Sulfur and iron in shipwrecks cause conservation concerns

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Synchrotron-based sulfur X-ray absorption spectroscopy reveals considerable accumulation of organosulfur (*e.g.* thiols), pyrite and iron(II) sulfides in marine-archaeological wood preserved in seawater, *e.g.* for historical shipwrecks such as the *Vasa* and *Mary Rose.* In the museum, oxidation of the sulfur compounds in the presence of iron ions may cause severe acidity in the moist wood. This *tutorial review* discusses developments of conservation methods to remove acid and iron, and how to analyse and stabilise sulfur compounds in the wood.

1 Shipwrecks and conservation

Famous shipwrecks such as Henry VIII's favourite warship the *Mary Rose*, Portsmouth, UK (sank preparing for battle in 1545) and the Swedish warship *Vasa* (foundered 1628), are connected to dramatic historical events and constitute "time capsules" with numerous connected items preserved at one site from a specific occasion. They provide invaluable insight in the conditions of the contemporary society and the state of technology. However, long-term conservation of the waterlogged wood in various states of degradation requires careful analyses and proper chemical evaluation and treatment of the objects.

The conserved wreck of the *Vasa*, and the remains under conservation treatment of the *Mary Rose*,[1] recently have gained a new scientific interest (Fig. 1).[2,3] Modern analytical methods revealed that about 2 tons of sulfur in different reduced forms had accumulated in the timbers of both the *Vasa* and the *Mary Rose* during their time on the seabed. Synchrotron-based sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy, combined with microspectroscopic methods, localised organosulfur compounds (thiols and disulfides) chiefly in the lignin-rich middle lamella between the wood cells. In addition, particles of iron(II) sulfides and pyrite, FeS_2, have formed when iron(II) ions from corroding iron objects reacted with bacterially produced hydrogen sulfide in the waterlogged wood, and are together with elemental sulfur notably abundant in the partly mineralised timbers of the wreck of *Batavia* from 1629. By comparing the sulfur and iron distributions for the *Vasa* and the *Mary Rose* with that of the *Bremen Cog* (1380), which was preserved in river water and therefore almost sulfur-free, the mechanisms of the sulfur and iron accumulation are elucidated. After the established conservation procedures have been performed, oxygen access to the moist marine-archaeological wood can cause severe acidity by oxidization of the sulfur compounds, particularly in the presence of iron ions, as is occurring for the *Vasa*.

The *Vasa* capsized at the entrance of the Stockholm harbour on her maiden voyage, and is unexpectedly well preserved due to a combination of special circumstances.[4,5] The almost intact hull of about 1200 tons, mostly oak wood, was salvaged in 1961 from a depth of 33 m. For 17 years spraying with aqueous solutions of polyethylene glycol (PEG = $H(OCH_2CH_2)_nOH$, see Fig. 2 and below) was performed to replace water and prevent cracking when drying the waterlogged wood, in a

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Magnus Sandström obtained a PhD in 1978. He became Professor of Inorganic Chemistry in 1997 at the Royal Institute of Technology, Sweden and in 2000 Professor at Stockholm University, where he is currently Head of the Department of Physical, Inorganic and Structural Chemistry. In 2001 he became involved in the sulfur related problems of the Vasa and soon included other marine-archaeological shipwrecks. He is a member of the Vasa Conservation Council and is a project leader in the "Preserve the Vasa" project.

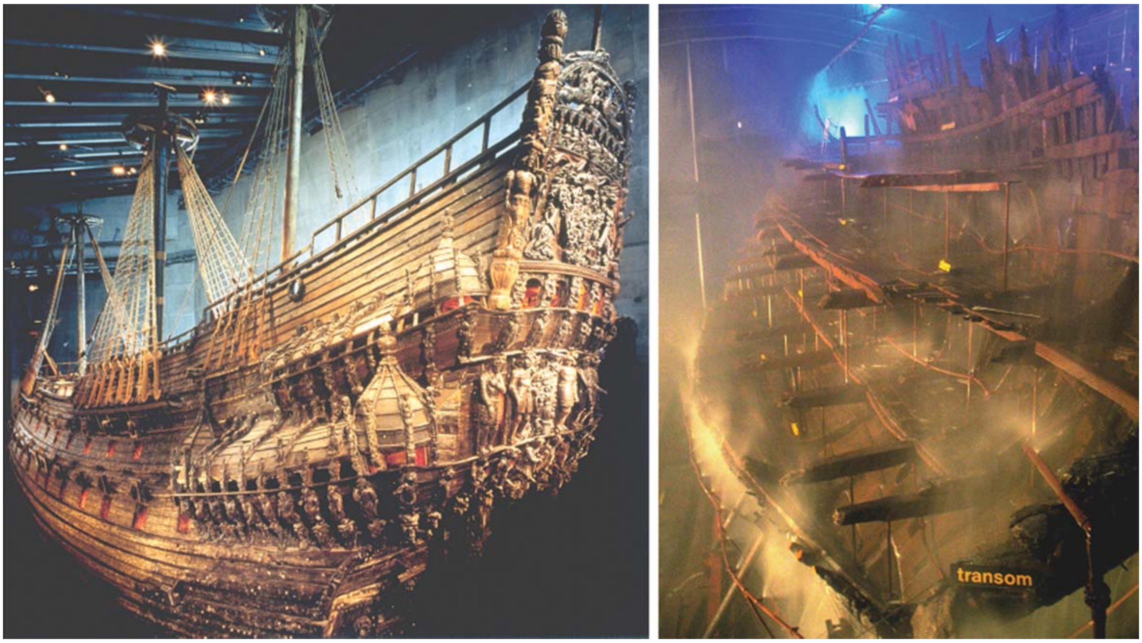

Fig. 1 (*left*) The *Vasa* after conservation in the Vasa Museum; (*right*) The hull of the *Mary Rose* is being sprayed with PEG 200 in aqueous solution. (The *Vasa* photograph reproduced by permission of © Hans Hammarskiöld and the Vasa Museum, Stockholm, Sweden; the Mary Rose photograph courtesy of Dr Farideh Jalilehvand, Department of Chemistry, University of Calgary, Canada.)

pioneering application of the PEG method for bulking treatment of degraded wood.[4,6] To start with, PEG with a mean molecular mass of 1500 ($n \approx 34$) was used for the *Vasa*, later replaced with PEG 600 ($n \approx 13$) that was found to penetrate the wood faster. The *Vasa*'s conservators sampled a large number of cores with a manual increment borer to monitor the penetration. In the final stage of the PEG treatment, some outer surfaces were hand-sprayed with PEG 4000, which is less hygroscopic. After slow air-drying, the *Vasa* has been on display since 1990 in the dedicated Vasa Museum, Stockholm.[6]

Recently, sulfur K-edge XANES spectroscopy at Stanford Synchrotron Radiation Laboratory (SSRL) was used to show that about 2 tons of sulfur in various reduced forms is slowly oxidizing to sulfuric acid in the *Vasa*'s timbers.[2] The investigation was started after reports of an alarming number of acidic salt precipitates forming on the hull timbers and on objects in storage when the wood dried up after an increase in the relative humidity in the Vasa Museum during the wet summer of 2000 (Fig. 3). Most of the salts were identified by X-ray powder diffraction (XRD) as sulfates. Hydrated iron sulfates, rozenite ($Fe^{II}SO_4\cdot 4H_2O$), melanterite ($Fe^{II}SO_4\cdot 7H_2O$)

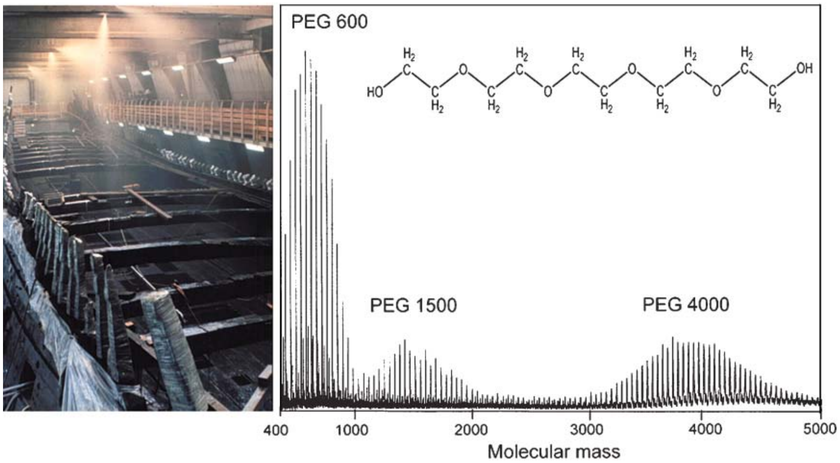

Fig. 2 The hull of the *Vasa* was sprayed with an aqueous solution of polyethylene glycol (PEG), $H(OCH_2CH_2)_nOH$, with mean molecular masses 600 and 1500 in a closed-circuit system from 1965 to 1979, followed by a surface treatment with a hand-sprayed PEG 4000 solution. The mass spectrum (MALDI-TOF) is obtained for a surface sample on the *Vasa*'s upper gun deck. Each peak corresponds to PEG molecules of a certain molecular mass, distributed around the expected mean values. The structure formula in the inset with $n = 5$ corresponds to PEG 238. (Photograph reproduced by permission of © the Vasa Museum.)

and natrojarosite ($NaFe^{III}_3(SO_4)_2(OH)_6$), are common, but also gypsum, $CaSO_4{\cdot}2H_2O$, and elemental sulfur (orthorombic S_8) occur as tiny crystals on the surfaces of hull timbers.[2,5] In March 2005 the conservators had registered 2075 areas in the *Vasa*'s hull with visible precipitates of acidic salts and with a surface pH of 3.5 or lower (as measured with wet pH indicator paper).[7] The special ion-conducting property of solid PEG (long-chain PEG has been tested as an ion-conducting medium for solid-state batteries)[8] is a probable reason why the salts largely precipitate on the surface, even though outbursts of expanding salts appear also underneath the waxy PEG 4000 layer (Fig. 3).

The concern for this unique historical artefact initiated the international "Preserve the *Vasa*" project, started in 2003. Methods are being developed and tested to remove or stabilise the sulfur and iron compounds, and to neutralise the acid that may continue to form in marine-archaeological wood after a PEG treatment.

Fig. 3 Outbreaks of acidic sulfate salts on a beam in the *Vasa*'s hold and on a stave-built bucket. X-ray powder diffraction (XRD) identified natrojarosite, $NaFe^{III}_3(SO_4)_2(OH)_6$, and gypsum $CaSO_4{\cdot}2H_2O$. The expanding salts beneath the waxy PEG layer of the bucket have detached pieces of the surface layer. The transformation from reduced sulfur compounds to sulfate salts often involves a large volume expansion, *e.g.* the oxidation of pyrite, FeS_2 (with a volume of 20 Å^3 per S atom), to gypsum (124 Å^3 per S atom) increases the volume of the solid compounds 6 times. (Photograph (top) by Magnus Sandström; (below) reproduced by permission of © the Vasa Museum.)

