Frontiers in Environmental Microbiology
2016; 2(6): 45-48
http://www.sciencepublishinggroup.com/j/fem
doi: 10.11648/j.fem.20160206.14
ISSN: 2469-7869 (Print); ISSN: 2469-8067 (Online)

Eco Friendly Recovery of Silver from Used X-ray Films by Alkaline Protease of *Bacillus Cereus* Strain S8

Lakshmi B. K. M., Hemalatha K. P. J.*

Department of Biochemistry, Andhra University, Visakhapatnam, India

Email address:

Kamalab42@gmail.com (Lakshmi B. K. M.), hemalathakpj@gmail.com (Hemalatha K. P. J.)

*Corresponding author

To cite this article:

Lakshmi B. K. M., Hemalatha K. P. J. Eco Friendly Recovery of Silver from Used X-ray Films by Alkaline Protease of *Bacillus cereus* Strain S8. *Frontiers in Environmental Microbiology.* Vol. 2, No. 6, 2016, pp. 45-48. doi: 10.11648/j.fem.20160206.14

Received: October 13, 2016; **Accepted**: October 31, 2016; **Published**: December 29, 2016

Abstract: Silver is a precious metal used in photographic/X-ray film industry. 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. In the present work an environmentally friendly recycling system to peel away the base coat from the X-ray film by using an proteolytic enzyme was developed. Alkaline protease from *Bacillus cereus* strain S8(MTCC NO: 11901) was studied for silver recovery from used X-ray films. Enzyme extract was obtained by using the medium supplemented with Molasses,1%(w/v); Potassium nitrate, 0.75%(w/v); salt solution- 5%(v/v) {$MgSO_4.7H_2O$, 0.5%(w/v); KH_2PO_4,0.5%(w/v)}; $FeSO_4.7H_2O$, 0.01%(w/v) and $CaCO_3$, 0.5% respectively which has optimum activity at pH 10.0 and 75°C. The silver having purity of 98.6% was recovered by smelting the obtained slurry in the presence of borax. The metal impurities (Al, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Sn) in the recovered silver were determined using the ICP-MS method.

Keywords: Alkaline Protease, *Bacillus Cereus* Strain S8, Used X-ray Films, Silver Recovery, ICP-MS

1. Introduction

Silver is a rare, precious, naturally occurring metal, often found deposited as a mineral ore in association with other elements. Fifty percent of silver produced is used in photographic and imaging materials. Silver is unique in its ability to react with light to produce images in applications such as photography and radiography (X-rays). Major emissions of silver are from the manufacture and disposal of certain photographic and X-ray films. X-rays films used in medical applications are made of a plastic sheet (polyester film) coated with a thin coating of gelatin (protein) impregnated with silver grain.

It has been reported that 25% of the world's silver needs are supplied by recycling, out of which 75% is obtained from photographic waste. The amount of silver varies between 1.5% and 2.0% by weight. With an increasing demand for silver in the world, recent attention is focused on X-ray/photographic films as one of the secondary sources of silver owing to the considerable amount of silver present in them. 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 jewellery. It is also used for silverware, electronic equipment and dental fillings [1]. 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.

Various studies have been carried out to recover the silver from photographic/X-ray film wastes and following methods are reported in literature: (a) burning the films directly (b) oxidation of the metallic silver following electrolysis (c) stripping the gelatin-silver layer using different chemical solutions (d) enzymatic hydrolysis of gelatin [2]. Recovery of silver by burning the films directly, a conventional method used at present is the most primitive method and generates undesirable foul smell. The method causes environmental pollution and polyester film on which emulsion of silver and gelatin is coated cannot be recovered. Stripping the gelatin-

