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Application of *Bacillus* sp. and its Keratinolytic Enzyme to Recover Plastic and Silver from Waste X-Ray Films

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The global adoption of microbial enzymes in various industrial processes has surged, driven by a desire to mitigate the adverse effects of chemicals, with proteases being the most prominent. In the same direction, the present study was dedicated for developing an efficient and eco-friendly way for hydrolyzing this gelatinous layer to leave behind a clean polyester film using isolated bacterial species *Bacillus* sp. ND6D and by the keratinolytic enzyme produced by it. The isolate tolerated up to 0.07 M silver nitrate and 0.2–0.3% (w/v) pure silver in its growth medium, indicating its potential for direct use in bioleaching. It could hydrolyze the gelatinous layer from X-ray films over a broad temperature range, from 20–70°C, with an optimum temperature of 50°C. The concentration of X-ray films causes variation in the time required, not in the efficiency of the isolate to hydrolyze silver halide embedded-gelatinous layers. Keratinase produced by *Bacillus* sp. ND6D is more efficient in this process; however, the enzyme is active under broad temperature, pH, and agitation conditions. The optimum conditions were found to be 7.4 pH, 45°C, and 40 rpm, and it is highly stable, allowing for repeated use until exhausted. At optimum conditions, 100% removal of the emulsion layer from the X-ray film was achieved in 40–50 sec with 0.5 U/ml of keratinase. Ultimately, both bioleaching by *Bacillus* sp. ND6D and enzymatic hydrolysis of silver halides embedded in a gelatinous layer from X-ray films were successful for the recovery of silver and plastic from radiographic films.

Keywords: Bioleaching, Enzymatic hydrolysis, Radiographic films, Protease, Waste management

Introduction

Proteases are types of biocatalysts that are substrate-specific. Depending on the type of proteolytic enzyme, they can easily break long, chain-like molecules of proteins into shorter fragments (peptides) and eventually into their components, amino acids. Proteolytic enzymes are produced by all types of cells, viz. bacteria, archaea, algae, plants, animals, and some viruses, and they are frequently used in detergent and food industries. Additionally, wherever possible, the replacement of chemicals with proteolytic enzymes for bioremediation and bioleaching is increasing, so research work in this area.[1,2] In similar directions, researchers are trying to replace the need for harsh chemicals employed to degrade the silver ions-embedded emulsion layer present on the X-ray films with enzymatic methods.

These include proteolytic enzymes produced by different types of organisms, for example, by myxobacterium *Pyxidicoccus* sp. S252, *Bacillus* sp ATP-P5,[(3)] *B. subtilis* sub-sp. subtilis,[4] *Purpureocillium lilacinum* LPS # 876,[(5)] *B. subtilis* ATCC 6633,[(6)] *B. brevis*,[7] *Bacillus* sp. B21-2,[(8)] *Bacillus* sp. Mar64,[(9)] *Conidiobolus coronatus*,[10] *Aspergillus flavus,*[11] and *Aspergillus versicolor*[12] are successfully tested for hydrolysis of the gelatinous layer from X-ray films. Bromelain and papain enzymes extracted from pineapple and papaya were also successfully tested for the same purpose.[13]

Although several types of proteases are produced by organisms across all domains of life, these days, keratinase is gaining more attention as compared to conventional proteolytic enzymes, which can be produced using microbial cells using animal waste as substrate. Keratinase is also a type of proteolytic enzyme. As per instructions of “The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB),” they are categorized into the “Hydrolase Class of Enzymes (EC 3)”. The Keratinases (EC 3.4.21/24/99.11) are potent enzymes with diverse biochemical and biophysical properties. Its ability to hydrolyze most recalcitrant structural proteins, i.e., keratin, makes it unique among other proteases and considered a “Protease of the Future Generation.”

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Most keratinases show broad protein substrate specificity, i.e., besides keratin, this enzyme can also hydrolyze other types of protein, viz. casein, collagen, gelatin, etc. Keratinase production has been reported in many animals, but microbes, especially bacteria, are preferred for industrial production.[19] That's why in this research work, the keratinolytic enzyme produced by *Bacillus* species was used to hydrolyze the gelatinous layer present on the radiographs to recover silver particles. The enzymatic process may be slower compared to traditional methods for recovering silver from X-ray films, but it is eco-friendly. It could be cost-effective for small-scale industries that are involved in the recycling of radiographic films. The higher the efficiency of the enzyme and its reusability, the more likely it is to increase microbial enzymes in such waste management processes.[14] As the enzymatic process is generally costly, especially on a pilot scale, this research also investigated *Bacillus* species directly for hydrolyzing the gelatinous layer from radiographs, similar to the bioleaching process and utilized the chicken feathers, waste of poultry industries, for enzyme production to make the process more sustainable and cost-effective.

