

# A Cleaner Deliming Process Using Sodium Gluconate for Reduction in Nitrogen Pollution in Leather Manufacture

by

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## Abstract

Ammonium salts used in deliming process are the main source of high ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) concentration in tannery wastewater. To reduce nitrogen pollution in leather manufacture without sacrificing quality of resultant leathers, a cleaner deliming agent prepared by mixing sodium gluconate (60 wt.%), citric acid (15 wt.%) and ammonium sulfate (25 wt.%) together was employed for deliming limed cattle hide pelt. The results indicated that this cleaner deliming agent penetrated the whole pelt very quickly and successfully formed a buffer system of pH 8-9 with the alkali from liming, which prevented acid swelling and damage to the fiber network of pelt. The delimed pelt had a cleaner grain surface with a higher extent of calcium removal than the conventional ammonium delimed pelt. The concentrations of  $\text{NH}_3\text{-N}$  and total nitrogen (TN) in the cleaner deliming effluent were dramatically reduced by 86% and 79%, respectively. Moreover, the cleaner deliming scarcely affected subsequent bating process with trypsin.

## Introduction

High concentration of ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) in tannery wastewater has received much attention in recent years due to the increasingly strict emission regulations. Since the concentration of  $\text{NH}_3\text{-N}$  in the untreated wastewater is usually higher than 100 mg/L and far from the wastewater discharge standard (15 mg/L in China), the tannery wastewater is normally biologically treated to remove  $\text{NH}_3\text{-N}$  pollution.<sup>1-3</sup> But unfortunately, the conventional tannery wastewater contains excessive nitrogen source in terms of the proper mass ratio of C:N:P for a satisfactory biological treatment, which results in a sharp increase in the difficulty and the cost of wastewater treatment.<sup>4-5</sup> Therefore, a reduction in nitrogen source or an appropriate introduction of additional carbon source in tannery wastewater will undoubtedly benefit the removal of nitrogen from the wastewater through biological treatment.

In leather processing, the deliming process is performed after liming to achieve a float pH of 8-9, release bound water from swollen collagen fibers and remove calcium from pelt for subsequent bating with trypsin.<sup>6</sup> Usually, 2%-4% ammonium salts (based on weight of limed pelt) are used in this process due to their effectiveness and cheapness in deliming. So, the conventional deliming process becomes the main source of  $\text{NH}_3\text{-N}$  in the tannery wastewater.<sup>1</sup> To reduce  $\text{NH}_3\text{-N}$  pollution from the source, many ammonium-free deliming technologies, such as  $\text{CO}_2$  deliming,<sup>4,7,8</sup> magnesium salts deliming,<sup>9</sup> weak acids deliming,<sup>10-11</sup> peracetic acid deliming,<sup>12-13</sup> sodium hexametaphosphate deliming<sup>14</sup> and leaf extracts deliming,<sup>15</sup> have been developed. However, although they can significantly reduce the  $\text{NH}_3\text{-N}$  pollution in wastewater as well as the unpleasant ammonia odor, these ammonium-free deliming technologies have not been widely applied in commercial scale due to some disadvantages, such as the requirement of special equipment for  $\text{CO}_2$  deliming, the risk of leading to an excessively low float pH in organic acid deliming, and in particular the unsatisfactory penetration rate of almost all the ammonium-free deliming agents that would increase the deliming time and bring the possibility of loose grain or coarse grain.

Sodium gluconate is widely used as a chelating agent for cement, plating and alumina dyeing industries and as a cleaning agent for steel surface and glass bottles because it has an excellent ability to chelate calcium, iron, copper, aluminum and so on.<sup>16</sup> So we speculate that a high extent of calcium removal from limed pelt can be achieved by using sodium gluconate in deliming. However, in our preliminary experiment, it was found that sodium gluconate is not acidic enough to decrease the pH of limed pelt from 12-13 to 8-9. Citric acid can effectively neutralize the alkali in limed pelt and is commonly chosen as a main component of commercial deliming agents in combination with its salt to form buffer system.<sup>11</sup> Thus, we planned to develop a cleaner deliming agent by mixing sodium gluconate with citric acid in a rational molar ratio. Moreover, sodium gluconate and citric acid are likely to act as a source of nutritive element carbon,

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Manuscript received October 4, 2017, accepted for publication November 3, 2017.

