doi:10.2533/chimia.2022.772 Chimia 76 (2022) 772–776 

# Microbes for Archaeological Wood Conservation

Sathiyanarayanan Ganesan, Sarah M. James, Mathilde Monachon, and Edith Joseph*

*Abstract:* This project focuses on innovative biological methods of extraction for the preservation of waterlogged wood suffering from salt precipitation and acidification. The principal investigator and her team proposed to exploit biomineralization capacities of some bacteria for anticipating the extraction of iron and sulfur compounds when wood is still wet. A comprehensive assessment of the extraction performances achieved on wood objects from lake and marine environments will allow a versatile extraction method to be proposed to end-users.

**Keywords**: Extraction · Iron · Sulfur · Waterlogged archaeological wood

In 2016, ***Edith Joseph*** obtained a prestigious SNSF Professorship proposing a breakthrough innovation, yet eco-friendly strategy for the conservation of wooden artefacts after excavation (MICMAC and Get on Board 2016–2023). In parallel, since 2012, she is employed as Associate Professor at the Haute Ecole Arc Conservation Restauration (University of Applied Sciences and Arts HES-SO) and developed her research activities on green technologies applied to cultural heritage involving both the University of Neuchâtel and this institution.

***Sathiyanarayanan Ganesan*** is a senior scientific collaborator working on the Get On Board! Microbes for the Archeological Wood Conservation (MICMAC II) since 2020.

***Sarah M. James*** is a PhD student working on the development and optimization of biotechnological approaches to cultural iron stabilization since 2019.

***Mathilde Monachon*** is a PhD student (2016–2020) and post-doctoral researcher since 2020 on chemometric applications for the preservation of waterlogged archaeological wood.

## 1. Introduction

### 1.1 *Background*

As most archaeological finds are composed of waterlogged wood, the phenomena of salts precipitation and acidification for these artefacts are a major concern for conservator-restorers. In fact, their preservation is complicated by the presence of iron and sulfur species, in particular iron sulfides, that can result in efflorescences, cracks, and finally loss of the structural strength of the objects (Fig. 1). Not only numerous everyday life items (*i.e.* chariots, buckets, utensils) from wrecks or wells suffer from these issues, but also important artefacts including the 17$^{th}$ century warship *Vasa* in Sweden or warship *Mary Rose* in Portsmouth (United Kingdom).[1] Also, freshwater artefacts can be polluted with iron and sulfur species and are now monitored, as the *Arles Rhône 3* in France.

To avoid weakening of structure and other alterations, interventions of conservation-restoration are then undertaken following a methodology that respects the aesthetic and historical values of the original artworks.[2] In particular, some basic principles are taken into consideration: the use of stable and safe materials, the

Fig. 1. Whitish efflorescences observed on the 17$^{th}$ century warship Vasa.

**Correspondence:* Prof. Dr. E. Joseph, E-mail: edith.joseph@unine.ch; edith.joseph@e-arc.ch; Institute of Chemistry, University of Neuchâtel and Haute Ecole Arc Conservation Restauration, University of Applied Sciences and Arts HES-SO, Neuchâtel, CH-2000 Neuchâtel

durability of the treatment, and its possible reversibility or at least retreatability.[2,3] So far, the approach commonly adopted for preventing shrinkage and breakdown is to consolidate the wooden structure. In particular, an impregnation with polyethylene glycol (PEG), replacing water in pores and vessels of wood, is performed and followed by slow air or freeze drying. Alternative systems involved polydimethylsiloxane[4] or polysaccharide materials.[5,6] However, in recent years, even consolidated objects became unstable and collapsed due to the presence of salts and acids in the core of the wooden structure.[7]

In order to remediate acidification, some successful experiments have been performed involving ammonia gas on the Batavia wreck.[8] Nevertheless, an identical protocol applied on the Vasa warship led to the degradation of cellulose and hemicellulose.[9] Furthermore, the use of alkaline nanoparticles was explored on the Vasa and Mary Rose wrecks and seems promising.[10,11] In parallel for removing sulfides, extraction methods with strong oxidants, complexing agents and reducing agents employed alone or in combination have been evaluated.[12] Some of these treatments pose serious health and safety risks.[12] Others have poor extraction rates or even cause bleaching and dislocation of wood.[12]

