AMMONIA NITROGEN IN TANNERY WASTEWATER: DISTRIBUTION, ORIGIN AND PREVENTION

by

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ABSTRACT

Tannery wastewater usually contains a high content of ammonia nitrogen (NH_3-N), which increases the difficulty of wastewater treatment. The objective of this work is to investigate the distribution of NH_3-N in wastewaters from beamhouse processes and analyze the origin of NH_3-N in each operation, so that the strategies that can reduce NH_3-N at the origin could be suggested. Not surprisingly, the results demonstrate that NH_3-N mainly concentrates in deliming and bating wastewaters due to the addition of ammonium salts in these two operations. This problem can be solved by using non-ammonia deliming and bating agents. A small quantity of NH_3-N arises from washing and soaking processes because of decomposition of proteins by microorganisms. But the NH_3-N in washing and soaking wastewaters may considerably increase when skins are badly preserved or the microorganisms in soaking float are not properly inhibited. Therefore, the effective antibacterial measures are important to diminish the production of NH_3-N in beamhouse processes. As for unhairing/liming process, NH_3-N in wastewater is mainly originated from hydrolysis of proteins, such as keratin and collagen, by strong alkali. Hair-saving unhairing and pelt swelling under milder conditions favor the decrease of NH_3-N discharge. In fact, the amount of organic nitrogen released from washing, soaking and liming processes is much more than NH_3-N. The experimental results indicate that the organic nitrogen mainly results from non-collagen proteins and their hydrolysates. This means the discharge of organic nitrogen is difficult to be avoided, and it may constitute a threat to end-of-pipe treatment of NH_3-N.

RESUMEN

Desperdicios acuosos de tenerías comúnmente contienen un alto contenido de nitrógeno amoniacal (NH_3-N), lo cual dificulta el tratamiento de efluentes. El objetivo de este estudio es investigar la distribución del NH_3-N en desperdicios líquidos de los procesos de pelambre, y analizar el origen del NH_3-N en cada operación, para así sugerir las estrategias que podrían reducir el NH_3-N en el lugar de origen. No es sorpresa, que los resultados demuestran que el contenido de NH_3-N principalmente se incrementa en los baños residuales del desencale y rendido debido a las adiciones de sales de amonio en estas dos operaciones. Este problema se podría resolver utilizando agentes desencalantes y rindientes exentos de nitrógeno amoniacal. Una pequeña cantidad de NH_3-N resulta del remojo y lavado por la descomposición de proteínas por microorganismos. Pero el NH_3-N en el remojo y lavado puede incrementarse considerablemente cuando las pieles han sido mal conservadas o la reproducción de los microorganismos en el baño de remojo no fue apropiadamente inhibida. Como tal, las medidas antibacterianas son importantes en la disminución del NH_3-N en los procesos de ribera. Es así como en los procesos de depilado/encalado, el NH_3-N en los desechos líquidos se origina principalmente por la hidrólisis de proteínas, tales como queratina y colágeno, por alcalinidad alta. La recuperación del pelo y la limitación al hinchamiento de la piel por medio de condiciones [alcalinas] menos extremas, favorecen la disminución de descargas de NH_3-N. A toda verdad, la cantidad de nitrógeno orgánico liberado en los procesos de lavado, remojo y encalado es mayor que la proveniente del NH_3-N. Los resultados experimentales indican que el nitrógeno orgánico principalmente resulta de las proteínas no-colagénicas y sus hidrolizados. Esto significa que la descarga de nitrógeno orgánico es muy difícilmente evitable, y que constituye una amenaza al tratamiento al final del procesamiento del NH_3-N.

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Introduction

Ammonia nitrogen (NH_3-N) is present in the forms of ammonia molecules and ammonium ions in water environment. As a nutrient salt, NH_3-N is an important component in the global biogeochemical cycle of nitrogen.[1] However, anthropogenic input of nutrients, especially NH_3-N, to aquatic ecosystem has enhanced greatly in recent years due to production and abuse of fertilizer, discharge of municipal sewage and other industrial effluents, etc.[2] These human activities can lead to the imbalance of ecosystem and subsequently eutrophication of water body.[3] Moreover, ammonia is highly toxic to fish and other aquatic animals. It is reported that ammonia can be fatal to fish in the range of 0.2~2 mg/L.[4] Therefore, excessive discharge of NH_3-N has been a serious environmental problem that we need to face and solve.

