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CAPABILITIES AND LIMITATIONS OF MICROBIAL ENZYMES IN WOOD CLEANING

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ABSTRACT

This work aims to evaluate applying microbial enzymes in cleaning archaeological wood and their effect on its chemical composition, using different concentrations of two types of microbial enzymes; the protease and α-amylase. Earlier, a preliminary study investigated the possibility of using enzymes in cleaning archaeological wood and concluded that enzymatic treatment can be a potential method in cleaning archaeological wood and recommended that further research is needed to evaluate their effect on wood. Here, the colour change measurement was undertaken to assess the effect of the chosen concentrations on the colour of the wood surface. Moreover, Fourier transform infrared (FTIR) was used to monitor the changes in the main components of wood; cellulose, hemicellulose and lignin, as a result of different concentrations of the chosen enzymes. The results proved that the lowest concentrations of both enzymes considered the safest concentrations that can be used in wood cleaning. Besides, the increase in the enzyme concentration causes partial remove of lignin and promote the degradation of cellulose especially after aging.

KEYWORDS: Wood; Protease, α-amylase, colour change, FTIR.

1. INTRODUCTION

The trend observed recently in wood conservation is intended to use materials friendlier to the environment (Hamed and Hassan, 2019). Therefore, researchers are paying more attention to the possible of using enzymes in cleaning archaeological wood (Hamed, 2012). There are three different sources for enzymes; animal, vegetable and microbial, but the microbial source is preferable due to many reasons among them: their availability, continuous supply, and stability (Gurung et al., 2013). Many enzymes from microbial sources including bacteria and fungi have a significant importance in various commercial processes and their application in industry have continuously progressed. In spite of wood degradation which can be caused by some fungi species due to their enzymatic system (Hamed, 2013; Hamed and Mansour, 2018), microbial enzymes have a biocleaning potential of cultural heritage generally and archaeological wood specially (Hamed, 2012). Concerning their importance, various techniques are applied in order to improve the quality and performance of the microbial enzymes (Nigam, 2013). Some of the commercially applicable enzymes were employed in cleaning of the cultural heritage (Ramirez et al., 2005), whether organic materials; such as paper (DeSantis, 1983; Larminie, 1992), oil painting (O'Hoski, 1976), textiles (Ahmed and Kolisis, 2011) and wood (Hamed, 2012), or inorganic materials; such as stones (Webster and May, 2006; Cappitelli et al., 2006; Cappitelli et al., 2007; Cappitelli, 2016) and wall painting (Beutel et al., 2002; Ranalli et al., 2005). Furthermore, many studies were imposed to evaluate the cleaning effectiveness of enzymes and the surface characterization of the different archaeological materials before and after enzymatic treatment (DeSantis, 1983; Hamed, 2012; Romão et al., 1990; Dyke, 2004; Pereira, 2012). FTIR is among the analytical methods that can be used to detect the chemical characterization and changes occurred in wood due to various treatments (Afifi et al., 2019; Hamed and Hassan, 2019).

As was noticed in a previous study which was concerned with using different enzymes for cleaning archaeological wood (Hamed, 2012), occasionally the low concentration of the enzyme which is intended to be used doesn't work out and doesn't remove the containments on the wood surface efficiently. Therefore, this experiment was undertaken to evaluate using higher concentrations of the different enzymes and their effect on wood.

2. MATERIALS AND METHODS

2.1. *Wood samples*

In this study, beech wood (*Fagus sylvatica* L.) were selected. Air-dried Beech samples have been used for color measurements, whereas beech samples from an archaeological artifact, that has been subjected to natural weathering, have been used for investigating the effect of two types of microbial enzymes used in cleaning on the chemical composition of wood.

2.2. *Microbial enzymes, treatment and aging*

The following microbial enzymes were used:

Protease: from *Aspergillus oryzae* P6110 - 50ML (Sigma-Aldrich): this product is offered as a buffered solution of the enzyme and may be used directly or may be diluted to a working concentration using 100 mM Tris HCl buffer, pH 8.0/ according to Sigma-Aldrich Technical Service recommendations.

α-amylase: from *Aspergillus oryzae* 86250-100G, Powder 1.5U/mg (Sigma-Aldrich): this product was diluted in sodium phosphate buffer, pH = 6.7 at 20°C.

