

BIOCONVERSION OF RECALCITRANT KERATIN RICH WASTES: A PRAGMATIC APPROACH TOWARDS SUSTAINABLE DEVELOPMENT

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ABSTRACT

A robust, sound, and comprehensive waste management system is a fundamental aspect to achieve environmental sustainability and development. Though as individuals, people are aware and conscious of this particular aspect of waste management, there have not been any concrete steps taken to tackle the issue. As per a statistical report from World Bank on waste, about 2.01 billion tonnes of municipal solid waste is generated annually at a global scale out of which about one-third of it is not managed in an environmentally safe manner. Keeping in mind the UN General Assembly goals with respect to sustainable development for the benefit of all by 2030, it is imperative to act in ways to manage and come out with ways to treat solid waste in particular. Keratin is a key structural protein that can be found in a variety of forms in nature, including hair, feathers, nails, horn, hoofs, scales, and wool. It is also found in several hard tissues that protect the organ by providing blockage with the surroundings. Keratin, a protein, fibrous in nature has solubility issues. Keratin is tolerant towards protease enzymes like pepsin, and trypsin is due to its structure, which is protected by bonds such as disulfide and hydrogen. The waste consisting of keratinous residues, like feathers, provides carbon, nitrogen, and Sulphur source that are transformable to certain products. Keratin-rich waste possesses a high content of amino acids and, if not correctly managed, can harm the environment, water supplies, and soil. This form of waste, on the other hand, can be used for less-cost amino acid sources, can be turned into animal feeds, or used as a fertilizer. This necessitates a thorough understanding of feather structure and properties. Feathers are composed of β -keratin and melanin pigments. In addition, to strategize their control and degradation, there is a necessity to differentiate α -keratin from β -keratin. The present article summarizes the various methods of waste treatment of compounds possessing keratin, limitations of conventional methods, ecological implications, the role of microbes in Bioconversion, and ways to improve enzyme production which can ultimately be used for the management of keratinous substances. This would also cater to Sustainable development goal number 12 which includes responsible consumption as well as production including the major focus on environmentally sound ways to manage waste through either its prevention, reduction, recycling, or reuse of such solid wastes including keratin.

Keywords: Bioconversion, Keratin, Solid Waste, Microbial Degradation, Ecology.

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INTRODUCTION

Structure of Keratin and Biochemical Characteristics

Keratin monomers show the formation of bundles to produce intermediate filaments that possess both toughness and insolubility and are present in various animals and mammals. Because of many hydrogen bonds and hydrophobic interactions, keratin is a strong and intact protein. Due to the presence of cysteine residues, the sulfur content is 3 to 5% and is responsible for cross-linking hair's polypeptide chains.¹ Chicken feathers are primarily made up of keratin protein, with minor amounts of lipids and water, and majorly consist of small and essential residues of amino acids such as alanyl, cysteinyl, glycyl, valyl,

and seryl. Even though they are proteins, they are not degraded by conventional proteolytic enzymes due to their hardness and recalcitrant character. The sulfur group composition, occurrence site, and functional significance related to polypeptide play a role in determining whether keratin is hard or soft. Based on its secondary conformation, keratin is divided into α -keratin and β -keratin.

Structure of α -keratin

α -Keratin (Fig.-1) is found in higher species, such as mammals. Keratin is found in animals' hair, nails, horns, and wool. Cysteine residues are abundant in keratin, which generates disulfide bonds that connect adjacent polypeptide chains. The insolubility of keratin and its resistance to stretching, two of its most essential biological features, are due to this. The sulfur level of keratin determines whether they are categorized as "hard" or "soft." Hard keratins, such as those found in hair, horn, and nail, are less pliable due to their high mercaptan content. Hair and wool fibers are springy because of the coiled coil's ability to untwist when stretched and revert to its original shape when the external force is released.² It is comprised of three structures: the N terminal end, a rod in the middle, and a C terminal end, altogether called a monomer. The two monomers form a heterodimer, which is a left-handed coil. The two heterodimers then clump together to create a tetramer, which is then linked by two sets of tetramer connections to form a protofibril. An intermediate filament is formed by the joining of four sets of protofibrils. Protofibril and microfibril connect to form an intermediate filament.³

