Hair-saving Enzyme-assisted Unhairing: Effects of Sodium Hydrosulfide and Peroxide

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Abstract: In a traditional dehairing process with sodium sulfide and lime, the hair is degraded to some extent so that it can not be recovered. Thus, the old process is a major contributor to wastewater pollution. Enzymatic dehairing is a cleaner process, but its results were not satisfactory. In this study, an attempt was made to modify the disadvantages of the two unhairing methods in the tannery. Several commercial enzyme formulations were chosen, and the effects of sodium hydrosulfide and peroxide on proteases activity were studied. Then, hair-saving enzymatic unhairing experiments with the two reagents were performed in paste and pile method. Activity of enzyme 2709 stabilized with the addition of sodium hydrosulfide; but peroxide would affect the activity of protease 3942. Hair was completely removed, which was confirmed with the scanning electron microscope analysis. Strength and bulk properties of the experimental leathers were also comparable to the controls. Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) significantly reduced with the two new processes. Therefore, the hair-saving enzyme-assisted unhairing is a cleaner technology to replace the traditional method.

Key words: enzymatic unhairing; hair-saving; goat skin; sodium hydrosulfide; peroxide

1 Introduction

Global concerns about the environmental impact of leather industry have led tanners to try to reduce the elements of toxicity in their effluents. Dehairing procedures are known for uncleanness and contribute to 60-70% of the total pollution load in leather processing. The conventional dehairing process with sodium sulfide and lime contributes a significant amount of BOD, COD, sulfide, and solid wastes. Extensive use of sodium sulfide bears unfavorable consequences on environment and the efficacy of effluent treatment plants. Consequently, it seems worthwhile to look for alternative dehairing processes, which could completely replace the lime and sodium sulfide and diminish the ecotoxicological parameters. Enzyme is a kind of bio-catalyst and no toxic itself, which can react with components of skin such as collagen protein, keratin, glycoprotein and fat, etc. Plenty of components useless to leather manufacturing will be removed so that collagen fiber can be moderately opened. Several researchers have tried and rationalized enzymatic dehairing as an alternative to sulfide dehairing. Even then, tanners are hesitant to use the enzyme because the quality and activity of proteases used in traditional process are so unstable that it can lead to loosened and tiny hairs. Therefore application of enzymatic unhairing is limited to some extent. Taking into account the superior effect of liming dehairing and the cleaner trait of enzymatic unhairing, enzymatic dehairing process assisted by sulfide or other auxiliaries is paid much attention to.

In this study, several commercial proteases were chosen, and the effects of sodium hydrosulfide and peroxide on their activity were analyzed. Those enzymes, whose activities have been slightly influenced or even activated by auxiliaries, will be selected to hair-saving enzyme-assisted unhairing experiments for goat skins in paste and pile method. The pollution load and the dehairing effects were evaluated.

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2 Experimental

2.1 Materials

The following apparatus and reagents were used in this experiment.

GSD-350 Type drums (300mm×160mm), TS2000-S Type multi-functional stress test machine, lastometer, shrinkage temperature tester, JSM-6360 Type scanning electron microscope (SEM). Several proteases used for enzymatic dehairing were Bacillus Licheniformis 2709 protease, Bacillus Subtilisin 3942 and A.S1398 proteases, Bacillus Actinomyces 166 protease, etc. Which were supplied by Enzyme Co., Ltd (Luniang, Yunnan), and all other chemicals were of commercial grade, such as sodium hydrosulfide, peroxide, lime, sodium sulfide, etc.

2.2 Methods

2.2.1 Characteristics of protease preparations

Protease activity was estimated according to Folin-Phenol method, with suitable modification. One unit was expressed as the liberation of 1 mg tyrosine equivalent of casein substrate in 10 min using tyrosine as standard. To obtain the optimum pH of one enzyme powder, a series of buffer solutions over a range of pH 6.0-12.0 were made, which were used to dissolve a quantitative enzyme preparation and then activity of one enzyme at this pH was determined by Folin-Phenol method. In this way, a series of pH curves were plotted as activities vs pH values.

