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Original Research Article

A new bio-oxidation method for removing iron deposits from waterlogged wood of Nanhai I shipwreck, Guangdong, China

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ABSTRACT

The widespread presence of iron and sulfur compounds such as pyrite in marine waterlogged archeological wood (WAW) can cause irreversible damage to the safety of its preservation. This issue has been a longstanding concern for cultural heritage conservation communities. In this study, we examined the distribution and phase composition of Fe and sulfur compounds in wood samples obtained from the Nanhai I shipwreck using ESEM-EDS, micro-Raman spectroscopy, and an X-ray diffractometer. The removal of iron from WAW samples of the Nanhai I shipwreck using *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) was evaluated using conductivity and ICP-AES analysis. The results showed that *A. ferrooxidans* effectively improved the removal of iron from WAW. The degradation of fresh healthy wood during treatment was also analyzed using infrared spectroscopy, and the results showed that the treatment had little effect on the samples over a short period. This study demonstrates, for the first time, the feasibility of iron extraction from marine WAW by *A.ferrooxidans*. This was also the first attempt in China to apply biological oxidation to the removal of iron from marine archeological materials.

1. Introduction

Nanhai I was a wooden ship that sank during the Southern Song Dynasty (1127–1279 AD) while transporting porcelain outwards on the Maritime Silk Road. The Nanhai I shipwreck was the first shipwreck site discovered in China, and is a precious cultural heritage site of the Maritime Silk Road. As the earliest dated, largest, and best-preserved ocean-going trading merchant ship among maritime shipwrecks found in the world to date, it is of great significance to the study of the ancient Maritime Silk Road [1]. It was discovered in the 1980s, then recovered intact in 2007 and is currently housed in the 'Crystal Palace' at the Maritime Silk Museum in Yangjiang, Guangdong Province, China (Fig. 1).

The anaerobic environment of the ocean and the actions of microorganisms can lead to the deposition of iron and sulfur in wood. Generally, wooden shipwrecks that emerge from marine environments contain large amounts of iron. The Nanhai I shipwreck was excavated with more than 130 t of iron [2]. The cold and dark underwater environment of the ocean provides near-anoxic conditions for the preservation of waterlogged archeological wood (WAW). This environment creates a relatively stable 'time capsule' and is the reason why some large wooden shipwrecks have survived intact [3]. Simultaneously, this anaerobic environment and the large amount of sulfate in the ocean create conditions for the reproduction of sulfate-reducing bacteria (SRB). SRB can use wood as their carbon source and participate in the sulfur cycle through their own metabolism, gaining sufficient electrons to completely reduce SO_4^{2-} to H_2S [4–6]. The hydrogen sulfide gas then reacts with large amounts of iron ions in the environment to form sulfur-iron compounds. Under the combined action of erosion bacteria (EB), iron and sulfur gradually accumulate in wood; therefore, iron and sulfur compounds are commonly found in marine WAW [7].

Studies on several wooden shipwrecks have shown that sulfur and iron compounds are prevalent in the wood of these wrecks. Research on the iron and sulfur compounds in WAW began with conservation work in Vasa. Since 2000, deposits have begun to erupt on the surfaces of many Vasa panels, along with a marked increase in the acidity of the wood surface [8]. The main components of these deposits are jarosite ($KFe_3(SO_4)_2(OH)_6$), green vitriol ($FeSO_4{\cdot}7H_2O$), sulfur (S_8), and gypsum ($CaSO_4$) [8,9]. In 2006, similar accumulations of iron and sulfur were found on wooden shipwrecks such as Mary Rose and Batavia [10]. In 2003, Jones [11] speculated that the hull of Mary Rose might contain at least two tons of sulfide and iron compounds. In addition, Fors' analyses of WAW from shipwrecks in the Baltic Sea area, Kronan, Riksnyckeln,Tattran, the Puck Bay Boat, and the Ghost wreck, and on the Scandinavian West coast, the Göta wreck, Stora Sofia, and the Viking shipwrecks of Skuldelev, show the accumulation of sulfur compounds [12]. Several wooden shipwrecks excavated in China, such as Huaguangjiao I

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Fig. 1. The excavation and preservation site of Nanhai I shipwreck in the "Crystal Palace".

Fig. 2. Wood samples of Nanhai I shipwreck.

at Hainan Province [13], Xiaobaijiao I at Fujian province [14], Nanhai I [15] and Nan'ao I at Guangdong [16], also show deposits of sulfur and iron compounds.

The presence of sulfur and iron compounds is detrimental to the long-term preservation of wood. In the presence of water and oxygen, iron and sulfur compounds are easily oxidized to produce sulfuric acid, which further degrades damaged cellulose in wood. Simultaneously, the redox reaction between Fe^{2+} and Fe^{3+} plays a catalytic role in the oxidation of sulfuric iron compounds and the degradation of organic matter. In addition, with the gradual oxidation of sulfur and iron compounds, the molecular volume of the mineral units expands, causing stress damage to wood fibers [17–19]. Therefore, sulfur and iron compounds in WAW must be removed or controlled.

Many treatment methods have been proposed to remove and control sulfur and iron compounds in wood. Because of the late recognition of the hazards and effects of sulfur and iron compounds in WAW, for most foreign shipwrecks (e.g., Vasa and Batavia), issues related to sulfur and iron compounds were investigated only after the wood was reinforced and filled. Therefore, these shipwrecks have mainly adopted different methods to control the negative effects from sulfur and iron compounds. For example, some studies have shown that the use of alkaline solutions [20,21], gasses [22], and nanomaterials [23] can neutralize acids in wood or that wood can be wrapped with polymers to prevent further oxidation and acidification of the hull [24]. In China, the conservation of many ancient wooden shipwrecks builds on foreign experience and focuses more on the removal of sulfur and iron compounds, aiming to reduce the accumulation of sulfur and iron compounds in wooden artifacts and the negative effects they bring. For example, Zhang [25] conducted a study in 2014 on the removal of sulfur and iron compounds from wood samples of the "Xiaobaijiao I shipwreck." A comparative study revealed that the addition of oxidizing agents to conventional chelating reagents is more beneficial for the removal of sulfur and iron. Therefore, this study uses EDTA-2Na hydrogen peroxide compounded with EDTA-HO to achieve significant results in seawater desalination.

In recent decades, bioleaching, which uses microorganisms to dissolve metals, has been widely used to produce nonferrous metals [26]. Therefore, the idea of microbial oxidative removal of sulfur and iron from wooden artifacts has been proposed. In 2019, Magdalena et al. [27] used *Thiobacillus denitrificans* (*T. denitrificans*) as a biomaterial for the removal of sulfides commonly found in artificially degraded wood. This study demonstrated the ability of *T. denitrificans* to use reduced sulfur compounds present in wood samples as an energy source, demonstrating the feasibility of biotechnology for the preventive removal of sulfides from wood, which is the first step in the study of the bio-oxidative removal of sulfur and iron compounds from wood. In 2021, the latest study published by the team [28] continued with the study of *T. denitrificans* in combination with natural iron chelator (iron carrier) approach for the joint removal of iron and sulfur from wood. It was concluded that the denitrifying sulfur Bacillus chemical removal method was more effective than the conventional chemical method.