silver layer by chemical methods using ammonium thiosulphate, sodium thiosulphate, nitric acid or reagents such as sodium cyanide, NaOH, nitric acid or organic compounds cause environmental hazards and are either time consuming or very expensive, while the use of NaOH at high temperatures poses a serious industrial safety problem. For this reason, the methods applied to recover silver from X ray/photographic waste should be cost effective and have minimal impact on environment and enzyme based methods can be an alternative option. Since the emulsion layer on X-ray film contains silver and gelatin, it is possible to break down the gelatin layer using proteases and release the silver [3]. The enzymatic hydrolysis of the gelatin layers on the X-ray film enables not only the recovery of the silver, but also the polyester base can be recycled. Hence in recent years, enzymatic methods using microbial proteases are being explored as alternatives to the burning and oxidation methods of silver recovery from photographic/X-ray films [4, 5]. Basically enzymatic processes are more specific and remove gelatin layer from X-ray film in few minutes without damaging the polyester film base. Gelatin molecules are cross linked with hardners and it is difficult for the usual proteases to degrade it in a short time. Most of the proteases used so far for silver recovery are of bacterial origin.

The present investigation involves a different method for silver recovery from the waste X-ray/photographic films with high purity by using the enzyme extract obtained from *Bacillus cereus* StrainS8, not used before for this purpose. The metal impurities (Al, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Sn) in the recovered silver were determined using the ICP-MS method.

2. Materials and Methods

2.1. Microorganism and Culture Maintenance

The microorganism used in this study was isolated from soil samples [6] screened using a skim milk agar plate depending up on the zone of hydrolysis. It was identified as *Bacillus cereus* strain S8 (MTCC No:11901) according to morphological, biochemical tests and 16S r RNA gene sequencing.

2.2. Production of Enzyme

Protease enzyme production was carried out using standard media glucose, 0.5%(w/v); peptone, 0.75%(w/v); salt solution-5%(v/v) {($MgSO_4.7H_2O$, 0.5%(w/v); KH_2PO_4 0.5%(w/v)}; and$FeSO_4.7H_2O$, 0.01%(w/v) at 160rpm. The culture medium was harvested and was subjected to centrifugation at 10,000 rpm for 20 min to obtain crude extract, which was used as enzyme source. The potential producer strain S8 was taken for further optimization studies to enhance the protease production.

2.3. Silver Recovery Method

The used X-ray/photographic films were supplied by the Radiology Department, Rangaraya medical college, Kakinada. The used X-ray films were washed with distilled water and wiped with cotton impregnated with ethanol, and was cut into 4 x4 cm^2 pieces after drying in an oven at 40°C for 30 minutes. Each of the film was rinsed in series 100 ml of stock enzyme extract and the pH of the solution was adjusted to 10.0. The solution and the film were stirred at 75°C in a water bath until the gelatin-silver layer was stripped completely. Twenty films were stripped and the obtained slurry was dried and smelted in the presence of borax at 1100°C in a furnace. The purity of the recovered silver was determined potentiometrically.

2.4. Determination of Trace Metal Impurities in the Recovered Silver

2.4.1. Sample Cleaning

The recovered silver (0.1-0.3 g) was transferred to a 100 ml PTFE beaker and 2.5 ml of cold 0.1 M HNO_3 was added with approximately 10 min agitation, followed by a thorough rinsing in distilled water. It was then dried and stored in a desiccator.

2.4.2. Sample Pretreatment

The cleaned silver was weighed and 3 ml of double distilled water and 3 ml of concentrated HNO_3 were added to it in a PTFE beaker. The sample was heated at temperatures below boiling point, and then 4 ml of 2.3M HCl was added progressively to form a fine precipitate of silver chloride. After the addition of 2 ml concentrated HCl and about 20 h of agitation, the solution was filtered by a G4 crucible under vacuum. The filtrate was made up to 25 ml with double distilled water in a calibrated flask, and then the trace metals in the filtrate were determined by ICP-MS.

2.5. Results and Discussion

Four major types of proteases are distinguished: alkaline (serine) proteases, thiol proteases, acid (carboxyl) proteases and neutral (metallo) proteases. Alkaline proteases have a serine residue at the active site and they exhibit activity in the neutral-alkali region with pH optima at values 8.0-11.0. *Bacillus* strains are the major source for alkaline and neutral proteases. The protease activity of the culture filtrate of *Bacillus cereus* strainS8was 192.35 ± 0.99 U/ml according to the method described in the protease assay section. The results showed that *Bacillus cereus* strainS8produced alkaline protease and this enzyme can be efficiently used for the recovery of silver from used X-ray films by degrading the gelatin layers on the films.

