

Microbial Degradation of Poultry Feather Wastes under the Influence of Temperature and pH – A Review



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Abstract

Poultry feathers require different approaches of disposal. Feather waste represents a huge resource of protein and the rational utilization of millions of tons of waste feathers will not only ease the shortage of protein resources but will also improve the environment. Conventional methods used for disposing or converting them to protein hydrolysates such as land filling, incineration, burning or the use of chemicals are costly, not environmentally friendly, and pose a danger to living organisms. Microbial hydrolysis is considered an alternative environmental-friendly method for recycling. As physiological and nutritional factors greatly affect feather degradation and keratinase production, the effects of various factors such as incubation temperature and pH on feather degradation by various microorganisms were reviewed in this study. Based on the various studies reported earlier involving microorganisms to degrade poultry feather and keratinase production, biodegradation of feathers is found to be an efficient, cost-effective, and environmentally friendly method for bioconversion of feather waste into useful products. The use of keratinolytic microorganisms to degrade poultry feather has emerged as a sustainable and alternative tool to sustainable use of feather waste.

Keywords: Poultry feather; Feather waste; Keratin; Keratinase; Biodegradation

Introduction

The daily consumption of chicken increases annually as one of the cheapest and healthiest sources of protein. About 3 billion pounds of chicken feathers are generated every year in the world with a great percentage disposed as waste [1,2]. Feathers account for approximately 5-7% of the total weight of a mature chicken and feather waste represents a huge resource of protein. 90% of feather biomass constitutes primarily β -keratin, azelon, and insoluble protein extensively cross-linked by disulfide bonds [3,4], 70% of amino acids, high-value elements, vitamins, and growth factors [5].

Since each chicken can provide meat, the generation of chicken feather wastes from slaughter and poultry houses will come from different coloured chickens such as white, black, brown and/or multiple-coloured broilers, layers and cockerels. These keratin-rich wastes are considered recalcitrant environmental contaminants and mixture of different coloured feather wastes is often disposed in dumb sites or landfills especially in developing countries. Majority of feathers are discarded or burned as waste, while a small proportion is used in down products and insulation material [6]. Despite the abundance, chicken feathers are more frequently treated as waste and often burned or taken to the landfill, polluting the environment.

Economic pressures, environmental pressures, increasing interest in using renewable and sustainable raw materials, and the need to decrease reliance on non-renewable petroleum resources behave the industry to find better ways of dealing with waste feathers. Burning poultry wastes may actually produce as much or more toxic air emissions than coal plants. Due to poor management, poultry waste especially feathers have become one of the major pollutants due to their recalcitrant nature [7,8]. The rational utilization of millions of tons of waste feathers in poultry factories will not only ease the shortage of protein resources but will also improve the environment.

Hydrolysis, reduction, and oxidation methods have been reported to isolate keratin from feather, wool, and hair [9-13]. Nevertheless, a large number of chemical reagents, i.e., urea, sulfite, thiol, acid, and alkali, were used in the extraction processes. However, the conventional methods used for disposing or converting them to protein hydrolysates such as landfilling, incineration, burning or the use of chemicals are costly, not environmentally friendly, and pose a danger to living organisms. Biodegradation of keratin is a process that involves a variety of biological factors and definitive mechanisms.

An alternative to reduce these environmentally unfavorable disposal options is the utilization of feather constituents as animal feed. Traditional methods to degrade feathers for subsequent use as animal feed include alkali hydrolysis and cooking under steam pressure. For example, the feathers may be hydrolysed, dried and ground to a powder to be used as a feed supplement for a variety of livestock, primarily pigs [14]. This is a fairly expensive process, however, and results in a protein product of low quality for which the demand is low [15]. These methods are not ideal in that they not only destroy the amino acids in the feathers but also consume large amounts of energy. Further, high content of β -keratin renders chicken feathers insoluble and resistant to intestine digestion by animals unless they are appropriately pretreated [16]. The keratinase produced performs keratinolytic function through breaking down of complex keratin structure of feathers, and subsequent releasing the major amino acids in the keratin, as well as soluble oligopeptides.

The management of feather wastes requires different approaches. The most commonly used method for feather disposal is incineration [17] and degradation by chemical methods. Compared with the physical and chemical treatments that frequently involve highly energy-consuming processes, feather treatments with biological processes are more promising because bioprocesses are advantageous in keeping the valuable nutrient components that are vulnerable to harsh conditions. Microbial hydrolysis is considered an alternative environmental-friendly method for recycling and valorising feathers [18-20]. Microbial processes are not only environmental friendly [21], but also maintain the original structure and activity of the products [22]. Studies on biodegradation are focused on the screening and identification of microorganisms that can degrade feathers. Diverse microbes have been identified as potent feather degraders. A number of feather-degrading microbes such as bacteria, *Streptomyces* and fungi [23-25] have been isolated from various environmental sources. Protease with keratinase activity produced by growing microbes of certain species decomposes the feathers, releasing the proteins, peptides, and free proteinogenic amino acids (and derivatives) that can support the growth of the living microbes. The products of keratinase hydrolysis of feathers are mainly amino acids and soluble peptides and display antioxidant properties [26,27].

