**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Assessment of the Applicability of Cellulolytic Enzyme in Disassembling of Caked Papers

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**Abstract**

The phenomenon of caked papers is one of the most serious problems faced the restoration and conservation of historical manuscripts, books, and paper documents. The papers reach a state of weakness and fragility make it difficult to deal with. Although various causes lead to this phenomenon, fungal bio-deterioration is the most important one. For the disassembling of caked papers, the traditional applied methods including mechanical separation and organic solvents are not completely effective. The present research aimed to find an applicable solution in the disassembling of caked papers via the evaluation of the use of locally produced cellulolytic enzyme. Therefore, different treatment conditions including the use of different enzyme activities for different treatment periods were examined. The amount of reducing sugars released in the treatment solution was initially determined. In addition, chemical, physical and mechanical properties of the treated samples were monitored using Fourier transform infrared spectroscopy, colour change measurement, tensile strength, and elongation. Digital and scanning electron microscopy were also applied to examine the topographic changes occurred on the surface of the treated papers. The results proved that the lowest concentration of the used enzyme able to separate the caked papers, improving its mechanical and physical properties.

*Keywords*: Cellulase, Caked papers, Fungi, Disassembling

## 1. Introduction

The phenomenon of caked papers is one of the most important problems facing the restoration and conservation of a lot of valuable documents and manuscripts, not only because of the arising difficulty in dealing with them but also due to the limited studies concerned about solving this problem. Although mechanical separation and organic solvents have been traditionally used in the disassembling of caked papers, they are not completely effective due to the intensity of paper cohesion and difficulty in separation without tearing the paper tissue [1]. Consequently, books and documents stored in many libraries, museums and archives are at risk of loss, reflecting the importance of finding applicable solutions with the least possible damage [2].

Fungi are considered important causative agents in the bio-deterioration of cultural heritage. They can stick and colonize on the surface of historical objects and under the appropriate environmental conditions they can grow causing dangerous damage [3]. Zhang and Lynd, [4] reported that the microbial bio-deterioration of cellulose-containing cultural heritage objects is mainly based on synergistic effect of cellulolytic enzymes leading to cellulose degradation. In addition, Lavin, *et al*., [5] reported that the fungus *Scopulariopsis* sp. and *Fusarium* sp. can survive and grow on cellulose fibers with bio-adhesion and pigment production properties.

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Receive Date: 01 July 2021, Revise Date: 26 July 2021, Accept Date: 28 July 2021
DOI: 10.21608/EJCHEM.2021.83497.4095

Enzymes are highly specific protein molecules act as catalysts in several biochemical reactions [6]. The use of enzymes in various fields is growing more and more in order to develop eco-benign and efficient processes. In the last few decades, the application of enzymes in the conservation of works of art had attracted the research focus. Hydrolytic enzymes are the most common class of enzymes used in conservation in which amylases, proteases and lipases found extensive applications [7].

Cellulases are complex group of enzymes that hydrolyze the β -1,4 linkages in cellulose chains. They are widely produced in nature by various fungal and bacterial species as a part of energy transfer and carbon cycle [8]. They are classified according to their mode of action into endo-glucanases, exo-glucanases and β-glucosidases. Endo-glucanases attack the internal bonds in the amorphous regions of cellulose fibrils and exo-glucanases degrade the chain from the terminal end leading to the production of cellobiose while β-glucosidases hydrolyze the endo- and exo-cellulase products into glucose units [9].

Enzymatic modifications of cellulose fibers have been reported to provide various benefits for cellulose-based industries via surface and pore structural changes [10]. The main advantages for cellulase treatment of cellulose fibers were; reducing the fiber coarseness, improving fiber brightness and enhancing strength properties [11]. Therefore, the current study aims the benign hydrolysis of cellulose fibrils of the seventeenth century AD manuscript preserved in the Central Library of the Sheikhdom of Al-Azhar Al-Sharif using locally produced endo-glucanase for its disassembling without adverse effect on its inherited physical and mechanical properties.

