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Microbiology for Our Cultural Heritage

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Abstract: Microbiology investigates different microorganisms such as bacteria, fungi and algae and can analyze these organisms and their effect as biodeteriogens. Any microbially caused damage is strictly the result of microbial growth and microbial metabolism.
After the diagnosis of a given situation, microbiology for the protection of our cultural heritage often has to set a prognosis regarding future ageing. Laboratory simulation experiments can help to provide answers to such questions. Different materials can be tested for their durability and resistance against bio-attack. Microbial growth results from the presence of organisms, availability of nutrients and favorable climatic conditions. Based on the analysis of the situation and taking further ageing into consideration, the need to intervene by conservation methods may be discussed. Chemical or physical methods can be applied to influence the natural durability and the deterioration process.
Not only the use of chemical preservatives but also the presence of microbial mats can present a health risk for the restorer, conservator and science consultant.
After the introduction (analysis, prognosis, and remedial treatment or conservation) some case studies are used to show the many possibilities of microbiology.

Key words: Biodeterioration · Conservation

1. Introduction

Microbiology is a discipline in biosciences that deals with different microorganisms such as bacteria, fungi and algae. These microorganisms are ubiquitous in our environment. Their spores or 'germs' are distributed – mainly linked to dust particles – by air and water or by people.

These organisms are an important source in the natural ageing of materials and buildings. In case of unexpected early ageing, terms such as 'damage', biofouling or biodeterioration are used. This microbial biodeterioration can only happen if the microorganisms present find good conditions for life and growth. Microbially caused damage is strictly the result of the microbial growth and the corresponding metabolism.

In the past microbiology mainly dealt with culturing methods, their proof and, with culturing as well as with laboratory techniques, establishing the presence of the microorganisms. To cultivate these microorganisms, knowledge about their optimal culturing conditions (nutrients, climate) is needed in advance. In the meantime new methods on a molecular or genetic basis have been developed to detect specific microorganisms. By these molecular tools microorganisms can be determined by means of their genetic code (nucleic acids). These molecular tools are useful to detect microorganisms which could not be cultivated up to the present (VNCs = viable but non-culturable organisms).

After the diagnosis of a given situation, the microbiology for the protection of our cultural heritage often has to set a prognosis regarding future ageing. Laboratory simulation experiments can help to provide answers to such questions. Different materials can be tested for their durability and resistance against bio-attack.

Based on thorough diagnosis of the situation and prognosis of the further behavior, the need to intervene by conservation methods may be discussed. Chemical or physical methods can be applied to influence the natural durability and the deterioration process.

During all this work it has always be considered that not only the use of chemical conservation agents but also the presence of microbial mats can present a health risk for the restorer, conservator and science consultant.

Some case studies are used to show the possibilities of microbiology.

2. Diagnosis of the Situation

Every diagnosis comprises different parts and aspects. The microbiologist working in the field of conservation and monuments has to describe the present microflora, in order to give an answer about their physiological state and to show evidence of their presence. This microbiological aspect has to be accompanied by the restorer's and the conservator's work and the description of the situation. Microbiology with the aim to protect our cultural heritage needs such collaborative teamwork.

One question is often asked, how detailed the microbiological diagnosis has to be done in the case of a particular study. Is it important for the restoration/conservation to know which type of fungi is present? The answer has different aspects. – In case of damage, where complete restoration is already planned and

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where later the climate will exclude fungal growth, the full determination of microorganisms is not necessary. But experience has shown that in many cases the prognosis derives directly from the diagnosis. There are existing fungi which indicate high humidity requirements, other species are indicators for only slight excess humidity. Some fungi grow at a material humidity corresponding to 65–75% rel. humidity. Other fungi need humidity values of approx. 95% rel. humidity. From the microbiological point of view and a lasting expertise the most complete diagnosis has to be postulated. Only this enables us to establish general rules from one individual study.