On the other hand, it was noted that it takes 15 min at 75°C to decompose the gelatin layer when *Bacillus cereus* strain S8was used, while the other alkaline proteases took more than 20 min to act and the enzyme rapidly became inactive at the temperatures above75°C. Table 1 shows the stripping capacity (SC) of enzyme extract at various temperatures; SC is high at 70°C. Therefore, 70°C was selected as the stripping temperature for gelatin-silver layer from the used photographic films by enzyme extract.

Table 1. *The stripping capacity of enzyme extract at various temperatures.*

S.No	Temperature (°C)	Stripped film number	Stripping Capacity (SC) (g)
1	30	20	0.8
2	40	20	1.8
3	50	20	2.9
4	60	20	3.4
5	70	20	4.8
6	80	20	2.1
7	90	20	0.6

Under the obtained conditions (pH 10.0 and 75°C), 0.3013 g silver was recovered according to the procedure described in the silver recovery method section. The purity of the recovered silver was determined potentiometrically and calculated to be 98.66 ± 0.11% and hence the silver content of the used photographic films was calculated to be 0.36 mg/cm^2. The X-ray films before and after enzyme treatments were shown in the Figure1.

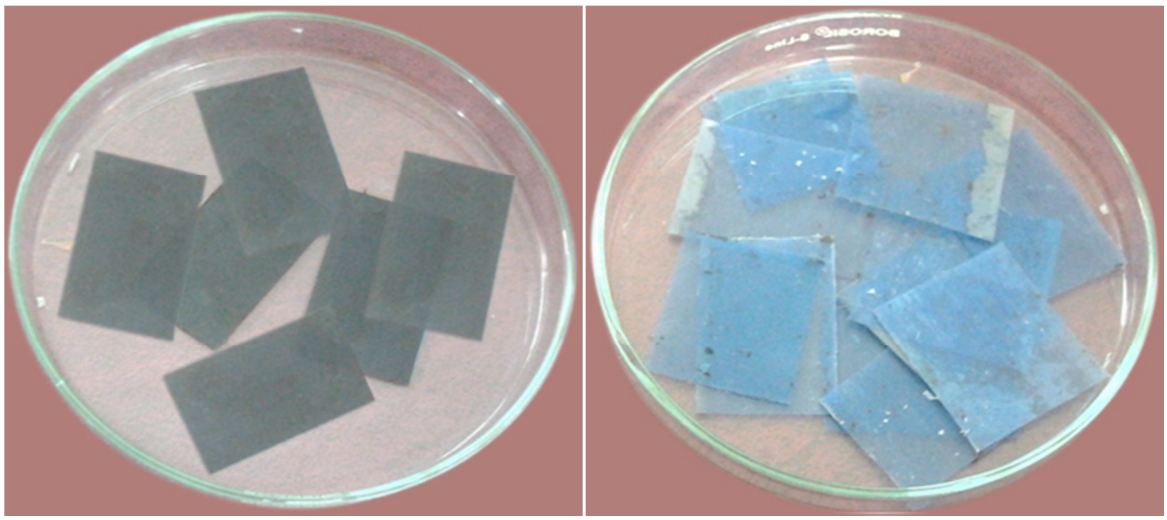

Figure 1. *Used X-ray film and film after enzymatic hydrolysis of gelatin-silver layer.*

Well-known enzymes used in silver recovery from films are alkaline proteases from *Bacillus subtilis.* It has been reported that it takes 30 min at 50 to 60°C to decompose the gelatin layer when Subtilisin BPN', an alkaline protease from *Bacillus subtilis* strain N', was used and treatment at 30°C increased the decomposition time to two to three hours. On the other hand all alkaline proteases from the neutrophiles took more than 20 min to act. Similar study was carried out by [7] alkaline protease from *Aspergillus versicolor* PF/F/107 successfully stripped and recovered silver (0.135) in good yield (0.337%). [8] successfully recovered 0.5012 g of silver from *Bacillus subtilis* (NCIM 2724) at pH 8.0 and 55°C. [9] had reported the use of alkaline protease from *B. subtilis* and *Bacillus* sp. B21-2 in silver recovery from the used X-ray films. [10] reported the recovery of silver from used X-ray film using alkaline protease from *Bacillus subtilis* sub sp. *Subtilis* with gelatin layer was stripped completely within 30 min with 97 U ml-1 protease at 50°C and pH 8.0.

