



Review

# Biodegradation of Keratin-Rich Husbandry Waste as a Path to Sustainable Agriculture

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**Abstract:** Every year, the size of the human population grows; with it, the need for agricultural products increases. This leads to an increment in the volume of waste, including hard-to-degrade keratin-rich ones, such as feathers. Currently, most of the agro-industrial complex protein by-products are utilized by incineration, landfilling, and chemical hydrolysis. Such methods do not meet modern trends in the development of a sustainable economy, negatively affecting the environment and humans, and preventing the reusing of waste. An alternative is biodegradation, which consists of the application of living organisms and their enzymes to recycle by-products. This approach is not only sustainable, but also makes it possible to obtain products of waste hydrolysis that are in demand for the manufacture of fertilizers and feed additives. This brings the development of agriculture closer to a circular economy and makes the recycling process more profitable. This review article emphasizes the significance of keratinolytic microorganisms and keratinases for the improvement of green methods for processing hard-to-degrade protein waste of the agro-industrial complex, which is necessary for sustainable economic development.

**Keywords:** keratinases; feather waste; enzyme technology; biodegradation; sustainability



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## 1. Introduction

According to the human population projections of the United Nations, the global population will reach 10.1 billion people by 2060 [1]. Such rapid growth creates several serious difficulties for humanity, one of which is hunger. In 2020, 690 million people, or nine percent of the world, were subjected to undernutrition and the absence of decent foodstuffs and meals [2]. One sustainable development goal is to improve nutrition, achieve food security, and end hunger [3]. However, circumstances such as climate volatility, pest invasions, and lately, the COVID-19 pandemic, prevent the establishment of efficient food systems. One way to supply sufficient amounts of food is by increasing the sustainability of agriculture [4].

Animal husbandry not only provides animal products but also has an enormous environmental footprint [5]. However, it is not yet possible to abandon animal husbandry due to the amount of food that needs to be produced. Therefore, it is necessary to research and further use technologies that do not interfere with sustainable development.

Poultry products are the most available among livestock and the cheapest in manufacturing, and are therefore the most represented [6,7]. However, many solid wastes and by-products are also produced by bird farming, e.g., used bedding, feed residues, sawdust, hatchery and mortality waste, and other biological waste such as feces and feathers [8]. The latter can be especially difficult for disposal since it mostly contains the hard-to-degrade fibrillar keratin protein [9]. There are several methods of disposing of waste feathers, including incineration, landfilling, chemical hydrolysis, and carbonization [9–14]. Incineration, or burning, results in gas emissions, including greenhouse gases such as CO<sub>2</sub> and CO, as well as particulate matter and soot [12]. Landfilling keratin waste requires a burial

area and may cause groundwater contamination, potentially leading to the infection of humans and animals [9]. Chemical hydrolysis is performed by harsh chemicals, either by acids or bases, followed by the recovery of substances used or disposal of undesirable salts [10,11]. Despite being in use, these approaches have significant disadvantages that do not meet current environmental trends and hinder sustainable development. Therefore, proper management of waste is required to optimize resource and energy consumption. It has also been shown that feathers can be carbonized to produce graphite materials and activated carbons that are in demand in various fields, including biocatalysis and bioremediation [13,14]. This method of waste disposal reduces the negative impact on the environment; however, it is energy intensive due to the need to apply a high temperature. Despite the obvious advantages of this approach, it also prevents the use of keratin-rich by-products as a source of amino acids and oligopeptides in agriculture, biomedicine, and pharmaceuticals.

Sustainability requires a set of approaches, one of which is the use of biotechnological methods in production and industry [15]. The use of biotechnology to dispose of poultry waste has been proposed already [16], but this approach seems to be the most relevant according to sustainability goals. Current investigations show a significant potential for the biotechnological treatment of waste, including keratin-rich waste [17]. The use of enzymes that break down keratin has several advantages over existing keratin-containing waste disposal methods. In contrast to the procedures mentioned above, enzymatic decomposition does not emit gases into the atmosphere, and there is no risk of contamination; enzyme treatment also allows the minimization of wastewaters and does not require land areas. In addition, enzymatically processed raw materials can be used for other industries such as fertilizers and feed additives [18,19], which is significant for sustainable farming and agriculture. The enzymes that perform the hydrolysis of keratin, or keratinases, may be produced by different organisms, mainly bacteria and filamentous fungi. These microorganisms can grow on various substrates, including wastes, while secreting extracellular enzymes with different substrate specificity and performance optima [20–22]. These characteristics make it possible to consider bacteria and micromycetes as promising producers of keratinases for sustainable agriculture. Using green catalysts for waste management is essential to meet current human needs while conserving the environment and resources.

