Chapter 10
Advanced Biocleaning System for Historical Wall Paintings

Giancarlo Ranalli and Elisabetta Zanardini

Abstract This chapter will focus on the potential role of safe microorganisms as biocleaning agents in the removal of altered or undesirable organic substances on historical wall paintings. Selected microbes can be adopted as biological cleaners to reduce and remove deterioration ageing phenomena, environmental pollutants and altered by-products of past intervention of restorations. The aim is to offer a comprehensive view on the role and potentiality of virtuous microorganisms pro-biocleaning of altered historical wall paintings. We also report four case studies in the CH restoration field, carried out in the last 25 years, with the innovative use of bacteria and different delivery systems, focusing the attention on the preliminary diagnosis and the monitoring of the whole process. The CH field represents a great challenge and Science and Art link together the work of conservator scientists and historians with researchers and scientists, sharing their diverse expertises and joining the knowledges to the preservation and the conservation of our artistic patrimony.

Keywords Conservation-Restoration · Cultural heritage · Biotechnology · Biocleaning · Organic matters · Bacteria

Cultural Heritage (CH) artworks are one of the most important elements of the identity in large part of the World, especially in the old European Continent and represent the cultural legacy from past generations defined as *tangible* (buildings, monuments, books, etc.) and *intangible* (as folklore, traditions, language, knowledge and natural as landscapes, and flora-fauna biodiversity).

The knowledge of our history has been subject to a continuous evolution over the time; that is true if we consider the modern potentialities of communications and the mobility of scientists and conservation specialists across the World. However, the

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knowledge in the conservation field, being relatively young, remains difficult both for the differences in terminology and for the lacks in the standardization of procedures, especially considering national-international multidisciplinary sectors (trades, professionals, administrative authorities, academic lectures, conservation students, workers involved in built environment, etc.).

Three examples are here cited: the terms *restoration*, *wall painting* and *fresco*. The first still linked to possible negative aspects originated by the restoration-reconstruction methods applied in the past centuries. The second defines *wall paintings* as paintings on inorganic substrates (plaster, stone), but in other cases, with *painting on the wall* are included paintings on wooden wall (EWAGLOS 2016). The third, the term *fresco* is limited to paintings on fresh lime plaster (Italy), but used in France for all kinds of wall paintings, including combination of fresco and secco techniques.

Artworks related to CH are exposed to weather and submitted to the influence of the environmental conditions: physicals, chemicals and biological factors interact with the constitutive materials, inducing changes both in its compositional and structural characteristics. In some cases the material transformation is due to the metabolic activity associated with the growth and the activity of living organisms, from microorganisms to plants and animals.

In the last decades, an important role of CH conservation has been mostly played by physics, chemistry and material science, but actually, the scientific scenery also includes biotechnology and applied microbiology, which can contribute to the development of new methods for the detection and identification of microorganisms altering stoneworks and for the bioremediation of weathered artworks (Fernandes 2006). In the last twenty years, the advanced methodologies have contributed to the knowledge of the structure (species composition and abundance) of the microbial communities colonizing artworks and their biodeterioration potentialities (Daffonchio et al. 2000; Gurtner et al. 2000; Piñar et al. 2001; Urzì et al. 2001; Crispim and Gaylarde 2005; McNamara and Mitchell 2005; Gorbushina 2007; Abdulla et al. 2008; Portillo et al. 2009; Ranalli et al. 2009; Scheerer et al. 2009; May 2010; Alonso-Vega et al. 2011; Giacomucci et al. 2011; Saiz-Jimenez et al. 2011; Zanardini et al. 2011, 2016, 2019; Cappitelli et al. 2012).

The biodeterioration mechanism and the role played by several biological agents "biodeteriogens" in the decay of stone monuments are well known. Different kinds of organisms (primary colonization) can grow using the stone mineral components and its superficial deposits. The main consequence of their metabolic activity, such as the excretion of enzymes, inorganic and organic acids and of complex forming substances, is the dissolution of minerals of the substratum. Moreover, the growth and the swelling of some vegetative structures (e.g. roots and lichenic thalli) induce physical stresses and mechanical breaks. The growth process and vegetative development of the living species of organisms can interfere on the conservation of CHs. More, the intensity of these damages is strictly correlated with: type and dimension of the organism involved; kind of material and state of its conservation; environmental conditions, micro-climatic exposure; level and types of air pollutants (Tiano 2002).