Table 2 shows the list of trace metal impurities (eleven metals) that are generally present in the recovered silver. The recovered silver contained low Mg, Pb, Sn and Cd impurities. High purity silver (5'9 or 6'9 grade silver) contained lower Fe, Cu, Pb, Ni and Sn impurities than FS 14.

Table 2. *Results (g/g) for the trace impurities in the recovered silver with comparison of some results for high purity silver given in the literature.*

S. No	Element	The recovered silver	EM9465	EM9343	FS 14
1	Fe	16.05	1.85	0.26	47.6
2	Cu	2.084	0.132	0.078	61.8
3	Mg	0.013	0.087	0.0640	-
4	Cr	0.471	-	-	-
5	Pb	0.301	0.597	0.011	33.8
6	Al	3.410	0.082	0.024	-
7	Mn	0.001	0.011	0.007	-
8	Co	9.891	0.0024	-	-
9	Ni	6.762	0.011	0.007	53.9
10	Sn	0.011	0.006	0.039	44.0
11	Cd	0.012	0.018	0.012	-

EM9465: high purity silver (5'9 grade silver), EM9343: high purity silver (6'9 grade silver), FS 14: Fine silver

3. Conclusion

Silver was successfully stripped and recovered in good yield with sufficient purity from the used photographic films by the enzymatic method. The method is easy and cheap. The enzyme obtained from *Bacillus cereus* strain S8, is thermophilic and its activity is high at alkaline pH (10.0). For

this reason, the hydrolysis speed of the gelatin with enzyme is high. Hence it can be thought that thermophilic and alkaliphilic enzymes yield good result in the stripping of the gelatin-silver layer.

References

[1] Bogner R. L., (1962). Snyder High dosage oral chymotrypsin as an adjunct in plastic surgery. J. Int. College Surg., 37: 289-295.

[2] Nakiboglu N., Toscali D., Nisli G., (2003). A novel silver recovery method from waste photographic films with NaOH stripping.Turk. J. Chem., 27: 127-133.

[3] Nakiboglu N., Toscali D., Yasa I., (2001). Silver recovery from waste photographic films by an enzymatic method. Turk. J. Chem.25: 349-353.

[4] Ingale S. S., Rele M. V., Srinivasan M. C., (2002). Alkaline protease production by *Basidiobolus*: effect of darmform morphogenesis and cultural conditions on enzyme production and preliminary enzyme characterization. World J. of Micro.Biotech., 18: 403-408.

[5] Masui A., Fujiwara N., Takagi M., Imanaka T., (1999). Feasibility study for decomposition of gelatin layers on X-ray films by thermostable alkaline protease from alkaliphilic *Bacillus* sp. Biotech. Tech., 13: 813-815.

[6] Lakshmi B. K. M., Ratnasri P. V., Ambika Devi K. and Hemalatha K. P. J., (2014). Screening, optimization ofproduction and partial characterization of alkaline protease from haloalkaliphilic *Bacillus* sp., International Journal of Research in Engineering and Technology, 3:435-445.

[7] Vaishali Ch., (2013). Recovery of Silver from used X-ray films by *Aspergillusversicolor* protease. Journal of Academia and Industrial Research., 2 (1): 39-41.

[8] Jayant P., Parpalliwar., Pallavi S., Patil I. D.,Patil and Umesh B., Deshannavar., (2015). Extraction of Silver from waste x-ray films using protease enzyme. International Journal of Advanced Biotechnology and Research. 6(2): 220-226.

[9] Fujiwara N., Tsumiya T., Katada T., Hosobuchi., (1989). Continuous recovery of silver from used X-ray films using a proteolytic enzyme. Process Biochemistry, 24: 155-156.

[10] Amira Hassan Al-Abdalall and Eida Marshid Al-Khaldi., (2016). Recovery of silver from used X-ray film using alkaline protease from *Bacillus subtilis* sub sp. *Subtilis*.