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Anish Selvin Inbaraj a,b, Gladstone Christopher Jayakumar a,b

a Department of Leather Technology, AC Tech Campus (Housed at CSIR-CLRI), Anna University, Chennai, India.

b Biochemistry and Biotechnology Laboratory, CSIR- Central Leather Research Institute, Chennai, India

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Abstract

Leather is a natural and premium material used in the manufacture of fashionable commodities and lifestyle accessories. The care and maintenance required for a leather material is higher compared to a synthetic material. Proper conditioning, cleansing and restoration is vital to address the microbial deterioration of leather and to extend its longevity. Traditionally, chemical-based formulations have been employed to cleanse the leather surfaces infected by microbial growth. However, this method poses significant environmental concerns and can disrupt the integrity of leather surface finishes, thereby affecting the aesthetics of the finished material. Biofilm formation on the leather surface by microorganisms is another challenge in leather sector which is restored using chemical-based cleansing agents. These cleansing agents is the temporary solution for a very short time interval. Utilization of bio-catalysts to achieve an effective cleansing method, would be a cleaner and sustainable approach for long-term leather maintenance. Enzyme-based bio-cleansing formulations is composed of multiple carbohydrase enzymes, which are known to effectively cleave the outer cell membranes of microbial species, eradicate microbial growth from the core, and resist the microbial growth and contamination comparatively for a longer period. Hydrolytic enzyme, cellulase was used in the present study to remove the microbial contamination from the vegetable tanned leather. Different concentration of cellulase has been used to optimize the effective removal of microbial contamination. From the results, it has been inferred that 2% cellulase concentration would result in effective leather cleansing.

Corresponding author email: anishselvininba@gmail.com

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1. Introduction

The skin/hide used as the primary raw material for leather manufacture is prone to microbial degradation. In leather processing, curing and tanning are the primary process to temporarily and permanently prevent the skin/hide from microbial degradation respectively [1]. However, certain microbial contamination, especially fungal growth is possible on the leather surface under appropriate environmental conditions. Excessive humidity and moisture conditions can accelerate the mould/fungal colonization on the leather surface which is prominent in the case of tanned/crusted leathers.

Species of Aspergillus and Penicillium genera were most prevalent and widely observed on leather surfaces contaminations [2]. These species can be often vulnerable and highly opportunistic in nature, known to act as disease causing pathogens in humans [3]. Persistence of such microbes will be leading to the production of different coloured stains alongside of microbial growth [4]. The stains of such kind are difficult to remove. The industrial practices of handling such complications involves using

chemical fungicides during processing. Chemical fungicides namely chlorophenols are been widely used as a method of treatment which leads to environmental impact and no longer a sustainable practice [5]. The current study focusses on utilization of bio-based cleansing agents which is capable of removing the bio-films and prevent the leather from microbial contamination.

2. Methodology

2.1. Material Selection

The microbial contaminated vegetable tanned crust leather was identified and taken for enzymatic microbial cleansing process. The commercial cellulase enzyme was procured from Shriji Exbix Pvt. Ltd., was used for the processing.

2.2. Enzyme Activity

Activity of the cellulase enzyme was studied using filter paper assay technique in which Whatman No.1 filter paper was used as the substrate. The catalytic activity of the total cellulase enzyme (endoglucanase, exoglucanase and â-glucosidase) upon the filter paper was studied at a

Table 1. Biocatalytic leather cleansing process

Process Chemicals/Reagents Percentage (%) Time (min) Raw Material: Microbes infected leather material

Enzyme Treatment Water 100

180 Cellulase X

X – 1%, 2%, 3%, 4% and 5%.

Table 2. Control leather cleansing process

Process Chemicals/Reagents Percentage (%) Time (min) Raw Material: Microbes infected leather material

Water 100

Control Treatment

180 Hydrogen peroxide 3

standard working temperature of 50 ºC and at different pH condition ranging from 3 to 6. Citrate buffer was used to maintain the pH. The enzyme solution (1 mg/mL) was added to the test tube containing filter paper strips, which was incubated for 60 min. The incubated mixture was added with 3 mL DNS reagent and was incubated in a boiling water for 5 mins. The test samples were diluted with equal volume of distilled water. Absorbance was measured at 540 nm, which will directly corelate to the concentration of glucose liberated in the reaction mixture [6]. Known concentration of glucose solution was used to derive the standard curve.

2.3. Enzymatic Cleansing

The cleansing behavior of the cellulase was studied on the microbial contaminated vegetable tanned leather. Vegetable-tanned leathers were previously allowed to get infected by a cocktail of microbial broth solution. The contaminated vegetable leather sample was incubated for 72 h at 37 ºC. The contaminated leather samples were taken for bio-cleansing process as shown in Table 1. Hydrogen peroxide was used as control cleansing agent (Table 2).

3. Result and Discussion

3.1 Enzyme Activity

Figure 1. (A) Glucose Standard graph (B) Effect of pH on enzyme activity

Enzyme activity studies have been carried out at different pH conditions viz., 3, 4, 5 and 6. The filter paper substrate was initially placed in the respective buffers with appropriate pH, to which enzyme solution was added followed by incubation, ceasing the catalytic activity and measurement of absorbance at 540 nm. From the Figure 1B., at pH 4 the cellulase enzyme shows an increased catalytic activity and found to be optimal pH condition for bio-cleansing process while the activity is decreased by increasing the pH to neutral condition. The standard curve for cellulase activity measurement using glucose is shown in Figure 1A.

Table 3. Leather trial images (A) Control; (B - F) Experimental samples

3.2. Enzymatic Cleansing

Carbohydrase enzymes are widely used in multiple sectors such as medical and food industry as an eco-friendly cleansing agent. The treatment of microbial colonies with carbohydrase enzymes will catalysis the degradation of the outer cell membranes of the microbes. The leather control samples treated with hydrogen peroxide and the experimental samples treated with the cellulase enzyme is shown in the Table 3 at different time intervals. The 2-5% cellulase enzyme treated contaminated leather samples shows complete removal of microbial contaminants at the end of 3 h, except for 1 % cellulase enzyme treated sample.

Cleansing

Concentration

Before Cleansing

agent

(%)

(0 h)

1

2

Cellulase

3

Enzyme

4

5

4. Conclusion

The enzymatic cleansing of the leather surfaces has resulted in completed biofilm removal comparable to that of the control surface cleaning technique. It was also found that the treatment of leather surfaces with 2% cellulase enzyme was an optimal concentration for

During Cleansing

After Cleansing

(1.5 h)

(3 h)

complete removal of microbial contaminants. In future studies, the combination enzyme system will be performed to study the synergistic action of cocktail enzymes on the microbial contaminants on the leather surfaces. Hence, enzyme mediated catalytic mechanism will be a sustainable and eco-friendly technique to effectively remove microbial contaminants on leather surfaces.

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