

## ENZYMIC TREATMENT OF CHROME SHAVINGS\*

by

M.M. TAYLOR, E.J. DIEFENDORF & G.C. NA\*\*

*U.S. Department of Agriculture*

*Eastern Regional Research Center\*\*\**

600 E. MERMAID LANE

PHILADELPHIA, PENNSYLVANIA 19118

### Abstract

A method was developed that separates solid chrome shavings into a protein and a chromium product. The method entails the proteolytic treatment of chrome shavings at a temperature from 60 to 65°C in the presence of 5-6% lime. Following the treatment, the protein can be separated by filtration, leaving a chromium cake. Extraction of the chromium cake with sulfuric acid produces a chromium solution which may be used directly in the pickle step. Alternatively, the chromium could be precipitated from the acidic solution and used to make up the tan. The protein hydrolyzate contains less than 4.5 ppm chromium and has potential as a feed, fertilizer, or as an additive in the cosmetic industry.

### Introduction

The disposal of chrome shavings generated from the production of blue stock presents a serious problem for the tanning industry. Chrome tanning generates approximately 54,000 metric tons of chrome waste annually in this country. Sanitary landfills are reluctant to accept chromium-containing waste because of the possibility of Cr(III) waste being oxidized to the toxic Cr(VI) and contaminating the ground water. Hazardous landfills are an expensive alternative. There is an urgent need to find an economical method of disposal.

Chrome effluent from the bluing stage can usually be recycled and the literature has many references on how the floats and washes could be treated, and then used again in the pickle or in the tan. Little has been done, however, with solid chrome shavings in the present form, other than pressing them to form sheet-like products, or possibly hydrolyzing them and recovering the chromium.

It has been demonstrated in the literature, including the work reported recently from our laboratory<sup>(1-6)</sup>, that waste products from fleshing and beaming operations can be treated with enzymes at low temperatures for short periods of time to give products with commercial values and/or acceptable by the sanitation department. There are a few references pertaining to the enzymic treatment of the solid chrome waste products, but the procedures described are usually time consuming, or require boiling of the chrome wastes before enzymic treatment<sup>(7-14)</sup>. Furthermore, chromium was frequently dissolved and became difficult to separate from the hydrolyzed protein. We recently have developed a method of treating chrome shavings under moderate temperatures. The method utilizes commercially-available enzymes, and gives essentially complete separation of protein and chromium. In this paper, we describe the methodology of the enzymic conversion of

\*Presented at the 85th Annual Meeting of the American Leather Chemists' Association, Pinehurst, North Carolina, June 21, 1989

\*\*Present Address: Sterling Research Group, 25 Great Valley Parkway, Malvern, PA 19355.

\*\*\*Agricultural Research Service, United States Department of Agriculture.

chrome shavings and the optimal conditions to achieve maximal separation. A subsequent paper will discuss the isolation, purification and analysis of the protein, and the chemical treatment of the chromium so that it could be used for recycling.

## Experimental

### MATERIALS

The initial experiments were run on blue stock prepared in our tannery. The sample was dried, cut into quarter-inch pieces and ground into a fine powder with a Wiley Mill. Subsequent experiments were run on chrome shavings which were obtained from two separate commercial tanneries. The shavings were stored at 4°C until use.

Alcalase® was obtained from Novo Laboratories, Inc.<sup>1</sup> (Danbury, CT). It is a proteolytic enzyme with its optimal activity found at pH 8.3-9.0, and a temperature of 55-65°C. It was standardized to contain 2.0 AU/g (Anson Units/g).

Enzeco® Alkaline Protease-L (alkaline protease) was obtained from Enzyme Development Corporation (New York, NY). Similar to Alcalase®, Enzeco® Alkaline Protease-L is heat-stable and functional at alkaline pH's. Its optimal activity was found at pH 7.0-11.0 and at a temperature of 50-70°C. It was standardized to contain a minimum of 400,000 D.U./g.

### PROCEDURE

Blue shavings were suspended in 500-1000% float. 1-12% lime was added and the samples were heated to 50-75°C and shaken at 85 RPM for 30 min. 1-12% enzyme was then added, and the samples were further incubated for 0-240 min. The samples were filtered under vacuum through Whatman #1 filter paper, using porcelain funnels. The protein hydrolyzate (filtrate) was stored at 4°C and the residue was weighed after being dried in a gravity oven at 50°C for 19 hr. The percent residue was calculated based on the dry weight of the shavings (measured separately). The Alcalase® enzyme was embedded in an inert carrier and sold in a granular form. Part of the lime remained insoluble throughout the treatment. Both the inert carrier and the insoluble lime contributed to the residue but controls were run to correct the residue weight.

### ANALYSES

Moisture content were determined by heating the shavings at 50°C for 19 hr. in a gravity oven. Chromium was measured with a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer<sup>(15)</sup>.

## Results

### INITIAL STUDIES WITH BLUE STOCK

Alcalase® was chosen for the initial experiments for two reasons. First, it is known for its hydrolytic activity on proteins from animal sources. Second, the enzyme is active at an alkaline pH. Since chromium is soluble at low pH's, but insoluble at high pH's, the enzyme can hydrolyze and solubilize the protein but not the chrome in the chrome shavings. Blue stock prepared at our laboratory was used in the initial experiments. Since the pH of the

<sup>1</sup>Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

shavings ranged from 3.5 to 4.2, a buffering system was needed to raise the pH to that of optimal enzyme activity. Tris and carbonate-bicarbonate buffering systems were tried. Under all conditions used, the pH dropped during the course of the enzyme treatment. Although the percent solubility of the chrome shavings went as high as 76%, all the filtered solutions showed a deep blue color, indicating solubilization of some chromium. 10% lime was used as an alternative to the buffering systems resulting in a final pH of 10.2. Our subsequent studies suggested that by adding a smaller amount of lime, the solution can be maintained at a pH near that of optimal enzyme activity, leading to better dissolution of the shavings. A study was carried out to determine the amount of lime needed to give a pH at which the enzyme would still remain active and at the same time would not fall too low for the chromium to remain insoluble. As shown in Figure 1, 0.1 gram of lime per gram of dry shavings dissolved up to 75% of the shavings.

