

SHORT COMMUNICATION

Recovery of Silver from used X-ray films by *Aspergillus versicolor* protease

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Abstract

Silver is an important industrial metal used in various sectors such as photographic films, x-rays, jewelries, silver wares and electronic items. There are three reasons as to why silver should be recovered from used X-ray films namely conservation of a precious metal, economic return and environmental concerns. A photographic film/X-ray film uses silver because of its unmatched quality as a light-sensitive material for creating a photographic image. Silver is not destroyed in the photographic process and can be reused and recovered. An alkalophilic fungal strain *Aspergillus versicolor* PF/F/107 isolated from poultry farm produced alkaline protease at 40°C and 9.0 pH. Alkaline protease successfully stripped and recovered silver in good yield from the used photographic films. The used X-ray film contains 1.5-2.0% (w/w) metallic silver (black in color) which can be recovered and reused.

Keywords: Silver, photographic films, *Aspergillus versicolor*, alkaline protease, metallic silver.

Introduction

Numerous studies carried out from time to time to recover silver from photographic films as well as from x-ray films are patented. The silver recovery methods from these wastes includes: burning the films directly (Ewell and Piper, 1970), oxidation of metallic silver followed by electrolysis (Ajiwe and Anyadiegwu, 2000), stripping the silver-gelatin layer using microbial enzymes specifically alkaline proteases (Nakiboglu *et al.*, 2001) and stripping the gelatin silver layer using different chemicals (Syed *et al.*, 2002). Recovery of silver by burning the films creates environmental pollution and health hazards. On the other hand, enzyme from microbial source breaks the gelatin layer embedded with silver in films creating pollution free stripping.

Enzymatic method although slow is free from pollution and cost-effective too. 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 50-60°C to decompose the gelatin layer and the treatment with same enzyme at 30°C took more time in decomposition (2-3 h). Alkaline proteases from the neutrophil have been found to take more than 20 min to act (Fujiwara *et al.*, 1989). Decomposition of x-ray films by partially purified extracellular alkaline protease produced by *Streptomyces avermectinus* NRRLB-8165 was shown by Ahmed *et al.* (2008). The methods used for silver recovery from x-ray wastes are based on transition of silver into solution i.e. ionic form and then by electrolysis producing silver metal. The amount of silver varies from 5-15 g/kg of film. Various studies have been carried out over a long period of time to recover the silver from these waste and most of them are patented.

The stripping methods using proteolytic enzymes are obtained from various microorganisms (Chwojnowski and Lada, 1985; Mueller *et al.*, 1990; Fujiwara *et al.*, 1991; Horikoshi, 1999; Masui *et al.*, 1999; Kibata and Nakamura, 1999), inorganic and organic chemicals (Garcia, 1987; Chwojnowski and Lada, 1985; Messerschmidt, 1988; Liu, 1989) and mechanical methods (Rettig and Buser, 1988) have also been used more often for recovery of the silver than burning and oxidation methods. In view of the above facts, alkaline protease produced by *Aspergillus versicolor* PF/F/107 was checked for its capability to recover metallic silver from used x-ray films.

Materials and methods

Alkaline protease production: Medium I containing (g/L) glucose-2.0, casein-0.5, peptone-0.5, yeast extract-0.5, KH₂PO₄-1.0, MgSO₄-0.5, FeSO₄.7H₂O-0.1 was used as production medium. Autoclaved erlenmeyer flasks containing 50 mL of medium I were inoculated with 1 mL of spore suspension (containing 2×10⁶ spores/mL) of *A. versicolor* PF/F/107. The flasks were incubated at 35°C for 4 d at 150 rpm in rotary shaker. The contents were then filtered through Whatman No.1 filter paper and the filtrate was used as a source of enzyme. The enzyme activity was determined by the method of Takami *et al.* (1989).

Hydrolysis of gelatin and release of silver: The used x-ray films were washed with distilled water and wiped with cotton impregnated with ethanol. The films were dried in an oven at 50°C for 30 min and were cut into 4×4 cm² pieces.

Approximately 40 pieces of the films were dipped in 100 mL of crude enzyme diluted in Tris-HCl buffer (pH 9.0) (Shankar *et al.*, 2010). The solution containing the films were constantly stirred at 50°C, pH 9.0 in a water bath with continuous shaking until the gelatin-silver layer was stripped completely. The turbidity of the reaction mixture increases with time and the reaction is considered complete as no further turbidity increases. The turbidity was monitored by measuring absorbance at 660 nm. The obtained slurry was washed 2-3 times and filtered to remove the gelatin from the slurry and then dried. To 1 mL of the solution containing silver, 1 mL of conc. HNO₃ was added followed by 1 mL of conc. HCl was added progressively. The formation of white precipitate indicates the presence of silver. The dried slurry was smelted in the presence of Na₂CO₃ and hard coked at 2000°C in a furnace. The silver settles and the waste burn out.

