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# Future prospects of biocleaning application in textile conservation

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**Abstract**  Studies of sustainable preservation methods are an important element of ongoing research into minimising the environmental impact of conservation treatment. Of these methods, the cleaning of antique surfaces using selected microbial cultures is attracting attention in the field of heritage conservation. Due to the highly specific nature of the action of these microorganisms, which is similar to enzymatic cleaning, it is generally assumed that individual cultures can remove dirt without endangering the complex structures of textiles. The emphasis is placed on the use of nonpathogenic microbial cultures that have proven to be effective in the cleaning of other historical materials, and which are active in a neutral environment and show relevant metabolic activity. The aims of this work were to study the application of *Pseudomonas putida* to clean iron gall ink staining and the feasibility of using a selected bacterial strain to clean historic textiles. A relevant procedure for the application of this method to the controlled biocleaning of textiles was also developed. The use of water-based gel systems as a matrix for microorganisms seems to be optimal in terms of providing suitable living conditions for the bacteria and maintaining controlled contact with the surface of the object while simultaneously ensuring efficiency. Tests were carried out on appropriately prepared model samples consisting of cotton and silk. The changes emerging on the surface were evaluated using optical microscopy, and the rate of cleaning was assessed using FTIR and colorimetric methods. In addition, FTIR spectroscopy was employed for microbial control after biocleaning. The research demonstrates the feasibility of cleaning iron gall ink from textiles with viable microbial cells. The selected microorganism was able to reduce undesired discolouration from iron gall ink on model textiles. The results indicate that *P. putida* has a profound impact on silk samples, and prove that microbial cleaning can achieve a high level of efficiency in the removal of concentrated dirt.

## 1 Introduction

Although the practice of conservation has changed over time, the main idea underlying the process, which is to address problems associated with changes in both material structure and cultural significance, has remained constant. Heritage objects are subject to many degradation-related changes due to ageing, and many other factors may also contribute to the further deterioration of the material. The removal of undesired potential triggers is therefore at the heart of any treatment. Cleaning has always been one of the most demanding processes in art conservation. One serious threat to textile materials is localised staining or soiling. Of the numerous types of spots, most of which need high volumes of water to be released, solid deposits cause particular problems, since they usually require the use of organic solvents [1]. The potential difficulties in this case arise from the high vapour pressures of organic solvents, which can lead to hazards, such as high flammability, toxicity and environmental pollution. Exposure to solvents is also typically associated with health issues [2]. Hence, the development and application of new cleaning methods is attracting attention at an international level. Continuous research is under way in this field, with the main trend being a transition towards environmentally friendly technologies [3]. Over recent decades, many novel and cost-effective biotechnological tools have been developed and subsequently optimised for the cleaning of historical surfaces [4]. Of these, sustainable cleaning by microorganisms immobilised in a multilayer biosystem has been particularly notable in the field of heritage science [5]. The term microbial cleaning has come to be used to refer to biological cleaning performed by viable microorganisms [6] or the application of enzymes of bacterial origin as cleaning agents [6, 7]. The advantages of methods based on microorganisms are associated with their selectivity, the possibility of local application and adaptation of the method of application to the specimen, and their gentle nature of action on the preserved material [8]. They are non-toxic to humans and the environment, do not generate waste, are inexpensive and can be combined with other cleaning methods from the field of green chemistry [9]. Although several studies have been performed so far, few have been published and almost none deal with historical textiles. Previous studies of organic materials are also limited. Some preliminary work was carried out in the middle 2000s on frescos from the Campo Santo, a cemetery in Pisa, by a group of researchers from Italy [10, 11]. This study focused on the removal of aged organic protein glue mixed with formaldehyde. Although formaldehyde was

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initially intended for use as a biocide, its reaction with animal adhesive over time was found to facilitate polymerisation, making traditional cleaning techniques ineffective. As a result, a decision was made to employ the *Pseudomonas putida* bacterial strain in this treatment [12]. In a major advance, Mazzoni et al. investigated the use of various microbial cultures for the removal of deposits of both organic and inorganic origin from mural paintings from the Casina Farnese (Palatine Hill, Rome), in 2014 [13]. The first group of cultures included protein-based compounds. Initial systematic investigations into the biocleaning of original carbon-based surfaces focused on microorganisms that could remove aged proteinaceous adhesive residues from paper artefacts [9], and microbial technology was proven to be effective in terms of removing undesired organic deposits, such as casein, egg yolk, oil, and animal fat [6]. However, the problem of staining affects a huge number of organic heritage objects, including highly degraded textiles. The reason for this is not only the heterogeneous structure of the fabric itself, but also the wide variety of different types of material found on the garment in the form of decorations. This means that special care is required, and also prevents the application of most mechanical methods that can be used effectively to clean other materials. The problem of staining of textiles with undesirable matter is therefore an important one, because in addition to aesthetic degradation, substances can migrate to the wider environment and cause total damage to textile fibres, leading to degradation and disintegration. The situation becomes even more complicated when coloured compounds are bonded to the fibres. A surprisingly challenging problem of deterioration arises in fibres dyed with tannins using an iron sulphate mordant. Dyes based on iron tannates have been used throughout history to colour various materials in shades of black, brown, grey, or blue/dark blue [14]. The acidic dye initiates degradation processes, for example through the catalysis of oxidation or the acid-based hydrolytic decomposition of the fibres. These reactions generate many problems, the most typical of which are brittleness, colour change and loss of tensile strength. Iron tannates are key components of iron gall ink, and are produced from the reaction between tannic acid and ferrous sulphate ($FeSO_4$), which are basic components of these dyes. This type of ink was the most popular in continental Europe for over ten centuries, until the twentieth century. However, it can be considered not only as a dyestuff but also as staining compound. Large proportions of free ferrous ions remain in the ink as a result of the formation of iron (II) gallate complexed with gallic acid, a product of the acid hydrolysis of tannins [15]. Aqueous treatment cannot therefore be considered, since any contact with water may lead to migration of the metal ions, followed by further degradation of the substrate. Thus, in order to investigate and evaluate the possibility of using microorganisms for stain removal from historical textiles, we used iron gall ink as a model damaging agent. The problem of removing this type of soiling from the surface of historical textiles has not yet been effectively solved, and current data highlight the importance of developing an alternative approach. The present study answers some of the questions associated with the negative aspects of using microorganisms in textile conservation, which are usually of concern regarding biodeterioration [16]. From this point of view, we present an alternative approach in which beneficial bacteria can be used for biocleaning, and explore the effects of using selected viable bacteria cultures as cleaning agents for the removal of solid iron gall ink staining from textile surfaces. Original ancient artefacts could not be used for this investigation, for ethical and practical reasons, and therefore a need to produce and analyse imitations of these objects arose. The materials used for the model samples were textiles composed of the types of natural organic fibres that are most often found among historical collections (cotton and silk). Production of model iron gall ink dyed samples was twofold: We developed both a staining procedure and a suitable method of accelerated ageing. The specific aim of this research was to examine the effectiveness of selected bacterial cultures against iron gall ink staining on textile surfaces. Although previous studies have based their criteria for the selection of microorganisms on those isolated from the surface of the examined material [17, 18], we instead chose individual cultures on the basis of a theoretical evaluation of their properties and the methodologies given in the relevant literature [19, 20]. Of the possible bacteria cultures, *Pseudomonas* spp. was determined to be the most functional species for polymer degradation due to its high adaptability to fluctuating environmental conditions [21]. This species is shown to have the ability to affect the cleavage of the ester bonds in both aliphatic and aromatic polyesters, leading to chain scission and ultimately giving water-soluble products that can be transported into microbial cells and then assimilated [22]. The capability of this bacterium to degrade aliphatic hydrocarbons means that it has already found applications in biocleaning, in relation to the removal of glue containing biocide. Its potential to remove adhesives of animal origin that are rich in collagen and casein is conditioned by its proteolytic activity. One important property of this bacterium within a natural environment is the degradation of oil derivates. This particular bacteria has also proved successful in metal cycling due to its high biosorption potential and resistance to metals in the environment [23].

