Biochemical Method for Extraction and Reuse of Protein and Chromium from Chrome Leather Shavings: A Waste to Wealth Approach

by

Anupama Pati,[1,2] Rubina Chaudhary[1]* and Saravanabhavan Subramani[2]

[1]*Schoolof Energy and Environmental Studies, Takshashila Campus, Devi Ahilya University,*
Khandwa Road, Indore-452001, Madhya Pradesh, India.

[2]*Research and Development, Tata International Limited*
Dewas-455001, Madhya Pradesh, India

Abstract

Chrome shavings are one of the major solid wastes generated during the leather making process. The presence of chromium in waste creates difficulty in disposing to landfill and incineration. Growing environmental concern about the toxicity and environmental impact of the chromium solid waste generated from the tannery has become key issue. In this work, a study has been made to extract protein from chrome shavings through a biochemical method. In this biochemical method the combination of chemical and enzyme processes have been employed to achieve the optimum extraction of protein. Optimization studies on enzyme and alkali concentration, time, pH and temperature on protein extraction were performed. Further, protein extraction by protease mixed with α-amylase has also been investigated. It was found that there was significant change in the protein extraction by protease in the presence of α-amylase. The protein extraction efficiency by conventional and biochemical method is found to be 60 and 80%, respectively. This study provides a biochemical method of hydrolysis for chrome shavings to protein and chromium.

Resumen

Las virutas de cromo son uno de los principales residuos sólidos generados durante el proceso de fabricación de cuero. La presencia de cromo en los residuos crea dificultades en la disposición en vertederos y su incineración. La creciente preocupación medioambiental acerca de la toxicidad y del impacto medioambiental de los residuos sólidos de cromo generada a partir de la curtiembre se ha convertido en tema clave. En este trabajo, se ha hecho un estudio para extraer proteína a partir de virutas de cromo a través de un método bioquímico. En este método bioquímico la combinación de procesos químicos y enzimas se han empleado para lograr la extracción óptima de la proteína. Se realizaron estudios de optimización de la enzima y la concentración del álcali, tiempo, pH y la temperatura de extracción de la proteína. Además, la extracción de proteínas mediante proteasa mezclada con α-amilasa también ha sido investigada. Se encontró que hubo cambio significativo en la extracción de proteínas por la proteasa en presencia de α-amilasa. La eficiencia de la extracción de proteínas por el método convencional y bioquímico fue de 60 y 80%, respectivamente. Este estudio proporciona un método bioquímico de hidrólisis de virutas de cromo hacia proteína y cromo.

*Corresponding author-e-mail address: rubinachaudhary.sees@gmail.com
Manuscript received April 20, 2013, accepted for publication June 8, 2013.

INTRODUCTION

Conversion of hides and skins into leather generates large quantities of liquid and solid wastes. Many cleaner processing approaches aimed at the reduction of liquid and solid wastes proved to be economically and environmentally beneficial.[1,2] The tanning process has been at the center of all attention with regard to pollution from this process. Despite research into alternative tanning agents in the last three decades, chromium remains the predominant tanning chemical. The management of tannery liquid wastes containing chromium through direct and indirect recycling methodologies has become popular.[3,4] However, the solid wastes containing chromium generated after the tanning process such as chrome shavings, continue to pose significant challenges.[1,5] It is estimated that solid wastes containing chromium are about 0.05 million ton/annum in United States of America alone.[6] The tanned wastes primarily consist of chromium and protein. It has been estimated that 0.02 million tons/annum of chromium shavings generated in India. Nearly, 0.8 million ton/annum of chromium shavings could be generated globally.[7, 8]

The use of chrome shavings in the manufacture of leather boards, insulators, building materials, fibrous sheets and shoe soles has been established.[6, 9, 10] However, presently the market for these materials has been replaced by synthetics. Hence, there is no demand for leather boards and shoe soles made from leather waste.

