# Recovery of proteins from wastewater of tannery beamhouse operations: influence on the main pollution parameters

A. Marsal, E. Hernández, S. Cuadros, R. Puig, E. Bautista and J. Font

## ABSTRACT

The recovery of proteins from effluents of beamhouse operations in a hair-pulping process of hides is proposed. Precipitation with sulphuric acid at the isoelectric pH was chosen for protein recovery. The precipitates were characterized in order to study their potential uses. Apart from the protein nitrogen, the precipitate also contained a considerable content of fats resulting from the co-precipitation of natural fat of the hide in the wastewaters. The precipitation of the protein fraction resulted in a reduction of 80–85% of COD, whereas the protein content decreased 68–78%. This diminution of the contamination load led to a notable reduction of the tax on wastewater. The content of protein material in the precipitate varied from 15 to 44%.

As expected, the protein in the precipitate did not result from the collagen decomposition of the hide but from soluble proteins such as albumin and globulin and remains of keratin.

The precipitates obtained met the maximum limits of heavy metals according to legislation on organic fertilizers. The potential uses of the protein fraction recovered from tannery wastewaters are currently being investigated.

Key words | beamhouse operations, contamination diminution, protein recovery, tannery

A. Marsal (corresponding author)
E. Hernández
S. Cuadros
E. Bautista
Department of Chemical and Surfactants
    Technology,
Institute of Advanced Chemistry of Catalonia
    (IQAC), Consejo Superior Investigaciones
    Científicas (CSIC),
Jordi Girona, 18-26,
08034 Barcelona,
Spain
E-mail: ammeco@cid.csic.es

R. Puig
J. Font
Leather Technology School,
College of Industrial Engineering of Igualada
    (EUETII),
Universitat Politècnica de Catalunya (UPC),
Plaça del Rei, 15,
08700 Igualada,
Spain
E-mail: jfont@euetii.upc.edu

## INTRODUCTION

Most of the contamination arising from the process of transforming raw material into leather is due to the beamhouse operation: 83% of $BOD_5$, 73% of COD and 76% of toxicity (Aloy 1979). Much of the contamination produced in the beamhouse operations results from the solubilization and/or dispersion of different components of hides that end up in wastewaters (Portavella 1995). Of these components, organic nitrogen from solubilized proteins accounts for a significant part of the organic load in wastewater. The elimination of solubilized proteins from wastewaters would considerably reduce the organic nitrogen content with a subsequent diminution of contamination.

The aim of the present work is to develop and implement an economical technique that is easy to apply in tanneries and that allows the recovery of solubilized proteins from the effluents of the beamhouse operation for hides. An effective recovery of proteins in the tannery would not only benefit the environment (less contamination) but also the tanners since two parameters of their wastewaters, COD and total nitrogen, would be markedly reduced resulting in a lower environmental tax.

The application of techniques with membranes (ultrafiltration) is one of the most usual methods for protein separation (Ghosh 2003; Feins & Sirkar 2004). However, ultrafiltration suffers from several problems such as membrane scaling and fouling, weakening of membrane structure (probably due to hydrolysis) etc. (Salmerón & Salmerón 2005) that shorten the time of the real life of the membranes. All this does not justify the adoption of ultrafiltration by the leather industry in very polluted and concentrated beamhouse wastewater. Nevertheless,

membranes have been successfully used as a tertiary treatment for reclaiming tannery waters after depuration in a biological treatment plant (Roig *et al.* 2009).

Likewise, the separation of proteins by precipitation has been widely used in the last decades. Different alternatives exist for the precipitation of proteins: the addition of i) miscible solvents (Rothstein 1993; Alzweiri *et al.* 2008), ii) metallic cations (Simpson 2006), iii) voluminous anions, and iv) polyelectrolytes (Dissing & Mattiasson 1996; Kumar *et al.* 2003), the precipitation by salting out (Shih *et al.* 1992; Leonil *et al.* 1994; Christensen & Anton 2009) and the precipitation at isoelectric pH (Hofland *et al.* 2000). However, in order to develop and implement one of these alternatives in tanneries, aspects such as technical and economic viability as well as the impact on the quality of the wastewater must be considered.

