Microbial Deterioration of Historical Textiles and Approaches for Their Control

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Abstract

Decaying books papers were collected in Erbil city, later decayed portions were excised and inoculated on nutrient agar plates, potato dextrose agar, dubo' s cellulose agar for microorganism isolation. Both *Bacillus subtilis* and *Acrodictys fimicola* were mainly isolated organisms and given positive test on Carboxyl Methyl Cellulose (CMC) media due to producing cellulase enzyme. Approaches were done to control microbial growth by modification of the ink which used in typing of the textiles. Since, the paper ink (pH =7) was mixed with lactophenol in different ratio 1:1, 1:2 and 2:1(volumes: volume) decreasing the pH in to 5, 3.7 and 4.2 respectively. As results which inhabit the growth of isolated bacteria and fungus because of unfavorable pH media, in addition to phenol composition which act as antimicrobials. The best result was obtained using mixture of two volumes of lactophenol with one volume of paper ink (pH=4.2) protecting textile surfaces from contamination and degradation.

KEYWORDS: Antimicrobial, degradation, decolourization, lactophenol cotton blue, cellulase.

Introduction:

Cellulose is the most abundant polymer found on the earth. Cellulases are a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobiose or oligosaccharides (Murad and Azzaz, 2010; Chinedu *et al*., 2010). Three types of cellulase enzymes are involved in the cellulose hydrolysis process including cellobiohydrolase, endoglucanase or carboxymethyl cellulase (CMCase) and β-glucosidases- (Bhat, 2000; Saber *et al*.,2010).

Cellulases enzymes produced mainly by microbial sources, starting from prokaryotic organisms like bacteria, and protozoan to eukaryotic organisms that catalyze the cellulolysis. However, there are also the cellulases produced by animal sources and plant materials. Cellulases are inducible enzymes that are synthesized by microorganisms during their growth on cellulosic materials (Ponnambalam *et al.,* 2011).
Cellulases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology, these enzymes produced by numerous microorganisms such as fungi, bacteria but the most common producer is fungi. *Aspergillus, Cladosporium, Fusarium, Geotrichum,Myrothecium, Paecilomyces, Penicillium* and *Trichoderma* species (Haight, 2005; Azzaz, 2009).It has been estimated that only about 5% of fungal species involved have been accurately described owing to culture limitations, misidentifications in culture collections, and unexplored habitats

(Hawksworth and Rossman, 1997).The level of cellulases activity and its application depend on the microbial producing strain, the media composition and process control (Ghose, 1987; Kheng *et al.*, 2006).

Fibers made of cellulose chains degrade when exposed to an acidic environment in the presence of moisture. Where in this acid hydrolysis reaction, cellulose chains are repeatedly split into smaller fragments as long as the source of acid remains in paper. This acid hydrolysis reaction produces more acid in the process, while the degradation accelerates in a downward spiral. (Shahani *et al.*2002)

Most modern book papers have a relatively short life span, which can be further reduced by improper storage environments. The exception to this general trend is alkaline paper, that is, paper contains an alkaline reserve. This alkaline reserve, most frequently chalks, neutralizes acids and also makes the paper look whiter. In addition to acid hydrolysis, papers are also vulnerable to photolytic degradation (damage by light), although newsprint papers are much more subject to this form of degradation than most other papers used to print books. Oxidation is also believed to play a role in the degradation of paper, although its role is limited as compared with acid hydrolysis, except probably in the presence of nitrogen oxide pollutants (Shahani and Harrison, 2002.).Paper deterioration is still a problem to libraries and archives which has to be result.

Bragulat, *et al.*, (1991) was tested the inhibitory effect of 13 dyes on the growth of mycelium of different fungi including members of the Deuteromycetes and Zygomycetes. He found that the dyes: aura mine, gentian violet, phenol red, methylene blue, dichloran and rose Bengal were performed, otherwise, the data obtained with malachite green show that the Zygomycetes strains were inhibited and the remaining organisms except *F. oxysporum* was completely inhibited.

An attempt has been made in the present study to isolate, identify, and optimize cellulases producing microorganism from decaying paper in addition to de-inking. The results obtained in this study have been reviewed with available literature.

Material and methods:

1-Isolation and preparation of pure cultures of bacteria from textile samples:

Decaying books papers were collected and the decayed portions were excised and inoculated on nutrient agar plates. The agar plates were incubated at room temperature (~35ºC). Pure cultures were prepared by streak plate method and they were maintained on agar plate by sub culturing once in a week.

Screening bacteria for cellulase activity:

After the pure culture formation, individual bacterium was inoculated on CMC agar medium containing carboxymethyl cellulose (5g/L), Peptone (5g/L), NaCl (5g/L), beef extract (3g/L),and agar (20g/L). The pH of the medium was adjusted to 7.0. The CMC agar plates were incubated at 37°C for 24 h.