Radiographic films, or radiographs, are light-sensitive materials used in medical imaging to capture X-ray images, typically containing a base material coated with a radiation-sensitive emulsion and a gelatinous protective layer. The emulsion is composed of a homogeneous mixture of gelatin and silver halide crystals, such as silver bromide or silver chloride, and coated on both sides of the base in layers. The radiographic film base is usually made from clear, flexible plastic such as cellulose acetate. Radiographs, also commonly known as X-ray films, are frequently used in medicine, including diagnosing injuries (for visualizing bones and joints), identifying diseases, guiding medical procedures, and in security and industrial applications.[15,16] Considering its utility and demand, especially from the medical sector, the production of radiographs is also increasing, as reflected in data provided by the World Health Organization on diagnostic examinations and statistical data on X-ray film market growth by Future Market Insights. The World Health Organization (WHO) estimates that 3.6 billion diagnostic examinations are performed yearly, and among them, X-rays are the most common imaging procedure, with 783 per 1,000 people.[17] The Global Industrial X-ray films industry's size is poised to reach USD 70 million in 2025. The Future Market Insights report "Industrial X-ray Films Market Growth Outlook 2025 to 2035" states, "Demand for industrial X-ray films will likely top USD 110 million by 2035, with an expected Compound Annual Growth Rate of approximately 5.3% over the next decade." This waste can also be categorized as hazardous depending on how much silver is on the X-ray films. Although hazardous waste, it is a good silver and polyester/plastic source. At the commercial level, considering the value of silver, the extraction of silver from X-ray film waste is prioritized over plastic films. Depending on the manufacturer, 1000 g of developed X-ray film may contain 14–17 g of silver.[18] Conventionally, methods employed for silver metal extraction from X-ray films include hydrometallurgy (leaching), pyrometallurgy (incineration), and electro-deposition, with hydrometallurgical processes, such as cyanide or nitric acid leaching, proving highly effective.[19] Incineration of X-ray films leads to environmental pollution and fails to recover the polyester film. At the same time, alternatives like electrolysis, precipitation, and adsorption involve harmful reagents and processes contributing to environmental issues and even causing harm to polyester film used for base formation in X-ray films. Alternative to these methods, biocatalysts can be employed, which may be not so cost-effective but definitely an eco-friendly approach. Typically, recovering silver from X-ray films consists of two main steps: first, separating the silver from the film base, followed by recovering the silver through smelting or electrolysis. For the first step, proteolytic enzymes can be employed. Gelatin, one of the main components of X-ray film emulsion, is a mixture of proteins and polypeptides obtained by the hydrolysis of collagen, typically by prolonged boiling of animal skin, tendons, ligaments, etc., in water and evaporation of the solution protein as defined by Oxford English Dictionary. So, the gelatin in the emulsion layer of X-ray film can be easily hydrolyzed by protease enzymes, facilitating the release of silver without damaging the recyclable polyester base.[20,21] Microbes are utilized for the bioleaching process, but as far as the literature was reviewed, no reports on the direct utilization of microbial species for removing gelatinous layers from X-ray films were found.

Materials and Methods

All chemicals and reagents utilized were of analytical quality and obtained from HiMedia; X-ray films (both used and new) from a local medical store and white chicken feathers used as substrate were collected from a local poultry farm, Mathana, Kurukshetra-136 119, India. The overall process is depicted in Fig. 1.

Bacterial Culture

A keratin-decomposing bacterium, Bacillus sp. ND6D was isolated from bird droppings and characterized, as previously discussed in our research paper.[22] The 16S rRNA gene sequence data of *Bacillus* sp. ND6D was submitted to GenBank, the National Center for Biotechnology Information (NCBI), and the National Institutes of Health (NIH) (SUB5426186 gbMK757681). This isolate could hydrolyze different protein substrates, including gelatin. In an incubator-shaker, the bacterial culture was cultivated in nutrient broth at 37°C for 18 h at 80 rpm. This active culture was used for further experiments.