In the last decades, the traditional microbiological culture-dependent methods based on the use of specific media for the cultivation and the identification of microorganisms have been coupled to the culture-independent methods. These allow a better understanding of the complexity of the microbial communities altering stoneworks, the detection of novel microorganisms (bacteria, fungi, algae, etc.) and their activity (Schabereiter-Gurtner et al. 2001; Portillo et al. 2009; Saiz-Jimenez et al. 2011; Villa et al. 2015; Zanardini et al. 2016, 2019).

Moreover, the molecular methods can provide an early detection of the biodeterioration process, even when the deterioration is not visible yet. So, the use of molecular techniques for a preventive diagnosis and in the conservation perspectives becomes necessary both for the scientific approach and the economical point of view. These new techniques combined with the traditional microbiological methods give a much better understanding of the number, activity, function of isolated or aggregate microorganisms, not only in the "barbarian" biodeterioration, but also in bio-restoration and bio-conservation process, for their "virtuosos" functions.

In addition, these new approaches can be useful to set up alternative biocleaning restoration methodologies and to control during the treatment the microbial growth and activity and after the treatment to prevent further colonizations (Palla 2004, 2013).

1 Biotechnologies Applied to CH

For these reasons, the use of biological systems, as living organisms or derivatives (e.g. enzymes), goes under the name of biotechnology in many fields including the recovery of CH artwork.

In natural environments (air, water, soil) microorganisms contribute to very important aspect of life in the Earth: the equilibrium of the "matter transformation cycle" or "carbon cycle". Some examples are the transformation of rocks in soils (pedogenesis) or the transformation of the complex substances (polymers) into simple components (monomers) until the complete degradation (mineralization). As we mentioned above, micro- and macroorganisms, ranging from microscopical bacterial cells to higher plants and animals (eukaryotes), can find as suitable habitats for their growth, the CH surfaces such as ancient archaeological remains, monumental buildings, works of arts made with different inorganic and organic materials (stone, wall paintings, metals, wood, textiles, paper, etc.).

In conservation and restoration practices, different procedures can be used and some of them require the use and application of substances (i.e. animal and vegetal glues, re-lining paste, varnishes, temperas and other materials for cleaning and soaking). These substances can contribute to the biological risk being nutrients for the microbial growth especially when the environmental conditions are favourable (high water content, air humidity, temperature, etc.).

Indeed, when in past restoration, organic matter has been applied to stone surfaces for various purposes, often stoneworks show alteration over time and, in these cases, their preservation poses serious questions (Alfano et al. 2011; Ranalli et al. 1996, 2005). The residual compounds, under certain environmental conditions, can act as an adequate growth substrate for microorganisms, as bacteria and fungi and they can deteriorate surfaces (Ranalli et al. 1997; Tiano et al. 2006).

Moreover all outdoor artworks surfaces are exposed to atmospheric inorganic and organic pollutants giving the formation of surface deposits and alterations (patinas) and these materials over the time tend to accumulate and interact with the stone surfaces.

Restoration practices, aiming the cleaning of altered surfaces, need to previously define and characterize the organic and inorganic components, the mineralogical properties and the level and extend of decay in order to understand the causes and mechanisms of the processes actually in place. In particular, CH wall paintings preservation and restoration can be complex and depends on the nature, state of conservation, environmental conditions (indoor and outdoor); therefore, an interdisciplinary technical and scientific approach is imprescindible.

Based on the chemical characterization results and their distribution on the artwork surfaces, the cleaning methods should be selective and specific in order to remove the altered materials and to respect the original materials avoiding irreversible damages (Cremonesi 2004).

During the last 20 years, the biological cleaning seems to satisfy this need of accuracy, since both cultural-dependent and molecular biology methods allow the selection of microbial strains with specific metabolic activities and safe to use. For the removal of patinas and deposits, which are the main form of alteration present in wall paintings, two approaches have been used until today: the application of purified enzymes and the use of viable microbial cells (especially bacterial strains), chosen for their selective action towards specific substrates.

