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Silver Recovery from Waste Photographic Films by an Enzymatic Method

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A different enzymatic method was developed for stripping the silver from waste X-ray photographic films. The silver having purity of > 99% was recovered by smelting the obtained slurry in the presence of borax. Enzyme extract, obtained from *Bacillus subtilis* ATCC 6633 by modifying Horikoshi medium, was used for stripping the silver layer. 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. The results were compared those in with literature for high purity silver using the same method.

Key Words: Silver recovery,waste photographic films, Bacillus subtilis

Introduction

The waste X-ray photographic films containing black metallic silver spread in gelatin are a very good source for silver recovery compared with other types of film. The amount of silver varies between 1.5% and 2.0% by weight. Various studies have been carried out over a long period of time to recover the silver from these wastes, and most of them are patented. The stripping methods using proteolytic enzymes obtained from various microorganisms[1−3] and alkali hydroxides[4−7] have been used more often for recovery of the silver than the burning and 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. Since the emulsion layer containing silver contains the protein gelatin, it is possible to break it down using a proteolytic enzyme protease. Well-known enzymes used in silver recovery from films are alkaline proteases from *Bacillus subtilis.* It has been reported that it takes 30 minutes 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 neutrophile took more than 20 minutes to act[1].

In this paper we report a different method for silver recovery from the waste X-ray photographic films with high purity by using the enzyme extract obtained from *Bacillus subtilis* ATCC 6633, 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.

Experimental

All of the chemicals were of analytical grade. Standard buffer tablets (4.01, 7.01 and 9.01 at 20°C) were supplied by B.W. & Co.

Horikoshi medium (8): 10 g glucose, 5 g polypeptone, 5 g yeast, 1 g KH_2PO_4, 2 g $MgSO_4.7H_2O$ and 10 g Na_2CO_3 were dissolved in distilled water and diluted to 1000 mL. $Na_2CO_3$was sterilized separately and added to basal medium after sterilization. This medium was used for cultivation of *Bacillus subtilis* ATCC 6633.

Standard buffer solutions were prepared by dissolving one tablet in a small amount of warm distilled water and making up to 100 mL with cold distilled water. *Bacillus subtilis* ATCC 6633 was obtained from Ege University Faculty of Science Basic and Industrial Microbiology Department. The used X-ray photographic films were supplied by the Radiology Department of Science of Medicine Faculty Hospital, Ege University, Izmir.

A Jenway 3040 pH-meter with combined glass electrode was used for pH measurements. A Webecke A 25 Autoclave, a Heraeus KB 600 Incubator and a New Brunswick Scientific G 24 thermostatic shaker were used in the activation and cultivation steps for *Bacillus subtilis* ATCC 6633. A Hettich Universal 30 RF refrigerated centrifuge was used to obtain clear enzyme extract (supernatant) from the fermentation medium. A Chiltern HS 31 magnetic stirrer and heater was used for stirring and heating. A Lenton furnace was used for smelting the slurry containing silver. A Tacussel TS 60/N potentiometer equipped with a Ag/AgCl electrode and a saturated calomel electrode connected with a KNO_3 (saturated) salt bridge was used to determinate the purity of the recovered silver. Trace metal impurities were determined by an HP 4500 ICP-MS in the laboratory of Izmir Reginal Sanitation Institute.

Cultivation and Preparation of enzyme extract

Bacillus subtilis ATCC 6633 was activated in Nutrient Agar (Difco) slants for 24 hours at 30°C[9]. Two loopfuls of activated culture were inoculated into 100 mL of modified Horikoshi medium in 500 ml flasks. One percent gelatin was added to the Horikoshi medium to induce production of protease and it was incubated by shaking at 200 rpm for 24 hours at 30°C. Also 5 mL of this culture was added to modified Horikoshi medium and incubated at the same conditions. After cultivation, cell-free enzyme filtrate was prepared by centrifugation at 10000 rpm for 10 min at 4°C[8].

Protease Assay

Determination of protease activity was carried out by the modified method of Anson described by Takami et al.[10]. A 0.5 mL aliquot of cell-free enzyme was added to 2.5 mL of 0.6% Hammerstein casein solution (pH=10.5 made up with 50 mL glycine, NaCl, NaOH buffer). After incubation for 20 min at 30°C, 2.5 mL of trichloroacetic acid (TCA) solution, containing 0.11 M TCA, 0.22 M sodium acetate and 0.33 M macetic acid, was added to stop the reaction. The mixture was further incubated for 30 min at 30°C and then filtered

with Toyo-Roshi filter paper No 5C. Then 2.5 mL of 0.5 N Na_2CO_3 solution and 0.5 mL twofold-diluted Folin-ciocalteau reagent was added to 0.5 mL of filtrate. After standing for 30 min at room temperature, the absorbance was measured at 660 nm. One unit protease activity is defined as the amount of the enzyme to produce the digest which is not precipitated by TCA solution and which gives an absorbance value equivalent to 1μg of tyrosine per minute at 30°C.

