SILVER PRODUCTION FROM WASTE X-RAY FILMS BY ALKALINE PROTEASE ENZYME FROM BACILLUS SUBTILIS

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Abstract: The waste X-ray photographic film containing black metallic silver spread in gelatin are a very good source for silver recovery compared with other types of film. This method was done using proteolytic enzymes obtained from microorganisms have been used more often for recovery of the silver than the burning and oxidation methods. Recovery of silver by burning the film 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 which was collected from soil sample from the roots of cotton plants. So now a media responsible for the growth of the enzyme called as Horikoshi media have been prepared for which bacillus subtilis stain was added and incubated for about 24hrs. After a period of incubation 1%gelatin was added to the media so that there occurs induction of alkaline protease enzyme and again incubated for 24hrs. To this culture containing bacterial enzyme x-ray films were added and incubated for 3days.After this period we observed that there occurs degradation of x-ray film by living the black metallic form of silver to one region, so we collected all these samples and have taken for casting, after this process of casting we observed the silver in mould forms .so finally by means of using bacillus subtilis and its alkaline protease enzyme we can degrade the x-ray film and produce silver.

Keywords: X-ray photographic film, Proteolytic enzymes, Bacillus subtilis, Silver production

Introduction: Silver is one of the precious and noble metals used in large quantities for many purposes, particularly in the photographic industry. The waste X-ray/photographic films containing black metallic silver spread in gelatin are very good source for silver recovery compared to other types of film. The amount of silver in the X-ray film varies between 1.5 and 2.0% (w/w). 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. With an increasing demand for silver in the world, recent attention is focused on X-ray/photographic films as one. 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 methods using proteolytic enzymes obtained from various microorganisms and alkali hydroxides 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 60C to decompose the gelatine layer when Subtilis in BPN', an alkaline protease from Bacillus subtilis strain , was used and treatment at 30C increased the decomposition time to two to three hours.

Experimental : Bacillus subtilis was collected from roots of cotton plants soil. Performed serial dilution and cultured the bacillus in agar medium

Horikoshi medium : 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 . waste x-ray films were collected from hospitals in Vijayawada.

Cultivation and Preparation of enzyme extract

Bacillus subtilis was activated in Nutrient Agar slants for 24 hours at 30C. 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 30C. 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 4C

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 50C 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 1100C in a furnace. The purity of the recovered silver was determined potentiometrically

Final output : General presence of silver in market would cost about 1gm=59 rs but this eco friendly silver can be obtained at 35 rs only by (calculating all raw materials cost). This was completely rectified by the silver preparation workers in Vijayawada 1 town silver market.

Results and Discussion : 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. Bacillus strains are the major source for alkaline and neutral proteases. Bacillus subtilis, used in this study, produces protease. The effect of pH on the waste film by using enzyme extract was investigated at 30 and 40C and the optimum pH was determined to be 8.0. The results show that Bacillussubtilis produces neutral and alkaline proteases and this enzyme mixture can be efficiently used for the recovery of silver from used X-ray fims by degrading the gelatin layers on the films. On the other hand, it was noted that it takes less than 15 minutes at 50C to decompose the gelatin layer when Bacillus subtilis was used. 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, 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 possible . Hence it can be thought that alkaline enzymes will yield good result in the enzymetic method of the gelatin-silver layer to produce silver.

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