ECO-FRIENDLY BIODEGRADATION OF SKINS AND HIDES BY KERATINOLYTIC FUNGUS *Cladosporium* SP.

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As a result of population growth and changes in lifestyles, livestock and meat production is increasing throughout the world. Therefore, a large amount of keratinaceous waste is generated annually from food and leather industry. The conventional method of hair removal in the leather industry through all the chemical processes used creates a great concern for the environment, being a major contributor to the production of waste water. The enzymatic process through microorganism is an eco-friendly option to reduce the oxygen demands in leather processing. In biodegradation and bioremediation processes, waste or polluting products found in different waste substrate can be transformed or converted into unpolluted end products. Our experiments are related to the biodegradative potential of fungi in reducing and reusing waste from the leather industry. The aim of the present study was to evidence the biodegradative ability of the fungal strain *Cladosporium* sp. on keratin wastes.

Keywords: biodegradation, keratinolytic fungi, leather industry

INTRODUCTION

Leather industry is one of the most polluting industries, harmful to the environment due to different dangerous chemicals that are used during the process (Awulachew, 2021; Silva, 2021). The produces high amount of dissolved and suspended organic and inorganic solids that are giving rise to high oxygen requirement. The presence of sulphide, ammonia and other volatile compounds are toxic to environment. At the same time, it also results in solid waste, including animal skin trims, animal hairs, flesh wastes, and keratin wastes, whose protein content is responsible for dangerous pollution problems to environment (Jaouadi *et al*., 2018; Călin *et al*., 2017). There are many countries where leather industry brings social and economic benefits in daily lives, but on the other hand, the industry has a negative impact on environment (Kanagaraj *et al*., 2015; Elhoul *et al*., 2020; Wu *et al.*, 2017). Accordingly, restricted environmental regulations have focused to develop an economic, social, and environmentally sustainable system, to manage, to minimize and finally to eliminate the hazardous chemicals.

Significant efforts were been made to develop sustainable alternatives, like, partially replacement of chemicals by biological and enzyme-based process, or combined technologies. Application of biotechnological methods has showed a reduction of environmental negative impact.

Our experiments represent an attempt to apply biotechnological methods in leather manufacture, and therefore, the present experimental study was dedicated to highlighting the biodegradative ability of the fungal strain *Cladosporium* sp. expressed against keratin wastes.

https://doi.org/10.24264/icams-2022.II.5

MATERIALS AND METHODS

Fungal Strain

The geophylic fungal strain *Cladosporium* sp. belonging to Microbial Collection of Microbiology Laboratory from INCDCP-ICECHIM was used in the experimental study. Prior to use the stock culture, *Cladosporium* sp. isolate was maintained at 4°C on potato-dextrose-agar medium (PDA, Scharlau) having the following composition (g/L): 4, peptone; 20, glucose; 15, agar; final pH = 5.6.

Keratin Substrates

Keratinous substrates used in the experiment were sheepskins and sheep leather, from the INCDTP - National Research & Development Institute for Textiles and Leather, Bucharest. All the samples of keratin substrate were sterilized, three times, at 121°C, 15 min.

Fungal Cultivation in Liquid Culture Media for Substrate Biodegradation

Cultivation in liquid culture medium was performed in Erlenmeyer flask, in mineral basal medium with the following composition (g/L): 0.1, CaCl2; 0.1, KH2PO4; 0.1, FeSO4 × 7H2O; 0.005, ZnSO4 × 7H2O; pH=7.5. The medium was autoclaved at 121°C, for 15 min. For the biodegradation ability test, an animal skin fragment (5x5 cm) was placed in culture medium. The flasks were incubated on rotary incubator Heidolph Unimax 1010, at 100 rpm and 28°C for 3 weeks. Control flasks used in tests were as follows: i) basal mineral medium with fungal strain; ii) basal mineral medium with keratin substrate and without fungal strain. After 3 weeks, the sheepskins and sheep leather were collected, dried in an oven at a temperature of 75°C for 48 hours, and examined. The degree of substrate biodegradation was evaluated with several methods, as follows: microscopic observations at light microscopy on Olympus BX51 and Scanning Electron Microscopy (SEM) on a FEI-QUANTA 200 microscope; FTIR (Fourier transform infrared spectroscopy) spectra recorded by using the FTIR Spectrum GX (Perkin Elmer), by ATR tehnique, 600 to 4000 cm^{-1}, with 32 scans.

RESULTS AND DISCUSSIONS

Following the contact between the keratin substrate and the fungal strain, several structural changes of the substrate were observed compared to the control specimens, like (Figure 1): loss of protein material, formation of mycelium networks around the substrate, exfoliation and partial destruction of the substrate (Figures 2-3).

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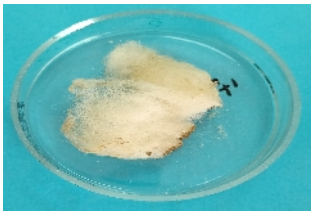
Control – sheep skin sample

Sheep skin sample after incubation with *Cladosporium* sp.

