Chapter

Origin and Control Strategies of Biofilms in the Cultural Heritage

Laura E. Castrillón Rivera, Alejandro Palma Ramos, Jorge I. Castañeda Sánchez and María Elisa Drago Serrano

Abstract

Biodeterioration is defined as the undesirable change in the properties of materials caused by the activity of biological agents. This process is complex and involves alterations in the physicochemical and mechanical properties by the action of organisms and depends on the microorganisms involved, type of substrate, and environmental conditions. The biodeterioration of cultural heritage is the physical or chemical damage caused by microorganisms on objects, monuments, or buildings that belong to the cultural heritage. Among the main materials that can be affected are: stone, metal, ceramic, polymers, and other materials. Among the main undesirable effects to these materials are: discoloration, dissolution, rupture, and efflorescence among others. Biofilms represent the usual form of growth of bacteria and consist of communities of microorganisms that grow attached to an inert surface or a living tissue, surrounded by an extracellular matrix that they themselves synthesize. The importance of biodeterioration by biofilms is mainly related to changes in pH values, ionic concentrations, oxide-reduction reactions in the biofilm thickness, and in the interface with the substrate and enzymatic degradation. This chapter presents evidence of the participation of biofilms and associated mechanisms in biodeterioration as well as the main prevention and control strategies.

Keywords: biofilms, cultural heritage, biodeterioration, art biodeterioration

1. Cultural heritage

The concept of cultural heritage refers to the cultural inheritance that corresponds to a given community and as such is protected and communicated to both the present and the future generations. This concept is subjective and dynamic, and it does not depend on the objects or goods, but on the values that society in general attributes to them at each moment of history and that determine which goods are those that must be protected and preserved for posterity. Therefore, the identification, protection, conservation, and dissemination of the world's cultural heritage are one of the most recognized tasks of the United Nations Organization for Education, Science and Culture [1–3].

Cultural heritage is made up of tangible or intangible assets that history has left to a country and those citizens in the present grant it a special and relevant historical, scientific, symbolic or esthetic importance. This inheritance left by our ancestors and received today is the clear testimony of their existence and vision of the world [4].

The tangible heritage is also called material heritage, and there are movable and immovable property such as the objects of the artistic collections such as the collection of religious, ethnographic, technological, historical, artistic, and archeological and artisan objects. The real tangible heritage is made up of archeological sites, engineering works, places, buildings, and architectural ensembles.

The intangible cultural heritage includes the wealth of knowledge, also living expressions inherited from our ancestors and transmitted to our descendants, such as language, oral traditions, customs, performing arts, ways of life, rituals, festive events, knowledge, and practices related to nature and the universe, as well as knowledge and techniques linked to traditional crafts [5].

Cultural heritage is a nonrenewable resource with regard to its past, and that is why it manifests itself tangibly as an untouchable and irreplaceable resource of a people. This heritage is always linked to the human collective, since it is men and women who produce it, and therefore it is what gives identity, origin, and continuity to our people. Hence, it is the responsibility of all its conservation and restoration for which it is essential to know what are its main threats in order to prevent, delay its deterioration and, if necessary, restore this heritage.

The alteration of cultural assets is the characteristic of the continuous cycle of disintegration and reconstruction, and it is a natural condition since all matter follows a process of alteration, degradation, or decomposition which means that original physical, chemical, and optical qualities are lost and enter a process of instability promoted by factors or agents of deterioration that are of two types:

Intrinsic: it depends on the nature of the material, manufacturing technique, and procedures that were used to perform the work.

Extrinsic: It depends on the sources external to the object such as environmental factors (light, relative humidity, temperature, and air pollutants), anthropogenic factors (handling, use, consultation, vandalism, tourism, etc.), biological factors (microorganisms, plants, rodents, and insects), and catastrophic factors (floods, fires, etc.) [6–8].

Among the main mechanisms of deterioration, three processes are known:

Physical or mechanical processes where the behavior of the material is modified, where several mechanical forces participate (compression, traction, etc.). These change the behavior of a material without modifying its chemical composition.

Chemical processes: are those that compromise a chemical reaction that transforms the matter.

Biological processes: where living organisms, such as microorganisms, insects, rodents, plants, etc., can chemically attack the material or its mechanical resistance. This process is also known as biodeterioration, which has been defined as "undesirable changes in the properties of materials caused by the vital activity of organisms" [9].

2. Biofilms as biodeteriogens

It is expected that works of art last for a long period of time; however, these suffer deterioration, and previously it was believed that chemical and physical processes were the dominant factors in the degradation of materials. Since 1967 and in latter decades, dogma has changed, and today it is assumed that microorganisms only by their very presence can cause damage by esthetic destruction of the materials since they inhabit them and penetrate causing their loss due to their acid corrosion, enzymatic degradation, and mechanical attack [10]. These microorganisms can grow in nature in large, silty colonies known as biofilms where relationships and dependencies are established [11–13].

Biofilms are microbial monospecies or multispecies (consortium) communities that have demonstrated the most successful colonization among microorganisms are ubiquitous in nature and responsible for many diseases. They are considered growing communities of microorganisms embedded in a self-produced exopolysaccharides matrix and are attached to an inert surface or living tissue [14–16]. The microorganisms in biofilms have properties that are not shared by free organisms, and the requirements for the formation of biofilms are simple: surface, moisture, nutrients, and microorganisms. This complex microbial organization that can consist mainly of bacteria and fungi, offers several advantages for its survival, such as resistance to environmental stress through the formation of stable microcolonies, facilitates the exchange of genetic material, and there is accumulation of nutrients and water in its matrix that offers protection against toxic substances (biocides and antibiotics) and against desiccation as well as immune defenses in the case of the formation of biofilms in higher organisms [17].

