Smithsonian
Museum Conservation Institute

Requested Report **MCI #6304**

EVALUATION AND TREATMENT OF MICROBIAL BIOFILMS ON THE NATIONAL MUSEUM OF THE AMERICAN INDIAN BUILDING

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November 2010

1. Introduction

The importance of microbial impacts in the alteration and deterioration of cultural artifacts made of mineral materials has been widely acknowledged (May et al., 1993; Warscheid and Braams, 2000). In the past, biodeterioration problems of cultural artifacts were mostly handled without an in-depth analysis; they were simply controlled with biocidal treatments. A greater interdisciplinary understanding of the environmental factors and material properties regulating biogenic damage is necessary to develop a specific and effectively tailored approach to reduce it.

The presence of microorganisms, e.g., algae, cyanobacteria, lichens, bacteria, and fungi, will influence the complex interaction between substrate materials and the surrounding environment and the consequent physical and chemical damage functions (Koestler et al., 1994). Microbial contamination is basically determined by the availability of water which may be provided by rainwater, rising damp, and/or moisture condensation, depending on the porosity and pore size distribution of the respective material.

Biofouling, i.e., the presence of colloidal microbial biofilms on or within the materials, will lead to both aesthetic changes through discoloration by biogenic pigments, e.g., green chlorophyll, brownish melanin, red carotenoids, and to the physico-chemical alteration of the materials by the release of enzymes. The mechanical properties, surface absorptivity, and thermo-hygric behavior of the material will be affected (Warscheid and Krumbein, 1996). Furthermore, the microorganisms may cause a biocorrosive attack, especially on carbonate containing substrates such as limestones, leading to chemical dissolution of the crystalline matrix. Microorganisms may also enhance (i) phototrophic enrichment of organic biomass and selective cellular enrichment; (ii) redox processes of cations such as iron and manganese); (iii) excretion of corrosive metabolic products such as organic and inorganic acids or chelating agents; and (iv) enzymatic mineralization of respective organic materials (Warscheid and Krumbein, 1996).

The material nature and structure (e.g., surface roughness, porosity, and specific surface) determines the adhesion, colonization, and spreading of the microorganisms on and within the material (Warscheid et al., 1993). In addition, the chemical composition of the substrate may support microbial succession by providing inorganic and organic nutrients. Nutrient sources may be created by light exposure that leads to the enrichment of photosynthetic biomasses, as well as the deposition of natural and anthropogenic aerosols (Warscheid et al., 1991; 1993; Saiz-Jimenez, 1995; Mitchell and Gu, 2000).

The control of biodeterioration processes on materials can be better and more safely achieved by measures that limit and inhibit the above mentioned growth conditions rather than by application of routine eco-toxic biocides (Warscheid and Braams, 2000).

2. Experimental Results

2.1 Sample Description

Following the 2010 Expert Meeting organized by the Museum Conservation Institute to evaluate the biocolonization problem at the National Museum of the American Indian (NMAI) and to propose methods for their control, four different samples from the building were received for analyses.

These were:

Sample # 2: From the 1st course of the loading dock wall at the NW corner of the building.

Sample # 5: Spalling flake with heavy black incrustation from 6th floor roof runoff; E face 5th floor, below scuppers near the Senator Daniel K. Inouye Terrace.

Sample # 7: Spalling flake with less biocolonization from the same area as #5.

Sample # 9: Slime material scraped from membrane found under the masonry by the 5th floor scupper near the Inouye Terrace.

Sample #10: Block of stone of the same Kasota limestone cut from spare blocks of the building that had been stored outdoors. The block had the exposed weathered face with a blackish appearance similar to the other three samples to be used in testing cleaning and biocide treatments.

Microscopical analysis and taxonomical assessment of the microflora within the stone was carried out followed by an assessment of their possible impact in deteriorationg the stone as well as testing the effectiveness of some biocides to control their growth.

