of the *Vasa* be removed, contained or deactivated?

7.3 Conservation advice

An important issue is what we can learn from the *Vasa* and the *Mary Rose* that could be helpful in preserving other marine archaeological wooden ob-

jects. As a part of an ideal preparation for future conservation tasks it is recommended if possible to analyse core samples (before and during the conservation treatment) to reveal the amount and distribution of possible contaminations, especially the accumulation of iron and sulfur compounds. The conservation procedure should then be adjusted accordingly.

All marine archaeological shipwrecks preserved in seawater that we have analysed show accumulation of sulfur and iron compounds in various amounts. This seems to be the natural consequence of the ubiquitous sulfur cycle carried out at the seafloor, in sediments and other marine environments. The only exception we have found so far is the *Bremen Cog*, which was preserved in river water with naturally low sulfate content.

The advanced spectroscopic methods described and used here are not generally available, but other useful methods are. Elemental analyses provide the amount of total sulfur. XRD-investigations are easily performed to identify crystalline sulfate and iron salts. An XRF scan along cores into the wood gives information about the sulfur and iron profiles (and other contaminants), which probably also show a fair correlation with the penetration of the EB that can be checked by light microscopy.

A stable climate, with small changes in temperature and especially a stable and low relative humidity (RH ~55% or lower), seem to be key factors in slowing down some of the processes. Fluctuations in the humidity enable migration of water, dissolved oxygen and salts, and will initiate different chemical reactions. Despite sulfur contamination in the Skuldelev Viking ships in Roskilde, Denmark the stable climate in the museum seems sufficient to prevent new outbreaks of sulfate salts. By reducing the changes in moisture content to a minimum, also the dimensional movement due to swelling or shrinkage of the wood will be minimised.[12] Surface coatings to reduce oxygen access could be useful, but must be permeable to water vapour.

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- The Swedish National Heritage Board (*Riksantikvarieämbetet, RAÄ*)
- The Bank of Sweden Tercentenary Foundation *(Stiftelsen Riksbankens Jubileumsfond, RJ*)
- The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (*Forskningsrådet för miljö, areella näringar och samhällsbyggande, FORMAS*)
- The Swedish Foundation for Strategic Research (*Stiftelsen för Strategisk Forskning, SSF*)
- The Swedish Agency for Innovation systems (*Verket för Innovationssystem, VINNOVA*).

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Yvonne Fors
Stockholm 2008-04-24

"Seldom, very seldom, does complete truth belong to any human disclosure; seldom can it happen that something is not a little disguised, or a little mistaken"

From Jane Austen's *Emma* (1816)

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