MICROSCOPIC STUDY OF WATERLOGGED ARCHEOLOGICAL WOOD FOUND IN SOUTHWESTERN CHINA AND METHOD OF CONSERVATION TREATMENT

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Abstract. Thousands of waterlogged wood pillars beneath crop fields were discovered during the 2008 excavation of an archeological site in southwestern China. Specimens were studied with scanning and transmission electron microscopes, and 2.5 cm × 2.5 cm × 5-cm specimens were dehydrated with methanol followed by treatment with neutral phenol–formaldehyde (PF) resin. The wood, identified as *Pinus kesiya* var. *langbianensis*, was severely degraded by bacterial surface erosion and tunneling of cell walls. Bacterial tunneling was more frequently observed near the cell corners with thick walls. Bacterial degradation of cell walls was accompanied by accumulation of degradation products and bacterial slime in cell lumens. Neither brown-rot nor soft-rot decay was detected in the wood. The wood samples gradually darkened after sampling, but removal of degradation products with methanol and a brief 2% oxalic acid treatment reversed the discoloration. The average specific gravity and crushing strength of the waterlogged wood were 0.25 and 7.1 MPa compared with 0.37 and 33.7 MPa of normal wood of the same species. Treatment of the waterlogged wood with neutral PF resin increased specific gravity to 0.44 and crushing strength to 12.8 MPa. The PF treatment minimized shrinkage and stabilized wood color of the waterlogged wood.

Keywords: Waterlogged wood, bacterial degradation, microscopy, phenol–formaldehyde, discoloration

INTRODUCTION

Bacterial degradation of wood at or near anaerobic conditions is a slow process. Schmitt et al (2005) found only surface deterioration of some of 400,000-yr-old spruce spears unearthed from a brown-coal mine in northern Germany while the interior portions remained relatively sound. Lignin in the cell wall has an inhibitory effect on bacterial degradation so that softwoods are more resistant to bacterial attack than hardwoods (Schmidt and Liese 1982; Holt and Jones 1983). In pine wood submerged under rice fields for more than 2000 yr, Kim and Singh (1999) found tracheids of normal wood were heavily degraded but tracheids of severe and mild compression wood suffered only S2 layer erosion

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with relatively intact S1 layers. Bacteria may degrade cell walls by surface erosion and by cavitation and tunneling in the cell wall (Blanchette 2000; Kim and Singh 2000). Schmidt et al (1987) suggested that the three patterns of bacterial attack occurred at different stages of attack with surface erosion at the beginning and cavitation and tunneling in the advanced stages.

In addition to bacterial degradation, waterlogged archeological wood also may contain other forms of biological deteriorations such as brown-rot, white-rot, soft-rot, and insect damage. For example, Björdal et al (1999), in examining hundreds of 9000- to 400-yr-old softwood and hardwood artifacts including sunken ships unearthed in Sweden, found that items associated with brown-rot decay were heavily degraded followed by those damaged by soft-rot fungi and tunneling bacteria, while those involving only surface-eroding bacteria retained a high degree of integrity. Growth of brown-rot fungi in wood is greatly arrested at moisture content over 50% and soft-rot fungi can tolerate higher moisture content, but bacteria degradation is the only form of biodegradation known to occur in anaerobic conditions in plain water-submerged wood. Based on growth requirements for various wood-degrading agents, Björdal et al (1999) were able to provide archeologists with additional information associated with unearthed wooden artifacts as to whether these items were suddenly submerged or by gradual submerging processes.

To conserve waterlogged archeological wood, the deteriorated cell walls need to be bulked and gaps filled before the objects are dehydrated. Hundreds of natural and synthetic materials have been used or considered for cell wall bulking and gap filling (Unger et al 2001). Because of the rigid reversibility/retreatability requirement concept in conservation of waterlogged archeological wood, polyethylene glycol (PEG) remains to be the preferred method (Christensen et al 2010). However, bulking and filling with PEG has a number of issues, including continuing wood deterioration by residual acidic agents in treated artifacts and iron staining (Godfrey et al 2012; Hocker et al 2012). These issues prompt the discussion of the reversibility/retreatability concept in conservation of waterlogged archeological artifacts (Hamilton 1999).

