Chapter 9

Keratin Waste: The Biodegradable Polymers

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Additional information is available at the end of the chapter

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Abstract

Keratins are everywhere, from being the major components of household dust to common contaminants of laboratory protein analysis. Keratin is the major structural fibrous protein belonging to the large family of structural proteins to form hair, wool, feathers, nails, and horns of many kinds of animals and has a high concentration of cysteine, 7–20% of the total amino acid residues, that form inter- and intramolecular disulfide bonds. Keratin wastes are considered as the environmental pollutants and produced mostly from the poultry farms, slaughterhouses, and leather industries. Keratin wastes are dumped, buried, used for landfilling, or incinerated and all these actions increase the threats of environmental hazards, pollution, negatively influence the public health, and increase greenhouse gases concentration. Nature has provided planet Earth with a variety of beneficial organisms. Soil is considered as a well-known source for the growth of keratinophilic microflora (fungi and bacteria), which have the capability to degrade the keratin waste. The keratin-degradation ability of keratinophilic microflora has been credited with the production of the microbial keratinase enzyme and biodegradation takes place (enzymatic degradation). So, the keratin wastes are the biodegradable polymers. Keratinase is the industrially significant enzyme that offers bioconversion of keratin waste, utilization as animal feed supplements, and dehairing agents in tannery industries and textile industries.

Keywords: keratin, environmental pollutants, keratinophilic microflora, biodegradable polymers, keratinase

1. Introduction

Keratin wastes are considered as the environmental pollutants and generated mostly from the poultry farms, slaughterhouses and leather industries [1, 2]. The poultry farms, slaughterhouses, leather industries and wool industries are constantly producing a million tons of keratin waste [3]. Leather industries throw out extensive amount of waste products and considered as polluting industries with negative environmental impact [4]. The main producer of keratin waste includes the United State of America, China, India and Brazil which produces millions of tons of keratin containing protein [5, 6].

Keratin protein is the major component of the keratin waste [7] and belongs to the scleroprotein group [8]. Keratin protein is greatly resistant to the action of physical, chemical and biological agents [9]. The poultry feathers and other keratin-containing waste is dumped, land filled and incinerated throughout the world [10, 11]. These activities cause soil, water and air pollution. Discarded feather furthermore causes different human diseases including chlorosis and fowl cholera [12].

Very few microorganisms are capable to break down the keratin protein and utilize them as a source of nutrition [13]. Nature has provided earth with an assortment of beneficial organisms. Keratinophilic microflora (fungi and bacteria) is nature's gift and the biggest group of the organisms which have the capability to degrade the keratin waste [14, 15]. Biological degradation of keratin waste is more efficient than the physical and chemical degradation, yielding more useful by-product which can be utilized in commercial applications. In this scenario, biological keratin waste degradation has received the consideration from the scientific research community in recent days [16, 17].

2. Keratin protein

Keratin is an insoluble protein that forms the major component of the outer layer of the epidermis and helps to prevent the loss of body fluids [9, 14, 18]. Keratin word initially comes into view in the literature around 1850 to describe that keratin is made up from the hard tissues [19]. Keratin is the most complex proteins of epithelial cells of vertebrates [20, 21]. Keratin protein is a tough, fibrous and the third most abundant polymer in the environment after cellulose and chitin [22, 23].

According to the sulfur content, keratin proteins are divided into (a) soft keratins – skin and callus (b) hard keratins – feather, hair, hoof and [24–26]. This protein belongs to the scleroprotein group [27, 28]. The durability of keratins is a direct consequence of their complex architecture with extremely high molecular weight [29, 30]. Keratin protein is not easily degraded by pepsin, trypsin and papain because of disulfide bonds, hydrogen bonding, hydrophobic interactions [31–34].

2.1. Types of keratin

There are two types of keratin.

2.1.1. Alpha keratin (α-keratin)

Alpha keratin is found in the epithelia of all vertebrates [35]. The α-helix in alpha keratin constitutes the environmental problem due to their resistance to degradation from microbes [36, 37].