## 2. Materials and Methods

*2.1. Isolation source*

The manuscript under investigation is considered one of the rare manuscripts given to Al-Azhar Library by those responsible for the library of Sheikh Hassane in Muhammad Makhlouf in the village of Bani Adi in the center of Manfalut, Assiut Governorate. It bears general number 200603 and special number 4086.

*2.2. Isolation and identification of fungi from the manuscript*

*2.2.1. Isolation of fungi*

Under aseptic conditions, sterile cotton swabs were used to wipe contaminant areas on the surface of the manuscript and directly streaked on Czapex-Dox Agar (CDA) medium composed of (g/L) Sucrose; 30.0, $NaNO_3$; 2.0; $K_2HPO_4$; 1.0, $MgSO_4.7H_2O$; 0.5, KCl; 0.5, $FeSO_4.7H_2O$; 0.01, Agar; 15.0 and adjusted to pH 5.5-6 then incubated at 25°C±2 for 7 days [12].

*2.2.2. Purification*

Fertile moulds were purified by spreading a few spores on the surface of CDA plates and incubated at 30°C for 7 days. A single colony was aseptically subcultured on a slant of CDA.

*2.2.3. Fungal identification*

Fungal isolates were identified according to morphological, cultural characteristics and microscopic examination of the fruiting bodies and spores utilizing standard manuals for *Aspergillus* sp. [13-14], *Penicillium* sp. [15-16] and *Cladosporium* sp. [17]. The isolates were stored on CDA slant agar at 4º C for further use.

*2.2.4. Cellulolytic activity of the isolated fungi*

Preliminary screening of the cellulolytic activity of the isolated fungal strains was estimated by plating pure culture onto CDA plates supplemented with 1% (w/v) cellulose instead of sucrose as the sole carbon source. For each strain, mycelium disc of 5mm in diameter from seven days old age culture was placed at the center of each agar plate then incubated at 30°C. The radial growth and its density were measured according to Sidkey *et al*., [18]. Three replicates were used for each treatment. The most potent organism was used in further experiments.

*2.3. Preparation of paper samples*

The experimental studies were initially carried out on Whatman filter paper no. 1 subjected to accelerated artificial aging in a closed climatic chamber at a temperature of 80ºC and at a relative humidity (RH) of 65% for a period of 4 weeks, which was equivalent to aging of the paper under natural conditions for 100 years [19]. The aging procedure was in conformance with the ISO 5630-3:1996 standard**.**

*2.4. Growth of the isolated fungal strain on Filter paper samples*

Initially, spore suspension ($1\times10^6$ spores/ml) of 7 days old age slant was prepared from the isolated fungal strain that identified as the most potent producer of cellulolytic activity. CDA medium without carbon source was sterilized at 1.5 pa for 20 min then poured into petriplates. After solidification, two pieces of the aged sterilized filter paper $10\times10cm^2$ (used as a carbon source) were aseptically placed on the surface of each Petri dish. After that, 10 ml of the prepared spore suspension were poured above the filter paper then incubated at 25ºC for 21 days.

*2.5. Application of cellulase on disassembling of caked paper*

*2.5.1. Production of cellulase*

The production of the enzyme was carried out under solid state fermentation (SSF) of rice straw using *Aspergillus terreus* RS2 (Accession no. MN368221) according to Ismail and Hassan, [20]. Briefly, a spore suspension (2mL) of 7 days old age slants was used to cultivate 3.75g of rice straw (with initial moisture content of zero percentage) moistened in the ratio 1:3 (biomass to moistening agent ratio) with moistening agent composed of (g/L) $(NH_4)_2SO_4$; 10, $KH_2PO_4$; 2, $CaCl_2$; 0.3, $MgSO_4.7H_2O$; 0.3 and adjusted to pH 7, then incubated at 30ºC for 8days. At the end of the fermentation process, the enzyme was extracted by adding 50mL distilled water to each flask, shaking at 150 rpm and 30ºC for 1h then centrifuged at 5000rpm for 10min. The culture free supernatant was air dried and used in further experiments.