The separation of chromium and valuable protein from chromium shavings through enzymatic alkali processes has also been reported.[11-15] The use of specific enzyme-based products as biocatalysts in place of chemicals is currently being explored in many areas of leather processing.[16-18]

However, the extraction of protein has not been achieved completely. It has also been shown that chrome shavings can be used as a reductant in the manufacture of basic chromium sulfate (BCS).[19] New parchment-like material from chrome shavings has been developed and found useful in the manufacture of home furnishing products.[20] Chrome shavings can be used for the preparation of pigment through controlled incineration.[21] It has been reported that chrome leather shavings can be used as adsorbent for the removal of toxic materials in wastewater.[22]

The cost effectiveness of solid wastes treatment and management in any industrial sector has for long remained the most important issue.[23] There is now an emerging recognition that the environmental issues in leather processing sector are better managed by new innovative approaches. The approach plan suitable for the need involves three parts viz., a) pollution mitigation through process adjustments, b) technological up-gradation of waste treatment systems and c) new avenues for effective utilization to create wealth from waste. The role and importance of technological innovative approach to meet the current environmental challenges from leather processing activity are now greatly recognized. This study explores the efficient way of separating protein and chromium from chrome shavings through biochemical method. The optimum separation of protein and chromium by laboratory grade protease and α-amylase at different conditions such as time, temperature, pH and alkalis has been investigated. The efficiency of protein separation from chrome shavings by enzyme and chemical method has been studied. The influence of protease and α-amylase on protein separation has been scrutinized for developing a biochemical process.

EXPERIMENTAL

Materials

Fresh chrome leather solid waste – chrome shavings were collected from Tata International Ltd., Dewas, Madhya Pradesh, India. Laboratory grade bacterial protease (15000 units/g) from *Bacillus licheniformis* and α-amylase (25000 units/g) from *Bacillus subtilis* were sourced from Sigma Aldrich. Busan 30L (TCMTB based microbiocide) was sourced from Buckman Laboratories (India) Private Limited. All the chemicals used for the analysis were of analytical grade.

Characterization of Chrome Shavings

The collected chrome shavings were characterized for moisture, chrome content, inorganic ash, nitrogen, oils and fats, pH and apparent density as per standard procedures.[24-30]

Optimization of Protease and α-amylase

Separation studies were carried out using predetermined quantities of chrome shavings, which were equilibrated in 200% water (w/w of dry weight of chrome shavings) for 2 h. The treatment studies were carried out in batch processes. The predetermined quantity of chrome shavings (25 g on dry weight basis) were α-amylase treated with protease (0 – 5% (activity 25000 units/g) (w/w on dry basis of chrome shavings)) and water 100 ml and 1% lime in a 500 ml triple neck round bottom flask equipped with condenser, agitator for mixing at 30 rpm and heating mantle for incubating at differential temperatures (45 – 55°C) for a period of 18 h. Further temperature was raised to 95°C for a period of 2 h and analyzed for protein content. The protein was separated out after the process by passing through the sieves and the protein content in the supernatant was determined using Biuret reagent.[31]

The protease to chrome shavings was optimized by treating predetermined quantities of chrome shavings (5, 10, 15, 20 and 25 g on dry basis) with 2% protease and 1% lime, above optimized temperature and duration. Studies on the effect of pH were carried out by treating 25 g of chrome shavings (on

dry basis) with 2 g of protease at various initial pH values (4.0 – 12.0) (pH was adjusted using 0.5 and 1% NaOH solution) with above optimized temperature and duration. The protein was separated out after the process by passing through the sieves and the protein content in the supernatant was determined using Biuret reagent.[31] Similar studies were conducted for α-amylase on protein extraction.

Optimization of Nature of Alkalis for Extraction of Protein

Experiments were carried out by treating predetermined quantity of chrome shavings (25g on dry weight basis), protease (2% (w/w), on dry basis of chrome shavings, different alkalis (0.5 – 1.5%) and water 100 ml in a 500 ml triple neck round bottom flask (as described above) with above optimized temperature and duration. The protein was separated out after the process by passing through the sieves and the protein content in the supernatant was determined using Biuret reagent.[31] Similar studies were conducted to study the effect of nature of alkalis on α-amylase activity on protein extraction.