The *Mary Rose* was for 35 years a principal warship in Henry VIII's navy, and the first known ship to carry gunports. In 1545 she suddenly went down at a depth of about 14 m while manoeuvring to engage a French fleet outside Portsmouth, UK. The open gunports contributed to the sudden disaster that took almost 400 lives. In the strong tidal flows the wreck served as a silt trap and a hard shelly layer of compacted clay was formed covering the timbers of the starboard side and parts of the decks. About ⅓ of the hull remains and was salvaged in 1982, followed in October 2005 by the raising of a 10 m long stem timber from the bow. The almost 20 000 objects recovered from the wreck site carry a wealth of information providing detailed insight in the life and conditions onboard a Tudor warship.[1]

Consolidation spray treatment of the hull timbers started in 1994 at the Mary Rose Trust, Portsmouth (Fig. 1), with aqueous solutions of PEG 200, which in 2006 will be replaced by PEG 2000 for a planned further five years of spraying, followed by slow air drying. After the discovery of accumulated sulfur in the *Vasa*, investigations were also started of the *Mary Rose* timbers to evaluate the effect of the different preservation conditions, *e.g.* the salinity of the seawater is about 10 times higher at the *Mary Rose* site.[3]

A recently conserved shipwreck is the *Bremen Cog* from 1380, discovered in 1962 during dredging operations of the river Weser downstream of Bremerhaven, Germany. This wreck is the best-preserved example of the cog, which was the dominating type of merchant ship during the time of the Hanseatic League. In 1982 the hull was immersed in a large tank for a two-step conservation treatment with PEG 200 followed by PEG 3000. After a final drying period the reassembled ship has from 2000 been on public display in the Deutsches Schiffartsmuseum in Bremerhaven.[10]

The trading vessel *Batavia* of the Dutch East India Company is contemporary with the *Vasa*. She was wrecked in 1629 on the Abrolhos islands off the Western Australian coast, also on her maiden voyage. This was an extraordinary tragedy, with 268 people abandoned on the small waterless islands, where a small group of mutineers massacred 125 men, women and children. Out of 316 people onboard the *Batavia*, only 116 survived.[11]

At the shallow site of the wreck ($\sim$9 m) an encapsulating marine concretion had protected some timbers of the port side that were covered by iron cannon and cannon balls. Starting in 1970, about 20 tons of oak and pinewood could be excavated and over a period of ten years conserved piece by piece with PEG 1500 in a heated bath treatment. The stern quarter of the ship's port side up to the top of the first gun-deck is reconstructed and displayed in the Shipwreck Galleries of the Maritime Museum, Fremantle, Australia.[12]

2 Sulfur accumulation

2.1 Distribution in marine-archaeological wood

From the hull timbers of the *Vasa* and the *Mary Rose* conservators sampled some oak and a few pine wood cores (about 4 × 150 mm).[2,3] Profiles of how the total sulfur and iron concentrations vary along the cores were determined by high resolution X-ray fluorescence line scans (Fig. 4), complemented with elemental analyses and multi-element X-ray photoelectron spectroscopy of wood slices. For the *Mary Rose* the sulfur concentration along all cores fluctuates around 1 mass% S, indicating a total of about two tons of sulfur in the hull of about 280 tons of oak wood. In the *Vasa*'s hull with more than 1000 tons of oak timbers, the sulfur concentration normally is highest in the first cm or so of the surface layers with a similar total amount, ~2 tons S, as in the *Mary Rose*.[3,13]

Core samples from a number of other historical shipwrecks reveal that accumulation of reduced sulfur compounds often can occur in concentrations up to several mass% S in marine-archaeological wood preserved in seawater. However, low concentrations were found for the wreck of the *Bremen Cog,* which was preserved in river water. Analyses for total sulfur and iron in core samples taken from a rib on the starboard side of the hull, which was deep into the mud, and through a plank from the port side, which was exposed longer and is the more degraded, revealed less than 0.05 mass% S in the inner parts of the cores, and slightly higher values (~0.15 mass% S) at the surfaces (Fig. 4).[13]

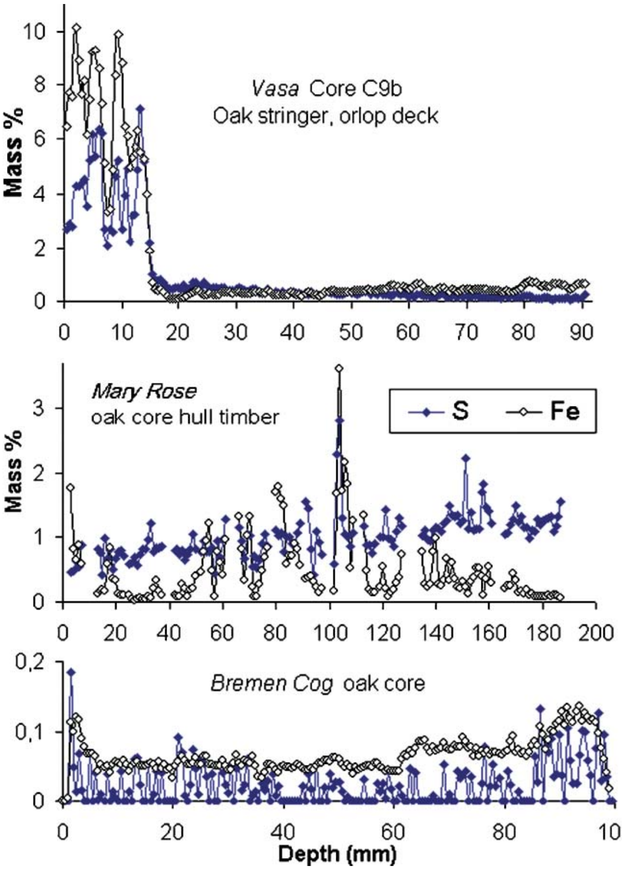

Fig. 4 X-ray fluorescence high resolution line scans (see Section 3.4) of total sulfur and iron in cores: (*top*) Vasa: Core 9b (oak bilge stringer, orlop deck) has very high sulfur and iron concentration close to the surface with similar profiles. (*middle*) Mary Rose: Oak core 3 from hull rider under PEG treatment shows a fairly uniform sulfur profile but fluctuating iron distribution. The gaps are due to pieces lost in destructive analyses. (*bottom*) *Bremen Cog.* The total sulfur and iron amounts are low.

The near anoxic environment that slows down degradation of waterlogged wood at or below the seafloor, also promotes bacteria metabolising organic debris by reducing the sulfate ions of seawater to dissolved hydrogen sulfide, $H_2S(aq)$.[14,15] Evidently, when the hydrogen sulfide enters the waterlogged wood, it can react to form solid reduced sulfur compounds. Comparisons of the types of sulfur compounds in wood from shipwrecks preserved under different conditions show that the distribution and amounts depend on the state of wood degradation, the concentration of hydrogen sulfide, and also the presence of iron(II) ions from corroding iron.[13]

In the wood of the *Vasa*, high accumulation, in some cases exceeding 10 mass% S and Fe, occurs only in the bacterially degraded surface layer, while for the *Mary Rose* all analysed cores display a fairly uniform concentration around 1 mass% S.[3] That difference is connected to the special conditions that prevailed at the *Vasa*'s site. Around the huge exposed hull of the *Vasa* the dissolved oxygen was largely consumed by decomposing organic matter in the increasingly polluted brackish water of Stockholm's harbour. Records of analyses can be found showing that in those near-anoxic conditions sulfate-reducing bacteria with some seasonal variations produced very high concentrations of hydrogen sulfide in all the surrounding water volume, certainly for more than a century before the salvage in 1961.[7]

The *Mary Rose* timbers were immersed in clay restricting the circulation of the anoxic seawater,[1] while over the wreck of the *Bremen Cog* the hydrogen sulfide concentration must have been low in the stream of river water with little sulfate. The iron and sulfur concentration profiles, obtained by high resolution X-ray fluorescence line scans along the cores (Fig. 4), sometimes show correlated variations for the *Vasa*, indicating that initially iron sulfides were formed when dissolved hydrogen sulfide and iron(II) ions penetrated the exposed waterlogged wood. From the *Mary Rose* a salt-infested gunshield, which originally was covered with segmented iron plates that corroded away, shows high total sulfur and iron content, up to 10 mass% S and 5 mass% Fe; otherwise the iron concentration fluctuates considerably in the timbers of the *Mary Rose*.[3,13]

The *Batavia*'s timbers, covered by iron cannon and shots, were exposed to high levels of iron corrosion products diffusing into the bacterially degraded wood. The hydrogen sulfide produced by sulfate-reducing bacteria when the environment became anoxic inside the encapsulating concretion, then promoted formation of pyrite and other iron(II) sulfides in the waterlogged wood.[12] Elemental analyses of a few samples indicate a high degree of mineralization, especially of the strongly degraded pinewood. The percentage by mass of sulfur and iron reached 6 and 25% at the surface of the pine samples, while the highest values for oak were 4 and 7%, respectively. X-ray powder diffraction confirmed that pyrite, together with its intermediate oxidation product elemental sulfur, S_8, and a range of hydrated iron sulfates, is present on the degraded and frequently very acidic *Batavia* wood.[12,16]

2.2 Organosulfur in geochemistry

The discovery of organosulfur accumulation within waterlogged wood in anoxic seawater also has a wider environmental implication. The high concentration of organosulfur in the lignin-rich middle lamella (see Section 4.2) of the wood indicates a nucleophilic reaction between the hydrogen sulfide (or HS^- ions) with active sites in lignin, *e.g.*, with activated double bonds,[15,17] and probably also with ether, carbonyl and α-hydroxy groups. Humic matter is often partly composed of lignin, and the early diagenetic formation of organosulfur species in anoxic marine sediments may occur *via* similar reaction mechanisms. Cross-linking of thiols *via* S–S bonds has been proposed to build macromolecular structures, which may enhance the preservation of organic matter in oxygen-depleted surroundings.[15,17–19] The accumulation and transformation of reduced sulfur compounds in organic material constitute a significant part of the natural sulfur cycle, in particular of the 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. When corrosion products of iron are present in sufficient amount, competing reactions with the hydrogen sulfide will also form particles of iron(II) sulfides, *e.g.* pyrite and pyrrhotite.[12,18]

Even though sulfur is ubiquitous and biochemically essential, details of the sulfur chemistry in natural or environmental samples are only partially investigated since so few tools are available for *in situ* analyses and speciation. The current progress is a result of new developments and applications of spectroscopic methods, in particular sulfur spectroscopy at synchrotron facilities. Therefore, a brief description of how sulfur X-ray absorption spectroscopic methods have been adapted and used for sulfur speciation in wood samples follows below.