NH_3-N is one of the main contaminants in tannery wastewater. In general, NH_3-N concentration in untreated total effluent is higher than 100 mg/L, which is far from the wastewater discharge standard (15 mg/L in China).[5,6] In addition, NH_3-N is more difficult to remove compared with other pollutants. This is because a considerable quantity of organic nitrogen will be transformed into NH_3-N during biochemical treatment of tannery effluent, resulting in the decrease of ammonia removal rate.[7,8]

There are two ways to reduce NH_3-N from tannery wastewater, i.e. end-of-pipe treatment and elimination at the origins. The latter strategy might be preferable in consideration of cost and operability. Of course, the combination of the two methods will be more effective. In order to reduce NH_3-N at the origins, the operations that generate NH_3-N in leather processing need to be analyzed at first. Considering the fact that NH_3-N is seldom generated from post tanning processes when chemicals containing ammonia are not employed, our investigation focused on the beamhouse processes. It is reported that up to 70% of NH_3-N in total wastewater is attributed to the use of ammonium salts in conventional deliming and bating operations, and thus many non-ammonia deliming technologies have been developed.[9,10] Beyond that, ammonia also occurs in other beamhouse operations. There are various microorganisms living in raw skins. They can destroy skin proteins under appropriate environment during preservation and soaking.[11,12] Unhairing/liming is the process in which hair, epidermis and corium are vigorously attacked by strong alkali like lime and sodium sulfide. Decomposition of skin proteins in these operations might also be the origin of NH_3-N,[13] although the detailed research has not been carried out yet. In this study, the distribution of NH_3-N in beamhouse processes of pig skin and cattle hide was built up, and the origin of NH_3-N in each operation was explored to find out some possible solutions for NH_3-N reduction from leather processing. Meanwhile, the total nitrogen (TN) in wastewater of each operation was detected since it is closely associated with NH_3-N treatment efficiency of tannery effluent.

Experimental

Materials

Wet salted pig skins and cattle hides were used as raw materials. The chemicals used for leather processing were of commercial grade. The chemicals used for the analysis of wastewaters were of analytical grade.

Determination of NH_3-N in beamhouse wastewaters

Five wet salted pig skins were treated according to conventional beamhouse processes as shown in Table I. At the end of each operation, wastewater sample was collected from the bath, as noted in the recipe (Table I). Then NH_3-N concentrations of the samples were determined as soon as possible. In the same way, five wet salted cattle hides were processed using the recipe given in Table II, and the concentrations of NH_3-N in wastewaters were measured.

Determination of NH_3-N content in leather chemicals

1 g of leather chemical used in the processes, such as bactericide, degreasing agent, liming auxiliary, trypsin and alkaline protease, was dissolved in 1 L ammonia-free water. Then NH_3-N concentration of the solution was determined.

The NH_3-N content of a chemical was calculated as:

$$NH_3\text{-N content} = \frac{NH_3\text{-N concentration of leather chemical}}{\text{concentration of leather chemical}}$$

Effect of curing time on NH_3-N concentration in washing wastewater

Five fresh pig skins were fleshed and painted by 40% salt (based on fleshed weight) on the flesh for curing. On the 10th, 30th, 90th and 180th days of the curing, one of the wet salted skins was chosen and washed according to process No.1 in Table I, respectively. The washing wastewaters were collected for measurement of NH_3-N. As control experiment, one fresh skin was directly washed in the same conditions, and NH_3-N concentration in washing wastewater was also determined.

Effect of soaking time on NH_3-N concentration in soaking wastewater

One wet salted pig skin was washed and soaked following the processes shown in Table I except that bactericide was not added in soaking float. The soaking time prolonged for one week, and a wastewater sample was taken from the soaking float each 24 h for measurement of NH_3-N.

TABLE I
Pig Skin Beamhouse Processes

No.	Process	Chemicals	%	Time	Remarks
1	Washing	Water	300	10 min	**Sampling**
2	Soaking	Water	200		
		Degreasing agent	0.3		
		Wetting agent	0.2		
		Bactericide	0.2	60 min. overnight	Next day run 30 min. **Sampling**
3	Degreasing	Water	50		
		Na_2CO_3	2		
		Degreasing agent	0.5	60 min	**Sampling** and washing
4	Liming	Water	60		
		NaHS	1		
		Na_2S	1.5		
		Liming auxiliary	1.2		
		Lime	2	90 min	Stop for 30 min
		Water	100		
		Lime	3	40 min. 5 min/h, overnight	Next day run 30 min. **Sampling**
5	Washing	Water	200	20 min	**Sampling**
6	Predeliming	Water	100		
		NH_4Cl	1	40 min	**Sampling**
7	Washing	Water	300	20 min	**Sampling**
8	Deliming	Water	100		
		NH_4Cl	2		
		Degreasing agent	0.3	60 min	**Sampling**
9	Washing	Water	200	20 min	**Sampling**
10	Bating	Water	100		
		Trypsin	0.2		
		NH_4Cl	0.2	60 min	**Sampling** and washing
11	Pickling	Water	50		
		NaCl	8	30 min	
		Formic acid	1	2×15 min	
		Sulfuric acid	2	3×15 min +1.5 h	**Sampling**

