Study of Various Degradation Methods of Keratin Wastes

[1]Siddhesh Warke, [2]Poorvi Kamble, [3]Vaidehi B. Mangalampalli

[1]Student, [2]Student, [3]Student.
Department of Biotechnology,
Thadomal Shahani Engineering College.

Abstract: Keratin waste in the form of human hair, animal hair, horns, feathers etc. Is generated in millions of tonnes globally. Currently, no efficient method is being practiced to deal with this enormous amount of garbage. Keratin and keratinaceous substances have a reputation for resistance in degradation, however, several studies have been conducted to devise different methods of degrading keratin waste. The objective for degradation of keratin wastes is to utilize of the valuable compounds present in keratin wastes and to create a system for managing the waste being generated. The methods discussed in this review include physical, chemical, enzymatic and microbial methods for the degradation of keratin. Due to degradation, the disulfide linkages present in keratin break thereby making the Nitrogen and Sulfur available as opposed to that in the resistant form of keratin. Keratin contains many valuable amino acids and is particularly rich in cysteine, lysine, proline, serine. When recycled, keratin waste has great value in terms of amino acid generation as well as a wide variety of industrial uses.

Index Terms - **Keratin, Cysteine, Degradation, Amino acids.**

I. INTRODUCTION

Keratin is an insoluble fibrous protein with a high degree of cross-linkage of disulfide and hydrogen bonds. It contains cystine, lysine, proline, serine and a trace amount of other amino acids making it very rich in nitrogen. Keratin wastes include human hair, animal hair, horns, feathers and nails that are generated in a million tons as by-products of agro-industrial processes, slaughter houses, leather and wool industries [1].

Current systems of managing keratin wastes include landfills, incineration, composting and mechanical grinding. Dumping keratin waste, the most common method of disposal, leads to the waste occupying a large area and other issues. Improper management of hair waste leads to various problems - clogging of drainage systems, eutrophication through contact with water bodies, foul smell from decay of natural matter (like skin, sweat and oils) adherent on hair, respiratory issues from prolonged inhalation of hair dust, and spread of diseases like chlorosis and fowl cholera [2].

Keratin sources contain up to 17% nitrogen by weight and are rich in sulfur and carbon. Though sulfur cross bonding makes keratin resistant to breakdown, methods have been devised for the same. Correct recycling of keratin wastes can not only cure a waste management problem but also provide great applications [2,3]. Due to its unique properties and ubiquitous availability, human hair can contribute significantly in many critical areas such as agriculture, medicine, construction materials, and pollution control. Methods like incineration and mechanical grinding of hair lead to the loss of several valuable amino acids.

Various methods of degradation of keratin, including physical, chemical, microbial, fungal and enzymatic methods are discussed in this review.

II. DEGRADATION METHODS

2.1 Physical methods of hair degradation

Pyrolysis

Pyrolysis is the change of chemical composition by the process of thermal decomposition of materials at elevated temperatures in an inert atmosphere. Researchers have found that when pyrolysis was performed on 10g keratin samples in a semi-batch glass reactor that was heated by 10 ∘C/min up to the final degradation temperature of 500 ∘C [5]. The volatile products are passed through a water-cooled condenser and then the condensed products are collected in a graduate cylinder. An organic fraction and a water fraction are separated in the liquid pyrolysis product. Refining of the aqueous fraction is done by extracting any remaining organic compounds with ethyl acetate and then the extracted solution is concentrated in vacuum. Pyrolysis of wool, hair and feathers produce nitrogen and sulphur containing compounds, coming from the amino acids in the protein composition of these keratin waste as well as from the disulphide bridges in keratin structure. Nitrogen is found mainly in aliphatic/aromatic nitriles, pyridines, pyrroles and amides. Sulphur is found mainly as sulphides, thiols, thiazoles and thiophenes [6,7].