All the approaches presented above are curative methods adopted to mitigate the visible damage in already consolidated and dried wood but the initial cause (presence of iron sulfides and iron and sulfur by-products) remains unchanged within the wood core. Preventive methods that would be carried out before consolidation when wood is still wet are crucial to achieve the long-term preservation of waterlogged wood.[13]

### 1.2 *Originality of the Research Project*

As expressed by conservation stakeholders, there is an urgent need to develop frontline research in conservation science.[14] In particular for waterlogged wood, novel conservation methods should be conceived so as to specifically remove sulfur and iron compounds, maintain the physical structure and chemical stability of wood as well as improve its appearance. In their design, some criteria should be taken into account in terms of effectiveness, durability and innocuousness for humans and the environment. The premise of taking a bio-based approach revolves around manipulating and optimizing natural microbial processes for the removal of various components (bioextraction) and for the conversion into a passivation layer (biomineralization). To this purpose, we will exploit unique properties of selected microorganisms, studying three different metabolic processes either leading to the oxidation of sulfur or the complexation or reduction of iron:

- Oxidation of sulfur and sulfides by selected chemolithotrophic bacteria;
- Removal of iron(III) species using microbial complexing siderophores;
- Stabilization of the iron parts by precipitation of biominerals.

Even if such metabolic processes are widely studied in nature, the use of microorganisms for preserving waterlogged wood has hardly been addressed.[12] The microbial mechanisms involved are first investigated for standard sulfur and iron compounds. Then, microbial cultures are specially designed and the application procedure defined and assessed on degraded wood samples. Finally, real wood artefacts are integrated in order to validate the newly elaborated conservation method. Based on the results achieved, we thus propose an innovative and eco-friendly strategy for the conservation of wooden artefacts after excavation. Real progress is expected in terms of stability, effectiveness, and decreased toxicity.

It is worth mentioning that there is a growing interest for environmentally friendly processes (close to ambient temperature and pressure, at neutral pH) that do not require the use of toxic materials.[15] For example in stone conservation, protective treatments using microorganisms were developed to induce carbonate mineralization or sulfate reduction.[16,17] Also, the utilization of microbial films in corrosion control was illustrated as a novel strategy for protecting metal substrates.[18] In particular, some metal-resistant fungi were demonstrated to convert copper corrosion products into stable and insoluble copper oxalates.[19,20] As a result, a ready-to-use fungal treatment (biopatina) was identified for protecting copper alloys.[21] Also regarding iron conservation, promising results were obtained for the stabilization of archaeological iron by microorganisms.[22]

## 2. Oxidation of Sulfur and Sulfides by Selected Chemolithotrophic Bacteria

Iron sulfides accumulated in archaeological waterlogged wood oxidize once the wood artefacts are recovered causing damage in the objects. Investigation was first carried out on sulfur- and iron-rich phases and a specific biological extraction method was designed together with wood conservator-restorers. In parallel, artificially degraded wood samples were produced as analogues and are currently employed to assess the newly developed conservation methodology. Attempts are made to apply the improved extraction method on ancient collections of waterlogged wood presenting the problematic iron and sulfur species.

Biological oxidation of these compounds in controlled anoxic conditions is suggested as a preventive treatment. A preliminary study with *Thiobacillus denitrificans* available at the host institution showed that this bacterium is able to oxidize iron sulfides commonly found in waterlogged wood into sulfur and iron sulfates. The bacterium *T. denitrificans* was cultured in medium 113 (DSMZ 2010) with small wood pieces (1x1x1 $cm^3$) artificially impregnated with iron sulfides (*i.e.* mackinawite). We successfully obtained some time allocation at a synchrotron source (I18 beamline, Diamond Light Source, UK) that confirmed the presence of sulfate species in the wood treated with the bacterium, resulting in a publication in collaboration with the Mary Rose Trust, expert in wood biodegradation processes.[23] Deeper investigations were carried out with *T. denitrificans* and additional sulfur-oxidizing bacteria to understand which types of iron sulfides are used as energy source. Pure pyrite ($FeS_2$), mackinawite (FeS), and elemental sulfur ($\alpha$-$S_8$) were then synthesized and their degradation under anaerobic conditions was assessed by production of sulfates (Fig. 2), allowing to select the most appropriate strains in term of sulfur oxidation.

