

BIOREMEDIATION OF POULTRY FEATHER WASTE- A REVIEW

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ABSTRACT

Poultry farming is growing at 8% Compound Annual Growth Rate (CAGR) in layer and 12% CAGR in broiler farming. Consequent to the growth of the poultry farming activity, eco-friendly approach to utilize the waste into value added products are of current importance. Waste on one hand is responsible for adding pollutants to the environment; on the other hand it causes major problems in handling. In domesticated birds, feather waste accounts to 7% of the body weight of the birds. At present it is being disposed off without any solid waste treatment. Feather is having advantage of containing valuable sulphur containing amino acids like cysteine and methionine. Over the years, feather meals are produced using thermal and chemical processes. However these methods are having disadvantages of energy expensive and poor end products. Now lot of research work are attempted through bioremediation of the feather meal. Even though much of the investigations are on the laboratory side, applications of this technology are yet to be taken up, either due to lack of detailed knowledge on these technologies or lack of information about various bacteria and fungi available for this purpose. In this paper various bacteria like *Bacillus subtilis*, *Bacillus pumilis*, *Bacillus altitudinis*, *Bacillus licheniformis*, *Pseudomonas microphilus*, *Leuconostoc*, *Thermus aquaticus* and *Fervidobacterium pennavorans* for their potential as keratinolytic activity are discussed. Similarly role of certain fungi like *Aspergillus niger*, *Penicillium citrinum*, *Trichoderma viride*, *Alternaria tenuissima*, *Chrysosporium tropicum* and *Fusarium culmorum* in bioremediation of the feather to feather meal are also elaborated. Altogether bioremediation of feather meal concurrently adhere to integrated eco-friendly waste disposal with due importance to maintaining biodiversity of the living organism in the ecosystem.

INTRODUCTION

Chicken feather is one of the major waste product getting accumulated without processing in the poultry processing industries. The quantity of feathers generated as waste cause major environmental hazards due to the decomposition and uncared disposal. Right now, chicken

feathers are disposed as landfills or burnt without any defied planning. Rather, feather could be converted as feather meal through different processing techniques like, chemical hydrolysis, thermal processing and bioremediation. Feather meal produced by this technique contains valuable proteins rich in hydrophobic amino acids and also other amino acids like arginine, cysteine

and threonine. Established feather meal production technology is a hydrothermal process. However, in this process there is every possibility for the reduction of amino acids like, methionine, lysine, tyrosine and tryptophan with further disadvantage of low digestibility and low nutritional value. As to work on the disadvantages, now microbes are being utilized for degradation of feathers into feather meal. Many microbes have been identified so far for efficient degradation of feathers. Some of the thermophilic and mesophilic microbes having feather degradation properties are, *Bacillus*, *Streptomyces* and *Chryseobacterium* strains. In addition, different mycotic flora are also responsible for the degradation of keratin by their metabolic product keratinase.

Feather constitutes 5-7% of body weight of the bird that is being disposed off in the massive quantity around the world. Raw feather without processing causes major ailments like chlorosis, mycoplasmosis etc. Because of the positive chemical composition, feather waste can be conveniently processed as valuable protein supplement, adding to the stock of protein rich feed stuffs to the livestock and poultry. Quiet important factor here however is to have cost effective ecofriendly technology to process feather waste into feather meal.

Chicken feather is having enormous tough keratinous materials in the shaft and other places. Disposal of feathers as such in the environment leads to prolific multiplication of mesophilic keratin degrading microorganism especially in keratin rich soil. Such mesophilic

keratinolytic organisms act as pathogens to human beings. Increased feather waste accumulation in the environment is a major disturbing ecological factor due to massive growth of the global poultry industry. Existing feather waste management could cause major health problems to the other human beings and animals.

Right now keratinase waste are getting accumulated in the environment as a final waste product from different industries, which are uncared due to lack of proper technology. Attempts are now being made through biotechnological means to hydrolyse these keratinase waste to soluble form by application of keratinase enzymes through microbes. These keratinase enzymes are powerful proteolytic enzymes capable of hydrolysing the insoluble keratins. In addition these enzymes have multiple applications in further processing of waste from poultry industry. Besides these enzymes are also used in detergent production, medicine, cosmetics, feather and feed industries and need areas like prion degradation as a treatment for mad cow disease. This utility enzymes are produced by many insects, microorganisms like bacteria, saprophytic and parasitic fungi and actinomycetes. Bacterial keratinases have special advantages of degradation of insoluble keratin substrates (Lin *et al.* 1995).