The activity of the enzyme was estimated by determining the released amount of reducing sugars according to Miller, [21] in a reaction mixture consisted of 500µL of 1% carboxymethyl cellulose (dissolved in 0.05M acetate buffer, pH 5) and 500µL of the enzyme solution that incubation at 50°C for 30min. One unit of the enzyme was defined as the amount of enzyme that released 1µmol of glucose per minute under the assay conditions.

*2.5.2. Selection of the suitable conditions*

The appropriate enzyme activity of the produced enzyme was selected by examining the use of different concentrations ranged from 0.025 to 0.2% w/v dissolved in 0.05M acetate buffer pH 5. The prepared solutions (200mL) were poured gently on the caked papers (~2 g) hold on a stainless-galvanized wire mesh holder inside Petri dishes 15 cm in diameter. The Petri dishes were incubated at 40ºC for 15 min then the samples were rinsed for several times with distilled water and dried at 70ºC for 30min. The appropriate concentration was selected on the base of the amount of reducing sugars released in the treatment solutions since the highest amount of reducing sugar indicated the maximum cellulolytic activity of the enzyme. The amount of reducing sugars was determined as described by Miller, (1959). Additionally, the appropriate immersing period was examined at 2.5, 5, 10, and 15 min [21].

*2.6. Mechanical Behavior Measurements*

The samples were conditioned for 24h in a standard atmosphere (23°C and 50% RH) prior measuring the tensile strength and elongation. Tensile and elongation measurements were performed on 15mm wide strips between jaws set 100mm apart [22-23] using QMat 5.37, Tinius Olsen according to Standard No. EN ISO 13934-1; 1999 Maximum Force & Elongation-Strip Method.

*2.7. Measurement of color change*

The color change in the treated papers has been measured according to International Commission on Illumination color space (CIE l*a*b*) system using Ultra-scan PRO, Hunter lab, USA, UV spectrophotometer as described by Abdel-Maksoud and Marcinkowska, [24], Rushdy *et al*., [25], and Fouda *et al*., [3]. L* value measured darkness to brightness, a* value measured red color to green and b* value measured yellow color to blue. The total color difference (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The results of ΔL, Δa and Δb were obtained from the difference between value of l*, a* and b* for the reference and treated samples respectively [26, 3].

*2.8. Digital microscopy*

The surface morphology of the paper, writing inks and color paints as well as assessing the paper making techniques employed to create the illuminations, a digital handheld microscope (ASIN: HJH001 B083TGGVPB) at 0x-1600x magnification was used [27]. The lens was 0.3m CMOS Sensor with an aperture of 2.0 MPIX.

*2.9. Scanning electron microscopy*

The samples were examined under scanning electron microscope at the Faculty of Agriculture, Cairo University, Cairo, Egypt (Quanta 250 FEG, FEI, Netherlands).

*2.10. Fourier transform infrared spectroscopy*

Fourier transform infrared (FTIR) analysis of the untreated as well as the treated samples were investigated using high-resolution attenuated total reflection - Fourier transformation infrared spectroscopy (ATR-FTIR) (JASCO FT/IR-4700 Spectrophotometer, Japan) in the region of 4000-500 $cm^{-1}$ with spectra resolution of $4cm^{-1}$.

*2.11. pH measurement*

The pH was considered the most important factor determining the stability of papers toward natural and accelerated ageing. Cold extraction measurements were carried out according to Tappi method [28-29] using Thermo Scientific Orion Star A111pH Benchtop Meter.

## 3. Results and discussion

*3.1. Evaluation of the historical manuscript*

Fungi play an important role in the adhesion of the papers of manuscripts and books via the secretion of enzymes that specifically hydrolyze its constituent components [4]. The danger aroused from the

sticking of fungi on the surfaces of manuscripts and books was that the fungal spores under the appropriate climate conditions especially temperature and humidity grow rapidly and at a very high rate of reproduction penetrating their component tissue causing the formation of their caked form [3, 30-31]. In addition, cohesion sticks them together until they become one block sticky manuscript like a stone (Fig.1).