However, the deficiency of iron oxidation and poor elemental sulfur oxidation capacities of *T. denitrificans* give rise to a low bioleaching efficiency of iron, sulfur compounds, and sulfide minerals [29]. *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*), a widely used bioleaching bacterium, is highly efficient in the microbial oxidation of iron and sulfur compounds [30]. *A. ferrooxidans* is an acidophilic and chemolithotrophic iron- and sulfur-oxidizing bacterium [31,32]. As a chemoautotrophic bacterium, it is generally accepted to be an aerobic chemolithoautotroph that derives energy for growth from oxidative respiration involving the oxidation of ferrous iron or various sulfur compounds. In addition to its strong adaptability to the environment, it is one of the most widely engineered strains. It has prominent applications in coal desulfurization [33], biometallurgy [34–36], and environmental treatment [37]. At present, although *A. ferrooxidans* has a significant effect on the removal of iron and sulfur compounds such as pyrite [38], this biomaterial has not been applied in the field of heritage conservation, like *T. denitrificans*. Therefore, it is highly relevant to use *A. ferrooxidans* to remove iron and sulfur compounds from WAW to provide new methods for its protection.

In this study, we analyzed the feasibility of iron removal from WAW by *A. ferrooxidans* using wood samples from Nanhai I and the impact of the treatment process on the safety of wood. This study offers new possibilities for the microbial oxidative removal of sulfur and iron compounds from wood, which is of great importance for the long-lasting preservation of WAW.

2. Materials and methods

2.1. Wood samples

A large amount of loose wood was excavated from sea mud at the Nanhai I shipwreck site. Often, such loose wood samples cannot be spliced back into the hull of a ship. To maintain its condition and remove soluble salts, the vast majority of this loose wood was immersed in a pool containing deionized water. At the end of 2020, we removed Sample K2NH-23 and Sample K2NH-26 (Fig. 2) from the pools to conduct microbial oxidative iron removal experiments.The WAW samples from the Nanhai I shipwreck were severely degraded, and the wood lost large amounts of cellulose and lignin [15]. Therefore, even if the biological oxidation process affected the lignin and cellulose components of wood, it would have been difficult to detect and identify them. Compared with the archeological samples, the healthy pine samples had a more complete preservation of cellulose and lignin, and the samples were in a more uniform state of preservation, which can better characterize the effects of the bio-oxidation process on wood. Pine was one of the main species used in the construction of ancient wooden shipwrecks and preliminary studies showed that the main species in the Nanhai I shipwreck was pine [39]. Therefore, to evaluate the degradation of wood by bio-oxidation, we selected healthy pine samples as treatment objects to assess the risk of the method.

The large wood samples were cut into cubes of size $1 \times 1 \times 1$ cm^3. Each sample was placed in a beaker, autoclaved at 121 °C for 15 min, cooled, and set aside on an ultraclean table.

2.2. Bacteria and culture conditions

Acidithiobacillus ferrooxidans(ATCC 23270) was obtained from the culture collection of the State Key Laboratory of Microbial Technol-

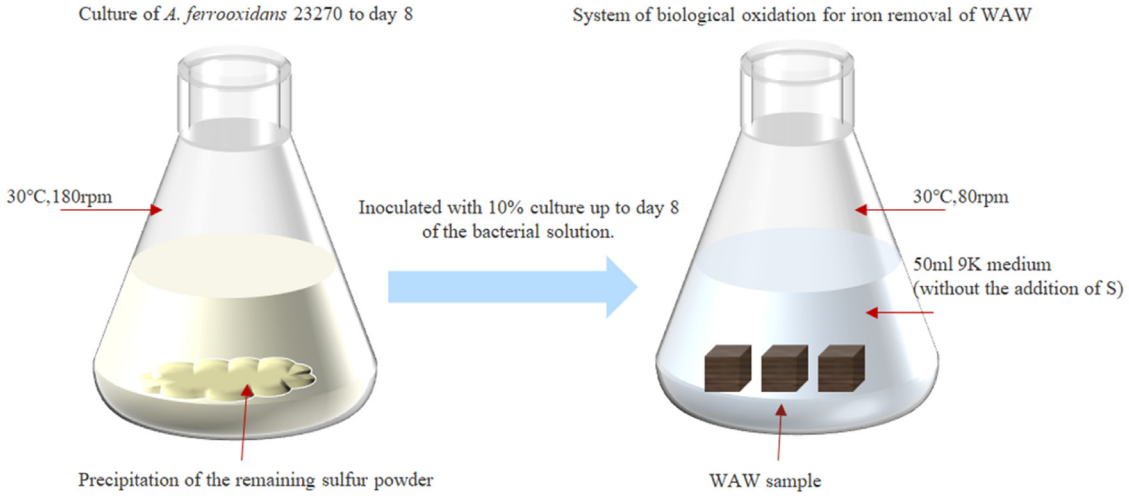

Fig. 3. Schematic diagram of inoculating *A. ferrooxidans* cultured to day 8 into the WAW microbial oxidation and iron removal system.

ogy at Shandong University, China. The strain was cultured using the aerobic culture technique at 30 ˚C with agitation (150 rpm) in 9 K medium. The composition of the 9 K medium was:2.5 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 g KCl,0.5 g K_2HPO_4, 0.02 g $CaNO_3$, and 5 g monomeric sulfur powder in a final volume of 1 L, with pH adjusted to 3.5. All chemical reagents used in the experiments were purchased from *Aladdin.*

2.3. Oxidation experiments

The 9 K medium used for WAW microbial oxidation for iron removal experiments must have the reduced sulfur powder removed to ensure that no additional sulfur or iron is added other than that in the WAW samples. A conical flask was filled with 50 ml of medium and autoclaved at 121 ˚C for 15 min. Three sterilized WAW samples were placed in each conical flask. The broth of *A.ferrooxidans* cultured up to day 8 was left for approximately 40 min, and after the unreacted sulfur powder in the conical flask had settled, 5 ml of the upper layer was carefully pipetted into the conical flask containing the WAW sample. The prepared reaction system was placed in an incubator and subjected to bio-oxidation experiments at 30 ˚C and 80 rpm for 25 days (Fig. 3). The same samples under the same experimental conditions, but without the system inoculated with bacteria, were used as negative controls, and the experiments and controls were performed in triplicate.

2.4. Analytical methods

2.4.1. Environmental scanning electron microscope (ESEM) with attached X-ray energy dispersive spectroscopy (EDS)

To determine the deposition and distribution of sulfur and iron compounds inside the WAW sample and the chemical composition of the salts deposited inside the wood, a Thermo Scientific Quattro S environmental scanning electron microscope equipped with an energy-dispersive X-ray analyzer was used to obtain high-resolution images and determine the elemental composition of the sample. The wood samples were first cut on the surface with a thin layer of wood slices in both the cross-sectional and longitudinal directions using a blade, vacuum freeze-dried, and then surface-sprayed with a gold layer. The observations were made under high vacuum, temperature 0 ˚C-35 ˚C, humidity 90% RH, operating voltage of +/−15 V (+5%), and 300 mA current.