Furthermore, this bacterium has minimal nutritional requirements in terms of a growing medium, and can be grown over wide range of temperatures. In view of the considerations set out above, the most versatile type of microbial culture was found to be *Pseudomonas* spp. [24]. In particular, the study was focused on *P. putida*. The findings of this investigation are intended to complement those of earlier studies by Troiano [18]. As noted by Troiano [18] the conservation approach based on biotechnology is in the developmental phase. Thus, every study that investigates the use of viable bacterial cells for cleaning is progress made in the conservation of cultural heritage. This particular research assesses the impact of bacteria on iron gall ink staining on textiles which has not been investigated so far.

More generally, we anticipate that this study will contribute to raising awareness of the need to choose highly specific cleaning methods, especially with regard to the type of staining, the environment and the health of the restorers. It can also be assumed that positive results from complex and sensitive forms of material such as historic textiles mean that the method will also be effective on different types of dirt and other types of surfaces of heritage objects; however, in this paper, we report only the preliminary outcomes of our laboratory studies.

## 2 Materials and methods

### 2.1 Textile materials

When selecting a reference for historical textiles in museum collections, various types of natural fibres were considered. In order to explore the feasibility of microbial activity on textile surfaces, potential materials were classified based on their structure and properties. Two different types of fabrics were chosen, in order to cover a wide range of historical textiles in museum collections; by using cotton and silk as model substrates, we were able to represent textiles consisting mostly of a carbohydrate polymer with high molecular weight, cellulose, and textiles whose main compound is a protein polymer high molecular weight. Standard silk fabric ISO 105-F06:2000 *Bombyx mori* with a satin weave and cotton fabric *Gossypium hirsutum L.* with a plain weave were used in this investigation. The fabrics were washed with pure water to remove any possible residues or sizing agents, both of which can affect the properties of the fibres, and the specimens were laid flat to dry [25]. The sizes and shapes of the model samples were determined based on the testing requirements.

### 2.2 Microorganisms

A pure strain of *P. putida* 2082 was chosen as a test microorganism for biocleaning investigation, and was purchased from CCUG (Culture Collection University of Gothenburg). In order to perform the test, *P. putida* was grown in a tryptone broth agar culture medium with the following composition: tryptone 5 g/l (Fluka, Germany), yeast extract 5 g/l (Gistex LS, DSM, Food Specialties, Netherlands), $K_2HPO_4$ 1g/l, glucose 1 g/l, agar 20.0 g/l (Merck KGaA, Germany). Prior to incubation, the pH of the solution was adjusted to 7 with 4 M NaOH. Colonies were collected via an inoculation loop and suspended in 100 ml of liquid medium in a flask containing the same nutrient basal solution but without agar. Before inoculation, the media were autoclaved for 20 min at 120 °C at 120 kPa and then cooled. The inoculated suspension was incubated for 24 h at 30 °C in a rotary shaker (at 200 rpm). After overnight incubation, we measured the optical density of the strain at 600 nm (OD600) using an automated BioSpectrometer (Eppendorf, Hamburg, Germany). Cell cultures reached $OD_{600}$ values of 0.333 (at tenfold serial dilution). The dilution for the measurements was prepared by mixing 100 μl with 900 μl of distilled water and then applying a vortex mixer. The colony-forming units were then estimated at a suitable serial dilution on agar plates. The final cell concentration was approximately $76 \times 10^8$ CFU $ml^{-1}$ of inoculum.

### 2.3 Staining procedure

One type of staining was created for the unaged samples, to imitate the authentic discolouration of historical textile objects that require conservation treatment. Although there is a vast number of recipes for making iron gall ink [26, 27], it was decided that the best method for this investigation was to study the impact of a dye made of natural ingredients [28]. Hence, we followed a recipe described in the Manuscript of Simon de Monte Dante (1422), in which one part powdered galls were boiled in ten parts water until the original volume was reduced by half. The mixture was strained twice through filter paper in a funnel and the extract was refilled to make up the original volume with white wine. During the heating process, a half part of Arabic gum (Sigma-Aldrich, St. Louis, USA) was added. When it had dissolved, the solution was left to cool down and a half part of vitriol ($FeSO_4 \times H_2O$, iron(II) sulphate hydrate, Sigma-Aldrich, St. Louis, USA) was added. It was left aside to blacken, and the ink was then filtered. In order to obtain a fairly even distribution of discolouration over the textile specimens, the samples were fully soaked in a cuvette containing the prepared solutions for 24 h at room temperature and then air-dried.

### 2.4 Accelerated ageing

Once both types of textile models had been artificially stained to mirror the natural degradation process, the specimens were aged. Ageing was carried out over two different cycles, due to the specific requirements of each type of surrogate. Each type of material was aged in two cycles. One for stained and one for unstained surrogates. The ageing cycles were planned according to Wilson [14]. Our setup bore a close resemblance to that used by Nilsson [29] although in the procedure [29], we applied thermo-oxidative ageing using a heating chamber with natural convection (ED 56 Avantgarde.Line, Binder, Germany). The exposure time to the ageing process was calculated based on the selected temperature and its correlation with the degree of degradation of the historical textiles that we aimed to mimic [30]. Nilsson's method [29] was modified to imitate the degree of degradation of eighteenth-century textiles, and the model silk samples were aged for seven days at a mean temperature of 125 °C in dry air. The results can be seen in Fig. 1. Due to the different activation energy for the deterioration of cotton, the cotton surrogates were aged for 18 days at 100 °C [31].

### 2.5 Application

To allow us to verify the cleaning ability of the selected bacterial strain, we used a gelling system as an appropriate carrier. We selected an agar-based hydrogel (Merck KGaA, Germany), as this was widely available and easy to prepare. The delivery system

**Fig. 1** Artificially stained silk model after accelerated ageing



was devised based on our experience and the recommendation given by Bosch-Roig [8] to use either agar gel or cotton wool as a delivery system for *P. putida*. A high concentration of gel was used to limit syneresis (leakage) of the aqueous phase upon prolonged contact with the surface of the object and resorption of the removed impurities. It was previously shown that 4% agar gel exhibits minimal absorption of water-soluble ink, in investigations performed by Schmitt [32]. The limited gel activity allowed us to observe the extent to which the microorganisms affected the biocleaning. The method used to prepare the gel was essentially the same as that described by Cremonesi [33]. Solid agar powder was dispersed in distilled water in order to obtain 4% gel, and the solution was brought to boiling on a heating plate, with regular rotating movements by an immersed magnetic stirrer [33]. Once the dispersive phase had been evenly dispersed throughout the water, the hot suspension was left to cool. At about 40 °C [34], the suspension inoculated with bacteria ($76 \times 10^8$ CFU ml$^{-1}$) at various volumes (1, 2 and 3 ml) was blended with the agar solution upon gelling, and each of the three solutions was then plated on a separate Petri dish. To provide maximum contact with the surface of the model samples, pure agar plates were smeared with the bacteria using a spreader from the liquid cell suspension, which was cultivated for 24 h at 30 °C and 200 RPM in rotary shaker. The second variants of plates were prepared from a mixture of agar (in a liquid state) and cell suspension, which solidified after mixing. Prior to the application, plates were spread with 1, 2, 3 ml of cell suspension and then were cultivated for 24 h at 30 °C. The findings of previous studies revealed that *P. putida* exhibits highest growth at 30 °C [35]. However, this temperature was too high for cleaning of historic textiles. In order to minimize damaging effect, thus the cleaning was performed at room temperature (approx. 20 °C).