An ancient village containing thousands of waterlogged wood artifacts was unearthed in 2008 beneath rice and corn fields in southwestern China (Fig 1). Min (2009) described wood artifacts in the 1400-m^2 excavation as follows. Remains of wood structures consisting of numerous posts 14-20 cm in diameter and approximately 130 cm in length and some planks 6-8 cm in thickness and up to 6 m in length were found at the depth from 45 to 120 cm below ground level. Many erected wood members were mortised, and C^{14} dating indicated these wood artifacts dated back from 3115 to 2565 BP ($\pm$75). Wood posts also were found from 120 to 160 cm below ground level, where they were 8-15 cm in diameter and from 55 to 95 cm in length. Investigation of other cultural artifacts indicated this deeper layer belongs to the Neolithic Age, from 5300 to 3900 BP.

The 2008 excavation was halted when wooden artifacts began to crumble upon drying. Most excavation pits were back-filled with soil leaving only three filled with water for easy access. In the present study, physical conditions and microscopic characteristics of the waterlogged wood posts were examined and the method of

Figure 1. Archeological excavation in southwestern China revealed thousands of waterlogged wood artifacts beneath rice and corn fields. Note that top ends of most wood poles were about 70 cm from the horizontal members (lower right insert) and that top end of poles crumbled due to drying.

using neutral phenol–formaldehyde resin as an alternative to PEG for treating and reinforcing the wood was investigated.

MATERIALS AND METHODS

Sampling and Sample Processing

The archeological site is situated at 26°12′ N and 99°33′ E and at 2300 m above sea level. During the site visit in 2009, a section of water-logged wood about 20 cm in diameter and 0.75 m long was allowed to be sampled from one of the water-filled excavation vats. The sample was wrapped in a plastic sheet and transported to the laboratory within 6 h where it was immersed and fixed in 4% formaldehyde at room temperature for 3 mo. The formaldehyde-fixed section was cut into 10-cm thick disks followed by polyethylene glycol (PEG 2000) embedding for long-term storage. During PEG embedding, formaldehyde was completely washed off with tap water followed by, at 60°C and under atmospheric pressure, five changes of 50% PEG in water and five changes of pure PEG at 3-wk intervals. Several dried and crumbled wood pieces discarded at the site during excavation also were sampled for species identification.

Microscopy

Microscopy specimens were taken at peripheral, ends, near cracks, and in the interior areas of PEG-embedded disks. For light microscopy, selected samples were removed from embedded disks and sectioned with a rotary microtome, and the sections were examined with a Zeiss Axioplan II microscope. For scanning electron microscopy (SEM), sample surfaces to be studied were prepared from PEG-embedded specimens by razor blade cutting. After removing PEG, the razor-cut specimens were dehydrated by the methanol–acetone–pentane solvent exchange method of Thomas and Nicholas (1966) and examined with a JEOL-5800LV SEM. For transmission electron microscopy, specimens about 1 mm × 1 mm in cross-section were stained with 2% $KMnO_4$ at room temperature for 2 h, dehydrated with graded ethanol series, and embedded in Spurr's resin. Ultra-thin sections were studied with a JEOL-2100 200 kV STEM.

Determination of Specific Gravity and Compression Strength Parallel to Grain

Due to extensive deterioration, only four specimens were obtained without visible defects for measuring crushing strength. Specimens approximately 2.5 cm × 2.5 cm in cross-section and 5.0 cm along the grain were cut with a band saw from PEG-embedded disks. Two each of these rectangular specimens were used for determining specific gravity and compression strength along the grain before and after reinforcing treatment. PEG in all four specimens was removed with 60°C deionized water followed by soaking them in 2% oxalic acid for 2 h at room temperature to remove discoloration. After washing with cold deionized water, the water-saturated weight of each specimen was determined. Two specimens not to be subjected to reinforcement treatment were dehydrated by the methanol–acetone–pentane solvent exchange drying in which three changes of each solvent were done at 3-da intervals. Solvent-dried specimens were kept in 60°C oven for 3 da to obtain oven-dry weights. Compression strength parallel to grain at the 0.4-mm/min loading rate was measured after conditioning in the laboratory condition for 2 da (about 6% MC). Specific gravity was determined by the maximum moisture content method of Smith (1954).