Regarding enzymatic treatment, the wooden samples divided to three groups; the first group treated with protease enzyme in different concentrations (5, 10, 20, 30, 40, 50 and 75 U/ml), the second one treated with α-amylase in the same concentration of protease at room temperature. The third one kept without treatments to compare the effect of both enzymes on the wooden samples (Fig. 1).

After treatment samples with the selected enzymes, the untreated and treated samples were subjected to accelerated aging that involved subjecting for moist heat aging at 80◦C and a relative humidity 65% for 240 hours at the National Institute of Standards (NIS) in Giza, Egypt. Then, all wooden samples were investigated.

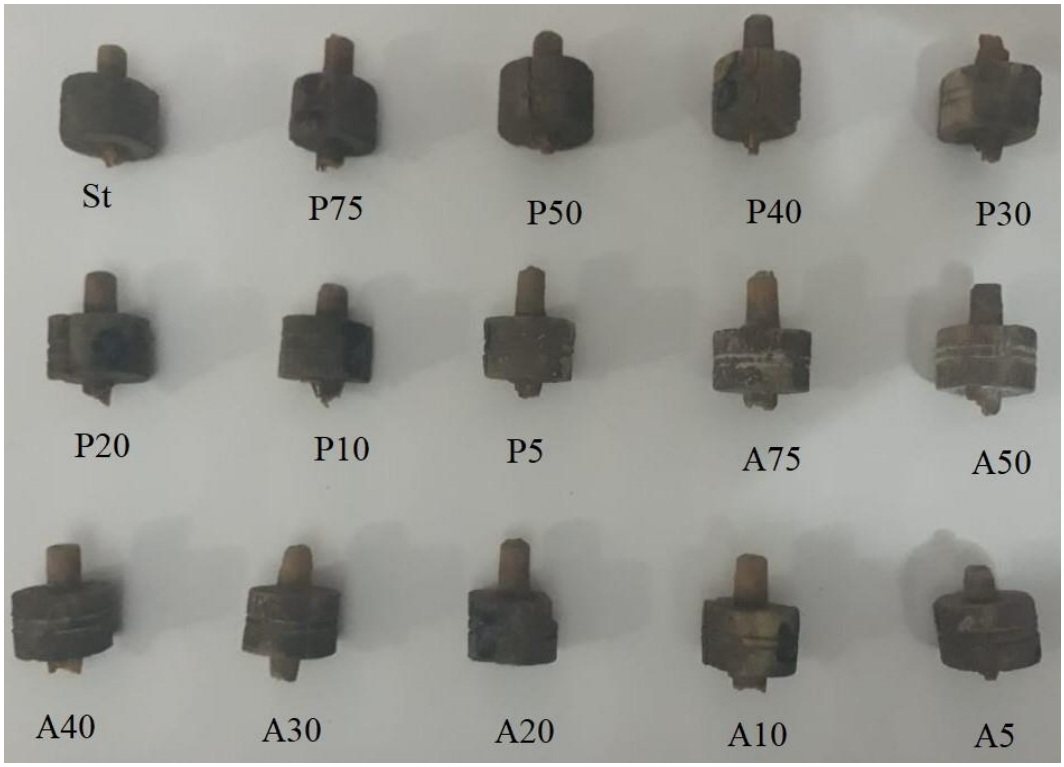

Figure 1. The wooden samples from an archaeological artifact after enzymatic treatment and aging

2.3. Colour measurement

The color coordinates were determined by using a Hunter lab colorimeter. The color parameters *L** (lightness), *a** (redness) and *b** (yellowness) as well as the overall color changes (Δ*E**) were determined in each wood sample before and after enzymatic treatment and aging according to the CIE-Lab color system. The total change in color indices (ΔE*), which were caused by the selected microbial enzymes used in wood cleaning, were calculated using the following equation (Mantanis and Lykidis, 2015):

$$\Delta \mathbf{E} = \sqrt{(\Delta \mathbf{l}*)^2 + (\Delta \mathbf{a}*)^2 + (\Delta \mathbf{b}*)^2}$$

Where: Δ*L**, Δ*a** and Δ*b** are the changes of the colour coordinates *L**, *a** and *b** for enzymatic treated and aged samples, compared to the control (untreated) sample

2.4. FTIR

FTIR spectroscopy was used for detecting the changes in the chemical composition of the wooden samples after enzymatic treatment and aging comparing with untreated sample. So, IR spectra of enzymatic treated and untreated samples were recorded by means of FTIR spectroscopy which was carried out on a Nicolet 380 FT-IR Spectrometer, in the frequency range of 4000 - 400 cm^{-1} with resolution of 4 cm^{-1}, in transmission mode using the KBr pellet technique. Peak heights and width of absorption bands were measured by essential FTIR software (version 310.041).

