

# Degradation Mechanism of Feather Waste By Keratinases with Promising Biotechnological Applications

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## Abstract

Feathers account for the major pollutant of the poultry industry due to its recalcitrant nature. They are composed of keratins that has numerous application in various fields. The untreated feathers production from poultry industry are considered major pollutant because of their resistance towards protease degradation. Chicken feathers can be enzymatically degraded by keratinases that are secreted by numerous bacteria and fungi and converted into value-added products. Degradation by keratinolytic bacteria represent as an alternative for the development of cost-effective, eco-friendly and cheap source of nitrogenous fertilizers that are needed for plant growth. The feather degradation also results in the release of tryptophan that acts as precursor for the phytohormone. The hydrolysate plays important role in the seed germination and plant growth. They also serve as an alternative tool to improve soil biological activities, organic farming and agro-ecosystems. The mechanism of degradation comprises of the three steps, sulfitolysis, proteolysis that is followed by deamination. This review summarizes the progress in the feather degradation by keratinases, its structure, mechanism of action and feather application.

**Keywords:** Keratinases, Chicken Feathers, Feather Degradation

## 1. Introduction

One of the important by-product of poultry industry is feathers that accounts for about 5-7% of the chicken weight. Annually, approximately about several million tons of feathers can be obtained from poultry industry globally [1]. Feathers are usually treated as there are chances of its mixing with meat, grease and meat. Therefore, they are must be kept at careful storage conditions such as duration and temperature. An effective method to destroy the infectious agents present in feathers are achieved by incineration. They can also be disposed through controlled landfilling or burial [2]. Chicken feathers are of great importance as it is used for decorative purposes, fertilizers, feedstock, bedding materials, dusters and medical devices [3]. Chemical treatments and steam pressure cooking method are the traditional feather processing methods by which feathers can be converted into animal feed but this processes usually huge amount of energy due to which some amino acids get damaged under treatment [4]. Feathers can be utilized in various field yet many of them are released into the surroundings without suitable treatment. Feathers due to its recalcitrant nature has become one of the source of pollutant that sustains many pathogenic microorganisms and results in the emission of pollutants such as hydrogen sulfide, ammonia, nitrous

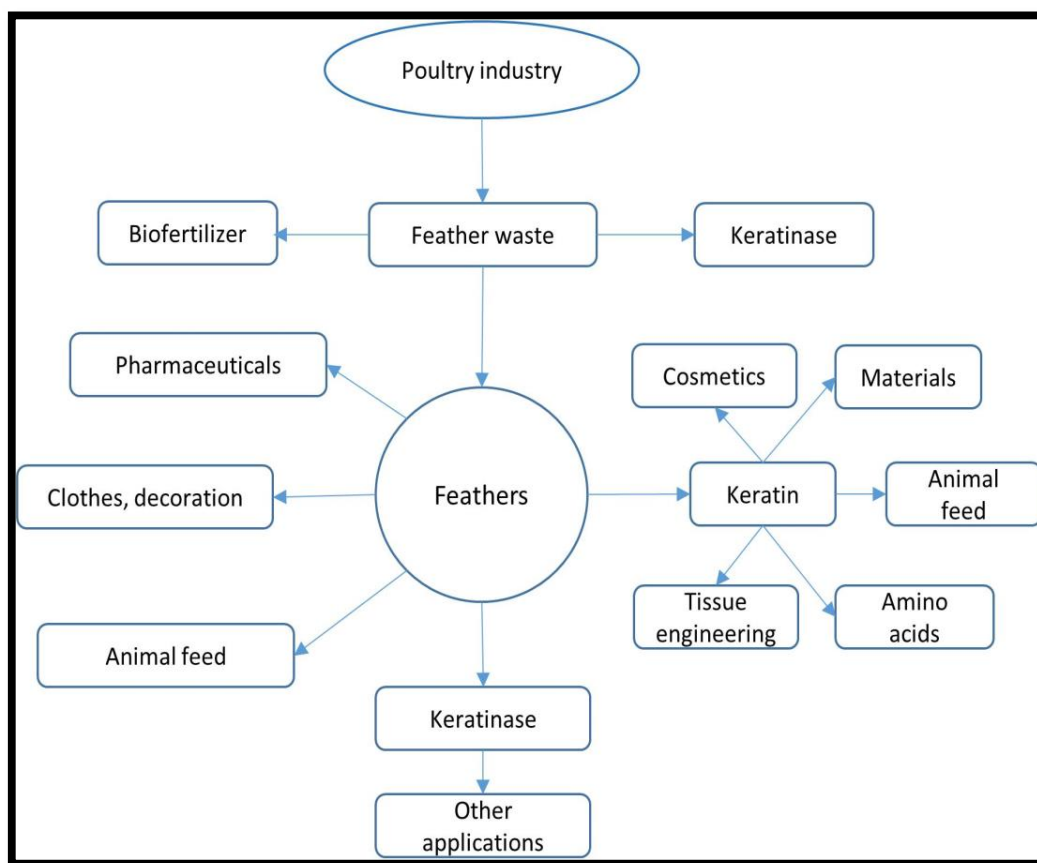
oxide that are toxic to the human health as well as for environment [5]. Therefore, converting these feathers into valuable material by the help of microorganisms is an efficient method. In poultry industries, microbial conversion of feathers into valuable products like animal feeds and bio-fertilizers are preferred. The enzymes that are secreted from microorganisms that follows the microbial consortium during treatment is effective degradation mechanism [6].

