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Original article

USING LIPASE ENZYME IN CLEANING OF ARCHAEOLOGICAL GLASS: AN EXPERIMENTAL APPLIED STUDY

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Abstract:
The main objective of this research paper is to study the effect of lipase enzyme on the waxy materials mixed with the carbon particles resulting from the burning of the candles, which were difficult to remove in one of the archaeological glass candle stands in the Coptic Museum, Cairo, Egypt. The paper is divided into an experimental study for the use of the lipase enzyme in glass cleaning and an applied study on cleaning an archaeological glass candle stand. The experimental samples were cleaned using lipase enzyme from Burkholderia sp. X-Ray Fluorescence (XRF) was used to identify the components of the archaeological glass candle stand in the colorless and colored parts. X-Ray Diffraction (XRD) and Raman spectroscopy analysis were used to identify the damaged products in the study sample. Then, the stereo microscope and USB digital microscope were employed to find out the efficacy of the lipase enzymes in the cleaning of experimental samples and the applied study. The results proved that there was a high efficacy in the dissolution of waxy fatty layers and the dissociation of carbon calcifications. USB digital microscopic images showed that lipase enzymes from Burkholderia sp. gave good results in cleaning the surface of the archaeological candle stand. Moreover, the enzyme was inhibited with the use of ethyl alcohol.

1. Introduction

The greatest virtue of archaeological glass is that its appearance is constantly transfo-rmed by the ever-changing light. However, dirt, soot, and grime can build up on both sides of the glass from pollution, smoke, and oxidation. In churches, the traditional burning of incense or candles can eventually deposit carbon layers. These deposits can substantially reduce the transmitted light and make originally bright candle stands and glass windows muted and lifeless [1]. Museums are rich in candle stands. Therefore, the present study covers one of these archaeological glass candle stands, which dates back to the Coptic era [2] when there were religious shifts in Egyptian culture to Coptic Chri-stianity from ancient Egyptian religion in the 1st century until the Muslim conquest of Egypt in the 7th century [3]. This era also suffered from various forms of damage concerning the remains of burning waxes used in the lighting process [4], such as bee wax and lanoline [5]. Their biotechn-

ology tools were large and largely untapped. To conserve our cultural heritage [2], enzymes have become increasingly available and used to clean artifacts. Enzymes have been used in cellulose compresses, as well as aqueous and organic solvent mixtures. Although it should be noted that the last procedure may not be recommended [4]. Enzymes are used in the preservation of oil painting [6] to help remove dirt [7], paper [8], and adhesives from previous reforms [9,10] in mural paintings [11], stones [12], textiles [13], and wood [14]. However, no study has explored the effect of enzymes on glass yet. The most common preserving enzymes are hydrolases, e.g. protease, lipase, and amylase. The lipase enzyme works on lipids, i.e., fats, that are organic sputtering substances found in living organisms [15]. Fats are the most important compounds of a general spectrum of lipids, which are compounds of living matter that are soluble in oils. They do not include sintered lipids only but also waxes and many other materials, as well. Fats and waxes are esters of long-chain carboxylic acids. Fats are semi-solid or liquid, such as oil [16]. However, all fats are esters of glycerol that has many alcohols (also known as glycerin). In contrast, candles are esters of other monohydroxy alcohols and contain a few impurities of various hydrogen coal, as well as industrial and aromatic substances [17]. The first commercial enzyme for lipase was not available until 1988 when there was increased interest in a solution to break down fats and waxes. Research papers have been published since the early 1990_s in 1994. The enzyme was obtained from the extraction of bacteria from Pseudomonas in a neutral medium (pH 7) at 50 °C [18]. It is sometimes used to treat and remove residual fats from the leather industry [19] as it was used as a commercial detergent that does not oxidize [20]. Moreover, some of its types can be extracted from fungi [21]. The lipase enzyme stimulates the decomposition of the long chain of triglycerides that makes up the sebum [22]. It also succeeds in removing the resinous layer of acrylic (Paraloid B72) as it does not pose any impact on the artifact and proves efficient in cleaning [23]. The present study was conducted to evaluate the use of enzymes in cleaning archaeological glass.