The importance of biofilms in the biodeterioration of cultural heritage has been reported for several decades and is related to: (a) modifications in pH values and ionic concentrations, (b) reduction oxide conditions in the interface of biofilms and substrate, (c) covering the surface and masking its properties, (d) increasing the leaching of additives and monomers outside the polymer matrix by microbial degradation, (e) releasing enzymes that lead to embrittlement and loss of mechanical stability, (f) accumulating water that penetrates the matrix causing swelling and increased conductivity, and (g) excretion of lipophilic pigments among others [18, 19].

3. Biodeterioration of stone

The mineralogical nature of the stone, its surface properties, and environmental conditions act synergistically for its bioreceptivity (ability to be colonized by microorganisms), and its intensity will depend on the concentration of pollutants, microclimatic conditions, and anthropogenic eutrophication of the atmosphere [20].

The climatic conditions in which the monuments or architectural structures are exposed can be the wind that wears the rock eroding it, the solar radiation causing discoloration, the temperature, as well as the rain, snow and humidity that induce the process of physical and chemical wear. These factors affect the stability of the matrix of the stone or act through chemical corrosion forming minerals by oxidation and hydration reactions as well as by the dissolution of carbonates and solubilization of some elements of minerals with silicates [21, 22].

The microbial communities after the interaction with biotic and abiotic factors are developed using the stone as a substrate and are partially responsible for the chemical and physical deterioration of the same and alter the esthetic appearance and physical integrity of the material through different mechanisms (**Figure 1**). The effects of microbial activities on historic buildings may be: discoloration, water retention, growth stimulation of heterotrophic organisms and higher organisms, material breakage, disintegration of the material, formation of patinas, degradation (corrosion), wear and dissolution of the structure, alkaline dissolution, and alteration of stratified silicates [23].

The microorganisms that colonize the stone monuments can be distinguished according to their location in the stone. The so-called epilithic that are located on the surface of the rock and those that live inside the rock within fractures and cracks and pores in granites are known as endolithic [12]. The main microorganisms that play a potential role in biodeterioration are autotrophic and heterotrophic bacteria, fungi, algae, and lichens. Phototrophic microorganisms such as microalgae, cyanobacteria, and lichens are considered the pioneer colonizers of the surface of stone monuments.

Figure 1.
Biodeterioration on stone (photo: Laura Castillón).

Cyanobacteria and algae such as chlorophytes, chrysophytes, and diatoms are a morphologically diverse and widely distributed group endowed with remarkable adaptability to variable environmental conditions and effective protection mechanisms against various abiotic stresses that enable them to colonize almost all classes of extreme lithic habitats [24]. These microorganisms form pigmented scabs (patinas) and incrustations that affect the substrate esthetically and cause physical and chemical deterioration of the rock. The epilithic cyanobacteria play an important role in the dissolution of the limestone carbonate, being able to cause the detachment of parts of it, due to a decrease in the coherence of the crystals around the colonies [25, 26].

The external stones are an appropriate niche for the growth and development of pioneering microorganisms that include photoautotrophs, lithophiles, and chemolithotrophs. The colonization begins with cyanobacteria and algae, probably followed by lichens that synthesize extracellular organic matter, in addition, dead cells release their constituents that form sources of nutrients for the growth of heterotrophic microorganisms which are considered secondary colonizers [27]. The phototrophic metabolism of cyanobacteria and algae facilitates their growth in oligotrophic environments such as stone forming biofilms on rocky surfaces, and it is the characteristics of the substrate that determine the speed of their growth and therefore the intensity of biodeterioration [28]. Lichens are highly resistant to extreme temperatures and desiccation that allow their easy growth on the surface of the stone. The microbial populations present in the rocky substrate are the result of successive colonization by different heterotrophic microorganisms.

It is well known that stone surfaces are exposed to high levels of solar radiation, high temperature, and to prolonged periods of desiccation alternating with rainy and damp periods. Many cyanobacteria are known to tolerate environmental extremes like UV light and their resistance to desiccation and tolerance for high level of light intensities and UV radiation provide them a distinct advantage for their survival on exposed surfaces, and they synthesize UV sunscreen pigments including scytonemin, mycosporine like aminoacids and biopterin glucosides. There are several reports on the effect of UV radiation on nitrogenase activity as it relates to the role of cyanobacteria in the nitrogen economy of ecosystem [29].

Pigmentation as a mechanism of deterioration depends on the nature of the substrate, the presence of trace elements such as iron, zinc, etc., the acidity or basicity of the medium, and even environmental conditions. The microorganisms produce two types of pigments (a) endopigments: they are located inside the cell and only leave after the lysis of the same as photosynthetic pigments, such as chlorophyll and phycobilins and (b) exopigments emitted outside the cell as fungal pigments (black, violet, blue, green, and purple). The black pigment known as melanin protects fungi against environmental threats and cellular lysis. Moreover, mycosporines and carotenoids (ß-carotene, s-carotene, phytoene, torulene, and torularhodin) may protect fungi against excessive UV radiation, act as antioxidants, osmoprotectants, and provide desiccation tolerance [30, 31].

The wear of materials is accelerated by the presence of biofilms containing active and latent microorganisms and their metabolic products, such as corrosive organic and inorganic acids as well as polymeric materials. Polymers, usually polysaccharides, act as gums that trap dust and other particulate materials increasing the disfiguring effects of the biofilm [32].

Beyond the type of microorganisms, the formation of the biofilm is a biodeterioration factor. The exopolysaccharide matrix plays a crucial role in this phenomenon since it produces mechanical stress on the stone through the pores of the mineral structure because it modifies the circulation of water within the material, its sensitivity to temperature variations, and the cycles of swelling and contraction dependent on the concentration of water within the matrix [27, 33].

Biofilms are also associated with the degradation of buildings and mural paintings by a phenomenon known as salt efflorescence, involves secondary minerals produced through the reaction of anions from excreted acids with cations from the stone which is available in the wall by the biological process or simply due to comigration with the infiltrated water. The solubilization of the calcareous material is detected by the presence of hygroscopic salts including carbonates, chlorides, nitrates, sulfates, etc., can be found on the surfaces of decayed monuments caused by chemical reactions (chemical agents in the air) or by enzymatic reactions of certain microorganisms. The most frequently isolated genus was *Bacillus*, followed *Staphylococcus*, *Kocuria*, *Micrococcus*, *Paenibacillus,* and *Arthrobacter* (bacteria of the sulfur and nitrogen cycle) [34].