3 Experimental methods

3.1 Sulfur XANES principles

Speciation of the sulfur compounds in the core samples was possible by means of sulfur K-edge X-ray absorption near edge structure (XANES) spectra measured at the dedicated SSRL beamline 6-2, operated in fluorescence mode and at atmospheric pressure (1 atm He), as previously described.[2,13,20] The energy of the incident X-rays is scanned over the K-edge, and absorption features appear in the spectra. The pre-edge peaks appear at the energies of X-ray induced transitions of S(1s) core electrons to unoccupied valence states localised around the sulfur atom with some sulfur 3p contribution in the molecular valence shell.[20,21] The lowest transition energy is approximately correlated with the formal oxidation number, as a higher effective nuclear charge increases the binding energy of the S(1s) orbital, resulting in a large shift of about 13 eV from sulfides(-II) to sulfate(VI).[2,18,22] The unoccupied molecular valence orbitals are more shielded and less affected, but are also influenced by the covalency of the bonds to the sulfur atom and the coordination geometry, which contribute to the transition energies and intensities. The intensity largely depends on the contribution from the sulfur 1s → 3p dipole allowed transitions, *i.e.* the overlap integral between the corresponding lowest unoccupied molecular orbitals with some sulfur 3p character. With increasing effective charge on the sulfur atom the overlap integral, and thus the intensity, increases because the 3p orbitals contract more than the 1s.[20] In this way the oxidised sulfates obtain a spectral intensity several times higher than that of sulfides (Fig. 5). Note that several schemes are in use for calibrating the absolute energies in the spectra.[20,22–24] In the current paper we display XANES spectra calibrated by assigning the first peak position of solid sodium thiosulfate, $Na_2S_2O_5 \cdot 5H_2O$, measured before and after the sample, to 2472.02 eV.[20]

Segments of the wooden cores, a few mm each, were filed in inert atmosphere to fine particles, mounted on sulfur-free tape and covered by 4 μm sulfur-free polypropylene film (Ultralene©).[3] The sensitivity at the SSRL beamline 6-2 was sufficiently high to determine the main sulfur species in a wood sample with a total sulfur concentration of about 300 ppm without radiation damage.

3.2 Sulfur speciation by XANES

When a series of spectra have been obtained from an object, Principal Component Analysis is helpful to establish the number of different types of functional sulfur groups that can be distinguished, *e.g.* by the DATFIT program in the EXAFSPAK software package.[25] Linear combinations of normalised XANES spectra of appropriate standard sulfur compounds describing such functional groups can then be fitted to the experimental spectra, allowing evaluation of the relative amounts under certain conditions (see below). As an example, a series of XANES spectra measured from segments along a 200 mm oak core from an oak beam (rider) in the hull of the *Mary Rose* is shown below (Fig. 5). The main absorption peak corresponds to reduced sulfur in several components. The most common could be characterised by standard spectra of disulfides R–SS–R, thiols R–SH, and elemental orthorhombic sulfur S_8 (Fig. 5 and 6). Note that for this core sampled from hull timbers being washed by the conservation liquid, oxidised sulfur(VI) compounds in significant amount occur only at the surface. Occasionally, the reduced sulfur peak in some cores also indicates pyrite in varying amount (Fig. 6), consistent with its occurrence in particles.[3]

Another series of XANES spectra has been collected at SRS, Daresbury, UK, from the wreck of the *HMS Dartmouth* that sank in 1690 and currently is awaiting conservation treatment at the National Museums of Scotland.[24] In one case a telltale shoulder appeared at ∼2471 eV, implying a substantial amount of iron(II) sulfide. The shoulder could be modelled by fitting with standard spectra that indicated ∼36% pyrrhotite, $Fe_{1-x}S$, $x \sim 0.1$, in the sample (*cf.* Section 3.3 X-ray micro-spectroscopy). We have occasionally seen similar low energy peaks and shoulders in sulfur XANES spectra of iron-rich marine archaeological wood from several shipwrecks. In particular, samples from the *Batavia* often show distinct prepeaks at ∼2470 eV. The low energy of this first transition with 1s → 3p character for such iron sulfides reflects a charge donation from the sulfide to the iron ions. Such an increase in bond covalency is substantiated by comparisons with theoretical evaluations of XANES spectra of Fe–S clusters with iron ions in different redox states.[20] Increasing charge

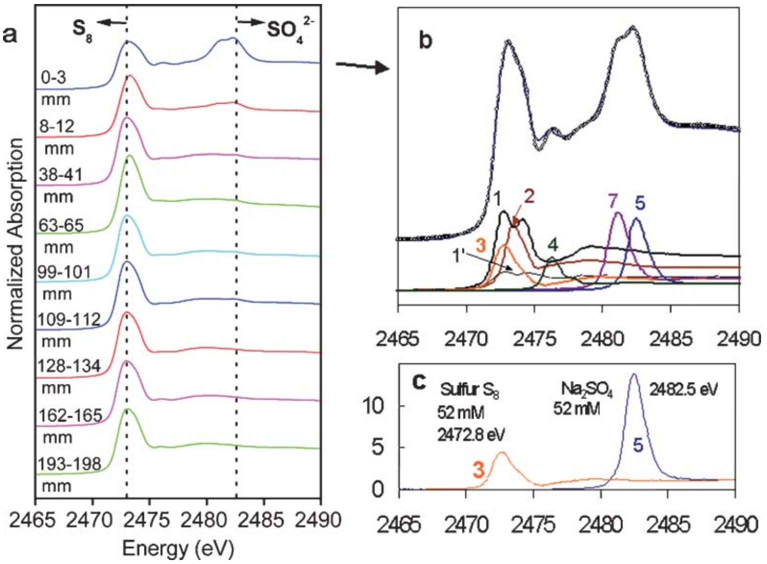

Fig. 5 **a)** The *Mary Rose*: Normalised sulfur K-edge XANES spectra of powdered core segments from PEG treated hull rider; core 3 (black-brown oak) *vs* depth from the surface. The major peak at 2473 eV originates from reduced sulfur species (elemental sulfur S_8, thiols R–SH, and disulfides R–S–S–R). Oxidised forms in significant amount, sulfates and sulfonates ($R–SO_3^-$) appear only at the surface (0–3 mm), as shown by a distinct double peak at about 2482 eV. For total analyses of sulfur and iron, see Fig. 4. **b**, Model fitting of standards (most in aqueous solution) to the surface spectrum (0–3 mm). The standard spectra of solutions used for the fitting are **1**: Disulfides R–S–S–R: (standard cystine with **1′** in solid state, peak maxima at 2472.7 and 2474.4 eV) 45%, **2**: Thiols R–SH (cysteine in aqueous solution pH = 7, 2473.6 eV) 23%, **3**: Elemental sulfur (S_8 in xylene 2473.0 eV) 10%, **4**: Sulfoxide R(SO)R′, standard methionine sulfoxide 2476.4 eV) 5%, **5**: Sulfate SO_4^{2-} (pH 6, 2482.6 eV) 7%, **7**: Sulfonate $R–SO_3^-$ (sodium methylsulfonate solution 2481.2 eV) 10%. **c.** The absorption feature for 52 mM sulfur in the standard solutions of aqueous sodium sulfate (**5**) is about 3 times more intense than that of elemental sulfur (S_8) in toluene (**3**).

donation, as indicated by the low energy 2470 eV prepeak, is consistent with an increased Fe(III)/Fe(II) ratio in the iron sulfides, as for greigite, Fe_3S_4.[7]

For the gunshield from the *Mary Rose*, which was subjected to a bath PEG conservation treatment, the XANES fitting was consistent with about ⅓ of the sulfur remaining in reduced forms, mainly as pyrite FeS_2. X-ray powder diffraction (XRD) confirmed pyrite and traces of mackinawite (Fe_8S_9), together with the hydrated iron sulfates, rozenite ($Fe^{II}SO_4{\cdot}4H_2O$) and melanterite ($Fe^{II}SO_4{\cdot}7H_2O$) and also natrojarosite ($NaFe^{III}_3(SO_4)_2(OH)_6$). Two years later XRD showed only sulfates in the sample, mainly rozenite and jarosite.[3]

The distinct peak appearing at ~2476.6 eV in almost all spectra can be ascribed to sulfoxides. However, for marine-archaeological wood the fitting of the sulfoxide peak often shows some deviation when using solutions of methionine sulfoxide or dimethyl sulfoxide as standards. The dimethyl sulfoxide spectra can have a rather different appearance when measured for free molecules or for ligands to metal ions. To investigate the reason, XANES spectra of dimethyl sulfoxide molecules, free and in different types of coordination, were simulated by means of the STOBE quantum chemistry code.[26] The transition energies from the sulfur 1s orbital to higher states were computed, with an individual orbital adjustment for each excited state for the stepwise increase in the effective nuclear charge when the core hole is created. The main pre-edge XANES absorption feature of the dimethyl sulfoxide sulfur atom comprised three electronic transitions within about 1 eV, which explains its asymmetry (Fig. 7). The experimental spectra of the hexasolvated trivalent ions of group 13, Al, Ga, In and Tl(III), showed that coordination, even to the oxygen atom, considerably shifts the transition energies and splits the 2476 eV dimethyl sulfoxide peak. Especially covalent bonding, as for the Tl(III) ion (Fig. 7), affects the energy and probability of the symmetry-dependent transitions and influences shape, intensity and position of the peaks in the spectra.[26] Hence, the slight shift of the sulfoxide peak in the current wood spectra may indicate some interaction, *e.g.* hydrogen-bonding, with the sulfoxide group of the compounds in the wood.

Generally, it is essential that the standard compounds used for the fittings adequately represent the characteristic sulfur groups of the compounds in the samples. In particular for the overlapping XANES peaks of the reduced sulfur groups, the standard spectra must be measured with careful energy calibration, the same instrumental resolution and with the sulfur groups in a similar chemical state and surrounding as in the sample. Also, the physical state is important. Standard spectra of sulfur species in dilute solution often display sharper features than for solids because of self-absorption of fluorescence X-rays, as *e.g.* for particles of elemental sulfur,[22,23] or by broadening in a "band" structure of transition energies for interacting species in a crystal structure. The amorphous organosulfur components in the wood are normally modelled better by spectra of standards in dilute solution than of solid standard compounds.[3]

3.3 X-ray micro-spectroscopy

The formation of acid in the wood depends on how accessible and reactive the different sulfur compounds are to oxygen