We chose to use a small sample size (diameter 0.5 cm), and plugs were punched from overgrown gel plates with a Miltex biopsy punch (Integra 3335) and collected with tweezers. These gel plugs were then ready for application to the model samples. The specimens were placed on blotting paper to remove excess water, thus demonstrating the ability of the method to draw out staining and limit the amount of residue on the treated surface. We investigated two application procedures. Table 1 shows the procedures. One set of gel plugs was located directly on the surface stained with iron gall ink, and the second set was placed onto a barrier layer made of naturally coloured Japanese tissue paper 6 g/m$^2$ (Ceiba, Slovakia). This is illustrated in Fig. 2. Next, both sets of gels were covered with blotting paper (300 g/m$^2$, acid free and pH neutral, PEL, United Kingdom) isolated from glass weights (Amber Glass, Poland) with polyester film (Melinex 401, 75 μm). This can be clearly seen in Fig. 3. The gels packed with bacteria were left in place for 4 h in total [36]. To avoid damaging the textile substrate after treatment with the microorganisms and to remove possible residues, the surface was treated by topical application of bacteria-free agar gel. These experiments were performed in triplicate on each type of textile, and the results were compared with a negative control (agar gel without bacteria). Figures 4 and 5 show the cleaned samples.

## 2.6 Analysis equipment

### 2.6.1 Optical microscopy

In order to characterise the surface morphology of the specimens, an optical microscope with universal LED illumination (DM2700 M, Leica, Germany) was employed. Samples were examined under both visible and polarised light and documented with a digital camera [37]. Image acquisition was performed using Leica Automated Suite (LAS) software. Samples were positioned on glass slides for observation, and analysed at various magnifications up to ×500.
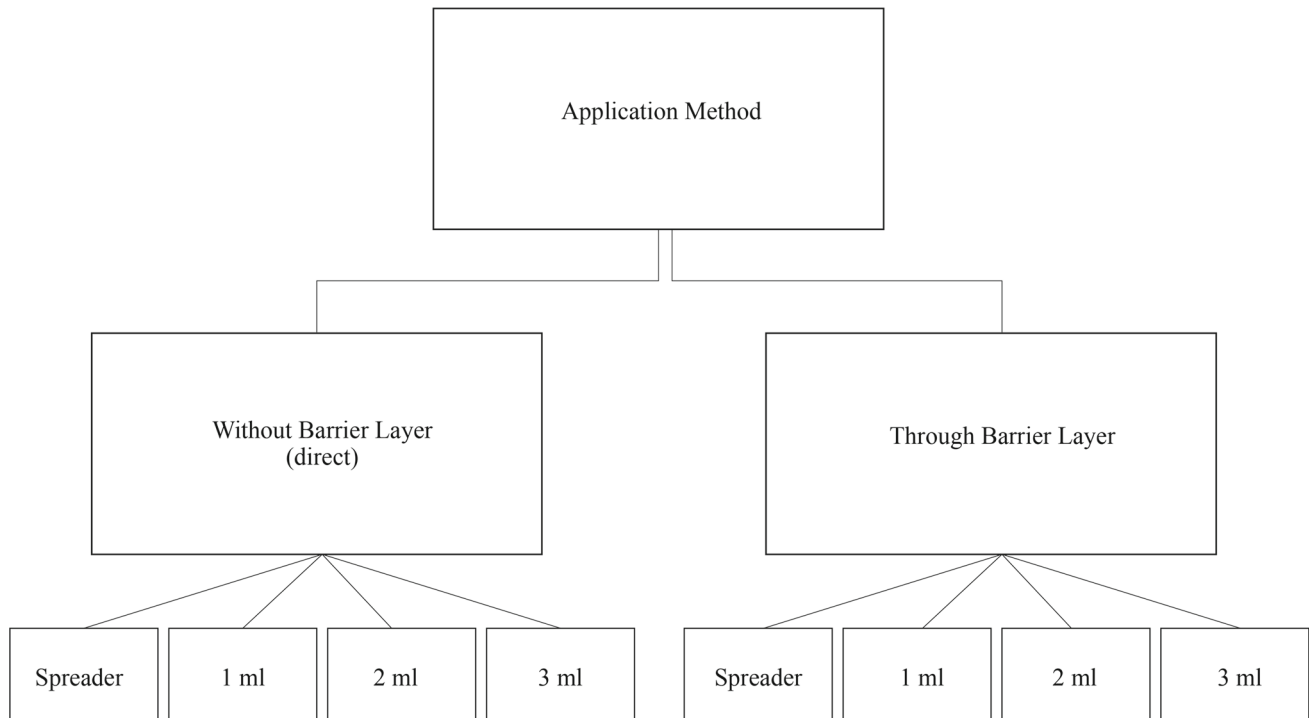
**Table 1** Application method

```
                    ┌─────────────────────────┐
                    │   Application Method     │
                    └─────────────────────────┘
                       │                    │
        ┌──────────────────────┐    ┌──────────────────────┐
        │  Without Barrier Layer│    │  Through Barrier Layer│
        │      (direct)         │    │                      │
        └──────────────────────┘    └──────────────────────┘
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| Spreader | 1 ml | 2 ml | 3 ml |    | Spreader | 1 ml | 2 ml | 3 ml |



**Fig. 2** Silk sample upon biocleaning. 1. Application procedure: Gel plugs applied directly on the surface. From left to right: inoculated with spreader, 1 ml, 2 ml and 3 ml suspension respectively. 2. Application procedure: Gel plugs applied on top of japanese tissue. From left to right: inoculated with spreader, 1%, 2% and 3% suspension accordingly
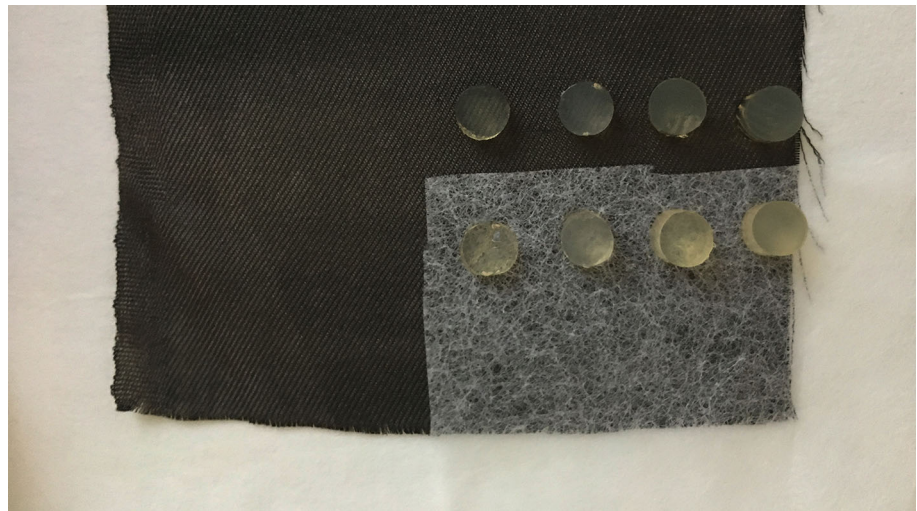
### 2.6.2 Colour measurement

A SpectroDens (Techkon, USA) portable spectro-photometer was used to measure the colour. These measurements were acquired in the spectral range 400–700 nm, with an acquisition step of 10 nm. The records were obtained using a D65 (daylight) illuminant and a 10° supplementary standard observer, and the specular component of the light was excluded. The measuring area was set to Ø3 mm, and the device was calibrated using a white ceramic tile standard. Measurements were made by placing samples on a paper background (Fabriano Tiziano 160 g/m$^2$, colour: white). Each colour measurement was performed five times for each gel system before and after biocleaning, which was shown to be sufficient to guarantee reproducibility [38]. The mean value of each set of measurements was calculated, and the total colour difference $\Delta E$ was evaluated based on these average values before and after biocleaning, in order to determine the biocleaning efficacy of each gel. The colour parameters $\Delta(L*a*b*)$ and $\Delta E$ obtained from the samples are listed in Table 2. The coordinate $L*$ gives information about the lightness of the colours. Its values range from zero (representing black) to 100 (representing white). The parameter $a*$ gives the red/green values, and $b*$ gives the yellow/blue values; both of these parameters range from + 60 to − 60. The total colour difference can then be expressed by a single number
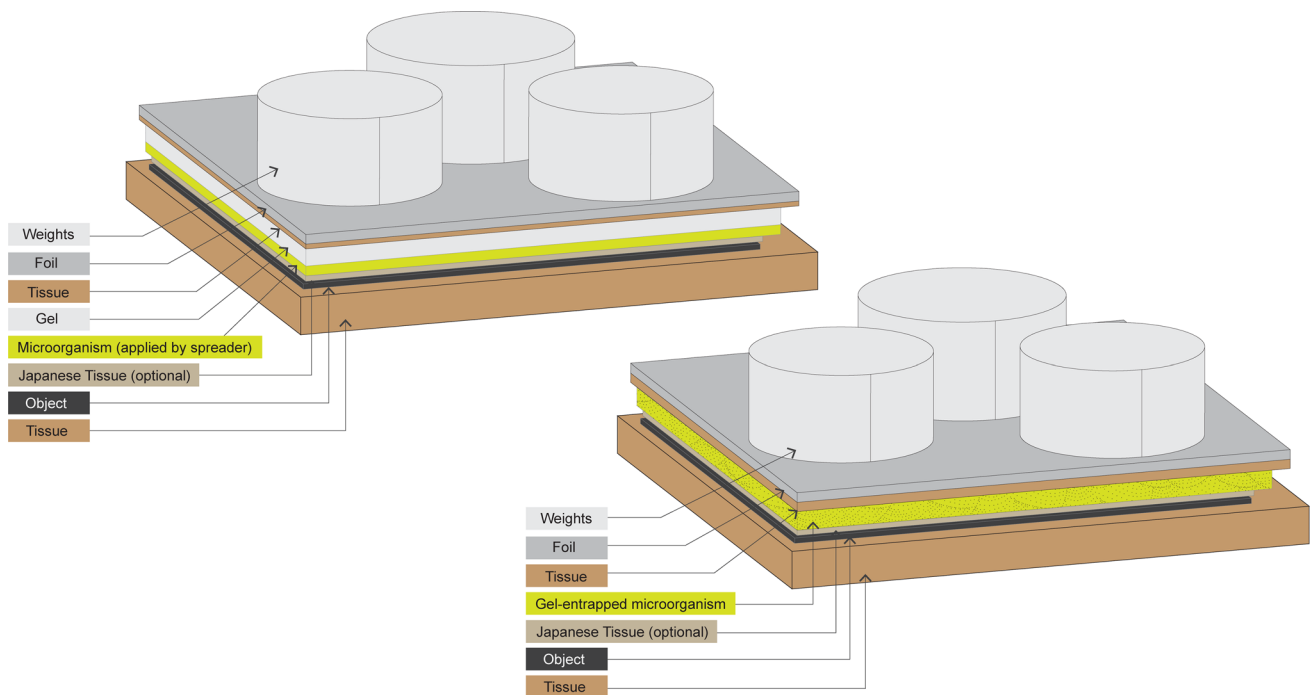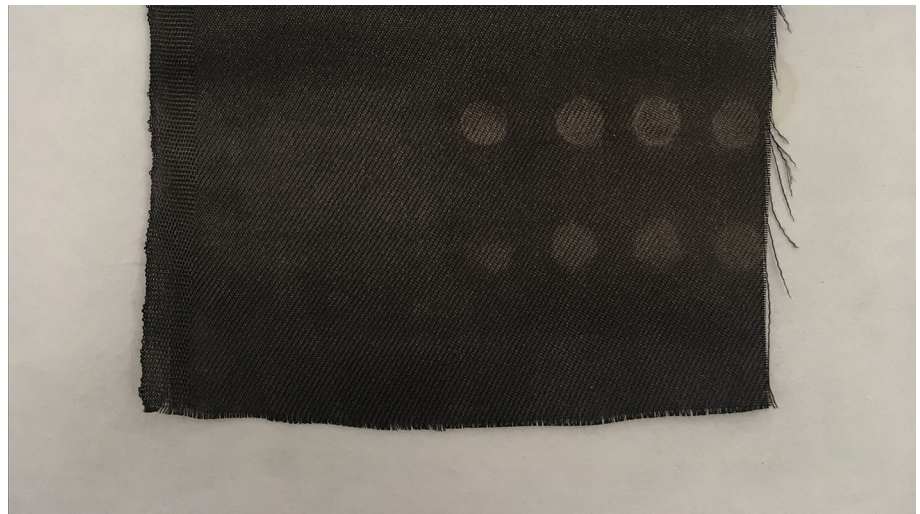