A preliminary qualitative assay for cellulolytic activity was carried out using Congo red dye. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (0.1% w/v) for 15 min. The excess Congo red solution was

poured off, and the plates were further treated by flooding with 1M NaCl for 15 min. (Sakthivel *et al*, 2010).

Preparation of ink mixture (or modification) of stain.

Paper ink pH initially measured by pH meter, later prepare mixture of ink with lacto phenol locally prepared in addition to lactophenol from BDH company, in ratio 1:1,1:2,2:1(v/v) and measure the pH again .

Testing the quality of mixed paper dyes with the lactophenol cotton blue:

The filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vials, sterilized by oven and allowed to cool. The solution were prepared fromprinter dye + lactophenol cotton blue in a ratio (1:1), (1:2) and (2:1)blank discs were soaked in known concentration of solution, then used for sensitivity test after incubated at 37^{o}C for 24-48h, Zones of inhibition were obtained by measurement of the radius from the centre of the disc to the edge of the inhibition of growth. Measurements were made from both sides of the slope and their average accepted (Al Refai, 2006).

2-Isolation and preparation of pure cultures of fungus from textile samples:

Various bio-deteriorated textile samples were collected from storage rooms in some library in Erbil city. All textile samples collected composed of linen fibers only. Samples mycoflora were isolated by using Agar Plate Method (APM), cotton swab technique and biodeteriorated textile part technique.

a. Agar Plate Method: Paper from books cuts into small cubes then transferred directly with sterile forceps into Petri dish contain sterilized Potato Dextrose Agar (PDA) and Dubo′s Cellulose Agar (DCA). Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics (Pitt *et al.,* 1992).

b. Biodeteriorated textile part technique: In biodeteriorated textile part technique very small paper separated from the original ancient textile objects, paper cuts into small cubes transferred using sterilized tweezers into SDW then 1ml of water added to the media (Potato Dextrose Agar (PDA) and Dubo′s Cellulose Agar (DCA)) and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics (Abdel-Kareem, 1997).

c. Cotton swab technique: In the cotton swab technique, the fungal species were isolated using sterile moist cotton buds swabbed over the surface of ancient textile objects where fungal growth or fungal structures were observed. Cotton swabs were then used to distribute fungi on the media PDA and DCA, in Petri dishes. The dishes were incubated at 25°C for 5-7 days (Abdel-Kareem, 1997).

Later the fungal genera were identified by transferred the isolated fungal to sterilized plates for purification and identification. The grown fungi were mounted on a slide, stained with lacto phenol-cotton blue to detect fungal structures (Basu, 1980) and identified on the basis of their colony morphology and spore characteristics.

Preparation of spore suspension: The spore suspension of the selected fungus was prepared by adding 10 ml of SDW on the fungal plate, the spore was scraped by using a sterilized glass rod, and the spore mixture was then placed in a small sterilized vial put stir for 10 minutes. The spores were quantified using Hemocytometer and a light microscope. The spore suspension was then adjusted to ideal concentration of $1x10^6$ spores / ml (William *et al.*, 1976).

Testing the quality of mixed paper dyes with the lactophenol cotton blue:

For testing the effect of printer and ink dyes separately and mixed with lactophenol cotton blue on the growth of the fungus Acrodictys, the stock solution of mixed dyes: (printer dye, ink, printer dye + lactophenol cotton blue (1:1), ink + lactophenol cotton blue in a ratio (1:1), (1:2) and (2:1), (or at concentrations 25, 50 75 and 100%) for testing the quality of the printer and the ink dyes and filter paper without any addition as control) were prepared then poured into sterilized petri dishes containing filter paper, the wetted sterilized filter paper was inoculated with lope full of spore suspension of *Acrodictys* sp., then incubated at 25^{o}C for 10 days, then the growth of the fungus observed at each plates for comparing the results.

Results and discussion:

As a result of this study on deteriorated paper textiles in Erbil city, common bacteria and fungus were isolated and identified in our laboratory. .

Figure (1) shows the growth of *Bacillus subtilis* after 24 hrs. of incubation on CMC agar and demonstrated positive results. The applied bacteria has shown positive results for dyes degradation/ decolourization, as that indicated by the change and disappearance of color of the dyes from the dye-containing media of the Petri plates which indicates the production of extracellular enzymes by the applied bacteria, during the biodegradation of tested dyes. This result is agreement with that found by (Crispen *et al*., 2000).