3. RESULTS AND DISCUSSION

3.1. Colour change measurement

The values of the determined colour parameters (*L*, a*, b**) of wood samples before and after enzyme application and aging are shown in table 1 and the overall colour changes are plotted in Fig. 2. The changes in lightness (*L**) were increased in wood samples treated with the selected enzymes (protease and α-amylase). Wood samples treated with protease showed the largest lightness increases compared to those treated with α-amylase. Also, samples treated with low concentrations of enzymes showed reduction in lightness compared to those treated with high concentrations of enzymes. Concerning the changes in *a** values (redness), all samples exhibited increasing in redness especially those treated with high concentration of the enzymes. Samples treated with protease showed the high values of redness compared to samples treated with α-amylase. Cleaning with microbial enzymes caused increasing in yellowing (*b**) in all treated samples; this was larger in samples treated with high concentrations of enzymes, but smaller in low concentrations. Notably, samples treated with α-amylase gives higher values in *b** than those treated with protease.

Wooden samples exhibited variable values of ΔE after enzymatic treatment and aging in range of 2.10 to 15.54 according to their concentrations. Generally, major changes in ΔE are observed in samples treated with high concentration of both enzymes. Using low

concentrations of these enzymes induced reduction in the total colour change (ΔE) especially in the lowest concentration (2.5%). So, the lower the enzyme's concentration, the less the colour change values. Furthermore, Protease caused the most notable change of colour compared to α-amylase in high concentration, but this change decreased to 2.10 in samples treated with protease 2.5%.

Table 1. The overall color values of the samples after enzymes treatment and aging.

Sample No.	Treatment	Δl	Δa	Δb	ΔE
1	P 75 U/ml	13.59	3.91	6.44	15.54
2	P 50 U/ml	12.86	3.40	6.86	14.97
3	P 40 U/ml	5.37	2.67	6.02	8.50
4	P 30 U/ml	4.35	3.71	3.51	6.71
5	P 20 U/ml	2.48	1.90	5.89	6.67
6	P 10 U/ml	2.48	1.47	5.33	6.06
7	P 5 U/ml	2.65	0.49	3.33	4.28
8	P 2.5 U/ml	0.74	0.28	1.95	2.10
9	AB 75 U/ml	4.37	2.71	7.54	9.13
10	AB 50 U/ml	3.69	1.19	6.13	7.25
11	AB 40 U/ml	2.64	1.35	6.14	6.82
12	AB 30 U/ml	2.78	1.14	4.86	5.71
13	AB 20 U/ml	2.26	0.69	3.24	4.01
14	AB 10 U/ml	2.15	0.66	2.54	3.39
15	AB 5 U/ml	1.33	0.49	2.79	3.13
16	AB 2.5 U/ml	0.35	0.46	2.18	2.26

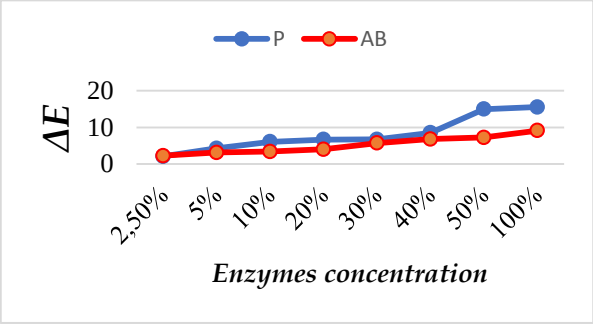

Figure 2. The total color changes after enzymes application and aging according to their concentration.

3.2. FTIR Spectroscopy analysis

Wooden samples were studied by FTIR spectroscopy after enzymatic treatment and aging and compared by untreated sample to detect any changes. The FTIR spectrum of enzymatic treated wood revealed significant changes in the fingerprint region (between 1800-600 cm^{-1}) notably in the samples treated with high concentrations of enzymes (Fig. 2, 3).