2.2.2 Effects of sodium hydrosulfide and peroxide on activity and stability of protease

The effects of sodium hydrosulfide and peroxide on activities of one protease was studied by mixing sodium hydrosulfide or peroxide in a certain proportion to protease and the mixtures were firstly dissolved by proper buffer solution, then, which would be adjusted to its optimal pH value point. Whereafter incubated at different intervals, described above enzyme admixtures were measured using Folin-Phenol method except for sodium hydrosulfide. Since reagents in Folin-Phenol method would be disturbed due to sodium hydrosulfide, thus enzymatic activities containing sulfide could be analyzed by UV-spectroscopy method. In order to conveniently demonstrate the influences of the two compounds, activities of each enzyme alone at different intervals were assayed as contrast and were also plotted as relative activity (%) vs time together with aforementioned enzyme mixtures in the same graphs.

2.2.3 Hair-saving enzyme-assisted dehairing and the control dehairing experiments

Prior to dehairing, fresh goatskins were soaked in water for 6 h and the soaked skins were taken for dehairing experiments. Three different groups of dehairing experiments viz., conventional lime–sulfide process, enzyme-assisted processes using a commercial enzyme and hydrosulfide, another commercial enzyme and peroxide were separately carried out using the paste method. Conventional dehairing (control) was performed using 2% sodium sulfide and 10% lime. Hair-saving enzyme-assisted dehairing processes (experimental) were carried out using a mixture of 0.2% sodium hydrosulfide and 0.3% protease 2709, 3% peroxide and 0.5% protease 3942, respectively and the mixtures were applied as a paste on the flesh side of the skin and left overnight, then the hairs were removed using a blunt knife. All the percentages were based on soaked weight.

Subsequently, both controls and experimental groups of dehaired pelts were relimed using 100% water with 10% lime (based on the weight of dehaired pelts) for 2 d with occasional handling. The relimed control pelts were then delimed, bated and pickled in a drum whereas the relimed experimental pelts were pickled without bating. The pickled skins and hides were finished as dyed crusts as per conventional procedures.

2.2.4 Scanning electron microscopic studies

Samples cut from experimental and control dehaired pelts were washed, fixed in buffered formalin,
dehydrated using a graded methanol series and finally with acetone. Subsequently, acetone was completely replaced by flushing with Freon Mafron R-22 gas and then samples were freeze-dried. The dried samples were cut into 3 mm thickness, mounted vertically and horizontally on copper stubs in order to view cross and surface details, coated with 20 nm of gold by direct current sputtering and examined in a JSM-6360 Type unit operated at an accelerating voltage of 15 kV.

2.2.5 Physical assessment of leathers

The crust leathers were tested for physical strength properties. After conditioning the crust leather at room temperature and at above 65% relative humidity over a period of 48 hours, the properties such as tensile strength, elongation at break, tear strength and grain crack were assessed in comparison with control samples using standard methods. The results are presented in the Table 1.

2.2.6 Analysis of effluents from the liming process

The effluent from the liming process for control and experimental groups of skins and hides were analyzed for various parameters such as BOD, COD, TDS, TSS. The results are expressed in terms of emissions per kg/ton of the raw material and are presented in Table 2 in comparison with control samples.

3 Results and discussions

3.1 Activities of several commercial proteases

A series of pH curves for several proteases were measured in Fig. 1. It is seen that every enzyme has its own optimum pH value; besides the enzyme 3942 has two optimum pH values at 7 and 10, respectively. The enzyme 3942 has an activity of about 40,000 units/g using casein substrate. Enzyme 2709 has optimum pH at 9.5 indicating that it was an alkaline protease and had an activity of 50,000 units/g using casein substrate. Enzymes A.S 1398 and 166 have optimum pH at 8 and 7 separately indicating as neutral proteases that have activities of 35,000 and 55,000 units/g respectively using the above substrate.

3.2 Effects of sodium hydrosulfide and peroxide on activity and stability of proteases

The Anson method was once employed to determine the enzyme differences. The activities of the enzymes were calculated as a percentage and compared to the enzyme activity in a buffer solution. The changes of activities in hydrosulfide or peroxide and each enzyme alone together are presented in Figure 2 and Figure 3. It is shown in Fig.2 that activities of the enzymes A.S 1398, 3942 and 166 are greatly influenced by hydrosulfide, attenuation of activity of those enzymes decreases especially rapidly by action of hydrosulfide compared to those enzymes alone. However, activity of the enzyme 2709 is stabilized by hydrosulfide and its activity attenuation fall slowly compared with enzyme 2709 alone.