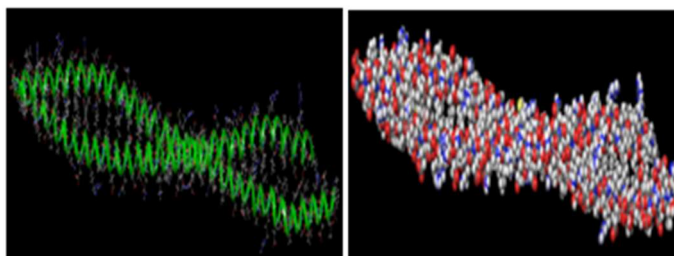


Fig.-1: 2D and 3D Structure of α -Keratin

Structure of β -keratin

β -Keratin (Fig.-2) is found in the feathers of birds and the scales of reptiles. The structure of the feather is hierarchical, with a central shaft, or rachis made up of a cortex that encloses a cellular core made up of equally sized cells. Barbs, the 2^o keratinous elements that generate the herringbone pattern of the vane, are supported by the rachis. Barbules 3^o features are also supported by the barbs. The cortex is primarily made up of fibers with a diameter of 6 μ m that are arranged along the shaft's length. Because β -keratin contains less cysteine and has fewer disulfide bonds than α -keratin, it is more easily degraded.

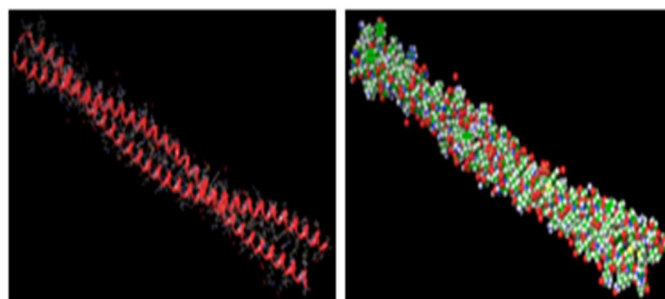


Fig.-2: 2D and 3D Structure of β -Keratin

Feather Composition

A feather is composed of moisture up to 6.72%; protein constitutes 81.46%; fat makes up 11.36% and fiber makes up 0.31%. It has α -helix and β -sheet configurations in its polypeptide chain, with α -keratin accounting for 2/3 of the total and β -keratin accounting for 1/3. It does not have the same elasticity or stretching as hair keratin. It is made up of 90% identical proteins. The molecular mass is estimated to be 10 kDa. In comparison to α -keratin, cysteine level is low. Barbules, barbs, and rachis make up feathers. The feather is made up of 32% α -keratin, 52% β -keratin, and 14% coils. Glycine levels in feathers are higher than cysteine levels.

Methods of Waste Treatment

Managing the massive amount of feather waste from industries like poultry is a concerning issue. The disposal of waste by, a variety of options are possible. Feathers are heated at high temperatures and pressure to make feather meal, which is utilized as an animal dietary element in traditional methods. Hydrothermal treatments and microbiological degradation are the two most used ways of treating feather meals. The hydrothermal approach, for example, is expensive and causes denaturation of certain important amino acids during processing, resulting in a product having poor digestibility and nutritional value. Thermochemical procedures like acidic and alkali hydrolysis; steam pressure cooking are included in hydrothermal pre-treatment.

Limitations Offered by Conventional Methods

The poultry feathers are either dumped, polluting the land, or burned, polluting the air once more. The current feather incineration technique also increases the release of some pollutants into the atmosphere, such as CO, SO₂, VOCs, and Nitrogen Oxides. As a result, these contaminants are associated with various disorders, such as respiratory disorders, cardiovascular disease, and cancer. To deal with the global problem of keratin waste, a comprehensive management system should be implemented.⁴ The feather contains higher protein and is mostly utilized in animal nutrition, and can be used as a biofertilizer. Increased temperatures and high pressure are usually required with the use of diluted acids like HCl or alkalis such as NaOH in thermal and chemical processes. Some amino acids, such as tryptophan, are lost when exposed to acidic liquids. Alkaline processes are slow, and some amino acids degrade more slowly when exposed to hydroxide. Furthermore, these procedures take more time, chemicals, and energy to process, as well as expensive industrial equipment. Treatments by thermal and chemical methods, as a result, cannot maintain sufficient nutritional value. Microbes with keratinolytic activity or the keratinase enzyme are involved in this process. Scientists and researchers have been drawn to microbial degradation of feathers as a cost-effective way to obtain feather powder that retains nutrients and key amino acids.