Every diagnosis starts from a clear question. This determines type and volume of the sampling. In most cases the sampling on our cultural heritage has to be performed using non-destructive methods. This is possible in the case of superficial mould growth or other surface phenomena, but it is not applicable when the microbial deterioration commences without exterior signs, as *e.g.* biodeterioration caused by biogenously produced acid. A microbiological sampling procedure has two different aims. Either microbial growth is prepared for microscopical analysis only, or the biofilm organisms on the surface are taken for isolation and cultivation, which is necessary for correct identification (Fig. 1)

A simple method exists for sampling for microscopy and at the same time the method can also be used to isolate and cultivate microorganisms: The surface growth can be sampled with a transparent scotch tape. Small pieces of this tape can be embedded in a coloring solution such as cotton blue dye in lactic acid and studied by microscopical examination at magnifications of up to 2000-fold. With this first microscopic analysis the main types of growth-forming organisms can be defined. With this knowledge selective methods for isolation and cultivation with special nutritive media are possible: A cotton swab, moistened with sterile water can first be brought in contact with the sample and then smeared on a nutrient agar layer. A second sampling method consists of using a 'Rodac' contact plate with agar medium without nutrients (*e.g.* 1.5% agar in sterile tap or purified water). Microorganisms sampled in this way cannot grow to normal colonies due to lack of nutrient. The germinated spores can be transferred to complete agar layers. By this sampling method and contact method without nutrient, no additional nutrients are transferred to the sampled object.

By comparing the situation sampled with the adhesive tape with the isolated organisms growing on agar medium it can be proven clearly that the isolated microorganisms are identical with the biofilm-forming and surface-altering microorganisms, respectively.

Microbiologists can also use an adhesive tape sample to isolate the organisms from the tape: a small piece of a spore-containing tape can be soaked in a small amount of nutrient broth for about half an hour. After intensive agitation, the resulting suspension is poured onto an appropriate nutrient. But in other cases it was found that isolation was much more successful if the isolation was already undertaken at the local site by inoculating different nutritive agars.

Only a few percent of all microorganisms present in our environment can be isolated and cultivated in the laboratory because their needs are not satisfied by usual routine laboratory methods. New molecular methods in biology offer the possibility to monitor the microbial 'flora' in its total diversity. These methods work on the basis of gene characters; the composition of their nucleic acids. This molecular tool is just beginning to be used for environmentally important organisms, but is already well-established in medical microbiology.

To make a correct diagnosis the restorer's observations are essential. Another aspect is the determination of any microbiogenous alteration of the materials. In this area microbiology still has much to do. Although we often know all the necessary requirements for microorganisms to grow (nutrients, climate) and we know the susceptibility of different materials such as paint films or binders, in practice only rudimentary knowledge about the very detailed effects of growth on paintings, different binders and varnishes exists. It can be seen that fungal hyphae can invade cracks, craquelures and hollow spaces. By such growth, they can loosen the paint layer or the material. But what processes are going on in the very early interaction between microorganisms, their enzymes and the nutrients in a painting?