Microorganisms are extremely versatile and show great potentialities to induce chemical transformations both using different substrates as energy and carbon sources and producing a large variety of enzymes. The enzymes specifically accelerate the chemical reactions, working with high selectivity; for example, amylases, proteases and lipases are widely used in CH field, for their ability to hydrolyze substrates such as starch, casein, animal glues, wax, oil, synthetic resins frequently used in past restoration practices.

In 1970, trypsin was used by Wendelbo, for the first time, for the detachment of book pages stuck with animal glue (Wendelbo 1976). Later, amylases and proteases were used by Segal and Cooper (1977) and Makes (1988) for the removal of animal glue from parchment and for the treatment of painted canvas, respectively.

The use of enzymes, pure or in mixture, has been reported for the cleaning of mural paintings, sculptures, paper, etc., evidencing the importance of the type of enzyme, operative conditions (pH, temperature, etc.) and the way of applications (Cremonesi 1999; Bonomi 1994; Wolbers 2000; Banik 2003; De la Chapelle 2003; Iannuccelli and Sotgiu 2009).

The use of the enzymes in CH requires the correct and deep detection of the materials to remove; in fact, proteases can work for the removal of proteic substances (e.g. casein and egg, animal glues and gelatines), amylases for starch and gum, and lipases for oils, waxes and fat.

If the substances to remove are complex, it can be useful to use a mixture of enzymes; so it necessary to individualize, case by case if the best choice is the use of purified enzymes or a crude mixture.

PH and temperature are important factors for the optimal enzyme activity.

In the CH restoration field, usually the enzymes are used at an optimal temperature ranging 30–37 °C, offer the possibility of a soft heating of the artworks surface, but only after a previous adequate evaluation. The pH values normally range between 4 and 9, excluding, for example, some proteolytic enzymes that require an optimal pH around 1 and obviously difficult to use.

Another important aspect is the application of the enzyme and the maintenance of the best conditions for its optimal activity, for example, the use of buffer solutions able to keep the pH stable and to guarantee the activity of purified enzymes, but the addition of some additives and/or gelling reagents could induce reduction in the effectiveness of the enzyme (Bellucci and Cremonesi 1994).

The same authors highlighted the importance of evaluating the economic impact of the use of the enzymes in the CH field, i.e. the cost depends on the grade of purity and the quantity needed. The formation of operators is also important being the use of the enzymes still not so widely accessible and optimized.

Among the biocleaning procedures, the innovative approach based on the use of viable bacterial cells has been proposed by Ranalli et al. (2005), especially when the traditional methods (solvents) cannot work for the removal of weathered and insoluble compounds.

Viable bacterial cells can produce both constitutive and inducible enzymes able to attack and to degrade several types of undesired organic substances.

The versatility and efficiency of specific groups of bacteria are well known since they are able to degrade a wide range of substrates resulting more effective compared to the use of a single enzyme that can work only specifically in the attack of certain substrates (Ranalli et al. 2005).

In the following section, different case studies are illustrated showing how in the last 20 years the use of viable bacterial cells has become an imprescindible step in the restoration practices aimed to the recover altered ancient frescoes (Table 10.1).

2 Case Study of the Conversion of St. Efisio and Battle (Conversione di San Efisio e Battaglia), Pisa, Italy

The restoration of one of the most important Pisa Monumental Cemetery's frescoes, *Conversione di S. Efisio e Battaglia*, a fourteenth century fresco at Monumental Cemetery at Pisa, Italy, painted by Spinello Aretino was the first pioneeristic study based on the synergic combination of bacterial metabolism with the hydrolytic

Table 10.1 Main decay agents on organic materials (wall paintings, manuscript, others), microorganisms and delivery systems adopted