2.4.2. Raman microspectroscopy

To determine the composition of iron and sulfur compounds in the wood samples and the changes in the composition of sulfur and iron compounds in the system, micro-Raman spectroscopy was performed on the WAW samples and the brown precipitates generated in the oxidation system. Micro-Raman experiments were performed using a RENISHAW inVia Laser micro confocal Raman spectrometer equipped with a research-grade Leica microscope with a spatial resolution of <0.5 μm. The spot diameter under the 50 × objective was set at 3 μm. The instrument uses a neon lamp as the signal source, and an 1800-line high-resolution grating, UV, and NIR simultaneously enhance the CCD detector. The spectral range is 100~1600 nm; excitation wavelength is 785 nm; laser power is 280 mW; laser power density is 0.5%, scanning time is 10 s, and scan time is 10 scans. The spectrograms were analyzed using LabSpec5 software (Horiba, Kyoto, J). The obtained spectra were compared to the reference spectra of pyrite and hematite(RRUFF library) [40].

2.4.3. X-ray diffraction (XRD)

The BRUKER D8Advance micro-area X-ray diffractometer was used to analyze the crystalline phase of the Nanhai No. 1 wood sample to add information to the Raman study. The angle of incidence was 5˚, with a CoKα wavelength (λ = 1.7903 Angström), providing a 90˚ curve detector. The intensity of the acquisition was 40 mA, and the sample acquisition time was 20 min. Finally, the diffraction patterns obtained were elaborated using Jade software.

2.4.4. Conductivity testing

To detect changes in the salt content of the solution during the biological oxidation process using a SevenCompact S230 conductivity meter, the conductivity of the stripped solution was measured at a constant room temperature of 25 ˚C and compared with the change in the conductivity of the negative control group without inoculated bacteria.

2.4.5. Inductively coupled plasma atomic emission spectrometer (ICP-AES)

To characterize the metabolism of iron during bacterial oxidation, the concentration of iron in the solution during treatment was measured using a MAT-YQ-012 inductively coupled plasma emission spectrometer (ICP-AES). Detection was performed at a temperature of 20 ˚C and humidity of 30% RH. The RF power for the detection was 1.2 kW, plasma flow rate was 12 L/min, vertical observation height was 8 mils, analysis pump speed was 12 r/min, stabilization time was 10 s, and reading time was 5 s.

2.4.6. Fourier transform infrared spectroscopy (FTIR)

Healthy pine samples were selected and subjected to FTIR analysis to assess the risk of microbial treatment of wood substrates. The cut and sterilized pine samples were placed in a bacterial culture solution and observed for aging degradation. The analysis procedure utilized a

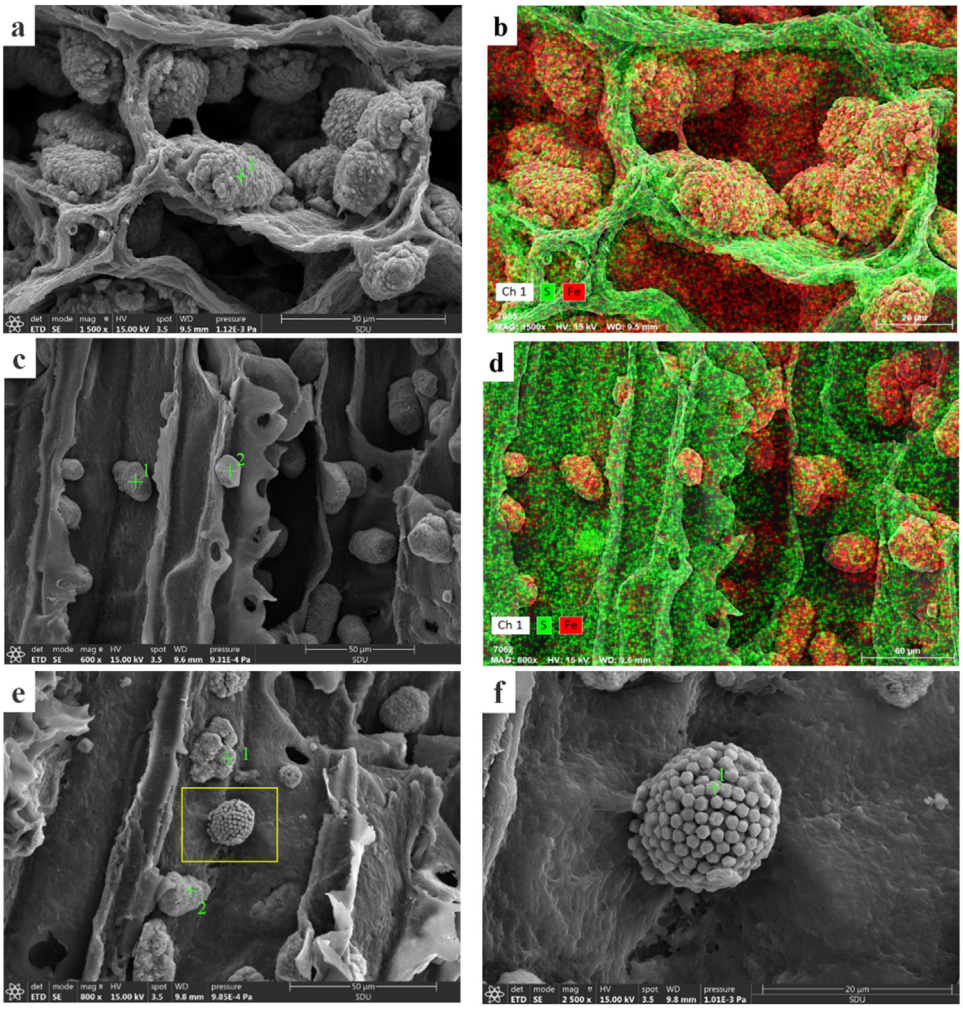

Fig. 4. SEM images of sulfur and iron deposit in wood structure of Nanhai I shipwreck **a** SEM image of cross-section of K2NH-23 sample; **b** K2NH-23 cross profile element mapping; **c** SEM image of K2NH-26 sample; **d** K2NH-26 cross profile element mapping; **e** SEM image of the longitudinal section of the K2NH-23 sample; **f** SEM image of pyrite framboid particles.

Fourier transform (micro) infrared spectrometer (Thermo Fisher Scientific) with a microscopic infrared mainframe (Nicolet iN10), attenuated total reflection (ATR) attachment, and transmission attachment (Nicolet iS10/iZ10 auxiliary optical stage). The surfaces of the treated wood samples were measured at ambient temperature. Averaged spectra were collected over 32 scans in the range of 400–4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Spectra were analyzed using the OMNIC™ software. To assess the final wood degradation, the intensity ratio between the lignin absorption band (1505 cm^{-1}) and the carbohydrate absorption band (1365 cm^{-1}) was calculated. These bands are assigned to aromatic skeletal vibrations in lignin, C–H deformation in cellulose and hemicellulose, C–O-C vibrations in cellulose and hemicellulose, and C–H deformation in cellulose [41].