The elimination/recovery of proteins from the wastewater of tanneries has been investigated by several authors. Sekaran *et al.* (1997) studied the removal of dissolved protein from soaking wastewater by adsorption with activated carbon. The main objective of the work of Vitolo *et al.* (1999) was the recovery of sodium chloride after the removal of proteins from the wastewater of several operations of the beamhouse process by acid precipitation and subsequent coagulation and filtration. Tünay *et al.* (2001) and Kabdasli *et al.* (2003) studied the recovery of solubilized proteins in different tannery wastewaters by acidification, metal salt addition ($Cu^{+2}$ and $Zn^{+2}$ salts), polyelectrolyte addition and coagulation/flocculation with $AlCl_3$ and $FeCl_3 \cdot 6H_2O$. According to these studies, acidification of the samples proved to be a satisfactory method to eliminate organic nitrogen from effluents. However, these studies focused neither on the influence of the elimination/recovery of proteins on the pollution/ toxicity values of the resulting wastewaters nor on the characterization of the precipitated protein fraction to evaluate its potential uses. Of these alternatives, Marsal *et al.* (2009) selected the precipitation of solubilized proteins at the isoelectric pH because of its technical and economic viability for the recovery of organic nitrogen from wastewaters of beamhouse operations for hides in a hair recovery process. The results obtained revealed that the prospects of the reduction of the contaminant load and the production of a valuable material were higher for the wastewaters of soaking, unhairing-liming, washing and conditioning.

This work focuses on the recovery of protein from the effluents of a hair-pulping beamhouse process for hides by precipitation with sulphuric acid at the isoelectric pH. The diminution of the pollution as a result of protein precipitation was also considered in addition to the characterization of the recovered protein fraction in order to study its potential uses. The study was carried out with wastewaters that offered higher prospects of reducing contamination and of obtaining a valuable material.

It should be noted that the present work refers to the protein precipitation for a hair-pulping beamhouse process, which differs from that previously reported (Marsal *et al.* 2009), where a hair recovery beamhouse process (with a prior hair immunization operation) was considered. This will enable us to determine the influence exerted by the immunization operation on the composition of the solubilized proteins.

## EXPERIMENTAL

### Materials and methods

In order to achieve the aforementioned objectives, the study was divided into three sections:

1. Formation and characterization of groups of wastewaters of beamhouse operations
2. Determination of the isoelectric point
3. Precipitation and characterization of the protein fraction

The influence of protein precipitation on the diminution of the pollution load and on the tax on wastewater is also presented.

### Characterization of wastewaters

*Formation of groups*. Samples from wastewater were provided by the Leather Technology School from Igualada (Catalonia, Spain). The wastewater generated from the following operations of a hair-pulping beamhouse process: soaking, unhairing-liming, washing and conditioning were used in the present work. Wastewater of other beamhouse

operations (deliming, bating, washing and pickling) was not taken since results of a previous work (Marsal *et al.* 2009) revealed that protein recovery from these latter operations was much lower.

For a more effective protein recovery, two groups of wastewater were formed taking proportional volumes of the total volume of each individual wastewater: Group 1: soaking; Group 2: unhairing-liming, washing and conditioning.

These two groups of wastewater were characterized by determining the following parameters: pH, chemical oxygen demand (COD), total Kjeldhal nitrogen, ammonium nitrogen, total soluble nitrogen (total Kjeldhal nitrogen of filtered bath), total solids and conductivity following the *Standard Methods* (1998). The following parameters were calculated from the results obtained: particulate nitrogen (total nitrogen – soluble nitrogen), organic nitrogen (total nitrogen – ammonium nitrogen) and dissolved protein (organic nitrogen $\times$ 5.62).

Toxicity was measured by means of the *Photobacterium phosphoreum* luminescence reduction test (Microtox Test) (DIN 1997).

## Determination of the isoelectric point

The value of pH of wastewaters was adjusted by a series of sodium acetate/acetic acid buffers that had a pH range from 2.8 to 8.0 to determine the isoelectric point (Miller 2001). The isoelectric point corresponds to the minimum of the graph protein concentration vs. pH. The protein concentration was determined in the supernatant resulting from the addition of the buffer and subsequent separation of the precipitated protein by centrifugation. The method used in the determination of protein concentration was the spectrophotometric method of bicinchoninic acid (Smith *et al.* 1985). This method is based on the reduction of $Cu^{+2}$ to $Cu^{+1}$ by protein and subsequent chelation of two molecules of bicinchoninic acid with one cuprous ion. The complex formed exhibits a strong absorbance at 562 nm, which is nearly linear with increasing protein concentration.

## Precipitation

*Characterization of the precipitate.* Precipitation of the protein fraction was performed by adding 2 M Sulphuric acid solution up to the isoelectric point. Once precipitated, the protein fraction was filtered off and the supernatants were characterized by determining the same parameters as for the original wastewaters.