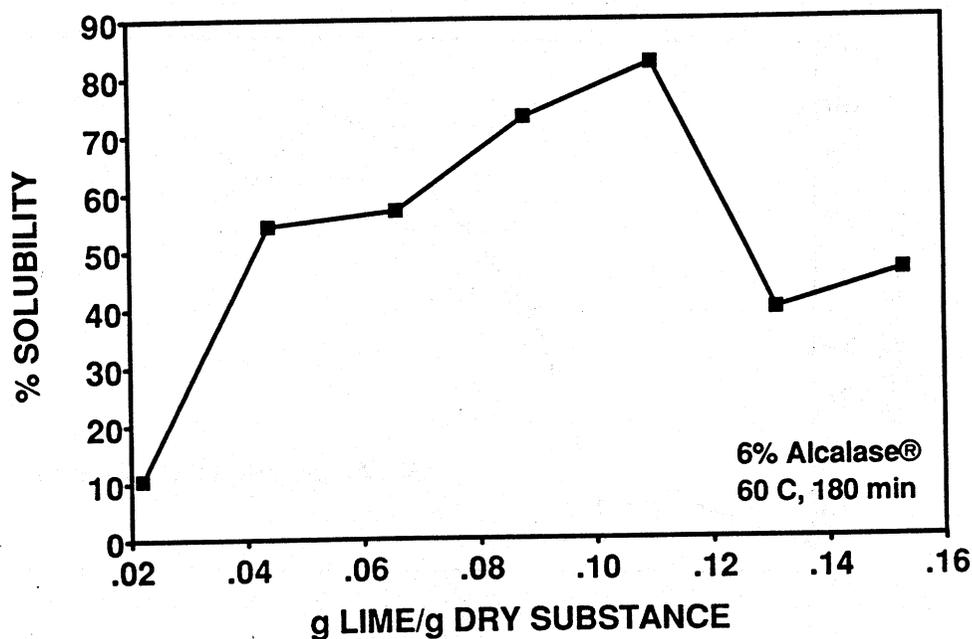


FIG. 1 — Effect of lime concentration, expressed as grams of lime per gram of dry substance, on the solubility of ground blue stock.

#### ENZYMIC TREATMENT OF COMMERCIAL CHROME SHAVINGS

Since we were successful in dissolving the ground blue stock prepared at our tannery, the next step would be to treat shavings from commercial tanneries. Since different tanneries usually employ different processes for chrome tanning, the pH of the final product could vary. Therefore, the first step in treating these shavings would be to determine the optimal amount of lime needed for the process. Figure 2 shows that 6% lime, based on the wet weight of shavings from one commercial source, will keep the pH in the optimum range. Since this batch of shavings contained approximately 55% moisture, this corresponds to 0.132 gram of lime per gram dry weight of shavings. This amount of lime needed to maintain the proper pH range for this particular commercial chrome shavings is clearly higher than that for our blue stock, indicating the importance of testing the pH.

The amount of enzyme needed for optimal conversion was also determined experimentally. The study was carried out with 6% lime and a 500% float. As shown in Figure 3,

6% Alcalase® (based on the wet weight) gave the highest solubility. Further increase of the enzyme did not result in increased solubility. It is interesting that even 1% of enzyme was successful in dissolving 65% of the shavings. Control experiments (no enzyme added) also showed 20% dissolution of the shavings.

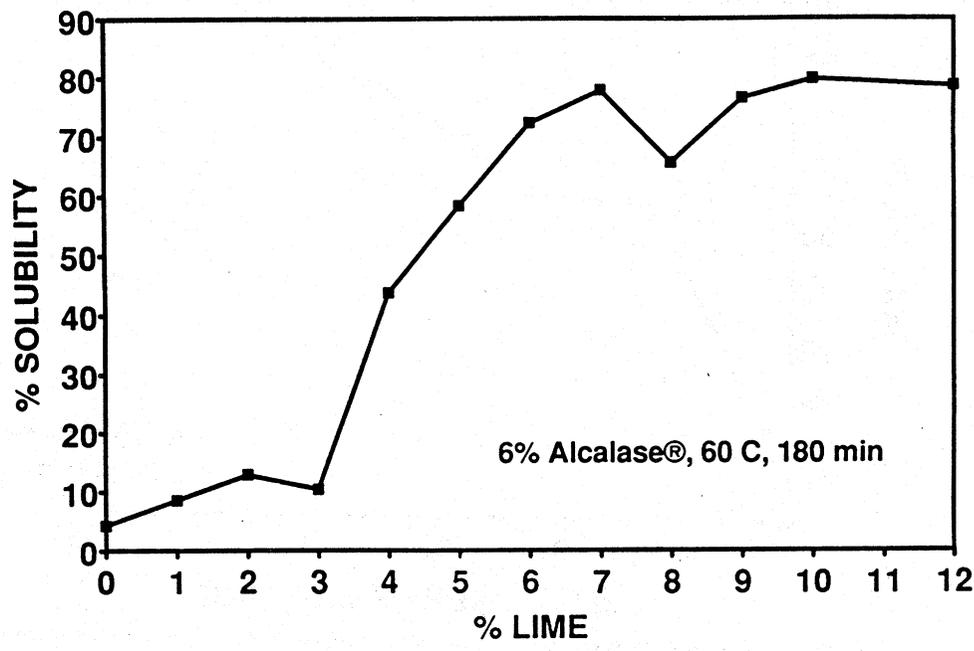


FIG. 2. — Effect of lime concentration, expressed as percent of wet weight, on the solubility of commercial chrome shavings.

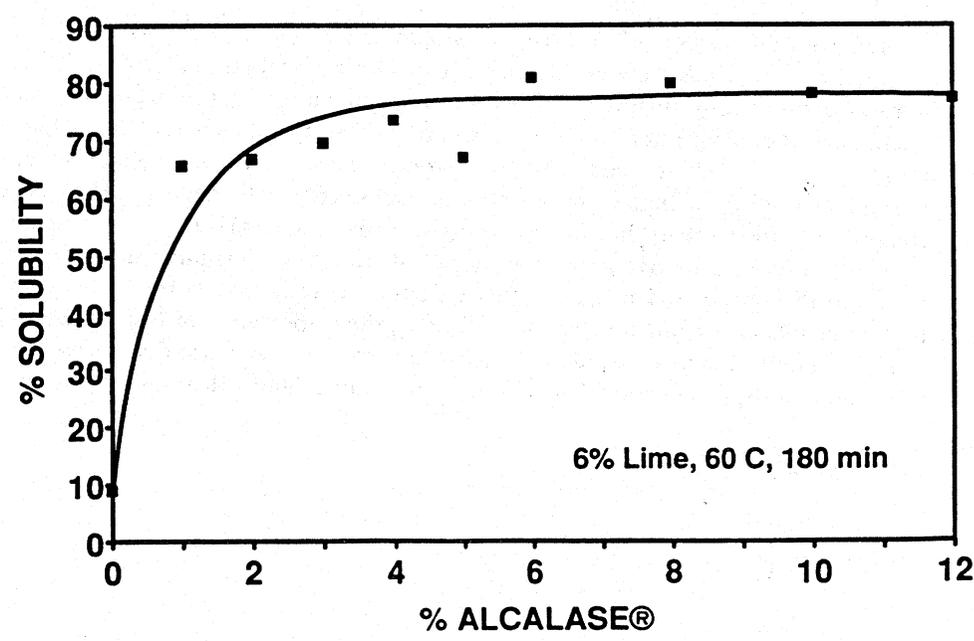


FIG. 3. — Effect of Alcalase® concentration, expressed as percent of wet weight, on solubility of commercial chrome shavings.