Preparation of X-Ray Film

For maintaining uniformity in experimental conditions, new X-ray films were used to study. X-ray films were cleaned by wiping them with sterilized moist cotton. The X-ray films were then dried in a hot air oven at 50°C for 25 min. These X-ray films were cut into 2 cm × 1 cm strips of almost equal weight and wiped with 70% ethanol, followed by surface sterilization by ultraviolet rays for 20 min in Laminar-air-flow. After that, these films were washed with sterilized distilled water and air-dried under laminar airflow.

Removal of Gelatinous Coating from the X-Ray Film

For hydrolyzing the gelatinous layer from the X-ray films, three methods were employed (a) chemical method, (b) directly by *Bacillus* sp. ND6D and (c) enzymatic method

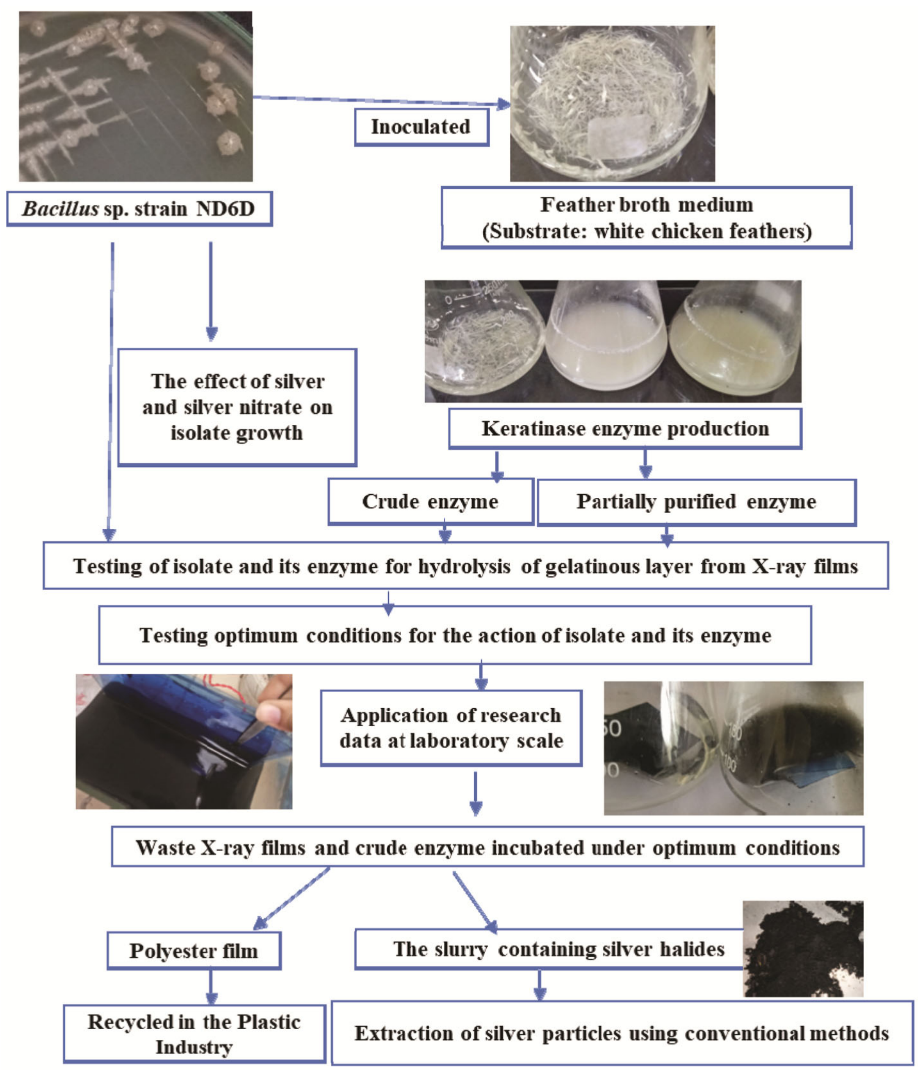

Fig. 1 — Graphical representation of Plastic and Silver recovery from waste X-ray films

(a) By Chemical Method (for reference)

Chemically,[23] the gelatinous layer was removed from X-ray films, and filtrate obtained after 100% removal of this emulsion was used for reference. In this method, the X-ray film strip was heated at 70–80°C in 10 ml of 0.5 N sodium hydroxide solution for 1 h under continuous shaking conditions and kept undisturbed overnight for complete removal of the silver-impregnated gelatin layer. From this solution, the plastic film was removed with the help of forceps. The progress of hydrolysis in the remaining solution was monitored in terms of turbidity by measuring the absorbance at 660 nm.[24] Maximum O.D. of the solution and colour of the film after emulsion removal were used as reference equivalent to 100% for all other experiments.

