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REVIEW ARTICLE

Biocleaning of Cultural Heritage stone surfaces and frescoes: which delivery system can be the most appropriate?

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Abstract The use of the advanced biotechnology of microbiological systems for the biological cleaning of Cultural Heritage (CH) has been recently improved and optimized taking into account different factors. Biocleaning systems have been indeed applied to historic buildings, statue,s and frescoes. Such application has developed new techniques and optimised and refined the existing systems. These systems remove altered forms like sulfate and nitrate crusts and organic substances like animal glue in a more effective, less invasive way than the traditional cleaning techniques. This review focuses on several delivery systems (sepiolite, hydrobiogel-97, cotton wool, carbogel, mortar and alginate beads, agar, and arbocel) used for the biocleaning of Cultural Heritage, comparing their main properties and characteristics, making a critical evaluation on how easy they can be applied, and on their future potentiality as ready-to-use and risk-free formulates. Therefore, this review will help conservation scientists, conservator-restorers, and researchers in the field to choose the most appropriate delivery system for any specific applications.

Keywords Biocleaning · Cultural Heritage stone surfaces · Frescoes · Delivery systems · Microorganisms

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Introduction

Amongst the damage found on works of art in both outdoor and indoor environments, those relative to lithoid-type matrices (stone, frescoes) are well documented. Both natural and artificial (obtained from transformation) stones have been widely studied because they are the key element in terms of archaeological and monumental heritage (Pinna and Salvadori 2008). Physical, chemical, and biological agents act to deteriorate stone (Warscheid and Braams 2000). Deterioration of stones depends largely on their physical and chemical properties such as strength, porosity, hardness, durability, texture, absorption, etc. Therefore, stones with high porosity, high rates of swelling, and low strength tend to be poor building materials (Doehne and Price 2010). Before starting to clean any type of stone surface, conservator-restorers and researchers must define the mineralogical properties of the stones involved, characterize the decay, measure its extent, severity, rate of progress and try to understand causes and mechanisms of that specific type of decay. Stone decay is a complex phenomenon produced by several factors that can have rapid or slow effects. There are many factors that can contribute to stone decay, but the main ones are air pollution, presence and concentration of salts, and biodeterioration (Camuffo 1998; Doehne and Price 2010).

Exposed stonework in polluted urban areas shows "black crust", "sulfation" and "nitratation" decay mainly caused by atmospheric pollution. Sulphur oxides, nitrogen oxides (NO_x), and carbon oxides present in the atmosphere create acidic solutions in the presence of water, which are capable of reacting with calcareous materials. Air pollutants can therefore cause the transformation of calcium carbonate in the calcareous matrix into calcium sulfate dihydrate and calcium nitrate with consequent loss of structure and increased susceptibility to corrosion phenomena (Gauri et al. 1989; Rivadeneyra et al. 1991, 1994; Orial et al. 1993; Salvadori

and Realini 1996; Saiz-Jimenez 2004; Doehne and Price 2010). These transformations cause deterioration processes of the structural, morphological, chemical, and aesthetic properties of the original material and, by definition, lead to what are termed "decay" (Saiz-Jimenez 2003). The effect of acidic pollutants on stone depends on the environment where monuments are located. When stone surfaces are in an exposed location with frequent rainfall, products from the reaction with air pollutants are wetted, and the stone surface is progressively deteriorated. If instead, when the stone surface is in a sheltered location, products from reactions accumulate and can form black crusts on stone surfaces. The blackness of the crust is mainly caused by combustion of fossil fuels. The rate of air pollutant decay mostly depends on the level of pollution, the amount of rain, and its acidity (Doehne and Price 2010). Indirect effects of atmospheric pollution like climate change may also alter stone monuments in different ways, such as by increasing the biodeterioration of the stone due to higher temperature and rainfall (Duthie et al. 2008), or by increasing salt crystallization on the stone due to greater fluctuations in humidity (Brimblecombe and Grossi 2007; Grossi et al. 2008).

Salts can also cause serious damage to Cultural Heritage (CH) stone surfaces and frescoes due to the stresses generated by crystal growth in the pores in the stone. Crystallization pressure and, therefore, damage result when a growing crystal encounters a pore wall (Scherer 2000). Salt damage may occur in both indoor and outdoor environments, and salts can be present from various sources (air pollution, soil, wind from the sea or the desert, deicing salt, cleaning materials, garden fertilizers, etc.) (Doehne and Price 2010).

Biodeterioration of historical and artistic stone objects and frescoes is another important cause of stone decay (Dornieden et al. 2000; Saiz-Jimenez 2001). Different organisms (including bacteria, fungi, algae, and plants) can grow on CH stonworks by using the mineral components and surface deposits (Bhatnagar et al. 2010). Biological colonization can accelerate deterioration of the mineral matrix due to the metabolic activity producing physical, chemical, and aesthetic damage (Warscheid and Braams 2000). The degree of the biodeterioration processes is related to the type of organisms present, the kind of material, and its state of conservation, as well as the environmental conditions where the works are located (indoors, outdoors, semi-confined areas), etc. (Ranalli et al. 2009). In the presence of biofilm extracellular polymeric substances (EPS) can result in mechanical stresses to the mineral structure leading to alterations in the size of the pores in the stone and in changes in the moisture circulation patterns and temperature response. In addition the presence of biofilms on stone surfaces can accelerate the accumulation of atmospheric pollutants and, therefore, act as a preliminary precursor for the formation of damaging crusts (Warscheid and Braams 2000). The best preventive method has proven to be the control of water, nutrients, and light levels around the artwork. The use of biocides must only be considered as a last resort due to their toxicity (Blazquez et al. 2000; Martin-Sanchez et al. 2012; Gómez-Alarcón and Sáiz-Jiménez 2013; Sanmartín et al. 2014). Other studies show that not all biological organisms produce stone decay, but biological patinas in some situations may help to protect fragile stone surfaces (Caneva et al. 2005; De Muynck et al. 2010). Also, specific microorganisms, because of their metabolic activities, can be positively used to clean stone surfaces (Atlas et al. 1988; Ranalli et al. 2003).