Results and discussion

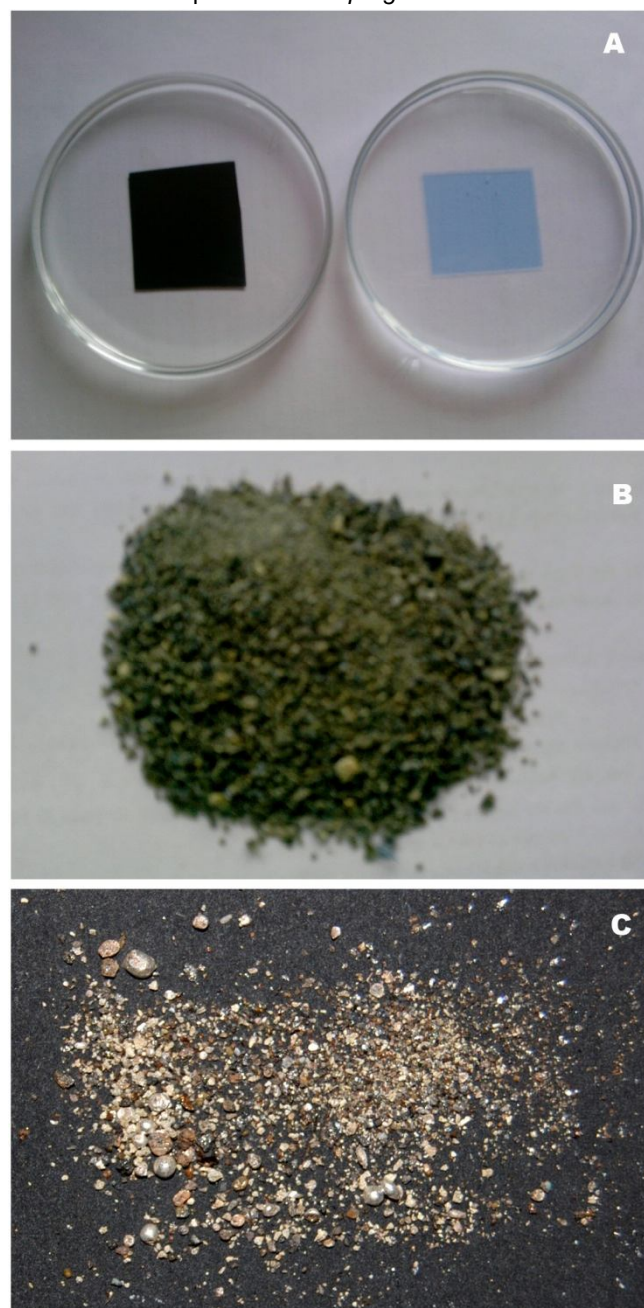
Four major types of proteases are distinguished based on their pH requirement for optimum activity i.e., alkaline (serine) proteases, thiol proteases, acid (carboxyl) proteases and neutral (metallo) proteases. Alkaline proteases have a serine residue at the active site and they exhibit activity in the neutral-alkali region range of pH with optima between at values pH 8-11 (Frost and Moss, 1987). Fungal strains are the major source for alkaline, acid and neutral proteases. Alkaline protease produced by *Aspergillus versicolor* PF/F/107 was used in this study. The protease activity of the culture filtrate used was 98.3 U/mL. It was noticed that it took 20 min at 50°C to decompose the gelatin layer completely at the given experimental conditions i.e., pH 9 and 50°C. Finally 0.135 g of silver was recovered with 0.335% yield following the described procedure for silver recovery (Table 1 and Fig. 1).

Nakiboglu *et al.* (2001) too selected 50°C as the stripping temperature for the enzyme from *Bacillus subtilis* ATCC 6633. Maximum gelatin hydrolysis was observed in the initial 15 min in above study. The protease from *Vibrio* sp. (V26) took 25 min for the complete removal of gelatin (Manjusha, 2011) whereas; the protease from a fungal isolate *Conidiobolus coronatus* ATCC PTA-4132 (Shankar *et al.*, 2010) took only 6 min. However, the enzymes from *Conidiobolus coronatus* showed hydrolytic action only at 40°C whereas the protease from *Vibrio* sp. functioned over broad temperature range (40-60°C). Masui *et al.* (1999) reported that alkaline protease from *Bacillus* sp. B21-2 mutant took 30 min for the complete hydrolysis of gelatin layer at 50°C. Gelatin hydrolysis was found to be best when Tris-HCl buffer of pH 9 was used indicating that it is the most ideal pH for hydrolysis of gelatin. Nakiboglu *et al.* (2001) showed that the best stripping of the waste film by enzymatic extract of *Bacillus subtilis* ATCC 6633 was found to be at pH 8.

Table 1. Recovery of silver from used x-ray films by alkaline protease of *Aspergillus versicolor*.

Alkaline protease (U/mL)	Weight (g)		Yield (%)
	X-ray film	Recovered silver	
98.30	40	0.135	0.3375

Fig. 1. Recovery of silver from used x-ray films by crude alkaline protease of *Aspergillus versicolor*.



A. Used x-ray film and film after hydrolysis of gelatin-silver layer.
B. Dried slurry after enzymatic treatment of x-ray film.
C. Recovery of silver particles from slurry.

Many have found pH 10 or above to be ideal for gelatin hydrolysis (Masui *et al.*, 1999; Shankar *et al.*, 2010). The conditions pH 9 and 50°C was found to be most ideal for the alkaline protease from *Aspergillus versicolor* PF/F/107.

Conclusion

Reuse or recycle of natural mineral resources remains most feasible option to slow down the exhaustion caused due to their depletion. This study indicates that the alkaline protease of *Aspergillus versicolor* PF/F/107 had the potential of being applied for reusing of silver from used x-ray films in an eco-friendly manner.

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