2.2 Optical Microscopy

Samples for microscopical analysis were stained with PAS (periodic acid-Schiff reagent) or with FDA (fluorescein diacetate) to evaluate metabolically active microorganisms. A stereomicroscope (Askania SMT 4) or a fluorescence microscope (Leitz) with a digital documentation equipment (Nikon Coolpix 950) was used.

The microscopical examination clearly indicated that the microbial biofilms consist mainly of phototrophic microorganisms, especially cyanobacteria, showing greenish (Figure 1) as well as encrusted structures (Figure 2).

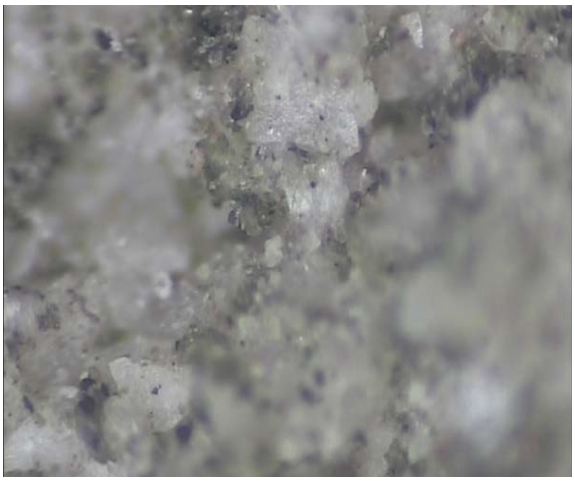

Figure 1. Green photosynthetic microorganisms on the stone surface of sample #2 from the low wall.

Figure 2. Encrusted cyanobacterial structures on the surface of sample # 7.

The microbial biofilms were mostly located on the surface of the stones and penetrated less than 1 mm beneath the surface (Figure 3) seldom reaching deeper (Figure 4). While sample #2 yielded a variety of microbial consortia, samples #5 and #7 were dominated by photosynthetic algae and cyanobacteria. Sample #9 consisted mainly of cyanobacteria (*Lyngbya sp., Gleocapsa sp.* and others), as found on the other samples mentioned above.

Figure 3: Superficial microbial biofilm on the stone surface of sample #5.

Figure 4. Detail of the previous figure showing that the microbial biofilm rarely reaches deep into the stone profile.

2.3 Cultural enrichment of microorganisms

Samples of the blackish colonization were inoculated onto solid nutrient media for selective enrichment of the microflora, as listed below:

- Malt extract agar and potato glucose agar for copiotrophic fungi;
- Dichloran-Rose-Bengal-Agar for oligotrophic fungi;
- Glycerin-Nitrate-Casein-Agar for actinomycetes;
- Plate count agar for heterotrophic bacteria; and
- BG 11 for photosynthetic algae (Ripka et al., 1979).

The results of culture enrichments revealed that the most cultivable fungi were found in sample #2 (*Paecilomyces lilacinus, Phoma glomerata* and to a lesser extent the ubiquitous *Cladosporium sp.*); and also bacteria were dominant. On the other hand, samples #5 and #7 were dominated by photosynthetic microorganisms, as revealed by the microscopical analyses and appeared to lack other heterotrophic microorganisms. Actinomycetes were only rarely found in all of the analyzed stone samples, indicating that the staining process on the rock is in its beginnings stage and has not reached the subsequent level of biocoenosis (i.e., community development).

2.4 Treatment Testing and their Effectiveness (CSTB-Test)

The testing of microbial control techniques was carried out on cut rock samples taken from sample #10. Tests were carried out at the LBW- Bioconsult laboratories and started at the beginning of May 2010, using the CSTB-Test and following the test protocol described below.

The cut specimens (5 cm x 10 cm) with typical microbial biofilms were pretreated with 70% isopropanol or a 5% stabilized hydrogen peroxide solution. After a slight desiccation the specimens were treated with different biocidal formulations, such as Keim Algicide Plus (based on a mixture of quaternary ammonium salts and isothiazolone), Schülke & Mayr Parmetol DF 12 (based on isothiazolone), and the LBW- Bioconsult's proprietary algicidal formulation called "*Mélange d´Angkor*" (based on copper compounds and undisclosed ingredients). The treated test panels were incubated in a moist cabinet with a 12/12 h light/dark cycle with periodic spraying and wetting (4 times per day), with different liquid algal solutions (Figure 5).