Even though feather are considered as a valuable biovalue amino acid resources, still cost effective technology to convert the feather waste into high value feather meal is yet to be established. Bioremediation process is one of the advanced eco-friendly emerging technology to utilize the feather

waste into feather meal, overcoming the limitations in thermo and chemical hydrolysis.

Composition

Chicken feather constitutes around 91% protein, 1% lipids and 8% water. Amino acid sequence of chicken feather is identical to that of other feathers and also mostly similar with reptilian keratins found in the claws. Amino acid composition of the feathers mostly are cysteine, glutamine, proline and serine. On the contrary, histidine, lysine, tryptophan, glutamic acid and glycine are absent. More than 16% serine is present in the feather and its hydroxyl group

is responsible for chicken feather stability (Kannappan and Bharathi, 2012).

Keratins are basically insoluble protein commonly seen in wool, hooves, scales, hair, nails (hard keratins) and also in stratum corneum (soft keratins). This specific proteins come under the classification of sclera protein groups which are highly resistant to the action of physical, chemical and biological agents. Disulphide bonds, hydrogen bonds, salt linkages and cross linkages present in the keratin are responsible for the mechanical stability and high resistance to proteolytic degradation of keratin.

Table 1: Amino acid content in keratin fiber from chicken feather

Functional groups	Amino acid	% Contents
Positively charged	Arginine	4.30
Negatively charged	Aspartic acid	6.00
	Glutamine	7.62
Hygroscopic	Threonine	4.00
	Serine	16.0
Hydrophobic	Tyrosine	1.00
	Leucine	2.62
	Isoleucine	3.32
	Valine	1.61
	Cystine	8.85
	Alanine	3.44
	Phenylalanine	0.86
	Methionine	1.02
Special	Proline	12.0
	Asparagine	4.00

Chicken feather fibres are basically having α - helical and some β sheet confirmations. However its outer quill

is different by having higher β –sheet conformations and lesser α - helical conformations. Higher cysteine content is

found in hard β -sheet keratins than soft α -helical keratins with greater possibility for higher disulphide chemical bonds responsible for linkage between keratin proteins. Strong covalent bonds present in the feathers are the main factors for the stability of the three dimensional structure which are difficult to break.

Feather Bioconversion

Existing application of technology limits the conversion of feather waste into feather meal in major way. Existing processing technique for feather waste requires steam cooking or chemical digestion to make it more degradable form constantly with more energy requirement. Bioconversion utilizing the micro-organism is an alternative cost effective technology to convert the feather waste into high nutritional value feather meal.

Advantages

Application of bioprocess technology involving keratinolytic organism gives three major advantages like ecological, economical and high nutritional values due to better digestibility.

Keratinolytic activity of several microbial species, such as, *Chrysosporium*, *Aspergillus*, *Alternaria*, *Trichurus*, *Curvularia*, *Cladosporium*, *Fusarium*, *Geomyces*, *Gleomastis*, *Monodictys*, *Myrothecium*, *Paecilomyces*, *Stachybotrys*, *Urocladium*, *Scopulariopsis*, *Sepedonium*, *Penicillium*, *Doratomyces* (fungi), *Streptomyces*, *Vibrio*, *Microbacterium* and *Bacillus* (bacteria), has been reported in the literature.

Source of keratinolytic organisms

Most of the keratinolytic organisms isolated from the soil collected near feather processing units are Bacteria, *Bacillus* Sp. FK46, *B. licheniformis*, *B. pumilus* sps, *Vibrio* sps strain Kr2, Actinobacteria, *Streptomyces pactum*, *S. albus*, and Saprophytic and Dermatophilic fungi, *Aspergillus* sps, *Rhizomucor* sps, *Trichophyton mentagrophytes*, *T. rubrum*, *T. gallinae*, *Microsporum canis* and *M. gypseum*. Here under, findings in different research trials for bioconversion of feather waste utilizing the microbes are presented.

