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Microbial biotechnology for sustainable preservation of cultural heritage: Bio-cleaning and self-healing preservation materials

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ABSTRACT

Microbial biotechnology offers innovative solutions for the conservation and restoration of cultural heritage (CH) materials, addressing biodeterioration and the limitations of traditional methods. This study explores the application of microbial biotechnology for bio-cleaning and self-healing preservation of heritage structures. Bio-cleaning refers to the use of microorganisms to selectively remove unwanted deposits such as dirt, biofilms, and encrustations, while self-healing preservation involves microbial-induced processes that enhance material durability by filling micro-cracks and improving surface integrity. These eco-friendly techniques offer promising solutions for sustainable conservation. Traditional methods often fail to prevent biofilm overgrowth and structural weakening, but advancements in microbial technologies present new approaches that use harmless microbes and avoid hazardous chemicals. Multidisciplinary collaborations are integrating microbial enzymes, bio-cleaning agents, and self-healing materials tailored to each artefact's unique composition. The study highlights microbial biotechnology's role in mitigating climate change impacts on CH preservation. While microbial biotechnology offers sustainable conservation solutions, potential risks include unintended interactions with heritage materials. Challenges such as microbial ecosystem complexity, lack of long-term studies, and regulatory uncertainties may impact its effectiveness, necessitating further interdisciplinary research. Despite these challenges, microbial enzymes and bio-cleaning agents can reduce costs and labor. The environmentally friendly nature of these methods ensures sustainable CH conservation. This paper emphasizes the potential of interdisciplinary collaboration in developing biotechnological solutions to address biodeterioration and climate change, enhancing the preservation of CH.

Keywords: microbial biotechnology, cultural heritage preservation, bio-cleaning, self-healing preservation, biodeterioration, multidisciplinary collaboration

INTRODUCTION

Heritage conservation stands as a pressing global concern, spanning continents and encompassing artifacts of profound cultural significance. In response, conservation scientists are at the vanguard of pioneering research, striving to refine practices with universal applicability. Against the backdrop of accelerating climate change, which poses an existential threat to cultural artifacts worldwide, researchers are fervently exploring solutions to safeguard our shared heritage. Despite their typically low nutrient content, artifacts harbor intricate microbial ecosystems, with microbial growth substrates permeating diverse materials such as textiles, paper, wood, and stone. These microbial communities, often overlooked until biofilm proliferation occurs, can contribute to discoloration and structural decay, ultimately diminishing the cultural and monetary value of artifacts. The imperative for efficient, non-invasive conservation techniques is underscored by the limitations and risks associated with traditional physico-chemical approaches.

Recent advancements underscore the need for a paradigm shift in conservation practices, particularly in integrating microbial biotechnology for preserving cultural heritage (CH). Studies have elucidated the complex microbial interactions within cultural artifacts, shedding light on potential mitigation strategies (Gadd et al., 2024; Mendoza et al., 2023).

However, the application of these techniques faces challenges, including microbial ecosystem complexity, lack of long-term studies, and regulatory uncertainties, necessitating further interdisciplinary research. The durability and stability of microbial-based treatments over extended periods remain uncertain. The interaction of introduced microbes with native microbial communities on heritage surfaces is not fully understood, leading to potential risks of unintended biological activity. While laboratory studies highlight the potential of microbial biotechnology, its transition into commercially viable, ready-to-use solutions for large-scale conservation projects is still underdeveloped.

One of the major concerns in CH preservation is the impact of climate change, which accelerates biodeterioration and alters conservation strategies. Researchers have increasingly explored innovative approaches to counteract these effects (Fidanza & Caneva, 2019; Nilson & Thorell, 2018). Biotechnologies, such as biological cleaning of CH artworks, have seen significant advancements and optimization over the past two decades. For instance, recent research highlights their application in restoring historic manuscripts by removing biological contaminants and degradation products (David et al., 2020).

