Chapter 9

Ancient Textile Deterioration and Restoration: Bio-Cleaning of an Egyptian Shroud Held in the Torino Museum

Roberto Mazzoli and Enrica Pessione

Abstract Ancient textiles are fragile and several factors can affect their integrity. In the present chapter, the main agents of deterioration of old and new textiles, namely physical-chemical (light, oxygen, heat, and humidity) and biological factors as well as human erroneous interventions will be explored. As far as the biological deterioration is considered, the effects of microbial growth, primary and secondary metabolites (acids, solvents, surfactants, pigments) and enzymes (lipases, proteases, and glycosidases) on textile strength and cleanliness will be described in details. The main fungal and bacterial species involved in the damage (textile discoloration, black and green spots, cuts) will be reported. Adhesive application during restoration procedures is discussed to highlight the risk of glue thickening giving rise to dull precipitates on the fabric.

The main strategies for oil-stain and glue removal (both animal glue, such as fish collagen, and vegetal glue, *i.e.* starch) will be described in the paragraph devoted to biorestitution. Finally, a case study concerning an ancient Coptic tunic housed in the Egyptian Museum of Torino, Italy, and biocleaned by means of gellan-immobilized alpha-amylase from *Bacillus* sp. will be largely discussed by reporting historical data, adhesive characterization, methods for artificial aging of simulated sample and glue removal from the artwork.

Keywords Microbial and physical-chemical deterioration · Human restoration · Starch glue · Immobilized enzymes · Amylase · Wool artificial aging · Gellan · Coptic period

R. Mazzoli · E. Pessione (✉)

Structural and Functional Biochemistry, Laboratory of Proteomics and Metabolic Engineering of Prokaryotes, Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy

e-mail: enrica.pessione@unito.it

1 Introduction

Among artworks, textile materials constitute important human history proofs. Specimens such as Pre-Columbian and Native Indian clothes, shrouds, tapestries, carpets, soldier uniforms, ecclesiastical vestments, Olympic winner swimsuits, and spacesuits constitute a rich collection of archeological fabrics that are precious but also fragile, generally revealing a bad conservation state.

Analyzing the causes of deterioration is of primary importance in order to protect the historical items and prevent further damage. However, restoration strategies are sometimes inescapable.

In the present chapter, we will first consider mechanisms of textile aging and deterioration, then the main bio-cleaning-bio-restoration techniques applicable to textiles and finally we will report a case study involving bio-cleaning of an ancient Egyptian shroud of the Torino Egyptian Museum (Italy).

2 Textile Aging and Deterioration

Ancient textiles can suffer from aging, deterioration and degradation events that deeply affect their original beauty and their ethnological and economical value. The environmental conditions (temperature, humidity, light exposure, microbial contamination) these artifacts face during their life strongly affect the item value including those of the museum exhibition rooms, which are crucial for a correct conservation. However, other factors can in case account for visible damage, such as operators' interventions aimed to partially restore the item (Ferrari et al. 2017). Here we consider three main types of stresses giving rise to deterioration: (i) physical-chemical environmental stressors; (ii) microbial degradation and deterioration; (iii) human erroneous treatments.

Physical-Chemical Factors as Cause of Textile Deterioration As far as physical-chemical agents are concerned, light, heat, and oxygen are among the main causes of textile alteration (Rubeziene et al. 2012). As regards solar radiation, it comprises visible, infrared (IR), and ultraviolet (UV) components. IR heats materials, but UV radiation causes most photo-chemical damages to textiles. Photo-decomposition is accelerated by the presence of heat and moisture. When also oxygen is present, oxidative reactions (such as chain scission and cross-linking) known as photo-oxidation may occur (Szostak-Kotowa 2004; Rubeziene et al. 2012). The effect of light radiation on colored textiles, such as discoloration, or generation of dark spots is easy to detect, nonetheless light also decreases tensile and tear strength of fabrics (Rubeziene et al. 2012). The effect of light can be different depending on the type of textile. Exposition of wool to radiation at 475 nm or shorter wavelength for more than 12 h decreases fiber strength (up to 20%) and causes color changes, especially in the presence of moisture (Treigiené and Musnickas 2003; Zimmermann and Hocker 1996). As regards color changes, UV irradiation generally causes

photobleaching followed by yellowing, likely owing to the presence of aromatic amino acids (phenylalanine, tryptophan, tyrosine) and natural yellow pigments in wool (Nicholas and Pailthorpe 1976). Cellulose is sensitive to farther UV radiation (200–300 nm). Photo-oxidation of hydroxyl side-groups and glycosidic bonds causes changes in color, solubility and mechanical properties (increase of rigidity and brittleness) of cellulose (Timár-Balázsy and Eastop 1998). UV rays cause linen textile elongation and fabrics to become darker and slightly lose tensile strength (Abdel-Kareem 2005). Silk fibers are the most sensitive to photo-oxidation by UV irradiation (220–370 nm), leading to significant color changes and to a modification of the textile that becomes more rigid and mechanically weakened (Shubhra et al. 2011). Also synthetic fibers are sensitive to UV light exposure at different extent depending on the kind of chemical polymer, namely polyacrylonitrile is more resistant while polyamides (nylon) are more sensitive (Rubeziene et al. 2012). In this case, UV exposure causes different degrees of yellowing and decrease of mechanical strength, as well. High water content also favors disruption of the fabric structure and color loss (Gutarowska et al. 2017).