Bacillus subtilis, *B.pumilis* & *B.cereus*

As per investigations carried out by Kim *et al.* (2001) it is found that feather degrading bacteria are ubiquitously present in feather waste. Among the isolates, *B.subtilis*, *B.pumilis* & *B.cereus* were commonly present. All these three isolates had degrading ability to the extent of 142, 96 and 109 units of keratinolytic activities respectively. It is stated that *B.pumilis* & *B.cereus* were inducible for production of keratinolytic proteases by feathers, whereas *B.subtilis* responded for the enzyme production with casein, feather and BSA. The optimum condition required for the maximum enzyme production activity were 40°C and pH 5-9 for *B.subtilis*, 40°C and pH 5-6 in respect of *B.pumilis* and 30°C and pH 7.0 for *B.cereus*. Time required for maximum keratinolytic activities for *B.subtilis* and *B.pumilis* were 84 and 72 hrs of incubation respectively with 161 and 149 units/ml enzyme production. *B.cereus* showed maximum enzyme activity of 117 units after 60 hrs of cultivation. It is also

reported that maximum enzyme activities were recorded in the late logarithmic growth phase or the beginning of the stationary phase. Similar trend was observed in production of soluble protein as that of keratinase.

Bacillus sp. MPTK6

According to the studies of Mukesh *et al.* (2012) *Bacillus sp. MPTK6* was found to degrade 30g/L raw feathers into feather protein hydrolysate under optimum pH 10.0 for 72 hrs of fermentation. Also the free radical- scavenging activity of feather protein hydrolysate was analysed using DPPH assay. Hydrolysed feather was found to have improved invitro digestibility than raw feathers.

Bacillus altitudinis

The importance of feather waste degradation by keratinolytic organisms was reported by Vijay Kumar *et al.* (2011) to supplement livestock feed and for production of protein hydrolysates. The keratin degrading bacterium *Bacillus altitudinis GVC11* was isolated and identified by him through morphological, biochemical and 16s RNA studies. The organism degraded white and dark chicken feathers in 48 and 96 hours respectively. Optimal production of keratinase enzyme by feather degradation was observed at 37°C, pH-9 and 200 rpm. During fermentation process, essential amino acids like phenyl alanine, valine, leucine, isoleucine and threonine were observed in 100 ml culture supernatant at 124, 88, 19, 17 and 11 µmols concentrations.

Bacillus licheniformis

B.licheniformis was the first bacterium to be identified for feather degradation and named as poultry waste digester-1 (PWD-1) by William *et al.* 1990. It is a non-pathogenic, gram positive, motile, spore producing, facultative anaerobe mainly present in soils and can be easily isolated due to highly resistant endospores. (Ponnusamy Konar Poovendran *et al.* 2011).

Brutt and Ichida , (1999) reported the presence of keratin hydrolyzing bacteria *B.licheniformis* in the plumage of living birds. They also demonstrated that inoculation of the bacterium intensified keratin degradation in poultry compost due to secretion of keratinase enzyme.

Ekta Tiwary and Rani Gupta, (2012) studied the keratinolytic property of *B.licheniformis* which produced the dimeric keratinase having the ability to degrade chicken feather. 25g of boiled chicken feather was converted into feather meal within 8 hours at 50°C, pH-8 and 150 rpm. 1200U of keratinase was required to degrade 2.5g of chicken feather. Feather meal produced after soaking and boiling was dried at 80°C and ground to fine powder. It contained 14% nitrogen and 44% carbon with all essential amino acids and showed 73% *in-vitro* digestibility.

Bacillus subtilis S1-4

Yong Bin *et al.* (2013) isolated a keratin degrading bacterial strain *Bacillus subtilis S1-4* from chicken feather and identified it by comparative genome analysis. 5% simple basal salt cultures at a narrow pH caused complete degradation of

chicken feathers resulting in the production of 11.61 mg/ml soluble peptides and 6.86 mg/ml amino acids respectively. During this process the organism secreted several proteases and keratinases along with disulphite reductase activity indicating their involvement in feather degradation.

Pseudomonas sp. MS21

As per investigations studies carried out by Tork *et al.* (2010) a keratinase producing organism *Kera MS21* was identified by morphological, physiological and biochemical characteristics as belonging to the genus *Pseudomonas*. The identification was confirmed by 16S rDNA studies. Optimal activity of the organism was observed at 37°C and pH-8.