**Fig. 3** Scheme illustrating variations of multilayer application procedure

**Fig. 4** Silk specimen after biocleaning



$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, which denotes the range between two points in CIE $L^*a^*b^*$ space [39]. Previous research has tended to focus on $\Delta E^*$ rather than the qualitative differences ($L^*a^*b^*$) in the colour changes [40]. The main limitation of $\Delta E^*$ is an overall evaluation of the changes. Thus, the obtained information cannot be considered as a model data. The purpose of colour measurements was in gaining objective and comparable results which are not limited by the human eye and do not depend on individual perception by vision. $\Delta E^* > 5$ is thought to be distinguished by the naked eye [41].

### 2.6.3 ATR-FTIR spectroscopy

The chemical and structural changes on the surface of the samples were examined using Fourier-transform infrared spectroscopy (FTIR) in attenuated total reflection (ATR) mode. The infrared spectra of the model samples were measured using a Digilab Excalibur FTS 3000 spectrometer (Digilab, Randolph, MA) equipped with a diamond single-reflection accessory for attenuated total reflection measurements. Each spectrum was recorded in absorbance mode, and an average of 30 scans were recorded over the range 400–4000 cm$^{-1}$ at 1 cm$^{-1}$ intervals with a 4 cm$^{-1}$ resolution at room temperature at constant pressure. The contact force between the sample and the crystal differed due to the type of material. Five spectra were taken for each sample, and the data

**Fig. 5** Cotton sample after biocleaning
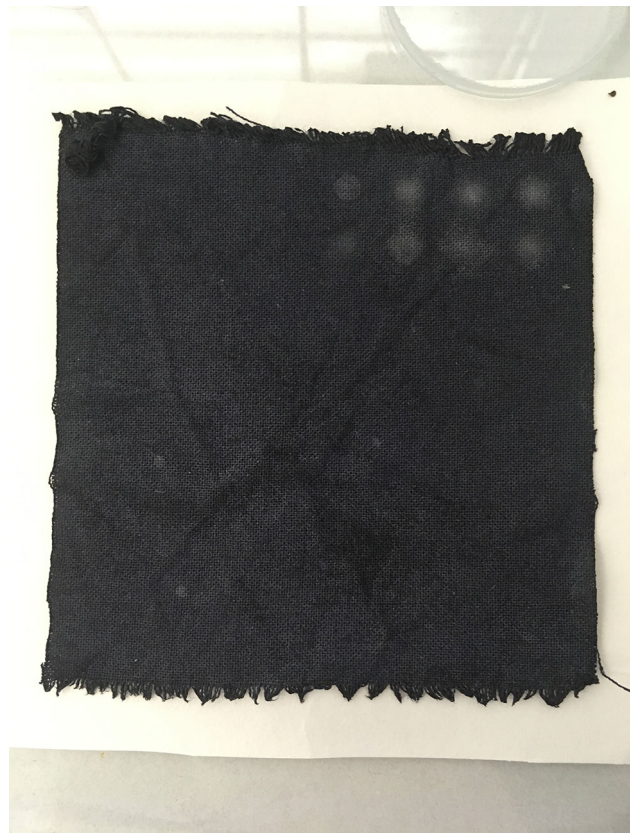


were acquired using Resolutions Pro FTIR Software by Agilent Technologies. The pre-processing of the obtained data consisted of de-noising followed by normalisation [42].

## 3 Results and discussion

### 3.1 Optical microscopy

An optical analysis of the surfaces of the specimens provided particularly useful information about the surface topography. The general appearance of the samples under a microscope revealed heterogeneous staining with iron gall ink that was locally impregnated deep within the fibres [43]. A varying absorption level was visible, due to tidelines caused by the evaporation of water from the iron gall ink stains upon drying [44]. In particular, the concentration of contaminant was higher at these locations, and formed a thick layer that glued the fibres. The images acquired are shown in Figs. 6 and 7.

### 3.2 Colour measurements

Colour measurements were used to determine the differences in the colour values at each location before and after biocleaning. These measurements gave objective, comparable results that did not depend on human perception or visual acuity. The aim of this analysis was to assess the effectiveness of the chosen method.

#### 3.2.1 Silk

*P. putida* showed the greatest colour change ($\Delta E^*$) when 3 ml of the suspension was gelled with agar and applied to a silk sample. The bacterial inoculum at 1 ml concentration gave the second highest $\Delta E^*$ of all the biocleaning methods employed, while the 2 ml suspension gelled with agar provided a similar $\Delta E^*$ to the agar surface inoculated with a spreader. The $\Delta E^*$ values demonstrate that differences in the bacterial performance depend on the method of application of the bacterial suspension to the surface. The indirect application of inoculated gels through barrier layer significantly reduced the bacterial performance.

**Table 2** Measured CIELAB colour values and calculated total colour change before and after biocleaning

| Material | Agar gel inoculated with *P. putida* | Δ(*L*\**a*\**b*) before cleaning | Δ(*L*\**a*\**b*) after cleaning | Δ*E* after cleaning | Δ(*L*\**a*\**b*\*) before cleaning through barrier layer | Δ(*L*\**a*\**b*\*) after cleaning through barrier layer | Δ*E* after cleaning through barrier layer |
|---|---|---|---|---|---|---|---|
| Silk | spreader | 23.43 ± 1.6 | 30.97 ± 1.2 | 8.4790 ± 0.8 | 23.64 ± 1.4 | 28.07 ± 1.2 | 5.4605 ± 1.8 |
| | | 2.88 ± 0.6 | 4.69 ± 0.9 | | 2.20 ± 0.7 | 4.36 ± 0.8 | |
| | | 5.65 ± 0.8 | 9.08 ± 0.7 | | 5.10 ± 0.6 | 7.46 ± 0.6 | |
| | 1 ml | 25.07 ± 1.0 | 33.61 ± 1.4 | 8.9423 ± 1.0 | 24.12 ± 1.6 | 30.49 ± 1.3 | 6.4946 ± 1.7 |
| | | 3.34 ± 0.3 | 4.76 ± 0.6 | | 3.44 ± 0.5 | 4.32 ± 0.7 | |
| | | 6.24 ± 0.5 | 8.48 ± 0.7 | | 6.43 ± 0.6 | 7.34 ± 0.5 | |
| | 2 ml | 25.27 ± 1.1 | 32.94 ± 1.1 | 8.3691 ± 1.1 | 27.11 ± 1.5 | 32.79 ± 1.4 | 5.8224 ± 2.1 |
| | | 3.43 ± 0.4 | 5.13 ± 0.7 | | 3.97 ± 0.4 | 4.73 ± 0.6 | |
| | | 6.45 ± 0.5 | 9.35 ± 0.6 | | 8.41 ± 0.7 | 9.44 ± 0.6 | |
| | 3 ml | 24.65 ± 0.8 | 33.67 ± 1.7 | 9.3232 ± 1.2 | 27.57 ± 1.1 | 31.97 ± 0.9 | 4.4078 ± 2.2 |
| | | 3.72 ± 0.6 | 4.73 ± 0.8 | | 4.32 ± 0.6 | 4.49 ± 0.7 | |
| | | 6.75 ± 0.7 | 8.49 ± 0.6 | | 8.94 ± 0.7 | 9.14 ± 0.5 | |
| Cotton | spreader | 26.99 ± 1.5 | 29.70 ± 1.1 | 3.9691 ± 0.9 | 24.17 ± 1.3 | 24.15 ± 1.4 | 2.1694 ± 0.9 |
| | | 1.54 ± 0.3 | 3.41 ± 0.4 | | 1.62 ± 0.2 | 2.86 ± 0.6 | |
| | | 4.34 ± 0.9 | 7.87 ± 0.8 | | 4.71 ± 0.8 | 6.49 ± 0.7 | |
| | 1 ml | 25.16 ± 1.2 | 28.53 ± 1.5 | 4.1338 ± 0.9 | 23.74 ± 1.2 | 26.37 ± 1.3 | 3.3413 ± 1.0 |