TABLE II
Cattle Hide Beamhouse Processes

No.	Process	Chemicals	%	Time	Remarks
1	Washing	Water	500	30 min	**Sampling**
2	Presoaking	Water	250		
		Degreasing agent	0.1		
		Wetting agent	0.1		
		Na_2CO_3	0.4		
		$Ca(ClO)_2$	0.2		
		NaCl	5	30 min., 5 min/h, overnight	Next day run 30 min. **Sampling**
3	Soaking	Water	250		
		Degreasing agent	0.2		
		Wetting agent	0.2		
		Na_2CO_3	0.3		
		$Ca(ClO)_2$	0.2		
		NaCl	1	30 min., 5 min/h, overnight	Next day run 30 min. **Sampling** and washing
4	Liming	Water	100		
		Liming auxiliary	0.8	20 min	Stop for 20 min
		NaHS	1	20 min	Stop for 40 min
		Na_2S	1	20 min	Stop for 40 min
		Lime	1	20 min	Stop for 20 min
		Na_2S	0.5		
		Lime	0.5	20 min	Stop for 40 min
		Na_2S	0.5		
		Lime	0.5		
		Wetting agent	0.5	20 min	Stop for 40 min
		Water	300		
		Lime	1.5	30 min., 5min/h, overnight	Next day run 30 min. **Sampling**
5	Washing	Water	300	20 min	**Sampling**
6	Predeliming	Water	300		
		$(NH_4)_2SO_4$	1		
		Degreasing agent	0.1	30 min	**Sampling**
7	Washing	Water	200	20 min	**Sampling**
8	Deliming	Water	200		
		$(NH_4)_2SO_4$	2.5		
		Degreasing agent	0.1	2 h	**Sampling**
9	Bating	Deliming bath	100		
		Trypsin	0.3	60 min	**Sampling** and washing
10	Pickling	Water	50		
		NaCl	6	10 min	
		Formic acid	0.6	2×15 min	
		Sulfuric acid	0.9	3×15 min + 2 h	**Sampling**

Analysis of origin of NH_3-N in unhairing/liming wastewater

Two wet salted pig skins were washed, soaked and degreased following the processes 1-3 in Table I. Then one piece was cut along the backbone in half. The hairs on one half (named sample A) were completely shaved by a razor, whereas the hairs on the other half (named sample B) remained. These two halves were separately limed using hair burning method (process No.4 in Table I), and the wastewaters were sampled for NH_3-N determination. Enzymatic unhairing (hair-saving unhairing) was performed on another piece of skin (named sample C) using 0.22% alkaline protease (an endo-protease by a genetically modified strain of *Bacillus alcalophilus*, activity 246,000 units at 25°C and pH 10), 1% sodium carbonate, 0.1% calcium chloride, 0.5% wetting agent and 20% water (based on weight of raw skin) at 25°C and pH 10. The duration was 30 min running per hour for three times and left overnight. Next day the wastewater was sampled for NH_3-N determination after running for 30 min.

Effect of reliming time on NH_3-N concentration in reliming wastewater

One limed pig pelt prepared according to processes 1-5 in Table I was relimed using 1% liming auxiliary, 3% lime and 300% water (based on limed weight). The duration of the treatment was firstly running for 60 min, then running 5 min per hour for 6 h and left overnight. Next day the reliming float was sampled after running for 30 min. The pelt was treated in the same bath for one week and a sample was taken from the reliming bath each 24 h for NH_3-N determination.

Determination of TN and hydroxyproline in beamhouse wastewaters

Five wet salted cattle hides were processed according to the conventional beamhouse processes as shown in Table II. After washing, presoaking, soaking, liming, predeliming, deliming, bating and pickling operations, volumes of wastewaters were measured and the wastewater samples were collected for determination of TN, NH_3-N and hydroxyproline (Hyp).

Analysis of origin of TN in unhairing/liming wastewater

Two wet salted cattle hides were washed and soaked following the recipe shown in Table II. Then one of the hides was treated by conventional liming process (Table II). The other piece was processed in two steps. Firstly the hide was unhaired using 0.48% alkaline protease (activity 145,000 units at 25°C and pH 7.2) and 50% water at 25°C and pH 7.2 for 2 h. Afterward, liming was carried out using 6% lime and 300% water and the duration was 5 min running per hour for 6 h and left overnight. The TN concentration of wastewater was measured at the end of unhairing and liming, respectively.

Analysis of origin of TN in deliming wastewater

One limed cattle pelt processed according to processes 1-5 in Table II was cut along the backbone in half for deliming trial. The left side was delimed by 3.5% ammonium sulfate, and the right one was treated by 2% non-ammonia deliming agent. The other operating conditions were identical. When deliming completed, wastewaters were sampled for TN determination.