Microbial Growth and Metabolism as Degradative and Deterioration Agents Humidity and relatively high temperatures favor the growth of microbial species, both bacteria and fungi, thus opening the way for release of molecules that can damage textiles (Mazzoli et al. 2018b). Among them, catabolites (surfactants, solvents, acids), secondary metabolites (pigments) and enzymes (proteases, lipases, and glycosidases) play a major role. Actually, microorganisms use the textile components (carbon, nitrogen, sulfur, phosphorous) for growth. Plant-derived fabrics host a microbial flora very different from that of animal-derived specimens, based on their different composition.

In general, the most recent proofs of human history such as synthetic fabrics are too hydrophobic to allow biodegradation and among them polyurethanes are the only suitable to bind water thus favoring microbial colonization. Exposure of polyurethane-made swimsuits from Olympic winners at museums should be carefully monitored since a huge number of fungal species bear extracellular esterases that can initiate polyurethane degradation (Rowe and Howard 2002). Other synthetic textiles, such as polypropylene, polyacrylonitrile, and polyamide, because of their hydrophobicity and the presence of ether chemical bonds (unusual in nature), undergo degradation only after light exposure since UV-induced photo-degradation generates shorter chain polymers that become available for bacteria. For this reason, textiles of historical and ethnological interest should be exposed in museums avoiding the use of intense light (Seal 1988).

Besides causing photo-oxidation, light also supports growth of phototrophic bacteria that can use textile-endogenous compounds as nitrogen source. As an example, the presence of light and a high degree of humidity can support the growth of microorganisms generating green pigments (Gutarowska et al. 2017). Other pigments (black, brown, red, orange, and yellow) are produced by non-phototrophic bacteria (*Bacillus*, *Corynebacterium*, *Achromobacter*, *Streptomyces*) as well as by fungi such as *Penicillium*, *Aspergillus*, and *Cryptococcus*. The

Fig. 9.1 Molecular structure of the three main proteins present in animal-derived textiles. (a) silk fibroin; (b) silk sericin; (c) wool keratin

opposite phenomenon, *i.e.* discoloration, can be due to microbial production of lipases, surfactants, and solvents such as acetone. Recently, Pre-Columbian textiles made of cotton and llama- or alpaca-wool were analyzed with the aim of identifying microbial discoloration agents. The most representative genera were *Aspergillus*, *Penicillium*, and *Cladosporium* among fungi and *Kocuria rosea* and *Paracoccus yeei* among bacteria (Pietrzak et al. 2017). Besides pigments and discoloring agents, microbial growth and enzymatic activity can also cause depolymerization of the fabric, generating loss of strength and elasticity of the textile that can undergo fragmentation (Mazzoli et al. 2018a).

Although sensitivity to biodeterioration is mainly related to the polymerization extent of the fiber, the type of weave, the fabric thickness, as a rule, also the vegetal or animal origin of the fabric is important: actually, plant-derived textiles are more susceptible to biodegradation than wool and silk because of the peculiar chemical bonds intrinsic to keratins and fibroins/sericins (Mazzoli et al. 2018a). Among plant-derived textiles, linen and cotton undergo the major risk of deterioration since they are rich in hemicellulose and pectin that are easily degradable by microbial hydrolases, namely endoglucanases (generating different length oligosaccharides), exoglucanases (generating di- or monosaccharides) and beta-glucosidases (converting oligosaccharides to monosaccharides) (Szostak-Kotowa 2004). Conversely, hemp and jute contain non-cellulosic components like lignin, which render the fabric more resistant to degradation, because only few organisms possess enzymes able to degrade it (Gutarowska et al. 2017). Cellulose depolymerization occurs differently in fungi and bacteria: the former directly penetrate into the fiber lumen generating a mycelium responsible for the secretion of extracellular cellulases, whereas the latter proceed from the fiber surface to the interior (Szostak-Kotowa 2004). However, the final effect carried on by cellulolytic enzymes is an impaired fiber strength. Silk and wool, being of animal origin, display a higher protein content (Fig. 9.1) and may undergo proteolysis. In silk, the first protein component to be used is sericin (Fig. 9.1b). However, most of the protein structure is made up of fibroin (Fig. 9.1a), a protein composed by four amino acid repeats (alanine, glycine, serine, and tyrosine) that is degraded slower. In spite of this slow biodegradation coefficient, fibroin is very sensitive to light: different bacteria

Fig. 9.2 Glue-damaged historical carpet dating back to the Ottoman period and exhibited in the museum of the Faculty of Applied Arts, Helwan University, Egypt (modified from Ahmed and Kolisis 2011)

(*Bacillus*, *Serratia*, *Pseudomonas* and *Streptomyces*) and fungi (*Aspergillus*) can modify fibroin structure after light exposure (Seves et al. 1998; Szostak-Kotowa 2004). Wool keratin is an extra-strong structure and it is generally degraded very slowly. Unfortunately, both insects and microorganisms (both bacteria and fungi, especially *Trichophyton* and *Trichoderma*) can attack the disulfide bridges (Fig. 9.1c) that hold together keratin chains (Szostak-Kotowa 2004).