3. Results and discussion

3.1. Sulfur and iron in archeological wood from Nanhai I

Analysis and knowledge of the distribution of sulfur and iron in WAW samples from Nanhai I and their physical phase composition are beneficial to our work on biological oxidation experiments. The SEM images show that a large number of deposited particles are distributed in the wood pores in the cross-section (Fig(s). 4a, b) and longitudinal section (Fig(s). 4c, d) of the sample. The elemental sulfur in the wood is relatively concentrated in the wood structure, and the results of its face scan distribution correspond to the wood structure. The distribution of elemental iron corresponds to the deposition of the particles. The main components of these deposits were iron, sulfur, and calcium, as detected by EDS (Table 1). The deposits near the wood surface have a high Fe content and are considered as Fe oxides [15]. The molar ratio of Fe to S in all deposited particles exceeded 2:1, and the oxygen content was high. This is mainly due to the fact that since Nanhai I was salvaged in 2007 and excavation began in 2011, the sulfur and iron deposited on the surface were gradually oxidized by the action of oxygen, water, and iron ions, so many iron oxides can be seen deposited in the wood. However, pyrite framboid crystal particles that have not yet been oxidized can still be observed inside the wood (Fig. 4f), which has a molar ratio of Fe to S of approximately 1:2 (Table 1). Micro-Raman spectroscopy confirms the presence of pyrite (FeS_2) in the samples (Fig. 5). The results of X-ray diffraction analysis (Figs. 6, 7) show that the sample may also contain iron sulfide (FeS), iron oxide hydroxide (FeOOH), pyrrhotite (Fe_7S_8), and hematite (Fe_2O_3).

Table 1
SEM–EDS results of deposit (at.%) in Fig. 4.

Sample	Analyzed point	C	O	Fe	Mg	S	Ca
K2NH-23	Fig. 4a-1	49.7	30.1	13.0	0.8	3.6	2.8
K2NH-26	Fig. 4c-1	33.8	10.5	19.3	0.8	0.7	2.3
K2NH-26	Fig. 4c-2	46.2	13.6	32.3	0.6	0.9	6.5
K2NH-26	Fig. 4e-1	47.8	25.6	18.9	0.9	2.2	4.6
K2NH-26	Fig. 4e-2	45.9	25.3	21.1	0.7	2.7	3.8
K2NH-26	Fig. 4f-1	18.4	——	27.2	——	54.5	——

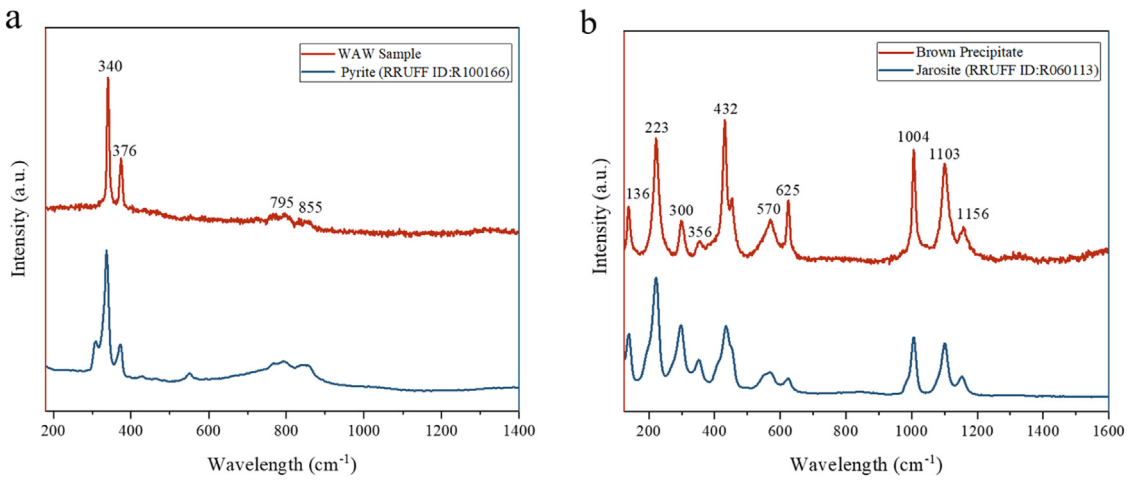

Fig. 5. The results of Micro-Raman experiments **a** Raman spectra corresponding to iron deposits in K2NH-23 samples (deep red line) and reference spectra of pyrite (FeS_2) (ink blue line). **b** Raman spectra corresponding to the brown precipitate formed within the bio-oxidation system (deep red line) and the reference spectrum of jarosite ($KFe_3(SO_4)_2(OH)_6$) (ink blue line).

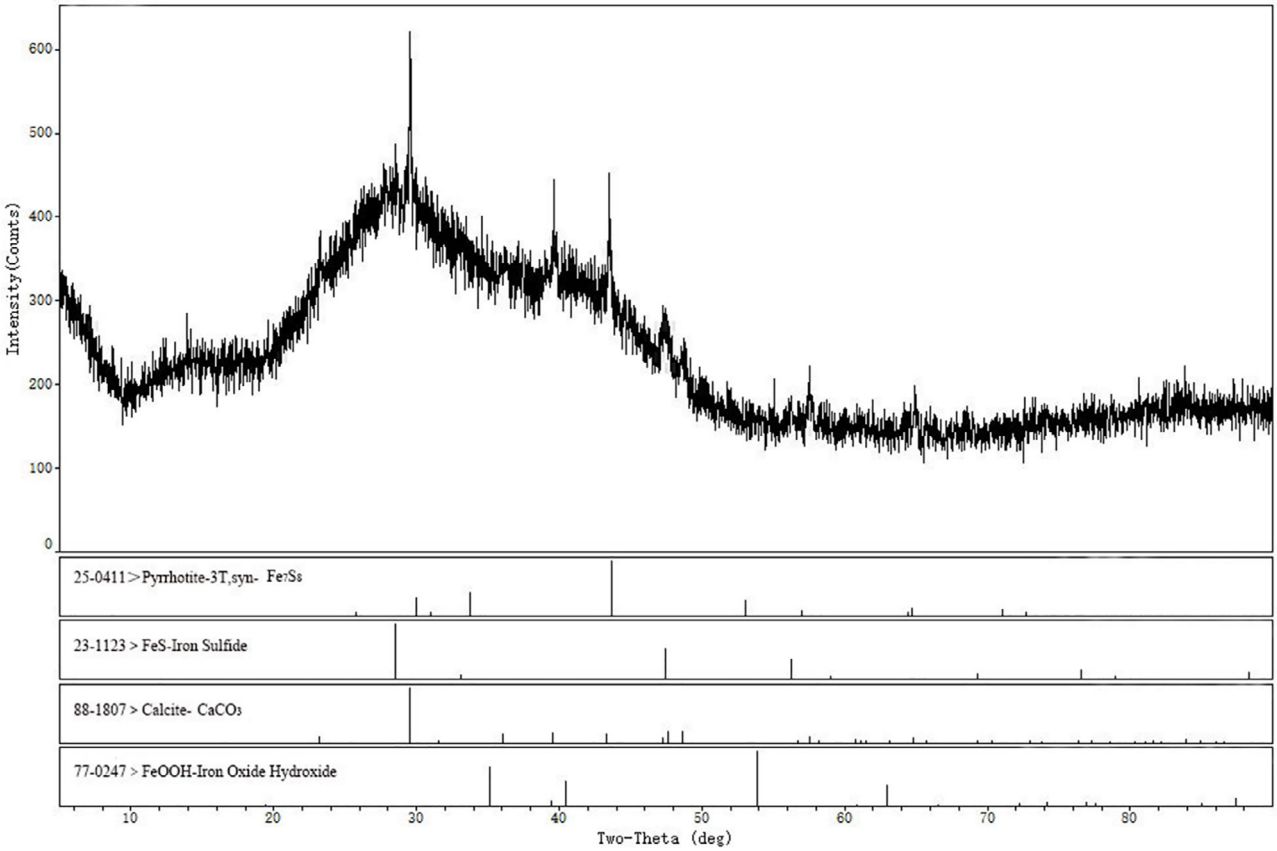

Fig. 6. XRD spectra of sample K2NH-23.