The precipitation of salts is due to changes in temperature or humidity, and the salts can precipitate on the exposed surfaces and cause a destructive effect. Some salts when hydrated occupy a large space causing additional pressure that eventually leads to the loss of material and destruction due to cracking and detachment of walls or calcareous structures. In studies conducted by electron microscopy of salt efflorescence zones on walls, biofilms have been reported by members of *Firmicutes, Actinobacteria,* and *Ascomycota* [13, 35, 36].

From the nutritional point of view, organisms show a wide range of metabolic modalities where they use different sources of carbon (organic or inorganic compounds) as well as light as an energy source, and they are classified as photoautotrophic, chemoautotrophs, chemoheterotrophs, chemoorganotrophs, and photosynthetic.

The main mechanisms of biodeterioration associated with the different types of stone-colonizing organisms according to their nutrition are presented in **Table 1** [20].

Microbial colonization on bare stone surface is thought to be initiated by pioneering microorganisms which includes photoautotrophs, lithophiles, and chemolithotrophs. These organisms may secrete carbohydrates and growth factors which help in the formation of biofilm (a three-dimensional structure regulating temperature and humidity) and support the growth of successive microbial communities that is predominated by heterotrophic bacteria and fungi [20].

Limestones are carbonate rocks composed of calcite, and their main uses are in construction, chemical products, smelting, agrochemicals, and glass. This material is highly porous and hydrophilic in nature, and it is highly susceptible to water (such as acid rain) and environmental contaminants. Water often penetrates the pores of the stones causing damage by corrosive ions such as chlorine and acids. Biofilms,

Mechanisms of Biodeterioration	
Organism	**Mechanism**
Cyanobacteria	Formation of biofilms, coloured patinas and crusts, bio-waste as a result of calcium uptake, calcium salt precipitation and mineral formation
Lichens	Extraction of nutrients from the surface of the stone, formation of oxalate, production of carbonic acid associated with biodeterioration, physical intrusions in small pores.
Algae	Biofilm formation, color alteration, black scabs
Mosses and liverworts	Discoloration, green-gray patches, extraction of minerals from the stone
Sulfur-oxidizing and nitrifying bacteria	Formation of black crusts, secreted acids Salt efflorescence
Heterotrophic bacteria	Scab formation, patina, exfoliation, color alteration Bio-waste by secreted acids
Actinomycetes	White powder - grayish, patinas, white salt efflorescence Biofilm formation
Fungus	Fungal diagenesis, color alteration, oxalate formation, bio-waste by secreted acids, chelating properties by secreted acids, physical intrusion of hyphal penetration, biofilm formation and destabilization of the texture of the stone
Sulfur reducing bacteria	Conversion of sulphate into sulfite that acts as a nutrient for sulfur-oxidizing bacteria, salt efflorescence
Superior plants	Intrusion of roots within the cracks and pores, collapse and detach the structure of the stone.

Table 1.
Mechanisms of biodeterioration.

industrial and persistent pollutants, particulate matter, ash and often smog are deposited on the stone, and as a result, its deterioration is accelerated [23, 37].

The wear of the rocks and monuments can also be a consequence of the removal and solubilization of cations present in the minerals of the stone in particular iron and manganese of the mineral network by the negatively charged exopolysaccharide (EPS) of the biofilms or by some microbial proteins called siderophores by organic transport complexes and metallic organic chelates. Under low iron stress, siderophores chelate iron and supply to bacterial and fungi cells by outer membrane receptors, and the role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cells [38, 39].

The ability to grow by the dissimilatory oxidation of inorganic electron donors (ferrous iron, hydrogen, sulfur, and reduced inorganic sulfur anions) is widespread among acidophilic prokaryotes. Both oxygen and ferric iron can act as electron acceptors from many species of chemolithotrophic acidophiles, enabling them to exploit anoxic as well as aerobic environments [40].

In aerobic conditions, electron donors may include ferrous ions or sulfur compounds which are oxidized into ferric iron and sulfuric acid, respectively, yielding high energy. However, during anaerobic conditions, ferric ions can replace oxygen as the electron acceptor with multiple substrates donating an electron. This pathway yields less energy than aerobic conditions, but energy can still be produced for growth. *A. ferrooxidans* is a chemolithoautotrophic bacterium which can use many different electron donors to support growth *Leptospirillum* spp. have been shown to use only ferrous iron as electron donor and are therefore (as a result of thermodynamic constraints) obligate aerobes.

The subsequent redox process is favored by the release of oxygen by photosynthetic bacteria, cyanobacteria as *Acidithiobacillus ferrooxidans, Bacillus* spp., *Leptospirillum* ssp., and chemoorganotrophic fungi such *Aureobasidium* spp. [24, 41, 42].

Mechanical damage to stone structures, monuments, and architecture is another type of biodeterioration mechanism which is due to the physical intrusion and penetration of bacteria, fungal hyphae in the gaps, pores, and fractures that destabilize the texture of the stone, causing mechanical deterioration or by the contraction, and expansion of the stem under fluctuations in humidity conditions. Also the mosses through the rhizoids can penetrate the rock causing holes (pitting) and the vascular plants through their mechanical deterioration through the growth of roots or chemically by the acidity and diverse exudates, alteration of the microclimatic parameters, increase of the risk of fires, and physical and visual obstruction [26, 43].

4. Biodeterioration of paintings

The chemistry of the manufacture of paintings and their function has now been transformed from art to science. The knowledge of the pictorial components allows to associate the type of microorganisms that can potentially colonize the paintings, and the different techniques used determines the final composition of the work.