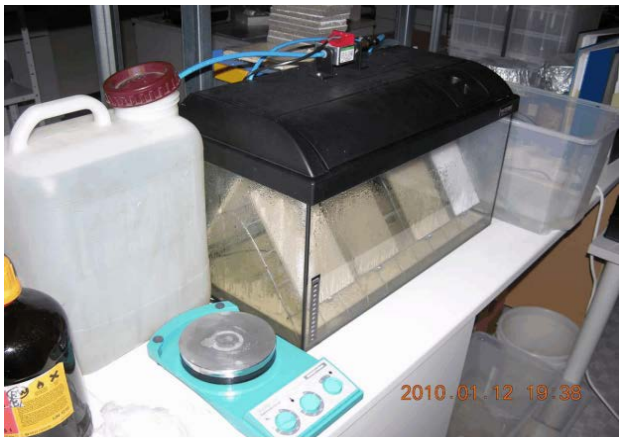

Figure 5. Principle of the dynamic CSTB-Test: (left) Inoculation solution with algae; (center) Moist incubation chamber with illuminated samples in front; (right) Collector for sprayed algal solution.

The spraying of the solution was carried out as follows:

- For the first two weeks the samples were preconditioned with a mixture of 1:1 rainwater and tapwater.
- During the following four weeks samples were sprayed with an *artificial mixture of green algae* (*Chlorella sp.*, *Chroococcus sp.*, *Nodularia sp.*, and *Stichococcus sp.*) to start the algal inoculation.
- Four weeks later, that is ten weeks after the beginning of the test, the panels were examined macroscopically for the first time to evaluate their appearance. They were then returned to the chamber.
- The panels were left another six weeks in the chamber with 12/12 h light/dark with periodic spraying (twice a day) with an algal mixture derived from the microbial biofilms from the above mentioned four samples obtained from the NMAI building.
- The test panels were examined after four months.

The effectiveness of the biocide treatments and consequent decrease in biocolonization (CSTB-Test) clearly demonstrated that a pre-treatment of the microbial biofilm with 70% isopropanol followed by the biocidal treatment with “Mélange d´Angkor” gave the best results in removing and controlling the darkening caused by cyanobacteria. This was also documented by the microscopical analysis, whereas the treatment with Parmetol DF 12 (isothiazolinone) gave the least satisfying result and Keim Algicide Plus (combination of quaternary ammonium compounds and isothiazolinone) offered a reasonable success (Figure 6).