The precipitates were characterized by determining the quantitative composition of the major components: *humidity* was determined by the weight loss after drying the sample at 102°C for 12 hours; *fats* were assessed by extracting the sample with dichloromethane for five hours and *ash* was determined by placing the sample in a muffle at 550°C for 2 hours. Humidity, fats and ash were determined by adapting the SLTC official methods of analysis (1996); *total nitrogen* was determined by the Kjeldhal method (*Standard Methods* 1998); HPLC was used for the evaluation of *free amino acids* by the AccQTAG method (Waters 2001). The AccQTag method is based on the pre-column derivatization with 6-aminoquinolyl-N-hydroxisuccinimidyl carbamate reactive (ACQ), which forms stable derivatives with primary and secondary amino acids. The derivatives are easily separated by reversed phase HPLC. The content of heavy metals, calcium, magnesium, sodium and potassium was determined by atomic emission spectrophotometry by Inductively Coupled Plasma (ICP-OES) after acid digestion according to EN 13650:2001 Standard. The composition of total amino acids was evaluated following the AccQTAG method after acid hydrolysis of the protein fraction with HCl 6N.

It should be noted that at industrial scale, the resulting acidic wastewaters obtained after protein precipitation must be mixed with those of subsequent industrial processes and must be neutralized after homogenization if the resulting pH is below 6. Thereafter, the neutralized wastewater is sent to the Biological Wastewater Treatment Plant.

## RESULTS AND DISCUSSION

Tables 1, 2, 5, 6 and 7 show the experimental results expressed as the mean values of three replicates ± standard deviation.

### Diminution of the contamination load

Tables 1 and 2 show the influence of acid precipitation on the characteristics of wastewaters of Group 1 and 2,

**Table 1** | Influence of acid precipitation on the characteristics of wastewaters of Group 1. Variation of the most significant parameters applicable to wastewater tax

| Measured parameters | Group 1 | | Variation (%) |
|---|---|---|---|
| | **Before acid precipitation** | **After acid precipitation** | |
| pH | 6.4 ± 0.2 | 3.4 ± 0.2 | |
| **COD (mg O$_2$/L)** | **13,700 ± 300** | **2,850 ± 50** | ↓ 79 |
| **Total N (mg/L)** | **268 ± 7** | **145 ± 3** | ↓ 46 |
| Ammonium N (mg/L) | 96 ± 1 | 90 ± 1 | ↓ 7 |
| Soluble N (mg/L) | 187 ± 3 | 145 ± 3 | ↓ 23 |
| Total solids (%) | 3.36 ± 0.01 | NA | |
| **Conductivity (mS/cm)** | **46.55 ± 2.35** | **48.35 ± 1.15** | ↑ 4 |
| **Toxicity (equitox/m$^3$)*** | **ND** | **ND** | = |
| *Calculated parameters* | | | |
| Particulate N (mg/L) | 81.7 | NA | |
| Organic N (mg/L) | 172 | 55.1 | ↓ 68 |
| Protein (mg/L) | 968 | 309 | ↓ 68 |

*Equitox/m$^3$: 100/EC50 (EC50 expressed in %).
ND: not detected; NA: not analysed. The most significant parameters applicable to water tax for wastewaters are in bold.

respectively. The characterization of the untreated waste-waters (before acid precipitation) shows a protein content, which is higher for Group 2 (Table 2) than for Group 1 (Table 1). The wastewater of Group 2 contains the residues of the dissolved hair produced by alkaline hydrolysis in the unhairing-liming operation (Merrill 1978). In the case of a beamhouse process with hair recovery (Marsal *et al.* 2009),

the immunization operation allows the hair to be physically recovered and, consequently, the value of protein content is markedly lower.

Table 2 also shows a COD value of Group 2 that considerably exceeds the value of Group 1 (Table 1). This is due to the increased protein content of Group 2. Likewise, the addition of a bactericide immediately before