The pictorial technique has multiple modalities: tempera, encaustic, fresco, mural, and oil among others. The first three techniques were used in the ancient Greco-Roman and Egyptian world. (a) Tempering consists basically of the mixture of earths or pigments with binder constituted by glue and water or by egg yolk and oils in any type of support, (b) encaustic or wax painting requires a previous preparation of the wall by means of wax, on which colors are applied to the tempera cast in wax using a hot palette, and (c) the fresco that consists essentially of painting on a surface of wet plaster, on the same wall, prepared by the artist himself [44]. This latter is the modality of frequent use and is performed on wet lime plaster that serves as a support for the various pigments dissolved in water which are chemically integrated into the wall, and so its durability is very high, and (d) oil is the best known pictorial technique and used on cloth or board. It consists of a mixture of colored pigments with oil, usually flax or walnut. Another technique related to painting on canvas is acrylic, which consists of a combination of acrylate molecules in emulsion with water [45].

The deterioration of a painting can have different origins such as: (a) alterations due to natural aging of the work that makes it more fragile, (b) defects inherent to the work such as low quality materials or bad techniques at the moment of being painted, and (c) influence of external conditions such as thermo-hygrometric conditions or other factors. The manifestations of the damage can be physical as lack of adhesion of the binder, or damage caused by the movements of the other, mechanical damage, etc. Chemical damage is manifested as a processes of gradual degradation and depolymerization or crosslinking of the materials of the work, damage caused by light, oxidation or biological attack [46–49].

The biodeteriorable character of the canvas is conditioned by the characteristics of the fabrics that are its support formed by cellulose fibers which is a polysaccharide whose constituent unit is D-glucose linked by glycosidic bonds β (1–4) forming linear chains, which in turn are links in parallel fibers called microfibrils. For cellulose, degradation involved different enzymes whose combined action allows obtaining glucose molecules that can be assimilated by microorganisms as a carbon source. The degree of polymerization and its orientation, the length of these chains, their crystallinity, and their orientation are detected by microorganisms and could be susceptible to biodeterioration. The susceptibility to biological attack depends on the percentage content of cellulose, lignin, and other organic components. The purest cellulose can hardly be attacked. *Alternaria, Aspergillus, Fusarium*, *Memnoniella*, *Myrothecium, Neurospora, Penicillium, Scopulariopsis, Stachybotrys, Stemphylium,* and *Chaetomium* are the main fungi associated to this process and as cellulolytic bacteria: *Cellvibrio, Sporocytophaga, Myxococcoides, Cellufalcicula,* and also *Clostridium* sp. as anaerobic bacteria has been reported [50]. This cellulolytic process is favored in conditions of relative humidity of high air or condensation water where the fiber of the fabric loses consistency and elasticity becoming brittle and falls apart.

The filmogenic substances act as a binder and as a vehicle for the pigments; they are mainly of organic origin; they are applied in liquid form, and with drying, they solidify forming a hard and flexible layer, with the passage of time and under certain adverse conditions, this layer loses its property of cohesion of pigments and causes dusty surfaces or the separation of the layers.

The most important component of the paintings on canvas is the pigments; they are either natural or synthetic origin, and have three main functions: they provide color, opacity, and brilliance, and protect the surface in which they are applied and protect the binder from its destruction by UV radiation.

The adhesives are a fundamental element for the final result of the work whose function is to facilitate a uniform distribution of color and prevent the paint layer from being absorbed by the fabric, and these adhesives have been changing in the course of history and have been classified depending on their origin in animal (gelatin, albumin, casein, and wax) and vegetal (starch, resins, gums, and gluten).

Gelatin is obtained from collagen which is an existing protein in the skin and cartilage, albumin (protein of egg or blood plasma), casein (protein of milk), and wax (secreted by bees composed of a mixtures of esters, hydrocarbons, and fatty acids). The starch is a polysaccharide of vegetable origin, which is formed predominantly of amylose and amylopectin. Vegetal resins are a mixture of organic compounds principally terpenes and derivatives. Gums consisting of mixtures of water soluble polysaccharides produced by exudation, usually from the stem of tree and gluten, refer to the proteins in cereal grains found in the endosperm plant embryos during germination (Coppen, 1995).

The organic composition of all these adhesives favors the growth of microorganisms due to their high nutritional content, and therefore they are easily attacked by them [30].

The use of varnishes is required to provide protection against environmental attacks. For them, natural or synthetic varnishes (resins) are used, which must be applied in such a way that they form a resistant, colorless, and transparent film. According to its chemical composition, the name of the natural resins (soft) depends on the number of isoprene units that contain such terpene molecules as monoterpenes, sesquiterpenes, diterpenes, and triterpenes. The deterioration of natural resins causes chemical changes such as polymerizations (crosslinking of polymer chains, hydrolysis of polymer chains, and oxidation of the main chain or side groups which causes the resin to become more insoluble, losing its resistance and changes in its coloring [51]. Synthetic (acrylic) resins have good adhesive properties and are currently widely used in preservation treatments, and the monomers of these resins are generated by the esterification of an acrylic acid with several alcohols. Its general formula is: $CH_2CR1COOR2$ and due to its hydrophobic nature are more resistant to microbial attack because they are not used as a source of nutrients [52, 53].

Among the forms of deterioration of pictorial heritage on canvas related to biodeterioration agents are [54]:

Alterations of the canvas: hydrolysis, colorations, loss of strength, loss of support, cracks, scales, and deformations.

Alterations of the binder/adhesive: enzymatic degradation, colorations, disintegration, and pulverulence.

Alterations of the varnish: yellowing, tiling, whitening, and peeling.