(b) By Bacillus sp. ND6D

Silver and its salts are known for strong antimicrobial activity against most microorganisms; therefore, tolerance of silver and its salt (silver nitrate) by the isolate *Bacillus* sp. ND6D was tested before testing its ability to hydrolyze the gelatinous layer from X-ray films.

Effect of Silver and Silver Nitrate on Bacillus sp. ND6D Growth

The susceptibility of *Bacillus* sp. ND6D was evaluated by determination of the minimum inhibitory concentration of silver nitrate by microtiter broth dilution method. Mueller-Hinton broth was used as the solvent. Silver nitrate with varying concentrations from 0.0–0.10 M was prepared in nutrient broth to analyze the effect of silver on isolate growth. Erlenmeyer flasks containing 50 ml of these sterilized media were inoculated with 2% active culture from the nutrient broth and incubated at 37°C for 24 h, 120 rpm. The growth of the isolate was assessed by measuring the optical density at 660 nm in a 50 mL Erlenmeyer flask. In another experimental setup, silver nitrate was replaced with silver metal powder at concentrations ranging from 0 to 5.0% (w/v). The conventional Disc-Diffusion method was used for testing isolate tolerance towards silver content in X-ray film by replacing the disc with an X-ray film strip.

Use of *Bacillus* sp. ND6D for the Hydrolysis of the Gelatin Layer

An Erlenmeyer flask with 50 ml of nutrient broth was seeded with 0.1 ml of a culture that was 18 hours old, then incubated for 24 h at 37°C while shaking at 80 rpm. The resultant culture was prepared as 1% cell suspension in 50 ml of sterilized tap water. A different number of sterilized X-ray film strips ranging from 2–10 were incubated in this cell suspension at various temperatures from 20–70°C for 72 h with intermediate shaking after an interval of 12 h for 30 min. The contents of this suspension were regularly monitored, and the total time required for the complete removal of the gelatinous layer from the X-ray film was determined.

By The Enzymatic Method

Keratinase Production

Feather Broth Meal (FBM) containing native white chicken feathers supplemented with $MgSO_4$ and NaCl, prepared in potassium-phosphate buffer (pH 7.8), was inoculated with 18-hour-old *Bacillus* sp. ND6D culture and incubated for 36 h at 45°C and 140 rpm for keratinase production (optimized media, data not published). After incubation, the broth was centrifuged at 10,000×g for 12 min at 4°C, and the supernatant was used as a crude enzyme. It was then partially purified with acetone (acetone protein precipitation method) and used to recover silver from X-ray films.

Assay of Keratinase Activity

The keratinase activity was assayed by replacing casein with soluble keratin in Sigma's non-specific protease assay method.[25] Soluble keratin was prepared from white chicken feathers.[22] In the keratinase assay, 5 ml of 0.65% keratin solution in test tubes containing 0.05 M potassium-phosphate buffer was first equilibrated in a water bath at 37°C for about 10 min. For the experiment, 1.0 ml of enzyme was added to it. A control was run simultaneously without the enzyme, keeping all other components the same. Both types of tubes were incubated at 37°C for exactly 10 minutes in a water bath under continuous shaking. Then, 5 ml of 0.110 M trichloroacetic acid solution was added, mixed vigorously for reaction termination, and incubated at 37°C for 30 min. In control, the enzyme was added after incubation with trichloroacetic acid. With 2 ml of the above solution, 5 ml of 0.5 M sodium carbonate solution, and 1 ml of 0.5 M Folin & Ciolcalteau's Reagent was added. The contents of all the tubes were mixed and incubated at 37°C for 30 min, and the absorbance at a 660 nm wavelength was measured. The concentration of tyrosine produced during the keratinase reaction was determined with the help of a standard graph of tyrosine. One unit (U) of keratinase activity is defined as the amount of enzyme required to liberate 1 µmol of tyrosine per min from soluble keratin under the assay conditions.

Factors Affecting Hydrolysis of the Gelatinous Layer from the X-Ray Film

Some experimental conditions, i.e., pH, temperature, time, agitation, and enzyme concentration, which affect the hydrolysis of the gelatinous layer and, thus, recovery of silver, were studied, considering one parameter at a time.