Decay agents	Artworks, Materials, Site	Biocleaning bacteria	Delivery systems	References
Animal glue	Camposanto Monumentale Pisa Frescoes, Pisa	*Pseudomonas stutzeri A29* (Ae)	*Ex-situ* Cotton Wool	Ranalli et al. (2005) Antonioli et al. (2005) Lustrato et al. (2012)
Proteins and inorganic compounds	Casina Farnese wall paintings (Palatine Hill, Rome)	*Stenotrophomonas maltophilia* (Ae) *Pseudomonas koreensis* (Ae)	*On-site* Laponite micro-pack	Mazzoni et al. (2014)
Animal glue and salt efflorescence	St. Nicholas frescoes, Valencia	*P. stutzeri* DSMZ 5190 (Ae)	*On-site* Cotton wool Agar	Bosch-Roig et al. (2010) Bosch-Roig et al. (2012, 2013)
Animal glue	Ancient paper	*Ochrobactrum* sp. TNS15	Agar	Barbabietola (2012), Barbabietola et al. (2012)
Animal glue and efflorescence	Archaeological frescoes, ceramic and bones	*P. stutzeri* DSMZ 5190 (Ae)	Agar	Martín Ortega (2015)
Black organic layer (mainly lichens)	Concrete	*Thiobacillus* sp. (Ae)	Immersion	De Graef et al. (2005) De Belie et al. (2005)
Graffiti paintings	Glass slides Stone	*D. desulfuricans* ATCC 13541 and others (An) *P. stutzeri* DSMZ 5190 *(Ae)*	Immersion Cotton, Agar	Giacomucci et al. (2011) Sanmartín and Bosch-Roig (2019)

Key: (Ae) Aerobic metabolism; (An) Anaerobic metabolism

enzymatic action for the restoration of medieval frescoes mural paintings (Ranalli et al. 2005).

The Camposanto Monumental Cemetery frescoes, a surface area of about 1500 m^2, were extremely damaged by a bomb in 1944 during World War II and a large number of frescoes were rapidly removed from the original walls under those extremely dangerous conditions.

As extreme intervention, the frescoes were detached using the "strappo" or "tear-off" technique; this procedure included the use of gauze and a layer of broth warm animal glue, followed by mechanical detachment by the walls. Then, by rolling them up without adding any rigid support, they were stored in deposits for about 50 years. The recovery of some frescoes became a challenge for the restorators as a consequence of humidity, atmospheric pollution and application of formaldehyde solutions to avoid microbial contamination (Antonioli et al. 2005). Most of them were also treated at the back with animal glue and casein as adhesives to the support.

After many years, the glue resulted polymerized and weathered and resistant to solubilizing agents and/or surfactants and the use of a mixture of the most performant proteolytic enzymes did not show significant effects.

In order to solve these problems and to identify suitable restoration practices and methodology to recover the frescoes, the approach required specific analyses to individualize the organic substances originally applied, as well as with those apported later in the restoration works.

For this aim, the use of sensitive, selective, and, if possible, non-invasive techniques that are minimally destructive, such as calorimetry, gas chromatography coupled with mass spectrometry (GC/MS) and pyrolysis/GC-MS, are suggested (Stassi et al. 1998; Colombini et al. 1999, 2002, 2003; Bonaduce and Andreotti 2009).

Once identified the substances to be removed, we decided that the use of viable cells could be the only way to restore the ancient frescoes.

The selection of microbial strains was a very important step, because the success of a "biocleaning" treatment depends on the efficiency of the used microorganisms. Both culture-dependent and -independent methods can help in the selection of the most effective microbial strain.

In our case, bacteria belonging to the genus *Pseudomonas*, Gram negative, asporigen, ubiquitary in nature and with extensive metabolic versatility, able in fact of using a large variety of different compounds as source of carbon and energy. Among the five species tested in vitro, *Pseudomonas stutzeri* A29 strain was selected because is not pathogenic to man and the environment and capable to grow using animal glue when supplied with it as sole organic carbon.

A direct application was carried out onto the artwork surface using viable *P. stutzeri* A29 bacterial cells in broth culture suspension (10^6–10^7 UFC/ml) embedded in sterile cotton wool, for 10–12 h at 30 °C temperature (Ranalli et al. 2005; Antonioli et al. 2005; Cappitelli et al. 2005, 2006, 2007). The costs of the bacterial suspension were quantified in about 50 euro/litre, and 100 euro/m^2 of fresco surface. At the end, a commercial Protease Type XIX was adopted to eliminate residues of glue on the surface.

3 Case Study of Stories of the Holy Fathers (Storie dei Santi Padri), Pisa, Italy

A second pioneeristic protocol based on the synergic combination of bacterial metabolism with the hydrolytic enzymatic action was performed for the restoration of medieval frescoes "*Stories of the Holy Fathers*", (size 6.10 15.65 m), painted by Buonamico Buffalmacco, in the fourteenth century (Lustrato et al. 2012).