An Alternative Approach- Microbial Degradation

The use of bacteria that produce extracellular keratinases as an innovative and environmentally acceptable means of converting this plentiful waste into low-cost, nutrient-rich animal feed is a viable option. Enzymatic and fermentation processes are examples of alternative ways. These techniques yield feather meal, which is high in unique amino acids including cysteine, serine, and proline, and can be used as a feed substrate. Poultry feathers contain a significant number of useful proteins and amino acids that proves to be beneficial as animal feed. As a result, animal nutritionists are interested in recycling feathers due to their potential as a low-cost, substitute protein feedstuff.⁵ But, the use of feathers is limited because of their poor digestion and low biological value, as well as a lack of nutritionally important amino acids like methionine, lysine, histidine, and tryptophan. Keratinolytic enzymes present an opportunity for a low-energy bioconversion of poultry feathers from a pollutant to a nutritionally enhanced protein-rich feedstuff for cattle. Feather waste is biologically degraded more efficiently than it is physically or chemically degraded, resulting in a more usable and toxic-free by-product.

Keratinase

Keratinase belongs to a class called protease that can hydrolyze keratin. In the presence of Keratin, a variety of microbes can synthesize keratinase, which can hydrolyze feathers, hair, nails, and fur.⁶ A variety of fungi and bacteria synthesize this enzyme, and several research have resulted in the identification of distinct *Bacillus* strains. Because fungus-produced keratinase is known to be harmful, bacterial keratinase is essential. The bacteria-derived hydrolyzed keratin substrate is utilized for nitrogen fertilizers, films, animal feed, and adhesives, as well as being a good source of serine, cysteine, and proline. Keratinase is also employed in industries like detergents, medicine, cosmetics, and the leather industry. Depending on the microorganisms utilized and the keratin-rich material, it has different features. They are mostly inducible enzymes, which means they are released when keratin-rich compounds are present. It shows the broad range of activity in different pH ranges from 4.5 to 12.5 and temperatures from 30°C to 85°C. It can digest a wide range of protein substrates, both soluble and insoluble.

Ecological Implications of Keratinase Producers

Feathers are discarded and accumulated in the environment indiscriminately due to their low economic value and strong mechanical stability. Because of their persistent nature, keratinous feathers would linger in the environment for an extended period of time, fostering the growth of various microbial strains while also emitting a foul smell from the release of airborne pollutants like NH_4 , N_2O , and H_2S . Similarly, incinerating would have a substantial impact on the greenhouse effect and other environmental issues. Because of the continued development and advancement of the leather and poultry industry, pollution by keratinous waste can lead to becoming a threat globally.⁷ Bacterial keratinases are dynamic proteases and in biotechnological areas are attaining popularity because of their ability to bio-convert recalcitrant keratin-rich wastes and provide a long-term solution for cleaner production. Because the redox system hydrolyzing disulfide-containing bonds of polypeptides is missing in traditionally treated feeds containing proteins of keratinous origin, animals are unlikely to metabolize them. As a result, an increase in protein digestibility and nutrient levels will improve the nutritional value of keratin-based feed products.

Keratinolytic Microorganism

Keratinolytic proteases are linked to a wide range of microorganisms isolated from various environments. Bacteria and fungi are found to be good decomposers of keratin substrates, by the production of keratinolytic enzymes extracellularly. *Bacillus*, *Vibrio*, *Chryseobacterium*, *Brevibacillus*, *Pseudomonas*, *Serratia*, *Fervidobacterium*, and *Microbacterium* have all been identified as bacterial sources of keratinase. *Aspergillus*, *Paecilomyces*, *Doratomyces*, *Trichoderma*, *Fusarium*, *Acremonium*, *Onygena*, *Cladosporium*, and *Microsporum* are recognized as natural colonizers of keratin substrate.⁸ *Bacillus* strains are the most common bacteria producing keratinase including *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus pumilus* to name a few. Keratinases are exoenzymes which means their release is in the extracellular matrix. Some bacteria produce both extracellular and intracellular keratinase simultaneously. Keratinase bound to cells is used for waste treatment and provides the benefit of being fixed on the surface of the cell, and hence proves to be of significant interest for industrial applications.⁹

Mechanism of Keratin Degradation by Microbes

The discovered keratinases belong to serine and metalloproteases which breaks peptide bonds, detect hydrophobic substrates, and impact disulfide bonds in peptide chains.¹⁰ The breakage of disulfide bonds by keratinase is done with the aid of other enzymes, and the degradation involves two steps: release of keratin peptide and degradation of peptide.¹¹

1. Sulfitolysis: For this stage, disulfide reductase and cysteine dioxygenase must break disulfide bonds to begin the degradation of keratin-rich compounds, and keratinases must cooperate synergistically with the above enzymes for successful degradation.