X-ray film strips were incubated with 0.5 U/ml keratinase enzyme adjusted at pH 2.6–11.0 with suitable buffers at temperatures 10–80°C and shaking at 0–100 rpm for 0–300 sec. Percent hydrolysis of the gelatin layer was calculated after measuring absorbance at 660 nm with reference to hydrolysis by sodium hydroxide. X-ray film strips were incubated with enzyme concentrations varying between 0.5 and 5.0 U/ml under optimized conditions to reduce the time required for removing the gelatinous layer.

Reusability of Keratinase

X-ray film strips were incubated with crude and partially purified enzyme (0.5 U/ml) separately under optimum conditions to analyze the reusability of the enzyme. X-ray film was replaced with a new one in the enzyme solution after completely removing its gelatinous layer, signifying the completion of the first cycle, and the time taken in the process was noted. This process is repeated with untreated X-ray film strips until a cycle is reached with incomplete hydrolysis. After this stage, the used enzyme solution was filtered through Whatman filter paper. The collected filtrate was reused for another series of cycles in the hydrolysis of the gelatinous layer from X-ray films. The duration needed for the total elimination of gelatin in every cycle was recorded.

Recovery of Silver and Polyester Films from used X-ray Films

Discarded X-ray films (500 g) were soaked in crude enzyme (0.5 U/ml) and incubated under optimized conditions. After the complete removal of the gelatinous layer from the X-ray films, the latter was removed, the reaction mixture was kept undisturbed overnight, and the slurry was settled in a container. The rest of the reaction mixture was decanted without disturbing the settled slurry. The slurry settled at the bottom of the flask was collected and dried in an oven for 4 hours at 180°C. The dried slurry (approximately 20 g) was smelted in the presence of Na_2CO_3 at 1200°C for 3 h in a furnace to recover silver from ashes as per the conventional method. Fine shiny particles were collected from the ashes and tested for silver by using aqua regia.

Statistical Analysis

All the experiments were done twice in triplicate; the average of the experimental results with the mean was used to represent the results in the table, with the standard deviation. Microsoft Excel 2021 was used for the analysis and graph preparation. In the graphs, the bar represents the standard deviation from the mean.

Results and Discussion

Bacillus sp. ND6D and the keratinolytic enzyme produced by it using white chicken feathers as substrate were used for the hydrolysis of the gelatinous layer from X-ray films so that recovery of silver particles embedded in it would release easily without using any harsh chemicals.

Silver and its salts are known for strong antimicrobial activity against most microorganisms; therefore, tolerance of silver by the isolate *Bacillus* sp. ND6D was tested by adding silver and silver nitrate in its growth medium and its antimicrobial susceptibility against the X-ray film strip. It was found that the isolate was able to tolerate up to 2–3% (w/v) extra pure silver (Fig. 2a) to 0.07M silver nitrate (Fig. 2b) in its growth medium and was also able to grow against X-ray film in the plate (Fig. 3a).

Hydrolysis of gelatinous layer by *Bacillus* sp. ND6D from X-ray films: The bacterial isolate *Bacillus* sp. ND6D hydrolyzed the gelatinous layer completely from X-ray (Fig. 3.b) films at a broad range of temperatures from 20–70°C, as shown in Fig. 4. At 50°C, hydrolysis took a minimum time of 680 min up to four X-ray film strips.

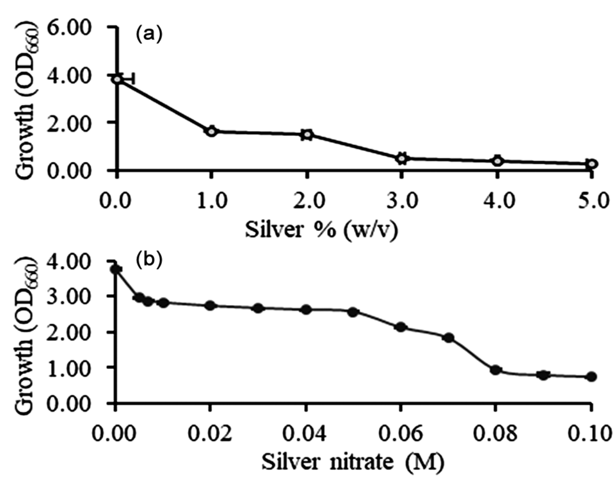

Fig. 2 — Effect of (a) silver and (b) silver nitrate on *Bacillus* sp. ND6D growth

Enzymatic Hydrolysis of the Gelatinous Layer from X-ray films

Keratinase produced by *Bacillus* sp. ND6D was also tested for the hydrolysis of the gelatinous layer from X-ray films, and it took far less time than using the organism itself.