**Table 2** | Influence of acid precipitation on the characteristics of wastewaters of Group 2. Variation of the most significant parameters applicable to wastewater tax

| Measured parameters | Group 2 | | Variation (%) |
|---|---|---|---|
| | **Before acid precipitation** | **After acid precipitation** | |
| pH | 12.0 ± 0.2 | 3.7 ± 0.2 | |
| **COD (mg O$_2$/L)** | **47,800 ± 850** | **7,254 ± 200** | ↓ 85 |
| **Total N (mg/L)** | **3,745 ± 176** | **930 ± 14** | ↓ 75 |
| Ammonium N (mg/L) | 490 ± 80 | 209 ± 3 | ↓ 57 |
| Soluble N (mg/L) | 2,970 ± 150 | 930 ± 14 | ↓ 69 |
| Total solids (%) | 5.3 ± 0.6 | NA | |
| **Conductivity (mS/cm)** | **31.40 ± 0.20** | **30.50 ± 0.21** | ↓ 3 |
| **Toxicity (equitox/m$^3$)*** | **5,000** | **67** | ↓ 99 |
| *Calculated parameters* | | | |
| Particulate N (mg/L) | 774 | NA | |
| Organic N (mg/L) | 3259 | 721 | ↓ 78 |
| Protein (mg/L) | 18,316 | 4,052 | ↓ 78 |

*Equitox/m$^3$: 100/EC50 (EC50 expressed in %).
NA: not analysed. The most significant parameters applicable to water tax for wastewaters are in bold.

the unhairing-liming operation resulted in a high toxicity of Group 2 in contrast to the non-toxicity of Group 1.

Although no large differences were observed in a wide pH range, the values of pH 3.8 and 3.9 were obtained for the isoelectric point of Groups 1 and 2, respectively. Tables 1 and 2 give the results obtained after protein precipitation. A clear reduction in most of the parameters is observed mainly for wastewater of Group 2. The protein content shows a 68% decrease for Group 1 (Table 1) and a 78% decrease for Group 2 (Table 2). Tünay *et al.* (2001) obtained 90% protein removal by acidification at a pH value of 4.6 for sheepskin wastewater, and 50% efficiency was obtained by acidification at a pH < 5 for bovine hide wastewater. A diminution of 79% (Group 1) and 85% (Group 2) of the COD values is noted. These values are consistent with those of Vitolo *et al.* (1999), who found values of COD reduction between 60–90%. The high reduction of COD could be attributed to the fact that emulsified and suspended fats in wastewater from hide fleshings underwent co-precipitation with proteins. This could be corroborated by the characterization of the precipitate.

Another important effect of the precipitation with sulphuric acid is noted in the toxicity of the wastewater of Group 2, which shows a decrease of approximately 99% (Table 2). This important diminution in toxicity mainly results from the elimination of the bactericide, which is produced in the precipitation and subsequent filtration. However, further studies will be performed to confirm this hypothesis.

## Influence of the diminution of the contamination load on the tax on wastewater

The 3/2003 Decree of 4 November governs the water tax, an environmental levy that takes into account the volume of water used and its level of contamination. (Generalitat de Catalunya 2003).

The contribution of the level of contamination to the water tax is calculated from the cost of the contamination parameters shown in Table 3.

The water tax is calculated from:

$$\text{Water tax}(\text{€}) = \text{taxable base}(m^3) \times \text{type of obligation}(\text{€}/m^3)$$

Table 4 indicates the price to be paid per $m^3$ for Group 1 and Group 2, respectively, as a function of the effluent

**Table 3** | Cost of the contamination parameter to evaluate the water tax

| Contamination parameter | Parameter cost (2009) |
|---|---|
| Suspended matter (SM) | 0.3980 €/Kg |
| Oxidizable matter (OM) | 0.7963 €/Kg |
| Soluble salts (SS) | 6.3705 €/(S cm$^{-1}$ m$^3$) |
| Inhibiting matter (IM) | 9.4438 €/Kequitox |
| Nitrogen (N) | 0.6045 €/Kg |
| Phosphorus (P) | 1.2092 €/Kg |

The units of the "Soluble Salts" parameter are "conductivity" (S cm$^{-1}$) multiplied by "volume" of water (m$^3$).

discharged by the industry. These figures are calculated with the prices for the year 2009 (VAT excluded).

As may be observed, the precipitation of wastewaters of the first beamhouse operations before discharge would mean a saving of 75.7 €/$m^3$ as far as the contamination parameters are concerned. Taking into account that the cost of precipitation is 0.5 €/$m^3$, the cost saved on taxes would be 75.2 €/$m^3$. This saving would be 28.6 €/$m^3$ if the bactericide is not added or if the tannery discharges the beamhouse wastewater that is mixed with wastewater of subsequent processes (this would eliminate the bactericide from the wastewater). In these cases the inhibiting matter parameter is not considered.

In any case, this means a considerable saving, especially for tanneries specialized only in beamhouse operations since they do not have other types of wastewaters at acid pHs for mixing.