Hydrothermal

Hydrothermal treatment processes operate in temperatures ranging from $100^{\circ}C$ to $150^{\circ}C$ and pressure of 1.5 atm. Frequently, acids (HCl, H_2SO_4, $HCOOH$ etc.) or bases ($NaOH$, KOH, Na_2CO_3, K_2CO_3, $NaSiO_2$ etc.) are added for about 2-3h [8]. This breaks peptide bonds of keratin and yields water soluble polypeptides, oligopeptides or even amino acids [9]. Drawback of using hydrothermal process is that hydrolysis may result in partial or even complete denaturation of amino acids. Due to this some essential amino acids are lost (lysine, methionine, tryptophan) and non-nutritive are formed (lysinoalanine, lanthionine) [10]. These problems created the need to search for other techniques which would preserve or even improve nutritional value of protein from keratinous materials. Keratinous materials: chicken feathers [8] and animal hair [9] can also be treated with lime to obtain digestible animal feed. Calcium as an end product is recovered by carbonating and soluble keratin is also obtained which can later have many applications in agriculture and food industry as well. [8, 9, 11, 12].

2.2 Chemical methods of hair degradation

KOH & TMAH

Hair has a significant amount of cysteine (1400-1500 micromoles/g hair), which are characterized by disulphide bonds and are known to provide a strong intermolecular link. Potassium Hydroxide and Tetramethylammonium hydroxide have proven to degrade hair when followed a proper protocol. The pace of reaction is controlled by the breakage of this disulphide bond, which is pH dependant since a pH over 10 causes alkaline chemicals to diffuse into hair and therefore controls the reaction rate. Hence, an alkaline environment is best suited for its degradation [13].

Studies suggest that TMAH is also a potential nitrogen source which then contributes to the total nitrogen content of the degraded hair. Whereas potassium is an essential macro element needed for plant life when added in sufficient amount [1]. Degradation of hair requires specific reagents, pH and instruments to be used. For this method, KOH and TMAH pellets are dissolved in water to prepare a 25% (w/v) solvent of different concentration. A measured amount of hair is taken, which is cleaned, washed and dried before adding it to the solvent. After addition, the mixture is heated and stirred. This cycle is carried out 3-4 times until complete dissolution of hair [14].

The end-product is expected to be a slurry after filtration is done through a sieve or a filter paper and can then have multiple uses. One of the best uses is the addition of this slurry in agricultural soil, and has proven to increase leaf number and vegetative development of plants, resulting in increased photosynthetic capability and higher dry matter in grown plants. These findings and observations are a direct outcome of increased nitrogen availability in the soil [15].
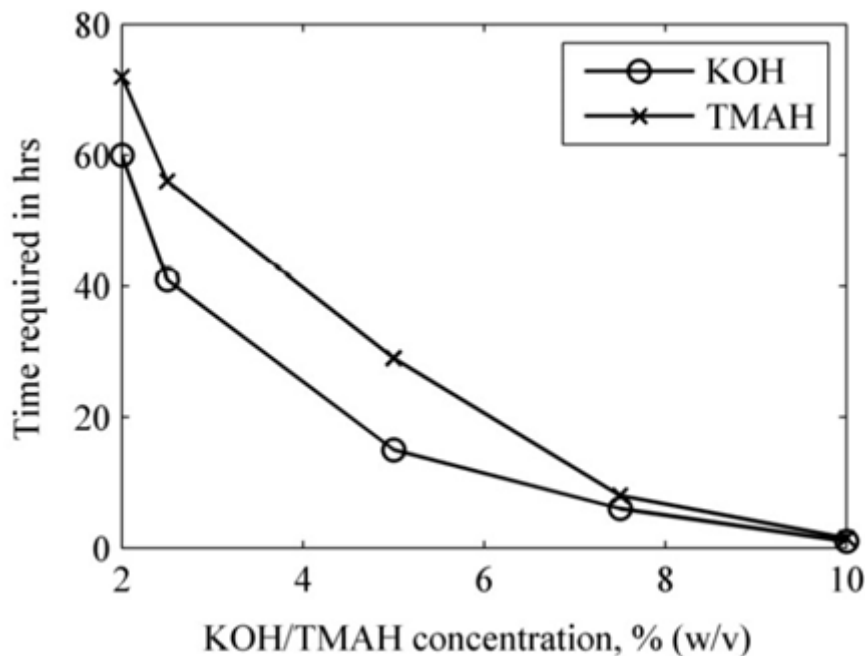
Fig. 1. Time required for dissolving hair inn aqueous solvent of KOH/TMAH [13]

Alkaline Hydrolysis

Waste from slaughterhouses, leather and fur industries (wool, horn, hoots, keratinous substances etc) are thrown away out in the open creating massive landfills. This inflicts havoc on the environment and public health all around the world. One of the most prevalent methods of getting rid of this waste nowadays is to bake it at high temperatures and then feed it to cattle after milling. This was also known as "animal flour" or an added protein supplement in the regular diet [16-17]. This was discontinued shortly after it was known that this was the carrier of an enigmatic cause (called prion). This resulted in diseases like (bovine spongiform encephalopathy, mad cow, swine fever, Creutzfeldt-Jakob disease etc). As a result, incineration is now the only viable alternative for dealing with such garbage [18].