which increases the C:N ratio in tannery wastewater and benefits the biological treatment of the wastewater. As we know, the delimiting process is generally required to be finished as soon as possible so as to avoid damage to the fine and tight grain surface of leather caused by prolonged mechanical action. The delimiting time is closely related to the penetration rate of delimiting agents in pelt. Until now, ammonium salts are found to be the delimiting agents that have the fastest penetration rate in pelt and make the pelt flaccid in the shortest time. Therefore, in this study, a mixture of sodium gluconate, citric acid and a small amount of ammonium sulfate was prepared as a practical cleaner delimiting agent, so that it can both significantly reduce  $\text{NH}_3\text{-N}$  pollution and keep the fast penetration in pelt. The delimiting effectiveness of this cleaner delimiting agent was evaluated by analyzing its penetration and pH buffering capacity, the extent of calcium removal from pelt, the concentrations of  $\text{NH}_3\text{-N}$ , total nitrogen (TN) and total organic carbon (TOC) in effluent and the effect of delimiting on bating process.

## Experimental

### Materials

Cow hides were used as raw materials. Limed grain split (pH 12.6, thickness 2.2 mm) prepared by conventional soaking, unhairing, liming and splitting processes was used for delimiting and bating trials. Cleaner delimiting agent was prepared by mixing sodium gluconate (60 wt.%), citric acid (15 wt.%) and ammonium sulfate (25 wt.%) together. Trypsin from bovine pancreas (activity 150,000 U/g at 30°C, pH 9) purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. and fluorescein isothiocyanate-labeled trypsin (FITC-trypsin) prepared according to the method described in our previous study<sup>17</sup> were used as bating enzymes. The chemicals used for delimiting and analyses of effluents and pelts were of analytical grade, and the other chemicals used for leather processing were of commercial grade.

### Experimental and Control Delimiting and Bating Processes

The limed pelt was cut along the backbone in halves. The left side was taken for experiment, and the right side was taken for control. The experimental cleaner delimiting was performed in a drum with 2.0% cleaner delimiting agent and 150% water at 32°C for 90 min. Subsequently, the experimental delimited pelt was washed with 100% water for 10 min and bated with 0.1% trypsin and 100% water at 30°C for 90 min. The percentage was based on limed pelt weight. The control delimiting, washing and bating processes were carried out by the same procedures described above except that 2.0% cleaner delimiting agent was replaced with 3.5% ammonium sulfate.

Besides, another four ammonium-free delimiting processes were also performed by the same procedure described above except

that 2.0% cleaner delimiting agent was replaced with 2.5% boric acid,<sup>18</sup> a mixture of 3% sodium hexametaphosphate and 1% boric acid,<sup>14</sup> a mixture of 0.85% acetic acid and 0.85% sodium acetate,<sup>19</sup> and a mixture of 0.8% citric acid and 1.6% sodium citrate,<sup>19</sup> respectively. Here, the dosages of these delimiting agents were optimized by our preliminary experiments.

### Analyses of Penetration of Delimiting Agents

The experimental, control and ammonium-free delimited pelts were cut with a knife during the delimiting process. Then these incisions were checked using phenolphthalein indicator to determine the "lime streak" in the pelts and to measure the penetration depth of the delimiting agents, viz., the depth of the delimiting action.

### Determination of Delimiting Float pH

In order to evaluate the pH buffering capacity of delimiting agents, the pH values of both the experimental and control delimiting floats were measured using a precise pH meter when the limed pelts were delimited for 5, 20, 40, 60 and 90 min, respectively. Here, the liming float pH of 12.55 was recorded as the initial pH of both the experimental and control delimiting floats.

### Determination of Concentration of Hydroxyproline (Hyp) in Delimiting Effluent

The experimental and control delimiting effluents were collected after delimiting for 90 min and filtered with 100 mesh filter cloth. The filtrates were hydrolyzed with 6 mol/L hydrochloric acid at 110°C for 10 h. The volume ratio of the filtrate to the hydrochloric acid was 1:10. The hydrolyzed samples were then taken for the analysis of Hyp concentration as described in the document.<sup>20</sup>

### Determination of Extent of Calcium Removal from Pelt

The limed pelts and the experimental and control delimited pelts were sampled and then analyzed for their calcium contents as reported in the documents.<sup>14,21</sup> The extents of calcium removal from the experimental and control delimited pelts were calculated as:

$$\% \text{ extent of calcium removal} = \frac{\text{calcium content of limed pelt} - \text{calcium content of delimited pelt}}{\text{calcium content of limed pelt}} \times 100 \quad (1)$$

### SEM and EDS Analyses of Delimited Pelt

The experimental and control delimited pelts were sampled after delimiting for 90 min and then freeze-dried. Subsequently, the topography and the elemental composition of their grain surfaces were analyzed using a scanning electron microscope (SEM, Phenom Pro, Phenom, China) and an energy dispersive X-ray spectrometer (EDS, Phenom Pro, Phenom, China), respectively.