The effect of the reduced contamination load on tax on wastewater in Catalonia (Spain), where the work was carried out, is regarded as an example of the saving that tanners can make by simply precipitating the protein fraction from the wastewaters of the initial beamhouse operations.

## Characterization of the precipitate obtained by addition of sulphuric acid

The precipitation at the isoelectric point generated 6 and 15 g of dry precipitate per L of acidified wastewater for Groups 1 and 2, respectively. The characterization of the precipitate obtained yielded the following results:

**Table 4** │ Tax calculated for each type of discharge

| Contamination parameter | Group 1 | | | Group 2 | | |
|---|---|---|---|---|---|---|
| | Initial waste water (€/m³) | Wastewater precipitated and filtered (€/m³) | Difference (€/m³) | Initial waste water (€/m³) | Wastewater precipitated and filtered (€/m³) | Difference (€/m³) |
| Oxidizable matter | 7.3 | 1.5 | ↓5.8 | 25.4 | 3.9 | ↓21.5 |
| Soluble salts | 0.3 | 0.3 | 0.0 | 0.2 | 0.2 | 0.0 |
| Nitrogen | 0.2 | 0.1 | ↓0.1 | 2.3 | 0.6 | ↓1.7 |
| Inhibiting matter | 0.0 | 0.0 | 0.0 | 47.2 | 0.6 | ↓46.6 |
| | | Subtotal | ↓5.9 | | Subtotal | ↓69.8 |
| | | | | | Total | ↓75.7 |

## Major components

Table 5 shows the composition of the precipitate obtained by the addition of 2 M sulphuric acid solution to wastewaters of Groups 1 and 2. The remarkably low protein content (15%) and the extraordinarily high content of fats (65.4%) in the precipitate of Group 1 should be noted. This confirms the occurrence of a significant co-precipitation of fats when the precipitation of proteins is performed. This co-precipitation of fats increases COD diminution.

The AccQTag method confirmed that the majority of the amino acids in the samples are combined in the form of proteins or peptides, especially in the case of Group 1 in which only 0.20% by weight of the precipitate was constituted by free amino acids. In contrast, Group 2 showed a higher content of free amino acids (6.4%), 37% of which corresponded to cystine.

**Table 5** │ Composition of the precipitate obtained with sulphuric acid

| Measured parameters | Group 1 (%) | Group 2 (%) |
|---|---|---|
| Humidity | 4.9 ± 0.5 | 9.6 ± 0.7 |
| Total nitrogen | 2.7 ± 0.3 | 7.8 ± 0.2 |
| Ammonium nitrogen | – | – |
| Organic nitrogen | 2.7 ± 0.3 | 7.8 ± 0.2 |
| Free amino acids | 0.20 ± 0.05 | 6.4 ± 1.0 |
| Fat and other substances soluble in dichloromethane | 65.4 ± 0.5 | 19.4 ± 0.1 |
| Ash | 15.9 ± 0.3 | 28.5 ± 0.1 |
| *Calculated parameters* | | |
| Protein | 15 | 44 |

## Composition of amino acids

The analysis of the total amino acids of the precipitated protein fraction revealed the remarkably low content of hydroxyproline. This amino acid is characteristic of collagen (it may contain up to 12% w/w) (Bowes *et al.* 1955). Given that hydroxyproline is usually employed as an analytical criterion to evaluate the amount of collagen present in protein derivatives, it may be concluded that the origin of the protein present in the precipitate is not the decomposition of collagen of the hide but the soluble proteins such as albumin and globulin.

The protein fraction of Group 2 also contains remains of keratin since 6.5% of the total amino acids corresponded to cystine. This value contrasts with the lower content of cystine in Group 1 (1.8%). The higher content of cystine in Group 2 results from the alkaline hydrolysis of hair caused by sodium sulphide and calcium hydroxide used in the unhairing-liming operation (Merrill 1978).

Amino acids can directly or indirectly enhance the physiological activities of plants (Rauser 1990; Naidu *et al.* 1991). The following eighteen amino acids were detected in the precipitated protein of Group 2: arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, cystine, glycine, proline, alanine, leucine, methionine, phenylalanine, valine, isoleucine, tyrosine and hydroxyproline. Some of them are recognized to have a very positive effect on plants. (Ashmed *et al.* 1986).