The feather degradation by keratinolytic bacteria is considered as efficient, environmental friendly and economical method for the bio-conversion of waste of feather into lysate which is nutritionally balanced and digestible. Feather lysate is comprised of peptides, free amino acids and ammonium ions that serves as a protein rich meal to animals. It can likewise use in horticulture industry as it is a source of nitrogenous manures for plants [7]. Bacterial keratinases are a cost effective, easily available alternative for the development of useful organic fertilizers. These keratinases due to their degradative properties considered as one of the novel approaches in the poultry, textile, pharmaceuticals and leather industries.

## 2. Feather Waste

Chicken feathers are comprised of 85% of the crude protein, 70% of amino acids, vitamins, high value elements and other growth factors. Great interests have been shown by researchers to obtain important products like fertilizers, feed and biofilm. Chicken cannot be hydrolyzed by the use of proteolytic enzymes because of its mechanical stability. The presence of large abundance of rigid keratin protein in chicken feather make its structure stable [8]. Keratin is the structure protein present in the epidermal appendages or epidermis of vertebrates such as skin, feathers and nail that are rich in disulfide bonds and cysteine residues. Cross-links are created by disulfide bonds among the peptide chains results in the production of polymeric structure having hydrophobic and hydrogen bonding. The bonding makes the keratin a structure with high mechanical strength. The chicken can be degraded with the help of physical methods such as puffing and pressurized hydrolysis and chemical methods like acid and alkali. The high energy consumption and extensive damage to the products during the process made these methods limited. Now, biotechnological methods have been preferred to degrade keratin. Degradative processes by using microorganisms are environmentally friendly and also maintains the activity and original structure of products [9].

The keratinase-treated feather is one of the possible source of dietary protein in feed and food supplements as the nutritive value is retained after its treatment with enzymes. The biodegradation studies focus on the identification of microorganisms for their ability to degrade feathers by bacteria and fungi [10]. The heterologous expression, purification strategies and enzymatic properties of keratinases have been studied to examine its property to degrade chicken feathers. Mainly soluble peptides and amino acids are produced from the keratinase hydrolysis of feathers and display the properties of antioxidants [11]. These antioxidants are essential in animals and humans and provide protection against scavenge free or free radicals. Antioxidants obtained naturally from animals and plants are widely preferred over chemically synthesized antioxidants. Degradation of chicken feathers can be a source of some antioxidant peptides and proteins. Keratinases are produced by various groups of microorganisms like fungi, actinomycetes and bacteria species. Various two keratinolytic strains can hydrolyze chicken feathers but they require different conditions for their growth and enzyme production [12]. It is believed that combination of more than one degradation strategy can be prove effective in feather degradation. rDNA technology can be used to increase the keratinase yield by using Ker A taken from *Bacillus licheniformis*.



**Figure 1:** Application of Feathers- Chemical treatment and microbial degradation are the different ways for processing of feathers.