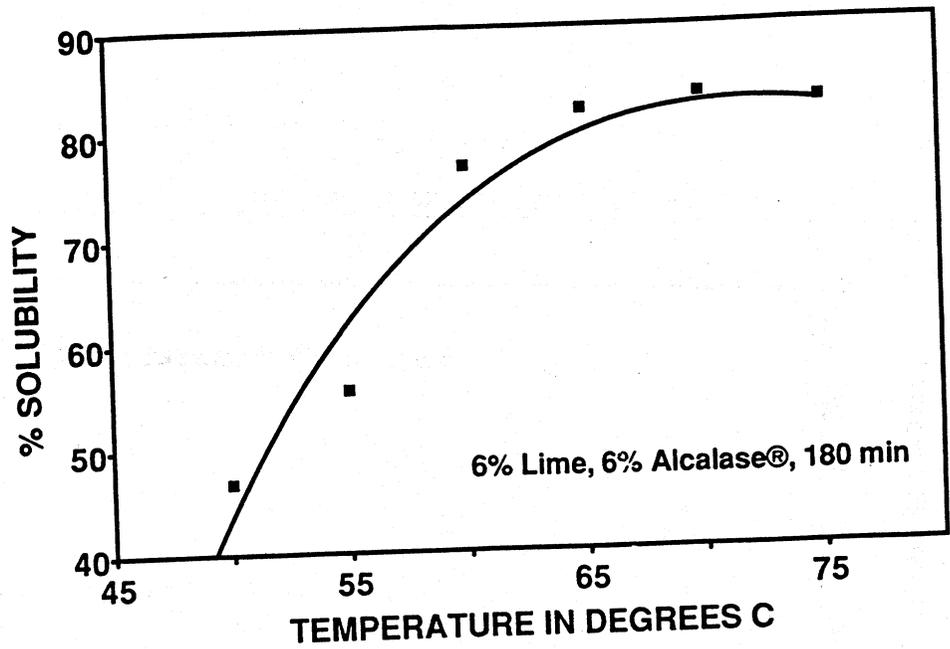


FIG. 4. — Effect of temperature, in degrees C., on solubility of commercial chrome shavings.

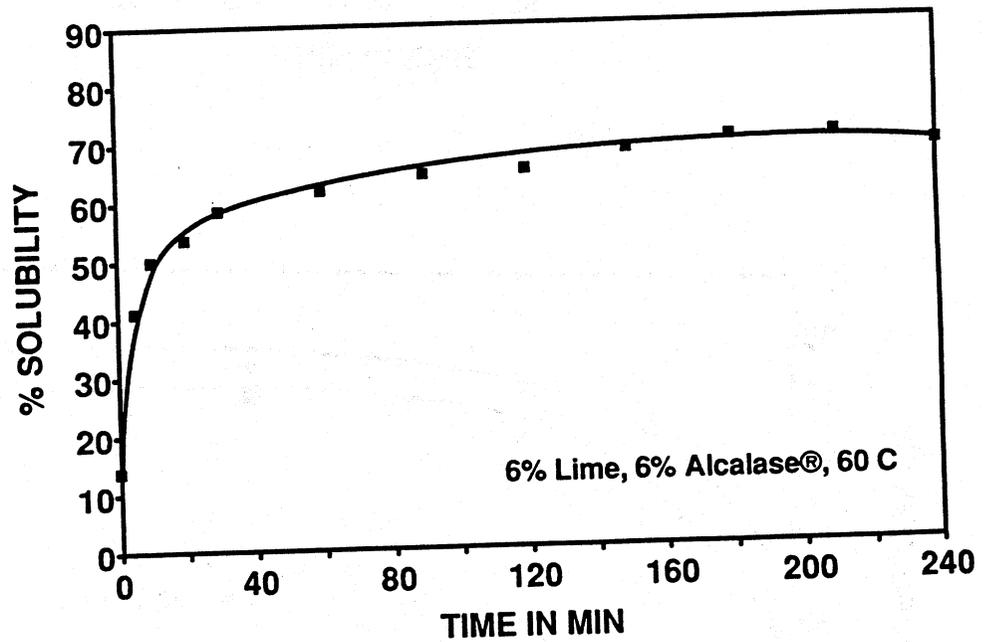


FIG. 5. — Effect of time, in minutes, on solubility of commercial chrome shavings.

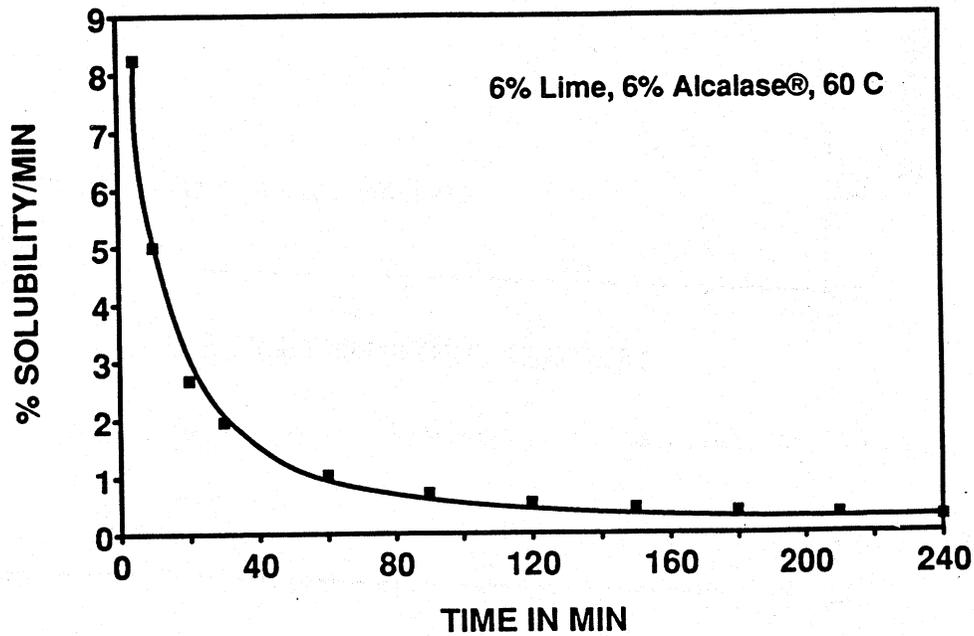


FIG. 6. — Variation of reaction rate with time.