### Determination of Concentrations of $\text{NH}_3\text{-N}$ , TN, TOC, COD and $\text{BOD}_5$ in Delimiting Effluent

After delimiting for 90 min, the experimental and control delimiting effluents were sampled and then taken for determination of concentrations of  $\text{NH}_3\text{-N}$ , TN, TOC, COD and

BOD<sub>5</sub>, NH<sub>3</sub>-N, COD and BOD<sub>5</sub> concentrations were determined according to standard methods.<sup>22</sup> TN and TOC concentrations were determined using a TOC/TN analyzer (vario TOC, Elementar, Germany).

#### Determination of Concentrations of Protein and Hyp in Bating Effluent

The experimental and control bating effluents were collected after bating for 90 min. For the determination of protein concentration, the effluents were centrifuged at 8000 RPM for 6 min, and the supernatants were measured according to the method described in the document.<sup>23</sup> The concentration of Hyp in the bating effluents was analyzed as described above.

#### Observation of Mass Transfer of Trypsin in Bated Pelt

The experimental and control delimed pelts (5 g for each) were washed with 100% water for 10 min and then bated with 0.1% FITC-trypsin and 100% water at 30°C for 90 min. The percentage was based on weight of delimed pelt. The delimed pelts and the bated pelts were sampled and cut into vertical sections with a thickness of 20 µm on a freezing microtome (CM1950, Leica, Germany). These sections were Weigert-van Gieson stained to identify collagen fibers and then observed using a biologic microscope (CX41, Olympus, Japan). Furthermore, the sections of the bated pelts were observed using an inverted fluorescence microscope (Ti-U, Nikon, Japan) to locate FITC-trypsin in pelt, and then the fluorescence micrographs were processed with Image J software to semi-quantify the relative content of FITC-trypsin in pelt.

## Results and Discussion

#### Penetration and pH Buffering Capacity of Delimiting Agent

A main purpose of delimiting is to neutralize the alkali (lime, sodium hydroxide, sodium sulfide, etc.) introduced in liming process, thus decreasing the pH of limed pelt from 12-13 to 8-9. Ammonium salts (e.g. 2%-4% ammonium sulfate, based on limed pelt weight) are normally used as they can penetrate limed pelt very quickly and form buffer systems of pH 8-9 with the alkali in the limed pelt to make the pelt become very flaccid.<sup>6</sup> Therefore, to replace the conventional ammonium delimiting, we first analyzed the penetration and the pH buffering capacity of the cleaner delimiting agent.

It was found that, after delimiting for 20 min, the whole vertical section of the pelt delimed by our cleaner delimiting agent reacted colorless to phenolphthalein indicator and became as flaccid as the ammonium delimed pelt. Meanwhile, the middle layers of the pelts delimed by all the ammonium-free delimiting agents reacted pink to phenolphthalein indicator and kept swollen (see Table I). These phenomena indicated that, compared with the ammonium-free delimiting agents such as boric acid, acetic acid, citric acid and sodium hexametaphosphate, the cleaner/low-

ammonium delimiting agent penetrated the pelt and completed the delimiting operation much more quickly. So, it could prevent excessive mechanical action on the pelt that may result in a rupture of the fibers and a looseness of the leather.

**Table I**  
Penetration of delimiting agents in limed pelts.

Delimiting agents <sup>a</sup>	Penetration depth after delimiting for 20 min	Time for complete penetration (min) <sup>c</sup>
3.5% ammonium sulfate (control)	Φ <sup>b</sup>	< 20
2.0% cleaner delimiting (experiment)	Φ	20
2.5% boric acid	5/6Φ	40
3% sodium hexametaphosphate and 1% boric acid	2/3Φ	60
0.85% acetic acid and 0.85% sodium acetate	1/3Φ	90
0.8% citric acid and 1.6% sodium citrate	1/3Φ	>90

<sup>a</sup>The percentage was based on weight of limed pelt.