The conservation of stone surfaces and frescoes needs a technical and scientific approach. The problem of conserving CH is not simple, decay is a natural process, and, therefore, we can only slow it down (Fassina 2000). Stones can accumulate different kinds of materials: atmospheric deposits, salts from efflorescence phenomena, and residues from past CH interventions. Deposits are due to different deposition mechanisms of materials from the environment; the main mechanism is inertia, so materials deposit as to their weight. On the contrary, black crusts are formed with an important contribution of material coming from the substrate. The main mechanism of black crust is sulphation reaction involving calcium carbonate. Therefore, black crusts and deposits are due to different mechanisms. These materials present on the surface may present a significant hazard for CH damaging their aesthetic, chemical and physical aspects, and properties producing fissures, exfoliation, disintegration, loss of original material, and so on. The removal of these deposits is, therefore, an important concern for conservator-restorers. Cleaning of CH is a complex issue and a critical part of conservation and attention must be paid to avoid altering the original surfaces during the treatments. To select the most appropriate cleaning method, the substrate characteristics and the chemical characterisation of the materials to be removed should be firstly studied (Gulotta et al. 2014). The basic evaluation criteria that the cleaning methodology should take into account are: physical and chemical harmfulness, homogeneity of the deposits removal, efficiency, selective cleaning, absence of aesthetic alteration, and durability (Vergès-Belmin 1996; Gulotta et al. 2014). The main cleaning methods usually utilized are mechanical (brushing and rubbing, washing and steaming, wet and dry abrasives,etc.) and chemical (alkaline treatments, acidic treatments or organic solvents,etc.) methods. Due to the risk of damage (like loss of original material) using some of these conventional cleaning techniques, most of the researches has been focused on alternative, more selective, and less aggressive techniques like laser and biological cleaning (Doehne and Price 2010). Laser cleaning allows high selectivity (lasers can discriminate between the soiling and the substrate) being a less intrusive, more easily controlled, method than the traditional ones. Laser technique can be sometimes unsatisfactory because it does not allow the complete removal of the deposits, and it can cause color changes. It can also cause

problems if applied to polychrome sculptures and the costs are significantly high when applied to large superficial areas (Salimbeni et al. 2003).

Biological methods that use microorganisms and enzymes as biological cleaning agents in the "biorestoration" of artworks are becoming attractive alternatives to the mechanical and chemical methods. They offer significant advantages in terms of soft intervention on the works themselves, lack of health risks for conservator-restorers, and also guaranteeing environmental safety (Saiz-Jimenez 1997; Cremonesi 2002; Ranalli et al. 2005; Valentini et al. 2010). Under optimal controlled conditions, biological methods reproduce the same processes that occur in nature (Boquet et al. 1973; Atlas et al. 1988; Ferrer et al. 1988; Heselmeyer et al. 1991; Tiano et al. 1996; Castanier et al. 2000; Maier et al. 2000; Rodriguez-Navarro et al. 2000; Zanardini et al. 2000; Ranalli et al. 2003; Biavati and Sorlini 2008).

The key idea of using living cells in the conservation and preservation of works of art is supported by the fact that microorganisms (mainly bacteria) are the most versatile and ubiquitous organisms found on earth, and they appear to be capable of colonizing almost any environment (Maier et al. 2000). Even if we know that some microorganisms have a negative effect, many of them are responsible for "positive processes" such as the degradation of unwanted organic substances (Sorlini et al. 2010a). Recently various Cultural Heritage stone surfaces have been cleaned of organic and inorganic unwanted materials. Biocleaning techniques have been performed on stone (marble, tuff, sandstone, limestone, etc.), on ceramic material (brick-work), on paper materials, and on concrete using specific bioformulations containing *Desulfovibrio* sp. and *Pseudomonas* sp. cells. (Ranalli et al. 2005; De Graef et al. 2005; De Belie et al. 2005; Cappitelli et al. 2006, 2007; Barbabietola et al. 2012). Until now, positive results have been obtained from experiments conducted on significant historical monuments like the frescoes at Camposanto Monumentale, Pisa, Italy (removal of a cloth firmly glued to the painted layer) (Antonioli et al. 2005; Ranalli et al. 2005; Lustrato et al. 2012), Milan Cathedral facade (removal of black crusts) (Cappitelli et al. 2006, 2007), and Matera Cathedral - both in Italy (removal of nitrates) (May et al. 2008; Alfano et al. 2011). Other positive results involve the colored lithotypes of Florence Cathedral (removal of black crust) (Gioventù et al. 2011), the frescoes on the Santos Juanes Church in Valencia, Spain (removal of animal glue residues and salt efflorescence) (Bosch-Bosch-Roig et al. 2013a, 2013b), and original paper specimens from the *Istituto Nazionale per la Grafica*, Rome (Removal of animal glue) (Barbabietola et al. 2012). Recently Gioventù et al. 2011 in a specific case study compared the biocleaning treatments on stone materials to chemical and laser treatments.They concluded that the most satisfactory cleaning treatment for sulfate removal was the biocleaning process. In another recent study, combined chemical and biological methods allowed efficient cleaning with a notable reduction in the treatment duration (Troiano et al. 2013).

Although considerable studies have been devoted to biocleaning strategies, there is still some work to do to obtain ready-to-use products for the biocleaning of organic and inorganic residues from works of art. In order to introduce a ready-to-use biocleaning product to the restoration market, two main basic aspects must be taken into account: the microorganism itself and the delivery system to be used.

Microorganism biodiversity includes Bacteria, Archaea and Eukaria, which can live in every habitat of the biosphere (soil, rocks, hot springs, oceans, etc.), since they are extremely adaptable to environmental conditions. Microorganisms are extraordinarily diverse in their requirements for growth, and their growth is greatly affected by the nutrients that are available in environment. However, they have common living requirements: energy (from light or from organic or inorganic compounds), macronutrients (carbon, nitrogen, hydrogen, oxygen,etc.), trace elements (Co, Zn, Cu, Mn, etc.), and water. A careful selection of the appropriate microorganisms that perform well in the removal of the desired substances (nitrates, sulfates, and organic matter) is one of the first steps to be made in the biorestoration strategies. Microorganisms can be isolated from the environment, like from the soil, that is one of the most abundant sources of microorganisms with an estimation up to 4×10^6 different taxa in a ton of soil (Curtis et al. 2002) ad These micoorganism display a wide diversity in enzymatic activities (lipases, proteases, oxido-reductases, etc.) as described by metagenomic studies (Neelakanta and Sultana 2013).

In order to use microorganisms for the CH biorestoration, the viability and efficiency of the selected microorganisms must be guaranteed, and it must be verified that they do not cause deterioration to the CH surfaces to be cleaned. Therefore, an appropriate "delivery system" for the application of microorganisms, providing them an adequate microenvironment to optimise their activity, is essential. The delivery system is, therefore, one of the most important aspects of biocleaning technology. To guarantee the best conditions, the ideal delivery system should have the following characteristics:

i) be able to retain the microorganisms and provide them the right conditions (aerobic or anaerobic) and the water that they need in order to remove the cause of decay, but without any damage to the art work itself and any undesirable changes in the color of the surface;

ii) be applicable to all types of surfaces (horizontal, vertical, oblique, rough, smooth, etc.).

and iii) be quick and easy to prepare, but also easy to apply and to eliminate at the end of the treatment, and using as far as possible a few cheap materials.