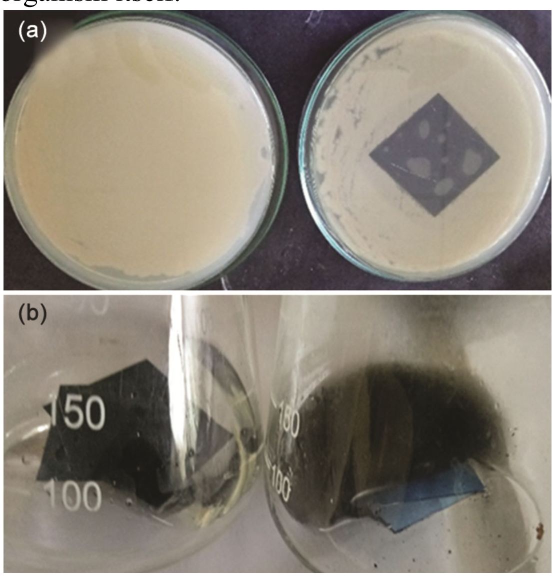

Fig. 3 — (a) Antimicrobial susceptibility of *Bacillus* sp. ND6D against X-ray film and (b) its effect on X-ray films after 24 h at 50°C (Emulsion layer dissolved, leaving clean polyester film)

The effect of various parameters on the hydrolysis is shown in Fig. 5 (a–e). As depicted in Fig. 5 (a & b), the optimum hydrolysis pH was near neutral, and the temperature was 45°C to 55°C. Agitation of the reaction mixture enhances the hydrolysis of the gelatinous layer from X-ray films several times with respect to static conditions. Still, agitation rate beyond 40 rpm did not improve the hydrolysis Fig. 5(c). However, 100% hydrolysis was achieved in 240 sec, keeping all other conditions at optimum levels, as shown in Fig. 5(d). The time of complete hydrolysis was further reduced to approximately 16% by increasing the concentration of enzyme from 0.5 U/ml to 2.5 U/ml, as shown in Fig. 5(e).

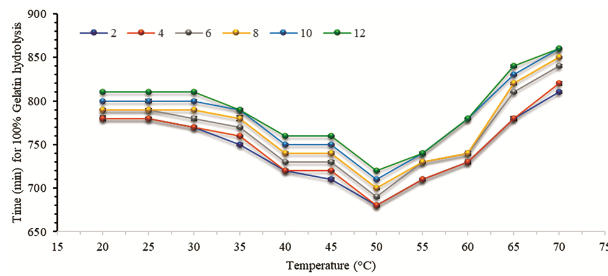

Fig. 4 — Time required by *Bacillus sp.* ND6D for 100% hydrolysis of the gelatinous layer from X-ray filmstrips at varied temperatures; The color lines represent a number of strips present in the medium