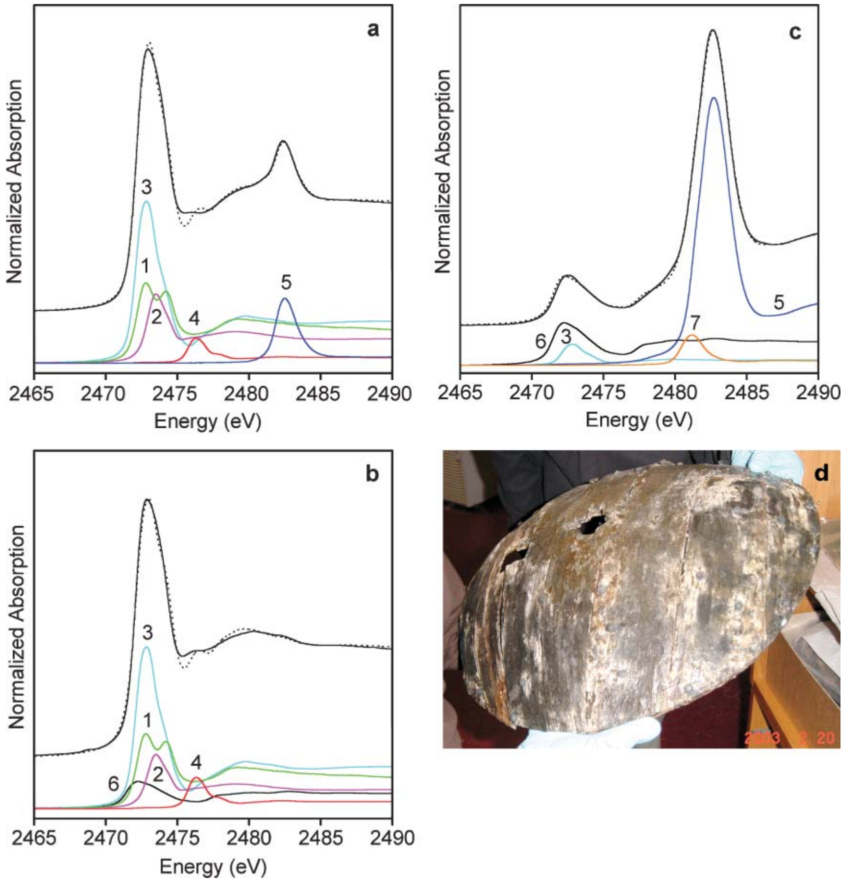

Fig. 6 The *Mary Rose*: XANES evaluation of sulfur species by fitting linear combinations of standard spectra. **a.** Core 1 from magazine stored oak timber (0–5 mm from surface): **1**: disulfides R–SS–R′ 31 atom% S, **2**: thiols R–SH 23%, **3**: elemental sulfur S_8 36%, **4**: sulfoxide R(SO)R′ 4%, **5**: sulfate SO_4^{2-} 6%. **b.** Oak core from hull timber under spray treatment (15–17 mm): **1**: R–SS–R′ 29%, **2**: R–SH 18%, **3**: S_8 35%, **4**: R(SO)R′ 5%, **6**: pyrite FeS_2 13%. **c**, **d.** Wooden remains of a gun shield from the *Mary Rose* with salt formation on the surface, **3**: S_8 6%, **5**: sulfate 63% (standard: melanterite $FeSO_4·7H_2O$), **6**: pyrite FeS_2 26%, **7**: sulfonate $R–SO_3^-$ 5%. XRD was used to identify the sulfur compounds on the gunshield as iron(II) sulfides: pyrite and mackinawite (Fe_9S_8), and hydrated iron sulfates: rozenite $Fe^{II}SO_4·4H_2O$, melanterite $Fe^{II}SO_4·7H_2O$, and natrojarosite $NaFe^{III}_3(SO_4)_2(OH)_6$. (Photograph by Magnus Sandström.)

penetration in an aerobic environment. This circumstance was investigated by means of scanning X-ray absorption microspectroscopy (SXM) at beamline ID21 of the European Synchrotron Radiation Facility (ESRF), which allows sulfur speciation at high spatial resolution (~0.5 μm). Raster scanning of thin wood slices (a few μm) perpendicular to the cell walls enabled mapping of reduced and oxidised sulfur species at energies of characteristic sulfur XANES resonances, *ca.* 2473 and 2483 eV, respectively, with a spectral resolution of ~0.5 eV over sample areas up to 100 × 100 μm. The SXM microprobe is operated under low-grade vacuum to avoid the strong air absorption at these X-ray energies.

The SXM images obtained of oak wood from hull timber at the X-ray energy 2473 eV consistently reveal reduced sulfur species in high concentration in the lignin-rich middle lamella between the cells and sometimes also in a thin layer at the rim of the lumen (Fig. 8–10). The lignin-reinforced wall of a vessel in freshly salvaged oak wood unexpectedly displayed a distinct double layer (Fig. 8). Complementary studies by means of scanning electron microscopy and X-ray fluorescence elemental analysis (SEM-EDS), showed sulfur but no iron in the middle lamella.[7,13]

The SXM microprobe also measures focused micro-XANES spectra without significant charge reduction. Such spectra from sub-micron spots in the middle lamella were consistent with sulfur in thiols at the peak energy 2473 eV, as for the unfocused SSRL XANES spectra of higher signal-to-noise (*cf.* Fig. 5, 7a),[3] and could also be used to identify a particle of iron(II) sulfide (probably pyrrhotite) at the X-ray energy 2471 eV (Fig. 9).

3.4 X-ray fluorescence line-scan analysis

High resolution X-ray fluorescence line scans along cores were performed by means of an automatic wood scanner (Cox Analytical Systems), to obtain concentration profiles of total sulfur and iron (see Fig. 4). The X-ray fluorescence was excited by means of a focused Cu Kα X-ray beam, and an energy dispersive solid state X-ray detector provided ~0.5 mm resolution and an analytical depth into the wood of about 0.1 mm. Standard samples consisting of pressed pellets of oak wood powder, PEG 4000, mixed with known concentrations of iron(III) sulfate and gypsum, were scanned to achieve quantitative calibration.[3,13]

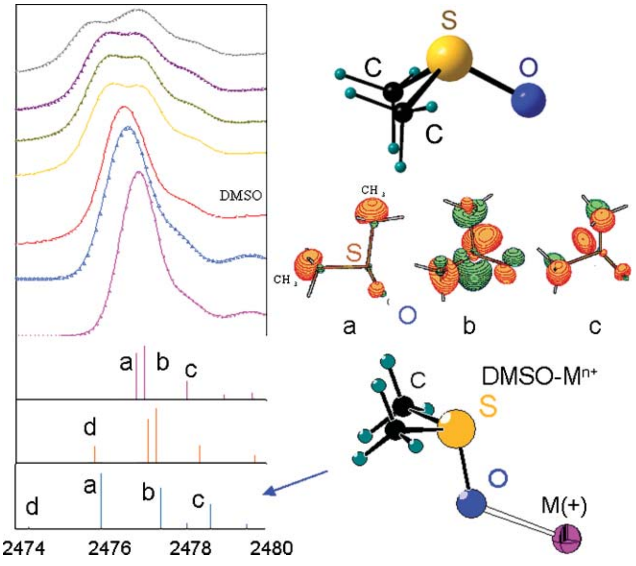

Fig. 7 Experimental sulfur K-edge XANES spectra (normalised intensities) of the hexakis (dimethyl sulfoxide)-M(iii) solvates of the group 13 metal ions (from the top *M* = Tl, In, Ga and Al), and of 50 mM dimethyl sulfoxide (DMSO) in acetonitrile. Below, spectra calculated for free DMSO, with the S–O distance 1.595 Å, and C2 with 1.495 Å, respectively. The rows of vertical bars below represent the calculated energies and cross-sections for the X-ray induced excitations of the sulfur 1s electron for (top) a free DMSO molecule, (*middle*) a DMSO connected point charge P+, and (*below*) DMSO coordinated to an Mg^{2+} ion. (*right*) To the top right the shapes of the antibonding molecular orbitals corresponding to the transitions **a**, **b** and **c**, are shown, and below free and oxygen coordinated DMSO molecules, $(CH_3)_2SO$.

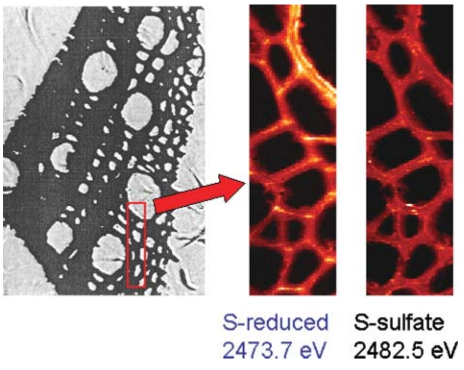

Fig. 8 The *Mary Rose*: The two scanning X-ray micro-spectroscopy (SXM) images to the right show freshly salvaged waterlogged oak wood after 459 years on the sea floor. The left image is an ordinary microscopy picture of a thin wood slice perpendicular to the cell walls, showing lumina (smaller holes) and vessels (larger holes). The S(red) image, at 2473.7 eV, displays two layers of thiols in high concentration in the lignin-rich walls of a vessel (lighter colour = higher concentration). Bright spots in the image to the right at 2482.5 eV correspond to sulfate particles; the diffuse background is due to seawater sulfate

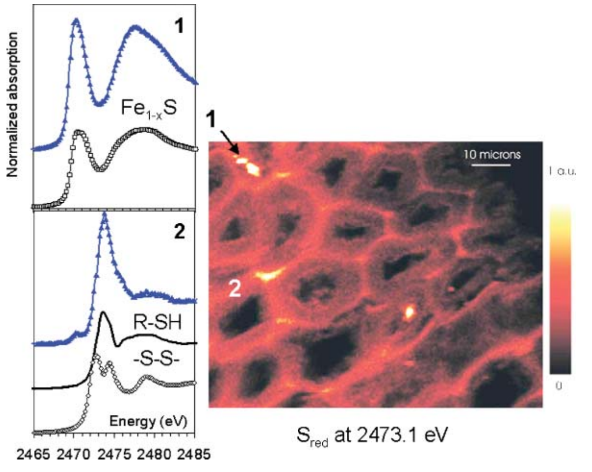

Fig. 9 The *Mary Rose*: Scanning X-ray micro-spectroscopy (SXM) images at 2473 eV of reduced sulfur (brighter colour → higher concentration): (*top*) oak core, sampled from hull timber under spray treatment. The micro-XANES curves below (blue triangles = experimental, black squares = standard spectra) indicate that the bright spot **1** is an iron sulfide (standard: pyrrhotite $Fe_{1-x}S$ peak at 2471 eV) particle, while spot **2** corresponds to thiols R–SH (standard: cysteine 2473 eV) and probably some disulfides R–S–S–R′ (standard cystine) in the middle lamella.

