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Recovery of silver from used X-ray film using alkaline protease from *Bacillus subtilis* sub sp. *subtilis*

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Silver is an important industrial metal used in several areas such as photographic and x-ray films, jewelries, silver wares and electronic objects. Silver is used for photographic film/x-ray film because of its matchless quality as a light-sensitive material for making a photographic image. Silver is not destroyed in the photographic process and it can be reused and recovered. Results have proven that, bacterial alkaline protease can be used to extract silver in 30 min, but its activity decreases with increasing incubation period. Gelatin hydrolysis was monitored by measuring the increase in turbidity of the hydrolysate, which was accompanied by release of protein and hydroxyproline. The protease of the culture filtrate used was 97 U/ml after 30 min, but it decreased to 86.5U/ml after 60 min. After 90 min, it reached 85 U/ml. A great inactivation was recorded after 120 min; it got to 39.5 and 36.5% (U/ml) after 180 min. Gelatin layer was stripped completely within 30 min with 97 U ml^{-1} protease at 50°C and pH 8. At the end of the treatment, gelatin layer was completely removed and the polyester film was left clean. In addition, silver was recovered in the hydrolysate, both of which can be reused.

Key words: Silver recovery, x-ray films, gelatin, alkaline protease, *Bacillus subtilis*.

INTRODUCTION

Alkaline protease is one of the most important enzymes in the commercial field and it occupies a large area in the field of enzyme production. It is widely used in leather industry, diagnosis process, extraction of silver, animal diet production and food processing. For these wide applications, it is now commercially produced (Singhal et al., 2012). Silver is a valuable metal used in photographic and X-ray film, which is considered as an important source of silver metal after recycling of used films compared to other types of films. X-ray films contain about 1.5 to 2% ratio of silver in gelatin-coated film made from polyester layer. And it can restore this quantity of silver by dissolving gelatin layer in alkaline protease to be used for other purposes (Nakiboglu et al., 2003). X-ray film is a rich source of silver, which is distributed in the gelatin layer. Burning is a traditional way of extracting silver. Silver oxidation is followed by electrolysis or chemical treatment of the gelatin layers of X-ray films. All these traditional ways are environmentally unsafe, so enzymatic analysis of the gelatin layer is preferable. For this reason, the considered methods of analysis for enzymatic gelatin are the best alternatives to reduce

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Table 1. Silver recovery from waste of X-ray films by alkaline protease.

Time (min)	Optical density ($OD_{660\ nm}$)	Protease activity (U/ml)	Std. error
30	0.2079	97	±10.94
60	0.1781	86.5	±9.88
90	0.1777	85	±9.53
120	0.0861	42.5	±5.12
150	0.0844	39.5	±4.96
180	0.0835	36.5	±4.50

their harmful effects on the environment (Nakiboglu et al., 2003). Gelatin is a protein from animal collagen, which contains a large number of glycine, proline and 4-hydroxyproline residues. Since the emulsion layer on X-ray film contains silver and gelatin, it is possible to break down the gelatin layer using proteases and to release the silver (Nakiboglu et al., 2001). X-ray films are made of polyester which cannot be recycled through traditional methods of silver extraction. Enzyme hydrolysis does not only extract silver from proteins, but also yields the polyester base to be recycled (Gupta et al., 2002). Nakiboglu et al. (2001) and Ahmed et al. (2008) used alkaline protease from *Bacillus subillis, Conidiobolus coronatus* and *Streptomyces avermectinus* to extract silver. Kumaram et al. (2013) found that alkaline protease from *Bacillus* grown on fish ruminants had a high activity in silver extraction. The aim of this work was to detect the use of alkaline protease to extract silver from X-ray films.

MATERIALS AND METHODS

The bacterial isolation

Bacillus subtilis isolated from soil in November 2009 at the Eastern Province of Saudi Arabia was used in this research. Isolation was done in Plant Protection Department, Faculty of Science and Agriculture in King Saud University by Biolog Systems (Al-Yahya et al., 2007). *B. subtilis* sub sp. *subtilis* was the highest isolate from which *Bacillus* alkaline protease was obtained. Identified isolations were evaluated for their ability to produce *Bacillus* alkaline protease (AL-Khaldi, 2014).

Cultural conditions and production of enzyme

The isolate was grown for enzyme production. This was done by incubating it at 37°C for five days in media containing fructose (10 g), potassium nitrate (5 g), NaCl (150 g), dipotassium mono hydrogen phosphate (5 g), magnesium sulfate (0.4 g), $CaCl_2$ (0.2 g) and Tween 80 (10 g) in one liter of sterilized water (AL-Khaldi, 2014). The enzyme was separated by centrifuging at 10,000 rpm.

