APPLICATIONS OF ENZYMES AS ECOLOGIC ALTERNATIVES IN THE LEATHER INDUSTRY

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ABSTRACT. The aim of this paper is to study the applications of enzymes, as ecological alternatives in the leather industry. The research proposes to create ecologic leathers/furs with additional functions (non-toxic, with high-quality of soft, well-degreased surfaces) through the use of original enzyme-based biotechnologies, as new additives for leather/fur processing intended for everyday use. Another application of the enzymes studied in this paper is the development of a novel technology based on enzymes and membrane technique, for purifying wastewaters from the leather industry. We selected and analytically characterized lipases, proteases, and also new enzymes: lysozyme, tryptophan deaminase for use in leather industry. The goal was to obtain leathers/furs for everyday use, with high-performance characteristics, by processing them with enzymes – as new additives that have not been used so far in the leather industry as an alternative to the use of potentially polluting chemical materials. Complex and original technologies of ecological bioprocessing of leather/furs were used. The innovation consists in developing new biotechnologies for leather/fur with enzymatic additives in order to achieve ecologic leathers or fur articles. These biotechnologies provided advanced performances for surface quality: intense and bright colors, soft, well-degreased, and resistant to abrasion and water.

KEY WORDS: original enzyme-based biotechnologies, ecologic leathers or fur articles with additional functions, technology based on enzymes and membrane technique for purifying wastewaters

APLICAȚII ALE ENZIMELOR CA ALTERNATIVE ECOLOGICE ÎN INDUSTRIA DE PIELĂRIE

REZUMAT. Scopul acestei lucrări este de a studia aplicațiile enzimelor, ca alternative ecologice în industria de pielărie. Cercetarea își propune realizarea de piei/blănuri ecologice cu funcții suplimentare (nontoxice, cu suprafețe moi, bine degresate, de calitate superioară) prin utilizarea biotehnologiilor originale pe bază de enzime, ca noi aditivi pentru prelucrarea pieilor/blănurilor de uz zilnic. O altă aplicație a enzimelor studiate în această lucrare este dezvoltarea unei noi tehnologii bazate pe enzime și tehnica membranara, pentru epurarea apelor uzate din industria de pielărie. S-au selectat și caracterizat din punct de vedere analitic lipaze, proteaze, dar și enzime noi: lizozim, triptofan-deaminază pentru utilizare în industria de pielărie. Scopul a fost obtinerea de piei/blănuri de uz zilnic, cu caracteristici performante, prin prelucrarea acestora cu enzime – noi aditivi care nu au fost utilizați până acum în industria de pielărie, ca alternativă la folosirea substanțelor chimice cu potențial poluant. S-au utilizat tehnologii complexe și originale de bioprocesare ecologică a pieilor/blănurilor. Inovația constă în dezvoltarea de noi biotehnologii pentru piele/blană cu aditivi enzimatici în vederea realizării unor articole din piei sau blănuri ecologice. Aceste biotehnologii au condus la performanțe avansate în ceea ce privește calitatea suprafeței: culori intense și strălucitoare, suprafețe moi, bine degresate și rezistente la abraziune și apă.

CUVINTE CHEIE: biotehnologii originale pe bază de enzime, articole din piei sau blănuri ecologice cu funcții suplimentare, tehnologie bazată pe enzime și filtrare prin membrane pentru epurarea apelor uzate

APPLICATIONS DES ENZYMES COMME ALTERNATIVES ÉCOLOGIQUES DANS L'INDUSTRIE DU CUIR

RÉSUMÉ. L'objectif de cet article est d'étudier les applications des enzymes, comme alternatives écologiques dans l'industrie du cuir. La recherche propose de créer des cuirs/fourrures écologiques avec des fonctions supplémentaires (non toxiques, avec des surfaces molles et bien dégraissées, de haute qualité) grâce à l'utilisation de biotechnologies originales à base d'enzymes, comme nouveaux additifs pour le traitement du cuir/fourrure à usage quotidien. Une autre application des enzymes étudiées dans cet article est le développement d'une nouvelle technologie basée sur les enzymes et la technique membranaire, pour purifier les eaux usées de l'industrie du cuir. On a sélectionné et caractérisé analytiquement des lipases, des protéases, mais aussi de nouvelles enzymes : lysozyme, tryptophane désaminase pour utilisation dans l'industrie du cuir. L'objectif est d'obtenir des cuirs/fourrures d'usage courant, aux caractéristiques performantes, en les traitant avec des enzymes – comme de nouveaux additifs qui n'ont pas été utilisés jusqu'à présent dans l'industrie du cuir comme alternative à l'utilisation de matériaux chimiques au potentiel polluant. Des technologies complexes et originales de biotransformation écologique des cuirs/fourrures ont été utilisées. L'innovation consiste à développer de nouvelles biotechnologies pour le cuir/la fourrure avec des additifs enzymatiques afin d'obtenir des articles en cuirs ou en fourrure écologiques. Ces biotechnologies ont apporté des performances avancées pour la qualité de surface : couleurs intenses et lumineuses, surfaces molles, bien dégraissées, résistantes à l'abrasion et à l'eau.

