Comparative Study on the Enzymatic Biodegradation of Synthetic and Tanned Leather Wastes

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This study presents an investigation concerning the influence of pH, enzyme type and concentration on biodegradation of three types of leather wastes (synthetic and tanned with chromium and natural tannin). In order to highlight the biodegradation processes, IR spectra have been determined for all leather samples after contact with enzymes mixture. A thermostat shake set-up has been used permitting the contact of enzymes with wastes at constant temperature. The results show an important influence of pH and enzymes concentration on leather wastes biodegradation. The leather waste tanned with natural tannin can be easily biodegraded by the enzymes.

Keywords: leather waste biodegradation, enzymes protease, lipase, amylase, IR spectrum of leather

Leather and leather-based industries represent one of the most important economic sectors. Huge amounts of solid leather wastes are discarded directly with the urban wastes [11]. The waste resulting from the leather industries according to the European legislation must be capitalized by the same methods: reusing, recycling, energy recovery, capitalization by chemical and biochemical degradation with recovery of the useful organic compounds [2-7]. Leather waste biodegradation encounters difficulties related to the decrease of water content in leather after its tanning, while the content of fats and proteins increase (70 % for the latter, mostly as collagen and small amounts of elasting) and new compounds are being introduced such as acids, alkalis, chromium salts, dyes, synthetic and natural tannins, solvents, sulphides, pesticides (biocide) etc. [4, 8, 9].

The cow hide usually has the following composition: 60-70% water, 30-35% proteins, 0.5-2% lipids and 0.35-0.5% mineral compounds. The fibril proteins are represented by collagen in proportion of 90%, elastine over 9% and keratins less than 1% [10 - 12]. In accordance with the composition of waste, specific enzymes can be used to sustain the biodegradation. Enzymes like proteases and lipases play an important role in leather waste biodegradation of collagen, keratin and fats, respectively [13]. Proteases are complex multi-enzyme systems which catalyze the hydrolysis of amide bonds in the protein molecule [14]. Collagen is a highly organized biopolymer, consisting of a large number of individual collagen molecules linked together in a periodic manner. The collagenase is a class of metalloproteases, which are broadly defined as enzymes that catalyze the hydrolysis of the native collagen at pH around 7 and at a temperature of 37°C [15].

The microorganisms that can synthesize the proteases are bacteria such as Clostridium species: *Cl. histolyticum*, *Cl. perfringens* and *Cl. capitovalae* [15]; Bacillus species: *B. subtilis*, *B. megaterium*, *B. anthracoides*, *B. pumilus* [16-18]; Pseudomonas species: *Pseudomonas aeruginosa*, [19-21]; fungi such as Paecilomyces species: *Paecilomyces ehrlichii*, *Penicillium klebani*, *P. aculeatum*, *P. purpurogenum* and *P roseopurpureum*, *P. chrysogenum*, *P. luteum*, *P. brevicompactum*, *P. decumbens*, *P. rugulosum*, *P. aculeatum*, *P. funiculosus*, [22-24]; Aspergillus species: *Aspergillus niger*, *A. fumigatus*, *A. ochraceus*, *A. wentii*, *A. flavus-oryzae* (group), *Mucormucedo*, *Rhizopus nigricans*, etc. [25-26].

The microorganisms that can synthesize the lipase are bacteria such as *Pseudomonas fragi* [27], *Aspergillus sp*. [28], *Bacillus subtilis*, *Proteus*, *Acinetobacter*, *Aeromonas*, *Escherichia*, *Myroides*, *Brevibacterium*, *Vagococcus*, *Staphylococcus*, *Mycoplasma* etc. [28-30].

Leather substitutes may be plastics such as polyurethanes (PU) and polyvinyl chloride (PVC) which, in addition to the basic monomer, also contain compounds increasing its elasticity and resistance, pigments and dyes [31]. Polyurethanes have similar structures to polyesters and polyamides. Thus, they are easily degraded by enzymes such as papain and subtilisin. These enzymes are synthesized by *Aspergillus Niger*, *A. fumigatus*, *Fusarium solani*, *Crytococcus lacerenti* [32].

In this paper we studied the enzymatic biodegradation of some leather waste samples function of the parameters like: the nature of leather waste, the nature and concentration of enzyme mixture, the pH of solution (medium), temperature and time of leather – enzyme contact.

The enzymatic mixture is a strong oxidant product for the organic matter and exerts an important influence on the formation of malodorous and toxic gases. It was found that the use of this product constitutes an effective biological antagonism against pathogens and significantly reduces the volume of wastes by a strong and rapid metabolic degradation of wastes produced by BIOMA AGRO ECOLOGY CO from Switzerland [33].

Experimental part

The wastes used in the experiment were: synthetic leather or substitutes (PS) and bovine leather tanned with

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chromium salts (PCr) and natural tannins (PT). Four types of enzyme mixtures were considered, as follows: E1 - mainly containing proteases, E2 - mainly containing lipases, E3 - mainly containing amylases, and E1 + E2 + E3 containing a mixture of the three previously mentioned types of enzymes.

The concentrations of enzyme mixtures E1, E2, E3 and the mixture E1 + E2 + E3 used were: 1 - 10 mL of E1 to one liter of distilled water, 1 - 10 g of E2 and E3 to one liter of distilled water, and a mixing ratio of 1 mL: 1 g: 1 g for the above concentrations of the E1 + E2 + E3 mixture. Seven numbered separate wastes were added to the mixture of enzyme and distilled water. They were dried at 50°C until reaching constant mass, and were then cooled and weighed. Samples were inserted in Erlenmeyer flasks provided with stoppers and thermostatic at 30°C. At various time intervals leather samples were taken and washed using distilled water, dried at 50°C until reaching constant mass and then weighed. The experiment took 250 h. The most representative moment for the comparative study was considered the enzymatic activity after 140 h, in relation to which the influence of all parameters was analyzed.