<sup>b</sup>Φ represented the phenomenon that the whole vertical section of pelt reacts colorless to phenolphthalein indicator, indicating that the delimiting agent thoroughly penetrates the pelt.

<sup>c</sup>Time of complete penetration was defined as the time that the whole vertical section of pelt reacts colorless to phenolphthalein indicator.

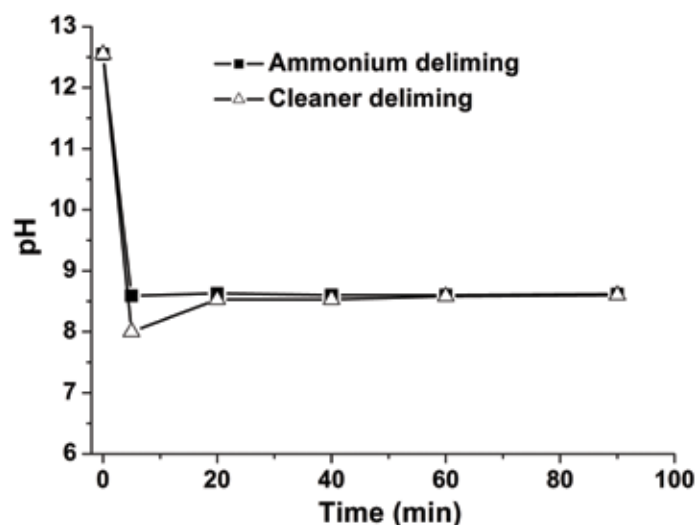


Figure 1. Variation of delimiting float pH.

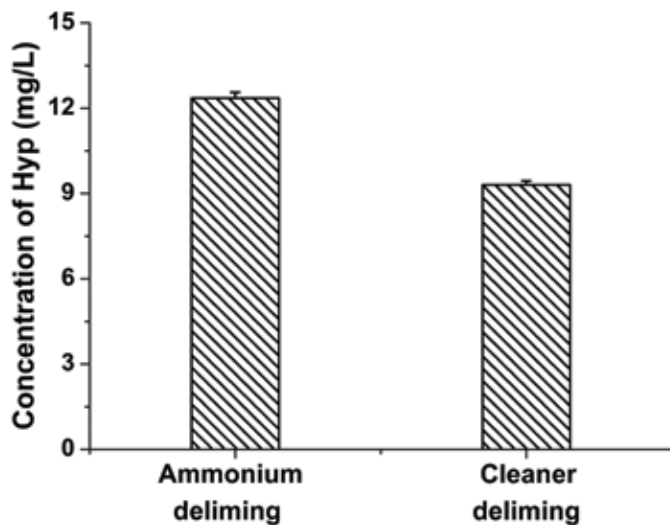


Figure 2. Concentration of Hyp in delimiting effluents.

As shown in Figure 1, the float pH of the cleaner delimiting was above 8.0 after delimiting for 5 min, and an equilibrium pH of 8.6 was achieved quickly in 20 min. The trend of the float pH of the cleaner delimiting was very similar to that of the typical ammonium sulfate delimiting. The results meant that the cleaner delimiting took place very gradually and could prevent acid swelling completely because the float pH could be buffered to 8-9 easily. The data in Figure 2 show that the concentration of Hyp in the cleaner delimiting effluent was even lower than that in the ammonium delimiting effluent, suggesting that the cleaner delimiting agent really caused little hydrolysis of pelt collagen.

#### Extent of Calcium Removal from Pelt

The extent of calcium removal from the limed pelt in delimiting process directly affects the action of subsequent chemicals on pelt/leather and the quality of resultant leather. For example, residual calcium salts in the delimited pelt would influence the bating effectiveness of trypsin, form insoluble calcium sulfate that precipitates in pelt during pickling process and cause an adverse effect on the evenness of dyeing and fatliquoring.<sup>24-25</sup> Hence, the extent of calcium removal from pelt by using the cleaner delimiting agent was analyzed.