Thus, although some sulfur and iron compounds in the samples were oxidized to produce iron deposits such as hematite and iron oxide hydroxide, unoxidized sulfur and iron compounds such as pyrite were still present. The presence of these unoxidized iron deposits may have contributed to the degradation of the wood [19]. *A. ferrooxidans* has good ability to oxidize and metabolize pyrite and other sulfur and iron compounds [42,43]. Therefore, it is reasonable to select *A.ferrooxidans* for Fe removal from wood.

During the microbial oxidation experiments, the stripping solutions were photographed and recorded from day 1 to 20. As shown in Fig. 8, the color of the stripped solution gradually changed from colorless to cloudy yellowish, then reddish brown, finally deepening to a brown-black turbid state as time increased. As the removal time increased, a brown precipitate was generated. By comparing the color of the stripping solution with and without bacteria, we found that the color of the solution with the addition of *A. ferrooxidans* changed more rapidly (Fig. 8a). Because the reaction needs to be carried out in a shaker in an incubator, centrifugal force is generated even though the shaker is set to a low speed (80 rpm) to match the fragile WAW samples. Brown colloidal precipitates were observed adhering to the

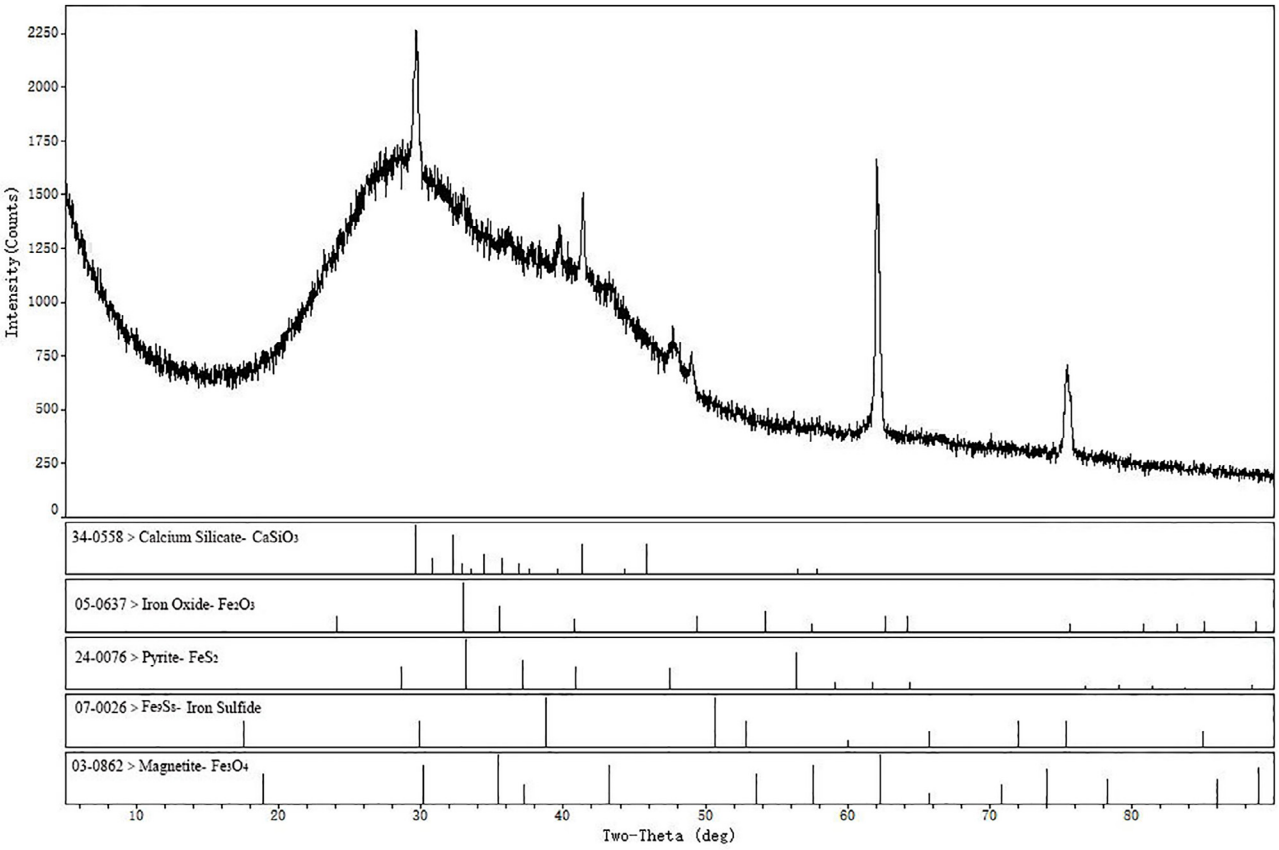

Fig. 7. XRD spectra of sample K2NH-263.2. Oxidation experiments.

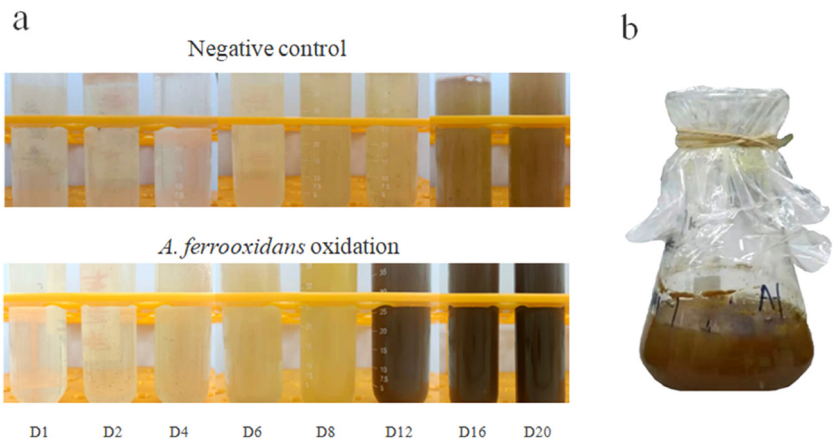

Fig. 8. a Variation of solutions corresponding to different treatment times during microbial oxidation treatment **b** treatment system on day 10 for sample 23 inoculated with *A.ferrooxidans*.

inner walls of the conical flasks during the later stages of treatment (Fig. 8b).

The reaction broth was filtered and a brown precipitate was collected. Using micro-Raman spectroscopy, the main component of the precipitate was identified as potassium alum pyrite. The presence of jarosite, a product of the biological oxidation of pyrite, is evidence of the oxidation of pyrite in the WAW sample by A.f. [43]. After bio-oxidation, iron was separated from the wood to form a jarosite precipitate.