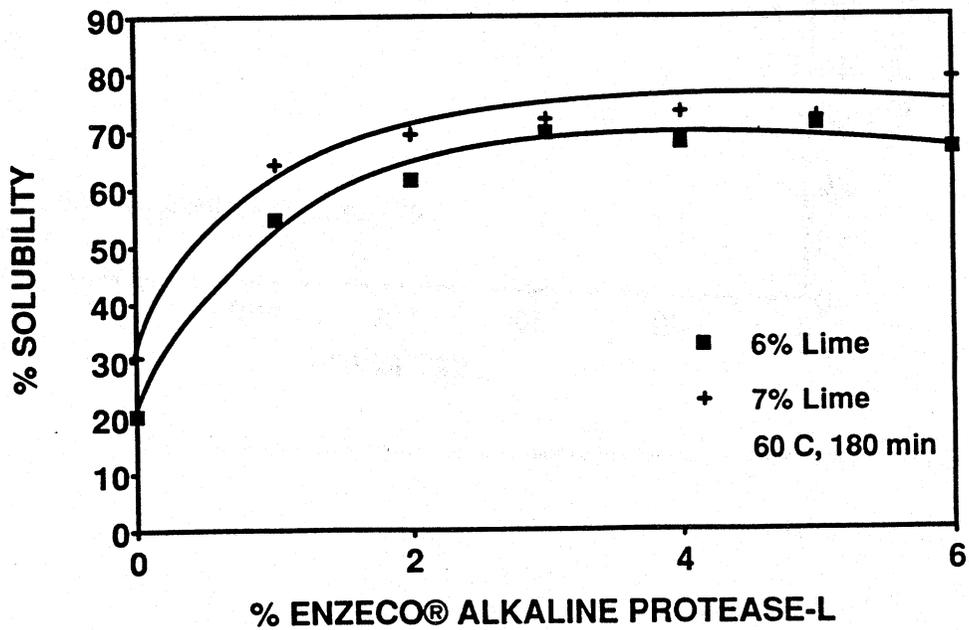


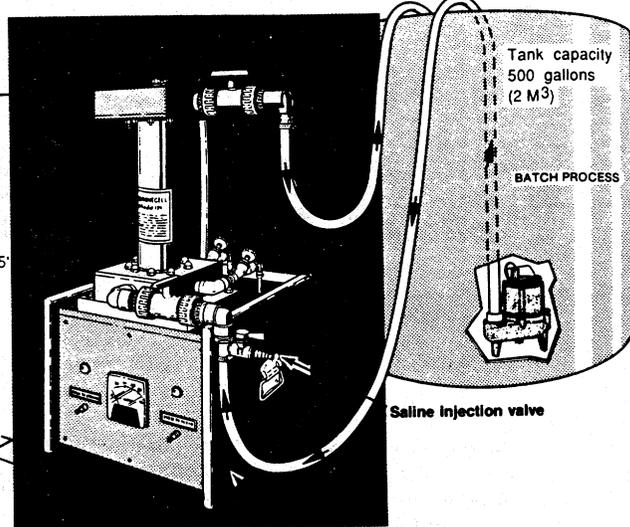
FIG. 7. — Effect of concentration of Enzeco® Alkaline Protease-L, expressed as percent of wet weight, on solubility of commercial chrome shavings using different lime concentrations.

**TREAT SEWAGE WATERS AND INDUSTRIAL WASTE EFFLUENTS, WITHOUT CHEMICALS!  
 DESTROY ANY AMOUNT OF BOD! REDUCE COD TO ACCEPTABLE LEVELS.  
 OXIDIZE EVERYTHING THAT IS OXIDIZABLE, CCL<sup>4</sup>, CHCL<sup>3</sup>, BROMACIL.  
 DE-INK, DE-COLOR, DEODORIZE AND STERILIZE, WITHOUT CHEMICALS!**

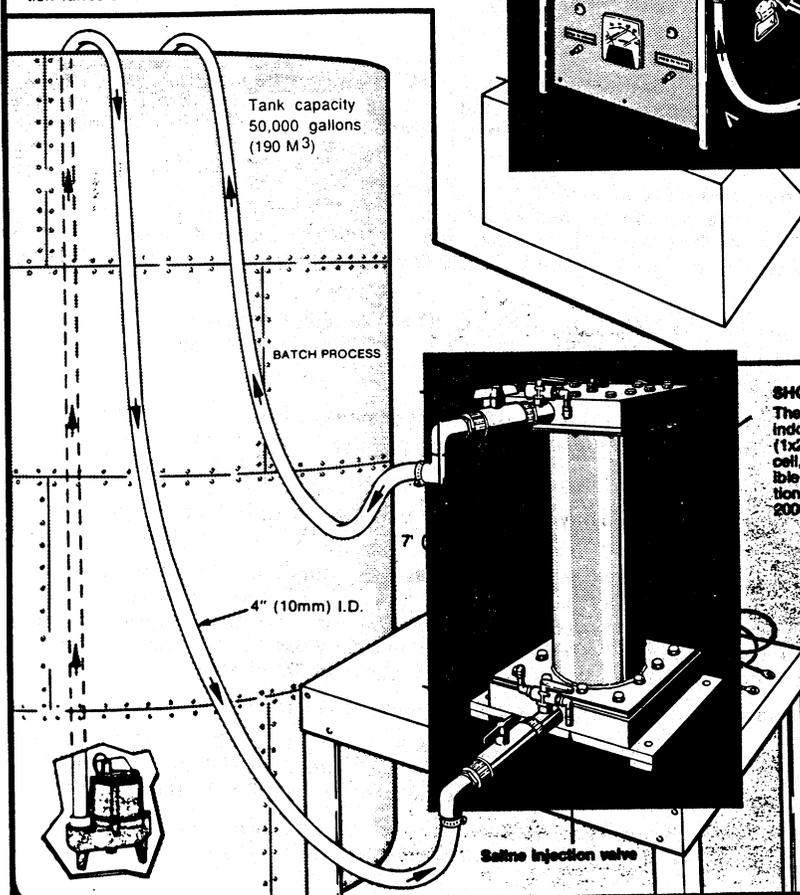
BRINECELL EQUIPMENT DOES NOT CAUSE WATER OR AIR POLLUTION

**HOW THE BRINECELLS WORK:** When municipal sewage or industrial sewage effluents that contain from as low as .1% (1 g/L-1000 ppm-mg/L) to as high as 10% (100 g/L-100,000 ppm-mg/L) salt (NaCl) pass through these world-multipatented **BRINECELLS**, nascent chlorine, ozone and their respective hydroxyl/free radicals are liberated. These agents are known to destroy, neutralize and oxidize all oxidizable organics, while they de-ink, decolor, deodorize and sterilize the effluents, rendering them safe and legal to be disposed of. Only these **BRINECELLS** have such capabilities!

