Edited by Eric May, Mark Jones and Julian Mitchell

Heritage Microbiology and Science

Microbes, Monuments and Maritime Materials

RSC Publishing

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Preface

Heritage at risk: a role for Heritage Science

Heritage Science is emerging as a discipline that brings together chemists, physicists, microbiologists, conservation scientists, archaeologists and conservators. Its scope, precise boundaries and the interfaces between its component disciplines may be in a state of flux but, above all, its interdisciplinary nature offers understanding of the causes, control and protection of heritage from ever-present environmental challenges. In particular, the activities of microbes play a central part in shaping the natural world of our planet but this awesome power constitutes a serious threat to the integrity of our most precious art, heritage artefacts, monuments and cultural treasures. Microorganisms show a remarkable versatility to colonise, utilise and transform a wide range of materials notably wood, stone, concrete, paper, cement, textiles and metals. Water and moisture fluctuations have a profound influence on the integrity of indoor and outdoor objects, causing physical change and initiating microbial growth. Extreme events and global warming will certainly change many of our assumptions about the hazards facing heritage materials at particular locations. Heritage artefacts that have been recovered from water, or that exist near the sea in maritime conditions, pose special conservation problems. This may be due to the combined effect of microbial activities and physical/chemical assaults that the environment can offer, but it will always require our vigilance to conserve and preserve our cultural inheritance for future generations.

HMS2005: a conference for heritage science

In June 2005, Portsmouth was the venue for a major international gathering of microbiologists, conservation scientists and conservators to discuss their research, review the state of the art, and exchange ideas about how to combat the challenges faced in heritage microbiology and conservation science. Portsmouth is home to the British Royal Navy and each of its ships is designated *HMS* or *Her Majesty's Ship*. The city and its dockyard are steeped in history with a rich range of immovable and movable heritage. *Heritage Microbiology and Science: Microbes, Monuments and Maritime Materials* (HMS 2005) was the third in a series, that followed successful meetings in Florence (*Of Microbes and Art,* 1999) and New York (*Art, Biology and Conservation*, 2002), each exploring different aspects of cultural heritage research. HMS 2005 was jointly organised by the University of Portsmouth and the Mary Rose Trust in the Year of the Sea, when Portsmouth hosted the International Festival of the Sea (IFOS) and celebrated the 200th anniversary of Admiral Lord Nelson's victory at the Battle of Trafalgar. His flagship *HMS Victory* (1756), has a permanent home in Portsmouth's Historic Dockyard along with several other historic ships including King Henry VIII's flagship *Mary Rose* (1535) and the first iron-clad warship, *HMS Warrior* (1860). So while media attention was focused on the celebrations in the Solent, the stretch of water between Portsmouth and the Isle of Wight, nearly 100 delegates from 16 countries around the world assembled for a heritage conference programme, that ranged from Scott's Antarctic hut to historic ships like

HMS Victory and the *Vasa* in Sweden. IFOS and the Trafalgar 200 celebrations provided an ideal social backdrop for a conference on heritage and historic ships.

Previous meetings in Florence and New York (at the Met) explored interactions between microbiology and conservation, each offering a flavour of the museums in their particular location. The need to integrate the work of experimental scientists and conservators was noted by Orio Ciferri[1] in his preface to the Florence proceedings. Bob Koestler[2] addressed this issue directly for the 2002 meeting and extended the scope to include archaeology and historic ships. HMS2005 focused explicitly on the emerging research field of Heritage Science, giving greater prominence to conservation of historic ships and their artefacts, covering wood, metal and textiles. It continued to highlight the role of microorganisms, but tried to reflect recent methodological developments in molecular microbiology, as championed by Cesareo Saiz-Jimenez[3].

Heritage scientists are currently addressing the need to improve public, and political, understanding of the role and importance of their research for conserving cultural heritage for future generations. At HMS2005, this was a key theme of the opening addresses by Professor John Craven, Vice-Chancellor

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[1] *Of Microbes and Art: the Role of Microbial Communities in the Degradation and Protection of Cultural Heritage (ICMC1999)*, eds. O. Ciferri, P. Tiano & G. Mastromei, , KluverAcademic/Plenum Publishers, New York, 2000.

[2] *Art, Biology, and Conservation: Biodeterioration of works of Art (ABC2002)*, eds. R.J. Koestler, V.H. Koestler, A.E. Charola and F.E. Nieto-Fernandez, the Metropolitan Museum of Art/Yale University Press, New York, 2003.

