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Advances in Use of Keratinase from Feather Wastes for Feedstock Modification

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Abstract

Background and Objective: Enzymatic modification of protein-base materials is fast emerging as a promising tool for chemical catalysts based on increasing knowledge in enzyme reaction and devotion to achieve sustainable systems. Enzymes actively used in protein modification include proteases, especially keratinases, and their most interesting features include ability to degrade keratin to finer molecules. This review summarizes strategies for the modification of keratin using keratinase to increase functional protein-based feedstocks up-to-date.

Results and Conclusion: Keratinases are useful safe agents for feather waste modification in animal feeds. Modification can be carried out either using whole microbial cells or enzyme activities through fermentation processes in costeffective environmental-friendly manners. In this study, promising outcomes in feather waste management were achieved and hence studies can be continued to treat wastes of other sources.

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1. Introduction

More than 1 million tons of feathers are produced as byproducts from Indonesian poultry slaughterhouses in 2019 with a 1.5-kg broiler recorded to produce approximately ±6% of feathers [1,2]. Rises in concomitant and unwanted poultry feathers produced as solid wastes are associated with increasing poultry consumption. Moreover, increases in quantity of meat products need further feed-stocks and, therefore, increase demands for protein-rich sources. However, limited availabilities of protein sources and negative effects of poultry feathers on environments have urged needs to search for the methods of improving their uses as feed sources [3]. Poultry feathers majorly consist of more than 90% of crude protein keratin [4]. This is a product of epidermal tissues stiffening body structures with sulfur-rich protein fibers of α-helices (α-keratin) or pleated β-sheets (β-keratin) held together by hydrogen (H) bonds, cystine disulfide crosslink, and hydrophobic interactions within the molecule [5-8]. Moreover, the morphological structure, which is extraordinary rigid and stable, generally provides resistances to proteolytic enzymes such as pepsin, trypsin and papain [7-10]. Therefore, accumulation of keratin in poultry feathers creates serious-environment concerns [3].

It is possible to recover feather wastes, as feedstuffs or in production of rare amino acids such as cysteine and lysine [4,11], through further hydrolysis of recalcitrant keratin biomasses using pyrolysis, chemical and biological processes [4,9,12-14]. Pyrolysis processes are involved in feather meal production through steam pressure cooking and chemical treatment of feather wastes using reducing agents [12] or hot NaOH [13]. However, these methods are not friendly to the environment because they need high energy consu-

mption and includes ability to destroy certain essential amino acids to create products with poor digestibility and variable nutrient qualities [15]. In addition, they form non-nutritive amino acids such as lysine, alanine and lanthionine [16]. Keratin naturally accumulated in environment can poorly be degraded by microorganisms [17] utilizing the protein as a source of carbon and nitrogen to produce an extracellular enzyme of keratinase [14,16,17]. This term is derived from ability of the enzyme to hydrolyze keratin [17,18] by breaking disulfide bonds of cysteine moieties to yield soluble proteins [19,20]. However, these microorganisms are not able to effectively degrade keratin due to their vulnerability in natural environments.

Several keratin degradation biotechnology approaches are currently used to improve quality and quantity of feather meals. The first step is usually denaturation through disulfide-bond cleavage followed by proteolytic hydrolysis of the substrate, which is called hydrolyzing step [19-21]. Several reports have been published on possible hydrolysis of keratin through several methods such as fermentation using whole microbes (feather degrading species of bacteria such as actinomycetes and fungi) and extracted microbial enzymes [13,22]. Furthermore, keratinase can be produced through submerged or solid-state fermentation [13,22,23]. Development of keratin biodegradation methods as a poultry waste is promising due to its ability to solve two problems at the same time, including use of feathers to cover protein sources demanded as feedstocks and decrease of quantity of poultry wastes [24,25]. Moreover, enzymatic feather degradation offers an environmental friendly, low-cost less waste generation technology. This review presents an overview of current available information on keratin-degrading microbes and mechanistic insights of keratinase as a hydrolyzing agent. In addition, role of keratinase to improve feather keratin to become a bioavailable protein for animal feeding is also discussed.

2. Proteinaceous feather wastes

2.1 Feedstocks

Industrial biotechnology involves conversion of waste biomass materials to improve livestock industry and pre-serve environment. The novel bioeconomy has also focused on upgrading mechanisms to decompose these materials to limited extents. It has been reported that reuse of feather wastes generated from poultry abattoirs is extensively possible [14,24,26,27]. Meanwhile, keratinous waste streams such as chicken feathers provide interesting non-exploited sources of digestible dietary proteins for animal feeding and can be used as additives for chicken broilers [14]. Furthermore, it is possible to use feather meals as 40% substitute of the total composition of fish feeds and in chicken or fish meals with no negative effects on their performances [14,24,26,27]. Feather wastes from abattoirs are reported to include averagely 4-9% of the total weight of slaughtered chickens [1,2]. Hey are usually disposed, buried, burned or used as land filling, which include possibilities of emission problems and ash disposals [3]. This, therefore, means that inadequate management of the waste generation can cause environmental pollutions.

2.2 Biochemical basis of keratin

Keratin includes a group of filaments proteins naturally occurring in large quantities. It is a fibrous, tightly-wound, water-insoluble structural protein extensively cross-linked with thermally-stable disulfide bridges, hydrogen bonds and hydrophobic interactions [4-8]. In feathers, keratin includes a 20-kDa molecular weight [5] and 17 amino acids such as aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine [4,11]. Keratin crystal structure demonstrates a helical domain bonded and involved in tight binding of two subunits [28]. Figure 1 shows the biodegradable feather waste processes from poultry industries by microbial fermentation including bacteria and fungi as well as keratinases. Therefore, it is possible to use crude hydro-lysates as feedstock formulations after hydrolysis by keratinolytic enzymes. Moreover, cysteine contains high quantities of keratins, which creates its rigidity and plays a significant role in nature of keratin [8].