### 3. Keratin and Its Structure

Feathers are composed of 90% of the keratin that is also an important structural component of other organs. Both feathers and keratin rich materials consist of keratin associated proteins. Fibrous structures are formed by keratin and present widely in nature. Keratin is the third most abundant recalcitrant structural protein after the chitin and cellulose [13]. Keratin can be classified into two categories; soft keratin whose cysteine content is less than 10% while hard keratin that contains 10-14% of cysteine content. Soft keratin is usually present in the skin cells whereas, hard keratin is present in wool, claws, hair, hooves and nails [14]. The recalcitrant polymers are produced by keratin that are insoluble in organic solvent and water and resistant to proteolytic enzymes like trypsin and pepsin [15]. The presence of high fibrous structures through hydrophobic interactions, hydrogen bonds and disulfide bonds makes it recalcitrant. The secondary structures of keratins are like normal proteins that contains  $\alpha$ -helix and  $\beta$ -sheet. Keratins are of two types,  $\alpha$ -keratins and  $\beta$ -keratins. The central domain of  $\beta$ -keratin deposits preferring to shape  $\beta$ -sheet structures related with the fiber system, a N-and a C-terminal area which are related with the framework and structure crosslinking by means of disulfide bonds. The intermediate filaments are made during the  $\alpha$ -helix assembling results in coiling [16]. P Among various organs, the amount of  $\alpha$ - and  $\beta$ -keratins varies. The analysis shows various percentages of  $\alpha$ - and  $\beta$ -keratins at different positions in feathers [17].  $\beta$ -keratins are mostly present in the outer rachis of chicken feather; higher disulfide bonds are present in this region due to cysteine content [2]. The protein structure is stabilized by the formation of covalent bonds which makes degradation difficult by help of proteases. The post-translational modification of keratin such as glycosylation or phosphorylation involves in stability and structure [18]. The structure of some uncoiled keratins comprises of both serine and threonine amino acids. On the basis of pH, keratins are classified into acidic and basic types. The PI of the keratins are effected by post-translational modification. The unsuccessful filament assembly may arise due to lack in posttranslational modification [19].

### 4. Keratinases

Keratinases (EC3.4.21/24/99.11) belongs to proteases that are involve in keratinolytic activities. Serine or metallo protease are the identified type of keratinases that have capability to degrade keratin proteins. Different characteristics are shown by keratinases that are produced either from bacteria or fungi such as molecular weight, amino acid sequence, optimal pH and different origin. Keratinases have ability to degrade recalcitrant substrates mainly derived from keratin rich wastes including wool, feathers and hair [20]. This degradation capability has wide range of industrial applications. Keratinase activity can be used in various field such as leather industries, fertilizers, detergents, biomedical fields and cosmetics. *Licheniformis* PWD-1 produces keratinases that are able to degrade prions. Prions are resistant to protein destructive and proteolytic processes and known as infectious agent. The presence of prions in the animal feed can be removed by keratinolytic enzymes [21].

They also play important role in environmental and agriculture chemistry due to its potential of degrading keratins from different keratin sources. During algorithm phase, the proteolytic activity of keratins shows slow growth whereas, onset of either stationary or late algorithm phase, keratinases attain maximum growth [22]. The maximum keratinolytic activity is exhibited by some species of *Bacillus* such as *licheniformis*, *Bacillus megaterium* and *Bacillus pumili* exhibits maximum activity at the late logarithmic growth phase. The species of *Streptomyces* also showed the similar results like of *Bacillus* [23].

The genes of the keratinases from the fungi and bacteria can be cloned by the use of recombinant DNA technology and later can be overexpressed in the bacterial cells such as *Escherichia coli* [24]. Using recombinant keratinases is although not much effective way to degrade the wastes such as feathers as the purification step lead to production with high purity and yield rate. This method is considered as important method for enzyme characterization using biophysical, biochemical and structural methods. The yield of mutants can be easily examined while using this method in protein engineering. The production of recombinant keratinases under optimized conditions can be used for the industrial applications [25].