But as mentioned before all of this waste is keratinous in nature and therefore is being wasted if incinerated. Because of their high protein content, this might be a fantastic source of protein and amino acids for animal feed and a variety of other uses as well. These compounds have shown to contain 20% to 25% protein, which can provide a significant quantity of nitrogen to plants [19-20].

Microwave alkaline hydrolysis is carried out under circumstances similar to those used in an autoclave for alkaline hydrolysis. In a microwave oven (800 W), a certain amount of wool waste is combined with 100 ml of 0.15 mol 1-1 KOH 0.05 mol 1-1 NaOH. After centrifugation, the precipitates are dried at high temperatures and crushed to powder, while the supernatants were frozen. Amino acid analysis is performed on the supernatant of sample [21].

Reducing Agents

Other than animal and human hair waste, millions of tonnes (8 x 105 tonnes) of bird feathers are also generated each year across the world due to chicken slaughtering. Keratin makes up around 90% of the keratin in bird feathers and as stated above this waste is also incinerated. But due to high energy consumption and increased CO2 emission, this method is not preferred. Another method is composting bird feather with manure. A major drawback of this method is that its time consuming and a strong hydrogen sulphide stench which lingers in the air for a long time [21-23].

Researchers have come up with a better solution to the problem wherein the study's findings illustrate the potential of hydrolytic procedures for obtaining soluble keratin suitable for biodegradable film creation for food applications, which are both safer and more successful than disulphide bond reduction [24].

When these disulphide linkages are reduced and oxidised, they give rise to a soluble keratin form which contain undegraded amino acids. Likewise, free cysteine residues are generated when keratin is treated with 2-mercaptoethanol, dithiothreitol (DTT) or dithioerythritol, thioglycolic acid, glutathione, sulphites, and bisulphite and these cysteine-containing derivatives are known as "kerateines". Kerateines are more stable in acidic and alkaline conditions than the oxidative derivatives and contain amino acids which are capable of crosslinking [25].

Sulphitolysis is a key step in the reduction of keratin where sulphite breaks the disulphide bonds in cysteine, resulting in cysteine thiol (reduced keratin) and cysteine-S-sulphonate residue (Bunte salt). Usage of urea, thiourea, transition metal hydroxides, surfactant solutions, and combinations of these as denaturing solvents helps the trapped keratin to be solubilized as well [26]. This denatured compound is industrially usable. The yield of soluble keratin after a 2-h reduction with 2-mercaptoethanol and sodium bisulphite was almost identical, at 84 and 82 percent, respectively. The cheaper and safer sodium bisulphite also reduced the extraction time to 1 hour while maintaining the same yield. Furthermore, treating the feathers with 2.5 percent NaOH enhanced the extraction efficiency even further, boosting the yield to 94%. 2-Mercap-toethanol produces a high yield while causing little keratin damage. Unfortunately, due to its high cost, it is not economically feasible. Other thiol compounds have lower extraction yields, which are insufficient for commercial use [27-28].
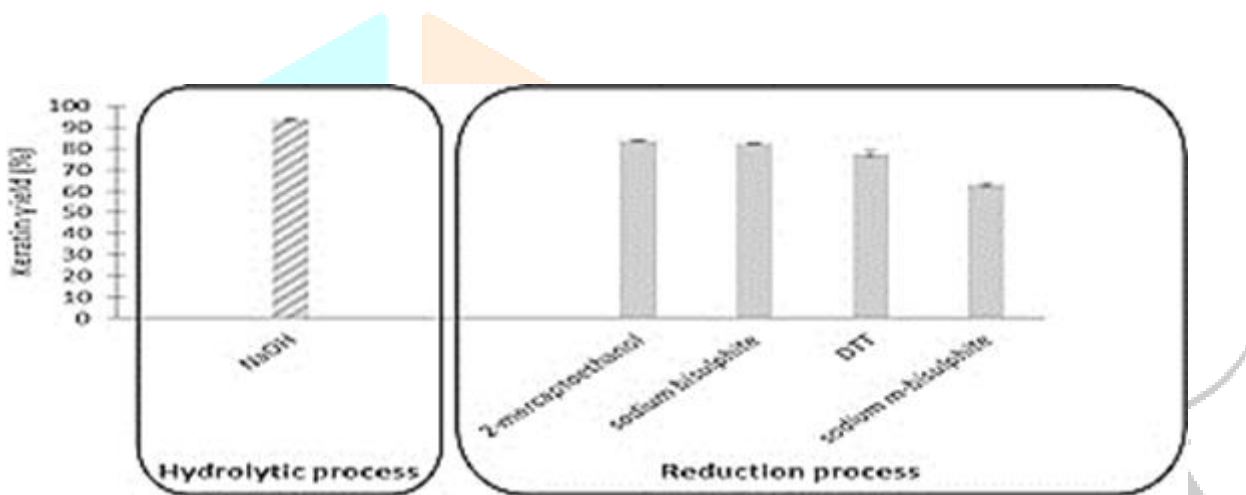