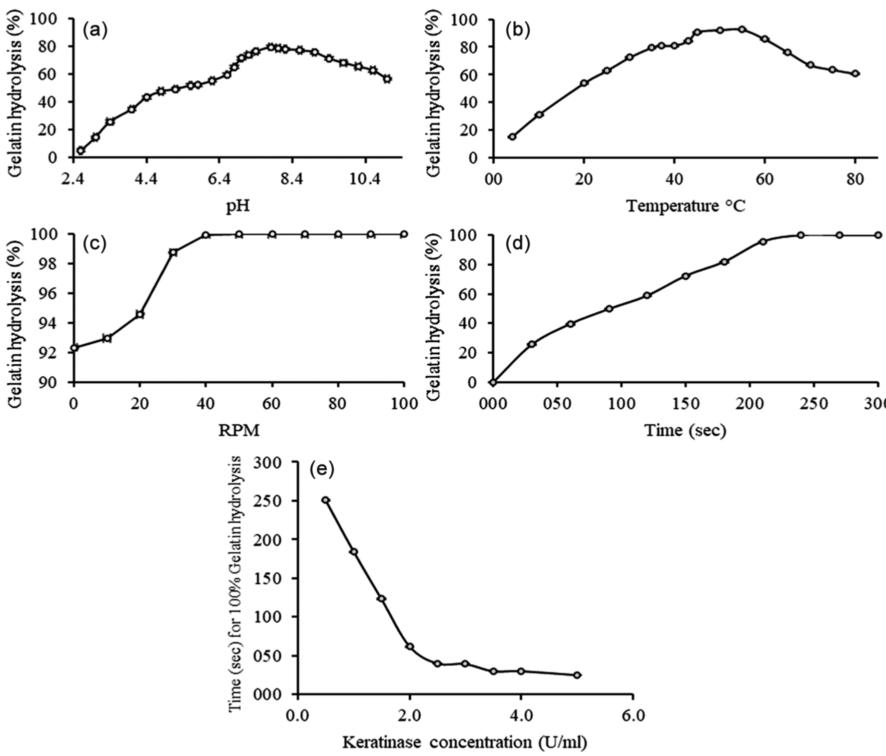

Fig. 5 — Effect of various factors (a) pH, (b) temperature, (c) agitation, (d) time, and (e) enzyme concentration on the removal of gelatin (% hydrolysis) from X-ray films by partially purified keratinase produced by *Bacillus* sp. ND6D

Table 1 — Reusability of the crude and partial purified keratinase from *Bacillus sp*. ND6D

Enzyme	Cycle1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle 10
	Time required for complete hydrolysis of gelatinous layer from X-ray film (min:sec)									
KE	6:00	6:00	6:30	6:45	7:00	7:10	7:10	7:30	10:00	13:00 (IH)
KE 2	6:45	6:45	7:00	7:10	7:10	7:30	8:00	8:10	8:30 (IH)	14: 00 (IH)
PKE	4:00	4:00	4:30	5:00	5:30	6:10	6:50	7:30	9:00	12:00 (IH)
PKE2	5:45	6:00	6:40	7:20	8:10	13:00	20:00 (IH)	—	—	—

KE- Crude keratinase enzyme obtained directly from fermentation
KE 2 - KE obtained by filtering the supernatant after 10th cycle of KE
PKE – Partial purified keratinase obtained by acetone precipitation
PPE 2 – PKE obtained by filtering the supernatant after 10th cycle of PKE
IH-incomplete hydrolysis

Table 2 — Optimum conditions reported by various researchers for hydrolyzing gelatinous layer from X-ray film to release embedded silver particles in the emulsion layer

Enzyme	Source	Optimum conditions				
		pH	Temp (°C)	Time (min)	Enzyme activity (U/ml)	Reusability of enzyme upto
Protease B18[26,27]	*Bacillus* sp. B18	10.0	—	02.0	1000.0	4 cycles
Bromelain[28]	Pineapple	06.5	55.0	35.0	—	—
Protease[10]	*Conidiobolus coronatus*	10.0	40.0	06.0	**1.35**	4 cycles
Alkaline protease[29]	*Aspergillus wentii*	08.0	50.0	20.0	—	—
Glycosylated cysteine protease[30]	*Euphorbia nivulia*	07.4	—	180.0	**5.0 U/mg**	—
Keratinolytic Serine Proteases[5]	*Purpureocillium lilacinum* LPS # 876	09.0	60.0	06.0	6.9	4 cycles (7 cycles with glycerol)
Alkaline protease[12]	*Aspergillus versicolor* PF/F/107	09.0	50.0	20.0	98.3	—
Alkaline protease[31]	*B. subtilis (*NCIM 2724)	08.0	37.0	4 to 6 days	—	4 cycles
Alkaline protease[4]	*B. subtilis*subsp. *Subtilis*	08.0	50.0	30.0	97.0	—
Protease[32]	*B. cereus* strain S8	10.0	75.0	15.0	192.35	—
Protease[33]	*Bacillus* sp. THZ14	09.0	40.0	40.0	—	—
Alkaline protease[9]	*Bacillus* sp. Mar64	11.0	55.0	150	120	—
Alkaline Fish visceral protease[34]	*Sphyraena obtusata*	8.0	50.0	45	152.4 U/ml	—
Alkaline protease[35]	*Bacillus licheniformis*-MA1	9.0	50.0	60	140 (U/ml)	—
Alkaline Protease[36]	*Engyodontium album* BTMF S10	10.0	25.0	120	165 (approx..)	—
Bromelain[37]	Pineapple waste peels	7.0	70.0	3.5	21.6	—
Keratinase	*Bacillus* sp. ND6D	7.4	45.0	6.0* 4.0**	**0.5**	18 cycles* 16 cycles**

*crude enzyme; *partially purified enzyme

From Table 1, it is observed that both crude and partially purified enzymes can be reused for up to 9 cycles without any filtration; however, the time required for complete hydrolysis of gelatin increased in successive cycles, which may be due to saturation resulting from repeated experiments until complete exhaustion. It was also observed that even after filtration, the keratinase enzyme, both partially purified and crude, could run 6 to 8 cycles for hydrolysis of the gelatinous layer from the X-ray films but again with increasing duration for hydrolysis.