In addition, in this case, the fresco damaged by a bomb during World War II was quickly removed from the original walls under extremely dangerous conditions.

In the 1960s, during the first restoration the fresco was cut into 17 sections (ranging from 1.3–2.0 m to 3.5–2.0 m) and attached onto asbestos-cement supports. The gauze and a large portion of the animal glue layer on the surface of the fresco were then removed using both traditional chemical and physical techniques, based on the application of ammonium carbonate solution and organic solvents.

Afterward the sections were recomposed, like a puzzle, on a metal frame at a distance of 5.0 cm from the wall in order to guarantee a better air circulation at the back of the fresco and to reduce the risk of vertical temperature gradient and condensation phenomena. However, unfortunately, alteration phenomena, such as swelling and detachment of the painting layer of the frescos, were noticed.

As for other frescoes of the Camposanto complex, degradation processes due to the synergism of the organic substances used as adhesive began and sulphation promoted by the lime putty, eternit and pollution determined the need to take again the *Stories of the Holy Fathers* frescoes down from the walls. In this case, traces of casein at the back of the fresco and glue residues ranging from a few granules (0.1 mm to about 1 mm thick) to a maximum of 20 mg/cm^2 were noticed on the painted surface.

Also in this case, the biotreatment with *P. stutzeri* A29 strain successfully removed animal glue and casein proteins from the altered fresco and the altered animal glue and casein were no longer detectable by visual inspection and confirmed by GC-MS and PY/GC-MS analyses (Bonaduce and Colombini 2003).

In addition, the results showed that the bioremoval procedure was quick, having a significant effect in the first 2 h after the application.

After the biocleaning treatment, the fresco treated surfaces were subjected to short- and medium-term monitoring to assess eventual microbial colonization, activity and presence of any viable *P. stutzeri* cells and the absence of viable cells was confirmed. Later, the fresco mounted on a frame was kept for one year in a confined and controlled environment in the restoration laboratory of Campaldo (Pisa) and then re-allocated in a semi-confined environment in the great hall of the Monumental Cemetery at Pisa. Periodic monitoring as well as surveillance and protection efforts continues in accordance with defined restoration protocols.

We highlight the advantages of the biocleaning process adopted: (i) the process was non-destructive and removed only extraneous substances or altered compounds; (ii) the use of safe microorganisms (not pathogenic and asporigen); (iii) safe procedure both for the operators and the environment; (iv) the biotechnological techniques were successful, low-cost, environmental friendly.

These positive results led the Technical Commission for Restoration (Pisa, Italy) to approve the use of the *P. stutzeri* strain for the biocleaning of all the ancient altered frescoes at monumental Cemetery, Pisa, that still remain to recover and the biological step has become essential in many cases.

In summary, the steps adopted were: (i) physical-chemical analysis for the characterization of the residual adhesive organic matter used in the past to detach the medieval frescoes; (ii) development and optimization of the advanced biocleaning system to remove the organic matters from the altered fresco surfaces; (iii) short- and medium-term treated surface microbial monitoring; (iv) costs–

Fig. 10.1 (**a**, **b**) Before and after biocleaning process on *Inferno* frescoes at Monumental Cemetery Camposanto, Pisa, Italy

benefits analysis of the biotechnological system employed (Lustrato et al. 2012) (Fig. 10.1).

4 Case Study of Casina Farnese on the Palatine Hill, Rome, Italy

In this study a bio-based procedure was employed to treat several hard-coherent deposits as aged proteinaceous matter mixed with gypsum, weddellite, calcium carbonate, apatite and nitrate was present on the wall paintings of the lower loggia of the Casina Farnese (Palatine Hill, Rome) (Mazzoni et al. 2014).

The microbial screening among three asporigen bacterial strains showed that at lab scale two were able to degrade proteins (*Stenotrophomonas maltophilia*) and to degrade protein material (*Pseudomonas koreensis*). For the biotreatment, the bacterial cells were suspended in a laponite gel, easy to apply and to remove, especially from vertical walls. The *in situ* application was carried out in multiple series at temperatures ranging from 6 °C to 37 °C, with contact times of 24–48 h.