This mini-review aims to summarize current data on the state of the poultry sector to emphasize the need to develop new sustainable keratin-rich waste disposal methods, as well as to discuss existing biotechnological approaches to solve this problem. To achieve this goal, information was collected and analyzed from open databases such as the Food and Agriculture Organization of the United Nations and topical articles of current interest.

## 2. Poultry Industry Dynamics and Analysis

Although pork is the most consumed type of meat worldwide, the poultry industry is growing most significantly [6]. The term “poultry” includes a wide range of domestic and indigenous birds, such as chickens, ducks, turkeys, guinea fowl, geese, etc. [23]. According to the Food and Agriculture Organization of the United Nations, in 2019, the poultry population reached almost 77 billion heads, of which chickens represented the majority, reaching 94%, followed by ducks (4%), and geese and guinea fowl (1%). The total amount of primary livestock poultry production in 2019 was estimated at 131,647,239 tons [24].

The coronavirus pandemic has disrupted supply chains due to production shutdowns and closed borders, but a compound annual growth rate (CAGR) of 3.8% is expected in 2021, provided by production recovery [25]. Moreover, according to The Business Research Company’s report, annual chicken meat sales will reach \$71.08 billion in 2023 [7].

In addition to the target compounds, poultry production generates by-products such as offal, heads, legs, beaks, skin, cartilage, bones, blood, and feathers [26]. The content of poultry by-products and their estimated amount of protein is presented in Table 1. These residues may be disposed of or somehow processed and applied in different fields. There are the following ways to meet sustainable development trends in waste management:

poultry by-products may be converted to edible material directly or indirectly via plant fertilizers manufacturing or processed into value-added products. The latter are feed additives, fertilizers, meals, biodegradable materials, biofuel, etc. [27,28]. For sustainable development, it is necessary to minimize the impact of waste on the environment, that is, to find a use for the resulting debris. Feathers are tough to utilize and process, although they have great applicable potential since they have a high protein content and make up a significant percentage of the bird's body weight.

**Table 1.** Types of poultry by-products and their protein content.

By-Product	Percentage of Body Mass per Component, %	Protein Content, %	References
Blood	6–7.5	28–31	[29,30]
Bones and cartilage	22–24	20–24	[31,32]
Feathers	7–9	up to 90	[33]
Offal	6	32	[8,34]

### 3. Chicken Feather Waste and Current Waste Management

Even though keratin-containing by-products are formed during poultry farming, pig farming, and cattle farming, chicken feathers constitute the most abundant type of keratin residues [35]. Therefore, proper management of produced feathers is necessary and relevant. Although a minor part of the feathers is proceeded to feather meal and fertilizers, it is considered waste. Assuming that the mass of feather cover is up to 9% of the bird's weight [24], the amount of feather waste can be estimated at 11,848,251 tons worldwide, according to data provided by the Food and Agriculture Organization of the United Nations for 2019.

Contaminated poultry by-products, particularly feathers, contain different species of microorganisms, including *Salmonella* sp., *Staphylococcus* sp., and *Clostridium* sp. [36], which are harmful pathogens. In addition, veterinary drugs, antibiotics, and other chemical compounds applied in poultry farming may also cause water and soil pollution [37]. Therefore, incorrect disposal of feathers can lead to environmental damage, water contamination, and the spread of diseases.

Their structure provides the complexity of the destruction of such wastes. The main compound of feathers is keratin—a hard-to-degrade fibrous structural protein found in vertebrates. There are three distinguished types of keratin:  $\alpha$ -keratins, which are usually found together with  $\gamma$ -keratins; and  $\beta$ -keratins [38].  $\alpha$ -keratins are present in all vertebrates and their secondary structure is dominated by alpha helices immersed in a matrix consisting of gamma keratin, for which the predominance of one of the secondary structures has not been described.  $\beta$ -keratins occur in reptiles and birds; their polypeptide chains form beta sheets [27]. Despite structural differences, it was found that keratins evolved simultaneously and are encoded by a gene family common to mammals, birds, and reptiles. Keratins are distinguished from many other biopolymers by a higher percentage of cysteine, and as a result, its dimerized form, cystine, provides keratin with increased resistance to hydrolysis [39]. Although cystine content is higher in alpha keratin, beta keratin molecules are stabilized by non-covalent interactions between beta sheets, so its structure complicates the hydrolysis and processing of feathers [40]. There are several ways to treat feathers. Table 2 describes some characteristics of these methods and explains the advantages and disadvantages of each.