Human Interventions as Cause of Textile Impairment Erroneous preservation attempts from human operators can play a role in cultural heritage item damaging (Beutel et al. 2002; Sterflinger and Pinzari 2012). As far as fabric is concerned, the use of glues has been largely employed in the effort to consolidate cuts. Actually, during aging, some parts of the textiles can be broken and both vegetal (starch) and animal (collagen) adhesives have been employed for pasting and consolidating specimens (Barbabetola et al. 2016; De La Chapelle et al. 1994; Ferrari et al. 2017). Animal-derived glue is of proteinaceous nature, namely collagen originated from fish swimming bladder or mammalian bones and cartilage. Plant-derived glues are made up of polysaccharides, namely starch (*i.e.* amylose and amylopectin) derived from rye, oat, barley, wheat, rice, corn, and potato (Barbabetola et al. 2016; Ferrari et al. 2017). Unfortunately, during time, both these adhesives can precipitate giving rise to dense and opaque material that enhances fiber distortion and fabric thickening, also forming intricate layers that cause structural fragility of the textile, besides producing a visible cloth difficult to remove (Fig. 9.2) (Blüher et al. 1995; Gostling 1989). In the case of animal glues, temperature, humidity, and light can promote protein cross-linking and peptide bond oxidation, whereas microbial attack can favor the production of unwanted pigments (Barbabetola et al. 2016).

In general, for these reasons, starch-based glues are more frequently and preferentially used for textile consolidation than collagen (Ahmed and Kolisis 2011; Whaap 2007). After aging, however, also starch paste can cause hardening, rigidity and yellowness of the textile, also constituting a nutrient-rich habitat for amylolytic bacteria and fungi, which promote textile deterioration over time. Such structural damages render ancient textiles fragile and unsuitable for exhibition. Therefore, cleaning and restoration interventions have to be applied.

3 Bio-Cleaning-Bio-Restoration of Textiles

Glue Removal from Ancient Textiles As reported in the previous section, adhesive application on damaged or broken fabric areas is the most frequent erroneous treatment causing textile impairment. To remove glue several approaches can be followed: (i) mechanical methods; (ii) chemical methods; (iii) wet cleaning (Table 9.1). The first two strategies are sometimes too aggressive and not always applicable to precious ethnographic textiles (Mazzoli et al. 2018a). As far as wet cleaning is concerned, although it represents a milder strategy, the main constraint lies in the long application times. Since this method is only based on humidification, it is not very performant on hardened and long-aged adhesives (Ferrari et al. 2017).

A promising alternative to these techniques is a bio-based approach exploiting living microorganisms or their enzymes, or sometimes a combination of both treatments when possible (Ahmed and Kolisis 2011; Barbabietola et al. 2016; Ferrari et al. 2017). Both microbial and enzymatic glue removal occur in a water environment without use of organic solvents, hence, these treatments are environmental friendly and free of risks for the operators (Barbabietola et al. 2016). The choice between a microbiological and an enzymatic approach strongly depends on the area to be treated: a large area suggests the use of bacteria since the treatment is cheaper and requires less stringent conditions. The living microorganisms approach is also the only feasible when a mixture of complex and heterogeneous compounds have been applied to obtain consolidation (Barbabietola et al. 2016).

Table 9.1 Strategies for adhesive cleaning from textiles

	Mildness	Toxicity/ damage	Operator skill	Specificity	Cost	Time	Efficacy
Mechanical	No	Yes	No	Yes	Low	Short	Yes
Chemical	No	Yes	No	No	Low	Short	Yes
Wet cleaning	Yes	No	No	No	Low	Long	No
Microbial	Yes	No	No	Middle	Middle	Middle	Yes
Enzymatic (solution)	No	Yes	Yes	Yes	High	Long	No
Enzymatic (Immobilized)	Yes	No	Yes	Yes	High	Long	Yes

Fig. 9.3 Enzyme immobilization strategies are necessary to treat textiles. (a) enzymes in solution; (b) immobilized enzymes

However, the limit of using microbial cells as removal agents is their low selectivity (Webster and May 2006). Therefore, more frequently, the enzymatic approach proves to be the most performant for cleaning adhesive-damaged cotton, linen and silk textiles being suitable for both protein-based and polysaccharide-based glues (Ahmed and Kolisis 2011; Ciatti et al. 2010). Actually, the very high catalytic specificity of proteases and amylases renders this method applicable on both collagen and starch. Furthermore, a wide range of microbial-derived enzymes allows the choice of the best fitting (optimum temperature and pH) according to the fabric to be treated (Germinario et al. 2017). These advantages overcome the relatively higher cost of enzymes with respect to microorganisms (Barbabetola et al. 2016). A further constraint of the protein-based strategy is the need to use aqueous solutions that can damage the textiles by excess of water (Hrdlickova Kuckova et al. 2014). During time, this humid environment can promote the growth of bacteria and fungi causing further impairment of the textile (Ahmed and Kolisis 2011). A successful alternative to bypass this bottleneck is the application of the enzyme preparations as a poultice (Bott 1990; Chapman 1986; Shibayama and Eastop 1996), using a gel as sorbent (Hrdlickova Kuckova et al. 2014) or after suitable immobilization on a solid surface (Fig. 9.3). The idea of using an enzyme-enriched poultice was developed to treat starch-damaged paper materials (Schwarz et al. 1999) and successfully translated to textiles. As far as enzyme immobilization is concerned, it has to be underlined that preliminary tests should be performed to evaluate (i) the immobilization yield (amount of enzyme really attached to the chosen surface); (ii) the catalytic activity after immobilization (K_m , V_{max} , K_{cat}); and (iii) the overall efficacy of the immobilized system to remove glue (Ferrari et al. 2017; Mazzoli et al. 2018b).