MOTS-CLÉS : biotechnologies originales à base d'enzymes, cuirs ou fourrures écologiques aux fonctions supplémentaires, technologie à base d'enzymes et technique membranaire d'épuration des eaux usées

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INTRODUCTION

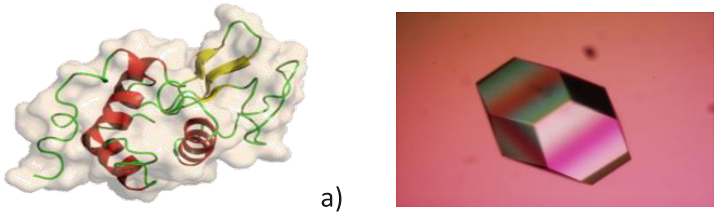
In the leather industry, chemical additives (tannins, retanning products, fatliquors, dyes, finishing products, etc.) play an important role, giving leather/fur properties and characteristics specific to each finite assortment, such as: resistance to mechanical stress (tensile, repeated bending, friction, compression), softness, suppleness, elasticity or stiffness, fullness, touch, color imposed by fashion, impermeability to water, capacity of water vapor absorption, etc.

Enzymes used as additives can have a complex role in the leather industry. Using enzymes to soak high-quality leather leads to more effective rehydration; better opening of the microfibrillar structure of the skin with effects on improving penetration of auxiliary chemicals; degradation and dispersion of fatty substances; more efficient removal of interfibrillar mucopolysaccharides [1-7]. Proteases and lipases are used to soak bovine and sheep skins. The most important effect of enzymes in soaking skins is the solubility of hyaluronic acid and dermatansulphate, basic components of interfibrillar substances from leather [2]. Another important operation in leather processing, in which enzymes are traditionally used, is leather bating. The main function of the bating operation is to eliminate interfibrillar non-collagen substances [3].

The enzymes used are of animal origin – proteases – or of bacterial origin. Bacterial enzymes are cheaper, their sources are more varied, they can degrade elastin and have a greater range of action, pH = 7-9. Bacterial enzymes have a significant action on elastin and may help achieve very soft leather [4].

Leather degreasing is an essential operation for hide processing. Conventional methods of degreasing sheep skins use organic solvents and lead to emissions of volatile organic compounds (VOC) into the atmosphere [2]. The action of the main enzyme types takes place on: hair, non-collagen proteins (proteases); dermis fats, fatty acids from glands in the grain side (lipases); elastin in leather structure and cell membranes (elastase). Lipases are enzymes that can remove fat and fatty acids, particularly those with moderate fat content. The most important effects of using lipolytic enzymes are: the effective removal of fatty substances with positive effects on the uniformity and intensity of leather color; reducing the tenside quantity used; reducing volatile organic compounds (VOC) [1, 4]. Alkaline and acid lipases are used in various stages of the technological process. Alkaline lipases are most often used in decalcifying and bating leather. Lipase activity is increased by the presence of calcium and sodium and is inhibited by the presence of tensides. Acid lipases can be used in degreasing sheep skins preserved by pickling. Research has also shown that degreasing with lipases can lead to an increase in tear resistance of leather, probably because of more uniform distribution of fatty substances in the skin [3]. Another crucial area for the leather industry concerns processing leather waste with proteases for protein separation and recovery of protein component. Lipases hydrolyze triglycerides to mono-and diglycerides, which are more hydrophilic, to fatty acids and glycerin, which are soluble in alkaline medium. In the case of bovine skins, lipases allow full replacement of tensides [1].