The biotreatment with colonized laponite was able to remove multiple hard-coherent deposits on the wall paintings showing no residues at the end of the restoration (Mazzoni et al. 2014).

5 Case Study of Animal Glue on Frescoes on the Santos Juanes Church, Valencia, Spain

Other studies showed the use of *P. stutzeri* DSMZ 5190 viable cells for the onsite removal of altered animal glue residues on frescoes of the central vault inside the Santos Juanes Church in Valencia, Spain (Bosch-Roig et al. 2010, 2012, 2013, 2015). The authors adopted in their experimental conditions, agar-gel colonized by bacteria as improved delivery systems. Agar showed to be the most appropriate carrier, both at lab scale and at onsite experiments. It was observed a good adhesive property when applied onto vertical and oblique surfaces, and an adequate water retention for bacteria without interfering with wall surfaces. Large amount of bacterial cell suspension (10^9 UFC/ml) was applied in an aqueous solution directly onto the fresco surface by soft brush, with short time of contact (less than 2 h) for each application. The positive results showed that the animal glue was completely removed, confirmed also by analytical pyrolysis and gas-chromatography–mass spectrometry analyses. The bacteria were able to degrade the organic matter present on the fresco compared with area of control treatments (only water).

The authors highlighted the positive results evidencing that the biotechnology is risk-free for the restorers since only non-pathogenic bacteria were used, and is not invasive for the artworks and easily monitored since these bacteria are unable to produce spores (Bosch-Roig et al. 2013).

In conclusion, the different case studies here illustrated show that the biocleaning steps are:

- Diagnosis and characterisation of the stone alteration.
- Laboratory selection of microbial strains able to remove the alteration.
- Evaluation of the degradation rate in order to individualize the most promising microbial strain (safe, efficiency, adaption to the *in situ* treatment, etc.).
- Selection of the appropriate delivery system to vehicular microbial viable cells.
- Delivery system colonization and evaluation of the better condition for the microbial metabolisms.
- Lab-scale application on specimens artificially enriched and on real altered fragments.
- Optimization of the *ex situ, onsite* application conditions.
- Evaluation of the environmental conditions at the time of the application.
- Monitoring during the application.
- Removal of the bioapplication and cleaning of the treated surfaces.
- Short– and long-term surveillance and monitoring after the biotreatment.

6 Final Considerations

After almost 25 years since the first use of bacteria in the CH restoration field, we believe it is important to highlight some consideration in order to individualize the aspects that still need to be improved and optimized (Fig. 10.2).

First, it is important to understand how laboratory studies have been successfully transferred *in situ* and how the evolution of technology can be further improved.

Another important aspect is the interest of the industries or the SMEs in the production and commercialization of bioformulates ready to use in the restoration field or if remains a niche sector.

A crucial question also arises: Are the conservation scientists sufficiently involved in the use of the biotechnologies? Moreover, can biodeterioration and biorecovery of cultural heritage provide a important focus for the development of informative and innovative activities in an educational setting? (Verran 2019).

Considering the frescoes at Monumental Cemetery, Camposanto (Pisa), the restorers at Campaldo Laboratories (OpaPisa) showed from the first time a great interest for these advanced and innovative methods. They decided to use the biocleaning step in many cases and in an extensively manner (for example, both for front and back of altered ancient fresco sections) to remove altered organic matters as glue, casein, etc. The restorers have been trained and prepared to recognize the potential of biotechnology but also the limits. A particular attention has also been given to the evaluation of the costs comparing the biological methods with the traditional ones and this aspect resulted positive.

Considering the scientific production in this field is worth to highlight that the number of research articles, reviews, books, proceedings of national and international conferences has greatly increased as reported in Fig. 10.3.