In samples treated with protease 75 U/ml and 50 U/ml, the broadening of the band at 3400-3426 cm^{-1} corresponding to the O–H group was detected as shown in (fig. 2a and b) which may be related to the presence of N-H amine group of the enzyme (Hamed, 2012). A high reduction of the absorbance of the typical bands assigned to cellulose at 1425, 1375, 1336, 1166, 897 cm^{-1} (Colom et al., 2003; Pandey and Pitman, 2003; Lionetto et al., 2012) was recorded with the absence of the band at 1317 cm^{-1}. Furthermore, a qualitative decrease of the intensity of the characteristic lignin bands at 1510, 1463, 1270 cm^{-1} (Lionetto et al., 2012) can be noticed. Hemicellulose bands at 1734, 1022 and 1059 cm^{-1} (El Hadidi, 2017) were significantly decreased. These differences started to decrease with the decrease the concentration of the enzymes to 40 U/ml (Fig.2c). The fingerprint bands remain somewhat similar to that seen for the untreated sample in the samples treated with protease 30, 20, 10, 5 U/ml (Fig. 2 d-g).

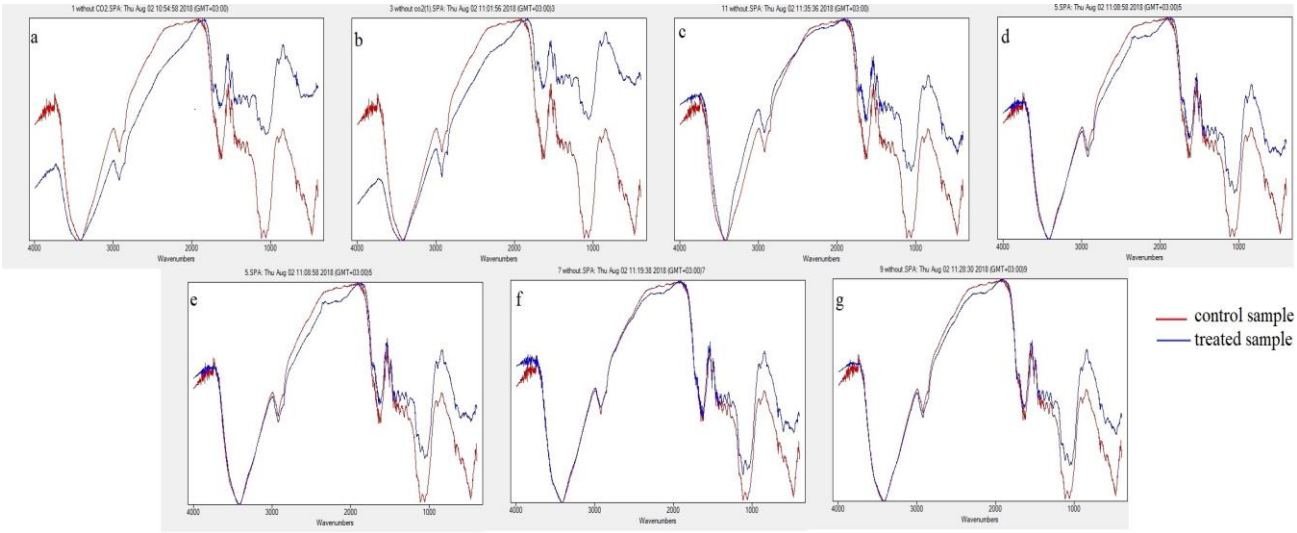

Figure 2. FTIR spectra of samples treated with different concentration of protease after aging compared to the control sample (untreated), where; (a) sample treated with protease 75U/ml, (b) sample treated with protease 50 U/ml, (c) sample treated with protease 40 U/ml, (d) sample treated with protease 30 U/ml, (e) sample treated with protease 20 U/ml, (f) sample treated with protease 10 U/ml, (g) sample treated with protease 5 U/ml.

The wooden samples treated with different concentrations of α-amylase show a similar trend to those treated with protease of the same concentrations, except for the broadening of the OH band, (Fig. 3) indicating that both enzymes have almost the same effect on the chemical composition of wood after aging since the hydrolytic enzymes are being active at higher temperatures (Nigam, 2013). On the other hand, the changes in the fingerprint region in samples treated with α-amylase were lesser than those which happened in samples treated with protease. This may be interpreted by the high activity of the protease especially in high temperature (Gupta et al., 2008; Nigam, 2013).