## Composition of ash

The high percentage of ash should be noted mainly in Group 2 (Table 5). The content of heavy metals (listed in

**Table 6** | Content of the main alkali and alkaline earth metals in the precipitate in the form of oxide and in the form of sulphate

**Alkali and alkaline earth metals (%)**

| Group 1 | | | | Group 2 | | | |
|---|---|---|---|---|---|---|---|
| $CaO$ | $0.23 \pm 0.02$ | $CaSO_4$ | 0.56 | $CaO$ | $4.4 \pm 0.5$ | $CaSO_4$ | 10.6 |
| $MgO$ | $0.040 \pm 0.002$ | $MgSO_4$ | 0.12 | $MgO$ | $0.050 \pm 0.002$ | $MgSO_4$ | 0.15 |
| $Na_2O$ | $3.92 \pm 0.02$ | $Na_2SO_4$ | 8.98 | $Na_2O$ | $4.2 \pm 0.2$ | $Na_2SO_4$ | 9.6 |
| $K_2O$ | $0.10 \pm 0.01$ | $K_2SO_4$ | 0.19 | $K_2O$ | $0.06 \pm 0.02$ | $K_2SO_4$ | 0.11 |
| Total | ~4.30 | Total | ~9.90 | Total | ~8.70 | Total | ~20.5 |

**Table 7** | Content of heavy metals in proteins obtained expressed on dry weight

| | Heavy metal (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Cd** | **Cu** | **Ni** | **Zn** | **Cr** | **Pb** | **Hg** |
| Group 1 | ND | $7.6 \pm 0.1$ | $1.8 \pm 0.2$ | $13.0 \pm 0.3$ | $63.8 \pm 0.5$ | ND | ND |
| Group 2 | ND | $11.3 \pm 0.5$ | $9.2 \pm 0.6$ | $89.4 \pm 0.5$ | $14.7 \pm 0.3$ | ND | ND |
| Maximum limits (EC 2003/2003) | 0.7 | 70 | 25 | 200 | 70 | 45 | 0.4 |

The bottom row shows the maximum limits permitted in class A fertilizers according to EC 2003/2003 European regulation; ND: not detected.

the section below) and alkali and alkaline earth metals were determined. The results obtained for the latter components are shown in Table 6, which are also expressed in the form of sulphates.

As expected, the ash composition shows a high concentration of sodium salts in the precipitate of Group 1, which comes from the salt used in the curing process (Heidemann 1993), whereas the material obtained in Group 2 is rich in calcium salts coming from the calcium hydroxide used in the liming operation (Lollar 1978).

The content of phosphorus expressed as $P_2O_5$ was 0.2% and < 0.1% (on dry weight of the precipitate) for Group 1 and Group 2, respectively.

### Content of heavy metals

Table 7 gives the content of heavy metals in the precipitates obtained for Groups 1 and 2 by precipitation with sulphuric acid.

Should this precipitate be used as a fertilizer, this parameter assumes an especial importance given that Spanish and European legislation demands the use of materials with negligible amounts of heavy metals to avoid

their dissemination in the environment through fertilizers that originate from recovered organic materials.

Table 7 shows that the precipitates obtained meet the maximum limits of heavy metals in class A fertilizers. These limits are the most restrictive of the EC 2003/2003 European Regulation governing fertilizers (European Parliament 2003).

The detection limits of cadmium, lead and mercury in the analysis conditions are 0.6 mg/kg, 3.0 mg/kg and 0.3 mg/kg, respectively.

### CONCLUSIONS

The isoelectric precipitation of solubilized proteins in wastewaters of the initial operations of a hair-pulping beamhouse process constitutes an alternative approach to the reduction of the contaminant load and the recovery of a valuable material (the precipitated protein fraction). This simple operation resulted in a major diminution of COD values and protein content. Emulsified and suspended fats in wastewater from hide fleshings experienced co-precipitation with proteins, increasing COD

diminution. Another important result was the drastic decrease in the toxicity of the wastewater due to the elimination of a bactericide added in the initial stages of the beamhouse process. This represents an important saving on the cost of wastewater treatment and associated taxes.

Peptides and proteins make up most of the precipitated protein fraction. They could be reduced in molecular size for use as organic fertilizers by means of controlled hydrolysis. The protein present in the precipitate did not result from the collagen decomposition of the hide but from soluble proteins such as albumin and globulin and remains of keratin.

Although our results show that the precipitated protein fraction meets all the requirements for use as organic fertilizers, further studies are warranted to confirm this hypothesis. In addition, the potential use of amino acids obtained by acid hydrolysis of the precipitated protein fraction for the production of amino acid-based surfactants as well as the application of this protein material as a retanning agent for tannery is currently being investigated.

## ACKNOWLEDGEMENTS

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