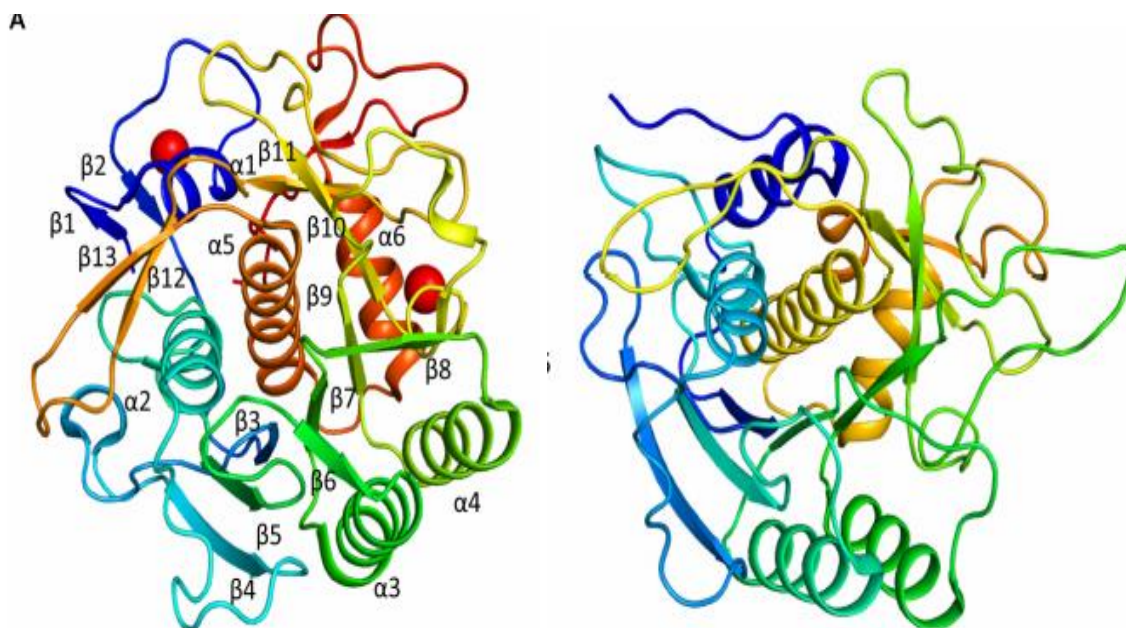
#### 4.1. Structure and Substrate Reorganization Site of Keratinase

Various studies have been carried out for various types of keratinases. *M. taiwanensis* WR-220 revealed the structure of keratinases (rMtaKer) that proved helpful in understanding the mechanism of the enzyme action. The rMtaKer comprises of N-terminal pro-peptide, single peptide and a domain of mature proteases containing amino acids such as His72, Ser224 and Asp39. In *E. coli*, rMtaKer was overexpressed after the cleavage of N-pro region [26]. The protease activity of purified c rMtaKer can be exhibited against several substrates such as elastin, feathers, casein and milk. Keratin structure is shown by the 1.5 Å crystal protease structure that was consist of six  $\alpha$ -helices, parallel  $\beta$ -sheets of seven stranded and four  $\beta$ -sheets adjoining around. The four  $\beta$ -sheets conserved catalytic triad by forming the two anti-parallel strands of  $\beta$ -sheets by arrangement of His72, Asp39 and Ser224 [27]. Two calcium ions present in the structure of keratinases make the whole structure stable. The presence of metal ions indicates its importance during enzymatic activity. The surface loop present between  $\alpha$ 1 and  $\beta$ 2 gets stabilized by the first Calcium ion (Ca<sup>2+</sup>) that coordinates by the presence of oxygen atoms taken from Gln15, Asp11, Ser21, Asp14 and Thr23. The contacts with oxygen atoms of Gly175, Val172, Thr177 and two water molecules is exhibited by second Ca<sup>2+</sup>. The metals ions present in them also belong to other members of subtilism superfamily. The residues of Cys165-Cys196 and Cys69-Cys101 are formed by disulfide bonds. The protein structure is stabilized by the disulfide bonds and the optimum temperature of rMtaKer is kept at 65°C. Thermal stability is also maintained by disulfide bonds. The protease thermal stability can be increased by the presence of disulfide bonds that may help in setting up strategy in

protein engineering of other keratinases [28].

Keratinases substrate specificity is not known yet. It is studied that *Nesterenkonia* sp. AL20 use chicken feather as the source of nutrient to produce alkaline protease. The hydrophobic residues present at the P1 site has ability to degrade the tetra-peptide substrates and self-cleavage was observed in the rMtaKer structure

[29]. RMtaKer occurs in the oligomeric form in the crystals due to crystal packing but appear monomeric in solution. The protease active site is occupied by the residues such as Glu279-Leu281-Tyr278-Asn280-Tye282 from the neighboring monomer. The residues are the critical substances involves in substrate binding. The optimal protease cleavage site can be determined by measuring the cleavage sequence from P1 to P4.