Reinforcement Treatment

Reinforcement treatment was done with laboratory-prepared neutral phenol–formaldehyde resin (PF). In the first stage of PF resin synthesis, 1 mol phenol was reacted with 2.4 mol formaldehyde and 0.1 mol NaOH at 65°C for 2 h followed by heating the mixture at 95°C for 0.5 h. The prepared low-molecular-weight PF resin, having final viscosity about 10 to 20 cps, was neutralized with 4N H_2SO_4 to pH 7.0. Upon complete phase separation, the top aqueous portion was decanted, and the light-colored organic

portion (PF resin) was dissolved in methanol to makeup 20.0 w % PF stock solutions for treating bacterial degraded wood. The two remaining water-saturated specimens as described previously were dehydrated by three changes of methanol at 3-da intervals between changes followed by three changes of 20% PF solution (3-da intervals), all at room temperature and under atmospheric pressure. After air-drying for 2 da, PF-treated specimens were placed in a 100°C oven overnight (about 16 h) to cure PF resin. Specific gravity and crushing strength of PF-impregnated specimens were determined in the same manner as for nonreinforced specimens.

RESULTS AND DISCUSSION

Microscopy

The sampled waterlogged wood section and wood debris gathered at the site are believed to be *Pinus kesiya* var. *langbianensis* (A. Chev.) Gaussen (Simao pine). There are two major pine species currently distributed in the region, Simao pine and Yunnan pine (*P. yunnanensis*). Simao pine distributes mainly from low to up to 1200-m elevations with growth ring width ranging from 0.504 to 0.240 mm, while Yunnan pine distributes from 1200 to over 3000 m above sea level with growth ring width ranging from 0.363 to 0.131 mm and average specific gravity of 0.37 (Wang et al 2003). The assumed species identification was not based on wood anatomical differences between the two species but based on the difference in growth rate. The growth ring width of sampled specimens ranged from 0.50 mm near the pith to 0.20 mm near the bark. It is interesting that Simao pine was used in the ruins while the archeological site, situated at 2300 m elevation, at the present time is surrounded by Yunnan pine forests. At the present time, Simao pine forests are located at lower elevations, about 300 km to the south of the archeological site. Although only a few specimens were sampled for species identification, it is possible that the region was rich in Simao pine forests 3000 to 5000 yr ago but replaced by Yunnan pine forests due to climate change.

Light microscopy examinations of $KMnO_4$-fixed specimens showed severe wood degradation, in which as shown in Fig 2 secondary walls were heavily degraded and the lumens were partially filled with materials described by Björdal et al (1999) as cell wall degradation products and bacterial slime. Without $KMnO_4$ fixation, the degradation products and bacterial slime in SEM specimens were removed during water washing and solvent drying (Fig 3). SEM observations showed that pit membranes of bordered pits were partially destroyed and the cell wall surfaces revealed the remains of S_1 microfibrils (Fig 3a). Figure 3b shows window-like cross-field pitting and fungal hyphae in the ray. Fungal hyphae without clamp connections were frequently observed in rays and in tracheids adjacent to rays. Under a light microscope, these fungal hyphae in unstained radial sections showed dark brownish pigmentation (Fig 4) similar to common blue stain fungi in the sapwood of many wood species. Based on these observations, the fungal hyphae observed in the rays of this waterlogged pine wood are believed to be pre-existing stain fungi prior to being submerged. Fungal hyphae also were observed in tracheids not directly in contact with rays (Fig 3c), but the rare occurrence of fungal hyphae in tracheids did not provide sufficient evidence to identify whether they belong to stain

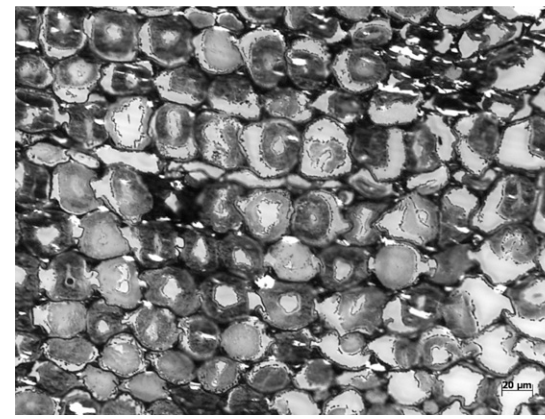

Figure 2. Semithin (0.5 µm) section of specimen taken from peripheral regions fixed with $KMnO_4$ showing severe cell wall degradation and accumulation of cell wall degradation residues and bacterial slime in lumens.