While biocides remain a widely used method for mitigating biodeterioration, their indiscriminate application poses ecological and health risks. To address these concerns, recent studies have focused on developing targeted biocide formulations with enhanced efficacy and minimal environmental impact (Chelazzi & Baglioni, 2023; David et al., 2020). Moreover, the integration of biocides with emerging conservation technologies, such as nanomaterial-based coatings, holds promise for achieving comprehensive preservation outcomes (Camargos et al., 2022).

Similarly, bio-cleaning techniques tailored for preserving wooden artifacts have gained attention. Ranalli and Zanardini (2021b) demonstrated the efficacy of enzymatic treatments in combating fungal colonization and insect infestation. Unlike harsh chemicals, enzymes such as laccases and cellulases selectively degrade biological contaminants while preserving wood integrity, demonstrating effectiveness in cases like mold removal from historic furniture and biodegradation of insect residues. These studies underscore the diverse applications of microbial biotechnology in addressing specific types of artifacts and alterations, enhancing the credibility and effectiveness of CH preservation efforts (Ranalli & Zanardini, 2021a).

Despite these advancements, there remains a limited understanding of microbial biotechnology's efficacy in bio-cleaning and self-healing preservation. This study identifies critical research gaps in conservation evaluation and provides a literature-based foundation to inspire further exploration of microbial conservation techniques. The research also highlights the underutilization of evaluation methodologies in conservation, particularly in the context of bio-cleaning and self-healing processes.

The primary aim of this study is to explore the role of microbial biotechnology in the conservation and restoration of CH materials, focusing on bio-cleaning and self-healing preservation techniques as sustainable alternatives to traditional conservation methods. This paper highlights recent advancements, challenges, and future prospects in microbial biotechnology for CH conservation. Given the limited availability of literature on conservation assessment, this study contributes by reviewing existing evaluation theories applied in CH conservation while emphasizing the need for more structured assessment frameworks.

IMPACT OF MICROBIAL BIOTECHNOLOGY IN PRESERVATION OF CULTURAL HERITAGE

Microorganisms, often referred to as "virtuous" microorganisms, have shown promise in addressing alterations on CH materials and artworks. These microorganisms offer innovative approaches to CH preservation, complementing or even replacing traditional restoration methods that may be ineffective, harsh, or hazardous. The role of microbial biotechnologies has expanded significantly, introducing sophisticated bio-based solutions for conservation.

For example, Alfano et al. (2011) demonstrated that microbial biotechnologies effectively remove nitrate and sulfate salts, black crusts, and altered organic compounds from paintings by employing specific bacterial strains capable of bioreduction and biosolubilization. These microbes metabolize harmful deposits, converting them into harmless byproducts without damaging the underlying artwork, making them a promising tool for CH preservation.

In addition to microbial activity, enzymes also play a crucial role in biorestoration by selectively breaking down substances with minimal risk compared to traditional chemical treatments. For instance, laccases and cellulases degrade organic contaminants on paper and wood without damaging the substrate, while proteases help remove protein-based stains from historical textiles. These targeted enzymatic treatments help minimize material deterioration compared to conventional chemical methods.

CH encompasses a diverse array of objects crafted from materials such as metal, stone, pottery, organic matter, paper, and synthetic constituents, housed in both indoor and outdoor environments. According to Berenguer et al. (2019), all artworks undergo chemical and physical deterioration over time, along with biological attacks where biodeteriogens pose significant risks, particularly in environments conducive to microbial growth. Various lithotypes exhibit differences in strength, permeability, solidity, and absorption, which influence microbial surface colonization and bioreceptivity. As microbial growth on stone surfaces alters their properties and aesthetics, biofilm formation can impact pore size, water absorption (capillarity), and temperature responsiveness. While biofilms are often associated with deterioration, under specific environmental conditions, some microbial communities may form protective layers, potentially reducing further degradation (Singh & Yadav, 2023). **Table 1** describes the delivery system for stone surfaces and fresco bio-cleaning by microorganisms.