2. Materials and Methods

2.1. *Materials*

2.1.1. Glass samples

The glass samples were taken from the excavation area, i.e., a particular set of the glass of the Coptic era. They were exposed to weathering effects and covered with waxes and carbon. They were chosen for studying the effect of lipase enzymes used for cleaning. The glass samples were prepared for the experimental study and tested for different concentrations to evaluate the effect of the lipase enzyme in cleaning wax materials with archae-ological glass.

2.1.2. Lipase enzyme

75577 Sigma-Aldrich Lipase from *Burkholderia sp*, as lyophilized powder, slightly beige, ~13.7 U/mg. one unit corresponds to the amount of enzyme, which liberates 1 μmol oleic acid per minute at pH 8.0 and 40°C [24]. It was applied as solutions (enzymes in distilled water) with different concentrations (10, 20, and 30U/ml) on the glass samples by brushing at room temperature. The samples were subjected to the enzymatic cleaning for 30 min, and the results were followed up every 5 minutes.

2.2. *Analytical methods*

2.2.1. Raman spectroscopy

Raman spectroscopy from Horeba was used to identify the components of the burning wax found on the archaeological candle stand preserved in the Coptic Museum.

2.2.2. X-Ray Fluorescence (XRF)

A glass candle stand was analyzed by NITON XLP 300A/700A series. The analysis was carried out on different damaged parts of the glass candle stand.

2.2.3. X-Ray Diffraction (XRD)

XRD was used for mineral identification by Burker Company model D8, including reflectometry, high-resolution diffraction, in-plane grazing incidence diffraction (IP-GID), and Small-Angle X-Ray Scattering (SAXS). It was used to analyze a sample of the damage manifestations found inside the archaeological glass candle stand.

2.2.4. USB digital microscope

It was used to examine and follow up the cleaning process of the glass candle stand under study (Specifications: Leuchtturm USB Digital Microscope (China) with 20 to 500x zoom 8 LED lights with Measurement Software.

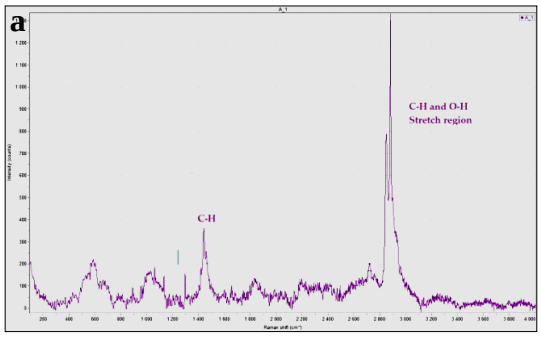
2.2.5. Stereo microscope

It was used in examining the surface of the glass samples before and after the cleaning process. All experimental glass samples were studied before and during the enzymatic treatment every five minutes until they completely treated using Stereo microscope "Germany" magnification 3.2 and light 2.5.

3. Results

3.1. *Raman spectroscopy analysis*

From Raman analysis, the components of the damaged remains were identified, as follows: It was clear that the damaged components matched the standard sample of animal wax, which contained lanolin as the basic substance fig. (1).



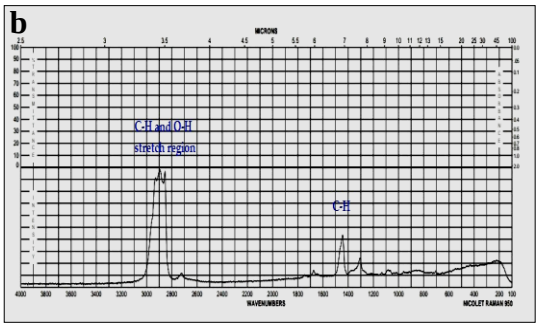


Figure (1) Shows Raman spectrum of **a.** damaged components, **b.** Animal wax (*After Sigma Aldrich*)

3.2. *XRF analysis*

The results of XRF showed that the transparent glass part of the archaeological candle stand elements contained Silica (Si) 62.78%, Alumina (Al) 4.70%, Sodium (Na) 12.65%, Magnesia (Mg) 4.74%, Potassium (K) 0.95%, Calcium (Ca) 8.20%, Antimony (Sb) 1.23%, Lead (Pb) 1.69%, Iron (Fe) 0.70%, Manganese (Mn) 1.82%, Chromium (Cr) 0.25%, and other elements 0.29%. on the other hand, the opaque glass part elements are the transparent glass part of the archaeological candle stand elements, including Silica (Si) 66.59%, Alumina (Al) 3.73%, Sodium (Na) 4.44%, Magnesia (Mg) 3.19%, Potassium (K) 3.96%, Calcium (Ca) 5.33%, Antimony (Sb) 1.12%, Lead (Pb) 2.57%, Iron (Fe) 6.43%, Manganese (Mn) 1.63%, and other elements 1.01%, all of these results are listed in tab. (1).