Table 1. The amount of reducing sugars released during the enzymatic treatment.

| Treatment condition | | Released amount of reducing sugars (µg/mL) |
|---|---|---|
| **Enzyme concentration (%)** | 0.025 | 24.4 |
| | 0.05 | 45.1 |
| | 0.1 | 112.9 |
| | 0.15 | 111.7 |
| | 0.2 | 110.6 |
| **Treatment period (min)** | 2.5 | 75.7 |
| | 5 | 88.3 |
| | 10 | 91.8 |
| | 15 | 112.9 |

*3.2. Isolation and identification of fungi from the. paper manuscript*

The isolation of fungi from the manuscript under investigation was carried out and the results revealed the presence of *A. niger*, *Cladosporium* sp., and *Penicillium* sp., identified on the base of their cultural and morphological features (Fig. 2). In libraries as well as museums, fungi have been reported to play an important role in bio-deterioration [3, 32-33]. The materials were deeply penetrated by the fungal hyphal networks, resulting in significant loss due to enzymatic degradation in addition to acid corrosion and mechanical attack [3, 34]. Sequeira *et al*., [35] reported that papers in general are a suitable carbon source for
fungal growth with the possibility of pigments secretion. These pigments could be coated in spores or mycelium or released onto the paper resulting in discoloration.

*3.3. Cellulolytic activity of the isolated fungi*

The growth of the isolated fungi on cellulose-based medium was examined. The results indicated that the isolated *A. niger* possessed the largest radial growth, so it was selected to cultivate the prepared paper samples as shown in Fig. (3) for further experiments.

Fig. 1. The caked manuscript under investigation.

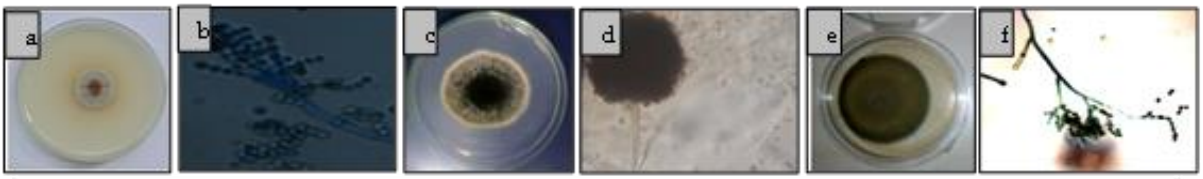


Fig. 2. Cultural morphological, and microscopic features of (a, b) Penicillium sp., (c, d) A. niger, and (e, f) Cladosporium sp.

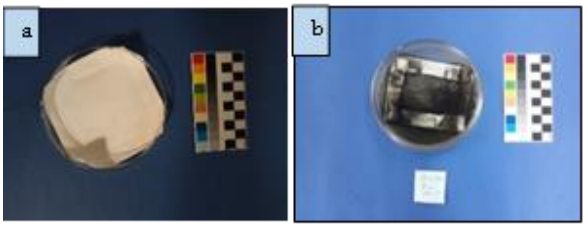


Fig .3. Filter paper (a) negative control and (b) after growing Aspergillus niger for 21 days.

*3.4. Application of cellulase on disassembling of caked paper*

*3.4.1. Enzyme production*

The fungus *Aspergillus terreus* RS2 had been previously reported to produce high carboxymethyl cellulase activity (1000U/g) by the fermentation of rice straw as a sole carbon source [36]. In general, the production of cellulases is very expressive because of their various biotechnological implementations. However, their industrial application is still restricted by the high cost of the produced enzymes in addition to their low production titer and low thermal stability [37]. As a result, economic cellulase production using local agricultural wastes has a bright future for industrial and archaeological conservation applications with a great environmental impact.

*3.4.2. Selection of the suitable conditions*

The use of different enzyme concentrations (0.025-0.2%) in disassembling of caked paper was initially examined. The amount of reducing sugars released in the treatment solutions was determined and the results were illustrated in table.1. The results indicated that the released amount of reducing sugars increased by increasing the enzyme concentration up to 0.1%. By additional increase in the concentration, no variation in the amount of reducing sugars was observed. Moreover, the treatment period was examined at 2.5, 5, 10 and 15min. The results indicated a slight increase in the amount of the released reducing sugar by increasing the treatment period. The released amount of reducing sugars can be attributed to the enzymatic hydrolysis of the surface cellulosic fiber fibrils that may lead to fibers de-adhesion and deterioration. Therefore, further investigations were carried out to examine the effect of the used enzyme on the treated samples.