[3] *Molecular Biology and Cultural Heritage*, Proceedings of the International Conference, Sevilla, Spain, 4-7 March 2003, ed. C. Saiz-Jimenez, Balkema, 2003.

of the University of Portsmouth, and Admiral Sir Kenneth Eaton, Chairman of the Mary Rose Trust. Kate Clark, Deputy Director of Policy and Research at the Heritage Lottery Fund (HLF) in the UK, outlined how funding through HLF has generated all sorts of benefits beyond heritage - to science, to regeneration, to communities and to the environment. The scientific programme explored wood, metal, stone and all the complexities of historic ships as museum objects. Several EU-funded projects were represented and Michel Chapuis from the European Commission reminded delegates of the 2004 "London Declaration" by heritage researchers that highlighted the essential need for continued future financial support for heritage research by the EU and its member states. *HMS Warrior* was the atmospheric venue for the conference dinner, with distinguished actor *and* longbow expert Dr Robert Hardy, fresh from providing commentary for Trafalgar 200, giving an entertaining after-dinner talk on the mysterious role of the Knights Templar in sinking the Mary Rose!

It was gratifying to the organisers that so many delegates, during and after the event, said it was a social and scientific success. This was, in no small part, due to the efforts of the many staff at the University and the Mary Rose Trust, but particularly Dr Alison Webster, whose organising skills, unflappability and unstinting commitment made it happen, Chris Dobbs, whose brilliance with social events shone through it all, and David Childs, whose financial acumen and advice achieved the impossible. To the International Scientific Committee, thank you for your suggestions and help in steering the programme towards such an interesting and stimulating mix of topics.

Although the sub-title of HMS2005 was *Microbes, Monuments and Maritime Materials*, this volume integrates these conference themes and organises the invited papers into 3 parts: Heritage buildings and materials, Molecular methods for heritage and Historic ships: archaeology and conservation. There was a point at which it was possible that this volume might not have been published because of the lapse of time but the editors believed that this is a unique combination of contributions that should see the light of day. The papers were submitted well after the meeting but have been updated accordingly. The range presents some exciting aspects of developing fields of heritage research, with many case studies. Some contributors chose to combine their efforts into a single article to provide a more comprehensive overview of the topic. The editors hope the final product is interesting and informative, and illustrates the diversity of activities in both heritage microbiology and heritage science in general. Thanks are due to Jeannette May who diligently read the manuscript and, as a historian, learnt a great deal about conservation science!

Finally, HMS2005 was made possible by an extremely generous donation by the Coral Samuel Charitable Trust that brought many of the invited speakers to Portsmouth and, along with ever-patient support from RSC Publishing, helped to fund the production of this volume.

Eric May
Chairman of the
Scientific Committee

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INTRODUCTION

HERITAGE MICROBIOLOGY, SCIENCE AND THE *MARY ROSE*. WHAT ARE WE TRYING TO ACHIEVE?

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1 INTRODUCTION

Even a brief glance down the titles of the papers in this volume displays the extraordinary diversity of research and investigation that is currently going into the science of conservation as it relates to heritage. The scope is truly multi-disciplinary and this paper will refer briefly to some of the diverse scientific programmes that have been carried out at the Mary Rose Trust.

The *Mary Rose* project has always been multi-disciplinary in approach and has much to learn from, and I hope offer to, many of the themes that are being presented in this volume. In addition, the paper will also consider what we are trying to achieve with our research and how it relates to Maritime Heritage and in particular to the work of the Mary Rose Trust.

2 BACKGROUND

The *Mary Rose* was built between 1509 and 1511 and served Henry VIII successfully for 34 years – much of this time as the flagship of his fleet and subsequently as vice-flagship. She sank in the Solent off Portsmouth and Southsea, during an engagement with a large French invasion fleet on 19 July, 1545. After some unsuccessful contemporary salvage attempts, the hull was left alone and gradually filled up with the silts that miraculously preserved her. The hull was briefly relocated by early pioneer divers in the 1830s but was otherwise left untouched until Alexander McKee located her during his 'Project Solent Ships' starting in 1965. He was joined by Dr Margaret Rule who directed the excavations that are still the most ambitious underwater archaeological excavations that have ever taken place. These led to the spectacular raising of the *Mary Rose* in 1982, watched on television by over 60 million people worldwide. The story of the life, loss, excavation and raising are told in a number of publications[1,2,3,4,5] and need not be recounted again here.