Fig2. Graphical representation of the efficiency of various reducing agents [22]

2.3 Bacterial

The bacterial species involved are aerobic in nature. These can be grown with keratinaceous substances such as feathers as a substrate, studies of which resulted in the complete degradation of the keratin protein. The presence of feather powder as keratin medium greatly increased the production of keratinolytic protease in B. subtilis, B. pumilis and B. cereus. The production of keratinolytic proteases can also be increased with other factors such as the increase in B. subtilis by using casein.

Extracellular production of keratinolytic proteases is inducible in B. subtilis. Keratinolytic proteases were primarily inducible, needing exogenous keratin as an inducer.

Three keratinolytic protease-producing isolates were thermophiles. B. subtilis and B. pumilis were able to grow at 55 °C, but the optimal temperature for enzyme production was 40 °C. Meanwhile, B. cereus was able to grow at 40 °C with the optimal temperature for enzyme production at 30 °C. In the feather medium, the best temperature range for the production of keratinolytic protease by B. licheniformis and B. brevis was between 40 and 45 °C [45]. Staphylococcus sp. could grow at temperatures as high as 50 °C, however 37–40 °C was the best temperature for enzyme synthesis. The temperature for maximum enzyme production was in all cases found to be slightly lower than that for growth.

Late in the logarithmic phase or at the start of the stationary phase, the highest enzyme activity was detected.

Table 1. Optimum Temperature needed for bacterial species

Bacterial species	Optimum temperature for production of keratinolytic proteases (in °C)
B. subtilis and B. pumilis	40 °C
B. cereus	30 °C
B. licheniformis and B. brevis	40 and 45 °C
Staphylococcus sp.	37–40 °C

Table 2. Optimum pH needed for bacterial species

Bacterial species	Optimum pH for production of keratinolytic proteases
B. subtilis	5-9
B. cereus	7
B. pumilis	5–6
Staphylococcus sp.	37–40 °C

The highest enzyme production in B. cereus was seen at pH 7.0. Whereas in B. subtilis, the highest enzyme production was obtained with a broad range of pH of 5–9. Synthesis of the enzyme by B. pumilis was better obtained in a weak acid pH range of 5–6. In the growth of bacteria and production of the enzyme, a raise in the pH was observed, such that, when initial pHs were between 6 and 7, the final pH of the media was between 8.5 to 9.0 after 3 days of cultivation.

Keratinolytic organisms metabolize free or combined cystein as a source of sulfur and nitrogen. The products of cystine metabolism by fungi were inorganic sulfur and other intermediate products. Any excessive sulfur is excreted back to the medium in the oxidized form as sulphate and sulphite. A neutral to alkaline pH sulphite reacts with cystine, cleaving it to cystein and S-sulphocystein [46, 47].

Factors like inoculum size and age of inoculum also play a role in the degradation of keratin and affect factors like the residual hydrolysates. These are composed of undigested feathers and bacterial cells. Cultivation conditions affect keratinase production and cell growth; thus, the amounts of residual hydrolysates vary with respect to different cultivation conditions. One such study found that the optimal conditions were: "initial pH 7.5, inoculum size of 2%, age of inoculum 16 h and temperature 23 °C" Actinomycetes and Streptomyces produce keratinases.