Oil-Stain Removal from Historical Fabrics Oil-stains on ancient fabrics mainly consist of unsaturated fatty acids that can undergo oxidation on the double bonds. During time, environmental oxygen can attack these double bonds generating oxidation products that can polymerize thus creating a network of molecules difficult

to be removed (Blüher et al. 1997). In this case, the application of lipases (especially those derived from *Candida cylindracea*) can efficiently solve the problem without any damage on the textile (Ahmed et al. 2010).

4 Case Study: Adhesive-Removal by Enzymatic Approach

Gellan hydrogel-immobilized α -amylases have been originally developed for removing starch paste from ancient paper documents (Mazzuca et al. 2014). A gellan-immobilized bacterial α -amylase (Fig. 9.4) has recently been used to clean a wool shroud, dating back to the Coptic period, from starch glue that had been used in the 1950s to temporary consolidate the textile (Ferrari et al. 2017). After selection of the most suitable enzyme among those commercially available, and optimization of the conditions for enzyme immobilization, the cleaning of the back of the two fragments (about 4 m² of textile) composing the tunic was completed in 160 h of work (Ferrari et al. 2017).

4.1 Description of the Coptic Tunic and Its State of Conservation

Since the end of the nineteenth century, the Egyptian Museum of Turin, Italy, has housed a wool-linen tunic (inventory number INV. S. 17490) that was probably a gift by the Egyptian Museum in Cairo to the Egyptian Museum of Turin.

As shown in Fig. 9.5, the tunic (hypothetically belonging to the fifth-century A. D.) was woven in a unique rectangular piece (with an opening at the center to allow the passage of the head) and folded in two at shoulder height. In the same Figure it is also possible to appreciate that the precious textile is abundantly decorated with squares (*tabulae*) and bands (*clavi*) woven with warps and wefts of different colors (green, purple, red, and yellow).

Unfortunately, some parts of the tunic (especially on the back side, Fig. 9.6a) are damaged, fragile, and fragmented. Recent investigations have suggested the idea that the tunic was not an everyday-life cloth but rather a shroud used in the Egyptian Byzantine Period for burying the bodies after abandon of the traditional mummification procedures corresponding to the spread of Christianity. This statement is based upon the evidence that organic deposits deriving from the dead body decomposition (Fig. 9.6b) largely contaminate the fabric (Ferrari et al. 2017).

Furthermore, other parts are stiffened and deformed, due to application of adhesives to contain cuts and fragmentation (Fig. 9.6c, d). During the 1950s, the Turin Egyptian Museum restorer Erminia Caudana extensively applied glue to both papyri and textiles to consolidate them, and it is probably because of these treatments that the wool shroud appeared rigid and hard in correspondence of previous fabric cuts.

Fig. 9.4 Immobilization of an α -amylase enzyme from *Bacillus* sp. on gellan. *Top*) Schematic representation of the enzyme poultice used. *0* Non-acidic blotter paper used to verify the amount of water released from the fabric during the cleaning procedure. *1* Coptic tunic. *2* Low basis weight (6 g/m^2) Japanese paper used to prevent the gel from seeping into or migrate onto the textile. *3* Gellan-immobilized amylase tablet. *4* Melinex layer used to create a wet room to prevent water evaporation from the tablet. *5* Glass weighting to ensure uniformity of the gel-textile contact and the gradual release of water from the gel. *(Bottom)* Application phases of the enzymatic poultice to the back of the tunic (a–f) (Ferrari et al. 2017)

Fig. 9.5 AutoCAD software reconstruction of the Egyptian Coptic wool-linen tunic belonging to the Egyptian Museum of Turin, Italy (inventory number INV. S. 17490) (a). Details of the *tabulae* of the tunic; (b). Details of the *clavi* (Ferrari et al. 2017)

Fig. 9.6 (a, b) Visible damages in the back side of the Coptic tunic of the Egyptian Museum of Turin (Italy); (c, d) Details of the damages due to adhesive application on the fabric (modified from Ferrari et al. 2017)

Such structural damages rendered this archeological artifact not suitable for exhibition since the flexibility of the wool fibers proved to be decreased, making the textile fragile. Therefore, our research group set up a method to solve the problem.