The recognition that the *Mary Rose* project would have to be multi-disciplinary in nature came early on during the excavation and, perhaps because she had a background as a chemist, Margaret Rule was particularly keen to involve scientists from other disciplines. As soon as she realised that the archaeology of the project could be better served by an

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expert from another field, she would find the relevant specialist and enthuse them about the benefits of applying their research to the *Mary Rose* project in a mutually beneficial way. This not only covered obvious links such as when Margaret and Alexander McKee brought in Dr Harold Edgerton from MIT to use the latest geophysical techniques of side scan sonar and sub-mud profilers to pinpoint the wreck site, but also with underwater video cameras, range-meters and sector scan sonars.[6] Some of the scientists became embedded in the team such as Dr Ken Collins and Dr Jenny Mallinson, marine biologists who came to dive regularly on the site, recording the marine flora and fauna.[7] This helped to build up a picture of the organisms that colonised the site and contributed, together with the geological and physical factors, to the site environment that eventually destroyed one half of the hull but preserved the other.

3 CONFERENCE THEMES AND THE *MARY ROSE*

There were ten sub-themes in the original programme for the HMS 2005 Conference on which this volume of papers is based and this author was delighted that aspects of the work at the Mary Rose Trust had very relevant links to at least seven of them. There were tentative links to two more of the sub-themes, namely 'Molecular Approaches' and 'Bioremediation' but the elusive tenth topic was 'Caves and Catacombs', for which a valid link would be hard to find.

Papers involving 'Historic Buildings' and 'Heritage Materials' and particularly discussions on mortar that were in the original programme had parallels with the analyses that Christopher Dobbs commissioned for the mortar samples in the brick-built ovens of the *Mary Rose.* This work, carried out by Dr Stubbs at the Bursledon Brick Conservation Centre, enabled us to discover the type of lime mortar mix used by the original builders of the brick ovens found on the *Mary Rose*. The mix could then be reproduced as accurately as possible when building a replica of the ovens (Figure 1), complete with brass cauldron.[8]

Figure 1 *The replica brass cauldron is inserted over the brick and mortar oven during the creation of the replica*

Sessions at the conference on 'Advanced Techniques for Conservation' and 'Sulfur in Historic Ships' have particular relevance to the Mary Rose Trust. Whilst the former reflects the research that Dr Mark Jones has been carrying out on *Mary Rose* timbers since he joined the Trust in 1983,[9] the latter is linked to the research that he has been doing more recently with the synchrotrons at Daresbury and Grenoble.[10,11] More recently, and subsequent to the HMS 2005 Conference, he has joined scientists from the Universities of Portsmouth and Kent, National Museum of Scotland, and STFC (Daresbury) to study the chemistry and microbiology of the iron-sulfur process, using molecular microbiology techniques and facilities at Research Station I18 with the new Diamond Light Source at the Harwell Science and Innovation Campus. Fortunately the sulfur chemistry in the *Mary Rose* is less likely to be a problem than with the Swedish warship *Vasa*. This is partly because the *Mary Rose* was generally constructed with wooden treenails rather than with iron bolts, but also because we have used titanium bolts and plates to clamp the hull together since the salvage rather than steel.[12]

The next conference theme was 'Maritime Archaeology and *in-situ* preservation'. Again this theme has enormous relevance to the *Mary Rose*, both because of the trials carried out in 1980 using the industrial geotextile 'terram' to wrap exposed timbers prior to the winter backfilling, and due to the need for a comprehensive re-covering of the site in 2005 after the new excavations at the wreck site. These new interventions into the bow area of the wreck site, that had not previously been excavated, started in 2003 as the Ministry of Defence in the UK, had proposed to dredge a new shipping lane next to the site. The excavations had to take place to assess the amount of heritage material remaining on the seabed, but fortunately the MoD later decided to enlarge the existing channel. Hence the site is not now under threat from potential development and could be reburied and left alone. Discussions with English Heritage took place and the site was again covered with 'terram' and infilled with 110 tonnes of sand to the required specification. The final conference themes were 'Preservation of Maritime Heritage' and the 'Preservation and Conservation of Artefacts' - subjects at the very core of the work of the Mary Rose Trust and covered in more detail in a later paper in this volume.

Other applications of a multi-disciplinary nature involved the study of the environmental remains found on board.[13] These varied from human material to foodstuffs, including casked beef, pork and mutton, as well as plant remains including plum stones, peppercorns and grape skins. A careful study of the human material has revealed both evidence of childhood diseases and possible occupational injuries.[14] A most promising area for future research is the use of DNA analysis. Very early work of this kind on a pig bone from the *Mary Rose* helped to prove that DNA could indeed be extracted from archaeological material.[15] More recent work on human DNA has been able to assign nineteen members of the crew to their mitochondrial haplogroups.[16] Future research may make it possible to determine the geographic origins for some of the crew.