Amalgamation of Protease and α-amylase

Treatment studies were carried out by treating predetermined quantities of chrome shavings (25 g on dry weight basis) with different dosages of protease and α-amylase (0 – 2% (w/w), on dry basis of chrome shavings) employing 1% lime and water 100 ml with above optimized temperature and duration. At the end of predetermined time interval a small aliquot was taken and analyzed for solubilized protein content using Biuret reagent.[31]

Pilot Study

About 2 kg (on dry basis) chrome shavings were taken for pilot study. Chrome shavings were feed in a pilot reactor equipped with an agitator 30 RPM and treated with optimized dosage of protease and α-amylase and alkali at optimized temperature and duration. Also chemical based hydrolysis was performed as comparison trial by treating 2 kg chrome shavings with 1.5% lime at optimized temperature and duration. The protein was separated out after the processes by passing through the sieves and the protein content in the supernatant was determined using Biuret reagent.[31] The chrome sludge was subjected to chrome recovery and for the preparation of BCS.[22, 23] The amino acid composition of the protein hydrolysate obtained from experimental process was determined using Hitachi amino acid analyzer model 835–50, Tokyo Japan, (1981).

Recovery and Reuse Studies

Protein hydrolysate derived from experimental process was concentrated to 40% solution and treated with 0.1% Busan 30L. The chromium bearing residue of chromium (III) hydroxide was treated with sulfuric acid till pH 2.8, left overnight and the basicity of the chromium (III) sulfate estimated by standard procedures.[32, 33] The chromium (III) sulfate solution was used in tanning as a 40% replacement for basic chromium sulfate (BCS) salt and followed conventional tanning process (E1). Control tanning (C) was done using BCS for comparison.

Concentrated protein solution was offered (3%) in rechroming of conventionally BCS tanned leather (E2). Experimental (E1 and E2) and control leathers (C) were made to crust leather following conventional post rechroming process. Experimental and control crust leathers were assessed for softness, fullness, grain flatness, grain smoothness, grain tightness (break) and general appearance by hand and visual examination. The leathers were rated on a scale of 0–10 points for each functional property by experienced tanners, where higher points indicate better property.

RESULTS AND DISCUSSION

The characteristics of chrome shavings from a commercial tannery have been analyzed. The amount of % moisture, % chromium (as Cr_2O_3 on dry weight basis), % inorganic ash, % nitrogen, % oils and fat present in the chrome shavings are 24.1±0.6, 3.12±0.12, 5.21±0.11, 16.4±0.1 and 1.5±0.05, respectively. The pH of aqueous leather and apparent density of chrome shavings used in this study was 3.4±0.2 and 0.94±0.2 g/ml.

Optimization of Protease, α-amylase Dosage and Temperature on Protein Extraction

The extraction of protein from chrome shavings by protease and α-amylase dosages has been studied to find optimum dosage for maximum protein extraction. Extraction of protein by protease and α-amylase on increasing temperature with varying concentration of protease and α-amylase is given in Fig.1. It is seen that protein extraction increases with increasing the concentration of protease and α-amylase. Further, protein extraction increases with the raise in incubation temperature from 45 to 55°C. These results indicate that optimum temperature is 55°C for both protease and α-amylase activity. The second step was carried out by raising temperature to 95°C for a period of 2h. Further it is observed from Fig.1 that there is no significant change in quantity of protein extraction by protease and α-amylase offer more than 2 and 3%, respectively.

Optimization of Weight of Chrome Shavings on Protein Extraction

The ratio of chrome shavings to protease and α-amylase concentration (2%) was increased from 5 g to 25 g. The quantity of protein extraction (Fig. 2(a)) is increased from 4.51 g to 18.45 g with increase in chrome shavings dosage 5 g to 25 g, respectively. Similarly, quantity of protein extraction (Figure 2(b)) is increased from 3.67 g to 15.01 g with increase in chrome

shavings dosage 5 g to 25 g, respectively in case of α-amylase. This shows that quantity of protein extraction is related to the ratio of chrome shavings to enzyme concentration.