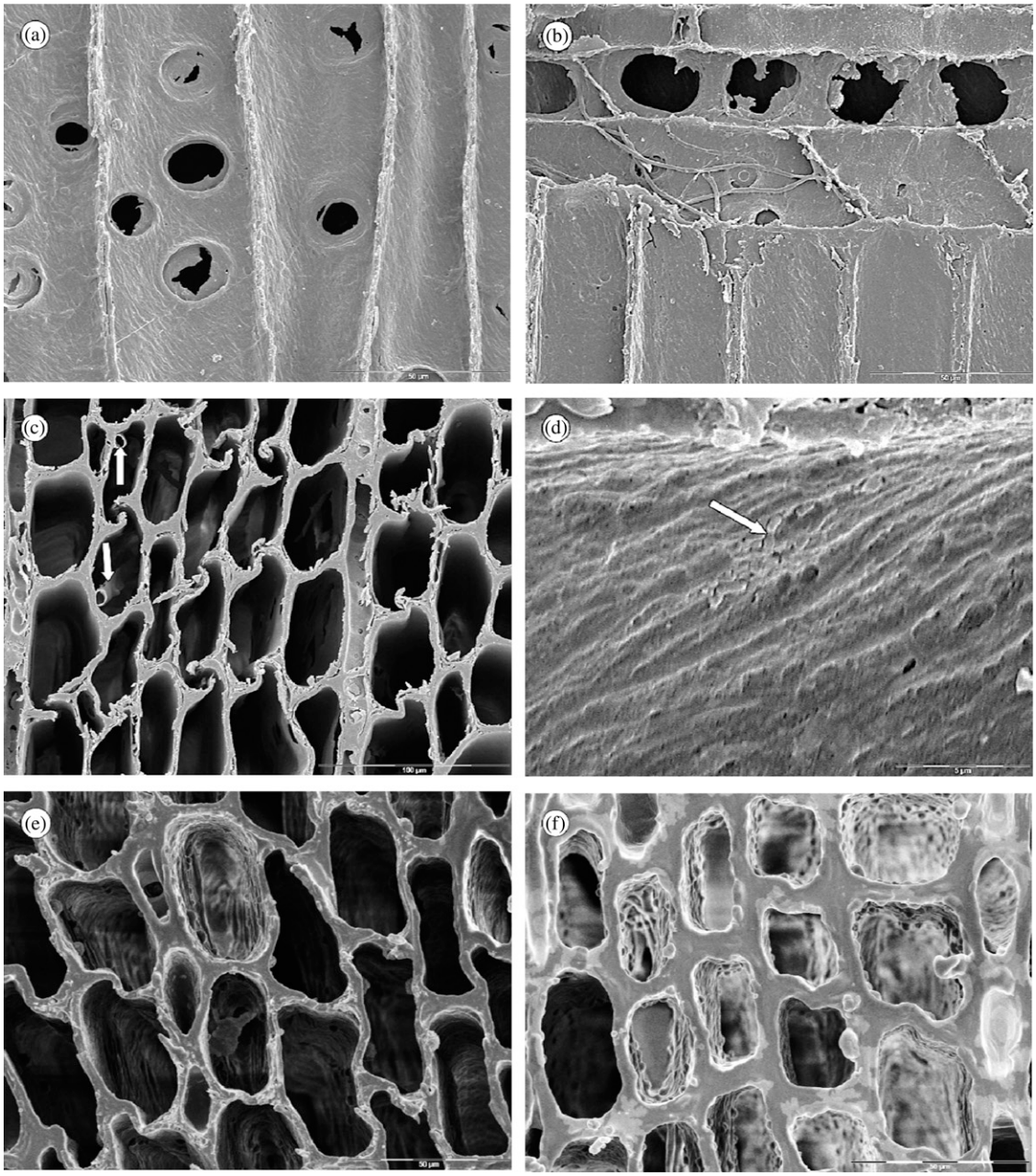

Figure 3. Scanning electron micrographs of waterlogged archaeological wood. (a) Bacterial destruction of bordered pit membrane (bar = 50 μm); specimen dehydration removed degradation products and bacterial slime observed in $KMnO_4$-fixed specimens shown in Fig 2. (b) Stain fungi in the ray and window-like cross-field pitting (bar = 50 μm). (c) Cell wall breakage in the earlywood region due to specimen preparation and fungal hyphae (arrows) in tracheids not directly in contact with ray (bar = 100 μm). (d) Bacteria lodged in troughs (arrow) of the remaining S1 layer surface (bar = 5 μm). (e) Earlywood tracheids of phenol–formaldehyde (PF)-treated specimen showing reduction of cell wall breakage (bar = 50 μm). (f) Latewood tracheids of PF-treated specimen, showing fullness of tracheid walls which indicates the effect of PF bulking (bar = 50 μm).

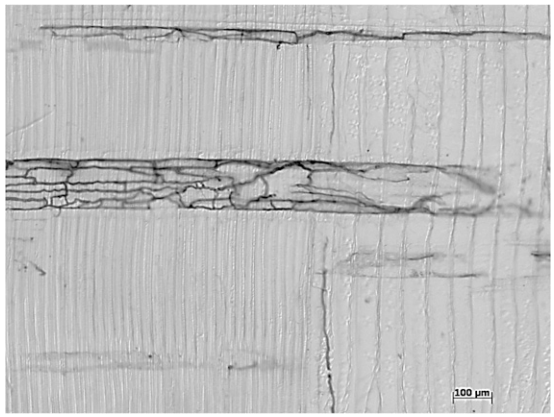

Figure 4. Unstained radial section of waterlogged wood, showing brownish pigmentation of fungal hyphae in rays and in tracheids adjacent to the rays.

or decay fungi. Because of very limited distribution, fungal hyphae observed in tracheids (Fig 3c) are believed to be an extension of stain fungi from nearby rays. Figure 3d shows surface eroding bacteria lodged in the cell wall trough parallel to S1 microfibrillar orientation. Surface eroding bacteria were observed only sporadically at this very advanced stage of bacterial wood degradation.

Transmission electron microscopy observations of thin sections indicated that bacterial wall surface erosion and wall tunneling often occurred simultaneously (Fig 5a). These observations support the assertion of Schmidt et al (1987) that the three forms of bacterial attack, surface