Outside the sciences, a multi-disciplinary approach yields further dividends in terms of understanding the collection, whether it is from working in close cooperation with musicians, carpenters, master mariners, wood-turners, numismatists, textile experts, furniture makers or the medical profession. Each of the multitude of classes of object found on board can benefit from analysis, comparison and comment from specialists outside the museum and archaeological world.

4 WHAT ARE WE TRYING TO ACHIEVE?

So, a multi-disciplinary approach can help us to preserve the past and can help us to extract as much information as possible from - in the case of the *Mary Rose* - a well-preserved Tudor ship and an extraordinary collection of 29,000 objects found within the ship. But what are we trying to achieve, or what should we be trying to achieve at the Mary Rose Trust beyond just saving what is there, or preventing it from deteriorating at such a fast rate? As archaeologists working in museums, I believe that we need to interpret the past in a way that ordinary people can connect with, adding the human element to the story we are trying tell[17] or personalising it so that it has meaning to those who access it.

To choose just one of the examples illustrated at the conference, we briefly reconsider the *Mary Rose* galley project.[8] Through scientific analysis of the bricks and mortar that were used in the construction of the ovens or in the brass of the cauldrons, we learn more about the technology and the crafting processes that were available in Tudor times. From the environmental remains we learn about the foodstuffs: the provisions on board, how the animals were prepared, how the meat was stored, how they made their food more palatable. From a replica made with integrity and authenticity, we can experiment with cooking methods to see how they could have cooked adequate food for 415 men on board (Figure 2), as well as more individual meals for the high-ranking officers and their retinues.

Figure 2a and 2b *Cooking and eating – the end products of an interpretation process that starts with both scientific and archaeological data and analysis (Photo 2b courtesy of Ben Lawrie, BBC)*

Traditionally this approach has been called 'bringing the past to life' although that phrase has become rather a cliché in modern times. Yet that is what we are trying to do: we are trying to present stories from the objects that people can relate to – that personalise the intensely personal objects that were found on board the *Mary Rose*. We can narrate stories about what they ate, what diseases and injuries they suffered from, how they controlled infestations of head-lice, how they flavoured their food. And through the different scientific disciplines within conservation, some of which is presented in this volume, it can be made sure that stories locked up in the building materials or archaeological assemblies in sites in the world survive for future generations.

5 CONCLUSION

Broadly speaking, the papers in this volume give more detailed scientific insights into two of the ways in which we manage our heritage; preserving the past and presenting it. Most of them relate to research that analyses the threats to our heritage or indicate how a threat can be ameliorated. They indicate how our heritage can be preserved for the future. Other papers and other research from many disciplines helps to give this heritage meaning so that we can recreate human stories from the past. Stories that people of today and tomorrow can relate to and yet have an authenticity based on fact, that is so rare in today's environment of virtual reality, fiction and fantasy.

Both of these outcomes, preservation and presentation are vital. It is by being outward looking and multidisciplinary that we, as maritime archaeologists, museologists or microbiologists, can best achieve the combined aims of both preserving the past and presenting it in meaningful ways to both existing and new audiences.

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PART 1: HERITAGE MONUMENTS AND MATERIALS

HERITAGE RESEARCH AND PRACTICE: TOWARDS A BETTER UNDERSTANDING ?

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1. INTRODUCTION

The importance of microbial impacts in the alteration and deterioration of cultural artefacts made of mineral, metallic or organic materials has been widely acknowledged in the course of many recent investigations.[1,2] In the past, biodeterioration problems on cultural artefacts were often approached without a detailed analysis and, as a result, simply controlled by biocidal treatments. A much deeper interdisciplinary understanding of the environmental factors and material properties regulating biogenic damage would allow actions that were more specific and practically adequate.[3] Thus long-term efficacy in the conservation of historical artefacts, whether derived from dirty, anoxic excavations, archives kept in dark and humid conditions or objects openly exposed to corrosive and nutritive atmospheric pollutants, is mainly dependent on a profound interdisciplinary anamnesis of the prevailing damage functions and the consequent formulation of conservation strategies.

In the course of the excavation of archaeological objects, made of glass, metals or wood, considerable changes of the prevailing physicochemical conditions assessed by *environmental site analysis* (i.e. temperature, moisture, redox potential, oxygen, nutrients) have to be taken into account in order to describe the possible (re-)activation of biodeteriorating impacts before and after exposure. In archives, the impact of microorganisms to the enzymatic deterioration of paper, parchment, leather and textiles is mainly determined by the availability of water determined by *building physics and climate control.* However, precise definitions of humidity levels favourable for the microbial growth are still missing and difficult to assess, since the climatic properties of the building and building materials (e.g. diffusion, absorbency), the maintenance of objects (e.g. cleaning) and types of materials affected differ from case to case and are presently not fully understood. In this regard *material science* helps to define the biosusceptibility of mineralic materials even for open exposed monuments (i.e. porosity, open surface, diffusivity, pH) and to understand the consequent function of biofilms as biodeteriorating (i.e. precursor of crusts) or bioprotecting (i.e. as protective barriers) factors.