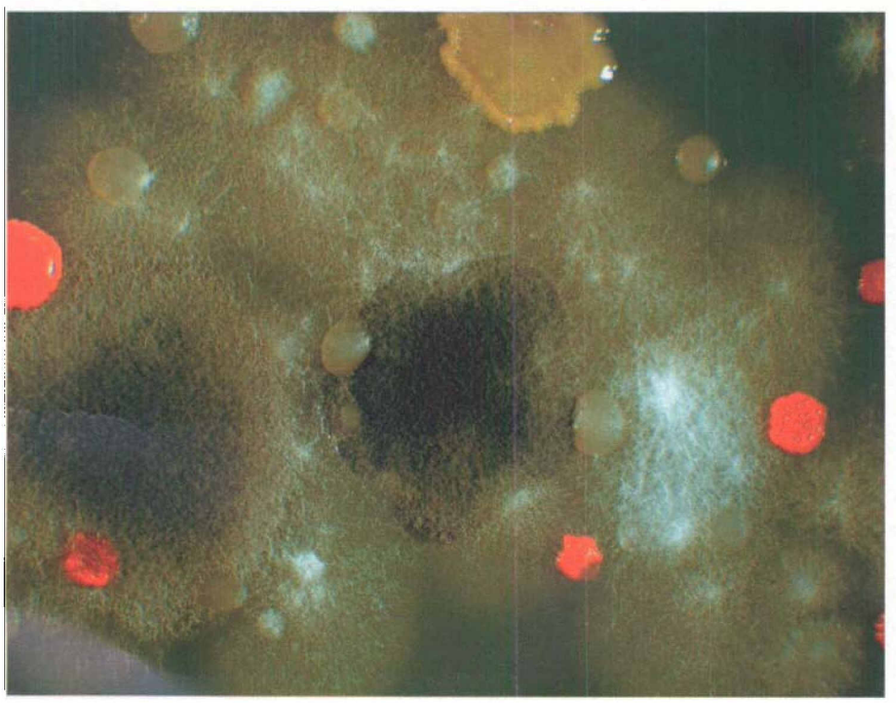

Fig. 1. Different microorganisms from a façade near Einsiedeln, collected by the Rodac contact method, growing on a nutritive agar (Bio_1582)

3. Prognosis and Future Development

The above-mentioned topics and many scientifically unsolved questions often make it difficult to give a clear prognosis. Much knowledge exists in the case of wood-destroying fungi. If these fungi find a convenient growth climate, the further development of damage is clear from the fungus name. In case of mould growth the prognosis is more difficult. The question about growth condi-

tions is easy to answer, but the question about the consequences of growth is difficult. In practice different risks exist and have to be considered in detail, but from many sides. Drying a plaster with wall paintings may lead to salt crystallization and loss of great areas of the paintings. On the other hand drying always stops fungal growth and fungal influence.

Microbial growth is the result of too much moisture in the materials and/or excessive air humidity. Two further conditions for growth are necessary: the presence of germs and enough nutrient for growth. These two prerequisites are easy to fulfill: microorganisms are always present in our environment and nutrients can be offered either by the material or airborne by dust deposition. To control growth, humidity control is always the easier option.

This explains the high value and influence of humidity in the prognosis of any development of the biogenous decay and especially of any further growth. If humidity is successfully eliminated, no further microbial influence on the cultural objects would occur. Any microbial influence is not caused by the presence, but by the growth of microorganisms. During growth, they excrete exoenzymes to make nutrients in their environment available. The production of soluble pigments that can discolor a substrate is also only possible under growth conditions. Metabolism and growth also create biogenous acids, which are often cause for microbial decay of materials.

To know the reasons for excessive humidity and to find methods and ways to avoid this are essential points in the prognosis of the further development of damage. Being indoors and, if there is no obvious building damage by water infiltration, condensation is the most frequent cause for excessive humidity, especially the formation of water drops.

Table 1 indicates the dew point temperature, calculated on the basis of saturation pressure of water vapor. At room climates of about 20 °C and 'critical' (>50%) relative humidity of the air, the following calculation indicates approximately the dew point temperature: Dew point temperature = ambient temperature x rel. humidity (%)/100. Example: if in a room at 20 °C and 65% rel. air humidity, there is a wall or object with only 13 °C, the air humidity will condense on this wall or object. The table value indicates the correct dew point temperature of 13.2 °C. This risk assessment by measuring climate and calculating the dew point temperature is easy to perform with a personal computer. By comparing the dew point temperature or absolute water content of the air indoors and outdoors, it is possible to judge whether opening the windows will lead to a reduction of the water content.