erosion, cavitation, and tunneling, occurred at different stages of bacterial degradation rather than specific attack patterns performed by different bacterial species. Cell wall tunneling was more often observed in the cell walls adjacent to the cell corners where cell walls were thicker than the tangential and radial walls (Fig 5b). To perform cell wall tunneling, bacteria must first excavate into the cell wall. Thus, tunneling bacteria also are capable to excavate cell walls. The tunnel recesses shown in Fig 5b may indicate where bacteria excavated into the cell wall. In the cell corners, however, the cell walls were more resistant to bacterial degradation (Fig 5c), where surface erosion was the main pattern of degradation and bacterial tunnels rarely entered into these regions. Mineral crystals were observed among cell wall degradation products and bacterial slime in cell lumen (Fig 5d), which indicates the initiation of mineralization of the waterlogged wood.

Since neither brown-rot decay nor soft-rot decay was detected in the sample, water submersion of the wood structures was most likely a sudden event. Figure 1 shows that the top halves of all wood structures were missing, suggesting the wood structures were not totally submerged at the time of the initial submersion. In that scenario, fungal decay might have occurred at the water level, causing all wood posts to lose top halves at the same level. Despite an exhaustive search, fungal decay was not detected at the top end of the sampled section, which may be due to the fact that the top end material was lost as a result of drying and crumbling during the 2008 excavation. Whether the structures were partially submerged in the initial event depends on future studies of less disturbed samples.

