BIODEGRADABILITY OF HAIR AS A WASTE OF LEATHER INDUSTRY

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Leather industry is one of the sources, which releases keratin-containing wastes. Still now, most of the tanneries are following hair-burning process, which contribute high amount of COD, BOD, TDS etc., to the effluent. This is a reason why numerous investigations are done reaching to replace the hair burning process by hair save unhairing: enzymatic or unhairing with hair immunization. The aim of the study was to investigate the biodegradability peculiarities of native hair, hair after enzymatic unhairing and hair obtained by unhairing with immunization effect. The soil burial test was carried out and the biodegradability of the wool was estimated. The biodegradability of the wool was estimated analysing their mass loss and fungal colonization. It was established that hair samples behave differently during the exposition in the soil: there were determined different species of fungi after 3-month exposition. The mass loss was different as well. Accordingly, the hair obtained after different method of unhairing are different in their properties as well. The different properties should mean different behaviour of the hair during biodegradation.

Keywords: unhairing, hair wastes, biodegradability.

INTRODUCTION

Leather industry is one of the sources, which releases keratin-containing wastes. Still now, most of the tanneries are following hair-burning process, which contribute high amount of COD, BOD, TDS etc., to the effluent. This is a reason why numerous investigations are done reaching to replace the hair burning process by hair save unhairing: enzymatic or unhairing with hair immunization. It is a paradox that the application of the hair save methods will lead to formation of huge amount of hair waste. It is known fact that hair/wool covering constitutes no less than 2 % of hide/skin mass. Compendium of Food and Agriculture Organization of the United Nations (2013) presented data which showed that during 2012 year about 7.2 billion ton of hides/skins (generally bovine, sheep, lamb and goat hides/skins) were processed into leather. Taking into account both facts, we can calculate that about 143 thousands ton (practically not less than amount of feather wastes: about 156000 ton annually as report Prasanti *et al.* (2016)) of hair/wool wastes in degraded form will not pass with tanneries wastewaters into environment, but it must be utilized in other way.

Despite the fact, that keratins find applications in cosmetic (Villa *et al.*, 2013; Sharma and Gupta, 2016), feedstuff (Martínez-Alvarez *et al.*, 2015), composites (Prochon *et al.*, 2016), pharmaceutical (Rouse and Van Dyke, 2010), fertilizer (Vasileva-Tonkova *et al.* 2009), sorbents (Wang *et al.*, 2016) industry etc., Tesfaye *et al.* propose that only small amounts of feather are processed into valuable products such as feather meal and fertilisers.

Unfortunately, the investigations related with the treatment of hair covering removed from hide/skin by enzymatic or immunization unhairing process are not

numerous. Here could be mentioned investigations of Barrena *et al.* (2007) devoted for composting of hair obtained using immunization unhairing method from bovine hide. Onyuka *et al.* (2012) present a study of a development of conditions favorable for the degradation of shaved form bovine hair (native hair) in a composting environment. Catalan *et al.* (2017) proposed two ways of utilization of hair obtained by enzymatic unhairing: composting and solid-state fermentation (SSF) for protease production. To similar investigations can be attributed research of Laba *et al.* (2017) describing process of bioconversion of pig bristle waste, after thermo-chemical pretreatment with sulfite, using keratinolytic enzymes of *Bacillus cereus* PCM 2849, and efforts of Prochon and Ntumba (2015) to use the cattle hair keratin derived from tanning industry wastes as fillers for Carboxylated Nitrile Rubber.

There are presented in a literature base few research works associated with the utilization of wool (mainly native wool) wastes obtained from leather and fur industry as well turning the waste wool into amendment-fertilizers (Zoccola *et al.*, 2015); to use the wool waste as the main medium for growing of a keratin-degrading strain (Fang *et al.*, 2013), or direct use of uncomposted native wool wastes as nutrient source and growth medium constituent for plants (Zheljazkov *et al.*, 2009; Gorecki and Gorecki, 2010).