GENERAL ASPECTS OF BIO-CLEANING TREATMENTS

Microbes are abundant in the biosphere due to their ability to adapt and thrive in harsh conditions. Bacteria, archaea, and microbial eukaryotes have a wide range of energy and carbon sources.

Table 1. Summary of the delivery system for stone surfaces and frescos bio-cleaning

Delivery system	Decay agent	Types of materials	Used microorganisms	Types of metabolism	Time (h)	REE (%)	References
Immersion	Sulfates & lichens	Marble (Georgia) stone and statue (USA), marble and sandstone (D), & concrete (B)	D. desulfuricans, D. vulgaris, & thiobacillus sp.	Anaerobic, anaerobic, & aerobic	60-84, ns, & 9 days	80, 100, & 40 ns	De Belie and Wang (2016), de Graaff et al. (2020), Gauri and Chowdhury (1988), Gauri et al. (1992), & Heselmeyer et al. (1991)
Sepiolite	Sulfates, black crusts, & bitrates	Marble (I), marble (candoglia stone) (I), & brick-works and calcareous stone (I) (marble and vicenza-stone)	D. desulfuricans, D. vulgaris, D. vulgaris sub.sp vulgaris, & P. stutzeri	Anaerobic, anaerobic, & anaerobic	36, 45, & 30	81, 98, & ns	Cappitelli et al. (2006) & Ranalli et al. (1996, 1997)
Hydrobiogel-97	Black crust	Marble (candoglia stone) (I)	D. vulgaris subsp. vulgaris	Anaerobic	7 days	28	Cappitelli et al. (2006) & Ranalli et al. (2000)
Cotton wool	Animal glue	Fresco, Pisa (I)	P. stutzeri A29+Protease	Aerobic	2-12	80-100	Antonioli et al. (2005), Lustrato et al. (2012), Ranalli et al. (2019)
Carbogel	Animal glue and salt efflorescence, black crust, & nitrate and sulfate	Frescos, Valencia (E), marble (candoglia stone) Milas (I), limestone sculpture, trento (I), coloured lithotypes, firenze (I), sandstone wall, & matera (I)	P. stutzeri, D.vulgaris subsp.vulgaris, P. pseudoaloalignes, & D vulgaris	Aerobics, anaerobic, & aerobic/ anaerobic	1.5-3.0, 45, 12, 36, 30-40, & 24-72	60, 98, ns, ns, ns, & 55.85	Alfano et al. (2011), Bosch-Roig et al. (2014), Cappitelli et al. (2005, 2006, 2007), Gioventù et al. (2011), & Polo et al. (2010)
Mortar and alginate beads	Nitrates	Sandstone walls & matera(I)	P. pseudoaloaligenes	Aerobic/ anaerobic	1 month	ns	May et al. (2008)
Agar	Animal glue and salt efflorescence	Frescos, Valencia (E)	P. stutzeri	Aerobic	1.5-2.0	92	Bosch-Roig et al. (2013a, 2013b, 2021)
Arbocel	Grey deposits and black crust	Marble column and statue, Cemetery of Milan (I)	D. vulgaris subsp. vulgaris	Anaerobic	68-110	ns	Troiano et al. (2013)

Note. REE: Removal efficiency evaluation & ns: not shown

Microorganisms require energy, water, nitrogen, carbon, and trace elements (from light or organic/inorganic molecules) for their survival. Carbon sources are particularly crucial for the survival and competition of heterotrophic bacteria. Microbes play a vital role in closing the organic matter cycle by mineralizing and degrading complex organic compounds such as carbohydrates, proteins, lipids, and cellulose.