*3.5. Mechanical behavior measurements*

The values of tensile strength and elongation percentage were illustrated in table 2.

The measurements revealed that the best time to loosen and separate the sheets was the immersion for 5 minutes. This period of immersion achieved the highest maximum force in compared to the reference sample and with a value almost similar to that indicated for the negative control sample.

Table 2. The effect of the treatment period on the mechanical properties of the enzymatic treated sample
FB: Filter paper

| Treatment | Maximum Force N | Elongation % |
|---|---|---|
| **Reference** | 34.49 | 1.518 |
| **Negative control** | 9.52 | 0.820 |
| **Positive control** | 1.433 | 0.863 |
| **FB 2.5 minutes** | 5.07 | 0.588 |
| **FB 5 minutes** | 9.12 | 0.890 |
| **FB 10 minutes** | 4.300 | 0.967 |
| **FB 15 minutes** | 8.18 | 0.899 |

*3.6. Measurement of color change*

The effect of cellulase treatment on the color change of the caked paper was examined and the results were illustrated in table. 3. The results of l*****-value indicated that the treated samples were near white color without the detection of a great variation between different immersion periods. This result indicated that the immersion period did not affect the paper color, but the variation may be attributed to the artificial aging as previously reported by Rushdy *et al*., [23, 25]. The results of a*****-value indicated that the color of the samples treated for 5 and 10 min tended to the green color while the color of the other samples tended to the red color that may be attributed to the artificial aging [38]. The results of the b*****-value of the samples before treatment by cellulase enzyme was near dark yellow, but after treatment for 5 and 10min, the color tended to the natural color of the reference standard sample and the negative control sample (yellow color). This result agreed with the results reported by Atodiresei *et al*., [39].

According to the obtained results, high color change (ΔE) was observed for caked papers inoculated with *A. niger* and for that treated with cellulase in which the sample immersed for 5 min possessed the best result in compared to other treated samples.

Based on the previous results, the use of 0.1% cellulase solution and immersion period for 5 min had been used successfully to facilitate the disassembling of caked paper without adverse effect on the mechanical properties of the treated samples in addition to color change improvement. Accordingly, these conditions had been applied in the disassembling of the manuscript under investigation (Fig. 4).

Table 3. Measurement of color change for the enzymatic treated samples for different treatment periods.

| Treatment | l* | a* | b* | ΔE |
|---|---|---|---|---|
| **Reference** | 90.42 | -0.20 | 0.70 | 00.00 |
| **Negative control** | 90.62 | -0.14 | 0.24 | 0.50 |
| **Positive control** | 72.19 | 0.67 | 9.21 | 20.14 |
| **FB 2.5 minutes** | 80.50 | 1.65 | 13.33 | 16.17 |
| **FB 5 minutes** | 79.18 | 0.09 | 5.11 | 12.08 |
| **FB 10 minutes** | 78.66 | 0.43 | 4.60 | 12.41 |
| **FB 15 minutes** | 79.35 | 2.08 | 14.24 | 17.64 |

FB: Filter paper; l* values measure darkness to brightness, a* values measure red color to green and b* values measure yellow color to blue. The total color difference (ΔE) was calculated as $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$ where ΔL, Δa and Δb are difference between value of l*, a* and b* for the negative control and treated samples.


Fig .4. Enzymatic disassembling of caked papers; (a) caked papers, (b) immersion in enzymatic solution and incubation at 40 °C, (c) and (d) disassembling of caked papers using needles with the help of magnifying lenses (e) Caked papers after separation and (f) Manuscript under investigation during separation.

*3.7. Digital microscopy*

It was found by digital microscopy that the enzymatic treatment using the produced cellulase improved the physical properties of the caked filter papers as well as that of the caked manuscript under investigation, as the cellulase enzyme separated the adhesion of papers, at the same time, it cleaned the dirt and color spots resulting from the process of natural and artificial aging of the papers, in addition to softening and straightening the papers by restoring their water content (Fig.5).