Thus, the utilization of keratin wastes from leather industry is somewhat studied but not enough thinking about possible amount of such wastes in future. Herewith, the researches, who investigate utilization of hair/wool obtained as waste after unhairing process, practically do not pay any attention to the method of unhairing while the unhairing method has vast influence on properties of keratin in the obtained hair/wool.

The aim of current research is to investigate an influence of unhairing method on biodegradability of the hair after unhairing process.

EXPERIMENTAL

Preparations of Hair Samples

Three pieces (20x20 cm) were cut from merino sheepskin (with medium wool) preserved by salting. The pieces were soaked under conditions: H₂O 1000%, sodium fluorosilicate 1 g/l, temperature 30°C, duration 6 h, run continuously, draining. The soaked samples were degreased: H₂O 1000%, sodium alkansulfonate „Volgonat“ (Chimprom, Russia) 7 g/l, alkyl sulphate „Novost“ (Chimprom, Russia) 5 g/l, temperature 42°C, duration 1 h, run continuously (draining at the end of process), and washed three times: H₂O 1000%, temperature 42°C, duration 20 min. Afterwards, the wool was cut from first piece, dried at ambient temperature and used for experiments as native wool (wool 1).

Second piece was unhaird according to enzymatic method: H₂O – 200%, temperature 25°C, enzyme preparation (EP) NovoBate WB (Novo Nordisk, Denmark) – 0.6 %, CH₃COONa – 2%, CH₃COOH – 0.3%, duration 6 hours (Valeika *et al.*, 2012). After the process the wool was removed from the skin sample using blunt knife, washed three times: H₂O 1000%, temperature 42°C, duration 20 min., dried at ambient temperature and used as enzymatically unhaird wool (wool 2).

Third piece was unhaird according to immunization method: H₂O 150%, temperature 20°C, Na₂SiO₃ 2%, duration 2 h (immunization stage), Na₂S (100%) 0.9%, duration 2 h (unhairing stage) (Valeika *et al.*, 2015). After the process the wool was

removed from the skin sample using blunt knife, washed three times: H₂O 1000%, temperature 42°C, duration 20 min., dried at ambient temperature and used as immunised wool (wool 3).

The chemicals used for the technological processes were of analytical grade and of commercial grade as well.

Soil Burial Test

Wool specimens of three different kinds were dried to a constant weight at 105°C temperature. The wool specimens (0.1g) were put into 50×60 mm bags of polyvinyl chloride with 0.05 mm mesh diameter buried into the soil in 5 L volume desiccators and incubated at 26±2°C temperature. The soil was from organic farming: sandy loam Haplic Luvisol; the moisture content 20–30%; pH 5.8; the soil activity was 30% after 3 months (estimated by cotton wool mass loss). Monthly the specimens were removed from the soil and were analysed regarding their mass loss and fungal colonization. The specimens were rinsed with sterile water and their replicas were made on Malt Extract Agar with chloramphenicol (50mg/L) to stop bacterium growth. The isolated fungi were purified and identified according to the morphological and cultural features (Domsch, 1980). The biodegradability of the wool was estimated according to their relative weight loss $(a - b) / a \times 100\%$, where a is the initial weight of the specimen (g); b is the specimen weight after incubation. The wool specimens were weighted by means of an analytical balance with high precision (±0.001).

RESULTS AND DISCUSSION

The wool biodegradability test showed the decrease of all specimens during three exposition months in the soil (Fig. 1).

Figure 1. Wool biodegradability in soil during 3-month exposition

The mass loss of the wool 1 and 2 was alike: their weight decrease made up 39.17 and 41.60%, respectively, after first month of the exposition, and after 3 month – 91.82 and 89.46%, respectively. However, after first exposition month the weight of the wool

3 decreased 1.4 times more than the wool 1 and 2, at the end of the experiment it was 1.4 times less degraded than the wool 1 and 2. Fungi from six different genera that likely took part in the wool decomposition were isolated from the specimens (Table 1). Fungi from *Trichoderma* genus were most often detected on the buried specimens.