Due to their ability to produce enzymes, carefully chosen microorganisms can be employed in CH cleaning, particularly in situations where the materials that need to be removed are complicated and worn and traditional procedures can be too invasive or harsh for the stone material, inspired the first innovative bio-cleaning study (Atlas et al., 1988). The effectiveness of bio-cleaning methods in CH preservation, particularly for complex and weathered substances, was inspired by the ability of appropriately selected microbes to produce enzymes. Abdel-Raouf et al. (2012) conducted a study to evaluate the effectiveness of bio-cleaning methods by identifying suitable bacterial strains and optimizing biotreatment protocols. Their research demonstrated that specific bacterial strains could selectively degrade unwanted deposits without damaging the underlying material. The study also highlighted key parameters such as the choice of bacterial carriers, treatment duration, and safety considerations, emphasizing the economic feasibility of microbial-based conservation techniques. These findings underscore the potential of bio-cleaning as a viable alternative to conventional chemical methods in CH preservation. These elements play a crucial role in influencing the bio-cleaning process. Palla and Barresi (2017) noted that biorestoration bacteria can be derived from the native microbial ecology of artwork, offering a targeted and ecologically sustainable approach to conservation. Utilizing indigenous microbial communities ensures better adaptation to the artwork's material composition and environmental conditions, reducing the risk of unintended damage or contamination. These native bacteria can effectively metabolize harmful deposits such as salts, biofilms, and organic residues while maintaining the integrity of the underlying structure. This method aligns with modern conservation strategies that prioritize minimal intervention and compatibility with the original materials of CH objects. International microbial culture collections prioritize non-pathogenic microorganisms to streamline isolation processes. However, given that the altered artwork does not reflect their actual surroundings, their efficacy as bio-cleaning agents needs proper validation. Various bacteria, including nitrate-reducing bacteria, sulfate-reducing bacteria (SRB), and organic substance-degrading bacteria, have been employed in bio-cleaning treatments on artworks. Nitrate-reducing bacteria play a crucial role in the removal of nitrate salts, which contribute to salt crystallization and surface deterioration in CH materials. These bacteria facilitate the conversion of nitrates into nitrogen gas through a denitrification process, effectively reducing salt accumulation without causing harm to the substrate. Studies have demonstrated their effectiveness in mitigating salt-induced damage on frescoes, stone surfaces, and historical artifacts, making them a valuable tool in conservation efforts.

Water plays a vital role in bio-cleaning techniques as it sustains the life and activity of microorganisms, whether free or in a water-based gel. In art conservation, water is essential for formulating solutions and cleaning processes. However, water diffusion can induce chemical, physical, or mechanical changes in porous, sensitive, or surface-modified materials like holes, craquelures, and lacunas. To mitigate such effects, it is crucial to control the addition and utilization of water in formulations, as highlighted in the study (Palla & Barresi, 2017).

Bio-cleaning requires water with high surface tension and low wetting power. To increase water viscosity, mineral or organic substances like cellulose chemical pulp, sepiolite, or cellulose ethers can be added. These low-cost and durable materials have adhesion limits of 10-15%. Additionally, polyacrylic acid byproducts such as carbogel and carbopol have been projected as delivery systems at concentrations of 1-15% (w/v), producing viscosities that are over 40-50 times greater than those of cellulose ethers. These derivatives offer benefits such as transparency, no color changes on materials, and informal removal post treatment (Banik, 2003). In bio-cleaning trials, restoration practice must begin with an initial characterization step. This involves analyzing the physical-chemical material composition, mineralogical qualities, degree and extent of artwork decay, and deterioration mechanisms (Barabesi et al., 2006). The stepwise procedure applied for bio-cleaning treatment:

1. Screening microbial strains for removal capability (isolating and/or using collections).
2. Diagnosing and describing artwork alterations.
3. Administering lab tests to determine the most efficient microbial strain and evaluate removal efficiency (considering safety, efficacy, treatment adaptation, etc.).
4. Employing a suitable delivery method for transporting viable microbial cells; evaluating the colonization time of the delivery system and improving microbial metabolism conditions.
5. Conducting bioapplication tests utilizing viable cells and delivery mechanisms on artificially supplemented specimens and authentically modified fragments.
6. Optimizing key environmental factors.
7. Monitoring cell capability and action during bioapplication.
8. Removing the bioapplication and cleaning surfaces that were treated.
9. Implementing therapy, there will be short-, medium-, and long-term surveillance and monitoring.
10. Evaluating budgets.