**Figure 2:** Left structure shows the rMtaKer, right structure shows the crystal structure of other keratinases

## 5. Mechanism of Keratin-Degradation by Keratinases

In vitro research on the keratinases and feather degradation has recommended that only one keratinase enzyme is not capable of degrading keratin as they cannot break disulfide bonds easily. Fungi and actinomycetes secrete the keratinases that invade into the body. The keratin degradation is facilitated by the hyphae which are the special structures of fungi [30]. The non-pathogenic fungi exhibited the degrading capability of feathers that has application in bio-fertilizers and animal feed [31]. Two steps are being recognized by several mechanisms that are involved in the keratinolytic process. Those processes are known as sulfitolysis and proteolysis. Sulfitolysis is a method for cleaving disulfide bonds, whereas proteolysis involves protein cleavage. Enzymes such as sulfide reductases are involved in the removal of disulfide bonds, whereas, sulfite is important for conformational changes of keratin [32]. The conformational changes of keratin develop available sites for the degradation of keratinases. The higher degrading rate of keratin is exhibited by the crude enzyme as compared to purified enzyme. Two enzymes are associated for keratin degradation. One degrading enzyme is responsible for reducing the keratin having cysteine residues, which expose the cleavage sites to protease.

### 5.1. Steps Involved in Keratin Degradation

The three steps are involved in the keratin degradation such as sulfitolysis, proteolysis and deamination. Keratin degradation is exhibited by bacteria and fungi through different mechanisms while polypeptides are cleaved by keratinases. Mechanical destruction also plays an important role in the degradation of keratin along with the addition of proteolysis and sulfitolysis. Studies have been made to identify the enzymes that are critical for the keratin degradation and its mechanism of action. The naturally occurring keratinolytic microorganisms indicate that keratins do not accumulate majorly in nature despite of the resistance to proteolytic enzymes. The degradation of keratinous waste such as hair or bird waste contributes to the recycling of nitrogen, carbon and sulphur [33].

The compact molecules such as keratins cannot be easily cleaved by peptide bonds due to the difficult access of peptide bonds and insolubility. Enzymatic degradation of keratin involves the following steps

- Hydrophobic and electrostatic interactions lead to the adsorption of the keratinases to the macromolecule surface.
- Catalytic action. It is a multistage process of keratinolytic degradation that involves two major processes known as proteolysis, reduction of disulfide bonds and sulfitolysis.



Sulfitolysis involves in reducing compounds such as cysteine, sodium sulfide, dithiothreitol (DDT), thioglycolic acid, mercaptoethanol or disulfide which acts in cooperation with the keratinases for the degradation of keratin molecules.

The keratinolytic bacteria includes the breakdown of disulfide bonds which involves in the degradation mechanism of feather along with proteolytic attack. Disulfide reductases (EC 1.8.1.8) are involved in the process of sulfitolysis by cleaving the  $\beta$ -sheet of disulfide bridges. *Streptomyces pactum* broth accumulates the sulphite substance. The species of *Streptomyces* and *Cryseobacterium* showed the phenomena of reduction of disulphide bridges. Disulfide reductase catalyzed the keratin in the initial step. The amino acid conformation changes due to the disulfide reduction of the  $\beta$ -sheet of keratin by affecting different hydrolytic sites. The keratinases are involved in proteolytic attack which renders the initial step.

Yamamura suggested the mechanism of degradation of extracellular keratin by the help of keratinolytic bacteria. The *Stenotrophomonas* keratinolytic strain is the crude enzyme that shows two peaks during purification step in ion exchange chromatography. The disulfide bond showed reducing activity in the second peak whereas the first peak showed only the protease activity. The keratinolytic activity is shown by none of the enzyme fractions [34]. The two enzyme fractions were mixed together after the keratinolytic activity was recovered. The keratinolytic activity was increased up to 50 fold after the mixing of two enzymes whereas in the absence of disulphide reductase the proteinase activity has increased to more than 2-fold. Mechanism was proposed that two combined enzymes are effective for keratin degradation having higher rate of keratinolytic activity [35]. The proteolytic activity is usually associated with subsequent deamination reactions and release ammonium ions during keratin degradation resulted in the increasing pH of the composting medium. *Bacillus licheniformis* is the strain of keratinases which involves in feather degradation by increasing the pH of the medium. The keratinolytic strain of *Bacillus* and the metabolite of feather showed increase in the pH [36].