The biological origin of deterioration in paintings has been widely reported in wall and easel paintings since the 1980s in different parts of the world [49, 54], and in general, the main fungal species associated with the biodeterioration of painted walls are *Penicillium* sps.*, A. niger, Rhizopus oryzae, Mucor, Trichophyton, Alternaria alternate,* and *Epidermophyton floccosum* [55, 56] and as biofilms *Acremonium*, *Cladosporium, Aspergillus, and Fusarium* [57]. In the case of canvases of oil paints, the bacterial strains of the phylum firmicutes such as *Bacillus* sp.*, Micrococcus luteus,* and *Paenisporosarcina* sp. and nonculturable bacteria of the phylum *Proteobacteria such as Stenotrophomonas* sp. [58] as well as *Halobacillus* sp.*, Halobacillus naozhouensis*, and *Nesterenkonia* sp. in wall paintings responsible for pigmentation by pink biofilms in Romanian monasteries [59].

The mere presence of microorganisms (colonization) in any type of surface does not determine their participation in the biodeterioration process, to be able to specify it, experimental strategies have been developed in the laboratory where traditional supports such as linen cloth prepared with layers enriched in gums of animal origin and linseed oil that are inoculated with suspensions of fungi and bacteria, later identifying the species that grow and deteriorate these materials, thus checking the postulates of Koch [60]. Another report associated with this proposal was made in the murals of the medieval church with the bacterium Arthrobacter responsible for the black spots as a result of the reaction of lead oxide of pigments and hydrogen sulfide produced by other bacteria responsible for spotting [61]. These models will allow to establish, under controlled conditions, which species colonize a given substrate and how the flora will change the substrate and how the substrate is modified by microbial colonization and how these modifications lead to the establishment of different microbial communities (microbial succession) [62].

There are several reports of the participation of biofilms as responsible for biodeterioration in the pictorial cultural heritage as in the case of the works of the Nerja and Treasure in Málaga [63], the church of St. Martins in Greene-Kreiensen, Germany [64], and the Mogao caves in Dunhuang, in Gansu Province of the People's Republic of China [65] among others. As an example of these works, mucilages have been analyzed in fragments detached from frescoes of the Santissima Annunziata Church in Siena Italy in damaged areas, and the presence of biofilms has been demonstrated where their growth is favored by external factors such as humidity, poor ventilation, and light which may be associated with biodeterioration, because their hydration retains particles and atmospheric pollutants that accelerate chemical corrosion by oxidation, reduction, and transformation of metal ions with the changes in the pigments in addition to the coexistence of saline efflorescence making the bioreceptives fresh and causing detachment of the paint layers [66].

Another threat has been reported to the cultural heritage of biodeterioration in frescoes caused by the presence of tourism such as the case of The Lascaux Cave, which is the most emblematic example of the damage that micro-organisms cause to mural paintings due to the amount of organic matter, respiration, and the sweat of visitors and workers that increased the concentration of CO2 favoring the growth of the fungus *Fusarium solani* and *Ochroconis lascauxensis* (black spots) and the alga *Bracteacoccus minor* forming a green patina (green disease) causing irreversible damage so it closed its access permanent visitors [62, 67].

At present, we must consider the use of commercial paints (canned) which, due to their composition, represent a carbon source for a large number of microorganisms and which can be a source of contamination and colonization for the surfaces in which they are applied. *Pseudomons, Flavobacterium, Escherichia, Bacillus, Enterobacter, Proteus, Micrococcus, Serratia, Aeromonas, or Stenotrophomonas maltophilia*, among others, have been reported in water-based paints, this contamination can occur during their production with the use of contaminated water or in the team [68–70].

5. Biodeterioration of textiles

Textiles are considered representative of cultural identity because they carry a significant value that transcends that of their materials and the work required for their manufacture. The desire of all cultures to express and communicate their social, esthetic and cultural values in their textile manifestations, and materials such as clothing or basketry (with ceremonial or ritual destination), are a unique cultural heritage, and the assignment of cultural value to a material object is the basis of conservation [71].

Textiles, such as clothing, fashion accessories, archeological objects, baskets, quilts, tapestries, embroideries, flags, funerary, and religious garb are often treasured for their artistic, technical, cultural and sentimental value, and for this reason, they are currently stored in collections in museums [72].

The textile heritage is very extensive, despite the loss to which it has been subjected throughout history, mainly due to the characteristics of its delicate materials and the interventions that have suffered and suffer from this type of pieces. The gradual deterioration of this material is very sensitive and can only be slowed down, the daily use of these fabrics, inadequate handling and bad storage conditions have caused the loss of unrepeatable textiles because the same materials and techniques are not available as well as the techniques that were used in their preparation that are already part of our past [73]. The state of preservation of textiles depends on the type of textile fiber, composition of the dye, age of the textile as well as its history of use and storage conditions.

It is called textile fiber to the set of filaments or strands susceptible to be used to form yarns (and of these fabrics). In the manufacture of the yarn for textiles, two types of fiber can be used: natural or synthetic. The natural fibers can be of animal or vegetable origin. In the first, they are generally of the protein type, such as wool from sheep's hair, goat, camelid, rabbit or another type of natural fiber such as silk from the silkworm. Among the natural fibers of plants include cotton, linen hemp, and jute among others. Synthetic fibers include polyester, polyamide polyurethane, polypropylene, polyacrylonitrile, and polyvinyl [74, 75].

In addition to the passage of time and the environmental characteristics of conservation as a possible source of deterioration, the development and presence of various types of organisms (microorganisms, rodents or insects) which are a threat to textiles and damage will depend on the type of fabric, its origin as well as storage conditions. The presence and permanence will depend on the availability of nutrients as well as light, humidity, and temperature conditions. The degradation of the materials that cause the damage by microorganisms is due to the processes of assimilation by fungi and bacteria that use these materials as a source of nutrients or to the degradation processes due to the effect of microbial metabolism.

The main manifestations of this process are the evident changes of the surface of these materials, discoloration, decrease in their resistance, changes in pH, and unpleasant odor. These damages can cause the total destruction of the material by the reduction of the degree of polymerization, decrease in its tensile strength, and elasticity. In general, natural fibers are more susceptible to microbial attack than synthetic fibers.