Degradation of feathers is the process of enzymatic degradation of β -keratin. The destroying of the hard protein by heating facilitates the function of enzymes, leading to more degradation [20]. Keratinase enzymes that have been purified from different microorganisms to date, such as fungi [28,29], bacteria [30,31], and a few *Streptomyces* species [32,33], all act as proteinases and have a high level of activity on insoluble keratin. Complete feather degradation ($98.3 \pm 1.52\%$) with high keratinase production ($373 \pm 4U/ml$) by *Bacillus pumilus* was observed [34].

As physiological and nutritional factors greatly affect feather degradation and keratinase production, the effects of various

factors such as incubation temperature and pH on feather degradation by various microorganisms were reviewed in this study.

Methodology

A systematic search was carried out in PubMed, Scopus and Web of Sciences using a combination of Boolean operators. Peer reviewed papers in English on the microbial degradation of poultry feather were retrieved and evaluated based on titles and abstracts. The retrieved papers were managed using Mendeley and the data were consolidated.

Morphological Structure of Feather

The chicken feather is composed of three distinct units: the rachis, the central shaft of the feather that runs the entire length of the feather to which is attached the secondary structures, the barbs and the tertiary structures, the barbules. The length of the rachis varies depending on the sampling position of the feathers on the body of the chicken; however, the lengths of barbs and barbules do not vary much except that sometimes barbs and barbules at the base of the rachis are longer than those at the tip of the rachis. The lengths of the rachis are about 1-150mm and barbs are about 1-45mm. The barbules are about 1-800mm long and have hook-like structures at their tips. Barbs display a fibrillar surface but no scale [35].

Keratin is the insoluble structural protein of feathers and wool and is known for its high stability. The composition and molecular configurations of its constituent amino acids warrant structural rigidity. The keratin chain is tightly packed in the α -helix (α -keratin) or β -sheet (β -keratin) into a supercoiled polypeptide chain, resulting in mechanical stability and resistance to common proteolytic enzymes such as pepsin, trypsin, and papain. In addition, cross-linking of protein chains by cysteine bridges confers high mechanical stability and resistance to proteolytic degradation of keratins.

Role of pH and Temperature on Microbial Feather Degradation

pH

The increase in pH during cultivation is an important characteristic accompanying keratin hydrolysis and the keratinolytic potential of micro-organisms. Organisms with a higher keratinolytic activity turn the media more alkaline, in comparison with other organisms exhibiting lower keratinolytic activity [36]. This observation is based on the fact that keratin degradation involves deamination reactions, which result in an increase in pH. Enhanced keratinase activity of 16.15U/mL was observed at pH 8 by *Alcaligenes* sp [37]. The bacterium grew well over a pH range of 7e9 using phosphate buffer, while high keratinase production was limited to pH 7.5-8.5 with 96% feather degradation. Sangali & Brandelli [38] reported that keratinase production was higher at pH 6.0.

The optimum pH of 7.8 for keratinase production by various fungi such as *Scopulariopsis brevicaulis* [39], *Microsporum canis* [40], *Doratomyces microsporus* and *Paecilomyces marquandii* [41,42] was reported earlier. Farag & Hassan [28] reported the purification and characterization of a keratinase secreted by *Aspergillus oryzae*. The effect of immobilization on the obtained enzyme was also taken into consideration and maximum keratinolytic activity of free enzyme was observed between pHs 7 and 9 with an optimum at pH 8.0 and the optimum pH of the immobilized enzyme was 7.4. The optimal conditions for keratinase production using chicken feather include initial pH of 7.0, inoculum size of 2% (v/v), and cultivation at 40°C by *Bacillus weihenstephanensis* [43].

Kojima et al. [44] had isolated the feather-degrading *Bacillus pseudofirmus* FA30-01 from the soil sample of poultry farm. The isolate completely degraded feather pieces after liquid culture at 30°C (pH 10.5) for 3 days. In another study, maximum feather-degrading activity by *Chryseobacterium* sp. was observed at pH 8.0 and 25°C [45].

Temperature

The interaction between temperature and feather degradation was studied by Demir et al. [25]. Keratinase activity was dependent on the temperature. For example, lowest keratinase activity (8.03U/ml) was with the lowest temperature condition (15°C) and the highest activity (405.5U/ml) was at highest temperature (27.5°C). Similar observations were reported previously with other bacteria [46-48] describing of the effect of temperature on the keratinase production. The growth, feather degrada-

tion, and keratinase production of *Alcaligenes* sp. were optimal within a room temperature range of 25-30°C [37]. The maximum enzyme production was observed by growing *Vibrio* sp at 30°C, although similar levels were observed at 25°C [38]. Anbu et al. [47] demonstrated that the keratinase produced by *Trichophyton* sp. was able to actively degrade the chicken feather as substrate. They found that highest enzyme production by *Trichophyton* was registered at 35°C (5.0KU/ml) and there was a decline in enzyme production beyond 35°C.