2. Proteolysis: Following the first stage, keratinase can hydrolyze the peptide bonds that are available.

Microbial degradation of keratin is influenced by a variety of factors, including pH, temperature, agitation, and energy sources.

Applications

Keratinases show applications in several sectors which include the production of leather, formulation of detergent, bio medications, and pharmaceuticals, not only due to their catalytic efficacy but also because of their sustained production by a cost-effective renewable resource. Their bio-additive significance in the formulation of detergents for hydrolyzing protein stains along with their toughness in the presence of chemicals proved to be beneficial in the industrial aspect. Additionally, keratinase possesses boisterous tendencies that are suited for greener technology. Hence, there is a continuous need for research directed towards novel producers of keratinase from the diversified environment.¹² Various pharmaceutical and industrial applications of Keratinase are depicted in Fig.-3.

Enhancement of Catalytic Efficiency of Keratinase

Lower tolerability towards industrial procedures and process parameters, like pH, salt, organic solvents, detergents, temperature, and oxidizing agents, are associated with enzymes produced by wild-type

microbes. Such variables significantly reduce catalytic efficiency and applications of enzymes. Whereas keratinase shows distinct properties which can be used in bioprocessing applications such as keratin bioconversion to usable residues, leather depilation, and textile treatment. For this, two key strategies are adopted. The first is to work with a good strain, and the second is to build an enzymatic system for keratin breakdown using optimal fermentation conditions.

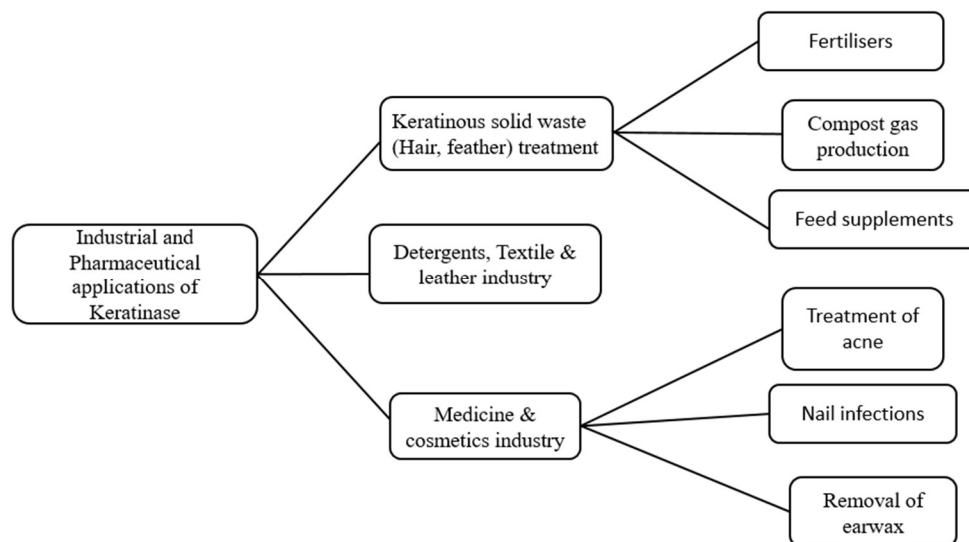


Fig-3: Pharmaceutical and Industrial Applications of Keratinases

Approaches for Strain Improvement

Techniques such as Protein engineering including site-saturation mutagenesis (SSM), direct evolution (DE), fusion, site-directed mutagenesis (SDM), and truncation have all been used to produce enzymes. SDM is focused on changes in protein structure, whereas DE is focused on a library of mutants generated randomly allowing a more extensive search for interesting variants. SDM alters proteins by modifying genes using target base substitutions, deletion, or insertion. DE employs non-specified and random mutations for building a variable genes library and then undergoes screening to select the better-performing variants. Such protein engineering technique facilitates amplification and integration of the desired potential within the single variant protein. Another unique laboratory strategy for producing strong proteins is SSM, which entails the directed substitution of an enzyme's amino acid mostly with amino acids occurring naturally. Truncation is the process of eliminating certain domains of proteins that are not necessary for bio-catalysis to improve functioning.¹³ Fusion entails joining different enzymes' crucial domains creating a chimeric enzyme that is more catalytically active. These numerous protein engineering methods allow for the modification of biochemical characteristics along with fine-tuning enzyme activity.