Table (1) Chemical composition of the glass candle stand obtained by XRF

Elemental Ratios	**Candle stand glass**	
	Colorless	***Colored***
Si	62.78	66.59
Al	4.70	3.73
Na	12.65	4.44
Mg	4.74	3.19
K	0.95	3.96
Ca	8.20	5.33
Sb	1.23	1.12
Pb	1.69	2.57
Fe	0.70	6.43
Mn	1.82	1.63
Cr	0.25	0
Others	0.29	1.01

3.3. *XRD analysis*

XRD results showed that the components of the damaged remains on the candle stand were Carbon (C) according to the card code 9014004 with average about 51.7%, in addition to Retgersite ($H_{12}NiO_{10}S$); card code 1011189 with average about 41.2%. Finally, 7% of Schafarzikite (FeO_4Sb_2) according to the card code 9014933 fig. (2).

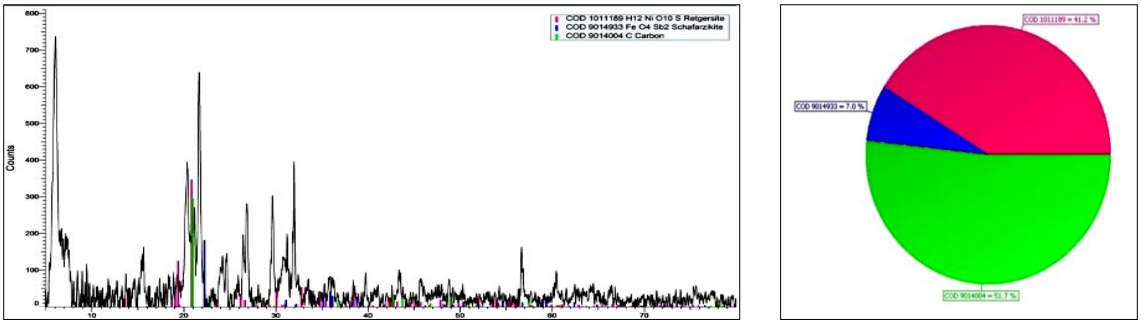
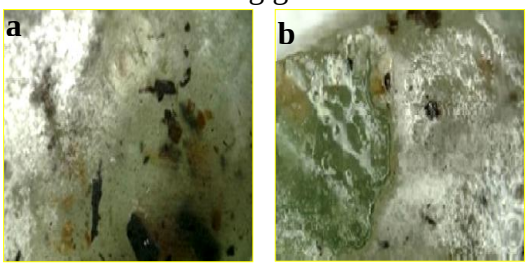


Figure (2) Shows X-ray patterns of the damaged components on the archaeological candle stand

3.4. *Stereo microscope*

Stereo microscope results of the un cleaned glass sample fig. (3-a) showed that the sample features changed after treatment according to the concentration of the *Burkholderia sp* lipase enzyme. In the cleaning process using 10 U/ml, fig. (3-b), and 20 U/ml concentrations, fig. (3-c), the waxes and carbon remains stayed on the surface. When using 30 U/ml concentration for 30 minutes, fig. (3-d) the enzyme was more effective and rapid in removing all waxes. The carbon remains were easily removed by traditional methods. The advantages of this cleaning method compared to the traditional methods include the disposal of the waxy layers, dissolution of the associated soot fractions, ease of application to the archaeological glass, and lack of effect on the archaeological surface or its internal composition. The cost is the most important disadvantage of this method compared to the traditional mechanical and chemical methods in cleaning glass.