Table 1. Fungi isolated from wool 1, 2 and 3 after month exposition in soil

Specimen	1 month	Exposition 2 month	3 month
1	<i>Aspergillus ustus</i>	<i>Aspergillus ustus</i>	<i>Aspergillus ustus</i>
	<i>Fusarium sp.</i>	<i>Fusarium sp.</i>	<i>Fusarium sp.</i>
	<i>Mucor sp.</i>	<i>Trichoderma spp.</i>	<i>Trichoderma spp.</i>
	<i>Trichoderma spp.</i>		
	<i>Trichoderma spp.</i>	<i>Aspergillus ustus</i>	<i>Cunninghamella</i>
2		<i>Fusarium sp.</i>	<i>elegans</i>
		<i>Trichoderma sp.</i>	<i>Fusarium sp.</i>
			<i>Trichoderma spp.</i>
	<i>Fusarium sp.</i>	<i>Aspergillus ustus</i>	<i>Aspergillus ustus</i>
3	<i>Penicillium sp.</i>	<i>Trichoderma spp.</i>	<i>Fusarium sp.</i>
	<i>Trichoderma spp.</i>		<i>Trichoderma spp.</i>

According to the results obtained, the wool 1 was the most degradable among tested wools and the wool 3 was decomposed at the least in natural environment during three-month exposition.

The results obtained from this test were somewhat surprising. It was expected, that mostly degraded wool should be after enzymatic unhairing process. The reason for such presumption is the damage of keratin by keratinolytic enzymes during the unhairing. Through the work of Lange *et al.* (2016), evidence was provided for the need for a combination of endoprotease, exoproteases, and oligopeptidase to bring about keratin degradation. Of course, one enzyme preparation acts as endoprotease or exoproteases, or oligopeptidase but not like all three sorts simultaneously. Thus, usually one enzyme preparation affects only one segment in macromolecule of keratin. Herewith, the conditions of the unhairing process are significantly milder comparing with conditions of complete hydrolysis of keratin. Accordingly, although the wool after the enzymatic unhairing visually seems not affected, nonetheless it is affected in macromolecular level somewhat.

The biodegradability test has shown (Fig. 1.) that wool after enzymatic unhairing is more stable than native wool independently on duration of exposition in soil. The mostly stable wool (as it was expected) was immunized one (wool 3). As is mentioned in literature, during immunization keratin becomes more resistant against action of reducers and enzymes (Heidemann, 1993). Therefore, the immunised wool becomes more stable against action of enzymes of microorganisms in soil during 3-month exposition.

On the other hand, the change of mass of the wool 3 in soil exhibits by odd character. The main loss of mass is on during first month of exposition. Further, the mass loss increases but negligible.

Six sorts of fungi were isolated from wool during the exposition in soil. There are reports that some species from *Aspergillus*, *Fusarium*, *Mucor* and *Trichoderma* develop on keratin and fat substrates and produce keratinase (Singh, 2014; Kim, 2003). The utilised experiment approved the mentioned proposition, and mainly *Aspergillus*,

Fusarium and *Trichoderma* species were detected on wool samples after 3-month exposition. By the way, there were determined different species of fungi after 3-month expositions as well: only on wool 2 specimen was detected fungi *Cunninghamella elegans*. The appearance of fungi *Cunninghamella elegans* is not surprising, because it is known as keratinophilic fungi (Fujii *et al.*, 2013).

CONCLUSION

The test has shown that each wool sample is characterised by different behaviour during the exposition in soil, and the behaviour depends on the method of unhairing. Accordingly, deterioration of any sort of waste keratin should be investigated thoroughly before the utilization by burial in landfills.

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