If a better understanding of the prevailing environmental, moisture-related and material-specific parameters of biodeterioration by interdisciplinary anamnesis could be reached, the formulation of effective countermeasure strategies would be far easier to access, and it would open possibilities for an environmental-friendly approach in conservation, based on physical, chemical and biological interventions. This paper will

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consider the value of such an approach and elucidate the need by reference to different case studies from China, Denmark, Cambodia and Turkey, discussing various conservation strategies for the protection and conservation treatment of objects at these historic sites under different environmental conditions.

2 EVALUATION OF BIODETERIORATION PROCESSES

2.1 Biodeterioration Mechanisms

Whether as a single or catalytic, enhancing factor, microorganisms such as algae, cyanobacteria, lichens, bacteria and fungi influence, due to their contamination, growth and metabolic activity, the complex interaction between various types of materials and the surrounding physical as well as chemical damage functions.[4]

In the course of biofouling (e.g. presence of colloidal microbial biofilms on or inside the materials), besides an aesthetic-impairing discoloration by biogenic pigments (e.g. green chlorophyll, brownish melanin, red carotinoids), the microflora leads to the alteration of physicochemical characteristics of the materials with regard to their (i) mechanical properties, (ii) surficial absorbency/hydrophobicity, (iii) diffusivity and (iv) thermal-hygric behaviour.[5]

Figure 1 *Sticky Biofilms force the accumulation of dust, aerosols and microbes*

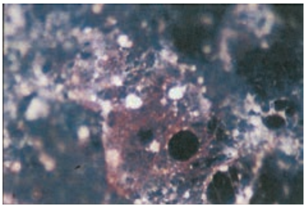

Figure 2 *Biofilms modify the capillary water uptake within the pore system of materials*

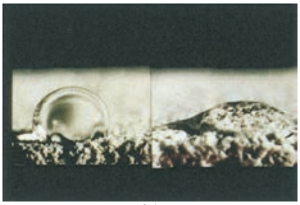

Figure 3 *Biofilms increase the wettability of material surfaces by mucous slimes*

Subsequently, the microbial consortia may cause biocorrosive attack (e.g. microbial induced respectively influenced corrosion on materials), leading to the alteration of the structure and stability of materials by (i) phototrophic enrichment of organic biomass, (ii) selective cellular enrichment and redox processing of cations and anions (e.g. iron, manganese), (iii) the excretion of immediate corrosive metabolic products (e.g. organic and inorganic acids) as far as the (iv) enzymatic mineralisation of respective organic materials.[6] Over and above these effects, germs, spores, dead cells and microbial toxins (e.g. endotoxins, mycotoxins) can all potentially exhibit allergenic, pathological effects, affecting restorers and conservators as well as visitors and users of cultural artefacts, especially in libraries and archives.[7,8]

2.2 Environmental Conditions for Biodeterioration Processes

During the anamnesis of biodeterioration processes on cultural artefacts it is important to signal at an early stage the environmental conditions that favour microbial infection, contamination and biodeterioration in particular, identifying the basic parameters in order to consider and establish effective countermeasure strategies.

2.2.1 Exogenic Parameters. The microbial contamination on and in materials is basically determined by the availability of water provided by rainwater, rising dampness and condensation moisture, depending on the sorption isotherms of the respective materials. Fungal growth will be enabled at a water activity (a_W)[a] >0.6 and a time of wetness TOW > 0.5 (e.g. more than 12 h during a day); optimal conditions for their growth will be given with $a_W \sim 0.75$.[9] Other microorganisms, such as algae or bacteria, probably need a higher moisture supply ($a_W > 0.9$), but in the widespread presence of moisture-conserving biofilms these microorganisms may survive in infected materials even under more unfavourable external moisture conditions.[10]

In the long-term, the material structure (e.g. surface roughness, absorbency / hydrophobicity, porosity and inner surface), determines the adhesion, colonisation and spreading of the microorganisms on and within the material.[11] Its chemical composition may additionally support the microbial succession, by providing internal inorganic and organic nutrients. Further decomposable nutrient sources may be offered by exposure to light, leading to the enrichment of photosynthetic biomass, as well as the deposition of natural and anthropogenic aerosols (e.g. ammonia[12], nitrate[11] or combustible, biogenic hydrocarbons[14]). When evaluating the nutritive conditions for a particular microbial consortia, it is important to consider that microorganisms settling on material surfaces are able to survive or even grow under oligotrophic conditions (i.e. low concentrations of nutrients).[1] The contamination process will even be extended when the material in question possesses a capacity to buffer biogenic metabolic compounds with acidic properties, since the optimum pH for most of the microorganisms studied on cultural artefacts varies around neutrality.