It is seen in Fig.3 that activities of the enzymes 2709, A.S 1398 and 166 are greatly impressed by peroxide, attenuation of activity of those enzymes descends especially rapidly by action of peroxide compared to those enzymes alone. On the contrary, activity of the enzyme 3942 is stabilized by peroxide
and its activity attenuation fall slowly compared with enzyme 3942 alone. So we can choose the enzyme 2709 and 3942 to manage enzyme-assisted dehairing process subsequently.

**Fig. 2 Effect of sodium hydrosulphide on the activities of the proteases**

3.3 **Analysis of SEM**

The scanning electron micrographs of control and experimental samples after dehairing showing the grain surfaces and the cross section at a magnification of ×100 and ×200, respectively, are shown in Fig. 4. It is seen that the grain surface appears to be more even and smoother in two experimental systems than control. In addition, hair pores are free of any hair residues in two experimental systems. Cut surface
features reveal moderate opening up of collagen fiber bundles in conventional system when compared to well open up fiber bundles in two enzyme-assisted experimental systems.

![Fig. 4 Scanning electron micrographs of control and experimental samples after dehairing showing the grain surfaces and the cross section: (a) grain surface of enzyme-assisted dehairing by sulfide at a magnification of ×100; (b) grain surface of enzyme-assisted dehairing by peroxide at a magnification of ×100; (c) grain surface of control dehairing at a magnification of ×100; (d) cross section of enzyme-assisted dehairing by sulfide at a magnification of ×200; (e) cross section of enzyme-assisted dehairing by peroxide at a magnification of ×200; (f) cross section of control dehairing at a magnification of ×200.](image)

### 3.4 Physical assessment of leathers

The physical properties of crust leathers from control and two experimental systems are shown in Tab. 1. It is seen that the two experimental systems (E1 and E2) show comparable physical properties in corresponding control samples (C). Except for tensile strength, the other properties, such as load elongation, elongation at break, tear strength and grain crack distention, etc., of two experimental systems are all slightly superior to that of the control samples. Moreover, tensile strength values of E1 and E2 still accord with standards of industrial application.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E1(2709+hydrosulfide)</th>
<th>E2(3942+peroxide)</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength/(N · mm⁻²)</td>
<td>25.3</td>
<td>24.5</td>
<td>25.8</td>
</tr>
<tr>
<td>Load elongation/%</td>
<td>34</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>Elongation at break/%</td>
<td>86</td>
<td>81</td>
<td>72</td>
</tr>
<tr>
<td>Tear strength/(N · mm⁻¹)</td>
<td>75.5</td>
<td>70.4</td>
<td>60.3</td>
</tr>
<tr>
<td>Grain crack distention/(mm)</td>
<td>17.3</td>
<td>16.8</td>
<td>15.1</td>
</tr>
</tbody>
</table>

### 3.5 Analysis of effluents from liming process

The pollution loads, such as BOD, COD, TDS and TTS, etc., generated in liming process are shown
in Tab. 2. The results showed that substantial reduction of BOD, COD, TDS and TTS existed both in two experimental systems (E1 and E2) compared with the control. The conventional unhairing methods lead to the destruction of the hair leading to increased BOD, COD and TDS loads in the effluent. However, the advantage of the two protease unhairing systems lies in the reduction in the pollution load as is evident from this investigation.

<p>| Tab. 2 Pollution load generated in liming process using different methods of unhairing |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>E1(2709+hydrosulfide)</th>
<th>E2(3942+peroxide)</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD(kg/ton)</td>
<td>30</td>
<td>32</td>
<td>43</td>
</tr>
<tr>
<td>COD(kg/ton)</td>
<td>80</td>
<td>56</td>
<td>121</td>
</tr>
<tr>
<td>TDS(kg/ton)</td>
<td>65</td>
<td>60</td>
<td>158</td>
</tr>
<tr>
<td>TSS(kg/ton)</td>
<td>42</td>
<td>38</td>
<td>53</td>
</tr>
</tbody>
</table>

4 Conclusions
In this studies, two sorts of commercial enzyme formulations, proteases 2709 and 3942, were found to be capable of being more stable on action of hydrosulfide and peroxide respectively and then chosen to explore hair-saving enzyme-assisted unhairing experiments. Scanning electron microscope analysis demonstrates that hair is removed completely in two experimental systems compared to the control. Strength and bulk properties of the experimental leathers are comparable to that of control leathers. Those two processes enjoy a significant reduction in BOD, COD, TDS and TSS. Therefore, those are cleaner processing technologies that could be chosen to solve traditional method’s disadvantage.

References