Among the recent studies carried out on CH biocleaning over the last few decades (see Table 1), eight delivery systems

have been utilized and reported: immersion (Gauri et al. 1989 and 1992; Heselmeyer et al. 1991), sepiolite (Ranalli et al. 1996a, 1996b, 1997, 2000; Cappitelli et al. 2006), hydrobiogel-97 (Ranalli et al. 2000; Cappitelli et al. 2006), cotton wool (Ranalli et al. 2005; Antonioli et al. 2005; Bosch-Roig et al. 2010), carbogel (Cappitelli et al. 2005, 2006, 2007; May et al. 2008; Polo et al. 2010; Alfano et al. 2011), mortar and alginate beads (May et al. 2008), agar (Bosch-Roig et al. 2012, 2013a, b; Barbabietola et al. 2012), and arbocel (Troiano et al. 2013). Table 1 reports a summary of the delivery systems used in the biocleaning (on stone surfaces and frescoes) literature, the decay agents, the used microorganisms, their type of metabolism, the application times, and the removal efficiency evaluation. The first study performed in this field used the immersion technique. Gauri et al. and Heselmeyer et al., immersed marble statues and sandstone blocks affected by sulfur dioxide crusts in liquid containing *Desulfovibrio desulfuricans*, under laboratory conditions, for about 60 h (Gauri et al. 1989 and 1992; Heselmeyer et al. 1991). After these studies, it has been recognized that the immersion technique in liquid culture can have many limitations for large, fragile artworks and that a portable application delivery system is needed to provide a suitable biocleaning strategy. Therefore, subsequent research strategies were based on the use of delivery systems (contact compresses), and new techniques were developed.

However, with the existing diversity in delivery systems found in the literature, often it remains unclear which one should be selected. Therefore, this critical review aims to describe and compare the main characteristics and properties of the delivery systems adopted until now in order to help conservator-restorers and researchers to choose the most appropriate system according to specific biocleaning application requirements.

Immersion

The immersion system consists of introducing the works of art into a liquid culture with the appropriate microorganism for a determined period of time (Gauri et al. 1989, 1992; Heselmeyer et al. 1991). The immersion system has been shown to have three main drawbacks: firstly, this kind of treatment cannot be applied to large objects, such as buildings, as it necessitates the complete immersion of the object in a recipient with a liquid medium; secondly, consolidation of the artwork prior to the treatment becomes obligatory in many stone types to prevent severe damages due to the immersion; and, thirdly, treatment efficiency has not been fully proved since gypsum removal was only evaluated by visual observation and not by a careful chemical analysis (Gauri et al. 1989, 1992; Heselmeyer et al. 1991). The type of damage produced to the work of art after immersion treatment and during the drying of the artwork can be due to salt migration that can produce efflorescence on the surface; the presence of diverse materials on the work of art can lead to water retention variation and unequal material expansion, causing exfoliation, fissures or fractures, for example, to the internal structure of the work of art. If the artwork is made up from different pieces linked together by metallic elements, these elements could be oxidized by the immersion and could produce fractures in the work of art itself. The laboratory works from De Belie et al., and De Graef et al., performed in 2005 compared the immersion and the sprinkling strategies to bioclean concrete (blast furnace slag cement and ordinary Portland cement) fouled by lichens using a mixture of bacteria of the genus *Thiobacillus* sp. with an appropriate nutrient broth for nine days. This study showed that the sprinkling treatments had about 50 % of the effectiveness of the immersion treatments. But both treatments had the drawback of the formation of a white gypsum layer on some of the cement specimens (De Graef et al. 2005; De Belie et al. 2005).

Sepiolite

The sepiolite mineral matrix is a clay mineral, which is a complex magnesium silicate that increases the total useful water fraction of a substrate, making the implementation of active liquids easier by transforming them into semisolids, permitting the gradual release of active ingredients.

Sepiolite is normally used in oil drilling, for cat litter, and in a solid form for carving. It is also used, due to its high water retention capacity, in construction lime mortars and in agriculture. It is used to control water and fertilizer loss in sandy soils.

Sepiolite was introduced for biocleaning strategies in 1996. Good results were obtained for the removal of nitrates using *Pseudomonas stutzeri* on brickwords and calcareous stones (marble, Vicenza-stone, etc.) in laboratory conditions (Ranalli et al. 1996b); sulfates were removed from an old marble sculpture and an old marble column using *Desulfovibrio* sp. under anaerobic conditions (Ranalli et al. 1996a, 1997); and black crusts on stone and marble materials were efficiently removed after 30 h to 72 h (Cappitelli et al 2006).

Its preparation for application for biocleaning consists of mixing the sepiolite powder (50-70 %) with a suspension of microorganisms and water under anaerobic conditions, allowing the microorganisms (bacteria) to colonize the sepiolite for 10 - 14 days until it is ready to be applied over Japanese paper to the work of art surface to be treated (Cappitelli et al. 2006) (Fig. 1). The Japanese paper is used to facilitate the removal of the delivery system and to reduce its penetration into the pores and cavities of the original surface reducing the possibility of residues on the cleaned artwork. Despite the high efficiency of the biocleaning treatments using sepiolite

t1.1 **Table 1** Summary of the delivery systems described in the stone surfaces and frescoes biocleaning literature

t1.2	Delivery system	Decay agents	Type of materials	Used microorganisms	Type of metabolism	Time (h)	Removal efficiency evaluation (%)	References
t1.3	Immersion	Sulphates Lichens	Marble (Georgia) stone and statue (U.S.)	D. desulfuricans	Anaerobic Anaerobic Aerobic	60–84	80	Gauri et al. (1989), 1992)
t1.4			Marble and sandstone, (D) Concrete, (B)	D. vulgaris Thiobacillus sp.		ns [a] 9 days	100, 40 ns	Heselmeyer et al. (1991)
t1.5								De Graef et al. (2005) De Belie et al. (2005)
t1.6	Sepiolite	Sulphates	Marble, (I)	D. desulfuricans, D. vulgaris	Anaerobic	36	81	Ranalli et al. (1996a), 1997)
t1.7		Black crusts	Marble (Candoglia stone), (I)	D. vulgaris subsp. vulgaris	Anaerobic	45	98	Cappitelli et al. (2006);
t1.8		Nitrates	Brick-works and calcareous stones (I) (marble and Vicenza-stones)	P. stutzeri	Anerobic	30	ns	Ranalli et al. (1996b)
t1.9	Hydrobiogel-97	Black crusts	Marble (Candoglia stone), (I)	*D. vulgaris* subsp. *vulgaris*	Anaerobic	7 days	28	Cappitelli et al. (2006);
t1.10								Ranalli et al. (2000)
t1.11	Cotton wool	Animal glue	Frescoes, Pisa (I)	*P. stutzeri* A29+Protease	Aerobic	2–12	80–100	Ranalli et al. (2005);
t1.12								Antonioli et al. (2005)
t1.13								Lustrato et al. (2012)
t1.14		Animal glue and salt efflorescence	Frescoes, Valencia (E)	*P. stutzeri*	Aerobic	1.5–3	60	Bosch-Roig et al. (2010)
t1.15	Carbogel	black crusts	Marble (Candoglia stone) Milan (I)	*D. vulgaris* subsp. *vulgaris*	Anaerobic	45	98	Cappitelli et al. (2006), 2007)
t1.16			Marble sculpture, Milan, (I)			12	ns	Cappitelli et al. (2005)
t1.17			Limestone sculpture, Trento (I) Coloured lithotypes*, Firenze (I)			36 30–40	ns ns	Polo et al. (2010)
t1.18								Gioventù et al. (2011)
t1.19		Nitrates and sulphates	Sandstone walls, Matera (I)	P. pseudoalcaligenes, D. vulgaris	Aerobic/Anaerobic	24–72	55, 85	Alfano et al. (2011)
t1.20								May et al. (2008)
t1.21	Mortar and alginate beads	Nitrates	Sandstone walls, Matera (I)	P. pseudoalcaligenes	Aerobic/Anaerobic	1 month	ns	May et al. (2008)
t1.22	Agar	Animal glue and salt efflorescence	Frescoes, Valencia (E)	P. stutzeri	Aerobic	1.5–2	92	Bosch-Roig et al. (2012), 2013a, b)
t1.23	Arbocel	Grey deposits and black crust	Marble column and statue, Cemetery of Milan (I)	*D. vulgaris* subsp. *vulgaris*	Anaerobic	68–110	ns	Troiano et al. (2013)