Optimization of pH

Fig.3 represents the protein extraction as a function of pH over the pH range 4 – 12. The influence of pH on the activity of the enzyme is given in Fig.3. It is seen that the activity is low in acidic region and increases significantly upon increase in the pH, especially in the alkaline region. It is clearly seen that the selected protease and α-amylase have maximum yield of protein at pH 12.0. Also, at pH values above 8.0, chromium present in the chrome shavings tends to precipitate as chromium (III) hydroxide.

Optimization of Nature of Alkali and its Dosage

The extraction of protein from chrome shavings by different alkalis such as lime, NaOH and MgO with different concentrations, with and without protease and α-amylase, has been studied to find suitable alkali for hydrolysis process. The protein extraction by different alkalis is given in Table I. It is observed that presence of protease and α-amylase yields more protein. There is no significant difference in protein yield above 1% alkali concentration in all the cases. Lime seems to be economical and also rapidly allows the chromium to settle as chromium hydroxide. Hence, lime has been chosen as a suitable alkali with 1% concentration as optimum.

Effect of Amalgamation of Protease and α-amylase on Protein Extraction

In order to find the effect of amalgamation of protease and α-amylase, the concentrations of both protease and α-amylase were varied and the protein extraction was analyzed. The protein yield by protease in the presence and absence of α-amylase with increasing concentrations of protease and

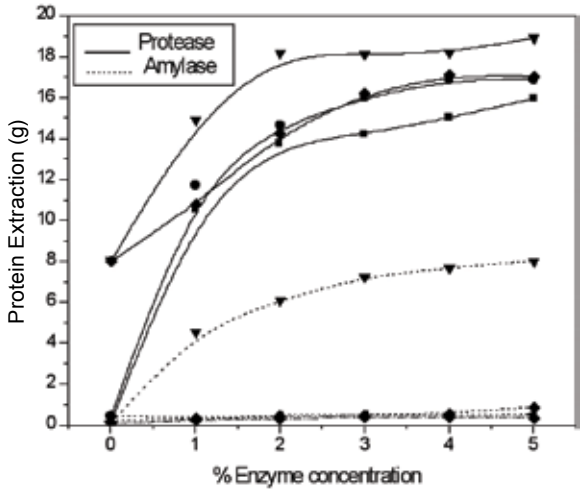

Figure 1. Effect of protease and amylase concentration and temperature on quantity of protein extraction (g), Treatment Duration – 18 h, Chrome Shavings – 25 g, Lime – 1%, Temperature ■ - 45°C; ● - 50°C; ♦ - 55°C; ▼- 95°C.