Mutagenesis

Mutation plays a significant part in the improvement of the yield of keratinase enzyme and with amazing keratinolytic impact. Most of the keratinase showing high units have shown unsuccessful application as they were unable to meet certain requirements of various industrial operations such as the enzyme exhibiting amazing capabilities of keratin degradation without harming surrounding tissues and within less time. Considering these facts, attempts were made in developing microorganisms with hyper-productivity with the application of various mutation techniques. Physical such as X-rays and UV rays and chemical-induced mutagenesis cause excitement of DNA molecules electrons resulting in a mutagenic effect. The effect on structural or regulatory elements of genes by random mutagenesis can show either a positive or a negative effect on the production and related properties of the target enzyme. UV radiation induces mutations such as deletion, recombination, base substitution, frameshift, and another type of genetic rearrangement. Mutagens examples are Radiation (X-rays, UV rays), intercalating agents (ethidium bromide (Fig.-4), acridine dyes), alkylating agents (*N*-methyl-*N'*-nitro *N*-nitrosoguanidine, ethyl methanesulfonate), and chemicals (5-chlorouracil (Fig.-5), nitrous acid).

Modification of Culture Conditions

Keratinase production is affected by various factors including the nutritional source of carbon, nitrogen, pH, aeration, and temperature, and the strategies to obtain optimized conditions enhance the yield of the enzyme. Fermentation is required for the production of enzymes on a big scale for meeting industrial demands.

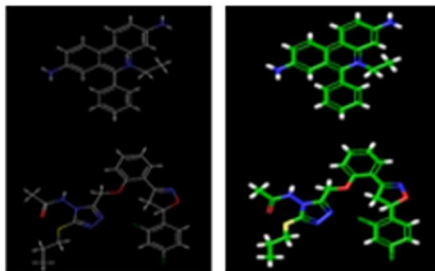


Fig.-4: 2D and 3D Structure of Ethidium Bromide

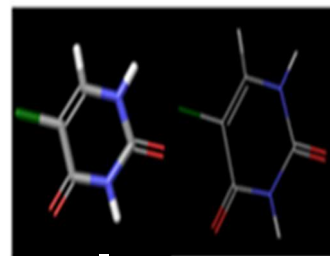


Fig.-5: 2D and 3D Structure of 5- Chlorouracil

According to research, different microbes require distinct environments for production at large-scale, implying the need for thorough experiments preceding enzyme synthesis at large-scale. Because keratinase synthesis differs from microbes to microbes, it is useful to identify and research the important nutritional and physical factors that affect keratinase production.¹⁴ The process of optimization, by classical and statistical methods, is used for the enhancement of keratinase yield from several wild bacterial isolates, since the production of keratinase is mainly influenced by physical and nutritional factors. Availability of nutrients, innate cell precursors, and cultural conditions, are some of the important factors influencing the metabolite production of microbes. The selection of physical and chemical parameters of fermentation is affected by the genetic-based diversity of keratin-degrading microorganisms. In addition, the lengthy period of fermentation for the production of keratinase by most wild microbes affects yield and sustainability. Hence, this demands the development of industry sustainable strains for enhancing productivity beyond the bench scale.

The one-variable-at-a-time approach is usually employed for screening the variables and studying the impact of the individual variable on microbial activities. In view of the growing demands for keratinases, optimization of process parameters like temperature, pH, carbon, and nitrogen supplementation is crucial for better and cost-efficient keratinase production. These factors play a major role in the promotion of the growth of microbes for the secretion of enzymes.

CONCLUSION

Sustainable development goals cannot be met until there is a sound mechanism in place to manage solid waste. Solid waste management is a cause of global concern and it should be on the top priority list of every individual. It is also expected that by the year 2050, 3.40 billion tonnes of waste is expected to get accumulated on an annual basis surpassing the 2.01 billion tonnes at present. Keratin found in many solid wastes such as feathers, hair, and wool to name a few add a huge chunk to the 70% of the staggering increase in the next 30 years. It is thus imperative from our side to manage this solid waste into something useful by various recycling methods or the use of enzymes to degrade this waste on a faster basis as well as to convert them into more useful byproducts. The careful and responsible management of goal 12 would guarantee a sustainable environment for the coming generations leading to a healthier life.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The PG students have contributed equally towards being co-first authors. The research profile of the authors can be verified from their ORCID ids, given below:

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