3.5 X-ray photoelectron spectroscopy

Electron spectroscopy for chemical analysis (ESCA) was performed on oak slices cut at various depths along cores, mounted and transferred in vacuum into the sample chamber of a Scienta ESCA-300 instrument for multi-element analysis. The kinetic energies of core shell photoelectrons, excited from all elements in the outermost surface layer (<100 Å) by means of high intensity monochromatic Al K_α X-ray radiation (1487 eV), were measured to obtain their electron binding energies. The photoelectron intensity is proportional to the atomic concentration of each element in the surface layer (<100 Å). The well-resolved $C_{1s}(CH_2)$ line, assumed at 285.0 eV, was used for charge-calibration, keeping the surface potential of the electrically insulating samples constant by an excess current of low energy electrons ($\sim$1 eV) from the Scienta flood-gun.[2,3]

The main purpose of this type of analysis is to get an overview of all elements in the samples, even the lightest (except for H). Table 1 and Fig. 11 clearly show that boric acid, $B(OH)_3$, used as fungicide, has efficiently penetrated throughout the wood. In the carbon C(1s) spectra the oxygen-bonded carbon can be distinguished, which in non-PEG treated wood allows an estimate of the cellulose : lignin ratio, and thus the degree of degradation.[3] Also, the reduced and oxidised sulfur compounds give resolved peaks at different energies that can be used for estimating the relative amounts by curve fitting (Fig. 12). However, the resolution is reduced because two peaks are required for each type of sulfur species, due to the excitation of sulfur 2p electrons from the $2p_{1/2}$ and $2p_{3/2}$ states in the ratio 2 : 1. Such fittings, performed as exemplified in Fig. 12, provided the results in Table 2, where also the minor amount of sulfoxides could be obtained at an intermediate energy. After calibrating the spectra by setting the $C_{1s}(CH_2)$ line at 285.0 eV, the mean S $2p_{3/2}$ energies obtained were: **1**: 168.8 eV, **2**: 166.3 eV, and **3**: 163.7 eV for the three components, consistent with the estimated energy differences for the components in the sulfur K-edge XANES spectra.[3]

This method of analysis is highly sensitive for contamination of the surface when preparing the samples. and smearing the

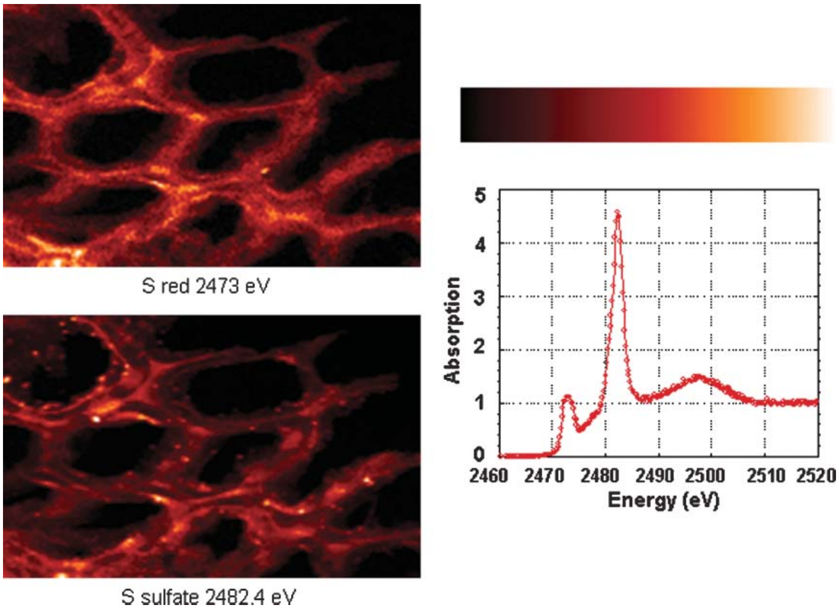

Fig. 10 The *Vasa*: (*top right*) Scanning X-ray micro-spectroscopy images of an oak core slice (core 9b) at a depth of ∼9 mm; (*top left*): reduced sulfur S(red) at energy 2473 eV, (*right*) sulfates S(VI) at 2482 eV. (*right*) The global XANES-spectrum of the slice shows partial oxidation (more than 50%) of the reduced sulfur compounds to S(VI), consistent with the acid production (NB, the intensity ratio S(red):S(VI) is ∼3 : 1, *cf.* Fig. 5c).

surface with waxy PEG when cutting wood slices could be a problem. Also, the high vacuum (below 10^{-8} mbar) needed for the electron spectroscopy requires pumping the samples for several hours, which means loss of volatile substances. Also elemental sulfur, which has a vapour pressure of about 0.5 × 10^{-4} Pa in crystalline form, could be affected, even though no obvious difference was found in the relative amounts of the reduced sulfur compounds (Table 2) when compared to the XANES results.[3,13]

4 Marine-archaeological wood

4.1 Preservation

Several concurrent factors are required to efficiently slow down the natural degrading processes of wood at the seabed. Some concern physical conditions (low temperature, darkness, protection by burial), others chemical (salinity of seawater, oxygen depletion and corroding iron), but lack of mechanical interference (human disturbance, strong currents, wave actions and sand movement causing abrasion), and lack of biological activity (marine bacteria, fungi and marine borers) are also essential.[4,27,28] The complex and interrelated effects mean that marine-archaeological wood can show considerable heterogeneity and each preserved object should be assessed individually.[28] Let us briefly examine the main circumstances that enabled the high levels of preservation in the present cases.

Table 1 Multi-element analyses with X-ray photoelectron spectroscopy (ESCA) of *Mary Rose* core 1 and of a surface sample from the gunshield (see Fig. 6)

Depth mm)	C	O	Fe	B	Si	N	Na	Mg	Ca	Cl
7.5	27.2	6.4	0.2	0.17	0.13	0.07	0.23	0.21	0.27	0.1
10	27.1	6.8	0.19	0.19	0.15	0.04	0.18	0.1	0.22	0.08
15	26	7.5	0.17	0.18	0.29	0.07	0.19	0.13	0.19	0.05
33	26.6	7.2	0.06	0.14	0.28	0.06	0.17	0.06	0.16	0.05
34	31.7	2.3	0.18	0.05	0.26	0.07	0.03	0.09	—	0.06
45	30	4	0.21	0.12	0.11	0.05	0.07	0.03	0.13	0.09
60	24.4	10	—	0.13	0.04	0.02	0.17	0.05	0.11	0.13
75	26.5	7.5	0.28	0.14	0.07	0.04	0.18	0.06	0.15	0.11
89	23.2	11	0.02	0.19	0.06	0.04	0.22	0.04	0.18	0.07
92.5	26.8	7	0.4	0.07	0.07	0.11	0.14	—	0.08	0.2
99	23.5	10.1	0.14	0.11	0.57	0.06	0.17	0.05	0.23	0.12
104	29.8	4	0.51	0.09	0.05	0.08	0.07	0.06	0.04	0.13
112	28.2	5.6	0.34	0.11	0.11	0.12	0.09	0.01	0.18	0.11
144	23.8	10.3	—	0.26	0.07	0.05	0.25	0.1	0.29	0.08
Gunshield	16.3	13.2	2.8	—	—	0.33	—	—	0.1	—

For the *Vasa*, the largest marine-archaeological find of its kind ever salvaged, the oak wood of the huge exposed hull (length 61 m excluding bowsprit, width 11.7 m, height of aftercastle 19.3 m) was in surprisingly good condition after 333 years at a depth of 33 m.[4,6] Severe mechanical damage had been inflicted, mostly by anchors; otherwise the wreck site was well shielded without strong currents or tidal water movement. All iron bolts had rusted away causing many sculptures and fastened wooden objects to fall down into an anoxic thick layer of silt. The hull timbers were still held together by thousands of wooden pegs.

A very special reason for the *Vasa*'s unique state of preservation must have been that the chemistry of the surrounding seawater was made unfavourable for biological degradation of the exposed wood. Firstly, the low salinity in the Baltic, ∼10% of that in the oceans averts marine borers such as the shipworm, *Teredo Navalis*, an efficient wood degrader. Secondly, the low oxygen levels controlled marine microbial attack on the cellular level of fungi and bacteria, which degrade cellulose and hemicellulose.[4,28,29] The marine fungi require dissolved oxygen levels of at least 0.3 mg L^{-1}.[1] Some marine bacteria, *e.g.* the cellulose degrading "erosion bacteria", seem to be able to survive in an oxygen-depleted

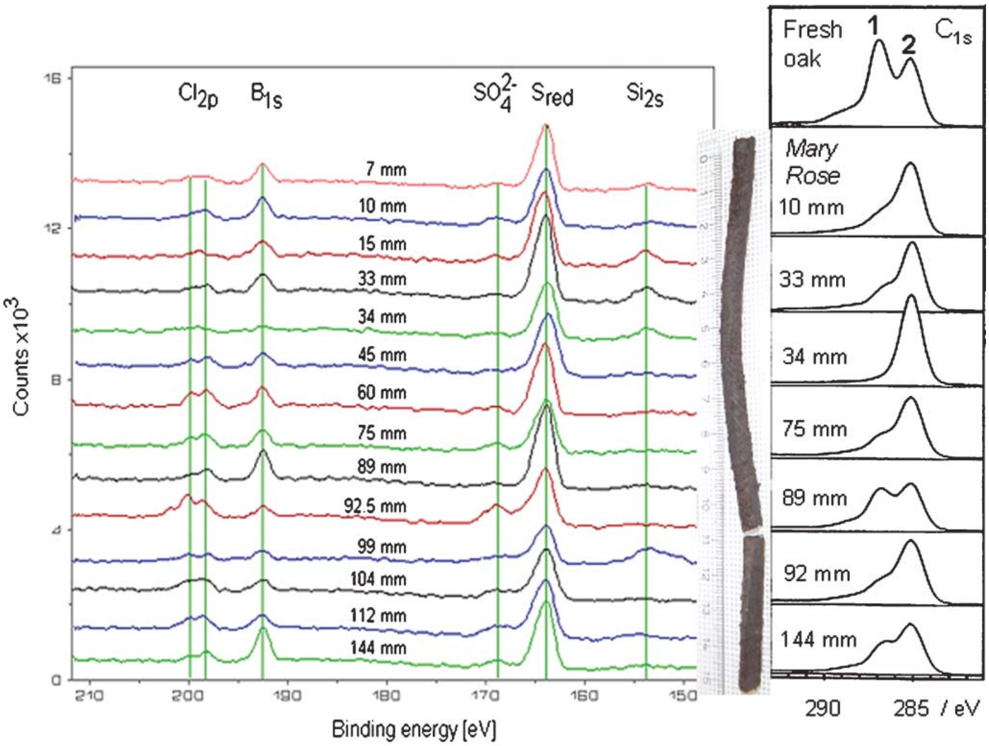

Fig. 11 (*left*) The *Mary Rose*: X-ray photoelectron spectra (ESCA) spectra along core 1 from magazine stored oak timber show an almost constant concentration of reduced sulfur compounds (S_{red}), with sulfates (SO_4^{2-}) occurring in cracks where the silicate (clay) and chloride concentrations also are significant. The B_{1S} peaks indicate that the fungicide boric acid $B(OH)_3$ has uniformly penetrated the wood. (*right*) The lignin to cellulose ratio increases when erosion bacteria degrade the cellulose. The amounts of carbon and oxygen from the ESCA analyses show an inverse correlation (high C ↔ low O, see Table 1), and the relative sizes of the two C_{1s} peaks at **1**: 286.5 eV and **2**: 285.0 eV, which mainly correspond to C–O (cellulose) and CH_2 (lignin) groups, respectively, indicate that the erosion bacteria degradation of cellulose extends throughout the *Mary Rose* timbers, consistent with the relatively uniform sulfur concentration profile, see Fig. 4a.

environment, and can slowly decompose the cellulose of the wood cells until only lignin-rich parts and lamella remain as a weak "house of cards" (Fig. 13).[14]

Huge amounts of organic waste from the growing city was deposited into Stockholm's harbour and created largely anoxic conditions in the polluted seawater all around the wreck of the *Vasa*. Sulfate-reducing bacteria use the sulfate ions (the SO_4^{2-} concentration is about 0.3 g L^{-1} in the Baltic Sea) as electron acceptors when metabolising the organic compounds. The end product of the sulfate reduction is dissolved hydrogen sulfide, $H_2S(aq)$. Analytical records exist almost one century back close to the *Vasa*'s wreck site, showing seasonal variations of interrelated low oxygen and high hydrogen sulfide concentrations from the sea floor almost to the surface.[7,13]

Thus, the near oxygen-free environment with high levels of hydrogen sulfide made the *Vasa*'s wood inhospitable not only to fungi, rot and other wood degrading micro-organisms during most of the *Vasa*'s 333 years on the seabed, but also discouraged the "erosion" bacteria. Also, the low, fairly constant water temperature (between 0 and 5 °C at the site of the *Vasa*) slowed down the chemical and biological deterioration processes.[4,6] Only the outer layers of the *Vasa*'s oak timbers were in this way bacterially degraded to a depth of about 1–2 cm, even less so at the keel which was down 3 m into thick clay.[3]

The anoxic surrounding created by the highly polluted brackish water is a special and important reason for the low extent of degradation of the *Vasa*'s exposed wood, but is also the origin of the accumulation of the sulfur compounds, which generally show the highest concentrations in the most exposed parts.[5,7] The high levels and close correspondence between the penetration profiles of iron and sulfur from the X-ray fluorescence line scans in the surface regions of the cores (Fig. 4), show that dissolved iron(II) ions from the corroding bolts could penetrate and react with hydrogen sulfide in the bacterially degraded surface layers of the wood.[3,7] It is also possible that consortia of wood-eroding and sulfate-reducing bacteria locally enhanced the production of hydrogen sulfide within wood under bacterial attack in oxygen depleted conditions.