CULTURAL HERITAGE AND AIR POLLUTION

The main cause of outdoor artefacts disintegration is air pollution, which causes material alteration as well as surface crusts and deposits. In the presence of water, inorganic pollutants such as sulfur oxides (SO_2 and SO_3) cause "when calcium carbonate and calcareous materials react, acidic solutions produce calcium sulfate dehydrate." As a result, a deterioration process known as 'sulfation' occurs, resulting in the creation of a thick 'black crust' (Barbabietola et al., 2016). The following are the chemical reactions involved in stone sulfation:

$2SO_2+O_2$---$2SO_3$

SO_3+H_2O---H_2SO_4

$H_2SO_4+CaCO_3$---$CaSO_4(2H_2O)+CO_2$

Barresi et al. (2015) demonstrated that SRB can effectively remove sulfates from masonry under anaerobic conditions. SRB convert gypsum into Ca^{2+} and SO_4^{2-} ions, with the sulfates then being reduced into H_2S (Heselmeyer et al., 1991). The Ca^{2+} ions subsequently reacted with CO_2 to create fresh calcites through a process called bioprecipitation:

$6CaSO_4+4H_2O+6CO_2$---$6CaCO_3+4H_2S+2S+11O_2$

Stone nitration, a process involving the process by which calcium carbonate and nitric acid combine to create, is another alteration induced by inorganic pollutants in the atmosphere. In addressing this issue, nitrate reducers, which are facultative anaerobic nitrogen cycle-related prokaryotes, have been investigated for their ability to remove nitrates from stone works (Webster & May, 2006). These microorganisms can reduce nitrates to N_2 in anaerobic environments and utilize nitrates in their metabolism as an electron receptor. The conversion of nitrate to N_2 can be outlined as follows:

$NO_{3-} \rightarrow NO_{2-} \rightarrow N_2O \rightarrow N_2$

ENZYMES IN BIO-CLEANING

Cleaning in CH conservation aims to remove undesired dirt deposits and incrustations from artwork surfaces, particularly those exposed to outdoor conditions over time. Using living bacterial cells and/or enzymes presents fewer risks to art, human health, and the environment. Microbes possess the ability to adapt to various environments and employ diverse metabolic pathways to transform substrates and synthesize enzymes.

Enzymes find applications in food, textiles, pharmaceutical, chemical, leather, waste management, and CH cleaning industries. Compared to chemicals, enzymes are more selective and gentle cleaners (Bellucci & Cremonesi, 1994). They play a crucial role in accelerating chemical reactions, particularly in the hydrolysis and removal of restoration substrates. Commonly used enzymes include amylases, proteases, and lipases, which are effective in breaking down substances like casein, egg, animal glues, and gelatins (Bellucci & Cremonesi, 1994).

Predominantly sourced from bacteria or fungi, proteases are the most commonly employed enzymes. They are categorized based on their acidic-basic characteristics, functional groups, and peptide bond locations. Among them, the extracellular alkaline protease produced by bacillus species (EC.3.4.21-24.99) exhibits high activity and efficacy. Beltrani et al. (2015) explored the use of enzymes for cleaning various CH artifacts such as wood, paper, sculptures, ceramics, mural paintings, and metals. Beltrani et al. (2015) investigated the type of enzymes, optimal working conditions including pH and temperature, as well as the application methods.

Mazzuca et al. (2014) presented an alternative method for removing starch paste from paper supports, employing stiff hydrogel. Mazzuca et al. (2014) put the gellan hydrogel containing the α-amylase enzyme to the test on a number of starch paste-soiled paper samples. The outcomes showed a considerable decrease in administration time, a more regulated water release to the artwork, and an easy and non-invasive formulation application and removal process.