The research groups working in this field are also expanded over the time involving a large number of countries and consequently different environmental

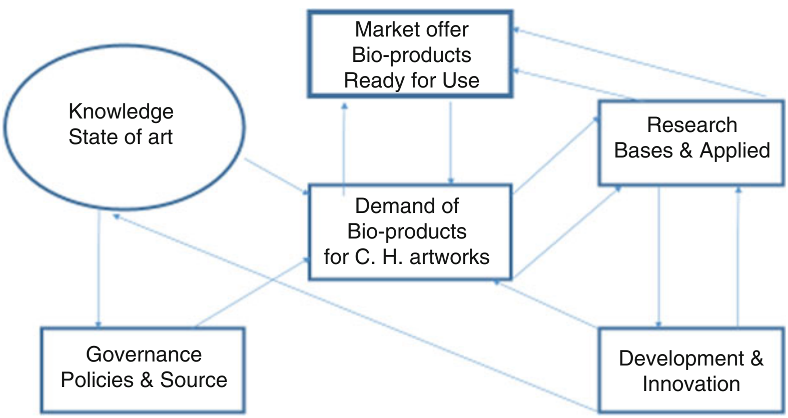

Fig. 10.2 Flow chart and interactions between the main actors in CH recovery

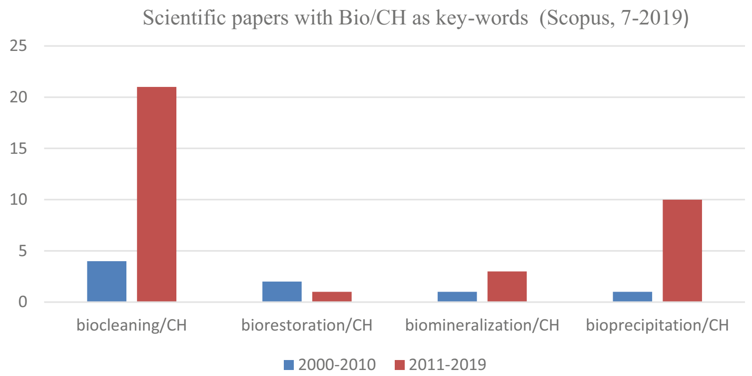

Fig. 10.3 Representative but not exhaustive literature data from Scopus, until July 2019

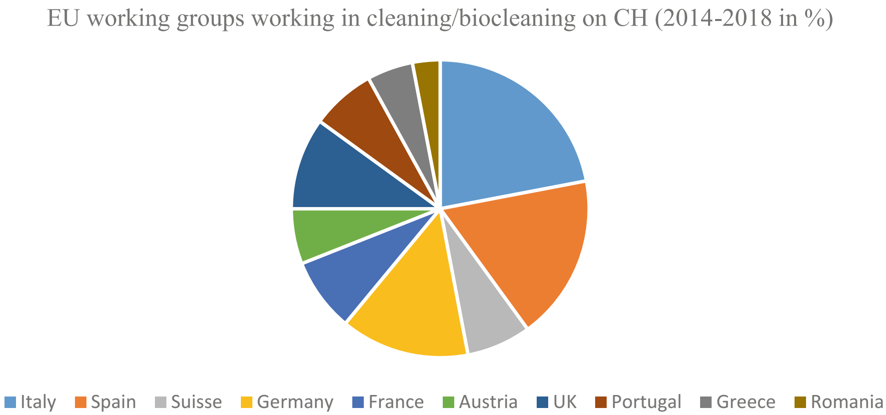

Fig. 10.4 European scientific research groups working in cleaning/biocleaning in the cultural heritage fields (2014–2018, in %)

conditions (climate, temperature, humidity, pollution, etc.), lithotypes, restoration uses and practices, cultures, etc. (Fig. 10.4).

Despite the increase number of scientific papers and research groups, the technological transfer on larger scale and the availability of products ready to use are still a problem to solve.

In addition, the dissemination especially to stakeholders and end users can be improved through the organization of workshops and seminars specifically targeted to conservation scientists. This will help worldwide the use of the correct terminology, the understanding of the potentialities of alternative methods and the preparation of guidelines and protocols available for all the operators in this field.

As evidenced in this context, numerous studies have been carried out in order to optimize the biocleaning strategies in terms of microorganism efficiency and delivery systems, ability to maintain and guarantee the microbial activity and ready to be used also on difficult altered stone surfaces (ornamented, vertical and vaulted). Different substances can be present as stone alterations and for this reason, the choice of the best microorganism is a crucial step in the biocleaning technology.

The CH field represents a great challenge and Science and Art link together the work of conservator scientists and historians with researchers and scientists, sharing their diverse expertises and joining the knowledges to the preservation and the conservation of our artistic patrimony.

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