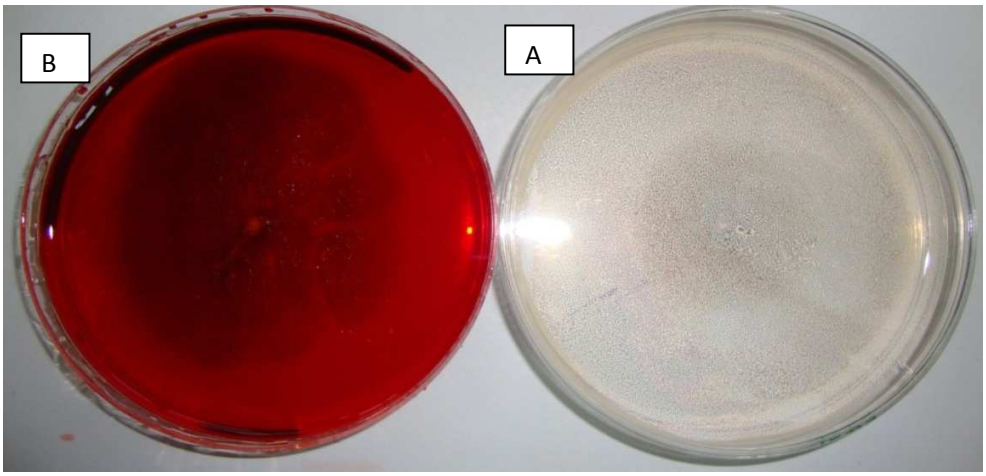

Figure (1) shows the growth of *Bacillus subtilis* on CMC agar (A) without and (B) with Congo red stain.

Congo red binds with carboxymethyl cellulose and turns into bright red. Cellulases produced by individual bacterium hydrolyzed carboxymethyl cellulose around the bacterial colony and the dye Congo red unable to stain it. Therefore, the hydrolyzed zone appears transparent while the unhydrolyzed regions appear bright red.

Table (1) and figure (2) show the inhibitory effect of bacteria growth in filter paper disc soaked in (ink :lactophenol cotton blue) in a ratio (1:1), (1:2) and (2:1) (v/v) , as shown in the figure(2) the filter paper used in the present study soaked in different concentration of mixed dyes for testing their inhibitory effects and the result was 2mm,4.2mm and 3.4mm respectively , filter paper stoked in ink used as control, the result shows that the pen ink allowed adequate colony development of the bacteria. That mean the dyes used in the printing and writing on paper have no any inhibitory effect. While in the case of plates contains filter paper soaked in the mixed dyes at different concentration which shows different inhibitory effect of bacterial growth that mean these dyes have been reported as anti bacterial effect.

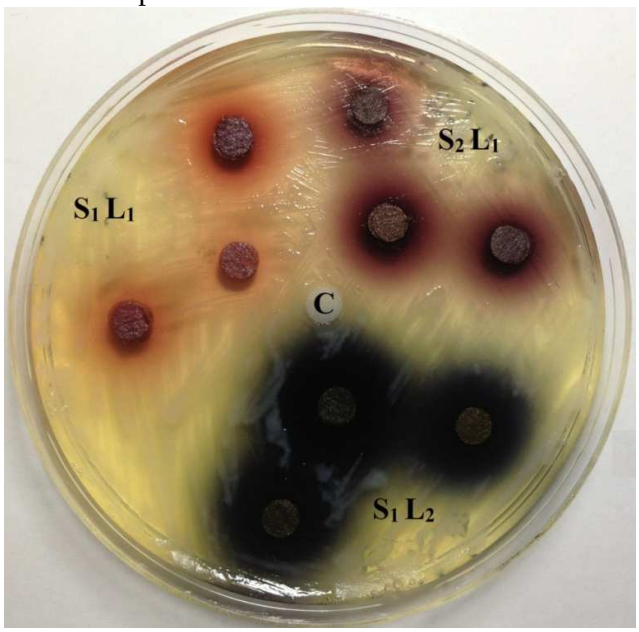

Figure (2) show the inhibitory effect of *Bacillus subtilis* growth in filter paper disc soaked in (ink(S): lactophenol cotton blue (L) (v/v)).

Table (1): Showing the inhibitory effect of lactophenol on the *Bacillus subtilis* growth.

	Ratio of paper ink (V): lactophenol (V)		
	S1: L2	S1:L1	S2:L1
Bacillus subtilus	4,2 mm	2mm	3.4mm

S: paper ink, L: lactophenol

Figure (3) shows the tested fungus *Acrodictys fimicola* after 7 days of incubation on CMC agar and demonstrated positive results in the Congo red test. The applied fungus has shown positive results for dyes degradation/decolourization, as indicated by the change and disappearance of color of the dyes from the dye-containing media of the Petri plates which indicates the production of extracellular enzymes by the applied fungus, during the biodegradation of tested dyes. This result is matches with that found by (Singh and Singh, 2010) who found *Aspergillus flavus* has which the ability for biodegradation of two commercially used textile dyes, Bromophenol blue and Congo red.