2.4 Fungal

Fungi also cause biodegradation of keratin. The most common fungi in doing so being Aspergillus, Penicillium, Fusarium, Microsporum and the fungal species already present richly in soil, Trichoderma.

When hair is inoculated with the keratinolytic fungi, tunnels are formed on the hair strand and capable hyphae deepen the tunnels thereby perforating the hair strand. Fungal filaments cover the hair tunnel, hyphae bundles surround the hair strand and surface erosion occurs. Aerial mycelium strongly adheres to the strand. The damage being produced to the cuticle is visible in an SEM [2]. Hyphae network detaches from the surface and attaches to the mantle. The mycelium forms an erosion pocket on the hair surface because of which the keratin macrofibril bundles separate. The perforated zone spreads causing lifting of the cuticle and s sleeve-like hyphae surround the strand. The hyphae penetrate the cortex below the scale cuticle and lift the scales. Digestion of the cuticle occurs and the cuticle layer is removed.
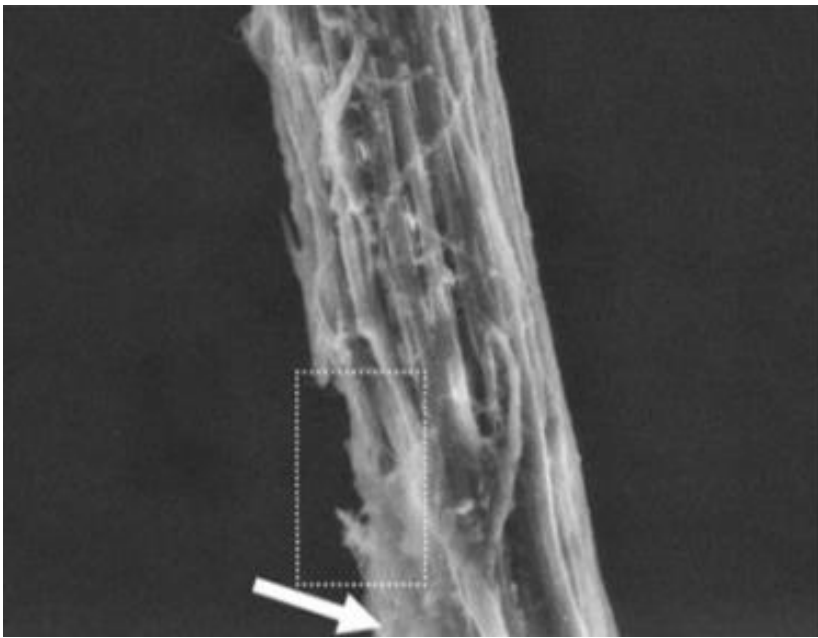
Fig. 3. SEM of mycelium hyphae create an erosion pocket, keratin macrofibrils separate [2]

2.5 Enzymatic

Papain

Papain is a cysteine protease enzyme, found in papaya and mountain papaya. It is also known as papaya proteinase I. In manufacturing, papain is used in cosmetics, toothpaste, contact lens cleaners, meat tenderizers, meat products etc. Studies have shown that papain is also capable of degrading human hair if followed a proper protocol [38].

The epicuticle is the hair's outermost layer, and it's made up of a monolayer of fatty acids, mostly 18-methyleicosanoic acid (18-MEA), that's covalently bonded to a protein matrix. This obstructs the enzyme's ability to disintegrate the hair. Ethanol and ether extraction remove all the protective layering of the hair leaving it naked for the papain to work on. Ethanol eliminates cell membrane material, which is thought to provide more enzyme access and so hasten hair breakdown. In addition, hot ethanol extraction has been demonstrated to remove a thin coating of electron transparent substance from the hair's surface. The root ends of the hair were extracted with diethyl ether in a Soxhlet device for 24 hours and then dried. Continuous hot extraction of the ether degreased fibres in a Soxhlet device for one week produced ethanol-extracted hair [39].

