

Research Article

Extraction of Silver from waste x-ray films using protease enzyme.

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ABSTRACT

The waste X-ray films contain 1.5 - 2 % (w/w) black metallic silver which is recovered and reused. Around 18-20% of the world's silver needs are supplied by recycling photographic waste. Since silver is linked to gelatin in the emulsion layer, it is possible to break the same and release the silver using proteolytic enzymes. Alkaline protease from *Bacillus subtilis* (NCIM 2724) was investigated for enzymatic hydrolysis of gelatin from waste X-ray films. At the end of the treatment, gelatin layer was completely removed leaving the polyester/cellulose film clean and silver was recovered in the hydrolysate, both of which can be reused. Various parameters such as pH, temperature, enzyme concentration, time etc. on silver removal from the film were studied. Gelatin hydrolysis was monitored by measuring increase in turbidity in the hydrolysate, which was accompanied by release of protein and hydroxyproline. Gelatin layer was stripped completely within 4 to 6 days with crude protease at 37⁰C, pH 8. Rate of gelatin hydrolysis increased with increase in protease concentration. The enzyme could be effectively reused for four cycles of gelatin hydrolysis. The purity of the silver was determined by Potentiometric method.

Key words: *Alkaline Protease, Bacillus subtilis, Silver recovery, Proteolytic activity.*

INTRODUCTION:

Silver is a soft, white, lustrous transition metal found in nature having chemical symbol Ag and atomic number 47. It is often combines with other elements, including oxygen sulfur chlorine and nitrogen. Pure silver has lustrous medium grey color. Silver is often extracted by mining or can be found at hazardous waste sites mixed with soil and or water. Silver has great industrial and economic applications and often used for making jewelry. It is also used for silverware, electronic equipment

and dental fillings [1,4]. Today, silver metal is also used in mirrors and in catalysis of chemical reactions. Its compounds are used in preparation of x-ray films and photographic films they are called as silver halides. Dilute silver called as silver nitrate solutions are used as disinfectants and micro biocides. If considered the medical approach silver is supplemented by antibiotics. The silver is used for the preparation of x-ray films, the two important ingredients of x-ray films are gelatin and

silver halide which is coated on both sides of the film but its thickness varies with the nature of the film, but it is usually no thicker than 10 mm. The materials used for the preparation of films are cellulose nitrate but more recently cellulose triacetate and polyester have been adopted. The waste x-ray films containing metallic silver spread in gelatin are a very good source for silver recovery compared with other types. The silver present in waste or used x-ray films can be recycled for industrial application by burning the x-ray films and by oxidation methods. Recovery of silver by burning the films directly, a general method at present, generates such a foul smell that it is desirable to replace burning by pollution-free methods. So to prevent air pollution, silver can be recovered by biological method that is from enzyme produced by bacteria. Since the emulsion layer containing silver contains the protein gelatin, it is possible to break it down using a photolytic enzyme protease which is extracellular enzyme produced by *Bacillus subtilis*[5,7]. A protease (also termed peptidase or proteinase) breaks down proteins. A protease is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Approximately one-fifth of the silver used each year worldwide ends up in X-ray film. The most common current process to recover silver from used X-ray film is to burn the film in a furnace and retrieve the precious metal from the ashes. However, this method is "rather expensive because of the cost of maintaining the furnace and treating the soot and foul-smelling smoke. Protease constitutes one of the most important industrial enzymes used. This enzyme accounts for 30% of the total world enzyme production. Among bacteria, *Bacillus* spp. is specific producers of extracellular alkaline proteases. Proteolysis enzymes can be classified as acidic, neutral and alkaline proteases, with regard to their pH working range. They are produced by a wide variety of microbial species like *Bacillus*

subtilis, *Aspergillus oryzae*, *Streptomyces cellulasa*, and *Aeromonas hydrophila* species. Most commercial proteases, mainly neutral and alkaline, are produced by organisms belonging to the genus *Bacillus*. Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms, and are essential for cell growth and differentiation. The extracellular proteases are commercial value and find multiple applications in various industrial sectors. A protease is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Bacteria secrete proteases to hydrolyse (digest) the peptide bonds in proteins and therefore break the proteins down into their constituent monomers. Bacterial and fungal proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated in by nutritional signals in these organisms.