We examined the conductivity of the treated solutions from days 1 to 20. Fig. 9 shows that the conductivity of the solution increased in the first 15 days with the precipitation of ions from the sample and decreased after the 15th day. The conductivities of the solutions inoculated with *A. ferrooxidans* were higher than those of uninoculated solutions. This indicates that the inorganic salt ions in the samples were continuously leached out during the early stages of treatment, which led to a continuous increase in conductivity. *A. ferrooxidans* can promote the oxidation of iron and sulfur in samples, producing Fe^{3+}, SO_4^{2-}, and other soluble salt ions in solution, which leads to an increase in conductivity. This indicated that *A. ferrooxidans* may have contributed to the removal of deposits from the samples.

The results of the ICP-AES analysis (Fig. 10) show that the iron content in the solution gradually increased during the first 15 days as the treatment time increased. Iron concentration increased more rapidly in the system inoculated with *A. ferrooxidans* than in the control groups. For example, on day 15, the iron concentration in the treated

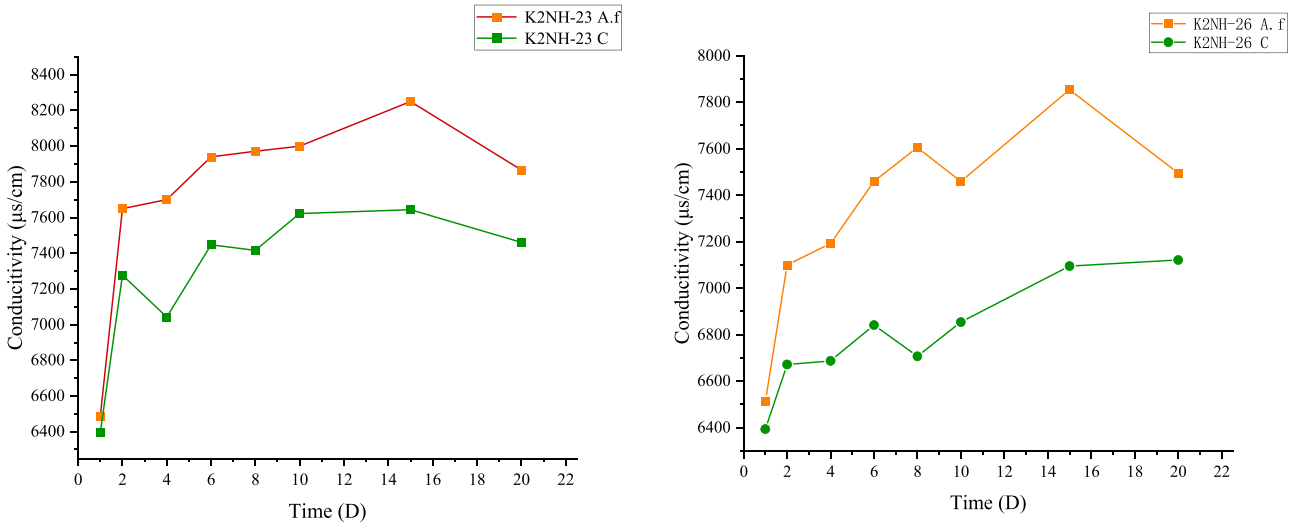

Fig. 9. Changes in conductivity in treated solutions.

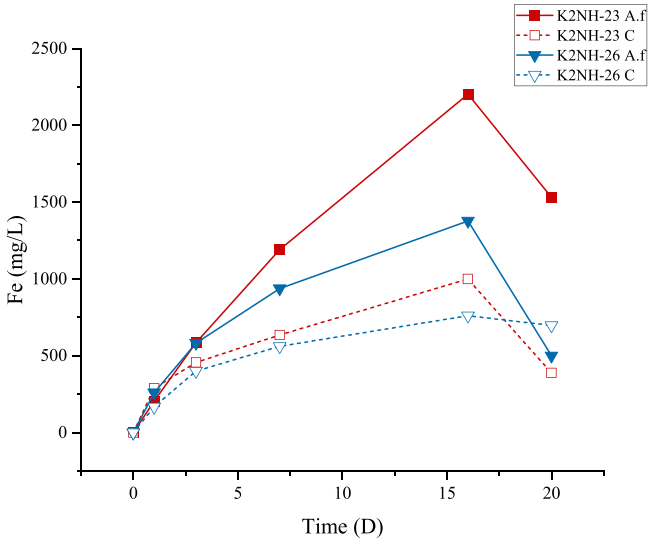

Fig. 10. Changes in iron content in the treated solution.

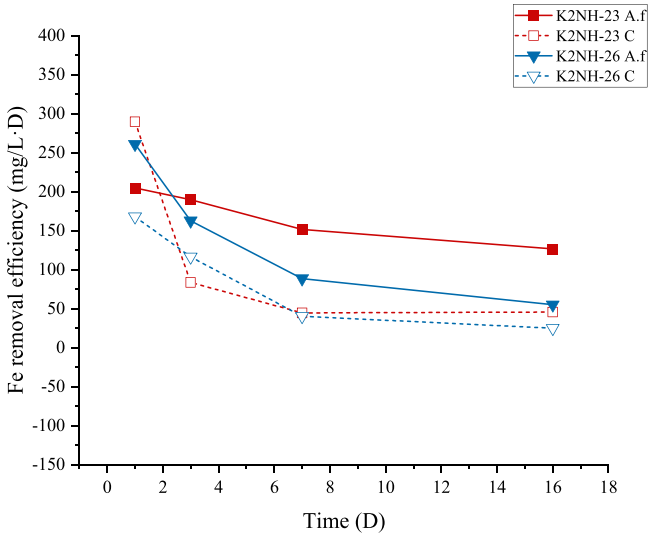

Fig. 11. Changes in iron removal efficiency in the treated solution.

solution of the K2NH-23 sample inoculated with bacteria reached 2153.2 mg/L, whereas the iron concentration in the treated solution of the corresponding negative control sample was only 1209.5 mg/L. The iron concentration in the treated K2NH-26 solution inoculated with bacteria reached 1377.6 mg/L, whereas the treatment solution of the corresponding negative control sample had an iron concentration of 760.8 mg/L. Fig. 11 shows the iron removal efficiencies under different treatment conditions. In the early stages of treatment, the Fe removal efficiency was relatively high, and the removal efficiency within the system inoculated with *A. ferrooxidans* was relatively high for the system inoculated with bacteria as a whole. These results suggest that this bio-oxidation method with *A. ferrooxidans* significantly promotes the removal of Fe from WAW samples, thus attenuating the negative effect of Fe on the preservation of WAW samples. At a later stage of the reaction, the conductivity of the solution and Fe content in the treated solution decreased, which may be related to jarosite precipitation produced during the bio-oxidation treatment. Some of the iron and sulfate ions in the solution were transferred to the potassium pyrite alum precipitate, leading to a decrease in the conductivity and iron content of the solution.