The result was also similar to biodegradation of Congo red and Bromophenol blue by the fungus *Trichoderma harzianam* in semi-solid medium (Singh and Singh, 2010). In the present study, dyes might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of *Acrodictys fimicola* during its growth in the dye-containing medium.

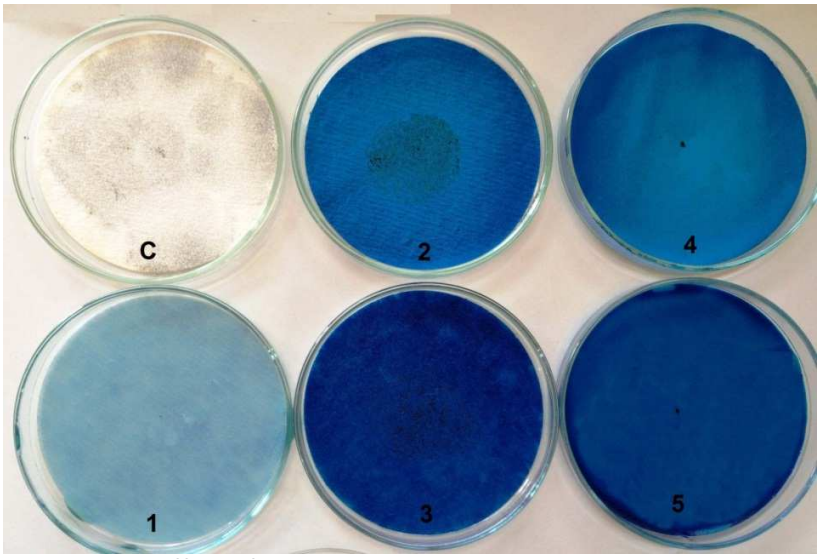

Figure (2): The inhibitory effect of lactophenol cotton blue stain with ink and printer ink on the growth of the fungus *Acrodictys fimicola* (C: Control, 1: lactophenol stain, 2: printer ink, 3: pen ink, 4:printer ink + lactophenol cotton blue stain, 5: pen ink + lactophenol cotton blue stain)

The results in figure (3) show the inhibition of fungal growth (*Acrodictys fimicola*) in filter paper soaked in lactophenol cotton blue stain, as shown in the figure (3) the filter paper used in the present study soaked in different dyes for testing their inhibitory effects, in the case of using filter paper soaked in different dyes: control (filter paper without any addition of dye), printer dye and pen ink allowed adequate colony development of the *Acrodictys fimicola*. That means the dyes used in printing and writing on paper have no any inhibitory effect, strains tested while controlling rapidly. While in the case of plates contains filter paper soaked in the dyes: lactophenol cotton blue alone, printer dye mixed with lactophenol cotton blue and ink dye mixed with lactophenol cotton blue and there was no any fungal growth that mean these dyes have been reported as mold-spreading inhibitors at different situation.

Generally, the paper ink is a complex medium, composed of solvents, pigments, dyes, resins, lubricants, solubilizes, surfactants, particulate matter, fluoresces, and other materials. The pH of paper ink and printing ink was (7) that is mean it is act as favorable

medium for micro organism contamination which cause of paper degradation by their extracellular cellulase enzyme.

While the prepared mixture (ink with lacto phenol) in ratio 1:1, 1:2, 2:1(v/v) were decreased the pH in to (5, 3.7, 4.2) respectively. That's mean producing of unfavorable pH for growth of isolated bacterial and fungal.

While the prepared mixture (ink and lactophenol) treated the pH effect by inhibition of bacterial and fungal growth because it acts as antimicrobial. However, pH 4.2 was the best in comparison to other pH of crude ink and ink mixtures.

As a result of analysis of carboxyl cellulose compose of the paper, that it enhances enzyme activity of cellulase which produced by bacteria and fungi, finally it causes degradation of paper. In addition to phenol composition of lactophenol which acts as antimicrobial for bacterial and fungal growth.

Results obtained in this study could make the development of new antifungal stain possibly happen. Lactophenol can be mixed with paper printing and writing ink for preventing paper and book deterioration in the libraries and archives.

Conclusion:

The sources of deterioration and degradation of historical textiles and stored book paper in Erbil city were the bacterial (*Bacillus subtilis*) and fungal (*Acrodictys fimicola*).It should be emphasized that mixture of paper ink with lactophenol is the best method to prevent fungal and bacterial growth on historical textiles in order to protect textile surfaces from any contamination.

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