*Thiobacillus denitrificans* successfully metabolized elemental sulfur to sulfates in all the conditions studied (*i.e.* anoxic and oxic atmosphere, extra sulfur source, *etc.*). Mackinawite was also oxidized but at a slower rate. Finally, pyrite oxidation is under discussion with this bacterium. Some oxidation was observed under some experimental conditions (*i.e.* extra sulfur source and addition of chelating agent), but further investigations are needed

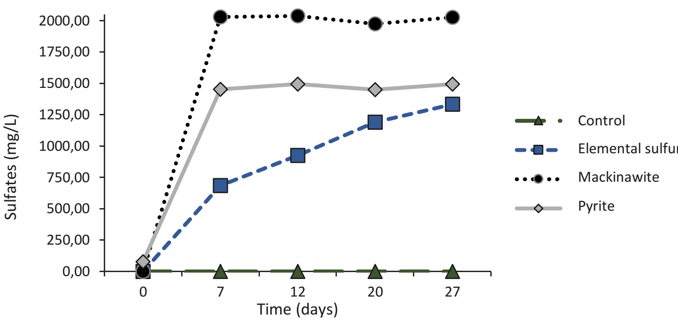


Fig. 2. Production of sulfates by the bacterium *Thiobacillus denitrificans* over time, as reaction of pyrite ($FeS_2$), mackinawite (FeS), or elemental sulfur ($\alpha$-$S_8$) with nitrates, in a culture medium containing a minimum amount of soluble sulfur sources (2 mM $Na_2S_2O_3$).

to ascertain the possibility of this bacteria to oxidize natural pyrite. *T. denitrificans* was then used to treat wood samples obtained from Swiss lakes. Sulfates production was observed over time.

## 3. Removal of Iron(III) Species Using Microbial Complexing Siderophores

Siderophores are the strongest iron chelators in nature, they can maintain iron(III) in solution making possible its extraction from wooden objects. An initial screening in chrome azurol S (CAS) agar medium and CAS liquid solution was carried out. From the tested bacteria, we selected *Pseudomonas putida*, *Serratia ureilytica* and *Escherichia coli* and the siderophore production was then evaluated with different iron(III) sources: $FeCl_3$ (bioavailable iron), hematite and goethite (no bioavailable iron). The extraction of iron from model wood samples artificially contaminated with iron and sulfur species was studied. First, a direct application of some of the bacteria mentioned above was conducted. However, siderophore-producing bacteria were not able to efficiently extract iron from artificially contaminated model wood samples.

The use of purified siderophores reported very good results. DFOM (deferoxamine mesylate, Desferal®) presented comparable results to the chemical chelator, EDTA (ethylenediaminetetraacetic acid). Even though the extraction times were longer with DFOM than EDTA, EDTA promoted the degradation of wood analogues. Therefore, DFOM is a safer alternative, not only for the user, but also for the wooden objects. DFOM also presented better results than our purified siderophore PVD. The use of commercial, stable siderophores can be, therefore, attractive for end users.