[a] (ns: not specified) * Green serpentine, red marlstone, and white Carrara marble

and the advantage represented by the high level of sepiolite specific surface (cm^2/cm^3), it has also the ability to offer the anaerobic conditions required during treatment by the microorganisms like *Desulfovibrio* sp. involved in the process (Ranalli et al. 1997). Nevertheless, there are some notable disadvantages when using it (Ranalli et al. 1996a, 1996b, 1997, 2000). The drawbacks in the use of sepiolite for biocleaning are: firstly, that it requires a long time (about two weeks) for the bacterial colonization before application, secondly, there can be a rapid loss of water after application that can lead to bacterial inactivity and, thirdly, there is a risk of hydrogen sulfide reaction with the iron in the sepiolite forming iron sulfide precipitates (Cappitelli et al. 2006).

Hydrobiogel-97

The hydrobiogel-97 delivery system is a polymer formed by an acrylic resin hydrogel (Ranalli et al. 2000; Cappitelli et al. 2006). This biogel displayed optimal hydration during a 9-day biocleaning treatment of stone material to remove black crusts. There were no chromatic changes and no physical or chemical modifications of the composition of the stone (Ranalli et al. 2000). Preparation for biocleaning system consists of mixing for ten min at room temperature by mechanical agitation of the two physic-chemical liquid components of the hydrobiogel-97. One of the components is the main agent, and the second is a catalyst that allows for obtaining the final gel (the composition of the gel is confidential, know-how of EniTecnologie, San Donato Milanese, Italy). Then, just before use, the suspension of microorganisms in water solution is added, and the biocleaning agent is applied over Japanese paper onto the work of art to be treated (Fig. 1) (Cappitelli et al. 2006). The disadvantages of this delivery system are: the difficulty in eliminating it after the treatment due to the intrinsic aspects of its high adhesiveness, and excessive fluidity that complicates its application to nonhorizontal surfaces.

Cotton wool

Cotton wool is a cellulose material obtained from the *Gossypium* sp. seeds with a characteristic microstructure that makes it very resistant, malleable, soft, and very absorbent. Cotton wool has been used in biocleaning since 2000. It was first used, with *Pseudomonas stutzeri* A29 strain and applied for 2 - 12 h, to efficiently bioclean (80-100 % animal glue removal) a fresco *Conversione di S. Efisio e battaglia* (The conversion of St. Efisio and the battle), which had been detached from the walls of the Camposanto Monumentale, Pisa, Italy (Ranalli et al. 2005; Antonioli et al. 2005). Interesting results were also obtained with cotton wool as a delivery system and *P. stutzeri* to clean nitrate salt efflorescence and animal glue from frescoes on the Santos Juanes church in Valencia (Bosch-Roig et al. 2010).

For the use of this delivery system in a biocleaning process, the live bacterial cell suspension at a high cell density ($5x10^8$CFU/ml) must be brushed on to the work of art and then entirely covered with a thin layer of hydrophilic, sterile, white cotton wool impregnated with the same bacterial cell suspension (Fig. 1). Although good biocleaning results were obtained, this delivery system has some drawbacks. It is not recommended for use on vertical surfaces because, due to the effect of gravity on the water and bacteria suspension within the cotton, there can be seepage through the lower part, leading to water loss through leakage and heterogeneous biocleaned areas (with a more intense degree of cleaning on the lower part). This is due to the high water retention and water release of the cotton wool, and long treatments (periods of longer than 15 - 17 h) can produce some risk of damage to the fresco, such as the swelling and detachment of paint fragments (Bosch-Roig et al. 2010, 2013b).

Carbogel

Carbogel is composed of a neutralized polyacrylic acid, which permits the gel to be prepared simply by adding water. Viscosity can be varied as desired. It has been used in restoration for the cleaning of wall paintings (Borgioli et al. 2001). Several biocleaning treatments using carbogel as a delivery system have shown that it can be used successfully to clean black crusts and calcite and gypsum deposits (from Matera Cathedral, Milan Cathedral, Florence Cathedral, from sculptures like the Rondanini Pietà base by Michelangelo Buonarroti in Sforzesco Castle in Milan, and from sculptures in the Courtyard of Buonconsiglio Castle in Trento) (Cappitelli et al. 2005, 2006, 2007; May et al. 2008; Polo et al. 2010; Alfano et al. 2011; Gioventù et al. 2011). It has also been successfully used to remove nitrates from stonework (at Matera Cathedral) (May et al. 2008; Alfano et al. 2011).

Carbogel preparation for biocleaning consists of mechanical mixing, preferably using an electrical device (automatic mixer) adding the carbogel powder (0.5-4 %) with a suspension of microorganisms (in water/P-buffer/DNT medium) at room temperature. The biocleaning agent obtained is then applied over a Japanese paper to the artwork to be treated. When a vertical surface has to be biocleaned with carbogel, the multilayer biosystem should be applied. This system consists of a Japanese paper in contact with the wall, a 0.8-1.9 cm thick layer of carbogel powder mixed with a bacterial suspension, a plastic reticulated net (PET with 25 empty space/cm^2) to facilitate adhesion and the distribution of the carbogel over the surface area, and, lastly, a plastic film to reduce undesired water evaporation (see Fig. 1) (Alfano et al. 2011). To avoid carbogel handling difficulties, some researchers added