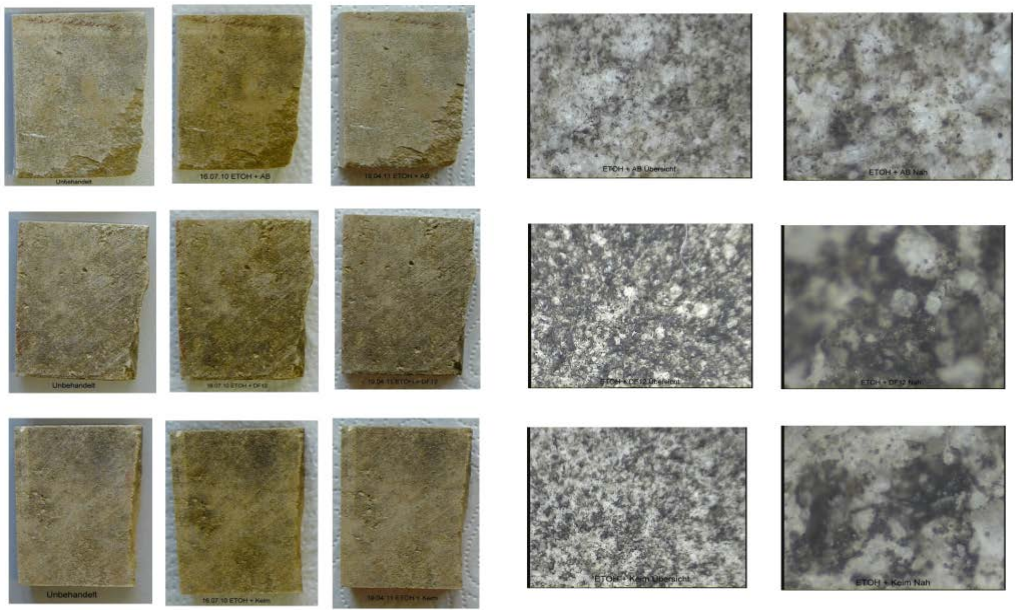

Figure 6. Final results of the effectiveness testing of biocidal treatments on rock samples from the NMAI building. From left to right: untreated; after 10 weeks of incubation; after 20 weeks of incubation; general appearance under the microscope, and relevant detail. From top to bottom samples were treated with "Mélange d´Angkor"; Parmetol DF 12; and Keim Algicide Plus.

An alternative biocide that should be tested is zinc-pyrithione (a zinc complex where the zinc is chelated to a derivative of pyridine N-oxide). The compound has low solubility in water and has been used in paints to protect from mildew and algae growth. This was not tested as it was not available at the time the tests were run. However, it should be considered as a possible alternative, since it is available on the American market, especially as the "Mélange d´Angkor" has not been registered as yet as a biocide in the U.S.

3. Conclusions and Recommendations

From the above study and the observations carried out onsite, it can be concluded that the black stains on the stone surfaces of the NMAI building are mainly caused by photosynthetic cyanobacteria (primarily due to the flow of water over the stones). Since the microbial biofilm does not reach deep into the stone, its current biodeteriorating impact can be considered as low.

Before any steps are taken towards the removal of this biocolonization it should be considered whether the impact of water could be eliminated as the main reason for biocolonization is the alternation wet-dry cycling to which the stone is subjected.

Since the most striking biocolonized areas are the result of architectural details, such as the slope of the coping stones and their overhang, these should be addressed first. Correction of

the water runoff in these areas might solve the biocolonization problem, or at least, significantly reduce it.

With regards to cleaning approaches, it should be emphasized that the elimination of the staining by high pressure water blasting may result in pushing microbes and organic particles deeper into the stone. A better approach would be to only clean the stained areas—not the entire stone surface—with a gentle particle cleaning system or gentle brushing, and to improve the cleaning effect by a gentle oxidation of any remaining organic residues by hydrogen peroxide or commercial products based on it.

The application of quaternary ammonium compounds (quats) may clean the stone surface but it will then have a higher wettability than it had before, and some nitrogen-containing compounds may be left behind that will serve as nutrients for future biocolonization . On the other hand, the most efficient biocide tested was the "Melange d'Angkor", followed by the KEIM Algicide Plus. The zinc-pyrithione should also be tested prior to application. The installation of zinc and/or brass plates should be considered but testing may be necessary to ensure that staining does not occur, particularly for the case of brass.

Water-repellent treatments should be avoided because their effectiveness on calcareous stones is unpredictable and they may foster growth of certain photosynthetic microorganisms, e.g., red-staining *Trentepholia sp.*, that develop under low water availability environments (Warscheid and Leisen, 2011). Furthermore, their application may result in the development of unsightly stripes on the treated surface (Delgado Rodrigues et al., 2011; Salvadori and Charola, 2011).

Finally, the use of "self-cleaning" products based on photoactive titanium dioxide requires preliminary testing, since they may also induce color changes to the surfaces (Warscheid and Leisen, 2011).

In conclusion, it is important to consider that control of biocolonization needs to take into account many factors, such as building design issues, environmental conditions, cleaning methods, and prevention measures. There are many variations that should be tested to find the most appropriate solution for this problem.