Table 1. Dew point (DP) temperature in relation to room climate, room temperature RT [°C] and relative air humidity RH [%]

[°C] [%]	6	8	10	12	14	16	18	20	22	24	26	28	30	32
20	−15.2	−13.6	−12.1	−10.2	−8.6	−6.9	−5.3	−3.6	−2.0	−0.3	1.3	3.0	4.6	6.2
25	−12.5	−10.8	−9.1	−7.4	−5.7	−4.0	−2.3	−0.6	1.1	2.7	4.4	6.1	7.8	9.5
30	−10.2	−8.5	−6.8	−5.0	−3.3	−1.6	0.1	1.9	3.6	5.3	7.1	8.8	10.5	12.2
35	−8.3	−6.5	−4.8	−3.0	−1.1	0.5	2.3	4.1	5.8	7.6	9.3	11.1	12.8	14.6
40	−6.6	−4.8	−3.0	−1.2	0.6	2.4	4.2	6.0	7.8	9.5	11.3	13.1	14.9	16.7
45	−5.0	−3.2	−1.4	0.4	2.2	4.1	5.9	7.7	9.5	11.3	13.1	15.0	16.7	18.6
50	−3.6	−1.7	0.1	1.9	3.7	5.6	7.4	9.2	11.1	12.9	14.7	16.6	18.4	20.2
55	−2.3	−0.5	2.9	3.2	5.1	6.9	8.9	10.7	12.5	14.4	16.3	18.1	19.0	21.8
60	−1.2	0.7	2.6	4.5	6.3	8.2	10.1	12.0	13.8	15.8	17.6	19.5	21.3	23.2
65	−0.1	1.8	3.7	5.6	7.5	9.4	11.3	13.2	15.1	17.0	18.9	20.8	22.7	24.6
70	0.9	2.8	4.8	6.7	8.6	10.5	12.4	14.3	16.3	18.1	20.1	22.0	23.9	25.8
75	1.9	3.9	5.7	7.7	9.6	11.5	13.5	15.4	17.3	19.3	21.2	23.1	25.1	27.0
80	2.8	4.7	6.7	4.5	10.6	12.5	14.5	16.5	18.4	20.3	22.3	24.2	26.1	28.1
85	3.6	5.6	7.6	9.5	11.6	13.5	15.4	17.4	21.2	21.3	23.3	25.2	27.2	29.1
90	4.5	6.4	8.4	10.4	12.4	14.4	16.3	18.3	20.3	22.3	24.2	26.2	28.2	30.1
95	5,2	7.2	9.2	11.2	13.2	15.2	17.2	19.2	21.2	24.1	25.1	27.1	29.1	31.1

SP(DP) = % rel. RH x SP (RT) / 100
SP = Saturation pressure of water vapor
DP values calculated and rounded to 0.1 °C

When we know the humidity requirements of the microorganisms responsible for damage, the critical air or material humidity can be calculated. Many mould fungi can grow already at an equilibrium moisture content of about 70–75% rel. air humidity. This minimum moisture requirement to the available water (water activity or aw-value) is material-dependant and different for different types of microorganisms. Below 0.65 aw no microbial growth is possible. At values of 0.70–0.75 some fungi can grow. At aw-values of about 0.85 and higher the spectrum of the potentially growing microorganisms increases.

The materials have an important role in making a prognosis. Do they act as nutrients for growth-forming microorganisms? Which parameters are limit growth and can thus be used to influence the ongoing damage development?

The prognosis as part of the risk assessment has to consider three different possibilities:

1) What will happen if there is no intervention
2) What will happen if the climatic conditions are changed in such a way that microbial growth can be regarded as impossible
3) What can be expected if a biocide is used and where are the limits?

4. Simulation

Microbiological research in material science can determine, under standardized laboratory conditions, which materials are bioresistant in a certain climate, or, which climatic conditions must be fulfilled to make a material susceptible and resistant, respectively, to biodecay. The

advantage of these experiments is the fact that without damaging the artifact, early stages or final stages of microbial influence can be simulated.

The simulation experiment starts with isolation and knowledge of the microorganisms causing the deterioration. Different strains of the same species do not behave identically. They can have different requirements in temperature and humidity. The isolated organisms can be used to analyze materials for their susceptibility to growth, or, if they even are nutrient for microorganisms.