The optimal temperature for most of the microorganisms involved in biodeterioration of cultural artefacts ranges between 16 and 35°C. The oxygen supply will not exclude microbial activity, but will determine the type of the respective metabolic pathways, whether oxidative or fermentative. Finally, the possible routes of contamination (e.g. air-borne, soil, vegetation, infected materials) have to be analysed and considered as potential causes of microbial infection and biodeterioration processes on historic objects.

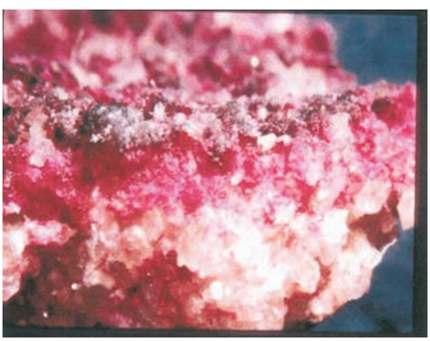

Figure 4 *Microbial biofilm penetrating into the pore system of a natural stone as visualized by reddish PAS-staining*

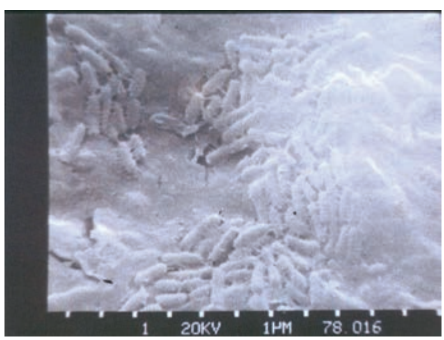

Figure 5 *SEM-micrograph of the microbial biofilm showing rod-shaped bacteria embedded in an EPS-matrix*

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[a] Water activity is the ratio of the vapour pressure of the air in equilibrium with a substance or solution divided by the vapour pressure at the same temperature of pure water.

2.2.2 Biofilm - a stabilising microniche. It is important to stress the fact that a material-specific microflora preferably is embedded in a colloidal slime layer, called biofilm (Figure 4 + 5). The biofilm protects the microorganisms by balancing changes in humidity, temperature, osmotic and pH, the latter due to the presence of colloidal polymeric substances. Based on its considerable ion-exchange capacity, the biofilm even resists the penetration of biocides, detergents or antibiotics, impeding the control of the microbial contamination and biodeterioration processes in the long-term.

Over and above the above effects, the arrangement of microbial consortia in a biofilm matrix leads to the stimulation of their metabolic activity by (i) the extension of the colonisation area, (ii) the deposition and enrichment of nutrients on the adhesive surface, (iii) the promotion of a microbial metabolic network (cross-feeding) and (iv) the support of the intracellular communication by the exchange of genetic information. Therefore, in contrast to medical microbiology, the 'pathogenic' impacts of microorganisms on materials refer only rarely to the activity of one species, but are more often caused by complex microbial consortia characterized by a high adaptability and flexibility during the biodeterioration process.[5,10]

2.3 Microbiological Assessment of Biodeterioration Impacts

The attention of restorers and conservators of cultural heritage to biodeterioration problems has revealed a growing demand for complete evaluation of the importance of microbial impacts interacting with material properties as well as natural and anthropogenic influences during the deterioration process.[15] According to the proposed analytical strategies of May and Lewis as well as Becker *et al.*[16,17], the appropriate analytical approach, in order, comprises:

- object anamnesis (e.g. damage description, object history, climatic/environmental conditions, material properties, previous protective treatments);
- non-destructive observations (e.g. videomicroscopy, remission spectroscopy, respiration/photosynthesis measurement, assessment of ATP-content);
- microscopical studies (e.g. biofilm staining procedures-PAS/FDA, light and fluorescence microscopy, SEM, CLSM);
- biochemical measurements (e.g. quantification of proteins/phospholipids as biomass, analysis of pigments); and finally
- microbiological investigations (e.g. enumeration of air-borne and material-associated microorganisms, characterisation and taxonomical classification of the microflora, simulation studies, toxicological studies).