In this paper new enzymes, lysozyme and tryptophan deaminase, were selected for use in the leather industry, in purification of wastewaters compared to a bolaform surfactant (sucrose diester). Lysozyme, Fig. 1, also known as muramidase or N-acetylmuramide glycanhydrolase, is a glycoside hydrolase, enzyme that damages bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins.



a) b)

Figure 1. Lysozyme: a) structures, b) single crystal [1]

Large amounts of lysozyme can be found in egg white. C-type lysozymes are closely related to alpha-lactalbumin in sequence and structure, making them part of the same family. Lysozyme was the second protein structure and the first enzyme structure to be solved via X-ray diffraction methods, and the first enzyme to be fully sequenced that contains all twenty common amino acids. Since lysozyme is a natural form of protection from pathogens like *Salmonella*, *E. coli*, and *Pseudomonas*, a deficiency due to infant formula feeding can lead to increased incidence of disease [1].

Tryptophan deaminase (Fig. 2) is an enzyme characteristic of groups of organisms belonging to genera *Proteus-Morganella-Providencia*. Tryptophan is one of the 20 standard amino acids, as well as an essential amino acid in the human diet. The distinguishing structural characteristic of tryptophan is that it contains an indole functional group. It is an essential amino acid as defined by its growth effects on rats [1]. Plants and microorganisms commonly synthesize tryptophan from shikimic acid or anthranilate.

a) b)

Figure 2. a), b)-Tryptophan or (2S)-2-amino-3-(1H-indol-3-yl) propanoic acid [1]

This paper also brings a solution to environmental pollution, by introducing membrane technologies (ultrafiltration) for the purification of wastewaters from the leather industry. Leathers and furs were treated with new enzymes and used for medical or everyday use, and the proposed articles are: medical fur belt, leather knee pads.

EXPERIMENTAL

Materials and Methods

The following materials have been used: sucrose diester from SERVA Feinbiochemica GmbH & Co; Pellvit AP from TFL; Esperase, Lipex 100L, NovoCor ABL, NovoCor AX, NovoCor ADL, Greasex from Novozymes; Perizym AFW from Textilchemie Dr. Petry GMBH; Protease produced at ICECHIM; Lysozyme and Tryptophan deaminase from Sigma-Aldrich.

The leathers and furs used come from The Leather and Footwear Research Institute (ICPI).

The experimental techniques used in this work consist in scanning electron microscopy – SEM and UV-VIS spectroscopy tests: a "SEM QUANTA 200" equipment from FEI company, with EDAX coupled. The samples for SEM investigations were prepared by slow evaporation in clean atmosphere at room temperature; a UV-VIS spectrophotometer GBC (model 918).

Ultrafiltration was done with internal cell stirring: Berghoff and with polysulfone membranes from Sigma-Aldrich.

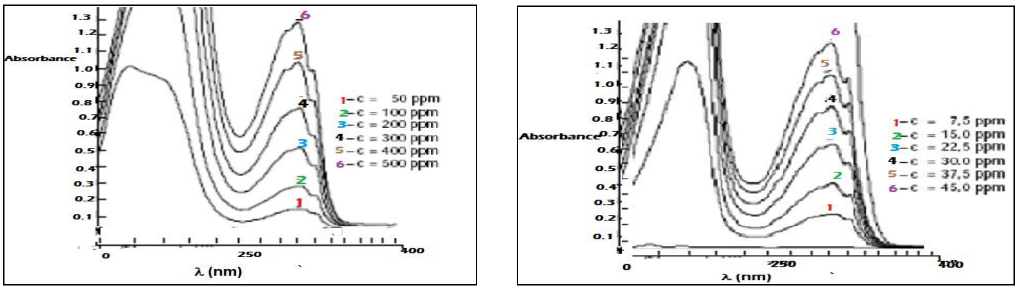
RESULTS AND DISCUSSIONS

The selected lipases and proteases (Pellvit AP, Esperase, Lipex 100L, NovoCor

ABL, NovoCor AX, NovoCor ADL, Greasex, Perizym AFW, Protease from ICECHIM) were used to develop a complex and original technology of ecological bioprocessing of leather/furs. In addition, new enzymes used in leather industry (lysozyme, tryptophan deaminase) were selected for their antibacterial effects in order to be used in purification of wastewaters compared to a bolaform surfactant (sucrose diester) by membrane technology.