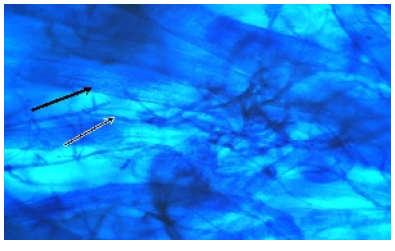


Control – sheep fur sample

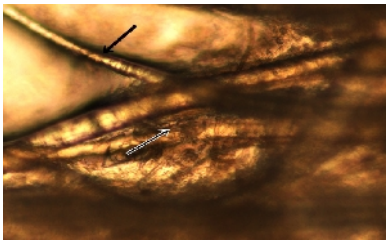
Sheep fur sample after incubation with *Cladosporium* sp.

Figure 1. Samples after incubation in liquid culture medium with (right) or without fungal strains (left)

Microscopic images of the substrates incubated with the fungal strain are presented in Figure 2. It can be observed the formation of hyphae networks of *Cladosporium* sp. on the substrate surface, intertwining among the hair strands of sheep skin sample.



Sheep skin sample after incubation with *Cladosporium* sp. Formation of hyphae networks of *Cladosporium* sp. (white arrow) on the substrate surface (black arrow) (10x)



Sheep fur sample after incubation with *Cladosporium* sp. Hyphae (white arrow) intertwining among the hair strands of sheep skin sample (black arrow) (10x)

Figure 2. Microscopic images (SEM) of the substrates incubated with the fungal strain (lactophenol blue cotton staining)

The SEM analysis highlighted some structural changes in samples incubated with fungal strain as compared to control samples (Figure 2). A dense network of hyphae tightly adhered to the surface of the hair strand from the substrate, producing exfoliation and even destruction of the substrate.

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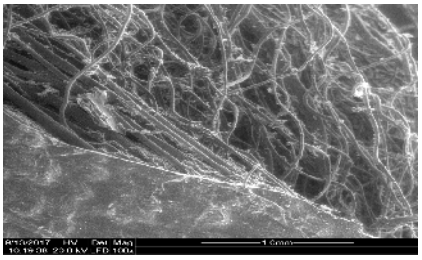
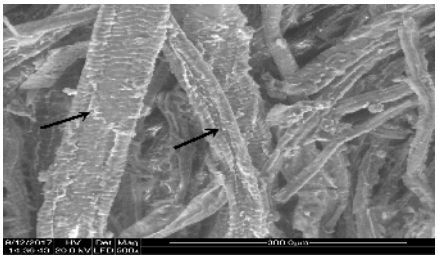
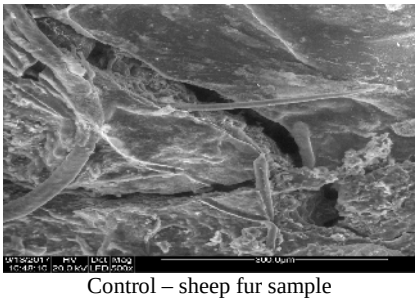


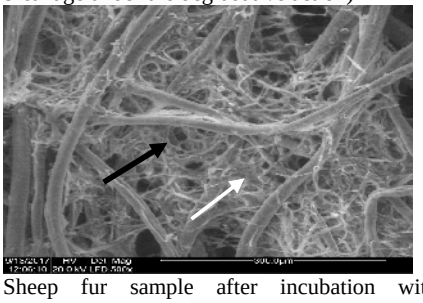
Control – sheep skin sample



Sheep skin sample after incubation with *Cladosporium* sp. (hair strands and exfoliation breakage under the degradative action)



Control – sheep fur sample



Sheep fur sample after incubation with *Cladosporium* sp. (dense network of hyphae (white arrow) tightly adhered to the surface of the hair strands from the substrate (black arrow)

Figure 3. SEM micrographs of the animal skins sample after incubation with *Cladosporium* sp.

FTIR results for sheep skin sample incubated with *Cladosporium* sp. strain are presented in Figure 4.

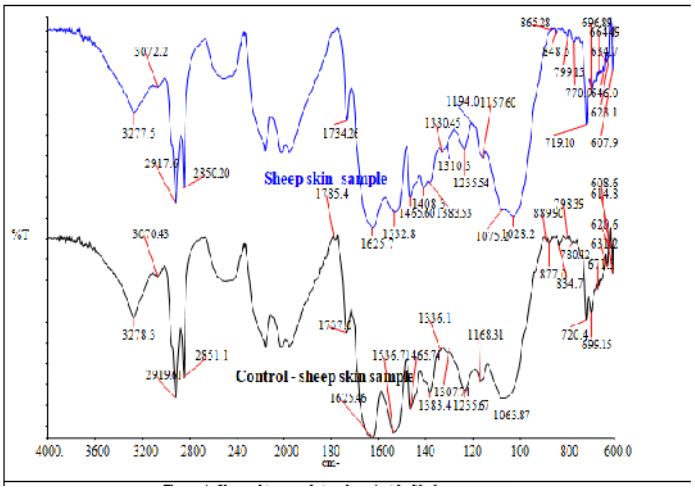


Figure 4. Sheep skin sample incubated with *Cladosporium* sp.