## 4. Stabilization of the Iron Parts by Precipitation of Biominerals

Regarding iron-reducing bacteria, some bacterial strains were isolated from iron coupons naturally corroded in a marine environment. In particular, their physiology (optimal growth pH and temperature), halotolerance and iron reduction capacities were ascertained and the most promising organisms identified through DNA sequencing. Individuated iron-reducing bacteria were domesticated to produce black iron carbonates (siderite) and greenish iron phosphates (mainly vivianite) and a first attempt of application to real artefacts was evaluated on corroded iron coupons and archaeological nails.[24–26]

Further tests were designed with *Pseudomonas putida mt2 (PPmt2)* based on its iron cycling indications, biohazard status, growth requirements (*i.e.* oxygen, temperature, salt, pH, and agitation), and potential for different application methods.[27] *PPmt2* was capable of growth in a poor nutritive culture medium without the addition of NaCl that is usually necessary for growth. Also, testing under agitation appeared to be mildly more effective than stationary testing. However, the latter would allow for different application modes (ex. solutions, gels) in practice. Oxygen requirement for growth was assessed with the addition of oxygen scavengers in the solution, as the oxidation of iron upon excavation is potentially damaging, and sodium thioglycolate proved successful in aiding the conversion of akaganeite. A 24 h pre-culture of *PPmt2* was incubated with four iron sources – iron powder, akageneite, ground archaeological mixture (composed of iron, iron oxyhydroxides, and traces of quartz from the soil). There was no conversion of pyrite, significant conversion of iron powder, and partial conversion of akaganeite. Increased conversion and rate of conversion for ground archaeological powder were also observed. Experiments are being conducted to test the effectiveness of both *PPmt2* and the natural biome on the conversion of iron powder, akaganeite, archaeological mixture (autoclaved), and a 50:50 mix of akaganeite and iron powder. The concept that the bacterial communities for archaeological artifacts may be reactivated for stabilization has been explored briefly at the Vindolanda Roman site in the UK, which identified that certain bacterial communities showed increased presence of more stable mineral formations than others.[28] These tests will help determine the impact the iron compounds treated, the intrinsic biome used, or a particular microorganism isolated from within the intrinsic biome has on conversion.

## 5. Biological Extraction Method for Waterlogged Wood Contaminated with Iron and Sulfur Species

A biological extraction application protocol (BT; 10 days extraction with siderophores + 20 days of incubation with *T. denitrificans*) as well as a traditional extraction method (CT) based on 1-day immersion in sodium persulfate + 7 days in EDTA and rinsing by ultrasonic baths were performed. The use of the selected microorganisms in association or not with isolated extracts was tested for iron complexation and sulfur oxidation. Alongside, a protocol to rinse the samples properly after the extraction treatment was tested.

The performance of the developed treatment was evaluated in terms of efficiency to remove sulfur and iron sulfides alongside the impact on wood color and morphology, and innocuousness of the used microorganisms. The visual appearance of the cubes treated with BT was closer to the appearance of untreated samples, while intense discoloration was observed on CT-treated samples (Fig. 3).

The effective iron and sulfur extraction were confirmed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Both extraction rates were highly independent, which means a high iron extraction rate is not necessarily correlated with a high sulfur extraction rate. BT samples had shown promising extraction rates for iron up to 75%. Similarly, sulfur extraction rates were also high in BT samples, up to 65% in some wood types. These rates are similar or more encouraging than the ones obtained for the CT extraction method. Raman spectroscopy analyses also validated the removal of sulfur species from BT samples (Fig. 3). In contrast, elemental sulfur was still detected on CT samples, while mackinawite and elemental sulfur were both detected on untreated samples. Finally, Attenuated Total Reflectance-Fourier Transformed Infrared spectroscopy (ATR–FTIR) proves that no further degradation of the wood matrix was detected after the application of either BT or CT extraction methods. After the extraction phase, all the wood model samples were consolidated with polyethylene glycol (PEG) impregnation followed by freeze-drying, and are currently being studied for their long-term stability.

## 6. Conclusions

Both applications on archaeological iron and waterlogged archaeological wood (WAW) require further exploration for practical application on heritage objects. A complete validation of the various procedures on iron artifacts or iron-wood objects from lake and marine environments will allow versatile methods to be proposed to end-users. At present, research is still being conducted for archaeological iron artifacts. The effectiveness of *Pseudomonas putida* mt2's conversion of iron powder over other oxidized iron species denotes the potential need for a secondary component to boost the desired reaction beyond oxygen stressing. For example, it could include the addition of iron powder to initiate a more uniform passivation layer or the use of siderophores produced by the same bacteria to aid treatment by removing thick concretions before biomineralization and increased availability of iron for conversion afterward. Additionally, experiments have been outlined to test the reproducibility of the natural biome, isolations from this natural biome, and *Methylophilus methylotrophus* (DSM 5691) based on indications from the archaeological biome study by Orr.[28] Once the critical control factors are recognized, a toolkit will be defined to determine the procedural steps necessary for uniform biomineralization.