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Alpha keratins in particular are remarkable for their strength, elasticity, toughness, insolubility and flexibility. Alpha (α) keratin has abundant quantities of hydrophobic amino acid, i.e. methionine, phenylalanine, valine, isoleucine and alanine [38]. According to the sulfur content, this protein is classified in hard and soft keratins [39].

2.1.2. Beta keratin (β-keratin)

Beta-keratin is structural protein and present in reptiles and birds [40]. Beta (β) keratin has high cysteine percentage and the cysteine readily forms disulfide bonds, which confer rigidity and provide enhanced resistance to degradation [41]. In a mature feather about 80–90% of β-keratin is present [42]. The molecular weight of individual keratin proteins is usually in the 10–14 kDa range [43, 44].

3. Major source of keratin protein

Keratin protein derives from living organism or from their body parts after death. The richest sources of keratin are feathers, wool, hair, hoof, scales and stratum corneum (**Figure 1**) [27]. Hair is the byproduct from tanneries during the haircut process [45]. Keratin protein is present in the human hair and offers flexibility, strength and durability to the hair in the form of different conformations [46, 47]. The bird's feather is made up of over 90% of keratin protein and produced as waste by poultry-processing industries [48].

Figure 1. Major sources of keratin protein (A) Bird's beak; (B) animal hair; (C) human nail; (D) horn; (E) human hair; (F) hoof; (G) nail; (H) chicken feather: the hosts for these sources include human, bird and animal.

The human hair is a natural filamentous biomaterial and chemically, approximate 80% keratin protein is present in human hair [49]. The accumulation of hair causes many environmental problems and considered as waste protein [50]. Feathers protect the birds from cold, rain, sun and injury [51]. The chicken feather is composed of about 90% of keratin [52], which is a fibrous and insoluble structural protein consisting of β-helical coils joined together by disulfide linkages [53]. This structural feature enables it to resist adverse environmental conditions and degrades by proteases [16]. Therefore, feathers are considered as a biological waste and cause serious environmental problems [54].

The human nail is an important organ of the human body and primarily composed of a highly cross-linked keratin network, a scleroprotein containing large amounts of sulfur (3.8%) with several disulfide linkages. This unique structure results in a highly effective permeability barrier [55]. The beak of the birds has an external shell of hard keratin which consists almost entirely of proteins [56]. Structurally, hoof keratin contains α-helical conformation with an admixture of β-sheet and possesses high thermal stability [57].

The horn is the tough animal tissue and has inflexible configuration due to the sulfur cross-linkages [58, 59]. Fundamental components of any horns are keratin, free amino acids, peptides, lipids, remain microelements: calcium, aluminum, chromium, copper, iron, manganese, and zinc [60]. Keratin protein in the animal horn is the tough-fiber, and its treatment is very difficult [61].

4. Impact of keratin waste on environmental pollution and human health

Industry has become an essential part of modern society, and waste production is an inevitable outcome of the developmental activities. Keratin wastes are produced in huge quantities from commercial poultry processing plants, leather industries, wool industry, textile industry, and slaughterhouses (**Figure 2**). These wastes may pose a potential hazard to the human health or the environment (soil, air, water) [1].

4.1. Keratin waste from the poultry industry

Feathers from the chicken generated in large quantity as a waste by-product of the poultry processing plant. Worldwide, around 8.5 billion tons of poultry feather is generated annually, of which India’s contribution alone is 350 million tons. Accumulation of chicken feathers will lead to environmental contamination [12, 62, 63]. Chicken feather causes environmental pollution as well as adversely affects the people’s life living in nearby localities [64].