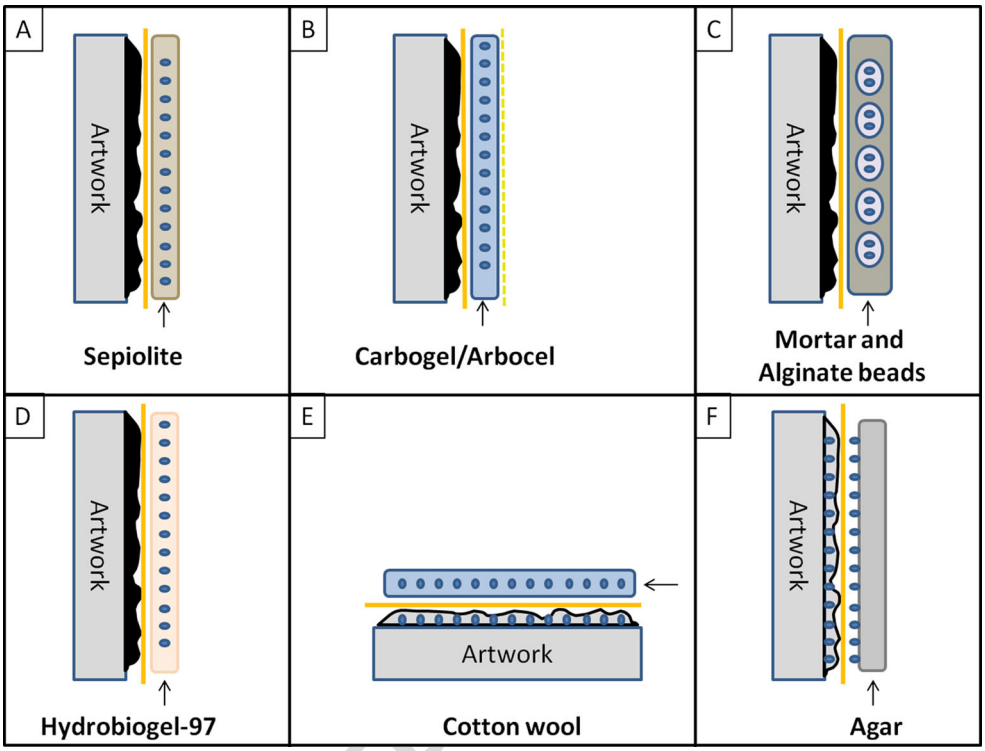

Fig. 1 Delivery systems for stone surface and fresco decay recovery. **a**: Sepiolite; **b**: Carbogel/arbocel; **c**: Mortar and alginate beads; **d**: Hydrobiogel-97; **e**: Cotton wool; **f**: Agar. Figure notes: delivery systems are indicated with a black arrow; alterations are indicated with a black area over the artwork; bacteria are indicated as blue spheres/~~circles;~~ Japanese paper is indicated as a yellow line; and plastic net is indicated with yellow discontinuous line

micronized silica to the carbogel, creating a more homogeneous, compact delivery system, which is, therefore, easier to handle and contributes to more anaerobic conditions when adopted to remove black crusts with *Desulfovibrio vulgaris* (Sorlini et al. 2010b).

The advantages of the carbogel biocleaning delivery system are that it has a high water retention capacity with consequent evaporation over longer periods, that no color change appeared on the stone, and no visual residue was found through SEM (Scanning Electron Microscopy) analyses after treatment. The drawbacks of this delivery system are that it can liquefy with high salt content making its handling very difficult, and it induced a few changes in the structure that can increase porosity and water uptake of the treated materials On long-term treatments it dries out, so it can only be used for brief applications (May et al. 2008); it has limited adhesive properties and; therefore, it can be difficult to use for cleaning vertical and hydrophobic surfaces, ceiling and vaults. Finally, carbogel, given its less compact structure, has been shown to be slightly difficult to handle (Beltrami et al. 2012).

Mortar and alginate beads

This delivery system is made up of a base of mortar that is mixed with alginate beads containing the selected microorganisms. Its preparation consists of mixing 100 ml gel (containing 90 ml mineral medium nutrient solution, 10 ml bacteria-tween-solution containing 10^6 cells/ml, and 3 % alginate beads) with 700 g of mortar. The alginate beads are formed as follows: mix the solution for 10 min on a magnetic stirrer, pick up the gel mix in a syringe and drip the gel mix into a 2 % calcium chloride solution, leave the alginate beads for 10 min in the solution, and, finally, wash them in purified water. The mortar is formed by 205 g of CEM III/C Portland cement, 301 g of standard sand (0 - 2 mm particle size), 91 g of pumice (0.3 - 1.5 mm particle size), 103 ml H_2O, and 0.5 g of air entraining agent.

This delivery system was used by May et al. (2008) in the European BIOBRUSH project (Bioremediation for building Restoration of the Urban Stone Heritage in European States, no. EVK4-2001-00055) to clean the external walls of Matera Cathedral in Italy.

The mortar gel system used for bioremediation of monuments consists of either applying it directly, or over Japanese paper, to the work of art surface to be treated (see Fig. 1). This delivery system has been shown not to be suitable for biocleaning strategies. This is due to the fact that the powerful adhesive properties of the mortar make its removal after treatment very problematic. Mechanical strategies have to be used, producing damage to the original matrix (May et al. 2008). Mortar performance is also reduced due to its long preparation procedure (May et al. 2008). May et al. (2008) in comparative studies between the carbogel and the mortar and alginate bead delivery systems showed faster capabilities of the carbogel over mortar in removing nitrates at Matera.

Agar

Agar is a complex polysaccharide extracted from a group of red purple algae of Rhodophyceae, usually from the *Gelidium* and *Gracilaria* genera. It is composed of two types of polysaccharides, agarose and agaropectine. It is water soluble, neutral, nontoxic, and able to produce gels with no liquid water release (Praiboon et al. 2006; Gulotta et al. 2014). It is commonly used in the food industry as a jelling and thickening agent; in microbiology and botanic fields as a support for the growth of certain organisms (Hesse and Hesse 1992); in pharmaceuticals for drug delivery (Santoro et al. 2011); and in chemical field as an electrode binder for electrolyte cells (An et al. 2013). For a few years, agar has also been used in restoration, for the cleaning of different types of works of art (mural painting, wood, stone, plaster, paper, and textiles), for its ability to control and limit water release onto artistic materials, and for its respect for the substrate (Iannucelli and Sotgiu 2009; Gorel 2010; Sansonetti et al. 2012; Casoli et al. 2013; Nualart-Torroja et al. 2013; Shaeffer and Gardiner 2013; Baglioni et al. 2014; Gulotta et al. 2014). Recent studies evidenced that after agar applications on porous art works no residue remains inside the porous materials (measured by FTIR spectroscopy and mass spectroscopy) (Cremonesi 2013; Tortajada Hernando and Blanco Domínguez 2013). Agar gel has also the advantage that it retains water-soluble dissolved substances like a sponge, reducing post treatment cleaning of the surface, and suggesting its ability to be used not only to clean surfaces but also to extract salts (Anzani et al. 2008; Cremonesi 2013; Gulotta et al. 2014).