*3.8. Scanning electron microscopy*

The results of the examination of the caked filter papers cultivated with *A. niger* for 21 days as well as the caked manuscript after de-adhesion using the produced cellulase in compared to reference, negative, and positive control sample were shown in figure (6). The results of cellulase treated samples indicated the cohesion of the fibers and their strong bonding, the absence of any sedimentation of the treatment solution on the surface of the fibers and the low level of fungal growth on the surface of the papers, indicating the success of the examined treating solution in loosening the adhesion and cleaning the papers without indicating an adverse damage on the fibers.

*3.9. Fourier transform infrared spectroscopy*

The characteristic bands for cellulose were previously reported as follow; at 3000–3600 $cm^{-1}$stretching vibration of hydrogen bonded OH-groups [40-41], 2780–2980 $cm^{-1}$CH stretching [42], 1635–1647 $cm^{-1}$OH bending of adsorbed water [43], 1420–1422 $cm^{-1}$ $CH_2$ bending [44],1315 $cm^{-1}CH_2$ bending vibrations related to the content of crystalline cellulose [45] and 1333–1337 $cm^{-1}$OH in-plane bending of amorphous cellulose [46] in addition to the finger print region of cellulose (900–1200 $cm^{-1}$) that included; at 1200 $cm^{-1}$ C-O-H in-plane bending vibrations, 1159 $cm^{-1}$ C-O-C stretching of the β-(1-4)-glycosidic linkage, 1105 $cm^{-1}$ asymmetric stretching of the glycosidic ring, 1055 $cm^{-1}$ C-O stretching of secondary alcohol, 1026 $cm^{-1}$ C-O stretching vibrations of primary alcohol and 872 $cm^{-1}$C-O-C in plane symmetric [40, 46-47].

In the current research, the FTIR spectra of the treated sample in compared to reference, negative, and positive control samples were shown in (Fig. 7a). A decrease in the band's intensity was observed in the cellulase treated sample in compared to that of the un-treated positive control sample (Table 4). This result confirmed that the cellulase treatment did not affect the paper as the intensity values approached that indicated for the reference sample. In addition, no discrepancies were recorded in the FTIR spectrum of the treated manuscript in compared to that before treatment (Table 5, Fig. 7b).

*3.10. pH measurement*

The pH measurements of the filter paper after cellulase treatment indicated that the treated paper tended to the degree of neutralization similar to the reference and the negative control sample (Table.6).

Table 4. Result of the FTIR analysis.

| Function Groups | Reference | | Negative Control | | Positive Control | | Cellulase treated sample | |
|---|---|---|---|---|---|---|---|---|
| | Absorbance Region | Intensity | Absorbance Region | Intensity | Absorbance Region | Intensity | Absorbance Region | Intensity |
| **O-H Stretching** | 3330.46 | 36.181 | 3331.43 | 42.0974 | 3331.43 | 55.5824 | 3329.5 | 21.3414 |
| | 3290.93 | 36.6908 | 3279.36 | 43.0117 | 3274.54 | 55.9918 | 3274.54 | 21.8329 |
| **C-H Stretching** | 2887.88 | 64.5966 | 2889.81 | 74.205 | 2898.49 | 73.5495 | 2900.41 | 48.4288 |
| **O-H bending of adsorbed water** | 1644.02 | 78.2301 | 1643.05 | 86.2222 | 1614.13 | 91.039 | 1643.05 | 84.8415 |
| **$CH_2$ bending** | 1426.1 | 81.252 | 1427.07 | 85.8438 | 1426.1 | 91.5492 | 1427.07 | 81.7243 |
| **O-H in-plane bending of amorphous cellulose** | - | - | 1332.57 | 83.3155 | 1334.5 | 90.2184 | 1334.5 | 78.9282 |
| **$CH_2$ bending** | 1314.25 | 78.9638 | 1314.25 | 80.8743 | 1314.25 | 88.4619 | 1315.21 | 76.6185 |
| **C-O-C** | - | - | - | - | - | - | - | - |