Reinforcement Treatment

Table 1 compares specific gravity and crushing strength of the waterlogged wood before and after the PF treatment. The first untreated specimen was 0.22 in specific gravity with 6.1-MPa crushing strength, indicating that bacterial degradation caused about 41% reduction in specific gravity while losing about 82% crushing strength relative to normal wood (Sun et al 2007). The second untreated specimen was 0.27 in specific gravity with 8.2-MPa crushing strength, which showed about 36% reduction in specific gravity and about 76% reduction in crushing strength relative to normal wood. On the average, the PF treatment of waterlogged samples increased about 78% in specific gravity (from 0.245 to 0.435) and about 76% in crushing strength (from 7.14 to 12.54 MPa), but the crushing strength of the treated sample is only about 37% of that of normal wood. Nevertheless, the attributes of the oxalic acid and PF treatment, in addition to bulking the cell wall and increasing strength,

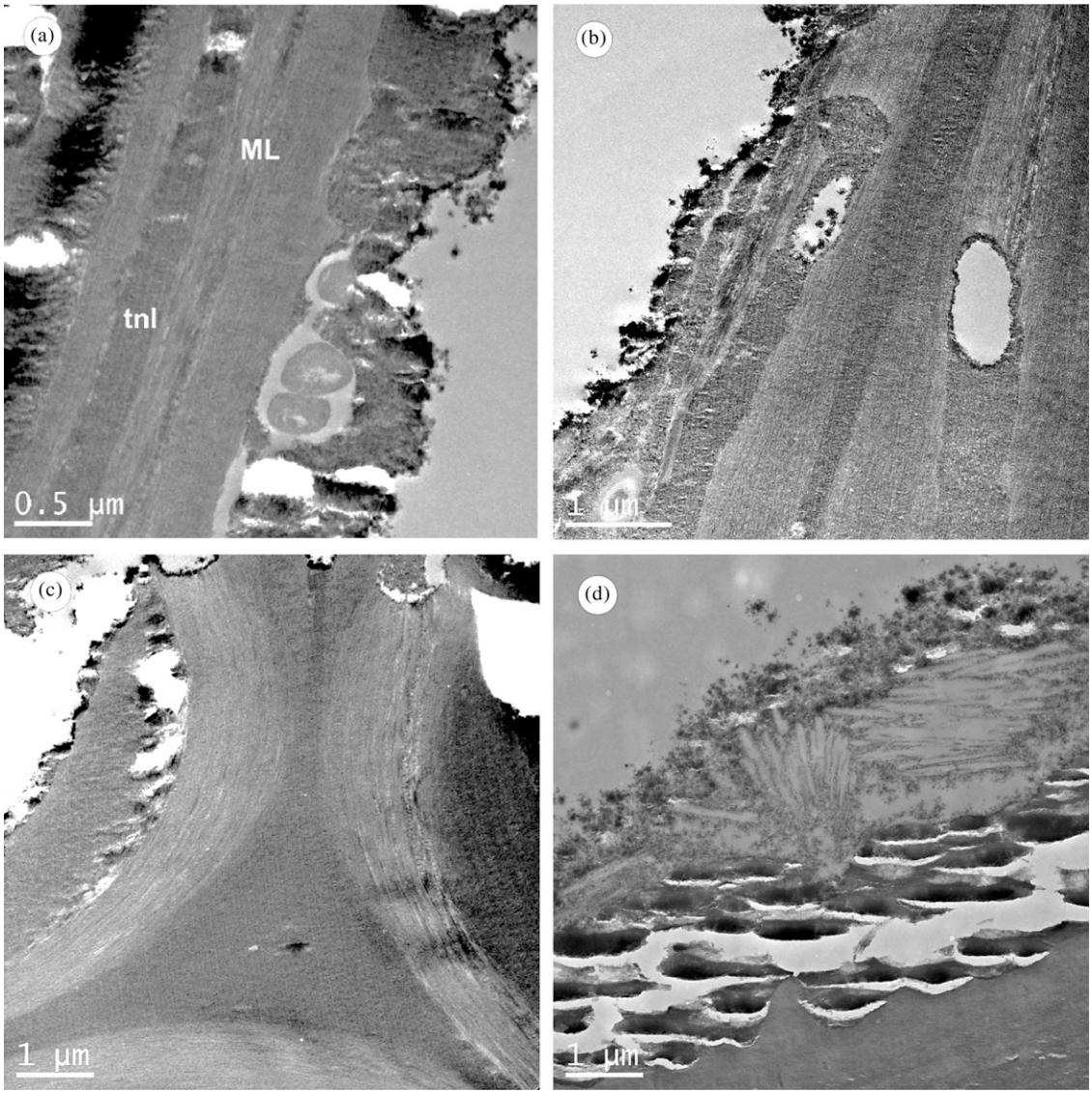

Figure 5. Transmission electron micrographs of waterlogged archaeological wood. (a) Simultaneous occurrence of bacterial surface erosion on the right side and tunneling (tnl) in the cell wall of a compound wall. (b) Three bacterial tunnels in the cell wall adjacent to the cell corner. (c) Surface erosion was the main mode of bacterial degradation; bacterial tunnels (top central) did not extend into the cell corner. (d) Presence of mineral crystals in lumen indicates initiation of wood mineralization.

also include removal of wood discoloration and color stabilization.

Figure 4e shows that the PF treatment greatly reduced cell wall breakage in the earlywood during specimen preparation in comparison with that in the untreated samples (Fig 4c). Figure 4f shows fullness of latewood tracheid walls, which indicates the bulking effect of PF. Figure 6 shows a discolored sample embedded in PEG 2000 and a sample after removing PEG followed by oxalic acid treatment, dehydration with methanol, and neutral PF treatments. The majority of the cell wall degradation products

Table 1. Specific gravity and compression strength parallel to grain of nontreated and PF-impregnated waterlogged wood samples.

Specimen	Treatment	Specific gravity	Ratio to normal wood	Crushing strength (MPa)[a]	Ratio to normal wood
No. 1	no	0.22	0.59	6.1	0.18
No. 2	no	0.27	0.73	8.2	0.24
No. 3	PF	0.42	1.14	11.45	0.34
No. 4	PF	0.45	1.22	13.63	0.41
Normal wood[b]		0.37 (0.27-0.44)		33.65 (23.6-49.2)	