**Table 2.** Feather waste disposal methods.

Method of Treatment	Advantages	Disadvantages	References
Incineration	Ash produced is safe and decontaminated Ash may be used as a fertilizer Allows the disposal of large volumes of waste	Air pollution Causes bad smell, fumes, and smog Special equipment required	[12,41–43]
Burial	Avoids unpleasant odors A relatively economical option	Groundwater contamination risk Soil and water pollution risk Landfill area required Burial is difficult when the ground is wet or frozen	[9,37,44,45]
Chemical hydrolysis	Results in the production of low molecular weight components	Requires dangerous, harsh chemicals Requires disposal or recycling of residues and undesirable salts needed Large amounts of wastewater produced	[10,11]
Biodegradation	Allows the obtainment of hydrolysate containing single compounds such as oligopeptides and amino acids A relatively energy-efficient process No emissions and environmental pollution Safe for people and animals	Potentially difficult to scale up	[18,46]

Incineration is the most common method of feather disposal [47], but it does not allow the reuse of waste to receive additional benefits and results in greenhouse gas emissions, which lead to global warming and must be avoided in the future. Thus, the existing methods, except for biodegradation, have significant drawbacks that do not satisfy sustainable conservation management. Continued use of these approaches can lead to the depletion of resources and damage to ecosystems. As an alternative green approach, biodegradation can replace unsustainable practices, but it requires additional research, optimization, and technological development.

#### 4. Biodegradation of Keratin Waste

Biodegradation of keratin waste is primarily the destruction of keratin by enzymes of microorganisms. Enzymes that can break down keratin are known and are called keratinases (EC 3.4.21/24/99). They are synthesized by various producers, mainly bacteria and fungi [48].

The ability to synthesize keratinolytic enzymes has long been associated with dermatophyte organisms, whose pathogenicity did not allow the biotechnological optimization of enzyme production [49]. However, further works showed that the formation of keratinases is not an exclusive property for pathogenic organisms [50,51], which made it possible to form a biotechnology research area aimed at studying and using keratinases that are in demand in many sectors of the economy, and at the same time do not pose a danger to humans.

Some keratinase-containing commercially used products are known. They are applied in biomedicine, pharmaceuticals, cosmetology, biodecontamination, and animal feed preparation (Table 3). However, these products have limitations in their use because of their prices, provided by the production, obtainment, and multi-stage purification of them. For biodegradation, scaling up keratinase production is essential since large volumes of feather waste are produced. Therefore, enzyme production costs should be kept to a minimum. The introductory price of keratinases, which does not include isolation and purification, is lower than other enzymes since feathers, which are waste, can be used as a food substrate to synthesize keratinases. The use of difficult-to-hydrolyze poultry waste as a nutrition substrate for the cultivation of microorganisms and the production of their

enzymes is a green and sustainable approach to the production of keratinases for their further application [52].

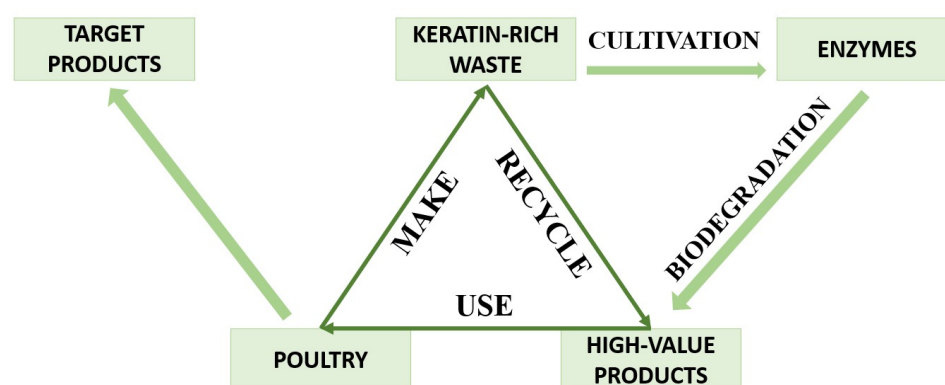
**Table 3.** Keratinase-containing commercially used products.