| | | 1.44 ± 0.2 | 3.36 ± 0.6 | | 3.25 ± 0.5 | 1.52 ± 0.3 | |
| | | 5.50 ± 0.8 | 6.93 ± 0.8 | | 5.94 ± 0.8 | 4.82 ± 0.8 | |
| | 2 ml | 22.99 ± 1.5 | 24.60 ± 1.2 | 3.3444 ± 1.0 | 24.38 ± 1.4 | 26.42 ± 0.9 | 3.4254 ± 1.1 |
| | | 1.51 ± 0.2 | 3.34 ± 0.3 | | 3.34 ± 0.6 | 1.45 ± 0.3 | |
| | | 4.34 ± 1.0 | 6.63 ± 0.5 | | 6.32 ± 0.7 | 4.32 ± 0.8 | |
| | 3 ml | 23.57 ± 1.1 | 25.42 ± 0.9 | 3.6264 ± 1.1 | 21.13 ± 1.2 | 23.65 ± 1.4 | 2.9994 ± 0.4 |
| | | 1.17 ± 0.1 | 3.42 ± 0.4 | | 1.61 ± 0.3 | 3.08 ± 0.7 | |
| | | 4.69 ± 0.9 | 6.85 ± 0.7 | | 4.07 ± 0.8 | 6.64 ± 0.9 | |

### 3.2.2 Cotton

With regard to the cotton samples, the highest values of Δ*E*\* were obtained with the 1 ml inoculums, followed by application via spreader and the 3 ml suspension. The 2 ml inoculum was the least effective, with the exception of the biocleaning conducted through the tissue isolating layer. Finally, application through Japanese tissue paper was the least efficient for both types of material. In addition, all of the gel systems investigated here were more effective on silk than on cotton. The cleaned surface became more yellow, which was confirmed by a raised value of *b*\* after biocleaning. The literature provides an array of possible explanations. The bacteria may have secreted organic acids which have a biocorrosive effect leading to discoloration [45]. Pinna [3], on the other hand, describes a case of pink and yellow discolorations observed on wall-paintings of St. Botolph's Church in Hardham and attributes the cause to the carotenoids and their protective mechanisms associated with anti-oxidation against degradation influenced by photooxidation, which may have been the cause also in this study [46]. Yet another possibility is that found in Szulc [46]. The article demonstrated that due to the enzymatic degradation of cellulose, protein and starch compounds, *Pseudomonas* spp. is able to synthetize diffusible yellow pigments [47]. Furthermore, Loeschcke [47] highlights that *Pseudomonas* spp. is reported to have the carotenoid biosynthesis gene [48]. Thus, the secretion of the yellow pigment might have been observed as a result of the biosynthesis. In addition, the value of *a*\* increased due to partial removal of the iron gall ink corrosion products. A similar effect in terms of the efficacy of the bacteria was achieved on the cotton samples.

The Δ*E*\* values with their standard deviations show that there were differences in the efficacy of biocleaning depending on the method of application of the bacterial suspension to the substrates. In general, the method was efficient for both types of materials tested. By analysing the data acquired from all three colour parameters, it can be seen that the lightness (*L*) increased in all cases. The colour became lighter after biocleaning except for the staining on the cotton specimens, which did not undergo notable changes in colour [49]. This can clearly be seen from Table 2. Although the experiments were carried out three times, it was not possible to draw definitive conclusions on how each inoculated gel system affected the process, as the results were not coherent for all samples [50]. Future studies should focus on this specific behaviour in order to validate the colorimetric measurements reported here.

**Fig. 6** Optical microscopy observation of artificially stained cotton specimen
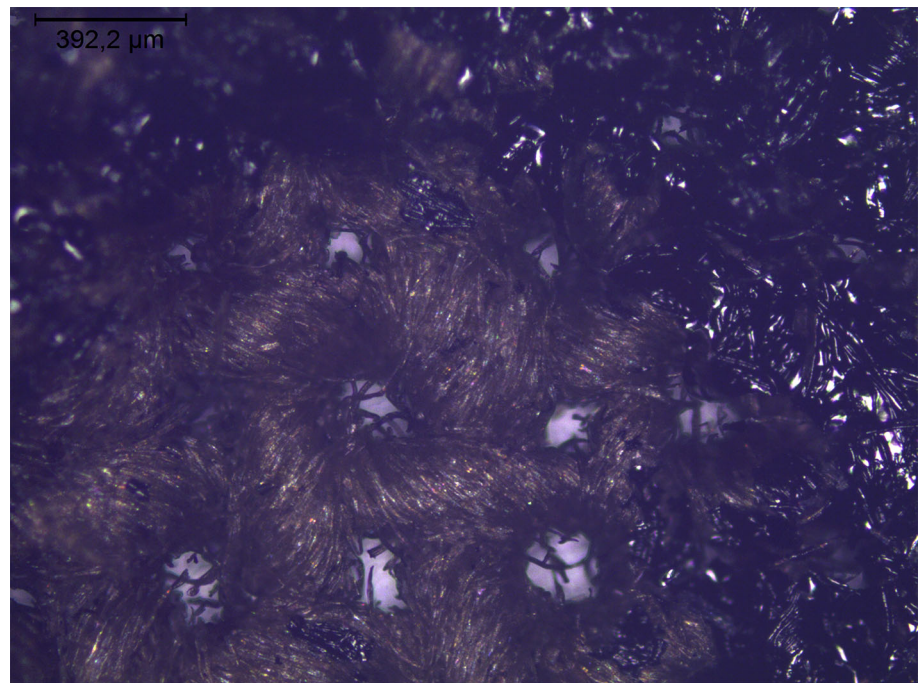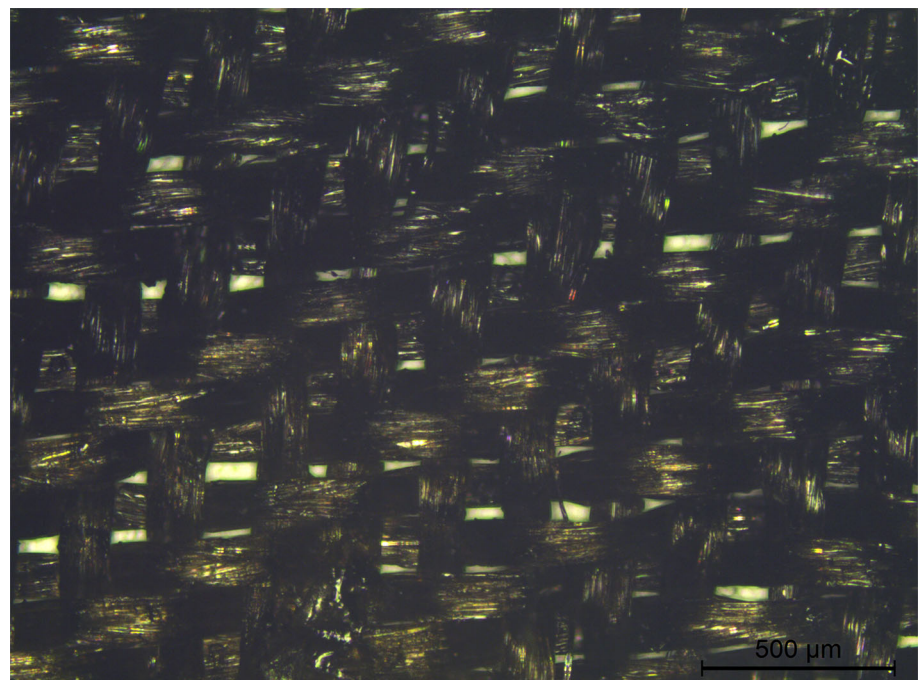


**Fig. 7** Optical microscopy observation of artificially stained silk sample



### 3.3 FTIR with ATR analysis

The aim of employing FTIR measurements in this research was twofold: Firstly, these can highlight the molecular structure of the model samples and any alterations occurring due to accelerated ageing, which would indicate a particular degradation process; and secondly, they were employed to evaluate the biocleaning performance of the selected bacterial strain. The first set of analyses examined the impact of accelerated ageing on untreated model materials. To characterise these changes, the spectra from the model textiles were correlated with those of aged materials. The correlation tables given below list the characteristic absorption bands of the relevant functional groups [51]. Due to space constraints, we cannot provide a comprehensive review of all spectra acquired in this step; however, the overall changes described above can be seen in Figs. 8 and 12, corresponding to the untreated specimens. In general, these spectra provide clear evidence of the occurrence of degradation processes during thermo-oxidative ageing.

**Fig. 8** Comparison of ATR-FTIR spectra obtained for silk before (blue line) and after accelerated ageing (red line)
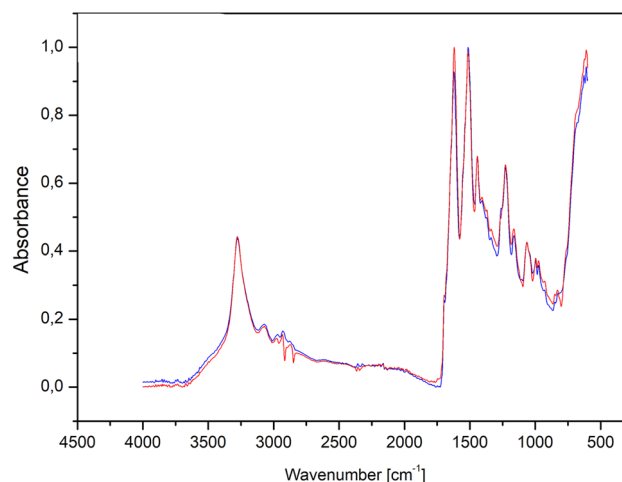