Microbial Metabolism in Conservation

Despite their potential to damage man-made objects, microbes can also play a role in maintaining stability and even aiding in the preservation of CH. Microbial activities such as bioweathering, biodeterioration, and biocorrosion can harm building materials and historic monuments, leading to significant economic losses, such as the replacement of cast-iron water pipelines. However, microbial metabolic pathways may also contribute to maintaining stability and extending the lifespan of construction materials, thereby making cities more sustainable.

Microbial biotechnology holds promise in the preservation of CH. Microbial metabolisms can protect buildings and cultural property, and organic coatings applied to inorganic substrates, such as metal sculptures, are a common method for conserving and restoring them. However, these coatings create a physical barrier that may eventually deteriorate over time (Valentín & Fazio, 2020).

Panella et al. (2019) reported that numerous bio-cleaning treatments were conducted to remove sulfates from murals by employing SRB as bio-cleaning agents. Mazzoli et al. (2018) from Palatine Hill, Rome, Italy suggested that more recent studies have primarily emphasized the usage of synthetic clay-like materials colonized with cellulose-producing bacteria to remove carbonate and calcium sulfate.

Lustrato et al. (2012) also reported the restoration of the Camposanto Monumentale frescoes during the same period. Lustrato et al. (2012) utilized viable Ps. stutzeri A29 strain cells on the surfaces in front and back, which allowed for the retrieval of many murals and their restoration on the former wall structure. A single bio-cleaning treatment lasting 3 hours was employed to get rid of animal glue. Other altered frescoes stored at Monumental Cemetery Camposanto (Pisa) and Campaldo Laboratories (OpaPisa) have recently undergone bio-cleaning procedures on both the front and back surfaces to remove organic matter such as glue and casein (Antonioli et al., 2005).

Bosch-Roig et al. (2019) introduced a new bio-cleaning protocol using Ps. stutzeri DSMZ 5190 strain immobilized into agar gel as a delivery system, instead of cotton and carbogel, to clean nitrate salt efflorescence. This protocol achieved shorter bio-cleaning contact times of 90 minutes. Using ionic chromatography, the biological treatment's efficacy was observed, which revealed a 92% drop in nitrates.

Advanced Biotechnologies in Cultural Heritage

Berenguer et al. (2019) continued the bioremoval of black crusts, aiming to improve the procedure by utilizing pre-washed sepiolite colonized by desulfovibrio vulgaris (ATCC 29579 strain) to minimize the risk of color variations on the artwork surface caused by the existence of iron precipitates or their release. For the chemical aspect, 10 ml of surfactant, 30 g of EDTA, and 50 g of ammonium carbonate were combined, while live D. vulgaris cells were suspended in Carbogel and mixed for a few minutes physiologically.

Alfano et al. (2011) conducted bio-cleaning on wall murals, during which they extracted apatite, aged proteinaceous materials, nitrate, calcium carbonate, gypsum, and weddellite from the bottom loggia of the casina farnese's wall paintings. They reported that

> "After six years of biotreatment using Ps. pseudoalcaligenes KF707 and D. vulgaris ATCC 29579 in carbogel to remove nitrates and sulfates, a monitoring investigation verified that the treated regions had constant nitrate concentrations, no microorganisms, and the stone surface did not differ in color from the untreated sections" (Alfano et al., 2011).

Comensoli et al. (2017) employed desulfitobacterium hafniense as a bio-cleaning agent in another study aimed at stabilizing corroding iron archaeological objects without yielding any detrimental waste products. This introductory bio-cleaning study suggests a viable substitute stabilizing technique for treating iron artwork corrosion (Comensoli et al., 2017).