Product Name	Source	Area of Application	Manufacturer
FEED-0001	<i>Bacillus licheniformis</i>	Production of feed additives for animals	Creative Enzymes
Valkerase		Production of feed additives for animals	BioResource International, Inc.
Versazyme		Production of feed additives for animals	BioResource International, Inc.
Prionzyme TM		Prion degradation	Genencor International, Inc.
Keratoclean PB		Cosmetology	Proteos Biotech
Keratoclean HYDRA PB		Cosmetology	Proteos Biotech
Keratoclean sensitive PB		Cosmetology	Proteos Biotech
Keratopeel PB		Cosmetology	Proteos Biotech
PURE100 KERATINASE	<i>Bacillus sp.</i>	Cosmetology, prion degradation	Proteos Biotech
Alcalase (protease P4860)		Scientific research	Novozymes Crop.
Esperase (protease P5860)		Scientific research	Novozymes Crop.
Savinase (protease P3111)		Scientific research	Novozymes Crop.
NATE-0853		Scientific research	Creative Enzymes
	recombinant strain <i>Escherichia coli</i> BL21		
Proteinase k	<i>Engyodontium album</i> (earlier <i>Tritirachium album</i> ) and recombinant strains	Scientific research	Various
FixaFungus	-	Treatments of toe nail	FixaFungus
Bioguard Plus	Proprietary blend of multiple microorganisms cultures including keratinolytic	Cleaning agents	RuShay, Inc.

From the data presented in Table 3, it can be seen that *Bacillus licheniformis* is the most common producer of keratinases in the industry. However, a large number of articles by researchers from different countries are published annually on the search and study of previously unknown producers of keratinolytic enzymes, as well as on the development of new approaches to increase the synthesis of target proteases [53–62].

The use of keratinolytic enzymes to destroy keratin waste is a promising method for obtaining fertilizers and feed additives for livestock due to the high protein content in feathers [33,63]. In addition, the substances obtained during hydrolysis can be used as a substrate for the cultivation of microorganisms, in particular, for the production of bioelectricity and biofuel [64–66], which has a positive effect on reducing the environmental footprint left by agriculture and also allows the reduction in energy costs within individual enterprises. Scaled-up production of low molecular weight compounds by biodegradation of keratin waste may create a closed-loop supply chain at poultry farms and agricultural complexes, significantly reducing farming's ecological trail. An independent transition from final disposal to feedstocks is an essential step toward sustainability. In addition, such resource use will reduce the cost of purchasing premixes, amino acids, and fertilizers, which is economically favorable. Figure 1 illustrates a potential strategy of the application of keratin biodegradation in terms of sustainable agriculture.





**Figure 1.** Biodegradation of keratin-containing waste in the poultry production cycle.

Hence, biodegradation is a robust approach to keratin-rich waste disposal. As it was previously illustrated, using green catalysts reduces the negative impact on the environment. Unlike existing feather-treatment methods, applying microorganisms and enzymes is affordable and does not lead to resource depletion. It also does not pollute the atmosphere and allows one to obtain many substances that can be further used in manufacturing. Therefore, this approach is vital in the transition to a circular economy that is necessary for sustainable development.

### 5. Keratinases, Their Sources and Use for Biodegradation

Keratinolytic microorganisms are found in all three domains (Bacteria, Archaea, and Eukarya); however, bacteria and micromycetes capable of secreting keratinases are of the greatest interest to the industry. Each of these sources of target enzymes has its own advantages and disadvantages.

Among non-pathogenic and conditionally pathogenic bacteria, representatives of many taxonomic groups exhibit the ability to secrete keratinolytic enzymes, but this property is most common among organisms of the genus *Bacillus*, for example, *Bacillus licheniformis* and *Bacillus subtilis*. In addition, filamentous bacteria, such as *Streptomyces* sp. and *Actinomadura* sp., are capable of the hydrolysis of keratin-rich substrates. Nowadays, only fungi-synthesized keratinases are known in the domain Eukarya. From time to time, reports have been published about this ability in some insects, but it was later shown that the assimilation of keratin is associated with the presence of keratinolytic microorganisms in the intestinal microbiota. Currently, the most studied ability to produce keratinolytic enzymes is in non-pathogenic and conditionally pathogenic fungi, among micromycetes of the genera *Aspergillus*, *Doratomyces*, *Myrothecium*, *Penicillium*, *Purpureocillium*, *Scopulariopsis*, *Trichoderma* and also ascomycete yeast *Candida* and the basidiomycete genus *Coriolopsis* [21,22].

