PELT WASTE DEGRADATION USING ACTIVE MICROBIAL CONSORTIA

RODICA ROXANA CONSTANTINESCU¹, GABRIEL ZAINESCU¹, MARIANA FERDES²,
IULIA CANIOLA¹

¹INCDTP – Division Leather and Footwear Research Institute, 93, Ion Minulescu Str., Bucharest,
Romania, rodica.roxana@yahoo.com

²University Politehnica of Bucharest, Faculty of Biotechnical Systems Engineering, Romania

In tanneries, environmental problems have special implications in terms of optimizing the consumption of used chemicals, applied technologies, the degree of recovery of useful substances from leather waste. Biodegradation is the process by which organic substances are broken down by microorganisms. From an ecological point of view, biodegradability assessments of new materials and compounds in the industry are essential to understand and quantify their impact on the environment. The sustainable development of the leather industry has focused on major environmental issues, such as clean production methods and waste management. Tanneries generate huge quantities of solid wastes as pelt waste. If these bio-waste materials are not utilized properly, they are potential source of pollution. Microbiological degradation of pelt waste is amongst the permanent concerns of leather processing units. The process may have the purpose of decomposing waste to exploit by-products as biocompost or to obtain proteases through a biotechnological process. These enzymes can be used after purification in various processes that have animal protein as a substrate. They can also be used in raw state for enzymatic hydrolysis. The paper aims at development of an experimental model on the bioenzymatic degradation process of protein waste from tanneries.

Keywords: tanneries, pelt waste, enzymes.

INTRODUCTION

Tanneries generate huge quantities of solid wastes as fleshings. If these bio-waste materials are not utilized properly, they are potential source of pollution. Leather has a complex composition comprising collagen, keratin, elastin, albumins and globulins (Sundar *et al.*, 2017). Each of these compounds can be degraded under certain environmental conditions (temperature, humidity, pH, O₂ concentration) under the action of enzyme complexes synthesized by a variety of microorganisms (bacteria and fungi). Leather waste degradation occurs by means of proteolytic enzymes (Tang *et al.*, 2021).

Worldwide research for leather waste recycling is aimed at obtaining protein composites, through biochemical treatments with microorganisms/enzymes and obtaining hydrolyzed proteins and biocomposites for various uses in forestry. Currently, the entire amount of solid waste resulting from leather processing is deposited in the landfill, leading to the accumulation of chemicals in the soil, with possible negative effects on the ecosystems (Thankaswamy *et al.*, 2018).

Microbiological degradation of pelt waste is amongst the permanent concerns of leather processing units. Microorganisms (fungi and bacteria) play an important role in solving these problems (Constantinescu *et al.*, 2015).

Molds or filamentous fungi are eukaryotic spore-forming microorganisms, feeding by absorption, easily adaptable, because they have the ability to form induced enzymes depending on the nature of the substrate they are found, causing degradation (Rangel Serrano *et al.*, 2003).

Enzymes are organic substances, generally proteins, known like biocatalyst to multiples chemicals reactions and are commercially exploited in detergent, food,

pharmaceutical, diagnostics and fine chemicals industry. They are between the more notable biomolecules due their extraordinary specificity and catalytic power, which are higher than the catalytic power of catalyst produced by man (Gupta *et al.*, 2019).

The main enzymes that are of interest to the leather industry are as follows:

- Proteases because they hydrolyze the protein fraction of dermatan sulfate, making the collagen more accessible to water and reducing the attachment of the basal layer. In addition, they act in the removal of globular proteins;
- Lipases, which hydrolyze fats, oils and greases present in the hypoderm;
- Keratinases, which hydrolyze the keratin of hair and epidermis and break down the disulfide bonds of this molecule (Hussain *et al.*, 2020).

Most degradations found on leather waste are due to the action of fungi, a microorganism group very rich in enzymes. The following strains have been identified: *Penicillium chrysogenum*, *P. rugulosum*, *P. brevicompactum*, *P. luteum*, *P. decubens*, *P. aculeatum*, *P. funiculosum*, *Aspergillus niger*, *A. fumigatus*, *A. ochraceus*, *A. ventii*, *A. flavus*, *A. oryzae*, *Mucor mucedo*, *Rhizopus nigricans*, *Paecilomyces varioti*, *Scopulariopsis brevicaulis*, *Verticillium glaucum*, *Trichoderma* species (Zhang *et al.*, 2017; Wosten, 2019).

Microorganisms (bacteria and fungi) are an excellent source of these enzymes and can be used as such for various purposes, including in the leather industry.

In recent years there have been numerous attempts to degrade hide waste by treatment with alkaline proteases in order to obtain protein products. Enzymatic methods use commercially available alkaline proteases, at moderate temperatures and for short periods of time. This relatively simple method can be a solution for the removal of potentially harmful waste. A variant of the method is based on the proteolytic activity of microbial cells, especially of microorganisms isolated from this waste.

The potential of these enzymes, used in the pure state or as extracellular enzymes elaborated by cultures of microorganisms, is much higher and it is considered that their application in other processes in the near future creates a major field of research advance in the field (Calin *et al.*, 2017).