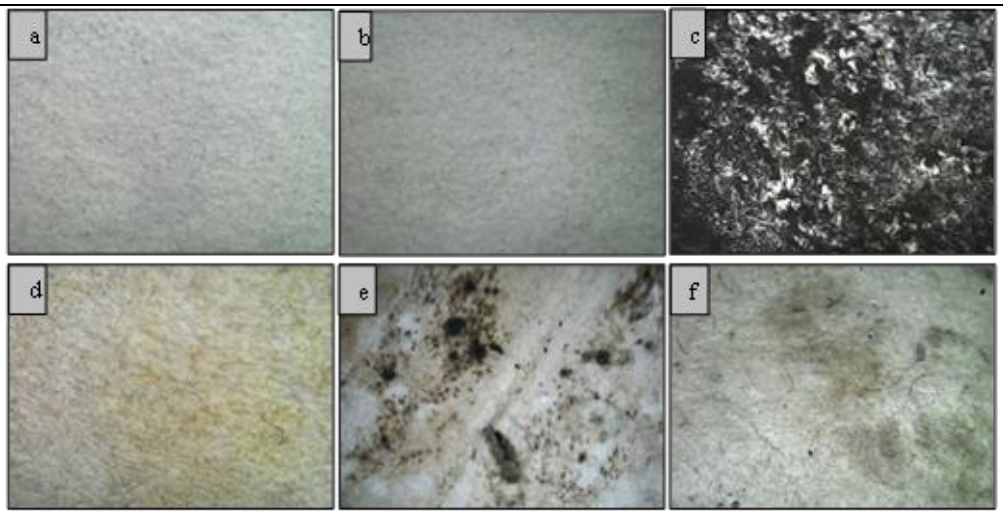


Fig .5. A digital microscope images (magnification of 1600x) of different filter paper samples (a) Standard filter paper, (b) Negative control, (c) Positive control of filter paper before cellulase treatment and (d) after cellulase treatment in addition to the caked manuscript (e) before and (f) after cellulase treatment.

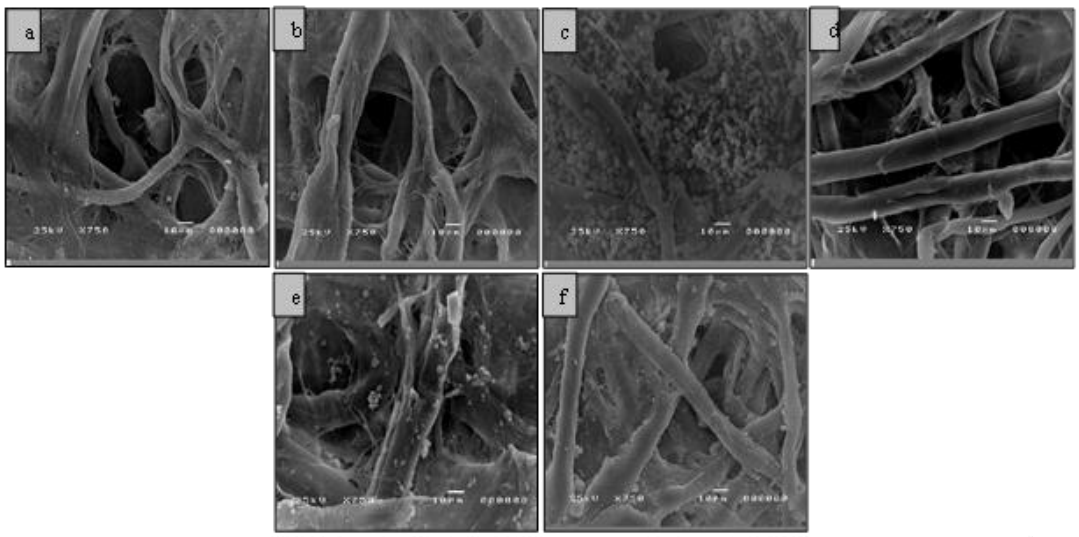


Fig .6. Scanning electron micrographs of different filter paper samples (a) Standard filter paper, (b) Negative control, (c) Positive control of filter paper before cellulase treatment and (d) after cellulase treatment in addition to the caked manuscript (e) before and (f) after cellulase treatment.