In general, microbiological experiments can be used to answer two different questions by the following test protocols. To find out whether a material is or contains nutrient for microorganisms, the material is offered as the only carbon source. Microbial growth would indicate that the test microbes are able to use the material or some components as sole carbon and energy source. To establish whether a material can be considered to be resistant against growth, the following experiment is used: In the laboratory it can be seen if microorganisms are able to attack a test material from a convenient nutrient agar.

Both questions (presence of nutrients or of an antimicrobial activity) can be answered either in a moist chamber test or in a test on an agar medium. In the moist chamber test the test material is inoculated with germs (spores, microorganisms) and incubated in a moist chamber at a climate of more than 95% rel. air humidity. This 'growth test' answers the question of the nutrient capability of a material. The same result is obtained from the growth test on an agar medium with the material as only carbon and energy source. As a general rule it can be said that the agar test results after four weeks incubation can be compared with moist chamber results of twice that incubation time. The 'less severe' moist chamber test can be used to obtain results with a finer gradation. This fact is useful to validate laboratory results under practical conditions.

Microbiological competence and knowledge in microbial nutrition is necessary to perform simulation experiments. Microbial life (for requirements, see Table 2) can be classified by the way they obtain vital energy and by the origin of carbon necessary to grow. Other fundamental differences depend on their oxygen needs, if they can grow without oxygen, which temperature is optimal for growth and limiting growth. Microorganisms can live under all possible conditions. Therefore the following remarks can only give an overview about microbial metabolism. It discusses those microorganisms that play some role in decay or conservation of cultural objects.

Each simulation experiment can be initiated by different questions. The main question is always: what is the value of a result obtained by a simulation experiment in regard to practice. If we can begin our work with the damage analysis, then it is necessary to use the damage-causing organism as well as those in routine test methods. Simulation cannot be considered as accelerated testing or bio-ageing. But the test conditions during the whole test period guarantees optimal growth conditions – growth conditions

Table 2. Requirements for microbial life

Mildew or ***mould fungi*** are respiring organisms and therefore need oxygen. They get energy and carbon to grow from the biodegradation of organic carbon compounds (sugar, starch, cellulose, but also proteins, *etc.*). Most mould fungi grow at an optimal temperature between 18 and 28 °C (mesophilic growth). Some strains prefer lower temperatures (psychrophilic conditions) or need a higher temperature (thermophilic organisms). All fungi need sufficient humidity for growth, but they can also survive long dry periods. Light is not necessary, fungi can grow in the dark or daylight. ***Wood-destroying fungi*** have more or less the same growth requirements as mesophilic mildew, but can use wood components as carbon and energy source. Brown rot fungi use mainly the cellulose part in wood, white rot fungi use first the lignin part of the wood.
Actinomycetes (bacteria with filamentous "fungus-like" growth) with relevance for our cultural heritage have in general the same growth requirements as mould fungi. Like other destructors they biodegrade organic carbon compounds.
Bacteria are a most heterogeneous group. Some destructors, biodegraders of organic matter, have significance for objects of art and culture. Some other bacteria can also grow anaerobically und also produce organic acids. When inspecting bacteria on our culture goods not only the carbon cycle but also the nitrogen cycle has to be considered: lithotrophic nitrifiers can generate aerobically strong mineral acids, nitric acid is a strong mineral solubilizator.
Algae are the producers among microorganisms because they take the necessary carbon from the air. They can reduce the carbon of the carbon dioxide by photosynthetic metabolism, using the sun's energy. This photosynthetic way of life allows growth on inert materials, because they use the host material only as support, but not as nutrient.
Lichens are the symbiotic life of an algae and a fungus together. By this symbiosis, the algal partner uses light and CO_2 as energy and carbon source to produce organic carbon compounds. The fungus uses the produced organic carbons as living source. In this way lichens can grow at very exposed sites (Fig. 2).

Fig. 2. Lichens at the Goetheanum on concrete (Bio_3253)

that could occur in practice, but mostly not for longer periods.