### 3.3.1 Silk

In the FTIR spectra for artificially aged silk specimens, we see a band corresponding to a free carbonyl group that is intensified compared to the untreated sample. This can therefore be used to assess the rate of degradation. In particular, free carbonyl groups appearing at 1700–1775 cm$^{-1}$ are characteristic of both aged and untreated silk, and the appearance of vibrational bands in this region indicate the formation of free carbonyl groups. This range is typical of oxidation reactions in both natural and synthetic polymers. Furthermore, due to the process of hydrolysis of the protein, an increase in the absorption band at 1650 cm$^{-1}$ was observed that corresponded to –OH bending in the amide I region (1600–1700 cm$^{-1}$). All of the compared spectra show modification of the distinctive band correlated with amide I. A characteristic band associated with the C=O stretching vibration was recorded at about 1615 cm$^{-1}$, which directly corresponds with protein conformation in the amide I region [52]. However, subtle changes in conformation appeared in both the amide II (1480–1580 cm$^{-1}$) and amide III (1200–1300 cm$^{-1}$) regions [12, 17]. The ratio of absorbance associated with decrease of crystallinity [43] has changed which was visible in the spectra. Detailed examination of the degradation markers of fibroin in silk described by Koperska [43] showed that crystallinity is determined by variations in the absorbance intensity ratio at 1620 and at 1656 cm$^{-1}$, or at 1620 and 1699 cm$^{-1}$. Oxidation was characterized by ratio of absorbance at 1620 and at 1514 cm$^{-1}$, and depolymerization—at 1318 and at 1442 cm$^{-1}$. As noted by the authors in their analysis of the degradation markers, hydrolysis and oxidation have the most meaningful influence on fibroin degradation upon accelerated ageing. In the acquired FTIR spectra, the variations might be observed mainly in the bands linked to depolymerization. Furthermore, the analysis of the spectra illustrates the intensification of oxidation. The spectra acquired after accelerated aging of silk are shown in Fig. 6.

Further analyses were carried out to examine the impact of iron gall ink staining on silk substrates. Spectra for silk stained with iron gall ink were collected and correlated with those of aged model samples. Common strong bands in the range 1620–1600 cm$^{-1}$ were found on stained silk samples that were assigned to overlapping between tannins and amide I. Reduced intensity of the vibrations characteristic of amides I and II due to hydrolysis was also observed. In contrast, the bands characteristic of hydrolysable tannins (1730–1700 cm$^{-1}$ and 1330–1320 cm$^{-1}$) overlapped with the hydrolysis of carbonyl groups and C–H bending in the amide III region, respectively. All of the other characteristic bands for tannins were clearly distinguishable, i.e. 1440–1450 cm$^{-1}$, 1300–1210 cm$^{-1}$ and 1050–1030 cm$^{-1}$. In addition, three distinctive bands were observed for gallotannins, which can be described as marker bands (1100–1000 cm$^{-1}$, 880–870 cm$^{-1}$ and 770–750 cm$^{-1}$). A further significant absorption band in the range 1300–1000 cm$^{-1}$ corresponding to ferrous sulphate was recorded [27]. Together with the large O–H stretching band at around 3340–70 cm$^{-1}$, these nine bands represent the production of iron gall ink staining [15], as shown in Fig. 8. After biocleaning, a comparison of the FTIR spectra for the stained samples revealed that the treatment process had no significant impact on the characteristic vibrational ranges of amides, although it did lead to a decreased intensity of the characteristic peaks for gallotannins [48]. A strong decrease was recorded in the vibrational band at about 1730 cm$^{-1}$, which was ascribed to gallic acid and free carbonyl moieties in silk. This can clearly be seen in Fig. 9. In 1951, Randall and co-workers were one of the first teams to investigate FTIR spectroscopy for studying of microorganisms [53]. Since the 1950s much more information on identification of microorganisms by FTIR spectroscopy has become available. A number of studies have found that FTIR is precise technique for identification of bacteria [54, 55]. The literature reports that *Pseudomonas* spp. bacteria cultivated in a minimal media gave unambiguous IR spectra [56]. More recent evidence highlights that analyses of microorganisms correlated to biodegradation of cultural heritage are being applied for the monitoring of microbial contamination [57]. Thus, the FTIR spectroscopy was employed for analyzation of microorganisms on cleaned surface of specimens [58]. Some minor differences in the spectra were observed depending on the cultivation medium, the particular species of *Pseudomonas* spp. and the method of biomass harvesting. However, no bacterial material was detectable, since there were no