The main molecules responsible for the attack on textile fibers are organic acids, extracellular enzymes released or by exopigments of bacteria such as *Achromobacer* sp., *Bacillus* sp., *Brevibacterium* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Rhodococcus* sp. *and Streptomyces* sp. or exopigments of fungi of the group *Aspergillus* sp., *Penicillium* sp., *Cryptococcus* sp., *Rhodotorula* sp., and others [76].

The biodeterioration mechanisms in textile objects will depend mainly on the type of fiber:

Cellulose fibers: The degradation of (1-4)-β-D-glucan or cellulose results from the activity of cellulolytic enzymes produced by several bacteria and especially fungi that hydrolyze cellulose to glucose by the enzymes: 1,4-β-D-glucan, cellobiohydrolase, endo-(1-4)-β-D-glucan glucanohydrolase, and glucohydrolase of β-D-glucosidases. These enzymes decrease the degree of polymerization of the long-chain cellulose molecules, resulting in a decrease in the strength of the fiber.

The presence of other components in fibers such as hemicellulose, pectins, other carbohydrates or substances added to fabrics (plasticizers) and even contaminants provide additional nutrients to microorganisms.

Among the genera of fungi associated with biodeterioration of cellulose are *Chaetomium, Myrothecium, Memnoniella, Stachybotrys, Verticillium, Alernaria, Trichoderma, Penicillium, Aspergillus, Aureobasidium, Cladosporium, Fusarium, Mucor, Paecilomyces, Rhizopus,* and *Trichothecium*. In the case of bacterial damage and with less significance, agents of degradation of cellulose are: *Arthrhobacter, Bacillus, Cellulomonas, Cellvibrio, Clostirdium, Cytophaga, Microbiospora, Nocardia, Pseudomonas, Sporocytophaga,* and *Streptomyces* [77].

Wool fibers: Keratin is the constituent protein of these fibers that form a polymer when disulfide bridges cross over this polymer. The mechanism of biodeterioration is by keratinolysis, sulfitolysis, proteolysis by peptidases, and deamination (metabolic processes with release of ammonia). The rate of degradation depends on the chemical composition, molecular structure, and degree of polymerization of the substrate and to a lesser degree on the structure of keratin [78].

Among the main biodeterioration agents are bacteria: *Arthrobacter, Bacillus (B. mesentericus, B. subtilis, B. cereus* and *B. mycoides), Cellulomonas, Cellvibrio, Clostridium, Cytophaga, Microbiospora, Nocardia, Pseudomonas, Sporocytophaga* and *Streptomyces* [79]. Degradation by fungi has been reported by the genera *Microsporum, Trichophyton, Fusarium, Rhizopus, Chaetomium, Aspergillus, Penicillium, Alternaria, Acremonium, Cephalothecium, Chrysosporium, Dematium, Oospora, Scopulariopsis, Stachybotrys, Trichoderma*, and *Ulocladium* [77, 80].

Silk fibers: They are produced by silkworms and are fibers of the fibroin protein that are joined to one another by rubber-like proteins, known as sericin that serves as protection from damage by light. This natural fiber is the most resistant to biodeterioration, and its decomposition depends on the proteolytic action on sericin and fibroin that are used as a carbon source by bacteria *Bacillus, Aeromonas, Arthobacter, Chyseomonas, Pseudomonas, Streptomyces, Serratia* and *Variovorax* and how biodeteriogenic fungal genera are: *Aspergillus, Chaetomium, Cladosporium, Penicillum* and *Rhizopus* [80].

There are very few works to which biofilms are directly associated as being responsible for biodeterioration in textile materials, and it may be the result that experimental designs have not been developed with these types of materials.

6. Biodeterioration of paper and parchments

The documentary production goes back in antiquity in different cultures that left numerous examples in different supports like tablets of mud, rolls of papyrus, parchment, sheet of amate and in more recent times the books. The invention of paper gave man a faithful support where the written memory will inhabit his journey through history, and thanks to its consistency and durability, the texts of our ancestors are still today, a faithful witness of his time [81].

The main components of paper are fiber or fibrous material (hemp, cotton, linen, bagasse, rice straw a,nd wood) and functional additives (sizing, optical brighteners, and consolidating agents such as gelatin, cellulose acetate and carboxymethylcellulose). In this chapter, cellulose fiber is the major component with a lower proportion of lignin, hemicellulose, and other macromolecules, its quality depends on the source of the raw material used, and the procedure applied to obtain the fiber. Its mechanical resistance depends on its degree of polymerization and its interfiber links.

The inks are an important component of the documents and consist of a liquid that is fixed to the support endowed with an intense, durable, odorless and variable pH, is composed of a pigment, a diluent and a binder. Among the oldest ones are ferrous ink, whose components are iron sulfate, gallotanic acid and a binder, usually gum arabic. Over time the components of plant and animal origin have been replaced by synthetic compounds [82].

The books are composed of a support (parchment or paper), supported elements (inks), binding elements (seams and adhesives), protective structures (covers), and each of them with particular chemical characteristics that can be elements of degradation [83].

The microorganisms that commonly appear in the documentary supports are bacteria and fungi (yeasts and filamentous fungi), which transport moisture and attract pests by modifying the nutritive environment of the substrate. Both colonize a susceptible medium when in a poorly ventilated place, with adequate pH and low illumination, where temperature higher than 25°C and ambient humidity greater than 65% with accumulation of dust and/or soot in the different types of surfaces.

Filamentous fungi are the most biodeteriogenic microorganisms because they have structures called hyphae that are vegetative and reproductive [14, 84]. Vegetative hyphae are intertwined in paper fibers and through enzymatic processes that degrade cellulose, absorb nutrients, produce acids, and affect the coloration of the support resulting in fragility of the paper and often its complete destruction (**Figure 2**). On the other hand, the reproductive structures (spores) are a potential threat because they can remain in a latent state, they can be airborne, and they accumulate in layers of dust as long as the environmental conditions for their germination are reached, such as the formation of condensation points and local microclimates due to poor ventilation and heterogeneous temperature on the surface of the material.