The paper aims at development of an experimental model on the bioenzymatic degradation process of protein waste from tanneries.

EXPERIMENTAL

Samples of ground protein waste were used in the experiments. They are gray-yellowish, with hard, slightly gelatinous, wet consistency.

Materials and Methods

The samples taken in the study were protein waste resulting from different hide processing stages from tanneries. Different-looking hide samples were selected, which were numbered as follows:

- P 1 - hide fleshings from Pielorex (white - gray), with smooth outer surface,
- P 2 - hide fleshings from Pielorex, with rough surface,
- P 3 - hide fleshings from ICPI, with smooth surface,
- P 4 - hide fleshings from ICPI, with rough surface.

In order to determine the pH value of the samples, from each protein waste were taken 1 cm² fragments, which were suspended in Erlenmeyer vials with 25 ml physiological serum.

<https://doi.org/10.24264/icams-2020.II.5>

Also, samples of the same surface were suspended in Erlenmeyer vials with 25 ml of nutrient broth and incubated at 28°C for 72 hours. The resulting culture liquids were used for decimal dilutions. These were seeded by incorporation into Petri dishes with nutrient agar, in three repetitions, in order to obtain isolated colonies and to determine the number of viable cells.

The plates were incubated for 24 hours at 28°C and then the number of isolated colonies was estimated by calculating the average of the values obtained in the three repetitions performed for each dilution.

The study of the morphological characters of isolated microorganisms was performed by optical microscopy observations on Gram stained smears, which were examined under a microscope.

The ability of isolated strains to hydrolyze proteins was determined by gelatin and casein hydrolysis assay.

Also, in all the gelatin and casein hydrolysis testing experiments, controls were used, represented by Petri dishes or tubes with non-inoculated media.

RESULTS AND DISCUSSION

Determination of pH value revealed the pH change of the physiological serum after immersion of hide samples, from pH 6.4 to values between 6.6-8.6.

The calculation of the number of viable cells obtained on plates with nutrient agar medium resulted in values between $4 \cdot 10^7$ for hide samples P 1 and P 2, and $5 \cdot 10^7$ for sample P 3, while sample P 4 showed a relatively lower number of bacterial cells - $2 \cdot 10^6/\text{cm}^2$ (Table 1).

Table 1. Determining the number of cells obtained on plates with nutrient agar medium

No.	Sample	pH	Number of bacteria / cm^2 sample	Number of isolated strains
1.	P 1	6.88	$4 \cdot 10^7$	6
2.	P 2	6.85	$4 \cdot 10^7$	4
3.	P 3	6.65	$5 \cdot 10^7$	5
4.	P 4	8.67	$2 \cdot 10^6$	5

From the four samples analyzed, a total number of 20 bacterial strains were isolated using the serial decimal dilution method, which was seeded on nutrient agar. Most strains showed colonies of white-beige color, with matte, flat, rough surface and irregular edges. In the case of sample P 3, the strains forming spherical colonies on the nutrient agar predominated, of white color, with smooth surface and even edges.

The P 4/4 and P 4/5 strains differed in color and appearance of the formed colonies, these being dark beige, semi-glossy, flat, with smooth surface and even edges.

The microscopic examination of the Gram stained smears enabled verification of the purity of cultures and showed that most of the strains were represented by Gram-positive bacilli, with rounded heads, arranged in short chains of 2 to 4 cells or in irregular agglomerations.

Pelt Waste Degradation Using Active Microbial Consortia

Table 2. Morphological culture and biochemical characters of isolated bacteria strains

No.	Strain	Colony morphology	Cell morphology	Gram reaction	Protein hydrolysis (after 96 h incubation)	
					gelatin	casein
1.	P 1/1	White, matte colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	+++
2.	P 1/2	Small white, matte, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
3.	P 1/3	White-beige, matte, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
4	P 1/4	White, matte, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
5	P 1/5	White-beige, matte, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
6	P1/6	White-beige, matte, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
7	P2/1	White, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
8	P 2/2	White, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
9	P 2/3	White, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
10	P 2/4	White, flat colony with rough surface and irregular edges	Bacilli with rounded heads, in short chains, with central spore	+	+++	++
11.	P 3/1	Large white, round, matte, flat colony with smooth surface and even edges	Bacilli with rounded heads, in short chains or agglomerations, with central spore	+	+++	+++
12	P 3/2	Large white, round, matte, flat colony with smooth surface and even edges	Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++

<https://doi.org/10.24264/icams-2020.II.5>

No.	Strain	Colony morphology	Cell morphology	Gram reaction	Protein hydrolysis (after 96 h incubation)	
					gelatin	casein
13	P 3/3	Large white, round, matte, flat colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
14	P 3/4	Large white, round, matte, flat colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
15	P 3/5	Large white, round, matte, flat colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
16	P 4/1	Cream-white, matte colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
17	P 4/2	White-beige, matte, flat colony with rough surface and irregular edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
18	P 4/3	Beige, matte, flat colony with rough surface and irregular edges	with central spore Bacilli with rounded heads, in short chains or agglomerations,	+	+++	+++
19	P 4/4	Dark beige, semi-glossy, flat colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains, arranged in pairs or isolated, with central spore	+	-	+++
20	P 4/5	Dark beige, semi-glossy, flat colony with smooth surface and even edges	with central spore Bacilli with rounded heads, in short chains, arranged in pairs or isolated, with central spore	+	-	+++

Gelatin hydrolysis testing revealed that of the 20 isolated strains 18 resulted in complete liquefaction of the culture medium whereas, two strains, P 4/11 and P 4/12 (isolated from sample P 4), did not show the ability to hydrolyze gelatin.