A special case of simulation is the microbiological monitoring of the environmental conditions. To increase prognostic value, samples made of special materials can be used and exposed with the sole aim of discovering growth conditions and a critical climatic situation at an early stage. Monitors are exposed which fulfill requirements regarding nutrients. Additionally, invisible germs or spores can be added to the monitors. This implies that such monitors will show visible growth as an indicator for specific growth conditions.

Simulation experiments by microbiologists can have quite different aims, *e.g.* to choose suitable materials, *i.e.* more bioresistant materials for a restoration. Screening experiments with different microorganisms and climatic conditions can reveal limit values for climatic conditions. *In situ* monitoring can be used to validate such limits under practical conditions.

5. Conservation

Conservation means to maintain the present status. With knowledge about the suppositions for microbial growth and biogenous damage, each of three needs (organisms, nutrients, humidity) can be used to exclude biogenous decay.

5.1. *Climate*

The indoor climate can be controlled. At a climate of max. 65% rel. air humidity no growth is to be expected. However, growth in archives and collections is a frequent occurrence. It is not the measured relative humidity, but the local humidity in a small area and local situation that is of significance. That depends directly on building-physics and climate-technical parameters. If there are construction faults such as bridges of coldness or room edges which cannot be aerated, quite different measurements can be registered at these sites, compared to the measurements in the middle of the room.

The outdoor climate cannot be controlled. But one of the reasons for growth – weathering and rain influence – can sometimes be controlled. Also if rain itself cannot be influenced, it might be possible to construct a rain protection (protecting roof), which will drain water and exclude water penetration into the material, or by a hydrophobic treatment.

5.2. *Possible Nutrition and Microorganisms*

In the case where conservation is not achievable by climate control, two other possibilities are applicable, either by nature or by the present organisms. These possibilities work in combination. The different organisms have partly different nutrient requirements. But because the possible damage-causing organisms are nearly omnipresent and because also dust deposits and surface mud act as nutrients, these two growth limiting factors have to be discussed together. Conservation is also possible by using a chemical preservative. In other words: a chemical preservative is used to protect a possible nutrient from getting degraded. In rooms the climate contributes much to the growth conditions. Climate depending, the spectrum of organisms is restricted, what makes the choice of a preservative easier.

The evaluation of chemical preservatives has to be supported by microbiological testing in a simulation experiment. Each product has a specific activity and action and defined leaks of action. It can be compatible or incompatible with other components. The characteristics of a chemical can either be known or unknown. Changing regulations within environmental protection laws, leads often to a loss of existing knowledge. In the past, some technical antimicrobials were used, which were active against a broad spectrum of microorganisms. This broad action against environmental microorganisms was the reason to eliminate these compounds from the list of chemical preservatives.

6. Maintenance and Care of Cultural Goods and Control of Success

Each treatment with the aim of stabilization and conservation is an intervention in the existence of the monument in its environment. Only examination of the success of the intervention at a later date can ensure the aims were achieved. For each restored/conserved object an inspection plan has to be created in order to monitor success and further development of the situation. Someone has to be responsible for these examinations. Especially after the use of chemical preservatives, it must be clear that no chemical protection will last for ever. Antimicrobials have to be available for the microorganisms. This means that chemical preservatives only have a limited time of action. This time can be determined either by chemical analysis of the presence of the antimicrobial or by microbiological monitoring. While chemical analysis needs knowledge about the minimum required amount of a chemical, microbiological monitoring indicates at the same time whether the chemical is still available for action. On the other hand it indicates the end of the effect and the protection. Maintenance and control after a treatment are also important aids in obtaining information about an intervention. With this knowledge it is possible to obtain deeper experience from a single case. Furthermore it will be possible to propose an intervention when there is not enough time to undertake simulation experiments.

7. Examples

All statements made until this point are demonstrated by means of case studies from my expertise work as a microbiologist and expert of the Swiss authorities for the care of monuments.