[a] Compression strength parallel to grain of waterlogged samples determined at approximately 6% MC.
[b] Data from Sun et al (2007).

and bacterial slime was believed removed during the processes of PEG removal and dehydration. The dehydration and oxalic acid treatment reversed discoloration, while the PF impregnation and subsequent PF curing bulked the cell wall and minimized shrinkage and warping. Furuno et al (2004) treated Japanese cedar wood with neutral PF to 30% weight gain and found that the treated wood retained original color, attained 60% antiswelling efficiency, and was fungal decay-resistant.

Discoloration of the waterlogged wood began immediately after sampling and proceeded slowly during the 4% formaldehyde fixation stage. PEG-embedding did not stop but decreased the rate of discoloration. Björdal and Nilsson (2001) attributed discoloration of PEG-treated archeological wood to surface microbial growth, and Fors and Sandström (2006) found that discoloration was caused by surface accumulation of organosulfur and iron sulfides and iron chelation of phenolic compounds in marine-archeological wood such as the *Vasa* ship. In this study, no microbes were detected associated with discoloration, and color reversion was achieved by removal of degradation products and bacterial slime and by the 2% oxalic acid treatment. Therefore, in this case, wood discoloration is likely to be associated with oxidation of degradation products and chelation of phenolic compounds in the degradation products with metallic ions.

Figure 6. (Left) Discoloration of waterlogged wood sample embedded in polyethylene glycol (PEG) 2000 and stored for more than 2 yr. (Right) Wood color reversion and color and dimensional stabilization after the oxalic acid and phenol–formaldehyde (PF) treatments.

CONCLUSIONS

The bacterial degraded archeological wood was identified to be *Pinus kesiya* var. *langbianensis*. Bacterial degraded cell walls by surface erosion and tunneling, but cell wall tunneling was more frequently observed near the cell corners where the cell wall was thicker than the tangential and radial walls. Neither brown-rot nor soft-rot decay was detected in the sample, suggesting the initial submersion of the archeological site occurred very quickly.

Gradual darkening of the waterlogged archeological wood occurred soon after sampling. Bacterial degradation products accumulated in the cell lumen was responsible for the discoloration. Removal of the cell wall degradation products with organic solvents such as methanol and a brief treatment with 2% oxalic acid reversed the discoloration.