4. Risk evaluation

In parallel with the characterization of the sulfur and iron species present before and after treatment with *A. ferrooxidans*, Fourier-transform infrared spectroscopy (FTIR) measurements were performed to evaluate the impact of microbiological treatment on the wood substrate. The relative contents of the cell wall fractions of archeological wood were significantly altered compared to those of normal wood. The relative hemicellulose and cellulose contents decrease, whereas the relative lignin content increases [44]. Therefore, the preservation status of WAW can be determined from the lignin/hemicellulose ratio and the relative ratio of hemicellulose to lignin [45,46]. In general, the higher the degree of degradation, the smaller is the lignin/holocellulose ratio. The areas of the characteristic peaks of lignin (1505 cm^{-1}) and holocellulose (1365 cm^{-1}) were measured using OMSNIC software, and the I_{1505}/I_{1365} ratio was calculated (Fig. 12). The results in Fig. 10 show that the I_{1505}/I_{1365} ratio did not change significantly in the first 10 days, indicating that the microbial removal process had little effect on fresh pine wood over a short period of time. And after the 15th day, the curve shows a small upward trend, indicating that the removal system

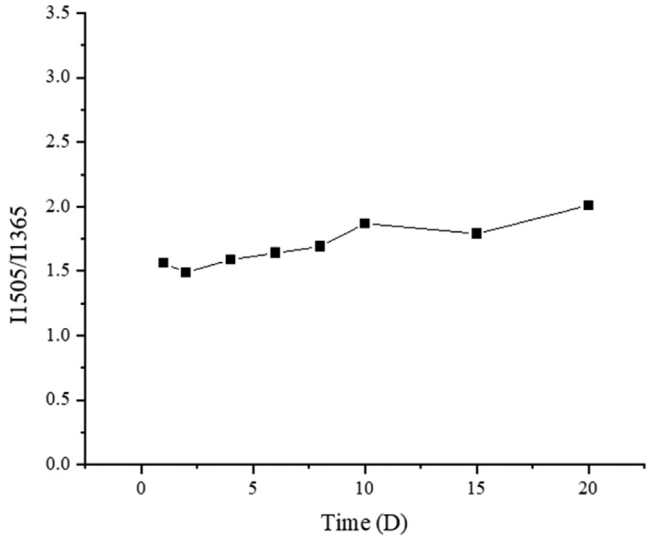

Fig. 12. Changes in I_{1505}/I_{1365} ratio of healthy pine wood during sample treatment.

may affect the degradation of wood as the treatment time is extended. The metabolic process of *A. ferrooxidans* does not consume organic matter; therefore, the reproduction and metabolic processes of *A. ferrooxidans* do not theoretically cause wood degradation. We speculate that this degradation trend may be related to the prolonged impregnation of wood samples and the acidic environment within the reaction system, where hemicellulose degrades under acidic conditions, which may eventually lead to the loss of holocellulose in the WAW samples.

5. Conclusion

The Nanhai I shipwreck, the largest ancient shipwreck in China, was analyzed. Its wood interior contained sedimentary salts of sulfur, iron compounds, and was found to contain pyrite that had not yet been oxidized. In recent years, although microbial oxidation treatments have been proposed for the removal of Fe and sulfur from wood, our study is the first to apply *A. ferrooxidans* for the removal of Fe from WAW. Through experimental and assay analyses, we showed that *A. ferrooxidans* could effectively improve the removal of iron from wood samples from the Nanhai I shipwreck. The degradation of fresh healthy wood during treatment was also analyzed using infrared spectroscopy, and the results showed that the treatment had little effect on the samples over a short period of time. However, after day 15, there was a slight upward trend in the ratio of I_{1505}/I_{1365}, indicating a certain degradation trend of wood, presumably related to the acidic environment within the reaction system. This requires follow-up and continuous attention. This study demonstrated the feasibility of iron extraction from marine WAW by *A. ferrooxidans*, providing a new approach to microbiological treatment for the removal of sulfur and iron compounds from marine WAW.

Since 2021, our team has been experimenting and working in the direction of the microbial removal of iron deposits from WAW. Currently, our team is systematically evaluating and improving this method, hoping to remove iron and sulfur from wood using a safer and more efficient treatment method. Relevant research reports should be published in the future. This study not only represents a significant innovation in the application of ferruginous bacteria for the removal of iron deposits from sea-sawn wood, but also a key step in advancing the application of green and sustainable materials for cultural heritage.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files or are available upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution Statement

Linxu Chen: Conceived and designed the study. Qinglin Ma: Directed the conduct of experiments and data analysis. Yishu Wang: Conducted experiments and wrote the manuscript. Zijun Zhao: Sorted out the data and assisted in the experiment. Jianqun Lin: Provided some of the materials and equipment needed for the experiments.

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References

[1] W. He, "Nanhai No. 1 Shipwreck" and "Maritime Silk Road", China Coast. Inspect. 10 (2019) 5.
[2] G. Tian, Explore the "Nanhai I" Water Iron Pot, Cult. Heritage World (2) (2020) 1.
[3] C.G. Bjordal, Microbial degradation of waterlogged archaeological wood, J. Cult. Heritage 13 (3-supp-S) (2012) S118–S122.
[4] Y. Fors, T. Nilsson, E.D. Risberg, M.M. Sandstr, P.J.I.B. Torssander, Sulfur accumulation in pinewood (Pinus sylvestris) induced by bacteria in a simulated seabed environment: implications for marine archaeological wood and fossil fuels, Biodegradation 62 (4) (2008) 336–347.
[5] A.B. Magdalena, M. Mathilde, J. Clémentine, J. Pilar, R. Céline, E.J. Schofield, J.J.I.B. Edith, Biological oxidation of sulfur compounds in artificially degraded wood, Biodegradation 141 (2018) S096483051731627X.
[6] J. Sorenson, D. Christensen, B.B. Jorgensen, Volatile Fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment, Appl. Environ. Microbiol. 42 (1981) 5–11.
[7] Rémazeilles C., Tran K., Guilminot E., Conforto E., Refait PJSiC: study of Fe(II) sulphides in waterlogged archaeological wood. 2013, 58(4):297.
[8] M. Sandstrom, I. Persson, F. Jalilehvand, U. Gelius, L. Gothe, Precipitation of salts on vasa: the sulfur problem, in: Proceedings of 8th ICOM Wet Organic Archaeological Materials (WOAM) Conference, 2001.
[9] Sandström M., Jalilehvand F., Persson I., Gelius U., Hall-Roth I.J.N.: Deterioration of the seventeenth-century warship Vasa by internal formation of sulphuric acid. 2002, 415(6874):893–897.
[10] Fors Y., Reviews MSJCS: sulfur and iron in shipwrecks cause conservation concerns. 2006, 35(5):399–415.
[11] Jones M.: For Future Generations: Conservation of a Tudor Maritime Collection.
[12] Fors Y., Jalilehvand F., Risberg E.D., BjRdal C., Phillips E., Sandstr M MJJoAS: sulfur and iron analyses of marine archaeological wood in shipwrecks from the Baltic Sea and Scandinavian waters. 2012, 39(7):2521–2532.
[13] Ma D., Zheng Y.: T T - Analysis of the iron sulfides in the shipwrecks Huaguang Reef I of the Southern Song dynasty. 2012.
[14] T. Jin, X. Ruan, J. Chen, Study on the underwater burial environment of the "Xiaobai Jiao No. I" site in Ningbo and its impact on the shipwreck, Res. Cult. Heritage China (1) (2016) 4.
[15] Zhang H., Shen D., Zhang Z., Ma Q.J.H.S.: Characterization of degradation and iron deposits of the wood of Nanhai I shipwreck. 2022, 10(1):1–13.
[16] Tian X.L., Nai-Sheng L.I., Zhang Z.G., Shen D.W., L. JJSoC, Archaeology: analysis and research on the wood from the Ming Dynasty shipwreck, Nan'ao No.1,in Shantou city, Guangdong. 2014.
[17] Vaclavkova S., JRgensen C.J., Jacobsen O.S., Aamand J., Elberling B.J.A.G.: The importance of microbial iron sulfide oxidation for nitrate depletion in anoxic Danish sediments. 2014, 20(4):419–435.
[18] K.M. Wetherall, and, R.M. Moss, and, A.M. Jones, and, A. DJJoAS: Sulfur and iron speciation in recently recovered timbers of the Mary Rose revealed via X-ray absorption spectroscopy. 2008, 35(5):1317–1328.