Analytical methods

Ammonia nitrogen

NH_3-N concentration of wastewaters was determined by distillation and titration method.[14]

Total nitrogen

Wastewater samples were centrifuged at 5000 RPM for 10 min. Then the supernatant liquors were taken and TN concentrations were measured using TOC/TN analyzer (LiquiTOC, Elementar, Germany).

Hydroxyproline

Wastewater samples were filtered by using 100 mesh filter cloth, and their Hyp concentrations were determined following standard procedures.[15]

RESULTS AND DISCUSSION

Distribution of NH_3-N in wastewaters of pig skin beamhouse processes

Figure 1 is the distribution of NH_3-N in wastewaters of pig skin beamhouse processes. NH_3-N concentrations in predeliming (#6), deliming (#8) and bating (#10) wastewaters are found to be the highest among all the processes. Ammonium chloride was used in these three operations, and the NH_3-N concentration in wastewater is in proportion to the dosage of ammonium chloride. Therefore, NH_3-N in deliming and bating wastewaters is mainly originated from the addition of ammonium salts. After predeliming and deliming, a considerable amount of NH_3-N was further washed out from pelt in the following washing operations (#7 and #9). Material balance was performed on deliming-bating section (#6 to #10). As the total amount of ammonium chloride added was 3.2% (based on weight of raw skin), NH_3-N input was 8.4 g/kg of raw skin. However, NH_3-N output was 6.8 g/kg of raw skin calculated by NH_3-N concentrations and volumes of wastewaters. This means some ammonium was left in the pelts and released in the following processes, or transformed into ammonia gas.

A little NH_3-N was generated in the initial washing operation (#1), which indicates that wet salted pig skin contains a small quantity of ammonia nitrogen. NH_3-N concentration in soaking (#2) wastewater is 34 mg/L. It was found that the bactericide used in soaking has a NH_3-N content of 14.5 mg/g as shown in Table III. Thus, the bactericide is one of the origins of NH_3-N in soaking wastewater. But in consideration of the small dosage of bactericide, there should be some other origins of NH_3-N. Similarly, NH_3-N in liming (#4) wastewater cannot be ignored, and calculation indicates that liming auxiliary contributes only a part of it (Table III). As we know, skin proteins can be decomposed in some beamhouse treatments such as liming and bating where ammonia nitrogen probably forms. To confirm this hypothesis, more investigations will be carried out in the following sections.

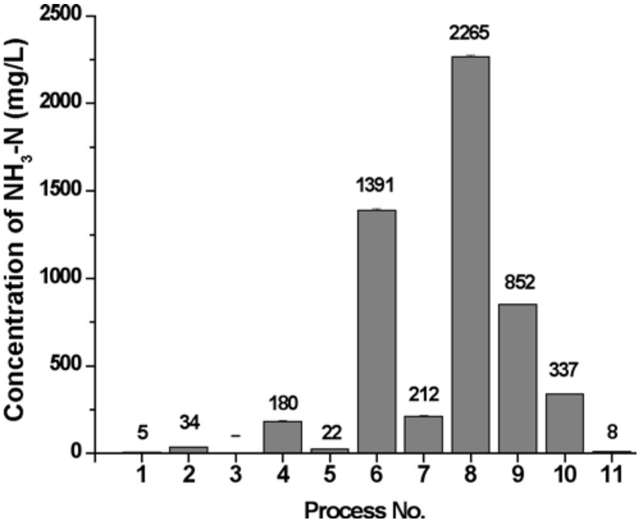

Figure 1. Distribution of NH_3-N in wastewaters of pig skin beamhouse operations

Distribution of NH_3-N in wastewaters of cattle hide beamhouse processes

Figure 2 is the distribution of NH_3-N in wastewaters from cattle hide beamhouse processes. Predeliming (#6), deliming (#8) and bating (#9) operations produced a great quantity of ammonia, which is similar to the distribution in Figure 1. Therefore, ammonium salt is the main origin of NH_3-N in tannery wastewater. Deliming (#8) and bating (#9) were performed in the same bath, and it was found that the NH_3-N concentration in the float after bating is nearly the same as in deliming float. This fact implies that trypsin catalysis on skin protein does not generate ammonia nitrogen during bating. Material balance on deliming-bating section (#6 to #9) indicates that NH_3-N input was 7.4 g/kg of raw skin, but output was only 5.5 g/kg of raw skin. Moreover, NH_3-N concentration in pickling (#10) wastewater is much higher than that in pig skin processing. These results can be explained by the difference of thickness between cattle hide and pig skin. The delimed cattle hide (thickness 2.5 mm) was observed to be much thicker than pig skin (thickness 1.4 mm). Although full washing was applied after deliming in both processing, the residual ammonium in cattle hide was probably more than in pig skin, thus resulting in a slower release of ammonium and higher NH_3-N content in pickling wastewater. Similar to pig skin processing, the initial washing (#1) and soaking (#2) of cattle hide also generated a small amount of NH_3-N. However, NH_3-N concentration of liming (#4) wastewater is 47 mg/L, which is much lower than that in pig skin processing. This may result from the milder unhairing system used in cattle hide processing (see Table I and Table II) that retards the transformation of proteins into NH_3-N.