4.2. Keratin waste from slaughterhouses

Keratin waste is generated from the meat industry (slaughterhouses) in the form of chicken feathers, beaks, mixture of bones, organs and hard tissues in very large quantity. Keratinous wastes are degraded very slowly in nature, and considered as hazardous wastes according to EU directives [65]. The contaminated waste water generated from such industries caused the problems of acidification of soils, eutrophication and decreased species diversity. The conventional methods employed for the disposal of keratin waste are not only costly, but also very

Figure 2. Major keratin waste producing industries.

difficult. Decomposition methods like incineration are employed [20], but these procedures are environment-polluting and pose risk to the environment [66].

4.3. Keratin waste from leather industry

Leather industries are the most polluting industries globally. The leather processing is responsible for unfavorable impact on the environment [67, 68]. Keratin wastes generated from leather industries in very large amounts include both solid and liquid waste, which is mostly of animal origin [69]. A considerable amount of keratin protein waste such as hair, horns and hoofs are thrown away by leather industries [20]. Tannery industries discharge the wastes and causing serious health problems as well as pollute the air, soil, and water [70].

4.4. Keratin waste from barber shops

Barber and hair stylist shops are also the most important keratin pollution sources. Human hair is considered as environment pollutant and found as the municipal waste in the world [71]. In the city area, it often accumulates in large amounts as solid waste and chokes the drainage systems. In rural areas, hair is thrown away in nature where it slowly decomposes over several years. Open dumps of hair generate hair-dust which causes discomfort to people residing in these areas and, if inhaled in large amounts, can result in several respiratory problems [50].

5. Traditional disposal strategies of keratin waste and their disadvantages

Each year, approximately 24 billion chickens are killed across the world and huge amount of poultry feathers produces globally [72, 73], in addition to the accumulation of human hair in waste treatment facilities worldwide [51]. Keratin solid waste generated from meat, poultry processing, fish industries, wool industries considered as harmful environmental pollutants [74, 75].

Due to pathogenic microbes on the keratin waste, efficient and immediate treatment of keratin waste has become necessary [76]. The tremendous volume of keratin waste creates a serious solid waste problem in many countries [77]. The keratin waste is linked with the evolution of odors and pathogens into the soil and water [78]. Disposal of keratin waste is quite challenging [79].

Considering the huge quantity generated, there are four methods for dealing with keratin waste: incineration, landfilling, composting, and mechanical grinding (**Figure 3**) [20].

5.1. Incineration

Incineration involves combustion of keratin waste and destroying potential infectious agents [80]. Incineration plant's temperatures are above 850°C and mostly waste is converted to CO_2 and water [81]. Due to the requirement of high temperature, the operating costs are not only expensive but also difficult to maintain [6]. Incineration leads to the release of pollutants into the atmosphere, causing foul odors and contribute to harmful runoff, which negatively impacts the surrounding and downstream areas including livestock and nearby ecosystems [50].

5.2. Landfilling

The traditional method for disposal of keratin wastes is land filling [20, 79]. Historically, landfills have been the most popular methods of organized waste disposal and continue to remain in several places around the world [82]. The improper disposal of keratin wastes by landfilling contributes to environmental damage and transmission of diseases [83]. Land filling also poses problems like landfill leachate and greenhouse gases [84]. Leachate increases the nitrogen concentration in surrounding areas, leading to algal blooms and harming the ecosystem [50]. So, landfilling is the less expensive way for discarding of keratins waste, but it is not an efficient method.

5.3. Composting

Composting is the additional economical method for recycling feather waste. Ninety percent of the feather-weight consists of crude keratin protein, and also contain 15% N [85, 86]. Composting is an aerobic biological process degrading organic material of poultry, slaughterhouse wastes, manure, and litter. This process reduces the pathogens, and compost product can be used as the soil fertilizer [87–89].

5.4. Mechanical grinding

The method to dispose of keratin waste is mechanically breaking it down into useful products. In this process, the poultry feathers are hydrolyzing under heat and pressure and then grinding and drying. The dried waste ground into a powder and later processed into useful products [90]. The ground powder can be used as a nitrogen source for animal feed (mostly ruminants) or as an organic soil enhancer [91]. There are certain disadvantages of the mechanical grinding method. Extremely high temperature and grinding result in the loss of several valuable amino acids [69, 92].