[19] Almkvist, P., - IJ: Extraction of iron compounds from waterlogged pine wood from the vasa. 2008.
[20] Macleod I.D., Kenna C.: Degradation of archaeological timbers by pyrite: oxidation of iron and sulphur species. 1991.
[21] F. YJIffk, O.K.O. strukturkemi: Sulfur-related conservation concerns in marine archaeological wood: the origin, speciation and distribution of accumulated sulfur with some remedies for the vasa. 2008.
[22] Chelazzi, G., S. Bjpmewe: Nanotechnologies for the conservation of waterlogged wood: the Vasa case studies. 2006.
[23] A EJS, B RS, B AM, A AMJ, D ASC, E JFWM, F AVCJJoCH: Strontium carbonate nanoparticles for the surface treatment of problematic sulfur and iron in waterlogged archaeological wood. 2016, 18:306–312.
[24] Multifunctional supramolecular polymer networks as next-generation consolidants for archaeological wood conservation, J. Proc. Natl. Acad. Sci. U. S. A. (2014).
[25] Z. Zhang, N. Li, X. Tian, J. Liu, D. Shen, Research on the removal technology of sulfur and iron compounds from the wooden shipwreck of Qing Dynasty in Ningbo "Xiaobai Jiao No. I", Heritage Conserv. Archaeol. Sci. (4) (2014) 9.
[26] Sugio, M., Inagaki, bacteriology TJJo: purification and some properties of sulfur:ferric ion oxidoreductase from Thiobacillus ferrooxidans. 1987.
[27] Zhang X., Chen Z., Liu H., Guo S., C. KJJoCSfC, Protection: effect of environment factors on the growth of sulfate-reducing bacteria. 2000.
[28] Monachon M., Albelda-Berenguer M., Lombardo T., Cornet E., J. Ejepjp: Evaluation of an alternative biotreatment for the extraction of harmful iron and sulfur species from waterlogged wood. 136(9):937.
[29] H.R. Beller, T.E. Letain, A. Chakicherla, S.R. Kane, T.C. Legler, Coleman MAJJoB: Whole-genome transcriptional analysis of chemolithoautotrophic thiosulfate oxidation by Thiobacillus denitrificans under aerobic versus denitrifying conditions, J. Bacteriol. 188 (19) (2006) 7005.
[30] Valdés, P., Quatrini, H. DSJH: comparative genome analysis of Acidithiobacillus ferrooxidans, A. thiooxidans and A. caldus: Insights into their metabolism and ecophysiology. 2007, 94(1–4):180–184.
[31] R. Dejarom: Heavy metal mining using microbes. 2002, 56(1):65.
[32] Naoya, O., Kazuhiro, S., Norio, M., Hiroshi, Bacteriology SJJo: anaerobic respiration using Fe(3+), S(0), and H(2) in the chemolithoautotrophic bacterium Acidithiobacillus ferrooxidans. 2002.
[33] I.C. Cardona, Biodesulfurization of two Colombian coals with native microorganisms, Fuel Process. Technol. 90 (9) (2009) 1099–1106.
[34] M. Vera, A. Schippers, W.J.A.M. Sand, Biotechnology: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A, Appl. Microbiol. Biotechnol. 97 (17) (2013) 7529–7541.
[35] C.L. Brierley, J.A.J.A.M. Brierley, Biotechnology: Progress in bioleaching: part B: applications of microbial processes by the minerals industries, Appl. Microbiol. Biotechnol. 97 (17) (2013) 7543–7552.
[36] Deveci H., Akcil A., Alp I.J.H.: Bioleaching of complex zinc sulphides using mesophilic and thermophilic bacteria: comparative importance of pH and iron. 2004, 73(3–4):293–303.
[37] D. Couillard, G.J.W.R. Mercier, Removal of metals and fate of N and P in the bacterial leaching of aerobically digested sewage sludge, Water Res. 27 (7) (1993) 1227–1235.
[38] L.V. Sian, N. Kelly, Q. Jamie, P.Y. Cheng, D.H. Wei, Y.L. Chan, H.J.M. Sarah, Surface chemical characterisation of pyrite exposed to Acidithiobacillus ferrooxidans and associated extracellular polymeric substances, Minerals 8 (4) (2018) 132.
[39] J.G. Reni Li, N. Macchioni, B. Pizzo, G. Xi, X. Tian, J. Chen, J. Sun, X. Jiang, J. Cao, Z. Zhang, Yafang Yin: characterisation of waterlogged archaeological wood from Nanhai No. 1 shipwreck by multidisciplinary diagnostic methods, J. Cult. Herit. 56 (2022) 25–35.
[40] J.A. Bourdoiseau, M. Jeannin, R. Sabot, C. Rémazeilles, Refait PJCe, Characterisation of mackinawite by Raman spectroscopy: effects of crystallisation, drying and oxidation, Corros. Sci. 50 (11) (2008) 3247–3255.
[41] Ganne-Chédeville C., JskelInen A.S., Froidevaux J., Hughes M., Navi P.J.H.: Natural and artificial ageing of spruce wood as observed by FTIR-ATR and UVRR spectroscopy. 2012, 66(2):163–170.
[42] J. Daoud, D.J.M.E. Karamanev, Formation of jarosite during Fe2+ oxidation by Acidithiobacillus ferrooxidans, Miner. Eng. 19 (9) (2006) 960–967.
[43] M. Gleisner, R.B. Herbert, P.J.C.G. Kockum, Pyrite oxidation by Acidithiobacillus ferrooxidans at various concentrations of dissolved oxygen, Chem. Geol. 225 (1–2) (2006) 16–29.
[44] Y. Xia, T.Y. Chen, J.L. Wen, Y.L. Zhao, J. Qiu, Sun RCJIJoBM, Multi-analysis of chemical transformations of lignin macromolecules from waterlogged archaeological wood, Int. J. Biol. Macrobiol. (2017) 407–416.
[45] N. Macchioni, B. Pizzo, C. Capretti, Giachi GJJoAS, How an integrated diagnostic approach can help in a correct evaluation of the state of preservation of waterlogged archaeological wooden artefacts, J. Archaeol. Sci. 39 (10) (2012) 3255–3263.
[46] Pizzo, G., Archaeometry F.J.: Evaluation of the applicability of conventional methods for the chemical characterization of waterlogged archaeological wood RID A-5399-2010. 2010, 2010,52(-):656–667.