Keratinase

Keratinase functions as disulfide reductase and polypeptide hydrolase. The degradation process of keratinase is divided into three steps, denaturation, hydrolysis and transamination. First, the disulfide reductase reduces cystine (-S-S-) to cysteine (-SH), causing the high-level structure of keratin to breakdown, resulting in degenerative keratin protein. Polypeptide hydrolase progressively hydrolyzes the degraded keratin protein into polypeptides, oligopeptides, and free amino acids. Finally, transamination produces ammonia and sulphide, which totally hydrolyzes keratin.

The precise mechanism of keratinolysis is yet to be elucidated. Deamination, which provides an alkaline environment required for substrate swelling, sulphitolysis, and proteolytic assault, has been postulated as the initial step in keratin breakdown [40].

As a result of microbial degradation of keratin sulfoxide bond (S_O) are formed due to the S–S bond breaking. The presence of sulphur oxides is necessary for the oxidation of monoxide to dioxide, which then progresses to complete oxidation and the creation of cysteic acid. Ammonia, CO_2, sulfur-containing inorganic compounds (SCS, SCO, and H_2S), water, thiols, nitriles, phenol, and 4-methylphenol compounds are among the degradation chemicals found. [2]

III. CONCLUSION

Studies show keratin waste poses as a hazard when left untreated. Many harmful effects of the same have been reported. Recycling of keratin wastes into a useable product provides a sustainable method for multiple applications, from offering a greener alternative to chemical fertilizers to decreasing the pressure on fossil fuels. There exists a large scope for expanding utilization of keratin wastes as its current percent utilization is very low. Proper management of hair waste needs guidelines created by legislatures all over the world. Standards must be created for the same inorder to make sure maximum possible utilization of keratin waste is being done. Awareness regarding its market scope must be made of its beneficial properties to stakeholders and safe collection and usage practices. This review thus provides information on how the resistance of keratin can be broken through different degradation methods thereby allowing its use.

IV. REFERENCES

1. Kumawat, T. K., Sharma, A., Sharma, V., & Chandra, S. (2018). Keratin waste: the biodegradable polymers. In *Keratin*. IntechOpen.

2. Călin, M., Constantinescu-Aruxandei, D., Alexandrescu, E., Răut, I., Doni, M. B., Arsene, M. L., ... & Lazăr, V. (2017). Degradation of keratin shindubstrates by keratinolytic fungi. *Electronic Journal of Biotechnology*, *28*, 101-112.

3. da Silva, Ronivaldo Rodrigues (2018). *Different Processes for Keratin Degradation: The Ways for Biotechnological Application of Keratinases. Journal of Agricultural and Food Chemistry, (), acs.jafc.8b04439–.* doi:10.1021/acs.jafc.8b04439

4. Gupta, A. (2014). Human hair "waste" and its utilization: gaps and possibilities. *Journal of Waste Management*, *2014*.

5. M. Brebu, E. Jakab, Y. Sakata, Effect of flame retardants and Sb2O3 synergist on the thermal decomposition of high-impact polystyrene and on its debromination by ammonia treatment, J. Anal. Appl. Pyrolysis 79 (2007) 346–352.

6. Mihai Brebu; Iuliana Spiridon (2011). *Thermal degradation of keratin waste. , 91(2), 288– 295.* doi:10.1016/j.jaap.2011.03.003

7. Zs. Éhen, Cs. Novák, J. Sztatisz, O. Bene, Thermal characterization of hair using TG-MS combined thermoanalytical technique, J. Thermal. Anal. Cal. 78 (2004) 427–440.

8. Coward-Kelly, G., Chang, V.S., Agbogbo, F.K., Holtzapple, M.T.: Lime treatment of keratinous materials for the generation of highly digestible animal feed: 1 Chicken feathers. Bioresource Technol 97, 1337–1343 (2006)

9. Coward-Kelly, G., Agbogbo, F.K., Holtzapple, M.T.: Lime treatment of keratinous materials for the generation of highly digestible animal feed: 2. Animal hair. Biores. Technol. 97, 1344–1352 (2006)

10. Choi, J.M., Nelson, P.V.: Developing a slow-release nitrogen fertilizer from organic sources. 2. Using poultry feathers. J. Am. Soc. Horticul. Sci 121, 634–638 (1996)

11. Chojnacka, K., Górecka, H., Michalak, I., & Górecki, H. (2011). A review: valorization of keratinous materials. *Waste and Biomass Valorization*, *2*(3), 317-321.