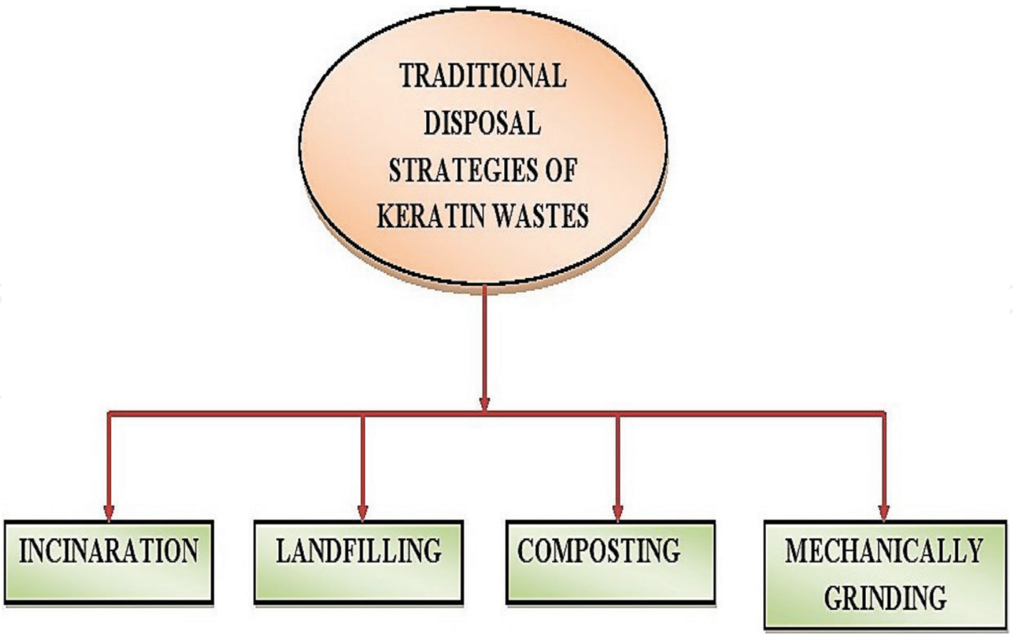

Figure 3. Traditional disposal methods of chicken feather waste.

Disposal of keratin waste from incineration, landfilling, composting, and mechanical grinding is restricted because of enormous production of harmful gases and poses the risk to the environment [66]. Considering the disadvantages of the above all methods, the management of keratin waste using microorganisms appears to be a viable option and it is therefore, attracting scientists for research in this field.

6. Techniques for hydrolysis of keratin waste

The management of keratin waste generated in the poultry industries, leather industries, and slaughterhouses is a major concern of many nations across the globe [93, 94]. Numbers of methods, including hydrothermal, chemical, enzymatic or biological treatments have been therefore investigated in the past few years to improve the digestibility of feathers (**Figure 4**) [20, 95, 96].

6.1. Hydrothermal method

The hydrothermal process usually employs high temperature (80–140°C) and high steam pressure (10–15 psi) with the addition of acids or bases for the degradation of keratin wastes [97, 98]. This method consumes the high quantity of energy and addition of acids (HCl) or bases (NaOH), which break peptide bonds of keratin [99, 100]. Hydrothermal hydrolysis of degradation also required a longer time (16 hours) for feather degradation [20].

Keratin protein is not degraded by trypsin, pepsin, and papain in its native state, because of multiple disulfide bonds [4]. Keratin waste is disposed of through thermal processing according to health regulations. The ash product that is obtained from this process is rich in macronutrients as well as micronutrients. These components have high fertilizing value [97]. The recent processes of hydrothermal treatment are costly as well as destroy amino acids and contain non-nutritive amino acids such as lanthionine and lysinoalanine [70, 101, 102].