These **BRINECELLS** operate by recirculating the effluents anywhere from 2 to 60 minutes, depending upon the effluent itself. They can also operate on a continuous flow basis by flowing from tank-to-tank or by installing them in a series formation from cell-to-cell. These **BRINECELLS** can operate at various voltages: 10, 20, 30, 40, 50 or 60, and at amperages from as low as 20 to as high as 800, depending upon the cell. **Operation can be low voltage and high salt, or high voltage and low salt.** If salt is needed, rock salt may be used. Or concentrated saline may be injected through the saline injection valves on the cells.



**SHOWN: A COMPLETE MODEL '129'**  
 Overall height: 2.5 ft. (.76 m)  
 Recirculate: 50 gpm (200 Lpm) 10 psi  
 Maximum energy consumption: 1 kWh



**SHOWN: THE CELL ONLY MODEL '158'**  
 The complete system comes skid-mounted in an indoor-outdoor enclosure, measuring 3x8x8 ft. (1x2x2.5 M) 2000 lbs. (1000 kg) consisting of the cell, DC power supply, acid washer and submersible pump. It comes ready for immediate operation. No installation needed. Recirculation at 2000 gpm (8 Mpm) at 90 psi, consuming 8 kWh.

**VERSATILITY...  
 MAKE YOUR OWN 10%  
 CONCENTRATION LIQUID CHLORINE**

...fill these tanks with plain water, add 10% salt (NaCl) and 2% sodium hydroxide (NaOH) and recirculate this mix...soon you will have the equivalent of 10% concentration liquid chlorine...

EPA registered

Manufactured in the United States of America by:

**BRINECELL® MANUFACTURING CORPORATION**

Mailing Address: P.O. Box 27488, Salt Lake City, Utah 84127 U.S.A.

Offices: 2109 West 2300 South, Salt Lake City (WVC) Utah 84119 U.S.A.

Telephone: (801) 973-6400 FAX: (801) 973-6463 Telex: 9102404492

June 1989

Litho in U.S.A.



# **BRINECELL**

**MANUFACTURING CORPORATION**  
Manufacturers of Patented Electrochemical Instruments

Gentlemen:

On the reverse side, we illustrate our multi-patented **BRINECELLS** for the treatment of all types of sewage effluents—residential, commercial, municipal and industrial. Our **BRINECELLS** treat effluents without using chemicals. And the treatment is guaranteed to meet all government's laws for its disposition.

Our **BRINECELLS** destroy, neutralize and oxidize all oxidizable organics (VOCs), such as: PCBs, bromacil, dioxin, carbon tetrachloride, pentachlorophenol, ammonia, chloroform, toluene, and of course, BOD and COD. At the same time, it de-inks, deodorizes, sterilizes and decolors! Below are the results of sewage effluent from a major U.S. Pulp & Paper Mill treated in our **BRINECELL '130'**.

**Untreated sample:** pH 8.3; TSS 2240; BOD 20,000; COD 64,000; color dark blue  
**Treated 30 mins:** pH 3.0; TSS 33; BOD 102; COD 439; color water-clear

**Here is how to test our BRINECELLS:**

- 1) Send us 15 gallons (60 liters) of your sewage effluent. We will treat it in our Model '130' and return samples with the test data. This service is offered without cost or obligation.
- 2) Visit us, bring at least 15 gallons (60 liters) of your effluent and test our **BRINECELLS**.  
If, within 12 months of your visit, you buy our Model '130' or '456', we will credit your purchase with \$5,000.
- 3) **We will visit you, with our Model '130' to provide on-site demonstrations.** We will stay in your facility the entire day. For this personalized service, we require a good-faith refundable deposit of \$5,000 payable ahead of the visit. If, within 12 months of our visit, you buy our Model '130' or '456', we will grant you \$5,000 credit for your original \$5,000 deposit. If you do not buy within 12 months from our visit, we will return your original \$5,000 deposit.

The following Models are available (we make smaller Models as well):

**Model '129'** 50 gpm (200 lpm) flow at 10 psi, input 120 VAC, output 15 VDC at 60 A 1 KWH  
**Model '130'** 200 gpm (800 lpm) flow at 10 psi, input 220 VAC, output 10 VDC at 200 A 2 KWH  
**Model '456'** 2000 gpm (8 M<sup>3</sup> pm) flow at 30 psi, input 220 VAC, output 10 VDC at 800 A 8 KWH

Delivery from 90-180 days, FOB our plant, packing included. Prepaid orders are allowed a 5% discount, guaranteed delivery, and freight prepaid. We also carry our own in-house, long-term financing, with 30% down.

Our **BRINECELLS** qualify for investment tax credits and grants from the Superfund. Samples, lab and field data, are available upon written request. Our 25 years experience is yours for the asking. Let us assist you!