The protease deamination of K S by the disulfide reductase  
 $S K \rightarrow K SH \rightarrow \text{Peptide} = \text{Amino acid} \rightarrow NH_3 \delta P$  Native keratin  $\delta P$   
 $P$  Reduced keratin  $\delta P$  Products

## 6. Biotechnological Application of Keratinases

Microbial enzymes are the important part of industries and acts as industrial catalysts, in which 60% of hydrolases make up the market. Due to broad applicability characteristic, proteases are considered as important group of enzymes. Keratinases are considered as important proteases due to its broad substrate specificity and general robustness that is valuable in many industrial applications.

6.1. Cleaning and Processing of Animal Hides – Leather Industry  
Leather industry is one of the important and fast growing industries

in the world that plays important role in economy. Leather processes results in the release of hazardous substances, toxic effluents and harmful compounds that are biggest source of pollution and are harmful to the industrial workers and environment [37].

A series of processes take place during treatment of hides in which pre-tanning is the leading source of pollution. The preliminary tanning results in the release of chemical such as lime, solid wastes and sodium sulfide that ultimately increase the chemical oxygen demand (COD), biochemical oxygen demand (BOD) and quantity of dissolved solids (TDS) in the waste water. Keratinases is considered as green alternative that improves the leather production by reducing the environmental pollution. Proteolytic enzymes are preferred for hide softening that improves the hides pliability and utilize in tanning process. The animal hair can be easily removed without damaging the skin containing collagen by using various keratinolytic enzyme preparations. The soft keratin tissue present in the follicle is selectively degraded by keratinases which helps in maintaining the tensile strength of the leather while pulling out the intact hair [38]. Keratinase originating from *Acinetobacter* sp, *Bacillus* sp, *Vibrio metschnikovii*, *Pseudomonas stutzeri* nad species of fungi such as *Penicillium chrysogenum*, *Aspergillus tamarii* and *Trichoderma harzianum* are considered important component for leather industries. The quality of the leather product is improved by the usage of keratinases and their use is much safer for environment as it reduces the pollution by chemicals [39].

## 6.2. Application in Detergent and Textile Industry

89% of the proteolytic enzymes are alkaline proteases that play a major role in detergent industry. The enzymes used in detergents industry must be compatible with other reagents of the washing agents. These enzymes should be stable and exhibit maximum activity at higher temperature or pH values. TKB2 is the alkaline keratinase isolated from *Paenibacillus woosongensis* that has wide range of application in laundry industry for the removal of composite strain alongside maintaining the fibres, texture of fabric and the strength of clothes [40].

Keratinases in textile industry is used for the processing of wool fibres. The structural protein present in wool consist of high degree of cross-linked disulfide bridges that proves resistant to degradation due to fibres mechanical strength. The epidermis layers are overlapping and keratin is present in exo and endocuticle whereas epicuticles are composed of lipids. During dyeing and washing processes, fibre contraction is associated with epidermis. Absorbable organic chlorides are traditionally used for shrinkage controlling. This chemical method has some disadvantages that includes the yellowing of material, loss of wood texture or character, affected biodegradability of fabric and waste water pollution by adsorbable organically bound halogens (AOX). Enzyme preparations composed of lipases and proteases are environmentally safe alternatives as compared to chemical treatments [41]. Proteases play an important role in removing the

outer layer of coarse fibre that lead to reduction in the rough texture of wool. Enzyme dose are taken carefully as some proteases cause damage, reduce the mass and tensile strength by penetrating deep into fibres. This is one of the reason why only enzymes are not involved for this processing. In some cases, few strategies are

adopted to limit the penetration rate such as increase of protease molecular mass, attachment of synthetic polymers and chemical crosslinking. Keratinases are the better alternative that selectively binds to the keratinous layers of wool while maintaining the fibrous part [42].

Commercially available keratinase products		
Application	Description of the product	Commercial product
Earwax removal	Successful, safe and effective removal of earwax from the external ear canal	Zymox
Corn and callus removal	Keratinases present natural alternative to the use of acids for corn and callus removal	Keratoclean® Hydra PB, PURE 100 Keratinase
Acne treatment	Acne is caused by blockage of sebaceous glands in the presence of large quantities of keratin, therefore keratinases can be used for successful treatment	Keratoclean® Sensitive PB, Keratopeel® PB
Commercial use (poultry feed)	Keratinase product improves the feed ratio and has a positive effect on chicken body mass	Versazyme®
Commercial use (poultry feed)	Enzyme product reduces cost of cooking and temperature processing of feather, therefore increasing the digestibility and nutritional value of feed	Valkeraze®
Prion decontamination	Effective decontamination of medical instruments from prions. It contains engineered protease with increased activity, broader specificity and thermostability	Prionzyme™
Biomedical, pharmaceutical and cosmetics	Keratinase product is supposed to regulate the concentration of keratin in pores, therefore helping to eliminate blisters, keratinized skin, it can be used for treatment of dermatophytic and nail diseases, scars and epithelial regeneration	PURE100 Keratinase
Cleaning agents	Cleaning pipes and tanks with different enzymes, including keratinases	Bioguard Plus