6.2. Chemical method

The chemical hydrolysis process of keratin wastes is based on the chemicals (acid, base, catalyst). Chemical hydrolysis requires more aggressive conditions of the reaction (high temperature and pressure) and carries a greater risk to the environment [103]. The chemical hydrolysis reaction is slower and highly efficient, but causes the loss of some amino acids, e.g. tryptophan [76]. The chemical methods require more time, chemicals and energy with expensive industrial equipment for processing. The product has low nutritional value because it contains small amounts of the essential amino acids. The solubility and stability of the hydrolysates depend on the degree of protein degradation [99].

The chemical hydrolysis process increases the emission of certain gases like CO, SO_2 into the environment and causes respiratory diseases, cardiovascular diseases, and cancer, among other illnesses [16]. Hence, there is an urgent need to develop biotechnological and eco-friendly alternatives for recycling of keratin waste.

6.3. Biological method

Considering the potent polluting implications and thermo-energetic cost of the above approaches for the treatment of keratin waste, microbial degradation/ biological method is an alternative, cost-effective and ecologically safe method [104, 105]. Keratinase enzymes produced by microorganisms are the possible alternative to convert keratin waste into the nutrient-rich animal feed [106, 107]. Very few microorganisms utilize keratin by enzymatic digestion as a source of nutrient substrate for growth. These microorganisms are called keratinophilic microflora.

Keratinophilic microflora represents a significant component of soil and an important group of fungi, bacteria and insects that degrade the highly stable animal proteins on earth due to the release of keratinases [14, 34, 108, 109]. Microbial keratinase is a proteolytic enzyme that

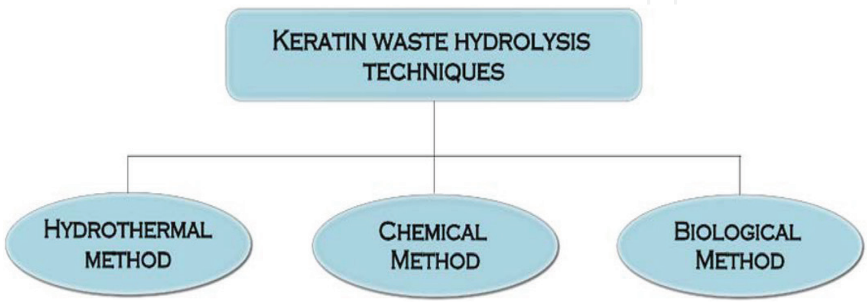

Figure 4. Keratin waste hydrolysis techniques.

posses the capability to degrade the insoluble keratin protein [110–112]. The enzymatic ability of keratin degrading microorganism to decompose keratin has long been interpreted as a key innovation [113]. Keratinase enzymes have molecular weights ranging from 18 to 240 kDa [114, 115].

Biological degradation of keratin waste is more efficient than hydrothermal and chemical degradation, resulting in more useful and toxin-free product. Thus, on employing this ability of keratinophilic microorganisms on an industrial scale, the environmental impacts of incineration and landfilling can be reduced to a great extent.

Microbial keratin degradation follows the sequence of adhesion, colonization, amplification of keratinase pursued by the breakdown and deprivation of the substrate [116]. In the process of microbial keratin degradation, microorganisms' preliminary consumes the lipids (non-keratinous elements) and then begin to degrade keratin [117]. Keratin degradation comprises two major actions, i.e., sulfitolysis (breakdown of disulfide bonds) and proteolysis (proteolytic attack) by keratinolytic proteases (keratinases) based on the complexity nature of keratin [16, 106, 109]. Sulfitolysis is the main process of keratinolysis [118]. In this process, microorganisms discharge sulfide, which is accountable for the breakdown of keratin's disulfide bonds [14]. In proteolysis, bacteria and fungi are able to degrade keratinous substrates proficiently due to their ability to secrete extracellular keratinase enzyme into the medium [22, 105].