Sincerely yours,  
**BRINECELL Manufacturing Corporation**

Tim Themy-Kotronakis, President

June 1989

Mailing Address: P.O. Box 27488, Salt Lake City, Utah 84127 U.S.A.  
Factory and Offices: 2109 West 2300 South, Salt Lake City, (WVC), Utah 84119 U.S.A.  
Phone: 801-973-6400 Fax: 801-973-6463 Telex: 9102404492

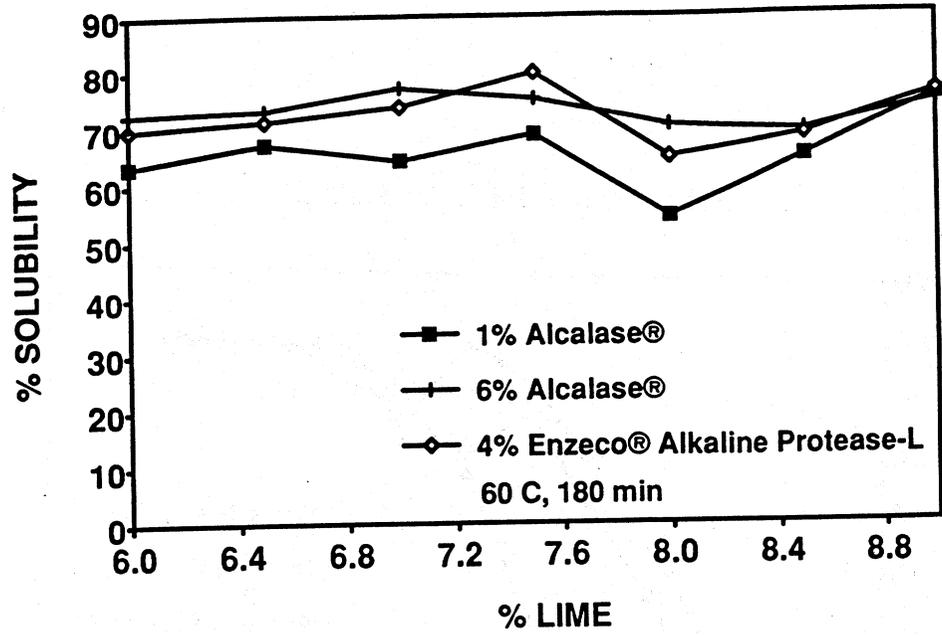


FIG. 8. — Effect of lime concentration, expressed as percent of wet weight, on solubility of commercial chrome shavings treated with different concentrations of Alcalase® and with Enzeco® Alkaline Protease-L.

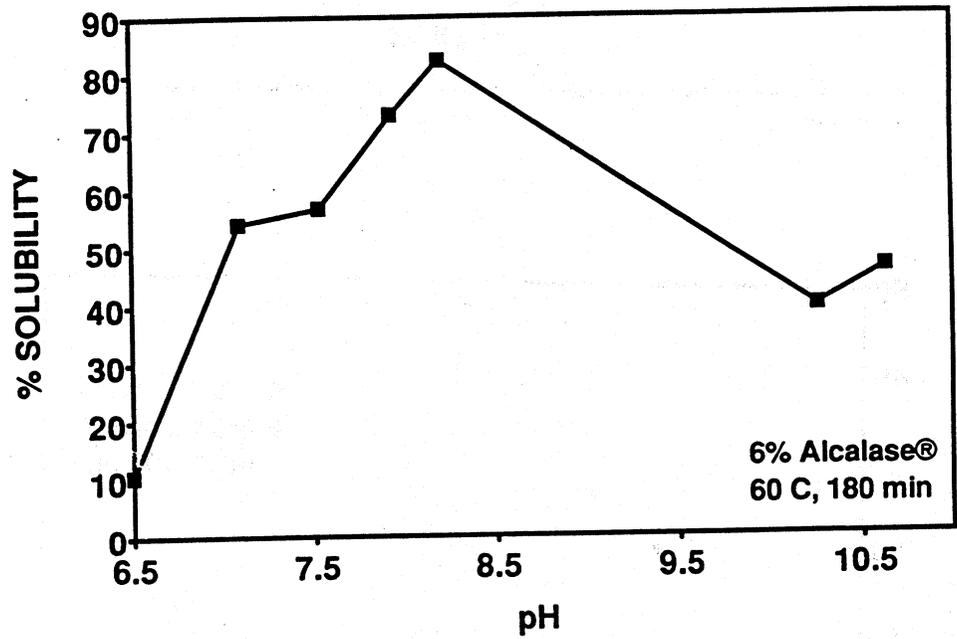


FIG. 9. — Effect of pH on solubility of ground blue stock treated with Alcalase®.

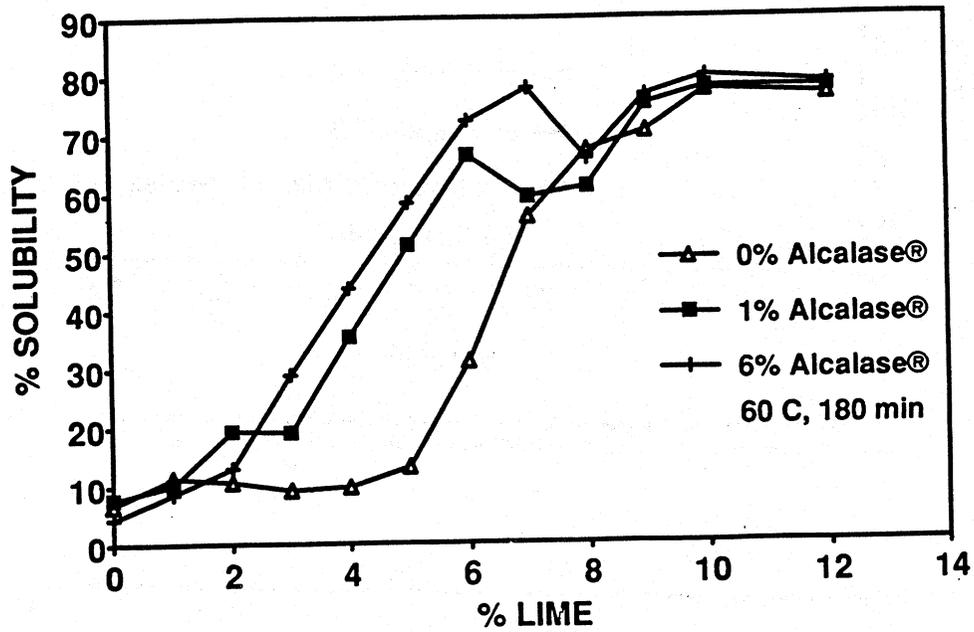


FIG. 10. — Effect of lime and Alcalase® concentrations, expressed as percent of wet weight, on solubility of commercial chrome shavings.