MATERIALS AND METHODS:

Step: 1

1.1. Collection of bacterial culture.

Bacterial culture (*Bacillus subtilis* 2724) was collected from NCIM, Pune. Culture was maintained by subculturing in time to time intervals. For this loopfull of suspension was inoculated on Petri plates by following streak plate method containing growth medium. The composition of growth medium is given as follows:

Sr. No.	Name of the Component	Concentration in (g / L).
1	Glucose	11.1
2	Peptone	5.5
3	Yeast extract	5.5
4	K ₂ HPO ₄	11.1
5	MgSO ₄ .7H ₂ O	0.22
6	Agar	16.6

Table 1. Composition of Growth Medium Required for Maintenance of Bacterial Culture.

The plates were incubated at 37°C for 24 h. After completion of incubation period the colonies were obtained. For checking proteolytic activity loopfull of culture was again inoculated on another

37°C for 24 hrs. The suspension was centrifuged at 10,000 rpm, for 20 min and the clear supernatant was used as the crude enzyme.

1.3. Estimation of Proteins by Folin Lowry method.

S. N	Working stock (ml)	Distilled water(ml)	Alkaline Cu solution (ml)		FC reagent (ml)		O.D at 650nm
1	Blank	2	5	Mix thoroughly & allow to Stand at RT for 10 min	0.5	The tubes were incubated for 30 minutes and O.D. was taken at 650nm.	0.33
2	0.2	1.8	5		0.5		0.53
3	0.4	1.6	5		0.5		0.64
4	0.6	1.4	5		0.5		0.77
5	0.8	1.2	5		0.5		1.16
6	1.0	1.0	5		0.5		1.00
7	Test(2.0)	-	5		0.5		0.98

Petri plates containing growth medium consist of peptone 5, beef extract 3 (or yeast extract 1), agar 15. Concentration is g / L. with substrate 1% of casein. The plates were incubated at optimal temperature for required time. After the completion of incubation period the zone of clearance was obtained around the colony. That is the casein as a substrate is degraded by the extracellular enzyme. By comparison of the activities of microorganisms for hydrolysis of gelatin, casein and starch at two different pH (7 and 10) depending upon the zone of clearance on the plate, an alkaline protease producer was be selected for further experimental studies.



Fig. 1. Zone of Clearance.

1.2. Production and optimization of enzyme along with activity:

For the production of enzyme the nutrient broth medium was prepared and the colony that gave best zone of clearance was inoculated in fermentation media. The process was carried out at

Table 2. Protocol for Estimation of the Crude Enzyme/Protein

1.4. Characterization of Enzyme:

• Dehairing property/ Dehairing of goat skin:

Goatskin was cut in to 5 x3 cm pieces and incubated with the protease (5U/ml) in 0.2 M glycine NaOH buffer pH 10.5 37°C. The skin was checked for removal of hair at different incubation times.



Fig. 2 Goat skin before the treatment of Enzyme



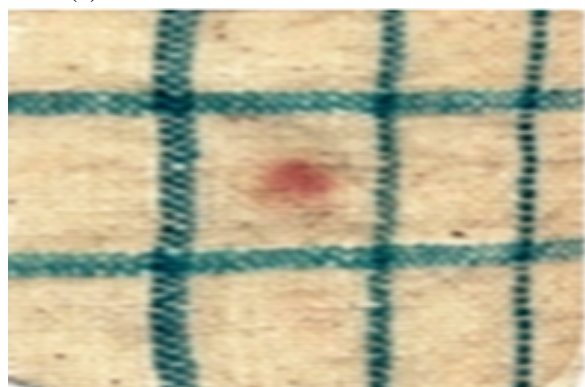
Fig.3 Goat skin after the treatment of Enzyme.