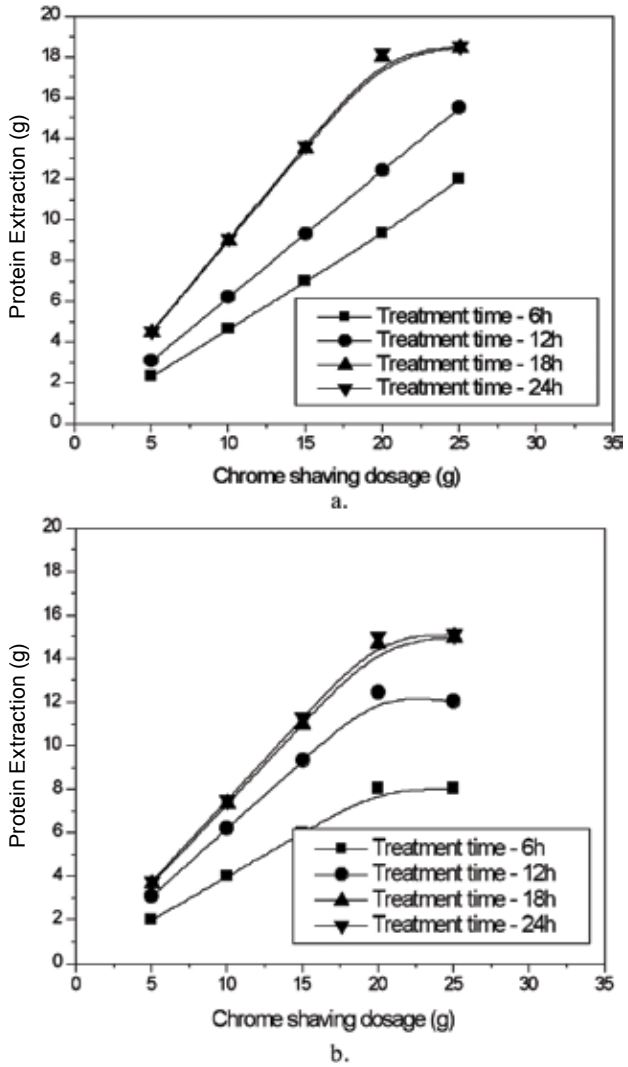

Figure 2a. Effect of chrome shavings concentration on quantity of protein extraction (g) by protease. Figure 2b. Effect of chrome shavings concentration on quantity of protein extraction (g) by amylase.

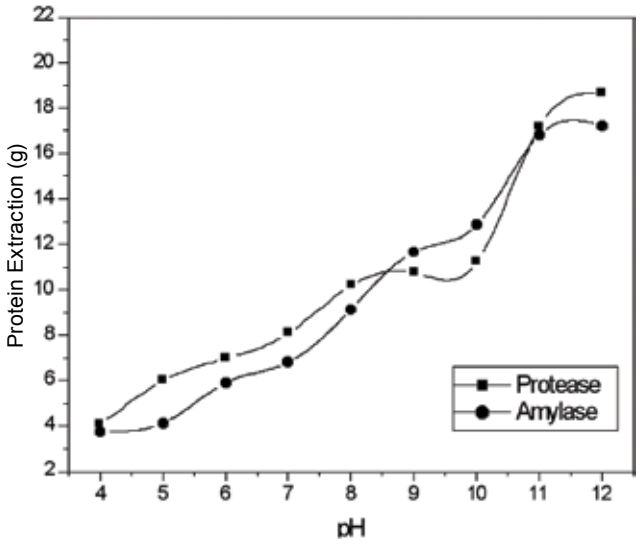

Figure 3. Effect of pH on quantity of protein extraction (g) by protease and amylase.

TABLE I
Effect of different alkalis and dosages on quantity of protein extraction.[a]

Alkali	Lime (%)			NaOH (%)			MgO (%)		
	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Without Enzyme	6.15	10.06	11.12	10.45	11.02	11.78	8.11	10.13	10.52
Protease (2%)	13.17	18.06	18.12	17.68	18.07	18.02	13.76	17.91	18.21
Amylase (2%)	12.82	15.62	16.12	16.35	17.1	17.1	13.03	15.12	16.25

[a]Quantity of protein extraction is expressed in grams. Chrome shavings dosage – 25 g (on dry basis)

TABLE II
Effect of amalgamation of protease and amylase on protein extraction.[a]

Protease (%)	Amylase (%)	Time (h)								
		2	4	6	8	10	12	14	16	18
0	1	3.83	5.42	8.32	9.32	10.30	10.63	10.70	10.78	10.91
1	0	4.54	6.42	9.86	11.04	12.19	12.59	12.67	12.77	12.92
1	1	6.63	9.37	14.39	16.12	17.80	18.38	18.50	18.64	18.86
1	2	6.51	9.21	14.14	15.84	17.50	18.07	18.18	18.32	18.54
2	1	6.54	9.25	14.20	15.91	17.57	18.15	18.26	18.40	18.62

[a]Quantity of protein extraction is expressed in grams. Chrome shavings dosage – 25 g (on dry basis)

α-amylase is given in Table II. It is seen that there is significant effect on protein extraction change in the presence of α-amylase. It is interesting to note that the yield of protein increases by protease with the presence of α-amylase. This is primarily due to the hydrolysis of glycan fibers by α-amylase that exposes sites on the protein to protease. It is seen that the activity of protease increases with all the concentrations of α-amylase. Hence, it can be clearly noticed that the α-amylase enhances protease activity in protein extraction.[34] The optimum quantity of protease and α-amylase is 1% respectively.