Characterization of Lysozyme and Tryptophan Deaminase by UV-VIS Spectroscopy

The enzymes were analyzed by UV-VIS spectroscopy. To obtain UV-VIS spectroscopy calibration curves (absorption depending on the concentration of enzyme solutions) we studied a set of two enzymes in a Na_2HPO_4-NaH_2PO_4 buffer solution (pH=7): Tryptophan deaminase (M=204 Da) and Lysozyme (M=14000 Da). This buffer is composed of two salts: monohydrogen phosphate (Na_2HPO_4), which is the basic component and system phosphate dehydrogenase (NaH_2PO_4), the acidic ones. UV-VIS spectra were recorded at room temperature 25°C and showed that certain enzymes are present only in UV absorption and others in visible spectrum. Lysozyme with M = 14 000 Da has four UV absorption peaks at: λ= 209 nm, λ=279 nm, λ =282.5 nm, λ= 292 nm (Fig. 3-a). Absorbance values at 279 nm depend on enzyme's concentration. Tryptophan deaminase has four absorption peaks in UV spectrum at: λ=220 nm, 275 nm, 280 nm and 290 nm (Fig 3-b). Absorbance at λ= 280 nm depends on the concentration of tryptophan deaminase, starting from an initial concentration Ci=45 ppm.



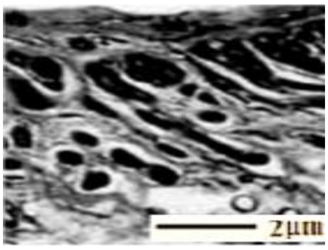
a) b)

Figure 3. UV spectra of Lysozyme (a) and Tryptophan deaminase (b) in buffer solution, for different concentrations

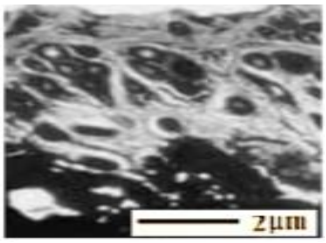
Complex and Original Technologies for Ecological Bioprocessing of Leather/Furs

The processing of hides requires the removal of interfibrillar substances such as soluble proteins, mucopolysaccharides and fatty substances before tanning. Soluble, interfibrillar proteins can be removed with the help of proteases and fats with the lipases or surfactants. Proteases are mainly used in the operations of softening, sanding, and graying of leather (repairing with the help of enzymes). Lipases are used for degreasing the skins by hydrolyzing the fats in the reticular layer and in the structure of the leathers. The use of lipases allows the reduction of pollution with surfactants or organic solvents. The use of lipases for dispersing and removing natural fats helps improve the tanning, retanning and dyeing processes of hides [1]. Lipases hydrolyze triglycerides to mono and diglycerides, which are more hydrophilic, to fatty acids (Fig. 4) and glycerin, which are soluble in an alkaline environment. Ultrafiltration technique was used to remove enzymes (lipases and proteases) from wastewaters from leather and fur industry.

Figure 4 shows SEM images of leathers treated or not with enzymes. Enzymatically hydrolyzed fatty acids from the structure of natural fatty substances in the leather are compared to non-enzymatically treated leather.



a) The structure of non-enzymatically treated leather with a few fatty acids



b) The structure of enzymatically treated leather with many fatty acids, hydrolyzed enzymatically

Figure 4. SEM images of leathers treated or not with enzymes

The dispersion of fats has the effect of a more uniform and brighter coloring of the skins. Lipases also improve the quality of hydrophobized leathers or leathers for car upholstery (by reducing the fogging effect developed on the car windshield by volatile fatty materials). In the case of bovine leathers, lipases allow the complete replacement of surfactants. For sheep leather, which contains up to 40% fatty substances, solvent degreasing can be replaced with an aqueous process in which lipases and surfactants are used. The development of enzyme preparation technologies allowed the creation of enzymes that allow the hydrolysis of non-collagenous proteins in the structure of leather tanned with basic chromium salts. Treating tanned leather with proteolytic enzymes allows better relaxation of the dermis, increased softness, reduced surface defects, and increased usable area by 3-9% (Fig. 5).

Figure 5. Image of leathers, malleable with a larger surface

The use of enzymes allows not only the reduction of polluting chemical materials, but also the reduction of water consumption. The use of surface-active materials requires numerous rinses and high water consumption, while the use of enzymes only requires a simple rinse. Enzymes also allow the elimination of foaming effects, which require the use of silicone-type antifoam materials, allow the reduction of nonyl phenol ethoxylated detergents by 1-3%, which can lead to a 20% reduction of CCO in wastewater.