Methanol-dehydrated specimens were treated with 20% neutral PF in methanol. The treatment

increased wood strength, prevented excessive shrinkage and warping, and stabilized wood color. The PF treatment is not reversible, but the wood structure remains porous to allow retreating.

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REFERENCES

Björdal CG, Nilsson T (2001) Observation on microbial growth during conservation treatment of waterlogged archaeological wood. Stud Conserv 46:211-220.

Björdal CG, Nilsson T, Daniel G (1999) Microbial decay of waterlogged wood found in Sweden. Int Biodeterior Biodegradation 43:63-73.

Blanchette RA (2000) A review of microbial deterioration found in archaeological wood from different environments. Int Biodeterior Biodegradation 46:189-204.

Christensen M, Hansen FK, Kutzke H (2010) New materials for the consolidation of archaeological wood—Past attempts, present struggles, and future requirements. Proc Wood Science for Conservation of Cultural Heritage, International Conference on Wooden Cultural Heritage, Hamburg, 2009. http://woodculther.com/wp-content/2009/christensen (5 September 2013).

Fors Y, Sandström M (2006) Sulfur and iron in shipwrecks cause conservation concerns. Chem Soc Rev 35:399-415.

Furuno T, Imamura Y, Kajita H (2004) The modification of wood by treatment with low molecular weight phenol–formaldehyde resin: A properties enhancement with neutral phenolic-resin and resin penetration into wood cell walls. Wood Sci Technol 37(5):349-361.

Godfrey IM, Richards VL, Cha MY (2012) The post-treatment deterioration of marine archaeological wood—Where to now? 13 pages in The MUA Collection. http://www.themua.org/collections/items/show/1246 (24 August 2013).

Hamilton D (1999) Methods of conserving archaeological materials underwater sites. Conservation Research Laboratory, Center for Marine Archeology and Conservation, Texas A&M University. http://nautarch.tamuedu/CRL/conservation manual/file6.htm (24 August 2013).

Hocker E, Almkvist G, Sahlstedt M (2012) The Vasa experience with polyethylene glycol: A conservator's perspective. J Cult Herit 135:S175-S182.

Holt DM, Jones EBG (1983) Bacterial degradation of lignified wood cell walls in anaerobic aquatic habitats. Appl Environ Microbiol 46(3):722-727.

Kim YS, Singh AP (1999) Morphological characteristics of compression wood degradation in waterlogged archaeological pine wood. Holzforschung 53(4):381-385.

Kim YS, Singh AP (2000) Micromorphological characteristics of wood biodegradation in wet environments: A review. IAWA J 21(2):135-155.

Min R (2009) The Haimenkuo archaeological site of Jianchuan County, Yunnan Province Archaeology 7:17-24 [in Chinese].

Schmidt O, Liese W (1982) Bacterial decomposition of woody cell walls. International J of Wood Preservation 2:13-19.

Schmidt O, Nagashima Y, Liese W, Schmidt U (1987) Bacterial wood degradation studies under laboratory conditions and in lakes. Holzforschung 41(3):137-140.

Schmitt U, Singh AP, Thieme H, Friedrich P, Hoffmann P (2005) Electron microscopic characterization of the 400,000-year-old wooden Schoningen spears. Holz Roh Werkst 63:118-122.

Smith DM (1954) Maximum moisture content method for determining specific gravity of small wood samples. Rept. No. 2014. Forest Products Laboratory, For. Service, U.S. Department of Agriculture, Madison, WI.

Sun Q, Chen T, Wang CM (2007) Study of variation of density and mechanical properties of plantation *Pinus kesiya* var. *langbianesis* with different ages. Wood Processing Machinery 2007(3):19-23.

Thomas RJ, Nicholas DD (1966) Pit membrane structure in loblolly pine as influenced by solvent exchange drying. Forest Prod J 16(3):53-56.

Unger A, Schniewind AP, Unger W (2001) Conservation of wood artifacts: A handbook (Natural science in archaeology). Springer-Verlag, Berlin, Germany.

Wang C, Wang J, Jiang H (2003) A study of the comparative anatomy of *P. yunnansis* Franch. stem under different habitats. Pages 345-352 *in* Proc. 9th Annual Meeting of Chinese Wood Science Soc., 30 May 2011, Harbin, China. The Chinese Wood Science Society, Beijing, China.