**Fig. 9** Comparison of ATR-FTIR spectra collected for silk before (red line) and, after accelerated ageing (blue line) and, after accelerated ageing and staining (green line)
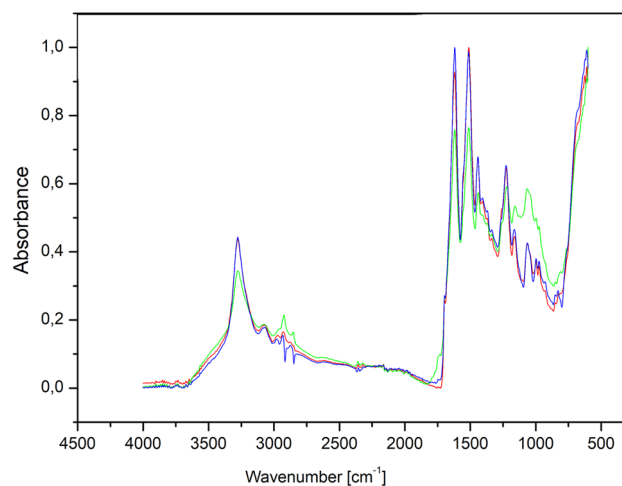


**Table 3** List of ATR-FTIR peaks characteristic of *Pseudomonas spp*

| Wavenumber (cm$^{-1}$) | Assignment |
| --- | --- |
| 3300 | H-bonded OH groups, NH$_2$ stretching (nucleic acids structures: adenine, quanine, cytosine) |
| 3000–2800 | aliphatic C–H stretching, cell membrane fatty acids |
| 1800–1500 | NH$_2$ bending, C=O, C=N stretching (amide I and II), cell proteins |
| 1500–1400 | C–H deformations of CH$_2$ or CH$_3$ groups in aliphatics, fatty acids |
| 1500–1200 | C-H bending, -CH3 stretch, proteins and phospholipids |
| 1200–900 | C–N stretching (amide III), C–O, PO$^-$2 stretching, glycopeptides and phosphate groups of nucleic acids |
| 900–550 | C–O–C, P–O–C, P–O–P bonding (RNA, aromatics, phospholipids), less defined constituents |

Assignments according to Filip and Hermann [35]

**Fig. 10** Comparison of ATR-FTIR spectra acquired for artificial stained silk before (black line) and after biocleaning (orange line)
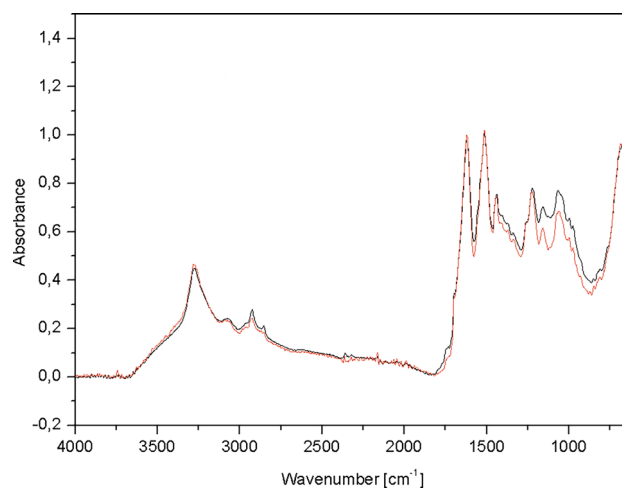


differences in the peak intensities of the distinctive bands for the cleaned samples. Since some of the characteristic peaks for the bacteria overlap those of amides, interpretation is not straightforward, although the difference in the peak intensities corresponding to iron gall ink and the lack of other bands characteristic of *Pseudomonas* spp. suggest that the biocleaning was successful and that no bacterial residues were left on the surface. In order to reduce the overlap from water bands, our analyses were performed 24 h after this treatment process, to allow for the evaporation of any water absorbed from the gel. The spectra acquired from the silk after biocleaning showed no signs of structural degradation of the fibres. Table 3 shows selected wavenumbers for the main vibrational bands for *Pseudomonas* spp. The spectra acquired after biocleaning are shown in Figs. 10 and 11.

**Fig. 11** Comparison of ATR-FTIR spectra obtained for artificially stained cotton before (black line) and after biocleaning (orange line)
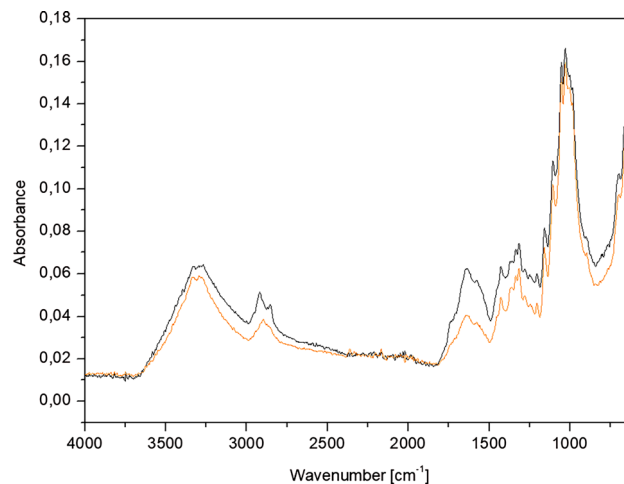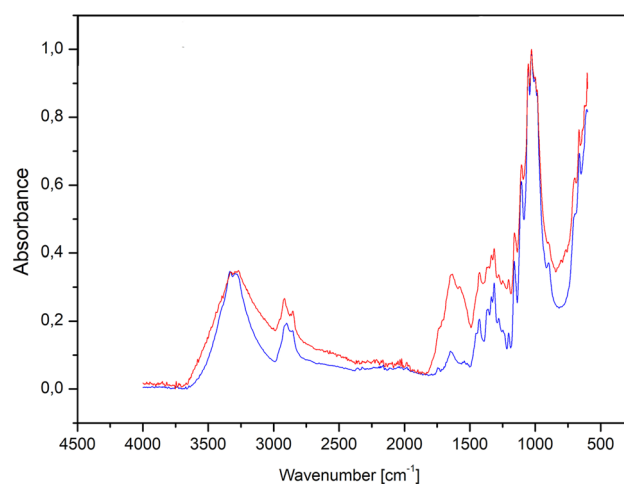


**Fig. 12** Comparison of ATR-FTIR spectra collected for cotton before (blue line) and after artificial staining (red line)



### 3.3.2 Cotton

FTIR analyses of the reference and aged cotton fibres mainly revealed characteristic peaks for the substrata [59]. The compared spectra were similar, and contained all marker bands corresponding to the characteristic constituents of cellulose. These bands are at $2900$ cm$^{-1}$ (related to C–H stretching vibration and ascribed to polysaccharides), $1635$ cm$^{-1}$ (attributed to water bonded within the fibres), $1365$ cm$^{-1}$ (associated with the C–H bond in cellulose), $1335$ cm$^{-1}$ (representing the CH$_2$ bond in cellulose), $1155$ cm$^{-1}$ (corresponding to C–C vibration, which is indicative of polysaccharides in cellulose), $1105$ cm$^{-1}$ (assigned to the (C–O–C) glycosidic ether band), and bands correlated to polysaccharide components, such as $1025$ cm$^{-1}$ (connected with the C–OH vibration of the alcohol bond in cellulose) and $895$ cm$^{-1}$ (corresponding to the C–O–C bond in cellulose) [60–62]. No specific deformations or fluctuations were observed in these bands after ageing. The typical signals at about $1600$–$1700$ cm$^{-1}$, assigned to the C=O stretching region, are related to hydrolysed and highly oxidised cellulose, and are characteristic of aged substrata [63]. Peaks corresponding to carbonyl vibrations at the ends of chains are related to depolymerisation [64].

In the next step, spectra for cotton stained with iron gall ink were collected and correlated with those of aged model fabrics. Unlike the silk specimens, all of the nine bands characteristic of iron gall ink were clearly observed [15]. Particularly notable were strong bands at about $1620$–$1600$ cm$^{-1}$ (attributed to gallotaninns) and peaks that were characteristic of hydrolysable tannins ($1730$–$1700$ cm$^{-1}$ and $1330$–$1320$ cm$^{-1}$). Please refer to Fig. 12. The intensities of the distinctive cellulose bands were slightly reduced.

The next set of analyses examined the samples after biocleaning. These measurements revealed considerable shifts in the vibrations attributed to both cotton and staining. All of the nine marker peaks were shifted, although the intensities of the vibrational bands typical of gallotannins ($1730$–$1700$ cm$^{-1}$, $1620$–$1600$ cm$^{-1}$, $1440$–$1450$ cm$^{-1}$ and $1300$–$1210$ cm$^{-1}$) were the most significantly reduced. The analysis was also used for microbial control. The spectra collected after biocleaning shows no characteristic bacterial peaks, which confirms the absence of *Pseudomonas* spp. Table 3 lists these typical wavenumbers for selected bacteria. Biocleaning effectivity and lack of microbial activity can clearly be seen in Fig. 13.