### 6.3. Prions Determination by Keratinases

Prions are the compact proteins that are responsible for neurodegenerative diseases for instance transmissible spongiform encephalopathies (TSEs). The increase in the transmission of prions from animals to humans through contaminated surgical tools affected the health and is responsible for the contamination of materials with prions [43]. The conventional methods require high energy and aggressive demand which can be overcome by enzymatic degradation of prions. Enzymatic degradation involves the destruction of aggregated proteins that structure is similar to keratin. Two keratinases, one isolated from *B.licheniformis* and other *Streptomyces* sp are used for the decontamination of prion [44]. The further treatment of the prion infection requires detergents, alkaline reagents, high temperature along with enzymatic treatment.

### 6.4. Applications in Medicine and Pharmacy

Keratinases has wide range of applications in pharmaceutical

industry as it is mostly used for the treatment of calluses, treatment of psoriasis, keratinized or removal of dry skin in cosmetic industry [45,46]. Keratinous nail surface improves the production of fungicidal drugs. Nail disorders are painful and associated with ranging from mild fungal infections to pigmentation and painful conditions such as nail dystrophy [47]. The onychomycosis is the treatment of fungal infection that is quite difficult and challenging. The treatment of onychomycosis requires the consumption of antifungal medicines and corticosteroids injections that cause various side effects such as liver damage and rashes. The antimycotic drugs is the alternative form of the traditional methods. The impermeability of nail surface is the main drawback that effects the treatment efficiency and drug penetration capability. Physical methods such as laser treatment, fast closing of nails, etching, mechanical methods such as separation and nail abrasions and chemical methods such as urea, salicylic and mercaptoethanol are used for the treatment of site of disease. These chemicals have pungent smell and effective in the high concentrations. Keratinases

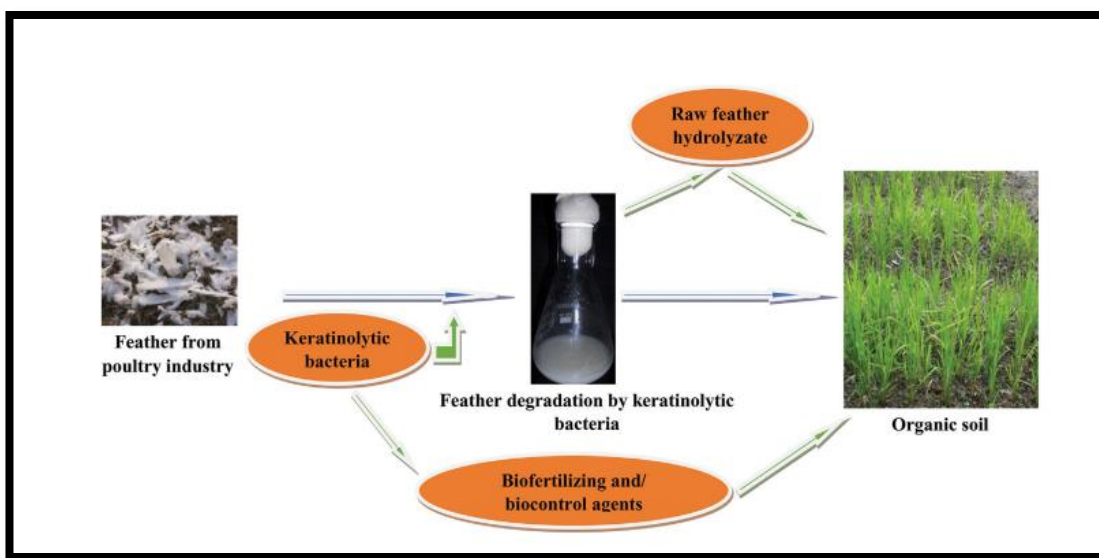
isolated from *Paecilomyces marquandii* acts on the intracellular matrix and very effective in low concentration for loosening of the nail plates. It increases the drug permeability [48].