4.2 Description of the Strategies to Fulfill the Objectives

First, since both protein-based and polysaccharide-based glues are employed to consolidate cuts in textile artifacts, it was necessary to establish the exact chemical nature of the adhesive to remove them by protease or glycosidase-mediated

enzymatic hydrolysis, respectively. Secondly, once established the subclass of hydrolase to be used, it was important to screen for the best-fitting enzyme, acting at the correct pH, ionic strength, and temperature. These are challenging factors when a high-value archeological textile has to be cleaned. Generally, plant and microbial enzymes are more suitable than animal enzymes that frequently display maximum activity at 37 °C, a too high temperature for textile integrity preservation. Moreover, a right immobilization approach on different matrices was to be set up, to avoid water flooding of the precious textile. Finally, before successful application of the cleaning procedure on the original Coptic shroud, a simulated sample was artificially aged and used as a test to prove the efficacy of the enzymatic treatment. The overall approach, based on bacterial α -amylases absorbed on gellan, allowed a satisfactory starch removal without any visible alteration of the fabric fibers thus resulting in a successful biorestitution of the precious artifact in study.

4.3 Step 1: Establishing the Nature of the Glue (Lugol Test and FT-IR)

The back of the shroud, as referred in a previous section, displayed significant damages consistent with precipitated opaque material, ascribable to a white adhesive (Fig. 9.6 c, d) because of an old restoration performed with the aim to consolidate cuts. Attempts to bio-clean the fabric with the traditional system of wet cleaning, namely vaporization-atomization, proved to be not suitable to remove the glue. In the perspective to use an enzymatic approach for removing the adhesive (Schwarz et al. 1999; Beutel et al. 2002), establishing the chemical composition of the glue was a priority. Analyses aimed to distinguish between vegetal (starch) and animal (collagen) glue. To this aim, a fabric sample of about $2 \times 5 \text{ cm}^2$ was excised from the Coptic tunic. Based on the assumption that textile restorers most commonly use starch glue (De La Chapelle et al. 1994) a Lugol test, which employs a iodine solution (0.1% w/v KI, 1% w/v I_2), was firstly performed. If starch is present, the sample turns dark blue. Actually, we detected a dark-blue stain on the paste thus revealing the presence of starch on the wool textile fiber (data not shown). In parallel, a Fourier transform infrared spectroscopy (FT-IR) evaluation on a very small area of the paste-damaged textile was set up, using a FT-IR Bruker Vertex 70 spectrophotometer coupled with an infrared microscope (Bruker Hyperion 3000) at a resolution of 4 cm^{-1} and 64 scans. The vibrational bands in the infrared spectra refer to different functional groups of a sample and help a general characterization of a material and, in some cases, the identification of specific compounds. This analysis furtherly confirmed that the glue was starch, by simply comparing these spectra with FT-IR spectra of standard compounds (Fig. 9.7).

Fig. 9.7 Fourier Transform Infrared Spectroscopy Analysis of the glue reveals the starch nature of the adhesive found on the Coptic tunic of the Egyptian Museum of Turin (Italy) (Ferrari et al. 2017)

4.4 Step 2: Selecting the Most Suitable Enzyme and Immobilization Strategy

Once established the starch nature of the adhesive, amylases proved to be the most suitable hydrolytic enzymes for bio-cleaning. Starch consists of two α -glucans, *i.e.* amylose (linear glucose polymer) and amylopectin (branched-chain glucose polymer), but amylopectin is generally considered the main responsible for paste-features. Both α - and β -amylases exist which hydrolyze amylose and amylopectin through different catalytic mechanisms. α -Amylases hydrolyze internal α -1-4 glycosidic bonds at random generating dextrins. β -Amylases are exoglucanases that cleave amylose and amylopectin starting from their non-reducing terminals and release maltose (Ray and Nanda 1996). Choice was addressed to microbial α -amylases because they are maltodextrin-generating endo-enzymes able to quickly eliminate the adhesive properties of starch (Schwarz et al. 1999) whereas β -amylases display a slower catalytic mechanism (Ray and Nanda 1996). Furthermore, prokaryote-derived enzymes display intrinsic higher stability in a wider pH and temperature range than animal-derived amylases (Mehta and Satyanarayana 2016). Among commercial α -amylases, that from *Bacillus* sp. was selected for starch glue removal, owing to its very high specific activity (Chand et al. 2014). More in details, lyophilized α -amylase from *Bacillus* sp. Type IIA (Sigma-Aldrich) with specific activity >1500 units/mg protein was finally chosen. A 0.1% w/v solution of the

enzyme in 20 mM sodium-phosphate buffer pH 6.9 was prepared (De La Chapelle et al. 1994; Cremonesi 2009) for tunic restoration. It is worth noting that pH 6.9 corresponds to the optimal pH for *Bacillus* sp. Type IIA alpha-amylase activity.