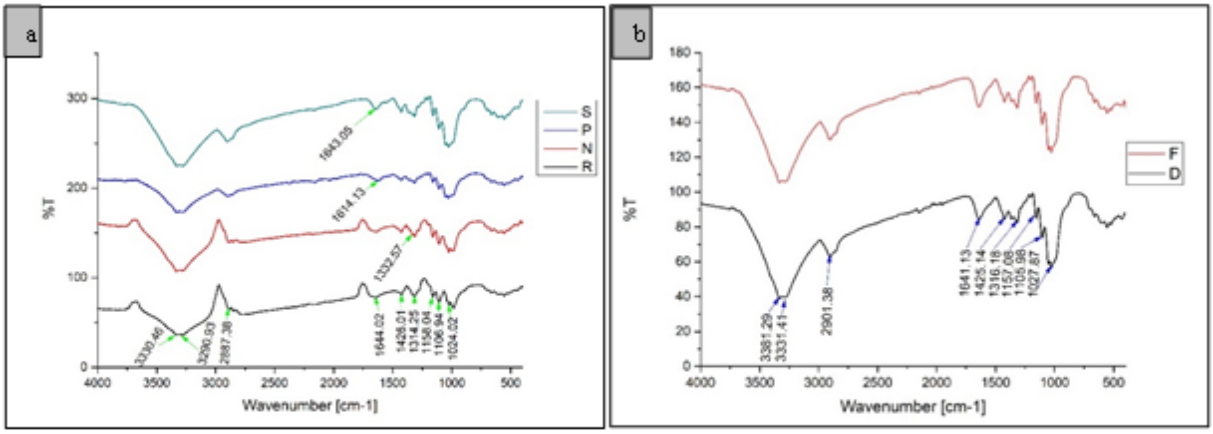


Fig.7. The FTIR spectra of (a) filter paper samples in which R was the reference, N was the negative control, P was the positive control and S was the cellulase treated sample in addition to the FTIR spectra of (b) the manuscript under investigation before (D) and after (F) treatment.

Table 5. Result of the FTIR analysis of the manuscript under investigation before and after cellulase treatment

| Function Groups | Un-treated | | Cellulase treated | |
|---|---|---|---|---|
| | Absorbance Region | Intensity | Absorbance Region | Intensity |
| O-H Stretching | 3331.41 | 39.0269 | 3332.39 | 38.2013 |
| | 3281.29 | 39.6383 | 3281.29 | 38.8233 |
| C-H Stretching | 2901.38 | 63.0226 | 2903.31 | 62.8871 |
| O-H bending of adsorbed water | 1641.13 | 84.6007 | 1642.09 | 81.6288 |
| $CH_2$ bending | 1425.14 | 84.3921 | 1425.14 | 84.5243 |
| O-H in-plane bending of amorphous cellulose | - | - | - | - |
| $CH_2$ bending | 1316.18 | 82.6079 | 1317.14 | 81.0397 |
| C-O-C | - | - | - | - |

Table 6. pH measurement results

| Sample | pH |
|---|---|
| Reference | 6.80 |
| Negative Control | 6.69 |
| Positive Control | 6.19 |
| Filter paper treatment by cellulase | 6.69 |

## 4. Conclusion

Enzymatic treatment using the produced cellulase had succeeded in disassembling of caked papers without affecting their physical or mechanical properties. The results of the tensile strength indicated an improvement in the properties of the cellulase-treated papers. In addition, surface examination manifested that the cellulase treatment loosened the adhesion of the fibers and at the same time cleaned the color spots resulted from the fungal infection. The FTIR analysis confirmed that there were no changes in the paper components as a result of cellulase treatment. On contrary, the changes that occurred in the functional groups of the cellulose were all in favor of the sample as they were often close to the reference sample. Accordingly, cellulase treatment is a suitable candidate in conservation of manuscripts, books and paper documents, not only in de-sticking of papers, but also in some other fields, such as cleaning them from the dirt that was stuck to them.

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