The exposed port side of the *Mary Rose* quickly eroded away in the strong tidal currents, weakened by wood-boring organisms such as the shipworm, and only the buried parts of the hull survived. The condition of the wood varies, as also the micro-environments created in the covering silt and clay layers, and especially the surface regions of the wood display decay patterns of fungi and marine borers.[1] However, the most extensive type of degradation is caused by the "erosion bacteria", with decomposition of the S2 and S3 cellulosic cell wall layers, but not of the lignin-rich middle lamella.[1,27] The rather uniform sulfur penetration profiles of all investigated

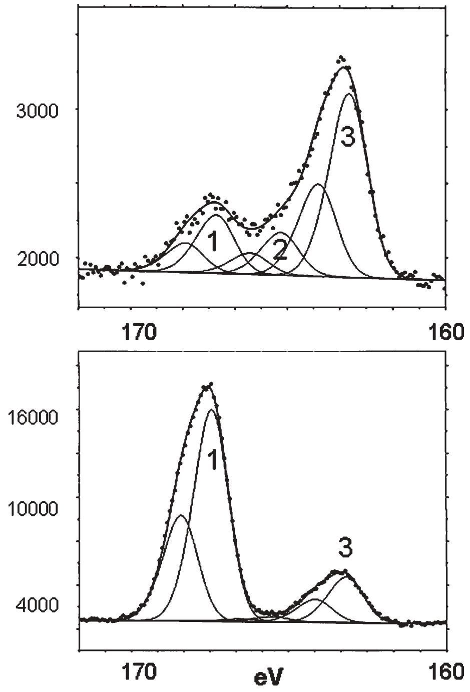

Fig. 12 The *Mary Rose*: Sulfur analyses of ESCA spectra of (*top*) oak core 1 from magazine stored timber at 92.5 mm depth and (*below*) the gun shield (*cf.* Fig. 6), by fitting three sulfur components, **1**: sulfate SO_4^{2-}, **2**: sulfoxide R(SO)R′, and **3**: reduced sulfur. The two peaks required for each component correspond to excitation of sulfur 2p electrons from the $2p_{1/2}$ and $2p_{3/2}$ states in the ratio 2 : 1. After energy calibration with the $C_{1s}(CH_2)$ line at 285.0 eV, the mean S $2p_{3/2}$ energies obtained were **1**: 168.8 eV, **2**: 166.3 eV, and **3**: 163.7 eV, respectively.

Mary Rose cores (Fig. 4),[13] and also the variations in the lignin : cellulose ratio (Table 1, Fig. 11), are consistent with severe attack by erosion bacteria that has facilitated penetration of dissolved hydrogen sulfide all through the timbers of the *Mary Rose*.[3,13]

The low sulfur content in the *Bremen Cog* can be connected to its preservation, where the low sulfate concentration and less anoxic conditions under streaming river water should lead to low bacterial production of hydrogen sulfide. The sudden increase in sulfur concentration just at the core surface (Fig. 4) may be caused by an event during the conservation. Dr Per Hoffmann, in charge of the treatment of the *Bremen Cog*, reported that aluminium sulfate was added as flocculant to facilitate removal of bacteria and algae growing in the PEG solution in the conservation tank. After some time, silvery bubbles with a foul smell started to pass by the windows of the tank, and black patches appeared on the wood surfaces! The water had become oxygen free so that sulfate-reducing bacteria could start to form hydrogen sulfide from the added sulfate, which then was replaced by aluminium chloride.[13]

Table 2 ESCA analyses of sulfur in *Mary Rose* core 1 and in a surface sample from the gunshield (*cf.* Fig. 12)

Depth (mm)	Total S	S reduced	Sulfoxide	Sulfate
7.5	0.78	0.68 (87%)	0.04	0.05
10	0.66	0.56 (85%)	0.03	0.08
15	0.89	0.77 (87%)	0.05	0.07
33	0.81	0.71 (88%)	0.05	0.05
34	0.74	0.66 (89%)	0.03	0.05
45	0.7	0.60 (86%)	0.06	0.04
60	0.64	0.58 (89%)	0.04	0.05
75	0.64	0.51 (80%)	0.05	0.08
89	0.69	0.63 (91%)	0.07	0.03
93	0.77	0.49 (61%)	0.12	0.16
99	0.68	0.59 (87%)	0.05	0.05
104	0.65	0.50 (77%)	0.07	0.08
112	0.72	0.54 (67%)	0.08	0.09
144	0.8	0.70 (87%)	0.03	0.07
Gunshield	3.5	0.60(17%)	0.07	2.8

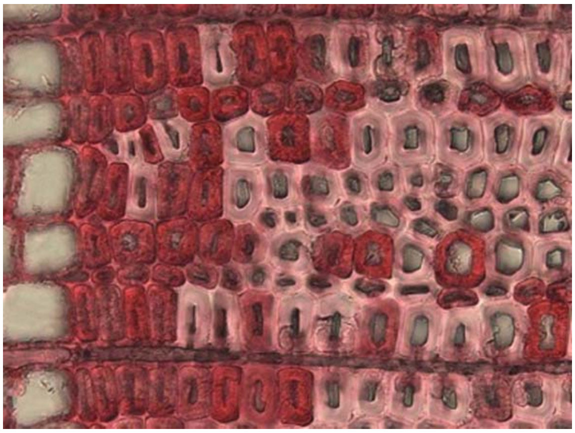

Fig. 13 Light microscopy of a transverse cross-section of wood partly degraded by erosion bacteria (darker cells), which may invade the cell wall through rays and pits. (Picture courtesy of Dr Charlotte Björdal, Department of Wood Science, Swedish University of Agricultural Sciences, Uppsala, Sweden.)

The wreck of the *Batavia* was located in a shallow site where heavy surf and turbulent wave action caused mechanical damage and only those timbers physically protected by the covering cargo and ballast, corroding iron cannon, cannon shots, *etc.*, survived initially. Eventually, the wood-degrading biological activity that must have been high in the warm sub-tropical seawater, ceased in the anoxic environment created beneath the protecting concretion. Sulfate-reducing bacteria could then produce hydrogen sulfide that diffused into the porous timbers, which became largely mineralised with iron sulfides that formed when hydrogen sulfide reacted with the iron corrosion products.[16]

4.2 Composition and structure of wood

Wood is mainly composed of hollowed, elongated cells running along the stem, which are held together by the lignin-rich middle lamella between the wood cells (Fig. 14).[27] The chemical composition varies in different parts of the stem of the tree and between different species of wood. Bundles of hydrogen bonded cellulose polymers are surrounded by cross-linking hemicellulose and lignin to form micro-fibrils, which provide mechanical strength and stability to the wood. The cell walls constitute a strong and sturdy construction, and are composed of several layers (*e.g.* S1, S2 and S3) reinforced by direction-altered cellulose fibrils.[27,29] Lignin is the least biodegradable wood component, as indicated by the ESCA spectra in Fig. 11.[27]

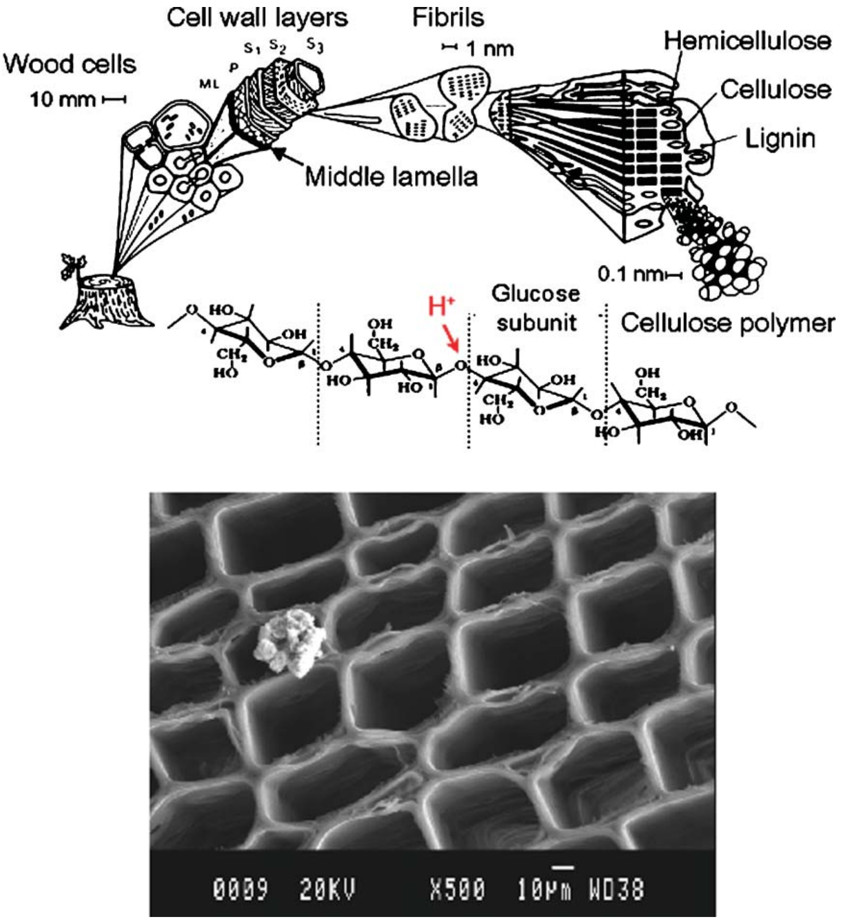

Fig. 14 a) The structure of wood, with bundles of cellulose polymers held together by lignin and hemicellulose in fibrils, which give strength to the cell walls. The cells are held together by the lignin-rich middle lamella. (*Below*) acid hydrolysis of a cellulose polymer is indicated (see text). **b)** Scanning electron microscopy transverse cross-section of the oak wood cells from *De Rob*, a merchant ship in the Burgzand Noord (BZN) wreck site 3, in Waddensea, the Netherlands. The particle has a Fe : S ratio of 1 : 2, and appears to be framboidal pyrite. (Top figure modified after Dr Per Hoffmann and Dr Mark Jones, cf. Ref. 29, Chapter 2, p. 36; SEM photograph by Yvonne Fors.)