Among the fungi identified are the Ascomycetes as well as mitosporic xerophilic fungi (which grow with a small amount of water) such as those of the genus *Aspergillus, Paecilomyces, Chrysosporium, Penicillium and Cladosporium* [85] and among the cellulolytic bacteria *Cellvibrio* and *Cellfacicula* as well as *Cytophaga* (myxobacteria).

In the case of the parchments that are composed of collagen (protein), its degradation depends on the oxidative chemical deterioration of amino acid chains and hydrolytic breakdown of the peptide structure and the production of pigments and organic acids that modify this material. The bacteria that have been described in this process are those of the genera *Bacillus, Staphylococcus, Pseudomonas, Virgibacillus* and *Micromonospora* as well as some alkalophilic bacteria such as *Actinobacteria* and among the proteolytic fungi reported are the Ascomycetes: *Chaetomium and Gymnoascus* and the genus *Acremonium*, *Aspergillus, Aureobasidium, Epicoccum, Trichoderma* and *Verticillium* [13, 78].

DOI: http://dx.doi.org/10.5772/intechopen.79617

Figure 2.
Paper biodeterioration. (Photo: Laura Castrillón).

7. Prevention, conservation, restoration, and control

To preserve the tangible cultural heritage, there are two ways of action: the prevention of deterioration (conservation) and the repair of damage (restoration). With the preventive conservation anticipates the damage generated by extrinsic causes, alien to the nature of the pieces to conserve, but that in more or less long term could degrade their cultural value.

Therefore, prevention methods and strategies are usually not directly applicable to the object to be treated, but are directed to the environment to control microclimatic conditions in order to eradicate harmful agents or elements that can temporarily or permanently influence degradation [82].

The prevention methods inhibit or slow down the biological growth modifying the factors that can condition or inhibit their presence (humidity, temperature, light, and ventilation), if these factors cannot be controlled as in the case of monuments or archeological zones can be modified eliminating dust, dirt and deposits of residues of plant or animal nature.

In contrast and in general terms, conservation can be defined as the set of operations that aim to prolong the life of the material, thanks to the anticipation of damage or the correction of deterioration.

In the field of the conservation of cultural goods, the purpose is to maintain the physical and cultural properties of what has reached the category of cultural property, with the purpose that its value does not diminish and lasts beyond a limited time segment. Preventive conservation, as a methodology aims to control the deterioration of works of art before they occur to reduce the need to intervene. The deterioration must be minimized and the optimum conditions of exhibition, transportation, handling, cleaning, and storage must be maintained [86]. Among the main measures applied for preventive conservation that have been incorporated in the facilities are: air conditioning free of biodeteriogens agents, environmental fumigations, humidity, and temperature control.

The restoration aims to recover the physical and functional integrity of the work, thanks to the correction of the alterations that it has suffered. Consequently, the curative methods are of direct application because they try to amend all the damages they have experienced through their own history, whenever these suppose mutilation or reduction of their documentary values. The restoration is more than an art is technical, thanks to the set of interdisciplinary scientific methods that give the work the authentic guarantee of the rigor of applied sciences to the field of conservation [87].

Restorers use the intervention techniques of biodeteriorated materials to eliminate the degradation products induced by microorganisms and if it is possible to delay their recurrence. The intervention treatment must be evaluated taking into account the identity of the biodeteriogens, degree and type of damage, safety of the treatment towards the materials of the object, risk for the worker, and possible environmental impacts. However, the growth of unwanted organisms will inevitably occur if the environmental conditions that favor their development persist.

Between the main methods of control of biodeteriogenic agents that grow as biofilms are:

Mechanical methods: they consist in the physical removal of fungi, bacteria, algae or any organism by shaving, abrasion, brushing, etc. Immediate but not lasting results are obtained, complete elimination is not achieved, and the results improve with the use of chemical agents.

Physical methods: modifying the temperature or pressure changes that are not suitable for the growth of organisms. Its biocidal effect depends on the denaturation or breaking of molecules of the organisms treated by breaking chemical bonds. Among these methods are electromagnetic radiation (microwave, ultraviolet rays, and gamma rays), anoxic treatments, and extreme temperatures. Its mechanism of action depends on its direct action with the genetic material or alteration of its structure and metabolic function.

Its main disadvantages are its high cost and the possible damage to the materials treated by its chemical alteration such as the pigmentation and hydrolysis of proteins and cellulose.

Chemical methods: these are the most commonly used intervention techniques through the use of biocides (disinfectants, bactericides or fungicides). Generally they are used in liquid or gas form, their mechanism of action is variable and they attack by disintegrating the bacterial or fungal membrane or by inhibiting their cellular processes, causing their death when they are used in the appropriate doses. Many products have been evaluated, however, due to their high risk and limited knowledge of the compatibility with the materials to which they apply their use has been limited. The selection of the biocide depends on the type of material, type of microorganism, and availability of the biocide. An additional problem is its long-term ineffectiveness [88].

A biocide can be a synthetic chemical, natural, of biological origin that is intended to destroy, counteract, neutralize, impede action or exercise control over any organism considered harmful to man. According to their action, they are divided into microbicides (bactericidal and fungicidal), growth inhibitors, and for the case of other organisms such as insects, rodents or birds, and there are also very toxic and lethal products such as pesticides, insecticides and/or repellents, acaricides, nematicides, avicides, rodenticides, etc.

A good biocide must have a broad spectrum of activity, be effective at low concentrations, be active over a wide range of pH, soluble in water, possess high persistence (effective over time), have low human and environmental toxicity, and have a low cost [89].

Among the main biocides used for the chemical treatment of tangible cultural goods are:

Thymol, orthophenylphenol (OFF), formaldehyde, pentachlorophenol, ethylene oxide, ethanol, etc., antibiotics and enzymes have also been used. For the best selection of the biocide, preliminary tests must be done to guarantee the innocuousness of the operation for the safety of the operator and absence of risk for the object not only immediately but in the long term. For this reason, the use of very strong and long retention solvents (glycols, formamide, turpentine, and butylamine) in the porous bodies has been ruled out.