Leuconostoc sp. and Pseudomonas microphilus

Tamil Kani *et al.* (2012) screened the ability of *Leuconostoc* and *Pseudomonas microphilus* to degrade feather keratin into feather meal. Among the two organisms maximum keratinase activity was 0.884 IU/ml for *Pseudomonas microphilus* in 30 days. 20% of the feather was degraded in 10 days and 70% in 30 days by *P.microphilus*.

Thermophiles

Complex nature of chicken feathers and pathogenicity mesophilic organisms has limited the use of mesophilic keratinolytic organisms. Some obligate thermophiles are also capable of degrading feather. One advantage of using thermophiles is that hard insoluble proteins like keratins gain plasticity at higher temperatures, so that they become more vulnerable to thermal

proteases. Anaerobic thermophiles such as *Thermoanaerobacter keratinophilus*, *Fervidobacterium pennavorans* and *Fervidobacterium islandicum AW-1* have been reported so far to have keratinophilic activity (Anupam Bhagat and Smitha Lele, 2012).

Thermus aquaticus

Thermus aquaticus, a non-spore forming, obligate aerobic thermophile has been analysed for keratinase production by Anupam Bhagat and Smitha Lele, (2012) . *Thermus aquaticus* produced 479U/ml of keratinolytic protease by digesting 0.4g/dl chicken feather in 48 hours. Hence *Thermus aquaticus* was found to have remarkable feather-degrading properties and could be used for protease enzyme production for industrial detergent applications. Vitamin K2 rich cells of *T.aquaticus* grown on chicken feather waste along with carotenoids can be used as feather meal for poultry.

Fervidobacterium pennavorans

This bacteria is novel thermophilic bacteria belonging to *Thermotogales* order isolated by Friedrich and Antranikian, (1996) from the hot spring of Azores islands. This is the first known extreme thermophile having ability to digest native feathers at high temperatures. It grows optimally at 70°C and pH 6.5. Based on the physiology, morphology and 16S rDNA studies, the new isolate was considered to be a member of the *Thermotogales* order and was referred as *Fervidobacterium pennavorans*. This strain was mostly related to *Fervidobacterium islandicum* and *Fervidobacterium pullulanolyticum*.

Chemical constituent of the keratinolytic enzyme produced by this organism is a serine protease with a molecular mass of 130 kDa and an isoelectric point of 3.8 along with optimal activity at 80°C and pH 10.0.

Fungi

In addition to several bacteria, certain fungi have been isolated having keratinolytic properties. Some of keratinolytic fungi types are reviewed here. Anbu *et al.* (2011), isolated a total of 20 fungal strains and 5 dermatophytes. He isolated another 20 strains of fungi from poultry farm soil of Namakkal, India. The organisms were screened for the production of extracellular enzymes like amylase, protease, cellulase, lipase and keratinase using plate assay. All the sporulating organisms were found to produce atleast one keratinolytic enzyme. *Chrysosporium keratinophilum*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium citrinum*, *P. frequentans* and *P. purpurogenum* were able to produce all types of examined enzymes. *A. versicolor* and *A. niger* produced the highest amount of enzymes amylase and cellulase. *P. citrinum* and *Trichoderma viride* secreted lipase enzyme and *Scopulariopsis brevicaulis* secreted the highest amounts of protease and keratinase. *Trichophyton mentagrophytes* and *A. niger* produced keratinase in greater amounts.

Feather associated Fungi

Sanjana and Geetha, (1999) isolated fourteen species of keratinophilic fungi namely *Chrysosporium*, *Malbranchea*, *Chaetomium*, *Sepedonium*, *Microascus*, *Scopulariopsis*, *Curvularia*, *Fusarium*,

Aspergillus and *Penicillium* from 100 poultry birds. The fungi were grown on basal salt solution containing natural keratin from human hair as a source of carbon and nitrogen and in another medium containing minor amount of readily utilisable carbon and natural keratin. All the isolated fungal species released sulphhydryl containing compounds cysteine and extracellular keratinase. Improved enzyme production was observed with addition of glucose and vitamins in the broth which also indicated the correlation between the mycelia biomass and proteolytic keratinases.