Recently agar 2 % has been used as biocleaning delivery system, performing effective removal of nitrate salt efflorescence and animal glue residues during in situ treatments, with *Pseudomonas stutzeri*, on the frescoes of the central vault of the Santos Juanes Church in Valencia, Spain (Bosch-Bosch-Roig et al. 2012, 2013a, b). Animal glues have been also successfully removed from paper materials after 4 h of biocleaning treatment with *Ochrobacterium* sp. bacteria immobilized in agar 1 % (Barbabietola et al. 2012). The preparation protocol of this delivery system consists of diluting the agar powder in distilled water to a final 1–2 % concentration, then heated to above 85 °C or autoclaved and placed under sterile conditions in plastic molds of the desired size producing agar layers. Once the solution is cooled, the polymer molecules assemble forming a thermoreversible rigid gel (Medina-Esquivel et al. 2008; Cremonesi 2013). The agar microstructure has a high number of homogeneous size pores enhancing its water retention (Pernodet et al. 1997). Some authors advised against the use of 4 % agar over 15 min and to avoid leaving the agar completely dry (at any concentration) (Tortajada Hernando and Blanco Domínguez 2013; Gulotta et al. 2014). The protocol consists of an initial application of Japanese paper to protect the altered paint surface; the bacterial suspensions are then applied with a sterile brush both onto the Japanese paper and onto one side of the agar surface and, finally, the side of the agar with the bacteria is placed onto the surface of the work of art (see Fig. 1). After treatment, the agar and Japanese paper have to be removed and the treated surface cleaned using sterile water and a sponge to avoid undesirable bacterial metabolic processes and damage to the original material. The painted surface must then be dried (Bosch-Roig et al. 2013b. Recent applications show that agar has good adhesive properties (it can be applied onto horizontal, vertical, and oblique surfaces); it gives a significant level of water retention and bacteria retention and produces homogeneous cleaning without leaving stain marks or residues on the surface (Bosch-Roig et al. 2013a). Another advantage of agar is that it is semitransparent, due to its lack of inherent color, which facilitates the control of the biocleaning process (Iannuccelli and Sotgiu. 2010; Cremonesi 2013; Gulotta et al. 2014. In using agar the fact that capillary forces tend to draw material from the substrate into the gel, may also be of interest (Wolbers 2000).

The rigid agar delivery system has shown two drawbacks, poor adhesion when used for the biocleaning of rough surfaces and the difficulty in finding plastic molds in which to prepare agar of the desired shape when the artwork shapes are complex. However, recent studies in restoration have solved these problems by using semisolid agar that consists of applying the warm gelling agar (40 °C–45 °C) just before it becomes cold and solid, thus, allowing it to cover rough and irregular surfaces and objects with complex shapes by brushing without damaging the surface nor releasing residues (Anzani et al. 2008; Cremonesi 2013; Tortajada Hernando and Blanco Domínguez 2013; Gulotta et al. 2014).

Arbocel

Arbocel is a natural cellulose fiber with a wide range of uses including: complementary food for animals (for regulation and support of the function of the gastrointestinal tract), paper and board production in the pharmaceutical industry (used as an economic, inert additive in tablets and capsule filling, improving tablet hardness, and disintegration time), in the production of modern detergents, for the coating of flower and vegetable seeds, in chemical construction products, and so on. In art restoration and conservation, it is used as a support for compresses for the cleaning of natural and artificial stone materials, frescoes, plaster, and stucco. It can also be used as inert filler for the preparation of mortars and plasters.

Arbocel preparation for biocleaning consists of mixing the arbocel powder (CTS, Vicenza, Italy) with a biomass suspension (in water/P-buffer/DNT medium) until obtaining a homogeneous mixture with the desired density. The system is applied to the work of art over Japanese paper previously

moistened with a phosphate buffer. When working with sulfate reduction bacterium like *D. vulgaris*, all the manipulations should be done under anaerobic conditions in an anaerobic box for a guarantee of the opportune anoxic-anaerobic metabolism (Troiano et al. 2013). The biological cleaning system is then covered with a plastic film to reduce undesired evaporation of water (see Fig. 1) (Troiano et al. 2013). This procedure is based on the one previously reported for carbogel (Alfano et al. 2011). The arbocel delivery system with *Desulfovibrio vulgaris* subsp. *vulgaris* entrapped biomass has shown good results for the biocleaning of black crusts present on stone artwork (columns and marble statues) (Troiano et al. 2013), but it has not yet been used for cleaning frescoes. Due to its similarities in composition to cotton wool, it is probable that the drawbacks of this delivery system will be similar to those described for cotton wool. Further studies should be performed with this delivery system to confirm its suitability for the biocleaning of polychromic works of art, such as frescoes.

Critical comparison of delivery systems main characteristics

A discussion and comparison of the main characteristics of the various delivery systems used for biocleaning of stone CH and frescoes is reported according to all published works (Fig. 2). The delivery system to use for restoration purposes must, therefore, be chosen depending on the water retention, water release, bacterial compatibility, adhesion capabilities, risks including CH undesired color changes, performance characteristics and difficulties, economical sustainability, and, of course, the state of conservation of the artwork to be cleaned.

In Table 2 the relationship between the main decay agents, the suggested biocleaning microorganisms, and the delivery systems benefits and drawbacks are shown. The benefits (including water retention and bacterial compatibility) and drawbacks (including adhesion capabilities, risks, and performance difficulties) are indicated in detail for each delivery system. To facilitate an easy reading and a correct understanding for conservator-restorers, on the basis of our past experience and of the data present on the literature, the following notes both for benefits and drawbacks have been attributed: absence; low; high; very high; and not applicable. Finally, an economical evaluation shows the costs of the delivery system alone (€/kg) and the total costs (€/m^2) including full biocleaning systems (microorganisms and delivery systems).

An ideal delivery system must have high water retention (water is necessary for the microorganism) while water release should be relatively low, in order not to damage the works of art themselves (salt migration, exfoliation, fissures, fractures, oxidation, etc.). The fact that the water release and water retention is related to the ratio raw powder/water must be taken into account when using cleaning gels. Gels, for example made with a high content of agar (4–6 % w/v), have a much slower water release and a lower water retention compared to gels made with lower agar content (1–3 % w/v) (Wolbers 2000; Iannuccelli and Sotgiu 2010). When 3 % semisolid agar gel is used for 1 h in the biocleaning of Candoglia marble sculpted stone, the contact of the water liquid and the treated surface is more limited compared with the use of sepiolite and cellulose pulp based delivery systems (Beltrami et al. 2011; Gulotta et al. 2014). These results are in accordance with a study showing that a thin layer of cotton wool soaked in water and applied to gypsum models for only 3 min show water penetration of up to 5 - 6 mm in depth, while agar water penetration varies from 1 to 4 mm depending on application times (3–20 min) and the percentage of agar (2–4 %) (Anzani et al. 2008). Given its water-related characteristics, cotton wool in contact with nonhorizontal porous surfaces or in the presence of surface alterations (and more probably also arbocel due to its cellulosic nature) can produce water marks on the treated areas (Bosch-Roig et al. 2010, 2013b). This undesired effect is reduced or entirely absent when using the other delivery systems tested (Bosch-Roig et al. 2013b).