The main areas of application of enzymatic treatments for fur skins are: when softening furs preserved by drying, washing-degreasing in the float of furs, acid pickling (application of enzymes in the pickling operation), and neutralization of chrome skins. In this paper, the purpose of enzymatic treatments is to replace polluting chemical materials, such as organic solvents, nonylphenolethoxylated detergents (in float degreasing and dry degreasing operations, in solvent), to hydrolyze mucopolysaccharide-type interfibrillar substances, elastin-type proteins, non-hydrolyzable in acid hydrolysis conditions. It is also proposed in this research to replace nonylphenolethoxylated detergents with bolaform sucrose diester, with is non-toxic, ecological.

In the complex technologies for sheep fur bioprocessing the following enzymes were used: Pellvit AP; Esperase, Lipex 100L, NovoCor ABL, NovoCor AX, NovoCor ADL, Greasex; Perizym AFW and protease produced at ICECHIM (Fig. 6).

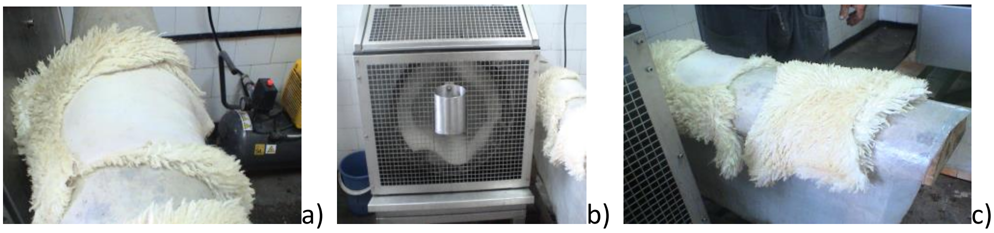


Figure 6. Bioprocessing of furs with enzymes at the micropilot level

Analysis of bioprocessed furs with enzymes (Fig. 7) and of exhausted floats are presented in Fig. 8. Two types of surfactants were used: a classic one, nonylphenol ethoxylate (sample Sc) and a bolaform surfactant, sucrose diester (sample Sb), by comparison. In this research, an original continuous alkaline-enzymatic wool hydrolysis bioprocess was also proposed, where the frequently used alcalase is replaced with lysozyme and the classic surfactant with a bolaform surfactant– sucrose diester. The filtration/separation was done by the membrane technique (ultrafiltration).

Figure 7. Image of fur samples bioprocessed with enzymes: samples P1-P6

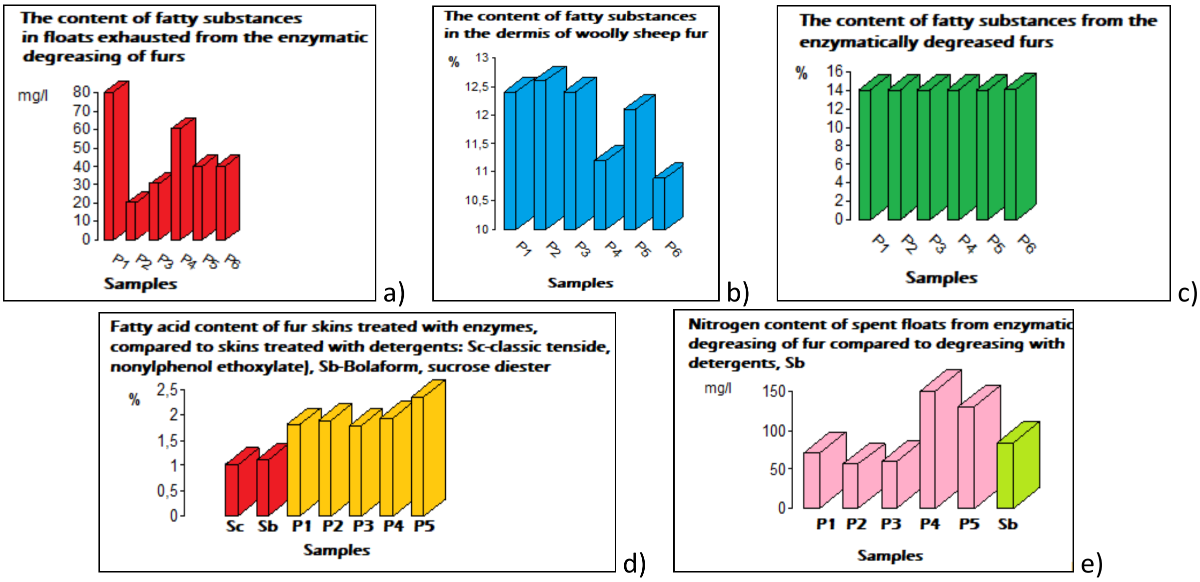


Figure 8. Analysis of bioprocessed furs with enzymes and of exhausted floats

In ultrafiltration an internal cell stirring was used: Berghoff and polysulfone membranes. The new biotechnology proposed for keratin solubilization by alkaline hydrolysis based on lysozyme/sucrose diester/ ultrafiltration is presented in Fig. 9.