However, as referred above, the use of water solutions of enzymes is not suitable for precious archeological artifacts since this treatment can flood the textile with a too high water load, favoring successive microbial contamination. Therefore, we designed an enzyme immobilization approach to restrict the amount of water needed for catalysis. Two immobilization matrices were tested: agar and gellan. Both are polysaccharides displaying high pH stability and highly hygroscopic properties that limit liquid transfer to the fibers, allowing a gradual release of the enzyme into the fabric. Furthermore, their un-adhesive nature and facility to form films render them suitable for application on textiles (Iannuccelli and Sotgiu 2007). Hence, one cm thick low-viscosity “soft” gels (2% w/v) agar (polygalactose and sulfurpolygalactose, Bresciani SRL) and 2% w/v Gellan gum (Phytigel, heteropolysaccharide, Bresciani SRL) in 20 mM sodium-phosphate buffer pH 6.9 were used for this purpose. Since a very good efficiency in glue removal can be obtained by adsorbing enzyme solutions at the interface between textile and support, the solution of α -amylase from *Bacillus* sp. was brush and immobilized onto the solid gel surfaces so as to have the maximum enzyme concentration at the interface and, hence, maximize efficiency of glue removal (Fig. 9.4a). However, the optimum catalytic activity for *Bacillus* sp. α -amylases is 60 °C and this temperature cannot be reached because of the risk of gel melting at temperatures higher than 40 °C. The overall enzyme immobilization procedure is illustrated in Fig. 9.4.

4.5 Step 3: Simulated Sample Preparation, Aging, and Damaging

To prevent any risk of damage to the original archeological tunic, a goat wool simulated sample, with similar chemical-physical properties to the original Coptic shroud fabric, was woven to compare the enzymatic cleaning and the cleaning effect of the immobilization gels alone, prior to application of the system on the real artwork. To have a simulated sample as similar as possible to the original textile, also aging should be considered. Reproducing the original aging of artifacts is a challenging procedure. In the present investigation, we tested an incubation temperature of 90 °C (maximum allowed for wool) (Quaglierini 1985) for 60 days to induce accelerated artificial aging (it has previously been established that 25 years of natural aging can be obtained by 3 days at 120 °C in silk) (Ahmed and Kolis 2011). Dry incubation was chosen and applied here because this condition attempted to reproduce the climatic conditions characterizing the ground where the Coptic shroud was buried in Egypt. However, some events occurring in the natural aging process (contamination by burial ground and dead body decomposition liquids) cannot be reproduced. After this time, a treatment with starch glue was performed to mimic the

previous inappropriate consolidation. Starch can be obtained from different plants, which cannot be distinguished by conventional diagnostic investigations. However, the use of barley, corn, oat, or rye starches in the fifties in Italy (where the restoration was made) is unlikely since these cereals were not of common use. The choice was then restricted to potato, rice, and wheat starches. For glue preparation, 3 parts of each starch were dissolved in 1 part of hot water so as to obtain a thick paste. The three glues were then applied to the simulated sample and subjected to three day-“aging” at 50 °C to further allow glue thickening, aging, and stiffening.

4.6 Step 4: Glue Removal Test by Immobilized α -Amylase on a Simulated Sample

After simulated sample preparation, the efficacy of agar and gellan alone was compared to the one of gellan- and agar-immobilized α -amylase in bio-cleaning the simulated samples. While the effect of gellan and agar alone was negligible, both macroscopic and microscopic evaluations have demonstrated that only after 15 min, enzymatic treatment (6×6 cm cold sheets) was successful in the removal of the starch glue from the simulated sample (Fig. 9.8). Furthermore, starch removal was also proved by performing the Lugol test after the enzyme application (data not shown). The liquefied glue residue was easy-to-remove and, after treatment, the wool sample was clearly more soft and flexible. Since this procedure did not cause

Fig. 9.8 Different cleaning strategies applied to simulated textile sample (Ferrari et al. 2017)

any damage to the simulated textile, it was suitable to be employed on the original Coptic tunic. However, the gellan matrix was better performing than agar also for monitoring adhesive paste removal since it is more transparent. Furthermore, the lower rigidity of gellan with respect to agar allowed a better adhesion to the discontinuity of the textile fibers. For these reasons, application of gellan-immobilized α -amylase on the original Coptic shroud was preferred.

4.7 Step 5: Glue Removal by Immobilized α -Amylase on the Original Archeological Shroud

Finally, the same enzymatic treatment described in the preliminary test on the simulated sample was performed on the archeological Coptic tunic. α -Amylase, immobilized on gellan sheets (that were those showing the best performances) was then placed on a thin Japanese paper sheet to avoid any seeping of the gel into the fabric (Fig. 9.4a, b). Afterwards, a “wet room” (Fig. 9.4c) was created by superimposing a Melinex sheet and a glass weight to prevent dehydration.

As shown in Fig. 9.4 e, f, 15 min were sufficient to swollen and liquefy the glue. It worth remembering that such a high efficiency was obtained although the temperature used during bio-cleaning procedure was lower than that for amylase optimum activity (60 °C) (Schwarz et al. 1999). The adhesive residue was then removed by means of a moistened cotton swab and a spatula (Fig. 9.4e). No more adhesive paste was detectable on the fabric (dried by using an absorbent paper (Fig. 9.4f), that also displayed improved elasticity and flexibility. After this bio-cleaning treatment, the needle consolidation of the textile was finally performed. The overall bio-cleaning of both sides of the textile (about 4 m²) was achieved in 160 h of work.

Other advantages of immobilized amylases are the very quick time of application and the overall milder mechanical treatment required as compared to traditional cleaning methods (*i.e.* wet cleaning). It is useful mentioning that α -amylases have been already used to restore wool/linen tapestry after suitable immobilization on methylcellulose matrices (Whaap 2007) but also (both in solution and as a poultice)

Fig. 9.9 The restored Coptic tunic-shroud exhibited at the Egyptian Museum of Turin, Italy (Ferrari et al. 2017)

for cleaning cotton and even silk textiles from starch (Ahmed and Kolisis 2011). Furthermore, α -amylases are largely used for fabric desizing (Chand et al. 2012).