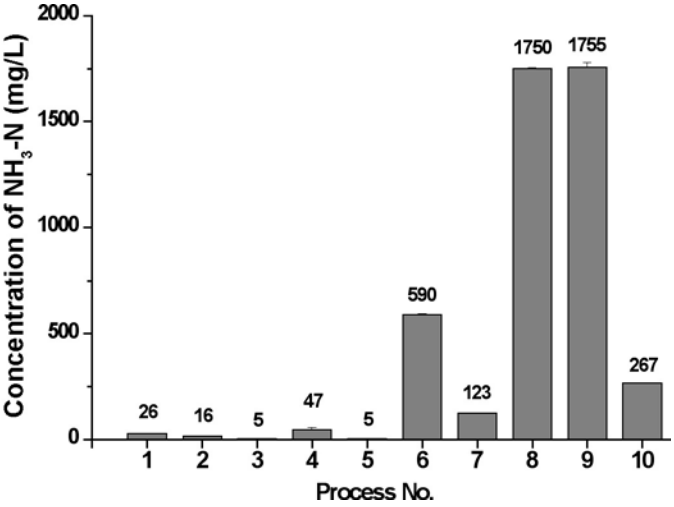

Figure 2. Distribution of NH_3-N in wastewaters of cattle hide beamhouse operations

TABLE III
NH_3-N Content in Leather Chemicals

Chemical	Process using the chemical	NH_3-N content (mg/g)
Bactericide	Soaking	14.5
Degreasing agent	Soaking, degreasing, deliming	—
Liming auxiliary	Liming	6.7
Trypsin	Bating	—
Alkaline protease	Enzymatic unhairing	—

—: No NH_3-N was detected

Origin of NH_3-N in initial washing and soaking wastewaters

Effect of curing time on the concentration of NH_3-N in initial washing wastewater is shown in Figure 3. It is found that curing time has little influence on NH_3-N concentration in washing wastewater for the skins in good preservation state, and the NH_3-N concentrations are all lower than 10 mg/L. In contrast, some raw skins began to decay due to poor preservation, e.g. curing without plenty of salt, or storing in a damp condition. During washing, NH_3-N released from the rotten skins was rather more than those in fair preservation. These facts suggest that raw skin contains a small amount of NH_3-N, and appropriate preservation can keep the NH_3-N content in a rather low level. However, bad preservation could leads to invasion of microorganisms, then decomposition of skin proteins and rapid growth of NH_3-N production. This process is called ammonification in microbiology.[16]

Ammonification also occurs in the soaking experiment. Figure 4 shows that NH_3-N concentration in soaking bath increased remarkably day by day. In addition, it was observed that the skins were gradually slippery and the bath became yellow and smelly during soaking. Bactericide was not involved in soaking, and water was not changed for seven days. Hence microorganisms reproduced rapidly in the soaking bath, and decomposed nitrogenous organics into ammonia. These results indicate that the effective inhibition of growth of microorganisms in soaking bath is important to reduce NH_3-N production.

Origin of NH_3-N in unhairing/liming wastewater

Hair-burning and enzymatic unhairing systems were used for pig skin processing in order to clarify the origin of NH_3-N in unhairing/liming process. Table IV shows that the hairy half of the pig skin (sample B) produced much more NH_3-N than the hairless half (sample A) under the same hair-buring unhairing conditions. This means hair-burning system can strongly destroy hairs and decompose keratin into ammonia. The NH_3-N derived from hairs contributes approximately 40% of NH_3-N in unhairing wastewater. The other origins of NH_3-N include use of liming auxiliary and decomposition of collagen and other skin proteins. At the same time, the concentration of NH_3-N in the wastewater of enzymatic

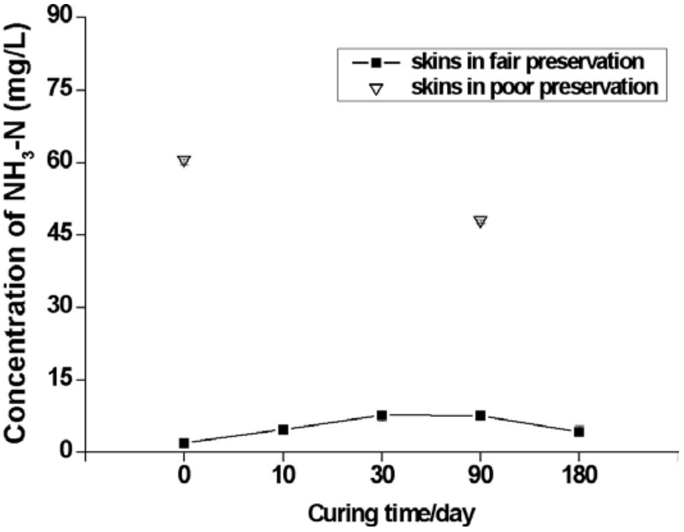

Figure 3. Effect of curing time on the concentration of NH_3-N in washing wastewater