The biological extraction treatment for iron and sulfur was successfully developed for WAW objects with efficient iron and

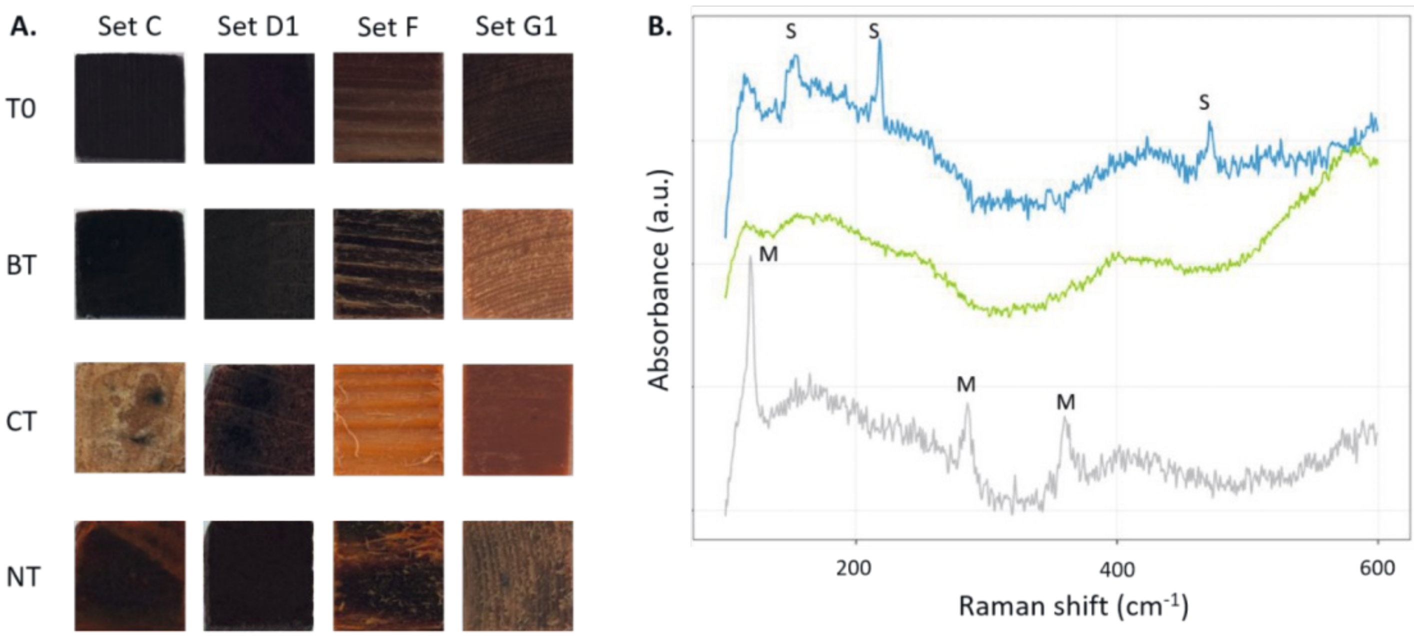


Fig. 3. Representative photographs of Neolithic and archaeological oak (sets C and D1) and lake and archaeological pine (sets F and G1) before extraction (T0) and after biological (BT) or chemical (CT) extraction, compared to untreated (NT) samples. B. Representative Raman spectra for chemically treated (CT, blue), biologically treated (BT, green) and untreated (NT, grey) samples, with characteristic bands of elemental sulfur (S) and partially oxidized mackinawite (M).

sulfur removal. Furthermore, the wood's appearance and structure were not disturbed. Therefore, the combination of iron chelators and sulfur-oxidizing bacteria can be used to remove iron-sulfides species from WAW. Further research is still needed to enhance the extraction of iron and sulfur since dissolution rates were not constant among the sets investigated, suggesting mainly a surface bioextraction. Experiments will be made to apply the improved extraction method on the ancient lake and sea waterlogged wood samples that possess the damaging iron and sulfur species.