Alkaline protease used for silver extraction (hydrolysis of gelatin and release of silver)

After washing the X-ray film, it was rubbed over with ethanol. The X - ray film was cut into small pieces (4 × 4 cm), and dried at 40°C for 30 min. Each piece was soaked in a solution containing 500 µl enzyme and 1000.0 µl buffer solution (0.2 M, pH 8). They were incubated at 50°C in a water bath and were shaken (90 rpm) at different periods (30, 60, 90, 120, 150 and 180 min). The turbidity of the reaction mixture (hydrolysate) increased with time and no further increase in turbidity was observed when the hydrolysis was complete. Hence, progress of hydrolysis, that is, turbidity was monitored by measuring the absorbance at 660 nm. Samples were removed at 1 min interval and time required for complete removal of gelatin layer was noted. The resultant color was determined spectrophotometrically at 660 nm (shankal et al., 2010).

Statistical analysis

The statistical analyses were performed in a complete randomized design of three replicates for each treatment. The results were analyzed and compared at 0.05 level of probability using the least significant difference (LSD) and SPSS 16 version of program according to the method of Norusis (1999).

RESULTS

Alkaline protease produced by *B. subtilis* was used in this study. The protease activity of the culture filtrate used was 97 U/ml after 30 min, then it decreased to 86.5% U/ml after 60 min, while after 90 min, it got to 85 U/ml. A great inactivation was recorded after 120 min; it got to 39.5 and 36.5% (U/ml) after 180 min. It was noticed that it took 30 min to decompose the gelatin layer completely at the given experimental conditions (50°C and pH 8). Table 1 and Figures 1 and 2 show the enzyme alkaline protease activity in extracting silver from X-ray films. At the end of the treatment, gelatin layer was completely removed leaving the polyester film clean, and the silver was recovered in the hydrolysate, both of which can be reused.

DISCUSSION

From the results obtained in this study, it was noticed that alkaline enzyme protease was effective in recovering the silver layer during the first 30 min. Subsequently, it went down with increase in the period of incubation. The reason for this decline is due to exposure of the enzyme

Figure 1. X-ray film used in this study as control which contains silver in gelatin-coated film made from polyester layer.

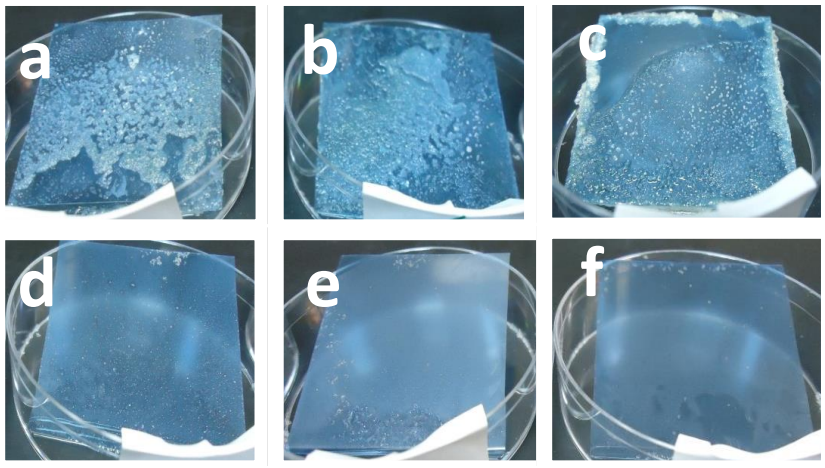

Figure 2. The extract silver layer from treated films by crude alkaline enzyme after: (a) 30 min, (b) 60 min, (c) 90 min, (d) 120 min, (e) 150 min, (f) 180 min, where gradual removal of gelatinous layer was noted.

to a temperature of 50°C, which is the optimum temperature in the different periods of time. This led to breakage of weak peptide bonds, making the enzyme to lose its activity (Bholay et al., 2012). The time factor is important for the stability of the temperature. Alkaline protease proved its activity in extracting silver from used X-ray films. Seid (2011) proved that silver could be extracted after 3 min treatment with alkaline protease at 55°C and pH 10.5, while Shankar et al. (2010) mentioned complete silver extraction after 6 min of using alkaline protease extracted from *C. coronatus*. Nakiboglu et al. (2003) could extract silver in 15 min after using the protease enzyme extracted from *B. subtilis* ATCC 6633; while using enzyme extracted from *Aspergillus versicolor*, Choudhary et al. (2013) extracted silver in 15 min. Also, Foda et al. (2013) extracted silver after 1 h of incubation with alkaline protease.

Silver extraction was also tested at 40°C for 20 min or incubation at 24 h with alkaline protease (Sangeetha et al., 2011). Furthermore, Pathaka and Deshmukh (2012) could extract silver after 24 h. Kumaran et al. (2013) mentioned high activity of the enzyme extracted from *Bacillus* grown on fish remains in silver extraction process.

Conclusion

Recycle of natural mineral resources especially silver metal remains the most practical option to slow down the exhaustion caused by their diminution. This study shows that the alkaline protease of *B. subtilis* sub sp. *subtilis*

has the potential of being reused for extracting silver from used X-ray films in an eco-friendly way.

Conflict of Interests

The authors have not declared any conflict of interests.

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