References

Delgado Rodrigues, J., M. Vale Anjos, and A. E. Charola. 2011. Recolonization of Marble Statues in a Garden Environment. In *Biocolonization of Stone: Control and Preventive Methods*, ed. A. E. Charola, C. McNamara, and R. J. Koestler, pp. 71-85. Smithsonian Contributions to Museum Conservation, No.2, Washington, D.C.: Smithsonian Institution Scholarly Press.

Koestler, R. J., P. Brimblecombe, D. Camuffo, W. S. Ginell, T. E. Graedel, P. Leavengood, J. Petushkova, M. Steiger, C. Urzi, V. Vergès-Belmin, and T. Warscheid. 1994. "How do External Environmental Factors Accelerate Change?" In: *Durability and Change - The Science*,

Responsibility and Cost of Sustaining Cultural Heritage, ed. W. E. Krumbein, P. Brimblecombe, D. E. Cosgrove, and S. Staniforth, pp.149-163. New York: John Wiley and Sons.

May, E., F. J. Lewis, S. Pereira, S. Tayler, M. R. D. Seaward, and D. Allsopp. 1993. Microbial Deterioration of Building Stone—A Review. *Biodeterioration Abstracts,* 7(2):109–123.

Mitchell, R., and J. D. Gu. 2000. Changes in the Biofilm Microflora of Limestone Caused by Atmospheric Pollutants. *International Biodeterioration and Biodegradation* 46:299-303.

Ripka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. 1979. Generic As-signment in Histories and Properties of Pure Cultures of Cyanobacteria. *Journal of General Microbiology,* 111:1-61.

Saiz-Jimenez, C. 1995. Deposition of Airborne Organic Pollutants on Historic Buildings. *Atmospheric Environment,* Part B. Urban Atmosphere 27:77-85

Salvadori, O., and A. E. Charola. 2011. "Methods to Prevent Biocolonization and Recolonization: An Overview of Current Research for Architectural and Archeological Heritage." In *Biocolonization of Stone: Control and Preventive Methods*, ed. A. E. Charola, C. McNamara, and R. J. Koestler, pp. 1-18. Smithsonian Contributions to Museum Conservation, No.2, Washington, D.C.: Smithsonian Institution Scholarly Press.

Warscheid, Th. 1996. Impacts of Microbial Biofilms in the Deterioration of Inorganic Building Materials and their Relevance for the Conservation Practice. *Internationale Zeitschrift für Bauinstandsetzen* 2(6):493-504.

Warscheid, T., and H. Leisen. 2011. "Microbiological Studies on Stone Deterioration and Development of Conservation Measures at Angkor Wat." In *Biocolonization of Stone: Control and Preventive Methods*, ed. A. E. Charola, C. McNamara, and R. J. Koestler, pp. 1-18. Smithsonian Contributions to Museum Conservation, No. 2, Washington, D.C.: Smithsonian Institution Scholarly Press.

Warscheid, T., and J. Braams. 2000. Biodeterioration of Stones - A Review. *International Biodeterioration and Biodegradation,* 46: 343–368.

Warscheid, Th., and W. E. Krumbein. 1996. Biodeterioration of Inorganic Nonmetallic Materials - General Aspects and Selected Cases. In *Microbially Induced Corrosion of Materials,* ed. E. Heitz, W. Sand, and H. C. Flemming, pp. 273–295. New York: Springer-Verlag.

Warscheid, T., M. Oelting, and W. E. Krumbein. 1991. Physico-chemical Aspects of Biodeterioration Processes on Rocks with Special Regard to Organic Pollution. Special issue "Biodeterioration of Cultural Property" *International Biodeterioration,* 28:37-48.

Warscheid, Th., T. W. Becker, J. Braams, S. Brüggerhoff, C. Gehrmann, W. E. Krumbein, and K. Petersen. 1993. Studies on the Temporal Development of Microbial Infection of Different Types of Sedimentary Rocks and its Effect on the Alteration of the Physico-chemical Properties in Building Materials. In *Conservation of Stone and Other Materials,* ed. M.-J. Thiel, RILEM Proceedings 21, pp.303 - 310. London: E& FN Spon.