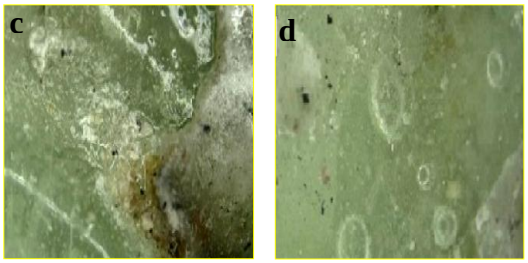
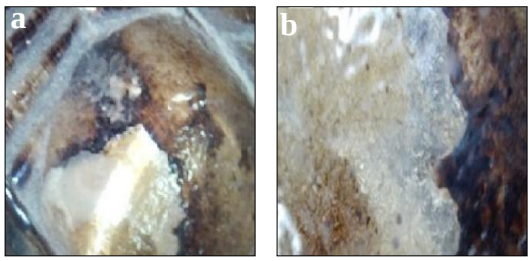


Figure (3) Shows stereo microscope images of the glass samples before and after enzymatic cleaning; as follows **a.** before cleaning, cleaning with lipase enzyme **b.** 10 U/ml, **c.** 20 U/ml, **d.** the best is 30 U/ml.

3.5. *USB digital microscope*

USB digital microscopic images of the candle stand showed layers of different colors (black, brown, and dark yellow), fig. (4) resulting from the residues of wax-burning materials and the calcifications of soot and dust on the glass surface. These residues calcifications and were concentrated between the decorative units on the external surface and inside the candle stand base.



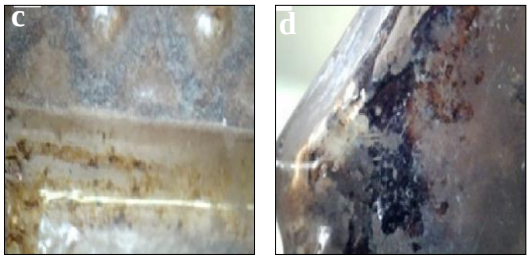


Figure (4) Shows USB digital microscope images of some parts of the archaeological candle stand show waxes remains, carbon deposits, as well as black and brown stains inside and outside the candle stand.

4. Discussion

The Raman spectrum showed that the wax was an animal wax, fig. (1-a, b) and the presence of lanolin as the main component of the animal wax. The characteristic Raman bands of lanolin (animal wax) were detected at 1056, 1139, 1288, 1443, 2822, and 2887 cm^{-1} [25]. The data illustrated that not just the waxes could be identified but also the carbon that was detected in the Raman spectra according to a previous study [26]. The transparency of the parts of the archaeological candle stand varied. The upper part was colorless, while the lower part was colored. The XRF analysis of the glass candle stand classified as (soda-lime-silica) glass was the common type of ancient glass for more than three thousand years [27]. There was a high percentage of silica in all colorless and colored glass samples. Manganese is always part of archaeological glass compositions, either as an impurity or as a deliberate addition during glass production when manganese dioxide is added to oxidize iron to iron and then provide a purple tint from Mn to offset the straw tint from the iron [28,29]. The high percentage of iron in the colored sample appeared in the lower part of the glass candle stand. In addition, the presence of lead and antimony was because the old manufacturer used lead antimons to obtain a dark yellow color [30], tab. (1). XRD results fig. (2) proved that the high percent of Carbon (51.7%), were caused by the burning of waxy materials as attested previously by Kotop [31]. in addition, the presence of Retgersite high ratio (41.2%) point to the damage remains caused by defects of use of the candle stand during the wax burning. Within the same context, the presence of while (7%) Schafarzikite is attributed probably to some traces of burial environment remains. The stereo microscope images showed that the concentration of the lipase enzyme 30U/ml was the best in cleaning calcified waxy remains, whereas the lowest concentrations at the same period of 30 minutes were less efficient. Thus, dilute concentrations of the enzyme should be used in the beginning and then gradually increase until the desired result is obtained [26]. The lipase enzyme helped reach the inner and narrow parts inside the candle stand as shown in the USB digital microscope images because these parts are difficult to clean by traditional methods. From the applied study, the use of the lipase enzyme proved to be highly effective in cleaning the damaged component of the glass decorated with prominent and sunken glass motifs that are difficult to clean by traditional methods due to the calcification of the damaged components inside them [32]. The results illustrated that enzymatic cleaning with lipase was an excellent method compared to traditional chemical cleaning for removing wax components and fats materials used for medical purposes [30]. Alcohol was used as an inhibiter to the enzyme residue on the surface [34].