Hamed (2012) discussed the potential of self-healing materials, particularly in situations requiring long-term reliability and difficult infrastructure access. Various techniques have been explored for substrates like metals, ceramics, and polymers, all centered on the concept of self-healing through the formation of a mobile phase that fills fissures. Advanced biotechnologies include solid constructions like bacterial concrete, crack remediation, biological mortar, and concrete that is self-healing.

Rosado et al. (2017) highlighted the use of biosurfactants, which are antibacterial compounds, in preventing the biological colonization of artworks. An advanced bio-cleaning system employing agar-gauze activated gel was applied to historical oil mural paintings with residual protein. The protocol was implemented at the Vatican Museum on the oil mural painting Cristo che salva Pietro dalle acque–La Navicella by G. Lanfranco (1627-28), and the oil mural painting L'Incarnato by O. and G. Riminaldi (XVII century) inside the Cupola at Pisa Cathedral. The results demonstrated a high level of effectiveness, as confirmed by Py/GC-MS and FTIR analyses (Ranalli et al., 2018).

STATEMENT OF PROBLEM

While numerous research papers have explored bio-cleaning and microbial biotechnology, this study focuses on the scarcity of information regarding the impact of microbial biotechnology on CH and the associated challenges and opportunities for preservation. Key challenges include poor physical planning mechanisms, insufficient development control and enforcement, unclear limits on permissible alterations at historical sites, low levels of public participation, ineffective preservation policies, and inadequate funding. Although advanced microbial technology has shown success in addressing the bio-cleaning process, the primary obstacle remains the lack of resources for implementing this technology, thereby posing difficulties in conserving and preserving CH.

CONCEPTUAL FRAMEWORK

CH artifacts offer a diverse habitat hosting various bacteria and fungi. While these microbes contribute to the degradation of artworks, some can also be utilized for efficient biotreatment of CH objects. Microorganism interventions prove effective in restoring antiquities while traditional chemical and mechanical treatments fail or yield unsatisfactory or temporary results.

The conservation and restoration of immovable cultural property involve preserving its material, historical, and design integrity through carefully planned interventions. Microbial genomics and biotechnology research play a crucial role in advancements related to biotechnology, value-added goods, human nutrition, functional foods, plant and animal protection, and basic agricultural research are all related to food safety and security.

Utilizing microbial metabolisms to safeguard cultural relics and man-made constructions have benefits and drawbacks. One significant advantage is the compatibility with the treated substrate. For example, while coatings of organic compounds on inorganic substrates are commonly used in metal sculpture preservation and repair, they create a physical barrier that behaves differently from the metal core and eventually becomes ineffective. However, the use of "consolidants" and "water repellents" in masonry is controversial due to their poor long-term efficacy and irreversible nature, with some findings suggesting that such treatments contribute to accelerated stone degradation.

Most multidisciplinary approaches to assessing the impact of microorganisms on historical items involve combining innovative dependent and independent analytical knowledge with traditional cultivation experiments and physicochemical characterization of artworks. Biotechnological techniques are initially utilized as part of a comprehensive strategy that includes microscope observation, in vitro culture, and genomic DNA analysis to diagnose and identify microbial populations. Various microorganisms such as green algae (chlorella), cyanobacteria (cyanobium and oscillatoria), and bacteria have been identified using these methods. Our advancing understanding of the intricate interplay involving microbial metabolism and bio-cleaning has revealed unexpected insights into microbial activity and its impact on building and heritage materials. The potential offered by these technologies has led to a surge in research in this field. These advancements are poised to harness the unrealized possibilities in nature's most talented chemists, microbes, for environmentally friendly synthesis of inorganic components, addressing existing challenges. Undoubtedly, this holds great promise as the biotechnological approach's most important feature (Ranalli et al., 2005).