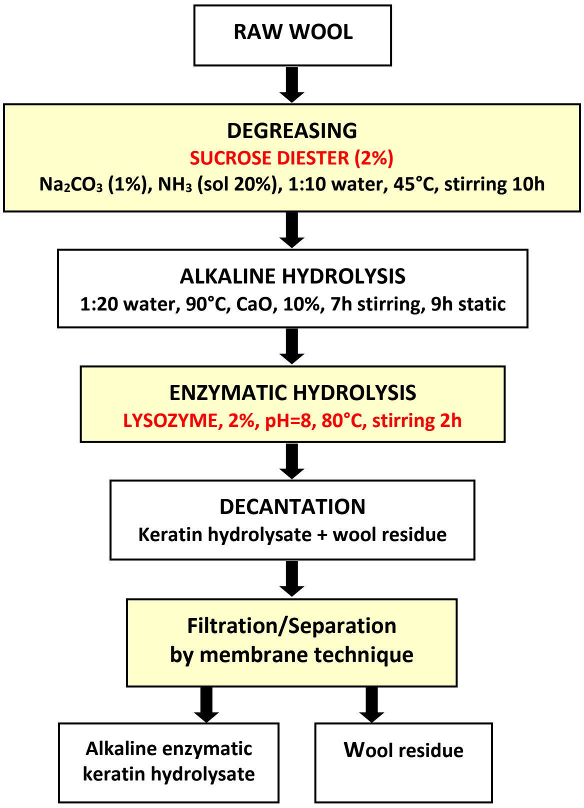


Figure 9. Biotechnology for keratin solubilization by alkaline hydrolysis based on lysozyme/sucrose diester/ultrafiltration

Biotechnology for Purifying Wastewaters from the Leather Industry, Based on Enzymes and Membrane Technique

Membrane techniques (ultrafiltration) can be used to remove lipases and proteases from wastewaters, from the leather industry. If a quantity of c=1% lysozyme or tryptophan deaminase in wastewaters is added before the ultrafiltration process, the glue retentions of enzymes (lipases and proteases) are improved. The addition of enzymes (lysozyme or tryptophan deaminase) improves the purification of wastewaters from the leather industry. Also, the presence of sucrose diester c=1% improves the purification of wastewaters.

There have been UV-VIS spectra of the initial solution and the concentrate, permeate samples,
before and after ultrafiltration. The calibration curves were determined for concentrations of permeate and retention. The retention of each enzyme (lipase or protease) from wastewaters was calculated on the ultrafiltration membrane, using the formula:

$$R = \left(1 - \frac{c_p}{c_c}\right) \cdot 100 \quad (1)$$

Ultrafiltration was done with internal Berghoff cell stirring and the experimental results are given in Table 1.

Table 1: Experimental results

Sample no.	Sample description	Permeate concentration (ppm)	Reject concentration (ppm)	Retention (%)
1	Wastewaters with lipase	95	110	13.63
2	Wastewaters with protease	60	100	40
3	Wastewaters with protease with lysozyme (c=1%)	60	130	53
4	Wastewaters with protease with tryptophan deaminase (c=1%)	30	499	94
5	Wastewaters with protease and lysozyme/ (c=1%)/sucrose diester (c=1%)	29	500	95

CONCLUSIONS

The conducted research has led to the following results:

- Selecting new enzymes with potential applications in the leather industry for their antibacterial effects: lysozyme and tryptophan-deaminase, besides lipases and proteases which are known.
- Selecting enzymes with a potential of stable interaction with collagen and/or keratin, in view of adding new functions to leather and fur.
- Characterization of enzyme solutions and analysis of concentrations by UV-VIS spectroscopy.
- Ultrafiltration technique was used to remove enzymes (lipases and proteases) from wastewaters from leather industry with ~ 13-94 % retention.
- Leathers/furs can be treated with enzymes and used for medical or everyday use and the proposed articles are: medical fur belts and leather knee pads.

Acknowledgments

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