Above all, the effects of biodeterioration need to be demonstrated by quantification of complementary changes in material properties (e.g. discoloration, loss of weight, weakened stability, increased roughness, altered structure/porosity, increased absorbency/hydrophobicity). In this approach, changes in the physicochemical behaviour of the material to the environment should be addressed, such as its thermal-hygric stresses due to the darkening of the material surface by biogenic pigments, its tendency for an increased deposition of pollutants due to the presence of a sticky biofilm and alteration in moisture transport due to the impact of pore-filling biofilms.[12,18] In specific cases, the potential hazardous impacts of microbial metabolites to human health (e.g. allergenic spores, toxins, pathogenic microorganisms) need to be considered, analysed and evaluated in parallel.[8,19,20]

3 MICROBIOLOGY AND ARCHAEOLOGY - CASE STUDIES

The microbial impacts at archaeological sites include three major phases: (1) initial decay of vulnerable organic materials soon after burial due to limited maintenance and care of the site in the initial months and years, (2) transforming biodeterioration processes during the burial and uncontrolled exposure to prevailing environmental conditions over centuries and (3) post-excavation biodeterioration after the uncovering, safeguarding and conservation of historical artefacts within days and months.

The preservation of archaeological sites and inherent historical artefacts is thus basically favoured by low temperatures, natural dry conditions, artificial and natural preservation (i.e. salts) as well as low oxygen content of the surrounding environment.

During the transformation period, natural mechanical processes (i.e. water flow, wind erosion, frost and silting), chemical processes (i.e. acids, aerosols) and biological processes determine the progress of alteration and deterioration, however, cultural transformations (i.e. ploughing, re-use) should not be underestimated in this regard. After the excavation of archaeological monuments and artefacts, microbial damage functions are stimulated again by considerable changes of redox conditions during the enhanced access of humidity, oxygen and nutrients supporting the contamination, infection and infestation of the archaeological artefacts by air- or water-borne microorganisms.[21]

Organic archaeological artefacts should not essentially be proclaimed as potential nutrient sources, since there might be a good reason why some have survived for centuries. In this regard, the microbiological investigations on the famous polychromic coatings on the warriors of the terracotta army in Lintong (China) have shown that the oriental lacquer layer with its phenolic compounds is barely attractive to the overall contaminating fungi in this particular excavation. The microorganisms present concentrate more and mainly on the mechanical detachment of the surficial paint layers rather then 'eating them up'.[22]

Mural paintings, whether based on fresco or secco techniques, offer a wide range of organic binders (e.g. casein, lime, oil, egg yolk) and, in the course of restoration treatments, consolidants and fixatives based on polymeric compounds (e.g. cellulose acetate, PVA, PMA). If sufficient water is available, biodeterioration will be expressed here as detrimental discoloration and/or decomposing biocorrosion, unless biocidal pigments (e.g. copper-containing malachite) might limit or even control microbial activity. If climate control can be achieved at these objects and their surrounding environments, biodeterioration processes could be limited and controlled even without, or reduced application of, biocidal additives.

The intensity of the microbial attack on historical glass objects and paintings depends mainly on the composition of the silica material. High concentrations of potassium will make the glass increasingly susceptible to microorganisms and their biocorrosive attack; additives of manganese- and iron-containing minerals will stimulate microbial oxidation processes. Nevertheless, the biodeterioration processes on glass mainly occur as secondary effects associated with corrosion processes caused by atmospheric pollutants. However, they can also be initiated and supported by organic oil and wax varnish residues derived from previous historical restoration treatments. In this respect, careful cleaning and sufficient ventilation in the latter will help to control the biodeterioration processes here.[23]

In archives, the impact of microorganisms on the deterioration of paper, parchment, leather and textiles seems to be a classical case of biodeterioration on cultural objects of historical value. Nevertheless, it has to be stated the fact, that, besides the nutritive nature of the material, considerable amounts of moisture and humidity are needed to sustain the biodeterioration process. Precise estimations of humidity levels that could be regarded as favourable for the microbial growth on archive material, are missing and difficult to assess, since the climatic properties of the building and building materials (e.g. building physics, absorbency), the maintenance of objects (e.g. cleaning) and types of materials affected differ from case to case and are presently not fully understood.[24] That said, our knowledge of the physiological behaviour of microorganisms (e.g. sporulation, germination) under particular conditions of exposure are scarcely known.[25] By reaching a better understanding of the prevailing growth conditions of microorganisms in archives, the formulation of effective and environmental-friendly countermeasure strategies would be easier to assess.

Thus, assessment of the threat of biodeterioration to cultural artefacts, whether archived indoors or openly exposed, requires clear proof and differential diagnosis of microbial impacts within the actual deterioration process. It will necessarily demand the development of integrated concepts with respect to long-term prevention of the objects under study.[3] The benefit of interdisciplinary and complementary cooperation of archaeologists, conservators and microbiologists in the evaluation and control of the impacts of biodeterioration on cultural artefacts will be demonstrated in the following case studies based on recent research activities of our laboratory with conservation practice.