For iron-wood composite artifacts, we could imagine developing a sequential protocol that includes iron extraction from the wood substrate and stabilization of corroded iron parts. One of the primary concerns with such a joint application is that one organism's needs and ideal parameters may be damaging or harmful to another organism or the object itself. For example, *Pseudomonas putida* is heterotrophic, while *T. denitrificans* is autotrophic. Secondarily, *P. putida* is a facultative anaerobe, which allows protocols to be developed using a less strict anoxic environment, whereas *T. denitrificans* is a strict anaerobe. This proves to be complicated for practical applications. Representative analogues with WAW and iron parts will be prepared through pre-aging procedures and in-depth characterization using spectroscopic analytical techniques for preliminary tests.

This article is based, in part, on an earlier review by Joseph and Junier.[29]

***Acknowledgements***

This work was supported by the Swiss National Science Foundation (grants no. PP00P2_163653/1, 2016–20, and PP00P2_190081, 2020-2023). The authors would like to thank the Archaeological service of Bern Canton, the Swiss National Museum and the conservation and research laboratory Arc'Antique (Nantes, France) for their support.

Received: July 15, 2022

[1] M. Ek, in 'Shipwrecks 2011- Chemistry and Preservation of Waterlogged Wooden Shipwrecks', Conference proceedings, Vasamuseet & Kungliga Tekniska Högskolan, Stockholm, **2011**, p. 193.
[2] B. Appelbaum, 'Conservation treatment methodology', Routledge, London, **2012**, p. 472.
[3] C. Brandi, 'Theory of restoration', Nardini editore, Florence, **2005**, p. 192.
[4] P. K. Kavvouras, G. Moraitou, C. Kostarelou, A. Zisi, M. Petrou, *Stud. Conserv*. **2009**, *54*, 65, https://doi.org/10.1179/sic.2009.54.2.65.
[5] M. Christensen, H. Kutzke, F. K. Hansen, *J. Cult. Herit*. **2012**, *13*, S183, https://doi.org/10.1016/j.culher.2012.02.013.
[6] Z. Walsh, E.-R. Janeček, M. Jones, O. A. Scherman, *Stud. Conserv*. **2017**, *62*, 73, https://doi.org/10.1179/2047058414Y.0000000149.
[7] M. Sandström, F. Jalilehvand, I. Persson, U. Gelius, P. Frank, I. Hall-Roth, *Nature* **2002**, *415*, 893, https://doi.org/10.1038/415893a.
[8] I. Mac Leod, C. Kenna, in 'Proceedings of the 4th ICOM Group on Wet Organic Archaeological Materials Conference', Eds. P. Hoffmann, J.A. Spriggs, T. Grant, C. Cook, A. Recht, Verlag H. M. Hauschild GmbH, Bremerhaven, **1990**, p. 133.
[9] Y. Fors, V. Richards, *Stud. Conserv*. **2009**, *55*, 41, https://doi.org/10.1179/sic.2010.55.1.41.
[10] R., Giorgi, D. Chelazz, P. Baglioni, *Langmuir* **2005**, *21*, 10743, https://doi.org/10.1021/la0506731.
[11] A. V. Chadwick, E. J. Schofield, A. M. Jones, G. Cibin, J. F. W. Mosselmans, *Solid State Ionics* **2012**, *225*, 742, https://doi.org/10.1016/j.ssi.2012.02.035.
[12] K. Tran, N. Bertout, F. Dalard, J. P. Magnin, in 'Proceedings of the 9th ICOM conservation Group on Wet Organic Archaeological Materials Conference', Eds. P. Hoffmann, J.A. Spriggs, T. Grant, C. Cook, A. Recht, Eds. Verlag H. M. Hauschild GmbH, Bremerhaven, **2005**, p. 227.
[13] G. Almkvist, I. Persson, *Holzforschung* **2006**, *60*, 678, https://doi.org/10.1515/HF.2006.114.
[14] P. M. Whitmore, 'Conservation Science Research: Activities, Needs, and Funding Opportunities: A Report to the National Science Foundation', Andrew W. Mellon Foundation: New York, **2005**.
[15] A. Bharde, D. Rautaray, V. Bansal, A. Ahmad, L. Sarkar, S.M. Yusuf, M. Sanyal, M. Sastry, *Small* **2006**, *2*, 135, https://doi.org/10.1002/smll.200500180.
[16] F. Cappitelli, L. Toniolo, A. Sansonetti, D. Gulotta, G. Ranalli, E. Zanardini, C. Sorlini, *Appl. Environ. Microbiol*. **2007**, *73*, 5671, https://doi.org/10.1128/AEM.00394-07.
[17] N. De Belie, *Mater. Struct*. **2010**, *43*, 1191, https://doi.org/10.1617/s11527-010-9654-0.
[18] R. Zuo, *Appl. Microbiol. Biotechnol*. **2007**, *76*, 1245, https://doi.org/10.1007/s00253-007-1130-6.
[19] E. Joseph, A. Simon, S. Prati, M. Wörle, D. Job, R. Mazzeo, *Anal. Bioanal. Chem*. **2011**, *399*, 2899, https://doi.org/10.1007/s00216-010-4279-2.
[20] E. Joseph, A, Simon, R. Mazzeo, D. Job, M. Wörle, *J. Raman Spec*. **2012**, *43*, 1612, https://doi.org/10.1002/jrs.4164.
[21] E. Joseph, P. Letardi, L. Comensoli, A. Simon, P. Junier, D. Job, M. Wörle, in 'Metal 2013: Proceedings of the Interim Meeting of the ICOM-CC Metal Working Group Edinburgh', 16-20 September, 2013., Eds. E. Hyslop,