The erosion bacteria decompose the cellulose of the cell walls, leaving a slime-filled skeleton of lignin-rich lamella behind (Fig. 13). Freshly salvaged degraded waterlogged wood may look intact when kept wet. However, when the internal water evaporates the weakened wood cell structure may irreversibly collapse, and the marine-archaeological object will shrink and crack.[28,29] The *Vasa* was the first large object for which polyethylene glycol (PEG) was used as a non-volatile bulking agent to provide mechanical stability to the degraded wood structure.[4,6] Nowadays PEG-stabilization procedures of marine-archaeological wood have become standard treatment, although alternative methods have been tested or are under development.[28,30]

5 Iron, sulfur and acid

5.1 Iron contamination

Marine-archaeological oak wood is often infested by iron(III) in (hydr)oxide particles and/or tannin complexes that create the characteristic dark "black oak" colour. The iron contamination originates mostly from the soluble iron(II) ions formed from corroding bolts, nails, cannon balls and other iron objects during the time on the seabed.[5] Microscopy (including SXM) of the *Vasa* shows that the PEG solution, which was recirculated for 13 years, has also carried small particles of iron (hydr)oxides into the larger cavities and vessels of the wood. The X-ray fluorescence and ESCA analyses reveal large fluctuations in the iron concentration throughout the wood of the *Mary Rose*, while there is high accumulation in the surface layer of the *Vasa*'s wood, sometimes up to several mass% Fe (Fig. 4).[3] However, deeper inside the *Vasa*'s wood, and also in the *Bremen Cog*, the iron concentration is low, generally lower than about 0.1 mass% Fe.[13] The *Batavia* is an extreme case being heavily contaminated with iron sulfides in mineralised wood, mostly pyrite (FeS_2) and pyrrhotite ($Fe_{1-x}S$).[16]

At the salvage of the *Vasa* in 1961 all original iron bolts had corroded away. Unfortunately, about 5500 new epoxy and/or zinc coated iron (mild steel) bolts were then inserted into the holes.[6] Presently, those iron bolts are corroding. The increased acidity has dissolved the zinc coating and the PEG impregnation accelerates corrosion of iron in the moist wood.[2,5] A program is now under way to replace accessible bolts with carbon fibre bolts. However, the hull has shrunk and subsided during the drying process and that will prevent the removal of most iron bolts.

Iron contamination of marine-archaeological wood is a well-known conservation concern. With oxygen access in humid conditions (as in moist PEG impregnated wood), the iron(II)/iron(III) redox couple can catalyse oxidation processes, *e.g.* oxidation of sulfides to sulfuric acid, but also direct oxidative degradation of cellulose and probably successive degradation of the PEG polymers.[2,5,12] PEG extracts from the *Batavia* wood in solution revealed NMR signals attributable to formates,[16] and formic acid from PEG degradation could also contribute to the total acidity in the wood. However, mass-spectrometry of PEG samples from the iron-rich surface of the *Vasa* does not indicate any extensive PEG degradation (Fig. 2).

Iron(II) sulfides are known to oxidise and produce acid relatively fast in a humid environment.[24,31,32] Thermodynamically, the stepwise oxidation process of iron sulfides may end at hydrated iron(II) sulfates while releasing acid, as for pyrite:

$$FeS_2(s) + {}^7/_2O_2 + (n + 1)H_2O \rightarrow FeSO_4{\cdot}nH_2O(s) + H_2SO_4(aq) \quad (1)$$

Other end products of the pyrite oxidation could be natrojarosite, $NaFe_3(SO_4)_2(OH)_6$, or goethite, α-FeOOH,[33] also commonly found in the wood:

$$FeS_2(s) + {}^{15}/_4\ O_2 + {}^5/_2H_2O \rightarrow FeOOH(s) + 2H_2SO_4(aq) \quad (2)$$

Microscopic investigations show particles loosely distributed within wood cavities (see Fig. 8–10, 14b), and iron(II) sulfides are probably the compounds primarily responsible for the production of sulfuric acid in the wood.[3,7,12] The presence of elemental orthorhombic sulfur, S_8, an intermediate compound in the pyrite oxidation process,[31] as well as the numerous hydrated iron(II) and iron(III) sulfates (*e.g.* rozenite, $FeSO_4{\cdot}4H_2O$), has been taken as proof that oxidation of pyrite has occurred in the wood of shipwrecks.[12] Such a sulfur oxidation could be microbially promoted.[12] During the current spray treatment of the *Mary Rose* the bacterial activity is monitored and shows various types of active sulfur bacteria.[1] However, in the *Vasa*'s wood, the low water content (the relative humidity is nowadays kept stable below 60%) is unfavourable for active microorganisms.

5.2 Acid in the *Vasa* and *Batavia*

Allowing high acidity to develop in conserved marine-archaeological wood could become a serious problem, since acid hydrolysis eventually would break up the cellulose polymer and reduce the mechanical strength of the wood. The catalytic degradation reaction starts with a hydrated proton attack on the glycosidic linkage between the oxygen and carbon atom in the cellulose molecule (Fig. 14a). Through a nucleophilic reaction, in which a water molecule binds to the carbon atom, the proton is released again. The rate of hydrolysis of the cellulose depends on several factors, *e.g.*, pH, temperature, humidity, presence of iron and the species of wood.[34] It is difficult to predict how fast marine-archaeological wood would deteriorate in ambient conditions, and it has not so far been possible to distinguish between degradation caused by acid hydrolysis and the bacterial degradation, which in turn facilitated the sulfur and iron penetration.[5,7]

During the spray treatment period of the *Vasa*, a 3 : 7 mixture of borax, $Na_2B_4O_7{\cdot}10H_2O$, and boric acid, $B(OH)_3$, was dissolved in the PEG conservation liquid as fungicide. The concentration (calculated as boric acid) varied between 1 and 4 mass%. The conservators noticed that the pH of the conservation liquid steadily decreased during the period of recirculation from 1965 to 1979. Acid was continuously leached out of the *Vasa*'s hull. Two possible reasons for that were discussed in the Conservation Council. "This might indicate oxidization and possible decomposition of the PEG molecule or that acid products had been dissolved from the wood of the hull".[6] Hence, PEG degradation or wood decomposition were suggested as possible reasons. To neutralise the acid, additional borax was dissolved in the recirculating liquid.[6] The reaction can be written:

$$\begin{gathered} Na_2B_4O_7{\cdot}10H_2O(s) + 2H^+(aq) + SO_4{}^{2-} \rightarrow \\ 4B(OH)_3(aq) + 5H_2O + 2Na^+ + SO_4{}^{2-} \end{gathered} \quad (3)$$

The effect of the added borax was thus a huge pH-titration of the *Vasa*. A total of 5 tons of borax was added during the 13 years of recirculation in the period 1965–79. In the light of the present results, the origin of the major part of that large amount of acid leached out from the hull must have been the formation of sulfuric acid, starting after the salvage when oxygen gained access to the reduced sulfur compounds in the wood. The amount of added borax was sufficient to neutralise 1.3 tons of sulfuric acid, which would mean that about 100 kg of sulfuric acid was produced annually.[5]

It seems possible that the rate of acid production may have slowed down somewhat due to reduced moisture in the wood and possibly lower oxygen access after the surface treatment with PEG 4000. However, the numerous acidic sulfate salts precipitating on the surfaces of the hull timbers, and the occasionally very low pH values obtained with wet pH indicator paper ($pH < 1$) show that formation of sulfuric acid still is in progress.

Assuming a current annual production of about 80 kg sulfuric acid, an equivalent of about 2 tons sulfuric acid would now be present in the *Vasa*'s wood. That amount of acid is consistent with the relative amount of sulfate in the XANES analyses of the core samples. The measured spectra indicate about 25% sulfate in the surface layer of totally 2.5 tons of sulfur, as estimated from the elemental analyses. Transformation of about 0.7 tons reduced sulfur to sulfate would correspond to 2 tons of sulfuric acid.[5] The SXM spectra show sulfur oxidation products in the form of sulfate particles distributed in cavities of the *Vasa*'s wood structure, which is a strong indication that sulfuric acid has been released (Fig. 10).[7]

The low sulfate content in the *Mary Rose*'s wood is expected during the spray treatment that washes away sulfates and acid (Fig. 9).[3,13] The large amount of reduced sulfur still remaining in the wood of the *Mary Rose* and in the surface layers of the *Vasa*'s timbers could potentially produce an additional 5–6 tonnes of acid if it all would oxidise to sulfates and acid. To achieve an immediate increase in pH before devising more efficient long-term treatments, the acidic sulfate salt affected wood surfaces of the *Vasa* have been treated with an alkaline solution ($pH \approx 9$) containing 5% bicarbonate ($NaHCO_3$) and soda ($Na_2CO_3{\cdot}10H_2O$) in the weight ratio 7 : 3. A more alkaline solution could be harmful to the wood by causing alkaline hydrolysis, in particular of the degraded lignin-rich surface layer.[35] The surface treatment, which was carried out by applying wet poultices or hand spraying, temporarily raised the surface pH, but many of the treated surfaces reverted to pH $\leqslant$ 3 within a few months. This treatment also removes some of the PEG, which leaves the wood surface with a dry and fragile appearance, and is clearly not an effective long-term procedure.[5,7]

Recently, deacidification introducing calcium hydroxide nanoparticles into the wood structure has been proposed as an efficient treatment for acidified wood, and in particular for the *Vasa*.[36] The method first requires dissolution and extraction of the bulking PEG material from the wood, followed by soaking with a suspension of calcium hydroxide nanoparticles in 2-propanol. The nanoparticles would then penetrate the degraded wood and react with the acid forming crystals of gypsum. A deposit of surplus calcium hydroxide and calcite particles in contact with the cell walls is reported in a test. However, it is difficult to comprehend that such a treatment can be anything but highly detrimental to marine-archaeological wood. With moisture returning to the wood an extremely alkaline microclimate would be created around the hydroxide particles that efficiently would hydrolyse the exposed lignin in the already degraded wood structure.[35] It is unlikely that carbon dioxide can diffuse into the wood structure and completely convert the calcium hydroxide to carbonate, as is claimed.