APPENDIX

Location of the samples taken from the NMAI building for analyses

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The samples were collected in April 2010, upon the occasion of the visit by the four invited scientists for their further analysis. Four samples were collected and subdivided into the specimens that the scientist subsequently analyzed.

Sample #2, a fairly large spalling flake with overall black surfaces on both front and back, was readily detached from the lowest course of the loading dock wall on the west side of the building. The sample area receives rainwater from sloping capstones without any overhang; some rainwater also splashes up from the black granite ledge below (Figure 1).

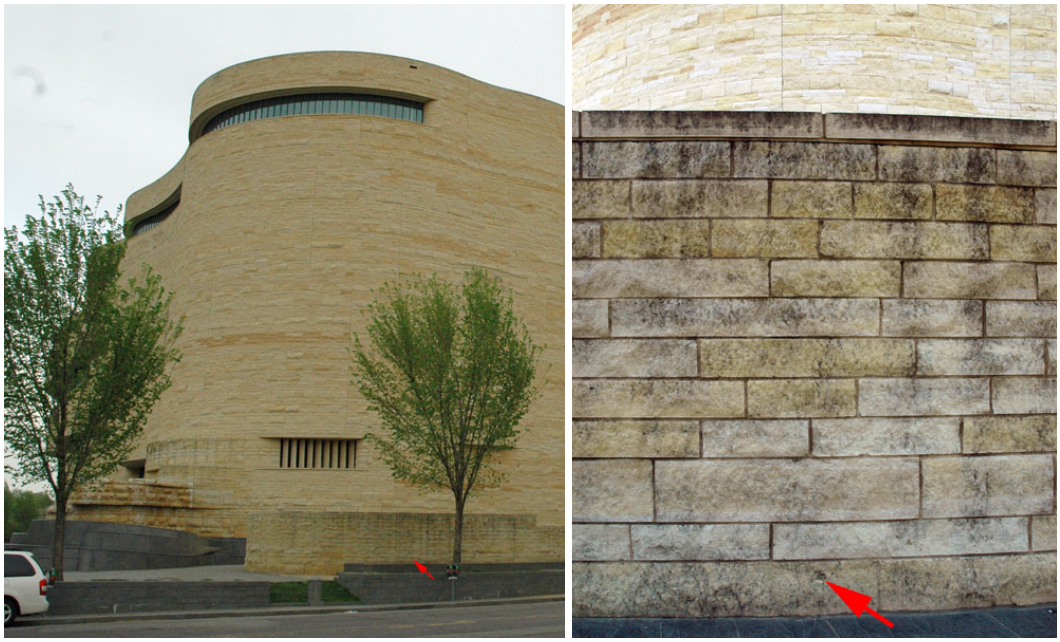

Figure 1. Sample #2 location indicated by arrows: left, general view of the exterior loading dock wall; right, detail (April 2010).

Samples #5, #7, and #9 were taken from areas of heavy biocolonization on the fifth floor terrace east wall below scuppers that drain the roofs above (Figure 2). Samples #5 and #7 were spalling flakes easily detached from below the same scupper: #5 from an area with a uniform black deposit and sample #7 from a slightly recessed area on an adjacent block with spotty surface biocolonization (Figure 3L). Sample #9 was scraped from dense black material on a membrane below a second scupper (Figure 3R).

Figure 2. Left, view of the east face of the 5th floor terrace showing locations of samples #5, #7, and #9 from darkened areas below two scuppers, which drain water from the small terrace above and, in turn, the main roof terrace with the dome (April 2010).

Figure 3. Details of sample locations shown in the previous figure. Left, samples #5 and #7 were flakes detached from areas indicated by the arrows. Note the uniform black colonization where sample #5 was taken, compared to the spotty area to its right where sample #7 was removed; right, sample #9 was scraped from the membrane at the bottom of the wall (April 2010).

The larger samples studied by May and Warscheid were pieces cut from extra blocks left over from the construction of the building.