The forms of application of the biocides are: sublimation, pulverization or fumigation, according to their possible solid, liquid or gaseous state. The solids in solution or dispersion have a longer time of action although less exterminating capacity. The sublimable solids have little penetrability and, unless they are applied in high concentrations, they become repellent air fresheners with little lethal efficacy.

Solvents that are unstable to light and have a tendency to yellow or polymerize are also eliminated (ethylenic compounds such as dipentene, turpentine, acetylacetone, N-methylpyrrolidone). These rules are applicable to all porous objects, paintings, polychrome sculptures, mural paintings, etc. In the area of textiles, the most suitable solvent is water. But there are fibers very altered or with very bad coloration that are too sensitive. Then we must resort to organic solvents but avoiding chlorinated solvents, taking into account an eventual acidity and the release of hydrochloric acid. Water-based solvents must be used with extreme prudence, since many materials can suffer deterioration [90, 91].

Because the control of pests that use chemicals that are generally expensive and have side effects in people and can deteriorate the material, the choice of a biocide is increasingly difficult, therefore alternatives substances with biocidal properties have been sought for many years such as the use of natural plant products for which more and more reports justify their use. Currently, there is already a database of the accumulated experience of a Spanish group of the use of natural extracts for disinfection and disinfestation of cultural goods [92], as well as the use of essential oils from medicinal plants such as *Mentha piperita, Thymus vulgaris, Origanum compactum, Salvia officinalis, Artemisa absinthium and Lanandula angustifolia*, among others [93–95].

The development of nanotechnology is currently an emerging field in the conservation of cultural heritage, consequently the FP7 NANOFORART project has arisen (nano-materials for the conservation and preservation of movable and immovable artworks) and as an example of its applications, there are reports of the use of zinc oxide nanoparticles to control fungal biofilms or nanosilver coated cotton fabrics application for antimicrobial textile finishing [96–99].

Biological methods: another option for the control and restoration of works of art has been the use of microorganisms in the processes of biocleaning and biomineralización that are presented below:

Biocleaning: The accumulation of organic material on surfaces either by deposition of atmospheric particles, traces of colonization of microorganisms and organic substances allow the growth of bacteria and fungi. This accumulation can cause damages to the art work in response to the growth of microbial and are therefore considered biodeteriorating agents, however, in recent years, bacteria have been used for the conservation and restoration processes for the elimination of these organic materials. This procedure is known as biocleaning.

One of the advantages of the use of microorganisms over physicochemical treatments (which are very drastic) is that they use substrate-specific enzymes that do not degrade complex substances and adapt easily to environmental conditions. The microorganisms selected should be nonpathogenic and nonsporulating so that it is not a risk for workers after application and not be able to produce forms of resistance (spores).

Examples of these treatments have been documented for the removal in stone of black scale (hydrated calcium sulfate and carbon residues) caused by sulfur dioxide, hydrocarbons, and particulate matter (soot) emitted by the exhaust pipes of vehicles, which were removed with the use of *Desulfovibrio desulfuricans* bacteria [100]. Good results have also been obtained for the elimination of nitrates in marble under anaerobic conditions with the use of *Pseudomonas denitrificans, Pseudomonas stuzeri, Pseudomonas pseudoalcaligenes* or *Paracoccus denitrificans* [101]. Biological cleaning of mural paints has also been explored to eliminate the remains of organic matter from old restorations or insoluble saline efflorescence with the use of *Pseudomonas stutzeri* [102].

Biomineralization: A modern and ecological alternative applicable to the restoration of historical monuments is the process known as biomineralization, specifically carbonatogenesis that can help in the restoration of cracks of statues or walls, since there are bacteria capable of mineralizing and filling these grooves when feeding them with means of culture that contain calcium salts in solution producing microcrystals of calcium carbonate that allow the restoration of damaged areas [103–105, 107, 108].

The carbonatogenesis or calcite production can occur either autotrophically or heterotrophically by the *Bacillus, Pseudomonas, Proteus, Myxococcus* and *Pantoea agglomerans* bacterial genera that allow obtaining a layer of a few millimeters thick by carbonatogenesis. Among the micro-environmental factors related to this process are: the concentration of dissolved inorganic carbon, the pH, the concentration of calcium ions and the presence of nucleation sites / or development of crystals for nucleation [23, 106].

8. Conclusion

In addition to the passage of time and environmental characteristics of the detriment of artistic and cultural heritage, this may also be the consequence of the microbial appearance favored by the enrichment of organic matter on the surfaces that colonize. The formation of biofilms is a strategy used by microorganisms to adapt to conditions that may be adverse for their growth. These biofilms are present in almost any type of surface, and the historical heritage is no exception. For the reason, it is important to know the characteristics that favor their formation, elimination and control to avoid being a threat to these objects.

As these biofilms represent a threat to this cultural heritage, the main challenges are to establish prevention measures for their appearance and to understand that if an intervention treatment is necessary, the resistance to treatment with biocides increases with this form of organization, which requires the use of high doses that compromise the physicochemical characteristics of the treated material.

The knowledge of the chemical composition of works of art made in different substrates such as stone, paintings, textile fibers, and paper composition allows us to understand what type of microbial colonization can be favored, and consequently be able to propose microbial growth as the responsible of biodeterioration. If these objects are valuable as a cultural heritage, it is the responsibility of all their conservation and restoration, for this reason the advanced techniques of identification and control of biofilms in cultural assets, currently, they are applied as strategies in the areas of preservation, restoration and control that will delay their natural deterioration and allow future generations to know this legacy.

Author details

Laura E. Castrillón Rivera*, Alejandro Palma Ramos, Jorge I. Castañeda Sánchez and María Elisa Drago Serrano
Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana, Mexico

*Address all correspondence to: lcrivera@correo.xoc.uam.mx

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