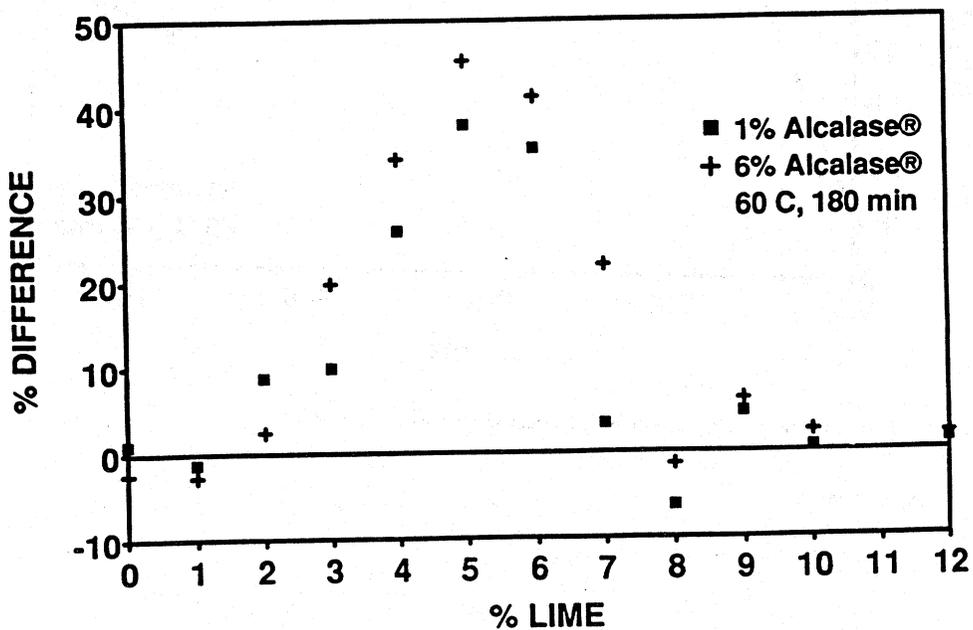


FIG. 11. — Improvement in solubility of commercial chrome shavings over treatment with lime alone by addition of Alcalase®.

The optimal temperature for the enzymic treatment was also examined. The optimal temperature range for Alcalase® was found to be 55-65°C. In this study, the reaction was repeated from 50-75°C in five-degree increments. The results are shown in Figure 4. The optimum temperature for treatment of the shavings appears to be from 60 to 65°C. Further increase in temperature did not lead to improvement in the percent solid dissolved.

The optimal time for the enzymic treatment was investigated. The reaction was run at 60°C, using 6% lime, 500% float and 6% enzyme based on the wet weight. The samples were incubated from 5 min. to 240 min. As shown in Figure 5, 42% of the blue shavings were dissolved after 5 min. Figure 6 shows reaction rate (the solubility divided by time) versus time. The optimal rate for the reaction was observed around 120 min.

Enzeco® Alkaline Protease-L also has its optimal activity in the alkaline pH range where the chromium would remain insoluble. A set of experiments was run to see if this enzyme could also dissolve the shavings. Lime was used again for maintaining the pH, and the reaction was run at 60°C, which was reported by Enzyme Development Corporation to be the optimal temperature for this enzyme. 2-6% of the enzyme was added along with 6% lime, both based on the total weight of the shavings. Figure 7 shows the results of this study. Five percent of the enzyme gave the highest solubility. The optimal pH of this enzyme was found around 9.5 and adjustment of the lime added was necessary to increase the efficiency of the reaction. These results are shown in Figure 8. Seven and one-half percent of lime gave the highest solubility. Varying enzyme concentrations were tried again at 7% lime. The results shown in Figure 7 indicated that the percent solubility leveled at approximately 4% concentration. Six percent gave only a slightly higher solubility.

#### SCALE-UP IN PILOT TANNERY

In the laboratory studies, 2.5-3.0 grams of blue shavings were used in each sample. In the pilot tannery, the experiments were scaled up to 100-1000 grams per sample. In the latter studies, the reaction took place in a heated shaker box, and the temperature was maintained between 60 and 70°C.

The filtered solutions were analyzed for chromium by atomic absorption spectroscopy, and were found to contain from 0.85 to 4.5 ppm of chromium. Disposition of the cake and the solutions will be discussed in a future publication.

### Discussion

Several papers have been reported concerning the enzymic treatment of chrome shavings. In one study from India, the pH at which the enzyme had optimal activity was in the acidic range and the chromium also dissolved along with the protein<sup>(7-11)</sup>. Also, this treatment needed to be run for up to 16 hr.

In the initial experiments using ground blue stock prepared in our tannery, 10% lime was used with the enzyme. The pH perhaps was too high for optimal enzyme activity, and only 47% of the shavings were dissolved. However, the resulting filtrate showed a pale yellow color indicating the absence of chromium and tested positive to ninhydrin, confirming the presence of protein or protein hydrolysate. In our preliminary experiments with this blue stock, in which we varied the amount of lime added, the chromium was also dissolved in the presence of the lower lime concentrations. We found subsequently that the pH had fallen below 7.6. Although the enzyme was still active, the chromium became soluble. Figure 9 shows the percent solubility of the shavings versus the pH of the solution after enzyme treatment. The sample to which 0.1 gram of lime was added gave the highest

solubility. The initial pH of this solution was about 10.2. As the reaction proceeded the pH fell continuously to about 8.2 where the chromium remained insoluble. It is therefore most critical that the correct amount of lime be introduced. Too little lime will give too low a pH for optimal enzyme activity and chromium will dissolve. Too much lime will give too high a pH leading to inactivation of the enzyme.

It should be noted that lime by itself can cause substantial dissolution of the chrome shavings. This effect has previously been reported in the literature<sup>(16-26)</sup>, and is most likely due to alkaline hydrolysis of the shavings. As shown in Figures 10 and 11, the advantage of the enzyme lies in that it can substantially reduce the amount of lime needed. If one plots the difference between using enzyme and no enzyme at various concentrations of lime, it is apparent that even the presence of 1% enzyme, as shown in Figure 11, will be more efficient in the solubilization of the shavings.

Figures 2 and 10 show the effect of lime concentration on the solubility of the shavings. There appears to be a dip in the data points around the 8% concentration. To confirm this interesting observation, experiments were carried out using 1% and 6% Alcalase®, with varying amounts of lime ranging from 6 to 9%. Figure 8 shows data from this experiment, in which the dip is most dramatic with the 1% concentration. The pH at this concentration of lime is a little too high for enzyme activity, but still too low for lime alone to solubilize the shavings.

In several recently-filed patents for treating chrome shavings, the inventors found it necessary to boil the shavings prior to the introduction of the enzyme<sup>(12,13)</sup>. One group found it necessary to maintain the pH at about 9.0, with sodium hydroxide<sup>(14)</sup>. These studies were carried out to obtain protein with specific molecular weights.

As it has been demonstrated from our data, we found that it was not necessary to boil the shavings before enzyme treatment. The lime is added for pH adjustment and the mixture is heated to 60-65°C over a period of 30 min. Also, we did not find it necessary to keep the pH at a level optimal for the enzyme, as long as it did not drop below 8.0 and dissolve the chromium.