The data in Figure 3 show that the extent of calcium removal from the cleaner delimited pelt was higher than that from the ammonium sulfate delimited pelt. The SEM and EDS analyses of the grain surfaces of the delimited pelts revealed that many calcium precipitates still covered the ammonium delimited pelt (Figure 4a), while the surface of the cleaner delimited pelt was much cleaner (Figure 4b). These results suggested that the cleaner delimiting agent prepared by mixing sodium gluconate (60 wt.%), citric acid (15 wt.%) and ammonium sulfate (25 wt.%) together could remove calcium salts from the limed pelt more

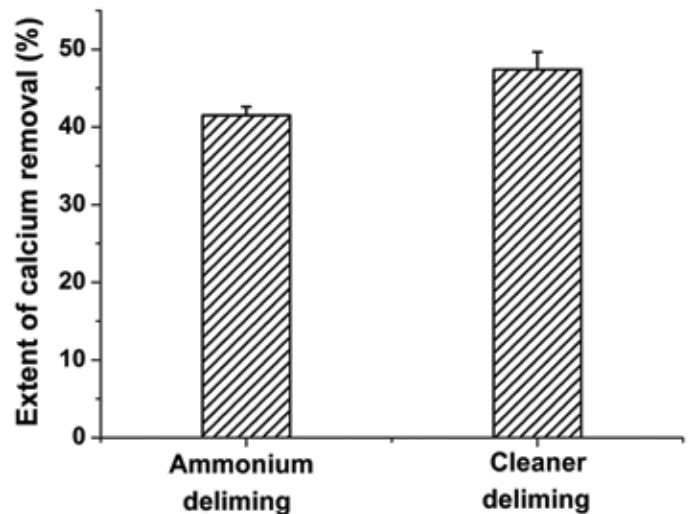


Figure 3. Extent of calcium removal from delimited pelts.

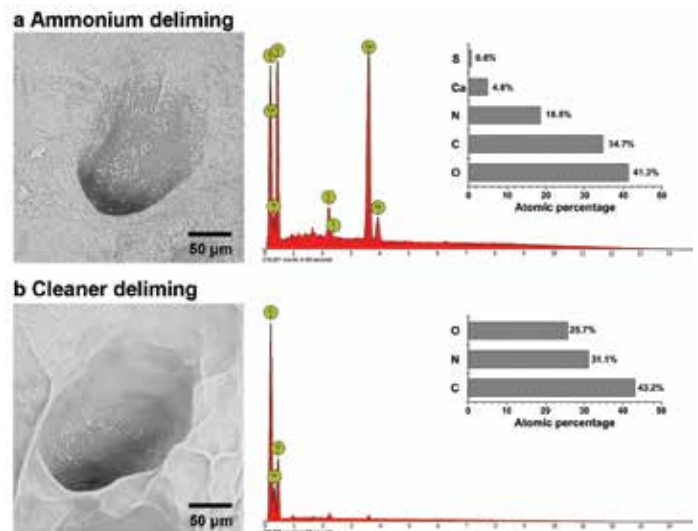


Figure 4. SEM micrographs, EDS spectra and elemental compositions of grain surfaces of delimited pelts: (a) the ammonium delimited pelt; (b) the cleaner delimited pelt.

effectively, which is helpful to the elimination of lime blast and the prevention of uneven dyeing and fatliquoring. The excellent ability of the cleaner delimiting agent to remove calcium is due to the fact that its main component, viz., sodium gluconate can form soluble complexes with  $\text{Ca}^{2+}$  very easily.<sup>16</sup> Moreover, as can be seen from the SEM micrographs of the grain surfaces (Figures 4a and 4b), the hair pores of the cleaner delimited pelt were as intact and clear as those of the ammonium delimited pelt, confirming that the cleaner delimiting agent with a fast penetration and a satisfactory pH buffering capacity did not damage the grain.

### Effect of Deliming on Bating

The deliming process aims to make the pelt suitable for subsequent bating whose main purpose is to further remove non-collagenous proteins from the pelt and open up collagen fibers for tanning.<sup>26</sup> Therefore, to determine whether the cleaner delimed pelt was well prepared for the conventional bating with

trypsin, we analyzed the concentrations of protein and Hyp in bating effluent and the collagen fiber networks of both delimed and bated pelts. It was found that the concentrations of protein and Hyp in the experimental bating effluent were close to those in the control bating effluent (Figure 5), indicating that the hydrolysis action of trypsin on non-collagenous proteins of the

**Table II**  
Concentrations of  $\text{NH}_3\text{-N}$ , TN, TOC, COD and  $\text{BOD}_5$  in control (C) and experimental (E) deliming effluents (unit: mg/L).