Silver recovery method

The used X-ray films were washed with distilled water and wiped with cotton impregnated with ethanol, and were cut into 4×4 cm^2 pieces after drying in an oven at 40°C for 30 minutes. Each of the films was rinsed in series 100 mL of stock enzyme extract and the pH of the solution was adjusted to 8.0. The solution and the film were stirred at 50°C in a water bath until the gelatin-silver layer was stripped completely. Seventy 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.

Determination of trace metal impurities in the recovered silver

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 minutes agitation, followed by a thorough rinsing in distilled water. It was then dried and stored in a desiccator[11].

Sample pretreatment

The cleaned silver was weighed and 3 mL of doubly 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 2.3 M HCl were 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 doubly distilled water in a calibrated flask, and then the trace metals in the filtrate were determined by ICP-MS[11].

Results and Discussion

Four major types of proteases are distinguished: alkaline (serin) proteases, thiol proteases, acid (carboxyl) proteases and neutral (metallo) proteases. Alkaline proteases have a serine residue at the active side and they exhibit activity in the neutral-alkali region with pH optima at values 8.0-11.0[12]. *Bacillus* strains are the major source for alkaline and neutral proteases. *Bacillus subtilis* ATCC 6633, used in this study, produces both types of protease: *Bacillus amylolliquefaciens* and *Bacillus cereus*[13].The protease activity of the culture filtrate of *Bacillus subtilis* ATCC 6633 was 22.35 U/mL according to the method described in the Protease Assay section. The effect of pH on the stripping of the waste film by using enzyme extract was investigated at 30 and 40°C and the optimum pH was determined to be 8.0 (Figure 1). The results show that *Bacillus subtilis* ATCC 6633 produces neutral and alkaline proteases and this enzyme mixture 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 less than 15 minutes at 50°C to decompose the gelatin layer when *Bacillus*

subtilis ATCC 6633 was used, while the other alkaline proteases took more than 20 minutes to act and the enzyme rapidly became inactive at the temperatures $\geq 60°C$. Table 1 shows the stripping speed (SS) and the stripping capacity (SC) of enzyme extract at various temperatures; SS and SC are high at 50°C. Therefore, 50°C was selected as the stripping temperature for gelatin-silver layer from the used photographic films by enzyme extract.

Under the obtained conditions (pH=8.0 and 50°C), 0.4013 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 99.16 ± 0.11 % (for the 95% confidence level, N=5) and hence the silver content of the used photographic films was calculated to be 0.36 mg/cm^2.

Table 2 shows the trace metal impurities in the recovered silver for eleven metals and a comparison of some results for high purity silver given in the literature[11]. The recovered silver contained low Mg, Pb, Sn and Cd impurities as well as high purity silver having 5'9 or 6'9 grade silver, and lower Fe, Cu, Pb, Ni and Sn impurities than FS 14.

In 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* ATCC 6633, is not thermophilic and its activity is high at a pH near neutral. For this reason, the hydrolysis speed of the gelatin with enzyme is low in contrast the thermophilic and alkaliphilic enzyme. Hence it can be thought that thermophilic and alkaliphilic enzymes will yield good result in the stripping of the gelatin-silver layer.

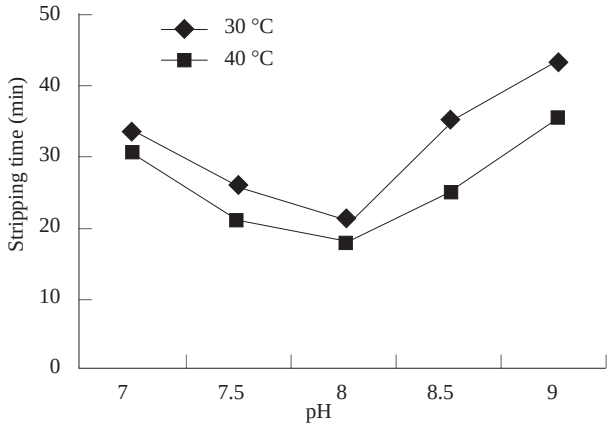

Figure 1. The effect of the pH on stripping of the waste film by using enzyme extract

Table 1. The stripping speed and the stripping capacity of enzyme extract at various temperatures

T°C	SFN_t	ΣSt (min)	SS cm^2/h	SC m^2/L extract
40	86	2025	509.6	1.72
50	103	2294	538.8	2.06
60	13	360	433.3	0.26

SFN_t,, stripped film number at the same time and St, overall stripping time

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

Element	The recovered silver	EM9465	EM9343	FS 14
Fe	20.01	1.85	0.26	47.6
Cu	1.784	0.132	0.078	61.8
Mg	0.033	0.087	0.0640	-
Cr	0.671	-	-	-
Pb	0.010	0.597	0.011	33.8
Al	4.620	0.082	0.024	-
Mn	0.021	0.011	0.007	-
Co	20.89	0.0024	-	-
Ni	9.962	0.011	0.007	53.9
Sn	0.021	0.006	0.039	44.0
Cd	<0.001	0.018	0.012	-

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

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