- **Destaining of blood.**

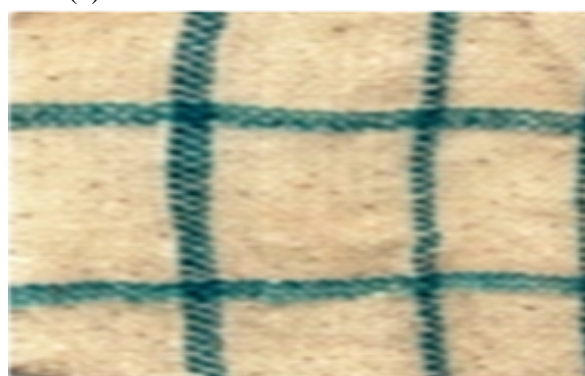
A clean piece of white cloth (5x5) was stained with blood and allowed to dry the cloth. The cloth was incubated with the protease at 37 °C incubation. After incubation time, cloth was rinsed with water for 0 min, 1 min, 2 min min and then dried. The same procedure was done for the control except incubation with the enzyme solution.



(a)



(b)



(c)

Fig.4. (a) 0 to 5 min. Fig. (b) 5 to 10 min Fig.(c) 10 to 15 min

Step 2:

Extraction of silver and degradation of gelatin by the treatment of enzyme suspension.

- The used X-ray films were washed with distilled water and wiping with cotton, impregnate with ethanol, and cut into $4 \times 4 \text{ cm}^2$ pieces after drying in an oven at 40°C for 30 minutes.(17)
- Each of the film was submerged in series 100 ml of stock enzyme extract and the pH of the Solution was adjusted to 8.0.
- The solution along with submerged film was stirred at 50°C in a water bath until the gelatin-silver layer is stripped completely. Required numbers of films were stripped and the obtained slurry was dried and smelted in the presence of borax at 900°C in a furnace.
- During the smelting ammonium chloride was added for getting the white color to the silver.
- After the completion of the smelting process the dried powder were washed with the water and the white colored crystals of the silver was collected.
- The purity of the recovered silver was determined potentiometrically or the purity can also obtained by NaOH stripping method with potentiometric titration.



Fig. 5. X-ray film submerged in Enzyme Solution.

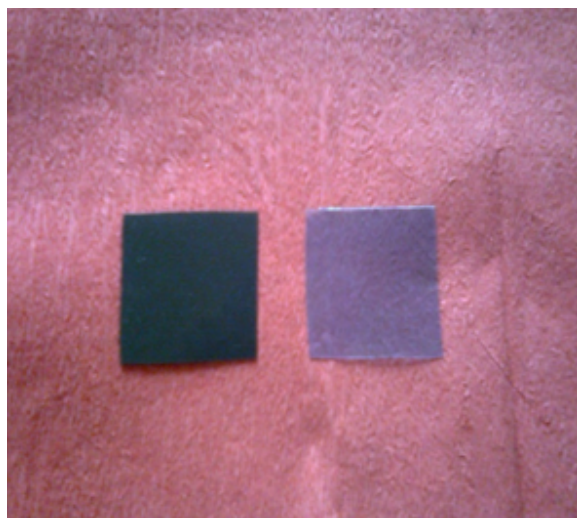


Fig. 6. Decolorization of X-ray Film after the treatment of Enzyme.



Fig. 7. Smelting Process for Silver Recovery with Continuous addition of Ammonium Chloride for removing the impurities and for getting the white color to the silver.



Fig. 8. Recovered Silver after smelting

Step 3.

Results:

- From step 1.1: The protein/ enzyme were estimated by Folin Lowry method and the amount of protein was obtained as 145 $\mu\text{g/ml}$.
- Incubation of the protease with skin for dehairing showed that after 2-3h incubation of the protease (5U/ml) with goat skin, hair was removed very easily (Figure-2 & 3).
- In case of removing blood stain from cloth, it was seen that the protease removed blood stain in 10 to 15 min duration of time.
- Silver Recovery :Under the obtained conditions (pH=8.0 and 55 $^{\circ}\text{C}$), 0.5012 g silver was recovered according to the procedure described in the Silver Recovery Method section, and hence the silver content of the used photographic films was calculated to be 0.49 mg/cm^2

CONCLUSION:

Silver was successfully stripped and recovered in good yield and sufficient purity from the used photographic films by the enzymatic method. The method is easy and cheap but it has some disadvantages such as the bad smell and burning step at high temperatures. Otherwise, the enzyme, obtained from *Bacillus subtilis* NCIM 2724, is not thermophilic and its activity is high at a pH near neutral. Hence it can be thought that thermophilic

and alkaliphilic enzymes will yield good result in the stripping of the gelatin-silver layer.

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