Filamentous fungi

Nadir *et al.* (2008) analysed 106 filamentous fungal strains from poultry farm waste mainly litter in Brazil. Among those, 13 species belonging to different genera namely *Aspergillus*, *Acremonium*, *Alternaria*, *Beauveria*, *Curvularia*, *Paecilomyces* and *Penicillium* were identified as feather keratin degrading organisms and were able to produce keratinase in the stationary phase of culture. A comparative study on four highest keratinase producing organisms was carried out in submerged and stationary conditions. The highest keratinase production was observed in 4 to 6 days in submerged conditions with 53.8 ± 6.1 U/mL in *Alternaria tenuissima*, 51.2 ± 5.4 U/mL in *Acremonium hyalinulum*, 55.4 ± 5.2 U/mL in *Curvularia brachyspora*, and 62.8 ± 4.8 U/mL in *Beauveria bassiana*.

Non dermatophytic filamentous keratinolytic fungi

Ingle *et al.* (2012) undertook systemic studies to investigate keratinolytic fungi in

poultry feather waste. During this study, 22 fungal species namely *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum*, *Myrothecium roridum*, *Penicillium sp.*, *Trichoderma hamatum* including certain non-spore forming fungi were recovered from poultry farm soil samples as keratin degrading organisms. Optimum growth was observed at 45°C within pH range of 5.0 to 6.5. These organisms degraded feathers completely in 11 days by producing keratinase enzyme.

Aspergillus

Aspergillus is a highly aerobic spore-forming structure, which grows as molds on the surface of its substrates. It has been reported to produce several proteolytic keratinases. Kim, (2003) isolated 14 species of feather associated fungi from soil samples collected from 10 different poultry farms. Fungi belonging to 10 different genera namely *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Monascus*, *Mucor*, *Penicillium*, and *Verticillium* were identified. Especially *Aspergillus sp.* like *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, and *A. terreus* degraded chicken feather keratin, producing sulphhydryl compounds which were detected as keratinase and cysteine. Among the five species *A. flavus* was reported to have higher keratinase activity of 12.9 KU/ml and *A. fumigatus* had the lowest enzyme production of 10.4 KU/ml.

Knachana and Divakar, (2013) also attempted to find a way to mitigate the increased output of keratin containing wastes from poultry industries. They reported that

keratinous wastes can be readily fermented and made into useful products like animal nutritional feed additives. A novel feather-degrading *Aspergillus sp.* was isolated from poultry farm soils of Goa, India. Maximum keratinase production was obtained when *Aspergillus sp.* was cultured at 37°C for 72 hours with pH 7-9.

Chrysosporium tropicum

Avasn Maruthi, (2011) assessed the degradation activity of feather and hair wastes using the fungus *Chrysosporium tropicum*. The fungi was highly keratinophilic and was cultured in mineral medium along with defatted feather. Maximum protein and Keratinase production of 6.9mg/ml and 8.56KU/ml was observed on 40th day sample with increase in alkalinity of the broth to pH-9.

Malbranchea aurantiaca and Fusarium culmorum

Piyusha, (2012) analyzed the feather degradation of hen feathers by 82 fungi isolated from Sagar, Dhar, Bhopal and Jabalpur districts of India by calculating percentage weight loss and growth of fungi on substrate. Among the isolated fungi 64 species were identified as keratinophilic fungi and *Malbranchea sp. S010* had the maximum keratinolytic activity of 37.81% feather degradation followed by *Malbranchea auranticum G012* of 35.57% and *Fusarium culmorum P010* of 28.66% degradation. Hence all these fungal isolates can be used for processing keratinous waste in large scale.

CONCLUSION

Owing to the fast growth of poultry industry with a CAGR of 8% in layer and 12% in broiler, there is enormous increase in poultry waste generation. There is a definite need to find an apt method to utilize the solid waste. Poultry feather contains valuable amino acids like cysteine and methionine. It was processed into feather meal using thermal and chemical methods. These methods had many disadvantages like loss of essential amino acids and energy expense. Bioremediation is a promising eco-friendly approach to utilize the poultry feather waste into value added products. This review highlights the use of bacteria and fungi as efficient waste utilization tools to process poultry feather waste. A number of keratinolytic bacteria and fungi in bioremediation of the feather are reviewed. Among the bacteria and fungi reported in this review, *Thermus aquaticus* a thermophile had the highest keratinolytic activity of 479U/ml. Thus Bioremediation can be used for best utilization of poultry waste to maintain the biodiversity.

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