**Fig. 13** Comparison of ATR-FTIR spectra acquired for artificially stained cotton before (black line) and after biocleaning (orange line)
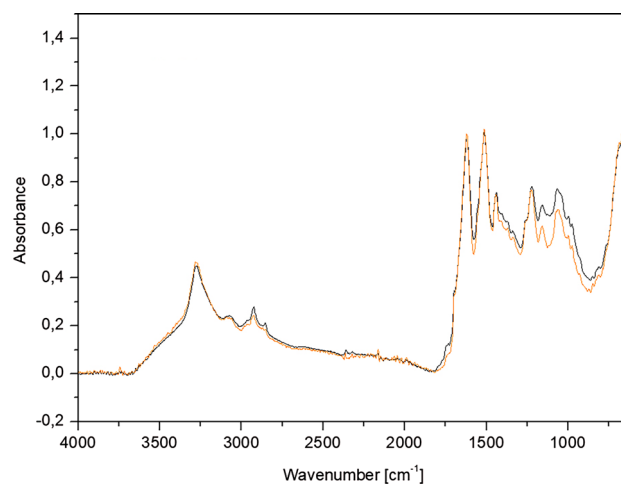


## 4 Conclusions

In this paper, a biocleaning procedure for historical textiles using microorganisms was investigated. Firstly, the reduction of iron gall ink staining using viable biomass in a gel carrier was evaluated. The results of off-site laboratory experiments carried out to evaluate this particular method were reported. The study represents progress beyond the current state of knowledge, and the approach is shown to have advantages over traditionally established methods based on destructive and challenging chemical interventions. Observations made using an optical microscope and colour and FTIR analyses demonstrated that the selected culture did not degrade the substrate material, and can therefore be considered safe and gentle for the treated object. The results show that under these experimental conditions, the monitored direct, short-term (4 h) application of *P. putida* in a multilayer biosystem based on agar gel can be effective in reducing iron gall ink deposited on textile surfaces. This can be noticed in Figs. 10, 11, 12 and 13. The performance of the bacteria was found to be influenced more strongly by the method of contact with the surface than their viability, and the application of gels on top of Japanese tissue paper did not demonstrate substantial difference. The use of a multilayer gel system limits the risks to the substrata by allowing the contact time to be monitored and any excess water to be eliminated, thus minimising the possible consequences of leakage. Hence, the concentration of the gel should be tested in advance. The qualitative results prove that the efficiency with regard to the biocleaning performance depends strongly not only on the type of staining but also on the type of material tested and the physical contact between the biosystem and the stained surface, as reported by Bosch-Roig [8]. The efficiency of the biocleaning treatment proved to be satisfactory. To evaluate the approach, we used a variation of the established procedure in which FTIR spectroscopy was applied rather than scanning electron microscopy. A preliminary optical microscope study of the topography of staining was confirmed by both colour measurements and FTIR spectroscopy [3, 5]. The acquired spectra proved that the intensity of the ratio of absorbance in degradation markers characteristic for silk fibers has not changed and no residues have been left on the surface, nor were any signs of microbial activity noticed after biocleaning treatment. FTIR showed that the selected bacterial strain was able to remove the staining to some extent [65], and a significant cleaning effect was evident for silk. It is possible that the cotton samples were not aged sufficiently. However, the overall appearance of both model materials was improved, even though the undesired particles were not removed completely. This study represents the first successful application of a cleaning biosystem based on *Pseudomonas putida* 2082 in the removal of staining caused by iron gall ink on textiles. This is an important issue for future research on bacterial activity against iron gall ink and its methods of application, and on a wider level, the applicability of biotechnology to textile conservation. The evidence from this study can form a starting point for the application of biocleaning to textile conservation. The results are promising, and should be validated using a larger surface and different accelerated ageing procedures. Further experimental investigations could be focused on the impact of staining procedure as well as application of bacterial culture and culture selection for biocleaning effectivity.

Presented study could be beneficial as a model treatment for on-site interventions. Further work needs to be carried out to establish whether FTIR spectroscopy is sufficiently sensitive to become a useful tool in terms of the differentiation of bacteria [54]. More broadly, research is also needed to determine other microbial cultures that might be effective against iron gall ink staining. The prospect of being able to remove iron gall ink staining from historical textiles serves as a continuous motivation for future work.

**Data Availability Statement** This manuscript has associated data in a data repository [Authors' comment: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.].

**Code availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare that this article content has no conflict of interest.

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