Gas treatment by ammonia would be a more gentle method to neutralise the acid in the conserved PEG-treated wood. The experiences reported from an ammonia treatment of the *Batavia*'s timbers seem promising,[12] and will be tested further. When the PEG conserved timbers were mounted on a steel support for exhibition purposes some timbers were found to be friable and very acidic. The acidity of some badly salt affected degraded timbers of the *Batavia* was occasionally found to be as low as pH = 1.2 and was ascribed to sulfuric acid derived from the oxidation of pyrite. An ammonia treatment was developed to reduce the acidity and also halt the oxidation and acid hydrolysis of the cellulose. Both gaseous ammonia and saturated ammonia solutions proved to be effective in changing the surface pH of the samples, and a plateau value of pH = 4.0 was reached after a few weeks, and was reported to be stable after several years.[12,16]

5.3 Iron removal with chelates

Extraction of iron from marine-archaeological wood by dissolving the iron compounds in slightly alkaline solution requires specially designed chelates that can form very strong and soluble complexes with iron(III) ions.[5,37] EDMA, derived from the well-known chelate EDTA, ethylenediaminetetraacetic acid ethylenediiminobis(2-hydroxy-4-methylphenyl)acetic acid, is such an efficient chelating agent, which forms particularly strong bonds with iron(III) in a well fitting cage of six surrounding donor atoms (Fig. 15). A similar chelate without the bond enhancing phenol rings is DTPA, diethylenetriaminepentaacetic acid.[5,37]

EDMA was developed to be used as micro-nutrient on carbonate-rich alkaline soils in the Mediterranean to increase the productivity of orange and citrus trees, since the natural sources of iron were unavailable for the plants. As shown by the stability constants, EDMA and DTPA can keep iron(III) ions dissolved in alkaline solutions up to pH $\approx$ 11 and pH $\approx$ 8, respectively.[5,37] The EDMA extraction process is easily followed visually because of the strong red colour of the iron–EDMA complex (Fig. 15).[5,7] The iron extracting ability of those chelates is being tested on archaeological wood. The efficiency is higher when the chelate is deprotonated in moderately alkaline solutions, but too high pH values (>9) may during prolonged exposure affect especially the exposed lignin in the surface layers of degraded wood.[35]

6 Summary

6.1 Sulfur and iron accumulation

Accumulation of sulfur is a common occurrence for marine-archaeological wood preserved under anoxic conditions in seawater. Samples from several historical shipwrecks reveal sulfur concentrations that could lead to post-conservation problems similar to those of the *Vasa*. The *Bremen Cog*, which was preserved in a riverbed, is an exception with low sulfur levels due to the low sulfate concentration in the flowing river water. The source of the sulfur accumulation can be traced to the oxygen-depleted conditions in the seabed, where sulfate-reducing bacteria produce hydrogen sulfide, H_2S(aq), which transforms to different reduced sulfur compounds in the waterlogged wood. High concentrations of both sulfur and iron are found in the bacterially degraded 1–2 cm outer layers in the *Vasa*'s wood. The lower ($\sim$1% S) but more uniform sulfur profile of the *Mary Rose* indicates that the cellulose degrading "erosion bacteria" have facilitated the penetration of Fe^{2+} ions and H_2S(aq) throughout the timbers.[5,7,14] With oxygen access after the salvage and promoted by the redox couple iron(II)/iron(III), the reduced sulfur compounds slowly

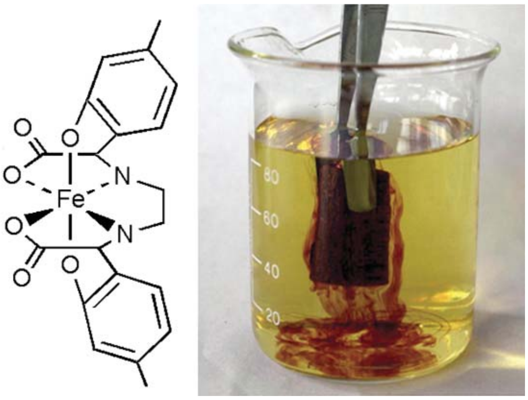

Fig. 15 The iron(III) ion in the $[Fe(III)–EDMA]^-$ complex is octahedrally enclosed by six ligating oxygen and nitrogen atoms. The electron-donating phenol rings increase the bond strength and are also responsible for the blood-red colour of the complex, which is evident when the iron compounds in a piece of *Vasa*'s wood start to dissolve in a solution of Na_4EDMA at pH $\approx$ 10. (Photograph reproduced by permission of © Stefan Evensen, the Vasa Museum.)

oxidise to sulfuric acid in the moist wood. Estimates from elemental and XANES analyses indicate that the present acidity in the *Vasa*'s wood corresponds to an amount of 2 tons of sulfuric acid, while the ongoing spray treatment washes out the acid from the timbers of the *Mary Rose*.

In the *Vasa*'s wood the sulfur and iron profiles in some cases correlate, which would indicate that iron(II) sulfides were originally formed, part of which already has oxidised to iron sulfates, both with iron(II), *e.g.*, rozenite and melanterite, and iron(III), *e.g.* natrojarosite, $NaFe_3(SO_4)_2(OH)_6$. In the *Mary Rose* samples no similar correlation is obvious, possibly because iron(II) ions were less abundant, except close to corroding iron objects. The high acidity in the *Vasa*'s wood probably originates mainly from the readily oxidised and more accessible inorganic iron(II) sulfides, while the organosulfur compounds may to a higher degree be protected in the lignin-rich middle lamella. The formation of acidic sulfate salt precipitates on the PEG impregnated wood surfaces are enhanced by fluctuations in the relative humidity.[13,24] This emphasizes the importance of keeping a stable climate when storing or exhibiting conserved marine-archaeological wood.

6.2 Conservation concerns

For devising long-lasting conservation methods of marine-archaeological wood with sulfur and iron accumulation, analyses are needed for speciation of the amounts of reduced sulfur compounds with different stability within the wood. The different rates of oxidation of the reduced sulfur compounds in ambient conditions, as well as the speciation and mechanisms of the sulfur accumulation, need further investigation. The organosulfur in lignin-rich parts bound as thiols and disulfides is likely to be more inert toward oxidation and less accessible for removal, than the iron(II) sulfide particles. Further studies will help decide whether the organosulfur and also the elemental sulfur, can be kept in the wood by creating a stabilizing micro-climate, or if removal during wet treatment, *e.g.* by the use of a selective oxidising agent, should be attempted. Also the combined degrading effect of the acid and iron in conserved marine-archaeological wood requires investigation.

FT-IR spectroscopy has previously been used to examine the degree of wood deterioration.[16] The polysaccharides generally degrade more rapidly than the lignin, and the ratio of lignin to cellulose and hemicellulose increases as the degradation of the wood proceeds. Wood components show characteristic infrared absorption bands in a "fingerprint" wave number region from about 900 to 1750 cm^{-1}, which can be used to diagnose the relative amounts of lignin, cellulose and hemicellulose, and thereby the extent of degradation of the wood. This technique, applied preferably with an FT-IR microscope at a synchrotron beamline in combination with X-ray micro-spectroscopy (*e.g.* at beamline ID21 at ESRF), can be used to follow the degrading effects of acid and iron in ambient or accelerated conditions on the marine-archaeological wood, but also to assess the outcome of the various treatment procedures, *e.g.*, the ammonia gas exposure and the iron–chelate extractions.

The neutralization of acid in the surfaces of the *Vasa*'s wood by aqueous bicarbonate and soda solutions does not give lasting effects. A more efficient and also gentler method would be neutralization by gaseous ammonia. The results reported from the treatment of the *Batavia* timbers are promising,[12] even though such a treatment would be a technically advanced task for the *Vasa*'s large hull. Oak wood is naturally acidic (fresh oak wood has pH $\sim$ 5) and even relatively high acidity does not seem to cause on its own very rapid degradation of the cellulose. However, the combined effects of PEG impregnation and the omnipresent oxidative redox couple iron(II)/iron(III) in marine-archaeological wood, are not well understood. On the other hand, alkaline treatments dissolving lignin have been well investigated.[27,35] Prolonged alkaline treatments could be detrimental especially to the surface layers of the artefacts, where bacteria have degraded the cellulose and exposed the lignin to chemical attack. For the *Mary Rose* the recirculated PEG solutions are monitored to assess when the strongly acid-forming sulfur compounds are exhausted. The low-molecular PEG solutions are also capable of removing some part of the iron content in the wood. Commercial antioxidants are being evaluated for subsequent stabilization of the remaining, lignin-bonded organosulfur compounds, and also to prevent PEG degradation.

Most of the iron contaminants of the marine-archaeological wood seem be to fairly efficiently removed by the Fe(III)-complexing agents EDMA and DTPA, and repeated washings can remove the red discoloration from the iron(III)–EDMA complexes. However, rather long extraction times (months) are needed and the alkalinity should then be kept sufficiently low ($pH < \sim 9$) to make sure that exposed lignin in the degraded outer layers is not significantly affected. The bulking agent PEG is also dissolved in the aqueous treatment, which makes it necessary to re-conserve objects treated with EDMA or DTPA solutions.

6.3 Outlook

All repeated treatments of archaeological artefacts must be well motivated and thoroughly tested. The methods should be chosen and applied with great care to minimise the strain on the weakened wood of the artefacts. Thus, before considering any major re-conservation for the *Vasa*, either with bath treatments for loose objects or treatments of the large hull, further investigations are necessary. In the international "Preserve the *Vasa*" project, such tests are being carried out to assess the rate of the detrimental processes in the wood, and to find ways to retard the oxidation and acid forming reactions. Renewal of the spray treatment for the *Vasa* would be stressful for the degraded wood surface. Neutralizing the acid with an ammonia gas treatment, followed by storage in stable and low ($\sim$55%) relative humidity seems to be a better way to appreciably slow down and hold back the production of acid and its effects. Variations in the relative humidity in the Shipwreck Galleries, Fremantle, housing the *Batavia* timbers, which are infested with iron sulfides (see Section 3.1), are found to promote surface salt formation.[12] The new climate system, installed in 2004 in the Vasa Museum, will by keeping a more stable relative humidity slow down the deterioration processes and allow the time needed to find and evaluate appropriate methods.

Until suitable treatments are established for the sulfur and iron related conservation problems for waterlogged wood, it is advisable before an intended salvage to analyse samples from the timbers of shipwrecks to anticipate the conservation concerns for that specific object. If possible the shipwrecks could be left in undisturbed sites for the time being, or reburied for future generations.

7 Bibliography

The conservation of waterlogged archaeological wood has been the subject of nine international conferences initiated by the International Council of Museums (ICOM), Committee for Conservation Working Group on Wet Organic Archaeological Materials (WOAM). The contributions, which together give a good account of the development in the field, are published in the WOAM Conference Proceedings (see *e.g.*, Refs. 9, 10, 12, 13, 16, 24), exclusively available from the German Maritime Museum, Bremerhaven (http://www.dsm.de/Pubs/woam.htm). More readily available general reviews are provided by Pearson,[28] Rowell and Barbour,[29] and Kaye.[30] Håfors has given a detailed account of the pioneering conservation work on the *Vasa,*[6] and Jones recently edited a volume providing a background and description of the conservation methods devised for the conservation of the hull and a huge variety of other recovered objects of the *Mary Rose*.[1] For an up-to-date description of the biological degradation of marine-archaeological wood the doctoral thesis of Björdal is recommended.[14] Accounts of applied sulfur XANES spectroscopy are given *e.g.*, in the articles by Pickering *et al.*,[22,24] with theoretical interpretations in an insightful recent review by Solomon *et al.*[20]

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