In Table 2, various reports on the reusability of protease enzymes to hydrolyze gelatinous layer are compared, the performance of keratinase from *Bacillus* sp. ND6D was found to be better than others as it can be reused up-to 18 cycles. Even the recently reported crude keratinolytic enzyme produced by *B. tropicus* LS27 also requires 6 min at 40°C to hydrolyze the gelatinous layer from X-ray films.[38]

The keratinolytic enzyme from *Bacillus* sp. ND6D is appears to take even less time for the removal of gelatinous layer from X-ray films from certain immobilized enzymes as well; for instance, the Ca-alginate-immobilized alkaline protease derived from *B. brevis* was effective in eliminating the gelatinous layer after 20 minutes in a pH 8 buffer at 40°C.[39] Even the crude extract from the leaves of 15 different plants viz. *Azadirachta indica*, *Hibiscus rosasinensis*, *Piper nigrum*, *Ficus religiosa*, *Ficus benghalensis*, *Tectona grandis* (Teak), *Pisum sativum*, *Carica papaya*, *Tridax*

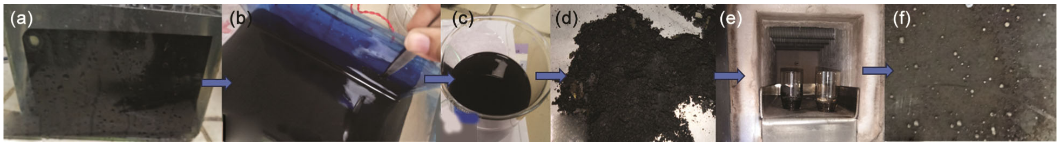

Fig. 6 — Process of silver recovery from used X-ray films: (a) X-ray films were dipped in a crude keratinase enzyme produced by *Bacillus sp*. ND6D, (b) emulsion layers completely removed the X-ray films, leaving behind a dark slurry and clean polyester film, (c) Slurry settled in the bottom was collected and dried in the oven, (d) dried slurry collected and (e) smelted at 1200°C, (f) ash containing silver particles obtained after smelting

procumbens, *Mussaenda erythro*, *Amaranthus cruentus, Psidium guajava*, *Myristic fragrans*, *Bougainvillae spectabilis,* and *Nerium oleander*, when used for hydrolysing the gelatinous layer from X-ray films, took a minimum 30 min to remove the emulsion layer from 20 g of exposed X-ray films at pH 7 and 50°C.[40]

Recovery of Silver from used X-Ray Films

From 500 g used X-ray films, approximately 30 g of dried slurry was obtained, from which a few shiny granules were obtained after smelting (Fig. 6). The addition of aqua regia on these minute granules results in an off-white creamy reaction, indicating the presence of silver in ashes. After removing the emulsion layer embedded with silver halides, the plastic film recovered from X-ray films was sent for recycling in the plastic industry via a local plastic collector. The above results were supported by several researchers, who employed protease from different sources to recover silver from used X-ray films.[27–38]

Conclusions

The isolate *Bacillus* sp. ND6D and keratinase produced either in crude form or partially purified efficiently, can hydrolyze silver halides embedded gelatinous layers from X-ray films at a broad range of temperatures and pH. Therefore, the need for chemicals at the initial steps during the recycling of X-ray films for silver recovery is eliminated. Besides, the polyester films left after removing the gelatinous layer can also be recycled, which is otherwise burnt and damaged in conventional methods. Replacing chemical methods with biological methods may be challenging at the pilot scale. Still, at a small scale and in areas where commercial methods for recycling X-ray films are not economically feasible, biological methods may be a good alternative, especially using microbes like *Bacillus* sp. ND6D and their proteolytic enzymes produced by them. Bacterial isolates like *Bacillus* sp. ND6D, which can tolerate silver-like metals in its growth medium, can be further explored for bioleaching.

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Conflict of Interest

There is no conflict of interest.

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