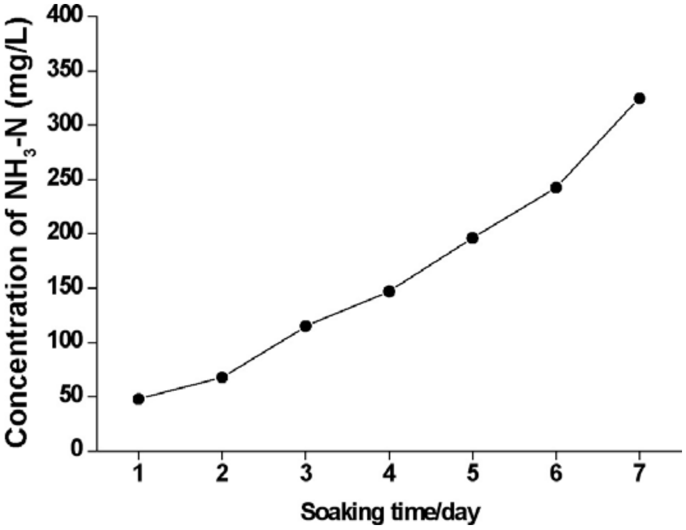

Figure 4. Effect of soaking time on the concentration of NH_3-N in soaking wastewater

TABLE IV
NH_3-N Concentration in Wastewaters from Different Unhairing Systems

Sample	Unhairing system	Concentration of NH_3-N (mg/L)
A (hairless)	Hair-burning	101
B (hairy)	Hair-burning	179
C	Enzymatic unhairing	11

unhairing (hair-saving unhairing) decreases to 11 mg/L (Table IV). This fact demonstrates that hair-saving system can significantly reduce NH_3-N discharge in unhairing/liming process.

Origin of NH_3-N in reliming wastewater

Figure 5 gives the relationship between reliming time and NH_3-N concentration in the reliming wastewater of pig skin. As reliming time extended, NH_3-N concentration underwent a slight increase at first, then remained stable. This small amount of NH_3-N should be produced by hydrolysis of collagen in the presence of lime. The reaction sites locate on the side-chain amido group of asparagine and glutamine and the guanidine group of arginine.[17]

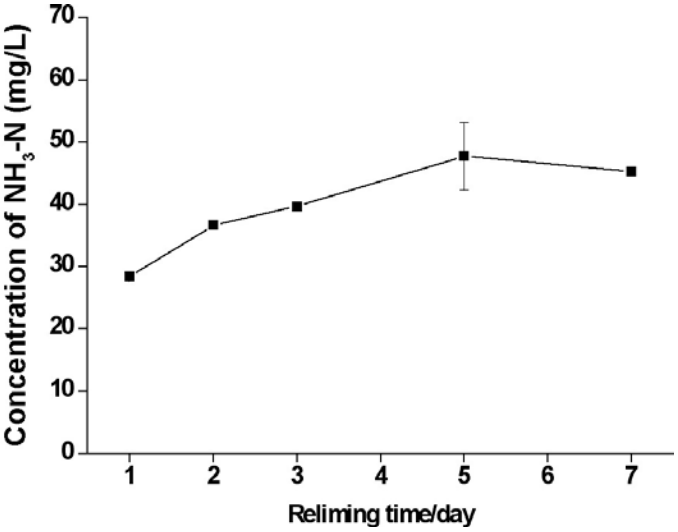

Figure 5. Effect of reliming time on the concentration of NH_3-N in reliming wastewater

Relationship between TN and NH_3-N in beamhouse wastewaters

The previous discussions suggest that the majority of NH_3-N in beamhouse wastewaters is attributed to the extrinsic ammonium salt, and a small part of NH_3-N arises from hydrolysis of proteins. However, Table V shows that TN loads in washing, soaking and liming wastewaters are in a high level, though NH_3-N only contributes a small proportion to TN. Nitrate and nitrite nitrogen are negligible due to their extremely low contents in fresh sample of wastewater. Hence TN is approximately the sum of ammonia nitrogen and organic nitrogen in this case. As we know, the non-collagen proteins in skin are removed during beamhouse processes. Dirt, blood and dung are cleaned out in initial washing and soaking processes. Some of the soluble proteins are removed from raw skins at the same time. The subsequent liming removes hair and epidermis and demolishes residual unstructured proteins to open up collagen fibre, accompanied by a strong hydrolysis of proteins. Therefore, the organic nitrogen compounds will considerably increase in wastewater, leading to a great rise of TN load.