### 6.5. Transformation of Keratinous Feather Waste to Value-Added Products and Bioenergy

Keratinous waste belongs to the third category of animal products that defines that it is not be used for human consumption, obtained from animal carcasses and should not be able to transmit diseases to animals or humans. Animal processing plants produce large amount of waste that is used as substrate for value-added products and bioenergy that is treated before use. The keratinous waste is effective degradation method that includes the acidic or alkaline hydrolysis take place at high temperatures (up to 150 °C) and pressures [49]. This method has many advantages but also have some drawbacks such as it consumes large amount of energy, expensive in nature and may results in the loss of some essential amino acids.

### 6.6. Feather Meal Production for Agriculture, Production of Bio-Energy and Feed Industry

Feathers are comprising of 7-10% of the chicken mass and major waste in the poultry industry. Feathers are composed of 90% of the keratin that consist of large amounts of glutamic acid, serine and proline and small quantity of histidine, methionine and lysine. Feathers are one of the biggest waste byproduct of the industry of poultry that ultimately responsible for environmental pollution. The transformation of feather to the feather meal by using valorization is an important in ingredient as animal feed or in bioplastic and as raw material in production of biodiesel [50]. The conventional method of feather degradation involves the high costs along with high pressure or temperature that ultimately cause destruction of amino acids (tryptophan, methionine and lysine. The final product has flexible nutritional value that are poorly digestible. Keratinases involves in the hydrolysis of feathers in the production of amino acids that can be added to ruminant, poultry and fish feeds. Hydrolyzed feathers is further converted into bio-hydrogen that acts as a fertilizer that helps in improving plant growth, release nitrogen slowly, encourages the microbial activity of soil, increases the water retention and enhances the soil structures [51].



**Figure 3:** Degradation of chicken feather by keratinolytic bacteria

### 6.7. Biogas Production by Anaerobic-Digestion of Keratinous Waste

The production of renewable energy from various types of waste substrates can be achieved by a process known as anaerobic digestion. Substrates are hydrolyzed during this process into amino acid that is later converted into ammonia, variety of organic acids, hydrogen, carbon dioxide and less amount of sulphur compounds. A microbial process, Methanogenesis requires these substrates that utilize hydrogen acetate and carbon dioxide to produced carbon dioxide and methane [52]. Under mesophilic or thermophilic conditions, the anaerobic degradation of feathers

involves different kind of waste such as leftovers, offal, manure and mixed bone fractions. Around 0.21 m<sup>3</sup>/kg of methane has been produced from the feathers waste. The compact keratin structure in feathers results in low yield due to less accessibility to nutrients that can be further increased by pretreatment. Various enzymatic, chemical and physical methods are used in pre-treatment. The keratinolytic strains development is considered as important method that improves the enzymes production, which causes decomposition of keratinous waste during anaerobic digestion. Chicken feathers can be hydrolyzed by the recombinant strain that carry the *B.licheniformis* gene of keratinases [53]. The feather

is degraded effectively degraded by the recombinant strain that results in the 80% of the methane production and biogas. This high degradation capability is due to the presence of the inducible promoter that manages the production of keratinase resulting in high amount of enzymes.

### 6.8. Other Applications

Keratinases has other wide range of applications that includes fibers structure modification in silk and wool, in the processing of edible nests of birds, bio-augmentation of keratin rich waste and in various cosmetic products. Pearl bleaching is one of its unconventional application. Impurities such as mucus cells, free cells and necrotic tissue at the bead formation time that is present in the mounting layer [54]. Hydrogen peroxide is used for the gentle bleaching and lightening and hence affect the color irregularity. Keratinases are used as better alternative for bleaching purposes.

### 7. Conclusion

Chicken feathers are one of the important by-product of poultry industry that is considered a high quality of protein supplement that contains about 85% of the crude protein content. Untreated feathers are source of pollution because of their resistant nature against degradation. Microbial process is a promising and eco-friendly technique that generates versatile products by degrading the feather waste. Keratinases are the valuable enzymes produce from various bacteria and fungi that degraded feathers waste into valuable products. Keratinases has potential in various field due to its degrading capability and its structure that efficiently degrades the keratin present in feather or other waste. Molecular properties of keratinases, keratinases characteristics and its novel application have made the degradation of feather a successful process. The recalcitrant structure of keratinases and its resistance to hydrolysis have applications in biogas production, textile, pharmaceutical, cosmetic and leather industries.

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