12. Karthikeyan R, Balaji S, Sehgal PK. Industrial applications of keratins – A review. Journal of Scientific and Industrial Research. 2007;66(9):710-715

13. Rahman, M. M., Kabir, K. B., Rahman, M. M., & Ferdous, Z. (2016). Quick Release Nitrogenous Fertilizer from Human Hair. *British Journal of Applied Science & Technology*, *14*(2), 1-11.

14. Mitu AM. Damage assessment of human hair by electrophoretical analysis of hair proteins (PhD Thesis): Bibliothek der RWTH Aachen; 2004.

15. Robbins CR. Chemical and Physical Behavior of Human Hair. Springer Berlin Heidelberg; 2012.

16. Gousterova, A., Nustorova, M., Goshev, I., Christov, P., Braikova, D., Tishinov, K., ... & Nedkov, P. (2003). Alkaline hydrolysate of waste sheep wool aimed as fertilizer. *Biotechnology & Biotechnological Equipment*, *17*(2), 140-145.

17. Lundgren H.P.,Siein A.M., Koorn V.M., O'Connell (I 948) J. Phys.Chem.S2, I 80-206.

18. Graham C., Waitkoff H.,HierS. (1949) J.Bioi.Chem.177, 529

19. Steinert P.M. (1975) Biochem.J.149, 39-48.

20. Dubois M., Gilles K.A., Hamilton J.K., Rebars P.A., Smith F.S., (1955) Anal. Chern., 28,350.

21. Gousterov G., Andonov P., Todorov Ts., Kominkov L., Gincheva-Starcheva M. (1977) in "Practice in microbiology and virusology" Pub!. Sofia University "St.Kl. Ohridski", p.I99, (in bulgarian).

22. Sinkiewicz, I., Śliwińska, A., Staroszczyk, H., & Kołodziejska, I. (2017). Alternative methods of preparation of soluble keratin from chicken feathers. *Waste and biomass valorization*, *8*(4), 1043-1048.

23. Pulidori, E., Micalizzi, S., Bramanti, E., Bernazzani, L., Duce, C., De Maria, C., ... & Tinè, M. R. (2021). One-Pot Process: Microwave-Assisted Keratin Extraction and Direct Electrospinning to Obtain Keratin-Based Bioplastic. *International journal of molecular sciences*, *22*(17), 9597.

24. Schrooyen, P.M.M., Dijkstra, P.J., Oberthu¨r, R.C., Bantjes, A.,Feijen, J.: Partially carboxymethylated feather keratins. 1. Properties in aqueous systems. J. Agric. Food Chem. 48, 4326–4334(2000)

25. Chojnacka, K., Go´recka, H., Michalak, I., Go´recki, H.: A review:valorization of keratinous materials. Waste Biomass Valor. 2, 317–321 (2011)

26. Wawrzkiewicz, K., Łobarzewski, J., Wolski, T.: Intracellularkeratinase of Trichophyton gallinae. J. Med. Vet. Mycol. 25,261–268 (1987)

27. Vasconcelos, A., Freddi, G., Cavaco-Paulo, A.: Biodegradable materials based on silk fibroin and keratin. Biomacromolecules 9, 1299–1305 (2008)

28. Hong SJ, Park GS, Jung BK, Khan AR, Park YJ, Lee CH, et al. Isolation, identification, and characterization of a keratin-degrading bacterium Chryseobacterium sp. P1-3. J Appl Biol Chem 2015;58:247–51. http://dx.doi.org/10.3839/jabc.2015.039.

29. Riffel A, Brandelli A, Bellato CM, Souza GHMF, Eberlin MN, Tavares FCA. Purification and characterization of a keratinolytic metalloprotease from Chryseobacterium sp. kr6. J Biotechnol 2007;128:693–703. http://dx.doi.org/10.1016/j.jbiotec.2006.11.007.

30. Gurav RG, Jadhav JP. Biodegradation of keratinous waste by Chryseobacterium sp. RBT isolated from soil contaminated with poultry waste. J Basic Microbiol 2012; 53:128–35. http://dx.doi.org/10.1002/jobm.201100371.