Reuse of Protein and Chromium in Leather Making

Pilot study reveals that chemical and biochemical hydrolysis process yields 1207 g and 1605 g of protein, respectively. In other words protein extraction efficiency of chemical and biochemical process are found to be 60.3 and 80.2%. The protein extracted through biochemical process was analyzed for amino acid content and results are given in Table III. The precipitated chromium was redissolved in sulfuric acid, aged overnight. The basicity of the chrome liquor was 35% and chromium content 7.6% (w/v). Recovered chrome liquor was used for tanning and leather is marked as E1. Concentrated protein hydrolysate (40%) was used in rechroming process as filler and leather is marked as E2. Control process was performed using BCS for tanning and rechroming without protein filler. All the leathers were made to crust.

Control Leathers vs Experimental Leathers (E1 and E2): an Appraisal Bulk Properties of the Leathers

The hand and visual evaluation has been done for both tanned and crust leathers. The tanned stage assessment values are given in Table IV. It is observed that the experimental leathers E1 have good bulk properties, which are comparable to that of control tanned leather.

Crust leather from both control and experimental processes (E1 and E2) were evaluated for various bulk properties by hand and visual evaluation. The average of the rating for the five leathers corresponding to each experiment was calculated for each functional property and is given in Table IV. Higher numbers indicate better property. The experimental leather (E2) exhibit better fullness compared to control leathers. This is primarily due to the fact that protein filler gives fullness to the leather. Other properties of such as grain tightness and smoothness of experimental leathers (E1 and E2) are comparable to that of conventionally processed leathers. The overall appearance of both control and experimental leathers (E1 and E2) are also comparable.

TABLE III
Amino acid composition of hydrolysate.

Amino acid	Collagen Type 1	Hydrolysate[a]
Thr	1.6	0.543
Val	2.3	3.411
Leu	2.5	2.721
Ile	1.2	1.101
Lys	2.8	2.112
Hyl	0.7	0.923
Tyr	0.4	0.312
Phe	1.3	1.812
Met	0.6	0.742
Gly	32.7	32.124
Hyp	8.6	6.012
Pro	13.0	14.356
Ala	11.4	13.124
Arg	5.2	3.867
Asp	4.6	4.512
Cys	0	0.452
Glu	7.5	7.121
His	0.5	0.473
Ser	3.1	1.925
Ammonia	-	1.213
Unknown	-	0.416
Total	100.0	99.272

[a]Hydrolysate obtained from biochemical process

CONCLUSIONS

Waste to wealth approaches are gaining importance for all the industries concerned about sustainability. In this study, an attempt has been made to extract and reuse protein and chromium from chrome shavings through biochemical method. Chemical and biochemical method shows that the efficiency of protein extraction is 60 and 80%, respectively. The concentration of protease and α-amylase is optimized as 1%, respectively. Amalgamation study reveals that α-amylase enhances the extraction of protein. The parameters such as pH and temperature are optimized as 12 and incubation temperature 55°C to achieve optimum yield of protein. Developed process is more efficient in recovering protein from chrome shavings compared to chemical based hydrolysis process. Tanning studies using chromium and protein reveal that the bulk properties of leathers obtained from experimental process (E1 and E2) are comparable to that of conventionally processed leathers (C). The developed process is environmentally friendly and efficient in extraction of protein from chrome shavings.

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TABLE IV
Hand and visual assessment data for control and experimental leathers.

Parameters	Evaluation at tanned stage		Evaluation at crust stage		
	Control	E1	Control	E1	E2
Chrome patches at tanned stage	No	No	NA	NA	NA
Grain smoothness	7.0±0.5	7.5±1.0	8.0±0.5	8.0±0.5	8.0±0.5
Color of leather at tanned stage	8.0±1.0	8.0±1.0	NA	NA	NA
Fullness	7.5±0.5	8.5±0.5	8.0±0.5	8.0±0.5	8.5±0.5
Softness	8.0±0.5	8.0±1.0	8.0±0.5	8.50.5	8.0±0.5
Grain Tightness	NA	NA	8.0±0.5	8.0±0.5	8.0±0.5
General appearance	8.0±0.5	8.0±1.0	8.0±0.5	8.5±0.5	8.5±0.5

NA – Not Applicable

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