## Conclusion

Chrome shavings can be treated with commercially available enzymes at moderate temperatures and for relatively short periods of time to give a chromium product which can be recycled into the bluing process and a protein product which has potential use as a fertilizer or is disposable by the sanitation department. This relatively simple treatment may provide a practical and economical solution to the disposal of a potentially hazardous waste. The present findings are the subject of a patent application.

## References

1. Novo Terapeutisk Laboratorium. Brit. Pat. 1,243,784 (1968).
2. Braeumer, K., Eckmayer, Z., Monsheimer, R. and Pfeleiderer, E., Ger. Offen. 2,705,671 (1978); *Leder und Hautemarkt. Gerbereiwissenschaft und Praxis*, **30**, 100 (1978).
3. Bronowski, K., Sagata, J. and Studniarski, K., *Leder*, **30**, 8 (1979).
4. Sauer, O., *Leder und Hautemarkt. Gerbereiwissenschaft und Praxis*, **36**, 70 (1984).
5. Iliskovic, N. and Mersed, I., *Koza Obuca*, **34** (6), 130 (1985).
6. Taylor, M.M., Diefendorf, E.J., Foglia, T.A., Bailey, D.G. and Fearheller, S.H., *JALCA*, **84**, 71 (1989).
7. Suseela, K., Parvathi, M.S., Nandy, S.C. and Nayudamma, Y., *Leder*, **34**, (1983).

8. Parvathi, M.S. and Nandy, S.C., *Leather Sci.*, **31**, 236 (1984).
9. Suseela, K., Parvathi, M.S. and Nandy, S.C., *Leder*, **37**, 45 (1986).
10. Parvathi, M.S., Suseela, K. and Nandy, S.C., *Leather Sci.*, **33**, 8 (1986).
11. Parvathi, M.S., Suseela, K. and Nandy, S.C., *Leather Sci.*, **33**, 303 (1986).
12. Monsheimer, R. and Pfeleiderer, E. Ger. Offen, 2,643,012 (1978).
13. Hafner, B., Sommerfeld, E. and Rockstroh, B., Ger. (East) DD 212,983 (1984).
14. Hafner, B., Rockstroh, B., Sommerfeld, E., Neumann, R. and Ingeborg, A., Ger. (East) DD 243,715 (1987).
15. Taylor, M.M., Diefendorf, E.J., Phillips, J.G., Fairheller, S.H. and Bailey, D.G., *JALCA*, **81**, 4 (1986).
16. Drugarin, C. and Cutui, M., *Tenside*, **6**, 197 (1969).
17. Blazej, A., *Oesterr. Leder Hautewirt.*, **5** (6), 37; (9) 57 (1969).
18. Halamek, C., *Kozarstvi*, **20** (3), 81 (1970).
19. Holloway, D.F., U.S. 4,100,154 (1978).
20. Antos, K., Markusovaka, E. and Hodul, P. *Kozarstvi*, **30** (1), 12 (1980).
21. Bataille, P., Faucher, R., Hamel, P. and Smith, W.E., *Technicuir*, **15**, 75 (1981).
22. Pecha, F., Tkac, J., Svoboda, V. and Klasek, A., *Kozarstvi*, **31** (9), 242 (1981).
23. Guardini, G., U.S. 4,483,829 (1983).
24. Bataille, P., Gagnon, F. and Smith, W.E., *JALCA*, **78**, 328 (1983).
25. Biedermann, M., Hille, H., Hussel, L. and Kiehn, H.J., Ger. (East) DD 218,549 (1985).
26. Becker, M., Ziebell, G., Bathke, B., Boehn, H., Schwarz, S., Wittstock, D. and Brandt, W., Ger. (East) DD 226,153 (1985).

## Discussion

MR. CHRIS EHRET, Garden State Tanning, discussion leader: Thank you Maryann for this fine presentation which holds the potential of great utility to the industry. I will open the floor up now for questions.

MR. SATYENDRA DE, Garden State Tanning: In your experiments you used chrome-leather shavings. How about other offals, such as larger pieces of chrome trimmings and treating them the same way you did?

MS. TAYLOR: It is highly desirable that your substrate be quite small. We have, however, taken pieces of blue sides, put them in a drum, and added the enzyme; after about three hours 50% of it was solubilized. Probably if it were run for a longer period of time it would be better, but it seems more satisfactory to cut the substrate into small pieces.

MR. ADEL HANNA, Paul Flagg Leather: Did you do any studies on the potential conversion of trivalent chromium to hexavalent chromium during the process?

MS. TAYLOR: No, we didn't.

MR. DAVID CROOKALL, Sentry/Custom Services Corp.: Can you give us any indication of the size of the vessel needed to treat a given number of pounds of shavings?

MS. TAYLOR: The reactor that I showed will hold eighteen gallons, and we were able to do twenty-five pounds of shavings, but you could certainly scale up from that. You just need a vessel with which you have good temperature control and good agitation.

DR. WILLIAM MARMER, USDA, ERRC: I don't believe that Maryann mentioned that we have applied for a patent for this process, and we hope to be able to license that patent at a future time. Right now we are interested in cooperating with the industry to scale up these experiments to a commercial scale, so if any of you are interested in cooperating with us on an experimental basis, please let us know.

MR. GEORGE STOCKMAN, Pfister and Vogel Tanning Company: Do you have any idea whether excessive temperatures and pressures would damage the enzymes?

MS. TAYLOR: It has 20% activity up to eighty degrees centigrade. If you brought it up to ninety degrees you would inactivate the enzyme. I don't know about pressure.

DR. NEILS SORENSEN (Novo Industries A/S): I am sure you inactivate the enzyme if you go over eighty degrees. We normally suggest that at eighty-five degrees you inactivate the enzyme in three minutes. I would be interested in knowing what the pH was, when you had this process running.

MS. TAYLOR: The initial pH was about 10.3. When the shavings start to dissolve, the sulphuric acid is exposed and peptides are hydrolyzed and the pH drops down to about 8.2. We recommend that the initial pH be no lower than 10.3, but if you go too high with the pH, the enzyme will be inactivated.

DR. KEN ALEXANDER, British Leather Confederation: Have you any ideas on how you are going to recover the protein from the solution? Is it possible for example to precipitate it under acid conditions, or is it too broken down?

MS. TAYLOR: I have not yet had success with precipitation. It has been suggested that it would be recovered by spray drying or lyophilization.

MR. CHRIS EHRET: Thank you once again Maryann for an excellent presentation.