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In FTIR spectra it can be observed the absorption bands at 3276 cm^{-1} and 3072 cm^{-1} which are assigned to the stretching vibrations of O-H and N-H. The strong absorption bands at 1626 and 1533 cm^{-1} are derived from the C=O stretching, N-H bending, and C-H stretching, respectively. The band at 1236 cm^{-1} resulted from the combination of C-N stretching and N-H in plane bending as well as some contribution from C-C stretching and C=O bending vibration (Ma *et al*., 2017).

During the degradation process, the C-S and S–S bonds were affected by the biodegradative activity of microorganism. The bands assigned to sulfide bonds S–S occured in the range 635–600 cm^{-1} and the bands assigned to C-S bonds occured in the range 670-646 cm^{-1}. As signs of degradation initiation can be considered the appearance of the bands at 1075–1028 cm^{-1} assigned to the sulfoxide bond (S-O), resulted form the breaking of disulfide S–S bonds. The presence of the oxide forms of sulfur is important for the oxidative process occurring as monoxide-to-dioxide, then proceeding to full oxidation with the formation of cysteic acid.

Figure 5 depicted FTIR results for sheep fur sample incubated with *Cladosporium* sp. strain.

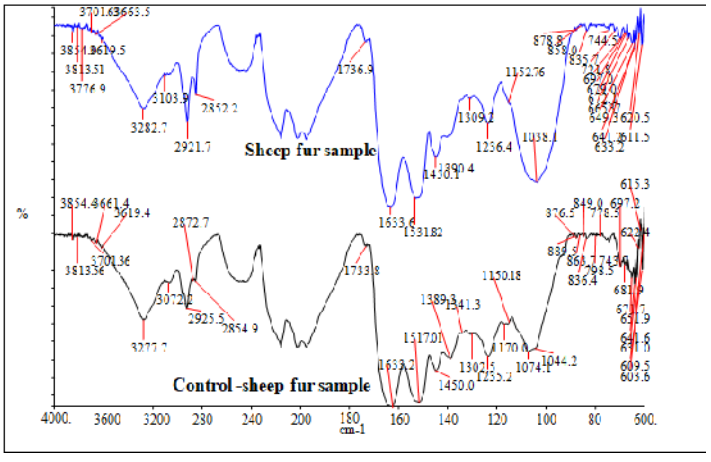

Figure 5. Sheep fur sample incubated with *Cladosporium* sp.

For the sheep fur sample, FTIR analysis shows significant change in amide region. The characteristic amide A band occurred at 3280–3270 cm^{-1}. Thus, amide I is related to C–O stretching and occurs at 1700–1600 cm^{-1}, while amide II is related to N-H bending and C–H stretching vibration and falls in 1540–1520 cm^{-1}. Amide III is related to a combination of C–N stretching and C–O bending vibration and occurs in the range 1300–1220 cm^{-1}. For the Ot2 sample, the amide I, II, and III bands were visible at 1634 cm^{-1}, 1532 cm^{-1}, and 1236 cm^{-1}, respectively in accordance with other reports on horsehair biodegradation.

In the sulphoxide region at 1074 cm^{-1}, the band corresponding to S-O bond was observed of medium intensity for undegraded sample (Ot, martor, reference), while for degraded sample (Ot2), this band disappeared and appeared as a large band and much intensive at 1038 cm^{-1}, corresponding also to S-O, due to S–S bond breaking (Kumar *et al.*, 2020).

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In conclusion, FTIR spectra of samples incubated with fungal strain showed that C-S and S–S bonds were affected by fungal strain enzymes, indicating the progress of the degradative process.

CONCLUSIONS

Incubation of keratinolytic fungal strain with keratin substrates allowed to evidence significant aspects. Thus, microscopic examinations showed several changes, like, dense network of hyphae tightly adhered to the surface of the hair strand from the substrate, hair breakage under the degradative action, and the exfoliation and even substrate destruction. FTIR spectra showed the presence of bands assigned to the sulfoxide bond, resulted from the breaking of disulfide S–S bonds from keratin. The corroboration of microscopic observations with FTIR results proved that *Cladosporium* sp. had the ability to exert a biodegradative activity on keratin substrates and may play an important role in leather manufacture for removing hair from animal skins.

Acknowledgments

The authors thank the Ministry of Research, Innovation and Digitization of Romania through Program 1 - Development of the national research and development system, Subprogram 1.2 -Institutional performance- Projects to finance excellence in RDI, Contract no. 15PFE / 2021.

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