The compatibility between the delivery system and the microorganism is another characteristic of the delivery system, which is vital for an efficient biocleaning process, because incorrect bacterial compatibility will lead to poor or inappropriate cleaning. All the delivery systems reported have shown high or very high bacterial compatibility. Sepiolite displays the disadvantage, among others, that the living cells have to colonize the delivery systems, and; therefore, additional time consumption is required before application (Cappitelli et al. 2006; Bosch-Roig et al. 2013b).

Delivery system adhesion capabilities are very important when cleaning vertical or oblique surfaces, but very high adhesion can be negative or problematic. The ideal delivery system adhesion capabilities must be enough to guarantee the effectiveness of the biocleaning treatment (limited adhesion can reduce biocleaning efficacy), and assure the complete removal of the delivery system after treatment with the absence of the original material's modification. Excessive adhesion can make the removal of the delivery system very complicated (as with the mortar system) and may cause decay to the works of art or make impossible the complete removal of the delivery system from the surface. Cotton wool's low adhesion capabilities cannot guarantee long treatments on vertical surfaces. Various strategies may be adopted to improve the limited adhesive capabilities of some delivery systems. Jelling polyacrylic acid materials like carbopol have recently been introduced for restoration cleaning strategies after their use in the cosmetics industry in last few decades (Beltrami et al. 2012; Baglioni et al. 2014). This interesting material seems to avoid the adhesive drawbacks of carbogel

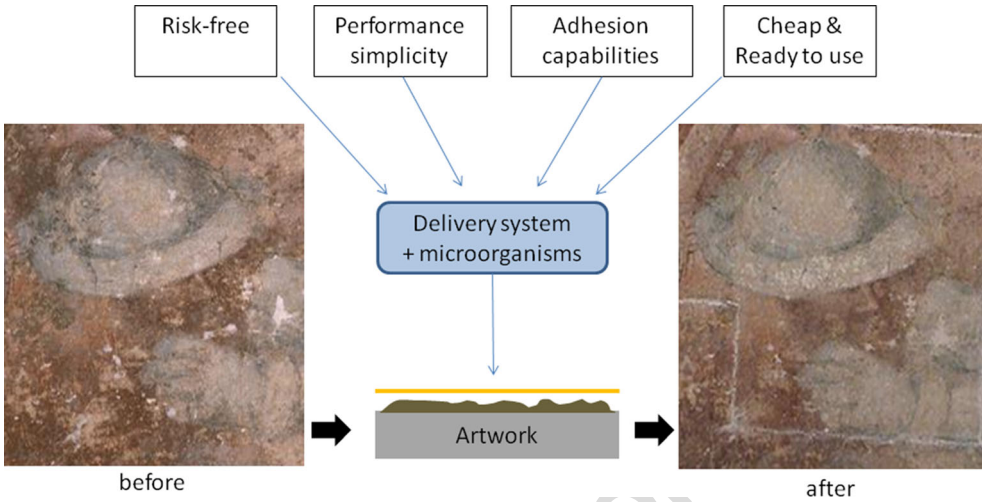

Fig. 2 Main characteristics of the most commonly used delivery systems as key components in biocleaning strategies of Cultural Heritage artwork adopted as a useful tool for researchers and conservator-restorers. Pictures show the effects of the biocleaning process on a fragment of the Conversione di S. Efisio e battaglia, fresco (XIV century) at Pisa Camposanto Monumentale, (Italy)

(low adhesion capabilities), and may, therefore, also be studied for biocleaning strategies. Generally the agar adhesion capabilities are high but when applied to rough surfaces adhesion can be low. To avoid these adhesion problems of solid agar, studies in restoration have solved this problem by using it as semisolid agar. Various restoration researches performed in 2013 (Tortajada Hernando and Blanco Domínguez 2013) have also shown the suitability of semisolid agar for cleaning fragile plaster materials (partially soluble in water) by using low concentrations of agar, short treatment times (2–30 min), and very thick layers of agar gel (to facilitate the removal). Several semisolid agar treatments did not show any removal of the original material, any changes in the surface roughness, and any residues of pores after the treatments and a constant vapor permeability (Tortajada Hernando and Blanco Domínguez 2013; Gulotta et al. 2014). This new application method using melted agar has been successfully used in restoration but has not yet been tested for biocleaning. Further studies must be performed to determine the effect of this type of application on the biocleaning process paying special attention to the temperature of the agar when applied to avoid bacterial death and any damage to the work of art. It should be also take into account the fact that the use of melted agar implies its preparation in situ. This is a fact that could be a drawback when restorations take place outside the laboratory and, for example, inside a church.

Among the different drawbacks, important factors to consider are the risks to CH works themselves (including undesired color changes and excessive water release), to conservator-restorers, and to the environment. When working with artwork materials, no unwanted color changes must be caused by the biocleaning treatment. Unfortunately, the liquid culture used in the immersion technique can dilute and dye artwork material. When using sepiolite, there is a risk of the presence of iron ions leading to discoloration. This disadvantage for sepiolite can be avoided by a pretreatment that eliminates the iron ions present (Ranalli et al. 1996a, 1996b). An absence of color changes is reported for the other delivery systems (Ranalli et al. 2005; Cappitelli et al. 2006; May et al. 2008; Alfano et al. 2011; Bosch-Roig et al. 2013a; Troiano et al. 2013). Previous studies have demonstrated that, among the techniques used for biocleaning, the immersion technique and the mortar system are the delivery systems with the highest risks for CH works, especially when preconsolidation treatments are not applied (Gauri et al. 1989, 1992; May et al. 2008). As discussed before, sepiolite that has not been pretreated has a risk of color change and cotton wool has a risk of leaving water marks when applied to nonhorizontal surfaces. Until now, when microorganisms associated with a delivery system have been used, no risks for the operators or for the environment have been reported for any biocleaning technologies. This is due to the fact that, on the one hand, the microorganisms used are adequately isolated from the environment (non-GMO) and are always nonpathogenic; and on the other hand, the cleaning strategies are nontoxic both for the environment and for conservator-restorers as they are based on water avoiding the use of toxic organic solvents (Wolbers 2000; Pietropolli 2001; Cremonesi 2004; Lustrato et al. 2012; Bosch-Roig et al. 2013a, 2013b.