Keratinophilic microbes attack keratin substrates in or on soil; therefore, biodegradation takes place [27, 119]. Several microbial strains could be valuable as they possess very significant degradation ability [94]. The keratin-degrading fungi are an environmentally important group of fungi and considered as soil saprophytes [120, 121]. The soil is rich in the keratin protein so the keratinophilic fungi easily occur and grow [122, 123]. Keratin degrading fungi colonize keratin waste and degrade them into low molecular weight [124, 125]. Most of the keratinophilic fungi belong to the families of Arthodermataceae and Onygenaceae in Ascomycetes. The keratinophilic species belong to the genera *Chrysosporium*, *Microsporum*, *Trichophyton*, *Aspergillus*, *Fusarium*, and *Uncinocarpus* [126]. These fungi are active producers of extracellular keratinase, they can be used in bioremediation of such waste and waste contaminated sites [14, 127].

A number of bacterial strains are capable of degrading keratins have been reported. Bacteria can grow faster than fungal species and therefore have potential in industrial applications. The degradation of keratin is predominantly confined by *Bacillus*, *Microbacterium*, *Lysobacter*, *Nesternokia* and *Kocuria* (Gram-positive bacteria) and *Vibrio*, and *Xanthomonas* and *Chryseobacterium* (Gram-negative bacteria) [128, 129]. The maximum feather-degrading abilities are observed mostly in the strains of *Bacillus licheniformis* [31, 130] and less frequently in populations of *Bacillus pumilus*, *Bacillus cereus* and *Bacillus subtilis* [131]. Keratin-degrading bacteria are *Burkholderia*, *Chryseobacterium*, *Pseudomonas*, *Microbacterium* sp., *Vibrio, Flavobacterium,* and *Thermoanaerobacter* [132, 133].

Studies on keratinophilic fungi started in 1952 with the discovery of hair baiting technique. This technique facilitated researchers for isolation of fungi from soil throughout the world [134].

Otcenasek [135] reported worldwide distribution of keratinophilic mycobiota in soil. Most keratin degrading fungi belong to Arthodermataceae and Onygenaceae families of the order Onygenales in Ascomycetes. The growth of fungi on temperature ranging from 15–35°C and some require a range of high temperature for optimum growth [29, 136]. Fungi grow at pH neutral to the weak acidic environment, with the highest production mycelial. Optimum pH 5.0–8.0 is suitable for conidial production and sporulation in liquid media [137, 138]. The screening of keratinolytic activity of fungi was tested through chicken feather degradation in Basal Salt Medium (BSM) [139].

Similar to isolates of fungi, lists of bacterial strains capable of degrading keratins have been reported [140]. Williams [141] isolated feather degrading Gram variable, endospore forming, motile, rod shaped bacterium and identified as *Bacillus licheniformis* PWD-1. This isolate demonstrated facultative growth at thermophilic temperatures with optimum at 45–50°C and pH 7.5. Deivasigamani and Alagappan [9] isolated keratinolytic *Bacillus* sp. from slaughterhouse and poultry farm and observed maximum keratinase activity (122.5 KU/ml) at pH 8.0. Cao [142] isolated a father degrading bacterium (*Stenotrophomonas maltophilia*) from decomposing poultry feathers, which showed the highest feather degrading activity at 40°C and pH 7.5–8.0. The keratin degrading microbes are widespread among the soil microbial population. These microbes have the ability to colonize and breakdown the complex keratinous waste.

The keratinophilic microorganisms effectively degrade the keratin waste and recycle them into valuable products [143]. The possible use of keratinase is in various applications such as in the poultry industries, waste bioconversion, leather industries, pharmaceutical industries, textile processing, detergent formulation, animal feed and fertilizers [144–146].

7. Conclusions

The keratinophilic microflora degrades the various keratinous waste effectively and showed the keratinolytic activity. The keratinous waste degradation by biological way is not only economical but also a possible process for better management of keratinous wastes. Keratin degrading microorganisms could be used for biotechnological application in recycling of poultry waste for environmental protection (production of nitrogenous fertilizer and animal feed) and its fermentation broth could be useful in leather industry and textile industry, etc.

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