31. Allure N, Madhusudhan DN, Agsar D. Enhanced production, purification and characterization of alkaline keratinase from Streptomyces minutiscleroticus DNA38. Int Lett Nat Sci 2015;43:27–37. http://dx.doi.org/10.18052/www.scipress.com/ILNS.43.27.

32. Syed DG, Lee JC, Li WJ, Kim CJ, Agasar D. Production, characterization and application of keratinase from Streptomyces gulbargensis. Bioresour Technol 2009;100:1868–71. http://dx.doi.org/10.1016/j.biortech.2009.08.026.

33. Lopes FC, Dedavid e Silva LA, Tichota DM, Daroit DJ, Velho RV, Pereira JQ, et al. Production of proteolytic enzymes by a keratin-degrading Aspergillus niger. Enzym Res 2011. http://dx.doi.org/10.4061/2011/487093.

34. Mazotto AM, Couri S, Damaso MCT, Vermelho AB. Degradation of feather waste by Aspergillus niger keratinases: Comparison of submerged and solid-state fermentation. Int Biodeter Biodegr 2013;85:189–95. http://dx.doi.org/10.1016/j.ibiod.2013.07.003.

35. Nwadiaro PO, Chuku A, Onyimba IA, Ogbonna AI, Nwaukwu IA, Adekojo DA. Keratin degradation by Penicillium purpurogenum isolated from tannery soils in Jos, Nigeria. Br Microbiol Res J 2015;8:358–66. http://dx.doi.org/10.9734/BMRJ/2015/16339.

36. El-Gendy MMA. Keratinase production by endophytic Penicillium spp. Morsy1 under solid-state fermentation using rice straw. Appl Biochem Biotechnol 2010;162: 780–94. http://dx.doi.org/10.1016/S0141-0229(02)00283-1.

37. Kannahi M, Ancy RJ. Keratin degradation and enzyme producing ability of Aspergillus flavus and Fusarium solani from soil. J Chem Pharm Res 2012;4:3245–8. http://dx.doi.org/10.1139/w06-067.

38. Holmes, A.W. (1964). Degradation of Human Hair by Papain: Part II: Experiments in the Isolation and Identification of the Protective Substance. Textile Research Journal, 34(9), 777–782. doi:10.1177/004051756403400907

39. Swift, J. A., & Holmes, A. W. (1965). Degradation of Human Hair by Papain. Textile Research Journal, 35(11), 1014–1019. doi:10.1177/004051756503501108

40. Sowjanya NC, Chary CM. Degradation of few avean feathers by Microsporum gypseum. J Phytology 2012;4:21–3.

41. Keratinase(n.d.).Retrievedfromhttps://www.creativeenzymes.com/similar/keratinase_382.html

42. Cao L, Tan H, Liu Y, Xue X, Zhou S. Characterization of a new keratinolytic Trichoderma atroviride strain F6 that completely degrades native chicken feather. Lett Appl Microbiol 2008;46:389–94. http://dx.doi.org/10.1111/j.1472-765X.2008.02327.x.

43. Maruthi AY, Lakshmi AK, Rao RS, Chaitanya AD. Degradation of feather and hair by Chrysosporium tropicum: A potent keratinophilic fungus. Afr J Biotechnol 2011;10: 3579–84. http://dx.doi.org/10.5897/AJB10.432.

44. Bohacz J. Biodegradation of feather waste keratin by a keratinolytic soil fungus of the genus Chrysosporium and statistical optimization of feather mass loss. World J Microbiol Biotechnol 2017;33:13. http://dx.doi.org/10.1007/s11274-016-2177-2.

45. Bockle B, Galunsky B, Muller R. Characterization of a keratinolytic serine protease from Streptomyces pactum DSM 40530. Appl Environ Microbiol 1995;61:3705–10.
46. Kunert J. Effects of reduced agents on proteolytic and keratinolytic activity of enzymes of Microsporium gypseum. Mycoses 1992;35:343–8
47. J.M Kim; W.J Lim; H.J Suh (2001). *Feather-degrading Bacillus species from poultry waste. , 37(3), 287–291*. doi:10.1016/s0032-9592(01)00206-0
48. Menon, S., Savur, B., Kasat, D., Movani, U., Singh, S., & Rane, M. (2020). Management and utilization of Keratin Waste–A review. *Int. J. Adv. Res. Ideas Innov. Technol*, *6*, 511-515.