Enzymatic unhairing/liming has been proved to be an effective approach to decrease NH_3-N production in the previous section, but it does not work effectively in terms of TN reduction (Figure 6 (a)). This can be explained by the introduction of enzyme and its catalysis on skin proteins. The hydrolysates of skin proteins like peptide and amino acid as well as enzyme itself (also a kind of protein) constitute most of the TN contamination. NH_3-N is undoubtedly the predominant component of TN in conventional deliming wastewater. However, TN concentration of deliming wastewater reduces by 78% when non-ammonia deliming technique is applied as an alternative (Figure 6 (b)). Therefore, both the NH_3-N and TN can be remarkably reduced at the origin of leather processing in this case.

TABLE V
Characterization of Wastewaters of Cattle Hide Beamhouse Process

Process	Volume of effluent (L/kg of raw skin)	Concentration (mg/L)		Emission load (mg/kg of raw skin)	
		TN	NH_3-N	TN	NH_3-N
Washing	4.6	398	77	1831	354
Presoaking	2.3	555	118	1277	271
Soaking	2.4	358	15	859	36
Liming	3.2	662	48	2118	154
Predeliming	2.2	1117	900	2457	1980
Deliming	1.0	2201	1584	2201	1584
Bating	1.1	2377	1590	2615	1749
Pickling	0.7	533	275	373	192

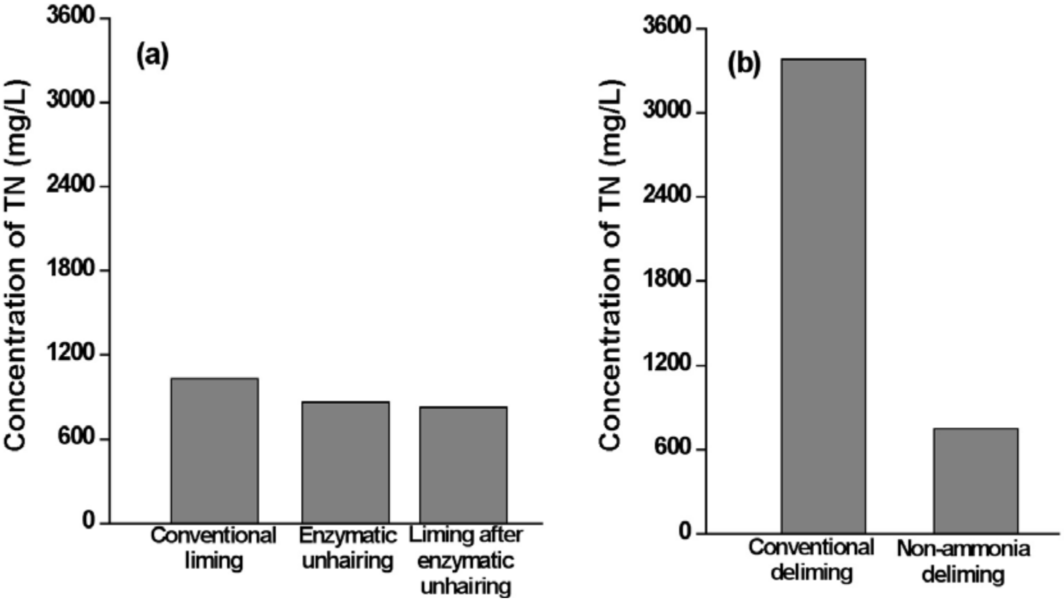

Figure 6. TN concentration in wastewaters from unhairing/liming (a) and deliming (b) operations.

TABLE VI
Hyp Content in Wastewaters from Beamhouse Processes

Process	**Hyp content (mg/kg of raw skin)**	**Nitrogen from collagen* (%)**
Washing	12.7	0.96
Presoaking	11.5	1.22
Soaking	8.2	1.29
Liming	49.8	3.23
Predeliming	5.2	0.29
Deliming	5.3	0.33
Bating	18.0	0.98
Pickling	3.7	1.26
Total	114.4	1.15

* Nitrogen from collagen is calculated as:

$$\text{Nitrogen from collagen}=\frac{\text{Content of Hyp}/12.9\%\times 17.8\%}{\text{TN}}\times 100\%$$

where 12.9% means the Hyp content in collagen of cattle hide, and 17.8% means the nitrogen content in collagen.[18]

Hyp contents in various beamhouse wastewaters can be employed to evaluate the damage of collagen during leather processing. Table VI indicates that nitrogen from collagen constitutes a tiny proportion of TN load even if the skins were treated with strong alkali, enzyme or strong acid in liming, bating or pickling. This means collagen basically remained intact throughout the beamhouse processes, and the organic nitrogen included in TN mainly results from non-collagen proteins that are unavoidable to be removed in leather processing.