Sample	$\text{NH}_3\text{-N}$	TN	TOC	COD	$\text{BOD}_5^a$
C - ammonium deliming	3780±14	4091±107	1180±6	2180±106	1300±100
E - cleaner deliming	532±8	876±24	3211±62	6790±14	3800±100

a -  $\text{BOD}_5$  was defined as the biochemical oxygen demand of wastewater measured over 5 days, which estimated the amount of oxygen required for biochemical degradation of organic matters in wastewater based on the metabolic activity of aerobic microorganisms.

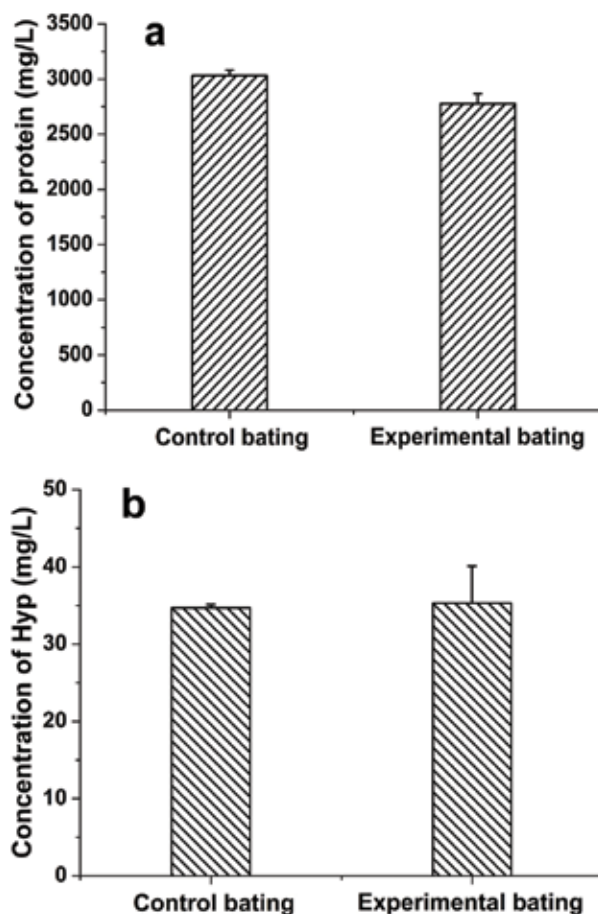


Figure 5. Concentrations of protein (a) and Hyp (b) in bating effluents.