RESEARCH METHODOLGY

The "dry bio-cleaning" approach, which employs dried microbial cells rather than free water or gel-based matrices, represents an innovative application of microbial biotechnology in cultural material restoration. This method is particularly advantageous for restoring highly intricate objects that are challenging to clean using conventional techniques. Initial laboratory studies involved infected stonework samples treated with dehydrated saccharomyces cerevisiae yeast cells as part of the experimental setup. The technique leverages the yeast cells' metabolic fermentation activity and the spontaneous rehydration process induced by ambient humidity. In contrast to the control and nebulization treatment tests, physical-chemical examinations after eighteen hours of bio-cleaning indicated a superior elimination of salt and contaminants (without cells). The novel on-site dry bio-cleaning process, utilizing living yeast cells, shows promise as a solution for recovering specific altered CH stone works. Further research and enhancements can be pursued to optimize its efficacy and applicability.

It is a non-invasive or micro-invasive process that can be utilized on historic surfaces to acquire samples such as stonework pieces. Further, micro-samples may be collected using a steel scalpel from the specimens in the lab. Sterile scalpels, swabs, membrane filters, or sticky tapes are used for sample collection.

RESEARCH MOTIVATION AND STUDY LIMITATIONS

Educational experience, heritage appreciation, and recreational engagement are significant motivations for researching bio-cleaning and self-healing preservation materials in CH. This new perspective on microbial biotechnology offers sustainable solutions for preserving and restoring materials of historical value, contributing to a broader understanding and appreciation of our CH.

The purpose of this work is to provide an overview of recent developments in the protection of historic artifacts from microbial growth and corrosion using antimicrobial products. We aim to compare traditional antimicrobial treatments with state-of-the-art applications in heritage conservation, showcasing the potential benefits of microbial activities while also identifying concerns and red flags highlighted in recent studies.

Additionally, we seek to analyze the increasing body of academic literature to identify relevant microbiological assays and characterization methods for assessing the in vitro and in situ antibacterial properties essential for safeguarding CH. Through this comprehensive examination, we contribute to the advancement of effective preservation strategies for CH artifacts worldwide.

Despite the promising potential of microbial biotechnology, several limitations must be addressed. Microorganisms are adept at infiltrating and deteriorating CH artifacts and structures. Unfortunately, existing chemical and physical methods to halt or eliminate microbial damage are often insufficient to thoroughly clean and prevent re-infestation. Our collective cultural history is an invaluable social, environmental, and economic asset on a global scale. To safeguard this material CH and ensure its preservation for future generations, we must confront the challenges of biodeterioration.

A key limitation lies in the limited application of antimicrobial nanoparticles in traditional preservation practices, presenting new research challenges for chemists and materials scientists. Each cultural object possesses a unique composition, distinctiveness, and value, necessitating an integrated multidisciplinary approach to preservation. The exploration of microbial biotechnology must therefore be carefully adapted to individual heritage materials while addressing long-term stability, potential ecological risks, and regulatory concerns.

CONCLUSION

Microbial biotechnology presents a transformative approach to CH conservation, offering sustainable alternatives to traditional methods. This study demonstrates that biological cleaning, by utilizing harmless microbes, eliminates the risks associated with hazardous chemicals while ensuring efficient removal of biofilms and encrustations from CH surfaces. With the development of ready-to-use bio-cleaning products, application and removal processes will become faster and more practical, making these techniques commercially viable. Additionally, microbial-induced self-healing preservation methods contribute to structural durability by filling micro-cracks and enhancing material integrity, mitigating the impact of environmental factors such as climate change.

The interdisciplinary integration of microbial enzymes, bio-cleaning agents, and self-healing materials holds promise for the future of CH preservation. A cost-benefit analysis highlights the economic advantages of these methods, reducing labor costs while ensuring long-term sustainability. However, challenges such as microbial ecosystem complexity, long-term efficacy studies, and regulatory considerations must be addressed through continued research. Moving forward, advancements in microbial biotechnologies, supported by multidisciplinary collaborations, will play a crucial role in safeguarding CH materials for future generations, ensuring both their preservation and accessibility in a rapidly changing environment.

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