Furthermore, it is important to take into account the performance characteristics and time consuming of each delivery system including preparation, application, elimination, and material needs. Cappitelli et al. (2006) reported a comparative study on sepiolite, carbogel, and hydrobiogel-97 for cleaning black crusts from stone materials showing that carbogel was better than sepiolite and hydrobiogel-97 because carbogel was the easiest to apply and remove, while maintaining also the best bacterial activity. But carbogel, due to its less compact structure, has been shown high performance difficulties because it is difficult to handle compared to agar and cotton wool, and it easily fragments leaving more residues on the artwork surface. However, arbocel, agar, and cotton have been

t2.1 **Table 2** Benefits drawbacks and economical evaluation of the used biocleaning delivery systems for Cultural Heritage stone surfaces and frescoes

t2.2 t2.3	Main decay agents and used biocleaning bacteria	Delivery system	Benefits		Drawbacks			Economical evaluation	
			Water retention	Bacterial compatibility	Adhesion capabilities	Risks*	Performance difficulties**	Delivery system costs*** €/Kg	Total cost**** €/m^2
t2.4	Black crusts, nitrates, sulphates; *D. vulgarisD. desulfuricans*	Immersion	Very high	Very high	na	Very high	Very high	100–300	300–800[#]
t2.5	*P. pseudoalcaligenes*	Sepiolite	Low	High	High	High	Very high	30–100	70–80
t2.6		Hydrobiogel-97	high	Very high	High	Absence	Very high	200–250	80–90
t2.7		Arbocel	Low	Very high	High	Absence	Low	10–50	70–80
t2.8		Carbogel	High	Very high	Low	Absence	High	30–50	70–80
t2.9		Mortar and alginate beads	Low	Very high	Very high	Very high	Very high	400–500	90–100
t2.10	Salt efflorescence, organic matters; *P. stutzeri*	Agar	Very high	Very high	High-low	Absence	Low	50–300	10–40
t2.11		Cotton wool	Low	Very high	Low	Low	Low	10–20	40–50

Legend: The evaluation criteria: absence; low; high; very high; na (not applicable) are related to benefits (water retention and bacterial compatibility) and drawbacks (adhesion capabilities, risks, and performance difficulties) of the delivery systems

* Risks refers to the hazard to art works (including undesired color changes and excessive water release)

** Performance difficulties include time consumption and handling complexity related to: material needs, preparation, application and elimination of each delivery system

*** Delivery system costs are referred only to the materials (do not include bacterial cells)

**** Average total costs are calculated including delivery systems and microorganisms according to the application modality reported on in Fig. 1 and Table 1; microorganisms costs are calculated assuming the use of bacterial cells (i.e., *D. vulgaris* and *P. stutzeri*) in relation to the delivery systems reported on the second column of this Table 2

[#] : Average total costs in €/m^3

shown to be very simple to apply due to their consistent and compact structure, therefore showing, low performance difficulties.

On the basis of the benefits and drawbacks evaluation criteria selected and given the notes previously described, we can do a classification to help conservator-restorers. In particular, the best evaluated delivery systems are: arbocel, carbogel, cotton wool, and agar. Among them, all have good evaluation on all the criteria except for low water retention of arbocel; carbogel has low adhesion capabilities and high performance difficulties; cotton wool has low water retention and low adhesion capabilities on vertical surfaces; and, finally, agar has low adhesion capabilities on rough surfaces. Immersion and mortar delivery systems must be avoided due to their high potential risk for damage to the CH works.

The economic aspects of the delivery systems are also very important, but few studies have been done. Ranalli et al. (2005) reported a cost study comparing the costs of cleaning 1 m^2 fresco surface with biological cleaning using viable bacterial cells (with cotton wool as a delivery system) and using enzymes. The costs of the biological cleaning using viable bacterial cells, comparing a *P. stutzeri* bacterial culture, protease enzyme, and a collagenase enzyme were determined. This analysis of the biocleaning costs showed a cost ratio of 1:3:10, respectively, demonstrating, therefore, that the use of bacterial cultures instead of enzymes was significantly cheaper (Ranalli et al. 2005). Lustrato et al. (2012) did another economic analysis, determining that, under real conditions and using cotton wool as a delivery system, the bacterial suspension (*P. stutzeri*) required to clean 1 m^2 of fresco surface, cost less than 200 euros (including the personal costs of the bioapplication). Recent studies have shown that, when using agar as a delivery system, a ten times smaller volume cell suspension is required per unit of wall painting area, compared to the cell volume needed for a cotton wool delivery system (Bosch-Roig et al. 2013a, 2013b). However, the higher cost of high purity agar, which can be even ten times more expensive than cotton wool, must be also considered. Another interesting study shows a cost evaluation of the use of bacteria for consolidation of stone CH with prices between 23-40€/m^2 considering the product and the application costs (De Muynck et al. 2010). An overview of the costs related to the different biocleaning systems has been summarized in Table 2 according to all the existing data. The costs show a wide range of prices for each delivery system due to the diverse market price. The total costs of the biocleaning treatments comprehend the price of the delivery system including the microorganisms' biomass, according to the application modality showed in Fig. 1 and the bacteria selection reported in the literature (see Table 1). Two microorganisms have been taking into account: *D. vulgaris* (cell suspension of 5x10^9 CFU/ml and 60 €/m^2, price of Micro4you) and *P. stutzeri* (cell suspension of 5x10^8 CFU/ml and 3-30 €/m^2 for agar and cotton wool, respectively). The microorganism *D. vulgaris* has been considered to calculate the prices of all delivery systems except for agar and cotton wool where *P. stutzeri* are adopted. The price of biocleaning systems based on *D. vulgaris* is affected by its intrinsic slow anaerobic metabolism compared to the price of the systems based on *P. stutzeri* (whose prevalently aerobic metabolisms permit an easy and fast growth determining a cost reduction). In addition, the application modality affects the biocleaning system total price because the quantity of needed microorganisms can vary substantially (as is the case of the agar which needs ten times less microorganisms in suspensions than does cotton wool). The final price reported in Table 2 doesn't consider the bioapplication time consumption and personal costs because they change depending on the type of artwork and country.

In comparing all the delivery system costs, the lower in cost are arbocel, carbogel, and cotton wool, and the lowest total cost is for agar, followed by cotton wool. The average of the total costs is under 100€, except by immersion whose price is ten times higher than the others.

To conclude, on the basis of the selected criteria and in order to help conservator-restorers to choose the appropriate system according to specific biocleaning application requirements, a useful classification is given: a) when black crust, nitrate, and sulfate decay agents must be bioremoved, *D. vulgaris* should be used, and the suggested delivery systems are arbocel and carbogel; b) when salt efflorescence, and organic matter decay agents have to be bio-removed, *P. stutzeri* and agar or cotton wool should be used, on vertical or horizontal surfaces, respectively.

Future studies must be conducted to verify and confirm the medium and long-term safety of these biocleaning methodologies (Bosch-Roig and Ranalli 2014). Attention must be paid to strategies for controlling and monitoring any new, posttreatment microbial interaction on biocleaned artwork. This monitoring should include advanced on-site technologies based on noninvasive tools to understand the potential risks for biocleaned tangible heritage (Rampazzi et al. 2011; Raimondi et al. 2013).

To conclude, intense research has shown that innovative microbiological systems based on microorganisms for removing decay on CH to be good alternatives for restoration. This review has reported and compared the characteristics of the existing delivery systems for biocleaning of CH giving information that will help conservator-restorers and researchers in the choice of the most appropriate methods to use in future applications.

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