5. Applied study

5.1. Documentation

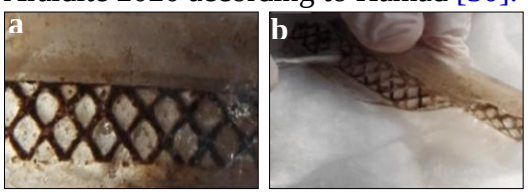
This candle stand is supported on a triangular base, which is decorated in the upper part with a braided motif and embossed

vertical lines topped by embossed dots. At the top of the base, there are three inflated spheres, each of which is surmounted by a column. Each column is decorated at the base with coiled branches. The area between the three pillars is decorated with geometrical designs, which consist of small embossed lozenges. The pillars get narrower towards the top and end at a cylinder, which is undecorated and forms the neck of the candle stand fig. (5).

Figure (5) Shows **a.** an archaeological glass candle stand from the Coptic Museum in Egypt, **b.** the study object that measures 8 cm wide and 25 cm long

5.2. Cleaning of the archaeological candle stand

The cleaning process was done in stages for each part of the candle stand. The lipase enzyme was prepared in concentration (30U/ml), and the cleaning process was carried out with a brush for 30 minutes. The enzyme gave high efficiency in removing waxy deposits. The carbon particles that were incorporated into the waxes fell apart, and the carbon was easily removed by mechanical methods fig. (6). Moreover, the enzyme was inhibited by ethyl alcohol [26]. Finally, the parts of the glass archaeological candle stand were joined with Araldite 2020 according to Hamad [30].



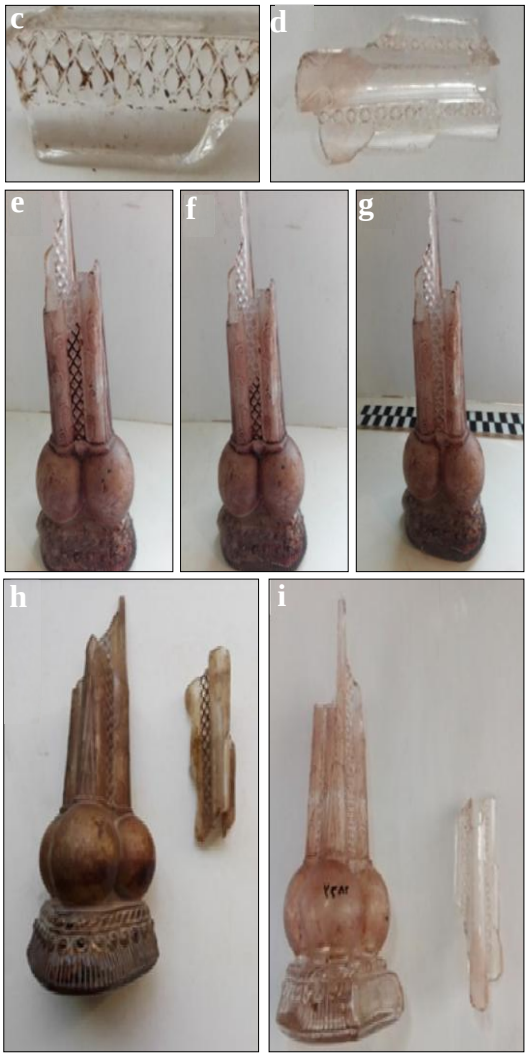


Figure (6) Shows **a.** the candle stand before cleaning, **b.** the first part of the cleaning stages of the candle stand through removing carbon disjointed from waxes, **c.** after using the lipase enzyme, **d.** after removing damage remains, **e., f., g.** stages of the second part of cleaning with the lipase enzyme, **h.** the archaeological glass candle stand before cleaning, **i.** after enzymatic cleaning

6. Conclusion

Lipase enzyme from Burkholderia sp. was used in cleaning the archaeological glass using different experimental and applied parts that included microscopic and analysis methods. The authors conclude that it is important to analyze the dirty surface layer covering the artifacts to choose the better enzyme. The efficacy of the lipase enzyme in cleaning

the glassy traces that contain waxy damage components combined with carbon granules gave better results than traditional methods. Therefore, further studies shall be conducted using various enzyme inhibitors.

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