In conclusion, the specificity, mildness, and efficacy of the enzymatic treatment render this strategy especially suitable for precious archeological textiles. The Coptic shroud, “hidden” from the community for a long time is currently exhibited at the Egyptian Museum of Turin (Italy) (Fig. 9.9) without any damage to its historical value.

5 Conclusion

This report demonstrates that textile artifacts are fragile and undergo aging and different kinds of deterioration. However, it is possible to face damages, such as those due to wrong restoration interventions, by using purified enzymes, suitably immobilized, to contain the risk of employing aqueous solutions. Among the wide range of commercially available enzymes, those displaying a good catalytic activity at low temperatures (lower than 30 °C) are very promising since they can be applied also to fragile items. The microbial world seems to be a good candidate to be explored for finding such enzymes. This field is also promising to find future solutions to contain microbial deterioration (Mazzoli et al. 2018b) avoiding the use of acids, solvents, and surfactants (dangerous for the artworks). For the latter purpose, the use of enzyme- or bacteriocin-mediated bacterial competition seems of particular interest. Interdisciplinary approaches and collaborations between art conservators and biotechnologists, biochemists, and microbiologists are the essential requisite to preserve objects that state the immense creativity of artists and the high value of human history.

Acknowledgements The restoration of the Egyptian shroud has been the Master thesis work of Martina Ferrari that we would like to thank. We are also indebted with the researchers of the Restoration School of Venaria Reale, Turin, Italy, which have contributed with their respective expertizes to the achievement of the experimental part concerning adhesive characterization and sample bio-cleaning.

References

- Abdel-Kareem OMA (2005) The long-term effect of selected conservation materials used in the treatment of museum artefacts on some properties of textiles. *Polym Degrad Stab* 87:121–130
- Ahmed HE, Kolisis FN (2011) An investigation into the removal of starch paste adhesives from historical textiles by using the enzyme α -amylase. *J Cult Herit* 12:169–179
- Ahmed HE, Gremos SS, Kolisis FN (2010) Enzymatic removal of the oily dirt from a Coptic tunic using the enzyme lipase. *J Text Apparel Technol Manag* 6:1–17
- Barbabetola N, Tasso F, Alisi C, Marconi P, Perito B, Pasquariello G, Sprocati AR (2016) A safe microbe-based procedure for a gentle removal of aged animal glues from ancient paper. *Int Biodeter Biodegr* 109:53–60

- Beutel S, Klein K, Knobbe G, Königfeld P, Petersen K, Ulber R, Scheper T (2002) Controlled enzymatic removal of damaging casein layers on medieval wall paintings. *Biotechnol Bioeng* 80:13–21
- Blüher A, Haller U, Banik G, Thobois E (1995) The application of carbopol poultices on paper objects. *Restaur Int J Preserv Libr Arch Mater* 16:234–247
- Blüher A, Grube A, Bornscheuer U, Banik G (1997) A reappraisal of the enzyme lipase for removing drying-oil stains on paper. *Pap Conserv J Inst Pap Conserv* 1997:37–46
- Bott G (1990) Amylase for starch removal from a set of 17th century embroidered panels. *Conserv* 14:23–29
- Chand N, Nateri AS, Sajedi RH, Mahdavi A, Rassa M (2012) Enzymatic desizing of cotton fabric using a Ca²⁺-independent α -amylase with acidic pH profile. *J Mol Catal B Enzym* 83:46–50
- Chand N, Sajedi RH, Nateri AS, Khajeh K, Rassa M (2014) Fermentative desizing of cotton fabric using an α -amylase-producing *Bacillus* strain: Optimization of simultaneous enzyme production and desizing. *Process Biochem* 49:1884–1888
- Chapman V (1986) Amylase in a viscous medium—textile applications. *The Conservator* 10:7–11
- Ciatti M, Conti S, Fineschi C, Nelson JK, Pini S (2010) Il ricamo in or nué su disegno di Raffaellino del Garbo. Aspetti storico-stilistici, tecnici, minimo intervento e conservazione preventiva. *OPD Restauro* 22:81–116
- Cremonesi P (2009) L'uso degli enzimi nella pulitura di opere policrome. Il Prato, Padova, Italy
- De La Chapelle A, Choisy F, Gallo M, Legoy M (1994) Emploi d'amylases et de protéases pour la restauration d'arts graphiques. In: *Environnement et Conservation de l'Écrit, de l'Image et du Son: Actes des Deuxièmes Journées Internationales d'Études de l'ARSAG*, 16 au 20 mai 1994. Association pour la recherche scientifique sur les arts graphiques, Paris, France, pp 217–229
- Ferrari M, Mazzoli R, Morales S, Fedi M, Liccioli L, Piccirillo A, Cavaleri T, Oliva C, Gallo P, Borla M, Cardinali M, Pessione E (2017) Enzymatic laundry for old clothes: immobilized α -amylase from *Bacillus* sp. for the biocleaning of an ancient Coptic tunic. *Appl Microbiol Biotechnol* 101:7041–7052
- Germinario G, van der Werf ID, Palazzo G, Regidor Ros JL, Montes-Estelles RM, Sabbatini L (2017) Bioremoval of marker pen inks by exploiting lipase hydrolysis. *Prog Org Coatings* 110:162–171
- Gostling K (1989) Bookbinders and adhesives: part 2. *New Bookbind* 9:30–39
- Gutarowska B, Pietrzak K, Machnowski W, Milczarek JM (2017) Historical textiles - a review of microbial deterioration analysis and disinfection methods. *Text Res J* 87:2388–2406
- Hrdlickova Kuckova S, Crhova Krizkova M, Pereira CLC, Hynek R, Lavrova O, Busani T, Branco LC, Sandu ICA (2014) Assessment of green cleaning effectiveness on polychrome surfaces by MALDI-TOF mass spectrometry and microscopic imaging. *Microsc Res Technol* 77:574–585
- Iannuccelli S, Sotgiu S (2007) La pulitura ad umido di opere d'arte su carta con gel rigidi di gellano: presupposti teorici, metodologia, applicazioni e verifica analitica. In: *Quaderni del Cesmar7*, Vol. 11. Il Prato, Padova, Italy
- Mazzoli R, Giuffrida MG, Pessione E (2018a) Back to the past: “find the guilty bug—microorganisms involved in the biodeterioration of archeological and historical artifacts”. *Appl Microbiol Biotechnol* 102:6393–6407
- Mazzoli R, Giuffrida MG, Pessione E (2018b) Back to the past—forever young: cutting-edge biochemical and microbiological tools for cultural heritage conservation. *Appl Microbiol Biotechnol* 102:6815–6825
- Mazzuca C, Micheli L, Cervelli E, Basoli F, Cencetti C, Coviello T, Iannuccelli S, Sotgiu S, Palleschi A (2014) Cleaning of paper artworks: development of an efficient gel-based material able to remove starch paste. *ACS Appl Mater Interfaces* 6:16519–16528
- Mehta D, Satyanarayana T (2016) Bacterial and archaeal α -amylases: diversity and amelioration of the desirable characteristics for industrial applications. *Front Microbiol* 7:1129
- Nicholas CH, Pailthorpe MT (1976) 50—primary reactions in the photoyellowing of wool keratin. *J Text Inst* 67:397–403