Keratinase from *Kocuria rosea* was active at a broad range of temperature (25-55°C) with an optimum of 40°C. The enzyme remained fully active when tested at temperatures between 10 and 60°C for 1h; additionally, at 90°C the enzyme was 40% active [31]. Disintegration of whole chicken feathers by incubation with keratinase from *Streptomyces pactum* was optimal in the range of 40 to 70°C [32]. The complete degradation of feather by *Bacillus licheniformis* was observed in the feather-peptone medium within 24h at 37°C [4]. *Fervidobacterium islandicum* showed the highest keratinolytic activity and degraded native chicken feathers completely at 70°C within 48h [49]. The effects of initial pH and temperature on feather degradation by *B. subtilis* were investigated [50]. An optimum effect at 37°C and pH 8.5 in a basal feather medium was found in the degradation process. *Bacillus licheniformis* from a polluted river exhibited high proteinase production when grown in chicken-feather media. Complete feather degradation was achieved at 37°C after 48hrs [51]. Werlang & Brandelli [52] reported that the temperature of 30-37°C was optimum for feather-degrading activity by *Bacillus* sp (Table 1-3).

Table 1: Bacterial degradation of Poultry Feather and Keratinase Production.

Organism	Temperature	pH	Keratinase Yield	Time	Reference
<i>Vibrio</i> sp strain kr2	30°C	6	32U/ml	72hrs	[38]
<i>Xanthomonas maltophilia</i>					[53]
<i>Fervidobacterium islandicum</i>	70°C	7	13U/mL	48hrs	[49]
<i>Bacillus licheniformis</i>	47°C	7			[7]
<i>Chryseobacterium</i> sp. kr6	25°C	8			[45]
<i>Microbacterium</i>	30°C	7	43U/ml		[46]
<i>Bacillus subtilis</i>	37°C	8.5			[50]
<i>Bacillus licheniformis</i>	37°C			24hrs	[4]
<i>Kocuria rosea</i>	40°C	10			[31]
<i>Bacillus pseudofirmus</i>	35°C	10.5		3 days	[44]
<i>Bacillus licheniformis</i>	37°C		37.35U/mL	48hrs	[51]
<i>Bacillus megaterium</i>	25-40°C	7-11	468U/mL	7 days	[14]
<i>Bacillus subtilis</i>	30°C	8	53.3U/mL	4 days	[54]
<i>Bacillus weihenstephanensis</i>	40°C	7			[43]
<i>Bacillus</i> sp MKR5	40°C	8	100%		[24]
<i>Nocardiopsis</i> sp. SD5	45-50°C	9	64.6U/mL	4 days	[55]
<i>Alcaligenes</i> sp.AQ05-001	27°C	8	88.4U/ml	72hrs	[49]

<i>Bacillus sp MBRL 575</i>	30°C		305U/ml		[56]
<i>Bacillus pumilus</i>	37°C	10	373U/ml		[34]
<i>Bacillus licheniformis</i>	42°C	6	72.4 U/ml		[58]
<i>Bacillus aerius</i>	35°C	7.5	127.63 U/ml		[18]
<i>Micrococcus luteus</i>			32.3 U/mL	15 days	[59]
<i>Pseudochrobactrum IY-BUK1</i>	30°C	7.5	95.25 U/ml	168 hrs	[57]
<i>Bacillus paralicheniformis</i>					[60]
<i>Bacillus licheniformis</i>	35°C			96 hrs	[61]
<i>Stenotrophomonas maltophilia</i>	23°C			96 hrs	[61]

Table 2: Fungal degradation of Poultry Feather and Keratinase Production

Organism	Temperature	pH	Keratinase Yield	Time	Reference
<i>Aspergillus niger</i>	50°C	8	71.43U/ml		[28]
<i>Beauveria bassiana</i>		6.0-8.2	62.8U/ml	4-6 days	[66]
<i>Alternaria tenuissima</i>	25-28°C	6.5	53.8U/mL		[66]
<i>Beauveria bassiana</i>	25-28°C	6.5	62.8U/ml		[66]
<i>Curvularia brachyspora</i>	25-28°C	6.5	55.4U/ml		[66]
<i>Trichophyton</i>	35°C	8	4.6U/ml	5 weeks	[47]
<i>Myrothecium verrucaria</i>	37°C	8.3			[62]
<i>Microsporum fulvum</i>	30°C	6.5			[63]
<i>Aspergillus fumigates</i>	NR	8.5			[25]
<i>Aphanoascus fulvescens</i>	28.7°C	7.58			[64]

Table 3: Degradation of Poultry Feather and Keratinase Production by Actinomycetes.

Organism	Temperature	pH	Keratinase Yield	Time	Reference
<i>Sreptomycetes fradiae</i>	60°C	8		0.5hrs	[65]
<i>Streptomycetes sp 2M21</i>	27.5°C	8	405.5U/ml	5.5 days	[23]

Conclusion

Due to poor management, poultry waste especially feathers have become one of the major pollutants. Traditional feather degradation reduces the overall quality of proteins and destroys essential amino acids. Biodegradation of feathers is found to be an efficient, cost-effective, and environmental friendly method for bioconversion of feather waste into useful products. The use of keratinolytic microorganisms to degrade poultry feather has emerged as a sustainable and alternative tool to meet this challenge.

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