7.1. *Diagnosis and Prognosis in the Case of a Red Layer on a Lime Plaster*

In the cloister of an earlier Carthusian monastery (Ittingen TG) great parts of the walls were homogeneously discolored by a red layer. The same phenomenon is frequently seen, *e.g.* in the cloister of an abbey which is today used as museum (St. Georgen, Stein am Rhein, Fig. 3). The same red crust is present in the cloister and a chapel of this cloister in the abbey of a convent in Mustair. This growth phenomenon can hardly be distinguished from a paint layer. When inspecting this 'color' with a hand lens, magnification 20x, it can be seen that this red color is not the result of pigment diffusion into the lime plaster. The color is located in the cell walls of bacteria/micrococci. In different cases the lime walls had been treated with different cleaning methods (dry with the 'wishub' sponge, moistened with alcohol or other toxic washes). After this surface cleaning, the red color had disappeared. Further observations showed that on cleaned surfaces growth was stopped. Experience from many cases revealed that under 'normal' or dry conditions no growth could develop. It was also seen that the plaster did not show any signs of deterioration. These micrococci react very sensitively to different biocides used for cleaning, such as quaternary ammonia compounds, strong oxidizers or 2-propanol. The same was reg-

Fig 3. Red micrococci in the cloister Stein am Rhein (St_041000_27)

istered by using more persistent compounds like organo-tins. Dry cleaning with the wishub sponge or moist cleaning with alcohol or oxidizers deleted growth and discoloring (remedial treatment), but could not control future growth. Toxic washes like quaternary ammonia or organo-tin compounds were also able to control new growth (remedial and prophylactic treatment). These could prevent the cleaned surface from developing new growth even under moist climatic conditions for certain periods.

7.2. *Simulation and Conservation*

The most frequently discussed question concerns the minimum humidity necessary for microfungal growth. The protection of the ceiling paintings on the wooden panels in Zillis GR was part of a microbiological study. For this purpose different monitors were incubated with *Eurotium (Aspergillus) amstelodami*, the same mould fungus which was determined as predominant species on the ceiling paintings. These simulation experiments in the moist chamber at different air humidities showed good growth at 75% air humidity, but no growth at 65%. Similar experiments are still running with wood panels coated by the same technique used for the ceiling paintings.

This simulation study should provide validated humidity limit values for growth conditions. The aim is to protect and conserve the paintings if possible without chemical conservation. The expert center for conservation of the ETH Zurich has therefore installed a registration and regulation device for air humidity.

A remedial treatment against existing growth and controlling the humidity near the ceiling at a level below 70% air humidity, which excludes fungal growth, will conserve the paintings without prophylactic use of chemicals. Observations at the painting show that hyphal growth leads to a loosening of the paint layer. This process must be stopped by successful conservation.

An associated problem is the conservation of glues, which are often excellent nutrients for microbial growth. Different binders that are used for the conservation of loosened paint layers were offered as sole carbon source for a simulation study by N. Billeter. Different glues, protected by different chemicals or biocides, were tested in the moist chamber with different mould fungi. The results showed that there was no 'best' conservation chemical available. The conservation effect is dependant on the material to be protected and the fungi present that are capable of growth under such climatic conditions. This result gives rise to the conclusion that any conservation treatment must be validated in advance by a microbiological simulation test. Of course, some chemicals are capable of protecting many different materials. But these 'powerful' biocides are also strongly ecotoxic and therefore no longer used for conservation.

7.3. *Maintenance and Care*

Some local parts of the exterior walls of a church which was renewed some years before, showed green algal growth. During a subsequent meeting together with the restorer and the designated representative of the canton for the care of monuments, it was seen that any places which were colonized by algae were easily accessible to an annual examination and local remedial treatment by the responsible restorer. Thanks to this organized maintenance and care procedure, a clean façade could be guaranteed without using great amounts of algal growth preventing biocides. By microbiological analysis of the growth phenomena, discussion of the possible treatments and subsequent prognosis, the most ecological way could be chosen.

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For more detailed information please refer to the following literature from the author and/or collaborators

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