Testing the capacity of casein hydrolysis led to similar results for both experimental variants used. Thus, all the strains tested determined the hydrolysis of the substrate and clarification of the culture medium around the bacterial growth areas.

CONCLUSION

Microbiological analysis of 4 samples of hide waste revealed the presence of bacteria and fungi. The density of bacteria developed on hide samples was estimated at $4\text{--}5 \cdot 10^7$ cells / cm² in the case of samples P 1 - P 3 and $2 \cdot 10^6$ cells / cm² in the case of P 4.

The vast majority of isolated colonies have a morphology characteristic of the species of the genus *Bacillus*, with irregular shapes. Microscopic observations revealed the presence of bacillary, sporulated, Gram-positive cell forms. The morphology of the colonies and cells, as well as the response to Gram staining, confirms that the bacteria belong to the genus *Bacillus*.

As a result of the isolation in pure cultures, 20 bacteria strains have been obtained, representing a first collection of bacteria that contaminate hide samples.

The proteolytic capacity of the tested strains was highlighted by the gelatin and casein hydrolysis test.

Acknowledgements

This project is funded by the Ministry of Research and Innovation within Program 1 - Development of the national R&D system, Subprogram 1.2 - Institutional Performance - RDI excellence funding projects, Contract no 6 PFE/16.10.2018.

REFERENCES

- Călin, M., Constantinescu-Aruxandei, D., Alexandrescu, E., Răut, I. and Lazăr, V. (2017), "Degradation of keratin substrates by keratinolytic fungi", *Electronic Journal of Biotechnology*, 28, 101-112, <https://doi.org/10.1016/j.ejbt.2017.05.007>.
- Constantinescu, R.R., Stefan, D.S., Meghea, A. and Zainescu, G. (2015), "Pelt waste degradation using fungi strains", *Buletinul Stiintific UPB, Seria B*, 77(4), 123-132.
- Gupta, S., Ponsubbiah, S., Gupta, S.K. and Mandal, S. (2019), "Sustainable Value Creation From Leather Solid Wastes: Preparation Of Shoe Soling Material Using Nano Fillers", *IULTCS Congress Dresden*, June 25-28, pp.87-92.
- Hussain, F.S., Memon, N., Khatri, Z. and Memon, S. (2020), "Solid waste-derived biodegradable keratin sponges for removal of chromium: A circular approach for waste management in leather industry", *Environmental Technology & Innovation*, 20, <https://doi.org/10.1016/j.eti.2020.101120>.
- Rangel Serrano, A., Maldonado, V.M. and Kösters, K. (2003), "Characterization of Waste Materials in Tanneries for Better Ecological Uses", *Journal of the American Leather Chemists Association*, 98, P43-484.
- Silveira, A., Moreno, R.J., Correia, M.J. and Ferro, V. (2019), "A method for the rapid evaluation of leather biodegradability during the production phase", *Waste Management*, 87(15), 661-671 <https://doi.org/10.1016/j.wasman.2019.03.003>.
- Sundar, V.J., Ramamurthy, G. and Sastry, T.P. (2017), "Study on plant growth promoter from proteineous wastes from leather industry", *Leather and Footwear Journal*, 17(2), 87-90, <https://doi.org/10.24264/lfj.17.2.2>.
- Tang, Y., Zhao, J., Zhang, Y., Zhou, J. and Shi, B. (2021), "Conversion of tannery solid waste to an adsorbent for high-efficiency dye removal from tannery wastewater: A road to circular utilization, *Chemosphere*, 263, <https://doi.org/10.1016/j.chemosphere.2020.127987>.
- Thankaswamy, S.R., Sundaramoorthy, S., Palanivel, S. and Ramudu, K.N. (2018), "Improved microbial degradation of animal hair waste from leather industry using *Brevibacterium luteolum* (MTCC 5982)", *Journal of Cleaner Production*, 189, 701-708, <https://doi.org/10.1016/j.jclepro.2018.04.095>.
- Wösten, H.A.B. (2019), "Filamentous fungi for the production of enzymes, chemicals and materials", *Current Opinion in Biotechnology*, 59, 65-70, <https://doi.org/10.1016/j.copbio.2019.02.010>.
- Zhang, J., Han, Z., Teng, B. and Chen, W. (2017), "Biodeterioration process of chromium tanned leather with *Penicillium* sp.", *International Biodeterioration & Biodegradation*, 116, 104-111, <https://doi.org/10.1016/j.ibiod.2016.10.019>.

<https://doi.org/10.24264/icams-2020.II.5>