Conclusions

We have investigated the origins of NH_3-N in wastewaters from beamhouse processes so that the strategy of reducing NH_3-N impact in leather production can be comprehensively considered. As expected, the conventional deliming and bating operations are mainly responsible for high NH_3-N impact in tannery wastewater due to the addition of ammonium salts. So non-ammonia deliming/bating is an efficient way to eliminate NH_3-N at the origin of leather processing. Moreover, our research indicates that a considerable amount of NH_3-N may be introduced into wastewaters by leather chemicals, and therefore, the determination of NH_3-N content in the unclear chemicals should be necessary before use. The ammonification of microorganisms on skin proteins also generate a large quantity of NH_3-N during preservation and soaking. But production of NH_3-N due to the ammonification can be controlled to an extremely low level by appropriate antiseptic treatment. It is commonly considered/speculated that the hydrolysis of keratin and collagen in soaking and unhairing/liming is one of primary origins of NH_3-N. But the determinations in this research indicate that only a small amount of NH_3-N results from hydrolysis of keratin and collagen in these processes. However, TN generated from these operations is in a high level, because a massive amount of organic nitrogenous compounds, particularly non-collagen proteins, are derived from hydrolysis of proteins. The high TN content in wastewater increases the difficulty in removal of NH_3-N since organic nitrogen would be converted into NH_3-N in the end-of-pipe treatment. Perhaps recovery of proteins from tannery wastewater is an available option to reduce TN pollution and lighten the burden of wastewater treatment.

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References

1. Russell, S.; Ammonia: WRc Instrument Handbooks, WRc, Swindon, UK, 1994.
2. Wiesmann, U.; Biological nitrogen removal from wastewater. *Adv. Biochem. Eng. Biotechnol.* **51**, 113-154, 1994.
3. Smith, V. H., Tilman, G. D., and Nekola, J. C.; Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **100**, 179-196, 1999.
4. U.S. Environmental Protection Agency; Quality Criteria for Water. EPA Publication 440/5-86-001, U.S. Gov. Prin. Office, Washington DC, 1987.
5. Feng, J. W., Sun, Y. B., Zheng, Z., *et al.*; Treatment of tannery wastewater by electrocoagulation. *J. Environ. Sci.* **19**, 1409-1415, 2007.
6. Ganesh, R., Balaji, G., and Ramanujam, R. A.; Biodegradation of tannery wastewater using sequencing batch reactor–Respirometric assessment. *Bioresour. Technol.* **97**, 1815-1821, 2006.
7. Tadesse, I., Isoaho, S. A., Green, F. B., *et al.*; Removal of organics and nutrients from tannery effluent by advanced integrated wastewater pond systems (R) technology. *Water Sci. Technol.* **48**, 307-314, 2003.
8. Ding, S. L., Li, L., and Zhao, M. J.; Study on the characteristics of nitrogen transformation in effluents from cattlehide leather-making process. XXX IULTCS Congress, Beijing, 2009.
9. Kolomaznik, K., Blaha, A., Dedrle, T., *et al.*; Non-ammonia deliming of cattle hides with magnesium lactate. *JALCA* **91**, 18-20, 1996.
10. Klaasse, M. J.; CO_2 deliming. *JALCA* **85**, 431-441, 1990.
11. Shede, P. N., Kanekar, P. P., Polkade, A. V., *et al.*; Bacterial succession on raw buffalo hide and their degradative activities during ambient storage. *Int. Biodeterior. Biodegrad.* **62**, 65-74, 2008.
12. Colak, S. M.; Soaking with tannins: the biocidal activity of vegetable tannins used in the soaking float. *J. Soc. Leather Technol. Chem.* **90**, 193-196, 2006.
13. Merrill, H. B.; Action of ammonia on calfskin. *Ind. Eng. Chem.* **19**, 386-388, 1927.
14. Clescerl, L. S., Greenberg, A. E., and Eaton, A. D.; Standard methods for the examination of water and wastewater 20th edition, American Public Health Association, Washington DC, 1998.

15. Reddy, G. K., and Enwemeka, C. S.; A simplified method for the analysis of hydroxyproline in biological tissues. *Clin. Biochem.* **29**, 225-229, 1996.
16. Ingraham, J. L., and Ingraham, C. A.; Introduction to microbiology (second edition), Science Press, Beijing, China, 2003.
17. Menderes, O., Covington, A. D., Waite, E. R., *et al.*; The mechanism and effects of collagen amide group hydrolysis during liming. *J. Soc. Leather Technol. Chem.* **83**, 107-110, 1999.
18. Gustavson, K. H.; The chemistry and reactivity of collagen, Academic Press Inc, New York, 1956.