The biodegradation ability was expressed as a ratio between the biodegraded waste mass, expressed in mg, and the initial mass of the waste, expressed in grams:

\[ a_E = \frac{m_o - m_b}{m_o} \]

where:
- \( a_E \) is the degree of enzymatic degradation, mg/g
- \( m_o \) the weight of initial leather waste sample dried at 50°C to constant weight;
- \( m_b \) the weight of leather waste sample after enzymatic biodegradation process, washing with distilled water and dried at 50°C to constant weight.

The leather samples were kept in thermostatic conditions in a Thermoshake with 12 plates (Eppendorf Innova 40).

The IR spectra for initial leather and waste samples after 140 h of biodegradation have been registered using a FT/IR-ATR spectrometer 4200 (Jasco, Japan) with 4000 - 400 cm\(^{-1}\) range of detection.

**Results and discussions**

The influence of the type of enzyme on the biodegradation of each type of waste, at various pH values, was plotted in figures 1-4. The influence of the mixing ratio of enzymes E1 + E2 + E3 on the degradation of PCr, PT, PS waste samples is presented in figure 5.

The biodegradation degree of tanned leather and substitute waste is influenced by the pH. More exactly, it increases with the increase of pH. Bovine leather waste tanned with natural tannins biodegrades the easiest under the action of all enzymatic systems, reaching biodegradation degree values of over 150 mg/g for E1 enzyme, of approximately 250 for E2 enzyme and of approximately 200 mg/g for E3 enzyme and E1+E2+E3 mixture, at a pH of 12.29 and after 140 h of contact with the enzymatic systems (figs. 1-4). In the acid pH range of 4.03-7.02, synthetic leather shows the highest biodegradation degree. Its value is of 40 mg/g for E1 enzyme, 25-30 mg/g for E2, E3 enzymes and E1+E2+E3 mixture. Leather waste tanned using chromium salts shows the lowest biodegradation degree, both in acid medium, reaching maximum values of 20 mg/g, and in alkaline medium, reaching maximum values of 60 mg/g for the E1 enzyme, the most efficient. Although the three enzymes used separately often have higher biodegradation ability than their mixture, when assessing biodegradation ability, it must be taken into account that their mixture ensures total biodegradation down to mineralization. This bio-

**Fig. 1. Influence of E1 enzyme mixture on the degradation of PCr, PT, PS waste samples at pH different values**

**Fig. 2. Influence of E2 enzyme mixture on the degradation of PCr, PT, PS waste samples at pH different values**

**Fig. 3. Influence of E3 enzyme mixture on the degradation of PCr, PT, PS waste samples at pH different values**

**Fig. 4. Influence of E1+E2+E3 enzyme mixture on the degradation of PCr, PT, PS waste samples at pH different values**
Degradation reduces the amount of gases which emanate an unpleasant odor throughout the degradation processes. On the other hand, the other enzymatic systems only provide partial biodegradation. This has the effect of slowing down the process or even stopping if specific compounds that they can biodegrade disappear from the system. Based on the results obtained (figs. 1-4) it can be noticed that an alkaline pH of 12.29 favors enzymatic degradation of leather wastes.

Analysis of figure 5 shows that, as the enzyme concentration increases in the system, the biodegradation degree decreases. Therefore, the optimal concentration for the systems used in this study is 5mL of E1, 5g of E2 and E3 concentration of enzymes to one liter of distilled water.

Degradation processes are highlighted by the changes occurring in IR spectra characteristic to samples. Thus, figures 6, 7 and 8 show changes in the PCr, PT and PS sample spectra in the presence of E1, E2, E3 enzymes and E1+E2+E3 mixture. Thus it can be observed a flattening of the peak in the 3450-3200cm⁻¹ range, several changes in the intensity of peaks at 1660 and 1550 cm⁻¹, 1210 and 1180 cm⁻¹ and also the occurrence of low amplitude peaks in the 2000-2500cm⁻¹ range, with higher intensity at 2380 and 2200 cm⁻¹, highlighting the degradation processes taking place.

The analysis of synthetic leather wastes IR spectra (figure 8) shows that its basic polymer is polyurethane, as expected from literature [34].
Conclusions

The biodegradation degree of tanned leather and substitute wastes is influenced by pH. More precisely, it increases with the increase of pH. Bovine leather wastes tanned using natural tannins biodegrade the easiest under the action of all enzymatic systems considered in this study. For total biodegradation of wastes in safe conditions (i.e., without gas emission and down to mineralization) it is recommended the use of the E1+E2+E3 enzyme mixture. The obtained results recommend that tanned leather wastes biodegradation to be performed at an alkaline pH of approximately 12.

The degradation processes occurring in leather and substitute wastes are highlighted on the IR spectra diagrams by the flattening of the peak in the 3450-3200 cm$^{-1}$ range, the changes in intensity of peaks at 1660 and 1550 cm$^{-1}$, 1210 and 1180 cm$^{-1}$ and the occurrence of low amplitude peaks in the 2000-2500 cm$^{-1}$ range, with higher intensity at 2380 and 2200 cm$^{-1}$.

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References

33. Sheet Product Overview: http://www.bioma.ro
34. BADILESCU S., TOADER M., GIURGINCA M., TALPUS V., Polymers and auxiliaries Infrared Spectroscopy, Ed. Tehnica, 1982, p.189

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