Keratinases can be intracellular, membrane-bound, and extracellular enzymes [67–71]. However, microorganisms more often release them into the external environment, which is biotechnologically beneficial since it increases the availability of the enzymes for isolation and purification and, therefore, reduces the cost of the process. Even though organisms with the constitutive synthesis of keratinases are known [72], most keratinolytics form target enzymes inducibly when growing on keratin-containing substrates acting as a carbon and nitrogen source [21,73]. The cultivation parameters of keratinolytic microorganisms vary and depend on the biology of the producer. For most keratinolytics, the optimal level of acidity of the medium, at which degradation of the keratin-containing substrate is observed, lies in the range of pH 6–9. An alkaline pH can increase the availability of keratin for enzymes since, at high pH values, lanthionine is formed from cysteine, which is more accessible for hydrolysis. In addition, under the conditions of submerged fermentation, many producers may experience an increase in the pH of the culture liquid caused by deamination reactions occurring during protein degradation [17]. The optimum temperature for keratinase synthesis varies from 28 to 50 °C for most bacteria and

fungi, except for some thermophilic and psychrophilic organisms [17]. The synthesis of keratinases is shown in submerged fermentation both under stirring and static conditions. In addition, the synthesis of keratinolytic enzymes can also occur during solid-state fermentation [51,74–77].

Solid-state fermentation requires little water consumption and, accordingly, creates little wastewater and does not demand the use of antifoams, which is an advantage of this method for preserving the environment. Hence, growing keratinolytic microorganisms in such conditions meets the trends of sustainable development. Furthermore, no harsh substances are used during biodegradation, as in chemical hydrolysis. Moreover, fermentation can also occur in a confined space, an advantage over burial, requiring particular areas for landfilling. In addition, as a result of enzymatic hydrolysis, low-molecular-weight proteins, oligopeptides, and amino acids are formed [9]. This makes it possible to use the waste from the agro-industrial complex, implementing the full potential of the biomaterial, which reduces the load on the environment and allows the partial closure of the life cycle of the by-products [78]. It is worth noting that hyphae of micromycete growing in such conditions can braid and penetrate the structures they destroy, facilitating and accelerating the decomposition process of the keratin-containing material [49]. For example, the growth of *Trichoderma harzianum* on feather meal, when is used as a substrate for solid-state fermentation, increases the production of keratinases by seven times compared to that of submerged cultivation, which is widespread in industry [79]. The efficiency of using feather meal for the synthesis of keratinolytic enzymes under solid-state conditions was also shown for the culture of *Aspergillus niger* [80].

For some keratin-degrading enzymes, broad substrate specificity is known. It has been shown that some keratinases have the ability to break down substrates such as casein, gelatin, bovine serum albumin, hemoglobin, silk fibroin, collagen, and elastin. Hence, their activity is not limited exclusively to the hydrolysis of keratin [21,73]. In addition, fungal enzymes may have a more diverse structure since the structure of the eukaryotic cell of the producer fungus allows the synthesis of proteins to be modified, for example, by glycosylation, which potentially increases the diversity, resistance, and range of conditions in which these protein catalysts can function [81].

Despite the vast possibilities of modern bioengineering and the relative simplicity of manipulating the genomes of prokaryotes [82–85], exploratory research to study new sources of keratinases remains relevant and in high demand due to the low level of accumulated knowledge and high prospects of keratinolytic micromycetes [80,86,87].

## 6. Conclusions

Industrialization and technological development have significantly impacted living standards and changed many of the usual production processes. The growth of the world's population has resulted in the problem of hunger, a solution of which leads to the depletion of natural resources and climate change. Production rates of animal products have grown tremendously, along with the amount of waste. Poultry farming leads to the formation of a large quantity of hardly hydrolyzable keratin-containing feather waste, mainly disposed of by incineration, burial, or chemical hydrolysis. These approaches exacerbate environmental problems and also hinder the rational use of resources and conservation. A green alternative in a sustainable environment is the use of green catalysts. Various microorganisms can synthesize enzymes capable of degrading rigid keratin, but fungal keratinases have vast biotechnological potential. While growing on feather waste, fungi can secrete target enzymes with various properties, substrate specificity, and working conditions. Enzymatic hydrolysis allows rational waste disposal and results in obtaining different biobased products, which can then be returned into the production cycle. Research and the further application of biodegradation of keratin-containing poultry waste is an essential and significant step towards sustainability.

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