3.1 Terracotta Army in Xian / China (polychrome coatings)

In order to improve the conservation techniques for the preservation and protection of the oriental lacquer coating on the terracotta warriors in the mausoleum of the first Chinese Emperor Qin Shi Huang in Lintong (Figure 6), the mechanisms of the alteration of the polychrome coatings were analysed in order to assess the consequences for conservation treatments. This work was done under the coordination of the Bavarian Institute for Preservation of Historic Buildings and Monuments in Munich (Germany) and the museum of terracotta warriors and horses of Qin Shi Huang in Lintong (China).

Figure 6 *Overview of the terracotta warriors in the mausoleum of the first Chinese Emperor Qin Shi Huang in Lintong*

Figure 7 *Whitish fungal contamination on terracotta fragments in pit No. 2 just after excavation and before climatic control*

Microbial contamination could be observed on nearly all materials inside the excavation in Lintong (painted layers, terracotta, wood, loam; Figure 7). The humidity ranged from 60 to 80%, reaching the dew point at the bottom of some parts of the excavation site. Accordingly, it was necessary to analyse the microbial contamination on the recovered terracotta fragments and in the excavation to determine its taxonomical composition, distribution and metabolic activity (e.g. impression plates, quantification of air-borne microorganisms, ATP-analysis), to evaluate the supporting growth conditions (e.g.

monitoring of climate data) and to develop effective countermeasures via specific climate controls and subsequent biocidal treatments (e.g. arrangement of test-sites).

Conservation procedures are concerned with the polychrome paint coatings on the terracotta statues and the important, inevitable role of decomposition of the purely organic priming coat. The coating is extremely sensitive to changes in its moisture content so that, as a consequence of drying, it shows extreme shrinking and deformation, leading to a steady loss of the ancient paints. The conservation procedure therefore starts with the reduction of dry shrinkage and consolidation of the paint layers; during this procedure, the microbial contamination of the fragments has to be controlled at those humidity levels (90-95% R.H.) necessary for the preservation of the non-fixed coatings. As a consequence, it was even necessary to test the effect of the proposed conservation treatments on microbial contamination, in order to minimise microbial attack of the fragments by selection of appropriate protectives.

The results of the microbiological investigations revealed that fungi are the most important contamination on the fragments analysed and in the excavation site itself; in soil samples the presence of actinomycetes was particularly common, and, from the *in situ* terracotta, various cyanobacteria could be isolated. The isolated microflora possessed a strong biocorrosive activity, including acid production and manganese-oxidation properties. Additionally, the microbial contamination caused health problems within the excavation fields. While the role of the lacquer layer as a potential nutrient source could not be proven, the underlying microflora tends to infiltrate and detach the paint layer from the fragments. This hidden contamination represents an important problem, especially for the preservation of the oriental lacquer layer on the terracotta warriors.

The regular application of organic biocides had to be evaluated critically. The clayish, loamy soil absorbs and neutralizes the active agents in the biocides very rapidly in its clay particles and, in the long-term, through microbial mineralisation, provides an important nutritive source for the reoccurring microflora. In order to control the biodeterioration problems on the terracotta fragments and in the excavation at Lintong, the provisional recommendations scheduled regular climate control and ventilation in the excavation area, allowing controlled drying of lacquer layers, and disinfection with subsequent biocidal treatment of the bulk loamy soil with medical alcohol and an inorganic biocidal solution (e.g. 5-10% borax in tap water).

The cleaning, impregnation and consolidation of the terracotta fragments considerably reduced microbial contamination and associated biodeterioration processes. Although organic biocidal treatments (e.g. 0.5% CMK in iso-propanol) were sometimes suggested during consolidation of the sensitive lacquer layers under high humidity conditions, in most cases the application of medical alcohol was quite sufficient to control the contaminating microflora during the conservation process.

3.2 Nydam Mose / Denmark (metals)

Recovery of archaeological objects leads to considerable changes in the environmental conditions to which the material is exposed, and here oxygen seems to be the most important factor, possibly initiating or accelerating biodeteriorating processes on metals, wood, leather or textiles. On the other hand, if objects remained in their buried environment, like sediments, soils and closed caverns, the biodeterioration could also continue due to the activity of anaerobic microorganisms, such as sulfate-reducing bacteria or fermentative microorganisms.

Within a Danish research project on *in-situ*-preservation, the possibility of safeguarding cultural artefacts *in situ* buried at the historic site was studied and evaluated