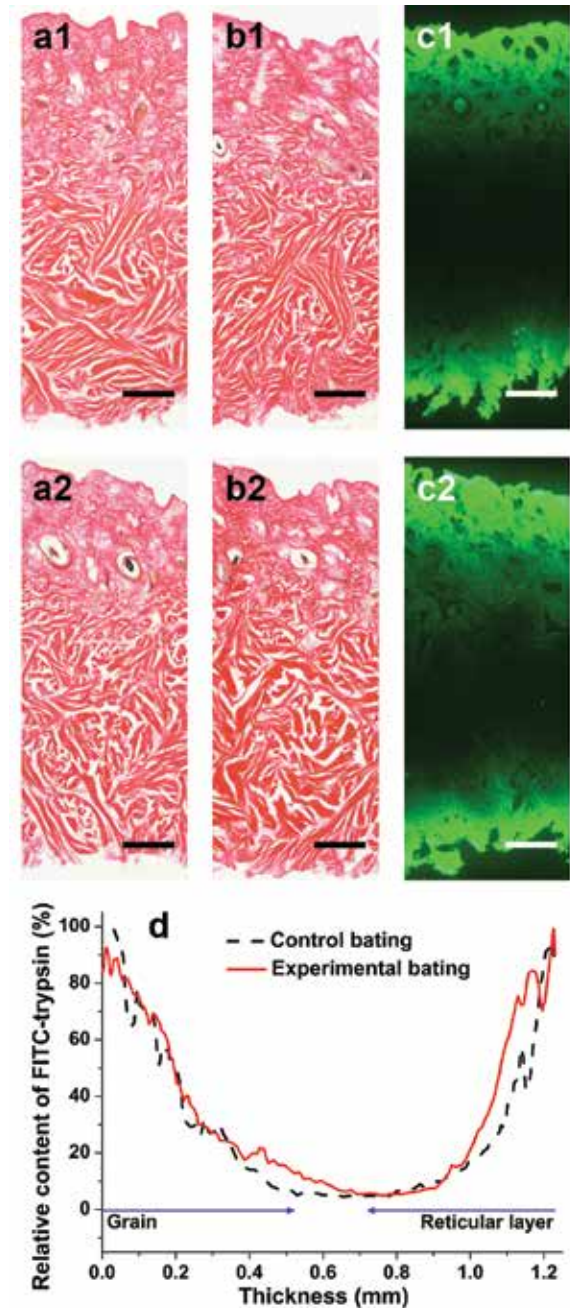


Figure 6. (a) Photomicrographs of vertical sections (Weigert-van Gieson stain) of delimed pelts from control (a1) and experiment (a2); (b) Photomicrographs of vertical sections (Weigert-van Gieson stain) of bated pelts from control (b1) and experiment (b2); (c) Fluorescence micrographs of vertical sections of bated pelts from control (c1) and experiment (c2); (d) Relative content of FITC-trypsin in the bated pelts that was semi-quantified by analysis of Figures 6(c1) and 6(c2) using Image J software. Scale bars, 500  $\mu\text{m}$ .

cleaner delimited pelt was similar to that on the ammonium delimited pelt. Comparing Figures 6(a1) and 6(a2), as well as Figures 6(b1) and 6(b2), it is shown that the dispersions of collagen fibers in the experimental delimited and bated pelts were almost the same as those in the control pelts. Moreover, it was observed that FITC-trypsin entered the experimental bated pelt more deeply than the control bated pelt (see Figures 6(c1), 6(c2) and 6(d)), which favored a more uniform enzymatic action for each layer of pelt and a higher quality of resultant leather. This is very likely due to the fact that the cleaner delimited pelt had a better removal of calcium. These results proved that the cleaner delimited pelt was suitable for the conventional bating with trypsin, and the cleaner delimiting scarcely affected the bating effectiveness.

### Environmental Benefits

To evaluate whether the cleaner delimiting, viz., a low-ammonium delimiting was useful for reducing nitrogen pollution, the concentrations of  $\text{NH}_3\text{-N}$  and TN in the cleaner delimiting effluent were determined and compared with those in the conventional ammonium delimiting effluent. As listed in Table II, the concentrations of  $\text{NH}_3\text{-N}$  and TN in the cleaner delimiting effluent were reduced by 86% and 79%, respectively, because of a remarkable decrease in the dosage of ammonium sulfate from 3.5% to 0.5%. The results demonstrated that the cleaner delimiting was very beneficial to the reduction in nitrogen pollution. Here, it is worth noting that although the concentrations of TOC and COD in the cleaner delimiting effluent were higher as most of ammonium sulfate was replaced by sodium gluconate and citric acid, the cleaner delimiting would not bring an aggravation of pollution based on the theory of nutrient balance. A rational C:N ratio of wastewater is necessary for a satisfactory aerobic biological treatment to remove both nitrogen and COD pollutions. However, the C:N ratio of conventional tannery wastewater is too low to successfully remove nitrogen.<sup>4-5</sup> It has been proved that an introduction of additional C source can dramatically improve the nitrogen removal, and the additional C source can be almost consumed at the same time.<sup>5</sup> Furthermore, according to the data in Table II, the  $\text{BOD}_5/\text{COD}$  ratio of the cleaner delimiting effluent was over 0.45, suggesting that the effluent is easy to be biodegraded. It is therefore reasonable to speculate that the cleaner delimiting, which can increase the C:N ratio by both a sharp decrease in the TN concentration and an increase in the TOC concentration, should benefit the removal of nitrogen pollution from tannery wastewater through biological treatment.

### Conclusion

The cleaner delimiting technology by using a mixture of sodium gluconate, citric acid and a small amount of ammonium sulfate can dramatically reduce nitrogen pollution in leather processing

with guaranteeing quality of resultant pelts because it keeps the advantages of conventional ammonium delimiting such as a fast penetration and a satisfactory pH buffering capacity, and thus makes the pelt flaccid in a short time and avoids excessive mechanical damage to the pelt. Furthermore, the cleaner delimiting is more effective in removing calcium salts from pelt than the ammonium delimiting, which is attributed to the great ability of sodium gluconate to form soluble complexes with calcium. Since the C:N ratio of conventional tannery wastewater is generally too low for the biological treatment to effectively remove nitrogen pollution, the cleaner delimiting that can cause a dramatic decrease in the TN concentration as well as an increase in the TOC concentration, would favor an increase in the C:N ratio and a better biological treatment of tannery wastewater.

### Acknowledgement

This work was supported by the National Natural Science Foundation of China (21406145, 21476149).

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