- Pietrzak K, Puchalski M, Otlewska A, Wrzosek H, Guiamet P, Piotrowska M, Gutarowska B (2017) Microbial diversity of pre-Columbian archaeological textiles and the effect of silver nanoparticles misting disinfection. *J Cult Herit* 23:138–147
- Quaglierini C (1985) *Manuale di merceologia tessile*. Zanichelli, Bologna, Italy
- Ray RR, Nanda G (1996) Microbial β -amylases: biosynthesis, characteristics, and industrial applications. *Crit Rev Microbiol* 22:181–199
- Rowe L, Howard GT (2002) Growth of *Bacillus subtilis* on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. *Int Biodeter Biodegr* 50:33–40
- Rubeziene V, Varnaite S, Baltusnikaite J, Padleckiene I (2012) Effects of light exposure on textile durability. In: Annis PA (ed) *Understanding and improving the durability of textiles*. Woodhead Publishing, Oxford, pp 104–125
- Schwarz I, Blüher A, Banik G, Thobois E, Maurer KH (1999) The development of a ready-for-use poultice for local removal of starch paste by enzymatic action. *Restaurator* 20:225–244
- Seal KJ (1988) The biodegradation of naturally occurring and synthetic plastic polymers. *Biodeter Abstr* 2:296–317
- Seves A, Romanò M, Maifreni T, Sora S, Ciferri O (1998) The microbial degradation of silk: a laboratory investigation. *Int Biodeter Biodegr* 42:203–211
- Shibayama N, Eastop D (1996) Removal of flour paste residues from a painted banner with α -amylase. *The Conservator* 20:53–64
- Shubhra QTH, Alam AKMM, Beg MDH (2011) Mechanical and degradation characteristics of natural silk fiber reinforced gelatin composites. *Mater Lett* 65:333–336
- Sterflinger K, Pinzari F (2012) The revenge of time: fungal deterioration of cultural heritage with particular reference to books, paper and parchment. *Environ Microbiol* 14:559–566
- Szostak-Kotowa J (2004) Biodeterioration of textiles. *Int Biodeter Biodegr* 53:165–170
- Timár-Balázsy Á, Eastop D (1998) *Chemical principles of textile conservation*. Routledge, Taylor and Francis Group, London and New York
- Treigienė R, Musnickas J (2003) Solvent pre-treated wool fabric permanent set and physical properties. *Fibres Text East Eur* 11:37–40
- Webster A, May E (2006) Bioremediation of weathered-building stone surfaces. *Trends Biotechnol* 24:255–260
- Whaap F (2007) The treatment of two Coptic tapestry fragments. *V A Conserv J* 55:11–13
- Zimmermann M, Hocker H (1996) Typical fracture appearance of broken wool fibers after simulated sunlight irradiation. *Text Res J* 66:657–660

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.