V. Gonzalez, L. Troalen, L. Wilson, Historic Scotland and International Council of Museums: Edinburgh, **2013**, p. 203.
[22] M. Albini, L. Comensoli, L. Brambilla, E. Domon Beuret, W. Kooli, L. Mathys, P. Letardi, E. Joseph, *Mater. Corros*. **2016**, *67*, 200, https://doi.org/10.1002/maco.201408168.
[23] M. Albelda Berenguer, M. Monachon, C. Jacquet, P. Junier, C. Rémazeilles, E. J. Schofield, E. Joseph, *Internat. Biodeter. Biodegr*. **2019**, *141*, 62, https://doi.org/10.1016/j.ibiod.2018.06.009.
[247] W. M. Kooli, L. Comensoli, J. Maillard, M. Albini, A. Gelb, P. Junier, E. Joseph, *Sci. Rep*. **2018**, *8*, 764, https://doi.org/10.1038/s41598-017-19020-3.
[25] L. Comensoli, J. Maillard, W. M. Kooli, P. Junier, E. Joseph, *Bio-Protocol*. **2018**, *8*, e3002, https://doi.org/10.21769/BioProtoc.3002.
[26] L. Comensoli, J. Maillard, M. Albini, F. Sandoz, P. Junier, E. Joseph, *Appl. Environ. Microbiol*. **2017**, *83*, e03478, https://doi.org/10.1128/AEM.03478-16.
[27] S. James, E. Joseph, *Corros. Mater. Degrad*. **2021**, *2*, 274, https://doi.org/10.3390/cmd2020015.
[28] C. H. Orr, R. Williams, H. H. Halldórsdóttir, A. Birley, E. Greene, A. Nelson, T. K. Ralebitso-Senior, G. Taylor, *Sci. Rep*. **2021**, *11*, 15837, https://doi.org/10.1038/s41598-021-94853-7.
[29] E. Joseph, P. Junier, *N. Biotechnol*. **2020**, *56*, 21, https://doi.org/10.1016/j.nbt.2019.11.003.

### *License and Terms*