It is interesting to notice the considerable differences in the spectra for samples treated with different concentrations of both enzymes, since the changes decrease with the decrease of the enzymes' concentration. These results are in agreement with the literature (Shamolina et al., 2004; Lipp-Symonowicz et al., 2004; Ahmed, 2013) which suggested that enzymatic treatment caused partial removal of lignin. Moreover, the increase in the enzyme concentration enhances the degradation of cellulose, leading to disordering of the cellulose macromolecules.

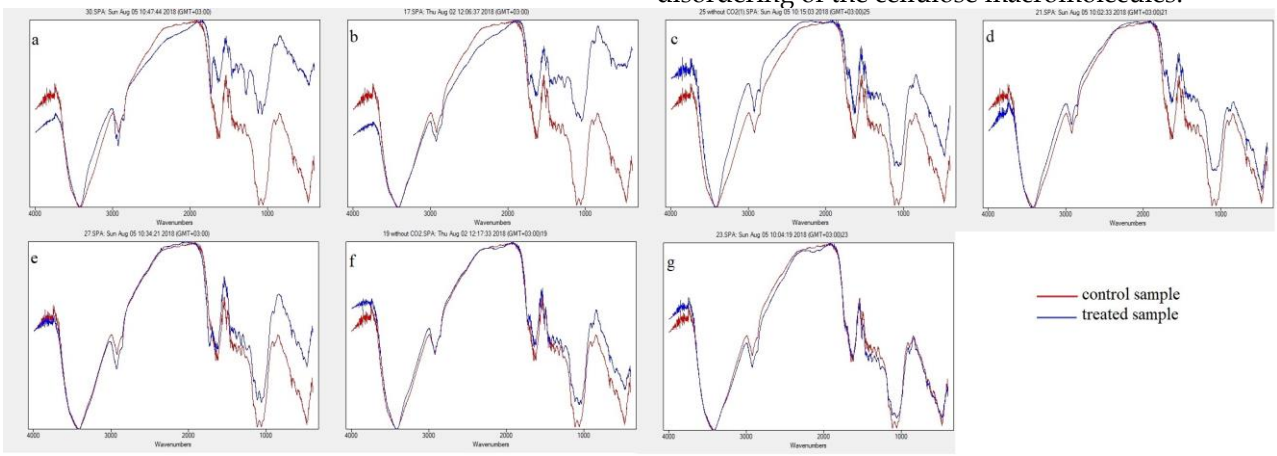

Figure 3. FTIR spectra of samples treated with different concentration of a-amylase after aging compared to the control sample (untreated), where; (a) sample treated with a-amylase 75U/ml, (b) sample treated with a-amylase 50 U/ml, (c) sample treated with a-amylase 40 U/ml, (d) sample treated with a-amylase 30 U/ml, (e) sample treated with a-amylase 20 U/ml, (f) sample treated with a-amylase 10 U/ml, (g) sample treated with a-amylase 5 U/ml.

4. CONCLUSION

Cleaning is a superficial process and performed most frequently in conservation the archaeological wooden artefacts, but it is irreversible and can be damaging or cause further alteration of the artefact. So, the chosen tools and materials influences the quality of the result. Searching for new materials that can be used in cleaning wooden artefacts is a contin-

uous process include evaluation their behaviour and their effect on wood substance. Therefore, this study concerning the assessment of the effect of two types of enzymes; protease and α-amylase with different concentrations on the wood colour and its chemical composition. In general, the high concentrations of both enzymes caused major changes in the overall colour values (ΔE) of the treated wood. Furthermore, the results confirmed that protease cause the most notable change of colour compared to α-amylase. Results of FTIR analysis of the enzymatic treated wooden samples provided a new perspective on the effect of enzymes on the wood composition that greatly differed based on their concentration. In this respect, the increase in the enzyme concentration affect the main components of wood; Cellulose, Lignin, Hemicellulose as it causes partial removal of lignin and promote the degradation of cellulose and hemicellulose. According to these results, one can agree with the researchers, who advocate considering enzymatic treatment as a last resort and use the lowest concentrations of enzymes since it considered the safest concentrations that can be used in wood cleaning.

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