Keratins belong to a super family of intermediate filaments, which are submerged in a sulfur-rich matrix [28,29]. The family primary function is to protect cells from mechanical and non-mechanical stresses as well as other roles such as cell signaling, regulating availability of other abundant cellular proteins and as a stress protein [8]. Keratins are structurally grouped into two families, including Type I which is acidic with α-helix configurations (hard trichocytic keratins) such as stratum corneum, wool, hair, quill, horn, hoof, nail, whale baleen, hagfish slime thread and whelk egg capsule [8,30]. Moreover, the substance contains 36% of cystine contents [31,32]. Type II is basic with β-sheet configurations (soft epithelial keratins) such as feather, beak and claw, containing up to 2% of cystine. However, the two types are intensively twisted to form micro and macro fibrils necessary for the formation of a highly stable left-handed super-helical motif to ensure stability against proteolysis [28-32].

2.3 Keratinous waste: sources and environmental hazards

Keratin wastes are majorly recovered from goatskins, sheepskins, cattle hides, buffalo hides and

poultries. They are generally derived as biomasses in Indonesia from the poultry industries with more than 1.1 million tons of feather wastes annually. In 2017, the Directorate General of Livestock and Animal Health Resources reported that an average farm size in Indonesia produced nearly 3,2 tons of chicken feathers every day and 327 tons every year in national scales [1]. Keratinous wastes are also generated from other human activities such as cut hairs in barbershops, which are abundant and recalcitrant in municipal drainages, causing environmental imbalances and stream pollutions. This may further lead to eutrophication, decreased nature diversity and soil acidification due to nitrogen deposition [8,33]. Moreover, burning of feathers and human hairs produces bad odors and toxic gases such as ammonia, carbonyl sulfides, hydrogen sulfides, sulfur dioxides, phenols, nitriles, pyrroles and pyridines [1].

The best way to address these problems includes developing systems to use wastes as alternative materials for poultry feeds. Meanwhile, the crude protein in chicken feathers is ±90%, which is greater than 42.5% in soybean and 66.2% in fish meals, meaning that it is appropriate as a substitute source of raw materials for feeds [10,34]. In contemporary feed industries, keratinous wastes are converted into rations using physics method [15], which involves use of extreme temperatures above 105 °C and high pressures of 3 Bars for 8 h [35] to produce chicken feather meals with protein contents up to 76%; however, prices are usually high due to the processing costs. The method includes ability to damage amino acid structures, including heat sensitive molecules such as methionine, lysine and tryptophan [15,16]. Therefore, the promising technique to improve extraction of nutritional quality of chicken feathers is to use bacterial keratinase due to the possibility of carrying out the process in mild conditions.

2.4 Feather protein biodegradation

Keratin, as a source of rich-proteins, should be decomposed to include added values due to its high resistance to chemical and physical agents. Microorganisms such as bacteria and fungi naturally work together to slowly decompose polymers by breaking down keratin structure [17]. However, alternative methods should be investigated to increase the efficiency and decrease carbon monoxide and carbon dioxide generation. This can provide adequate benefits to economy and global environment [36]. It is practically possible to degrade keratin through several ways using acids, bases, enzymes, high temperatures and UV radiations. However, biodegradable methods, involving whole microbes and enzymes, are common ways to decompose feather wastes [10,15]. Figure 1 shows that keratin protein with high mechanical and chemical agent resistance is easily degraded by enzymatic lysis (proteases). Moreover, one of the robust and popular methods for keratin decomposition is pyrolysis or high thermal degradation method; however, it is expensive and needs much energy [12].

Several studies have reported keratin biodegradation through microorganisms to produce simple basic compounds [2,14,37-39]. The two modes usually used for feathers, including microbial fermentation and enzymeatic reaction are shown in Figure 2. They are used to cut bonds in keratin to produce ammonium salts and free amino acids such as cysteine and methionine [40]. Feather degradation has been associated with substrate type, microorganism growth, pH, temperature and humidity, which is significantly linked to efficiency of the process [40]. There are correlations between the concentration of degradative products and degradation level of substrates [41,42]. Feather degradation process known as the keratin hydrolysis (Figure 1) has been studied under microscope and the first step includes adhesion of microorganisms to the feather, which is followed by penetration and separation of its barb that is broken down into a powdery gelatinous mass. In addition, spread to the shaft region causes its rupture and disintegration to needle-like structures [43,44].

This is the primary step in keratin decomposition known as the sulfitolysis, involving breaking down of disulfide linkages followed by the production of cysteine residues as thiol [21,44]. Several bacterial species have shown varied abilities to degrade. As reported by Laba et al. [21], quantities of soluble proteins released into culture filtrates vary within *Bacillus* strains; therefore, a variety of keratinolytic microorganisms have been studied intensively to optimize their feather-degrading capabilities. The molecular masses of degraded keratin as soluble products under optimal hydrolysis conditions range 3.55-3.60 kDa [44]. The low-molecular weight of keratin can be useful in anti-skin aging products [39], cosmetics [33] and animal feedstuffs [9].

3. Keratinases

Keratinases (E.C. 3.4.99.11) are a class of proteolytic enzymes sometimes called endopeptidases with the ability to break non-terminal peptide bonds inside the polypeptide chains [45-48]. They majorly hydrolyze disulfide bonds (-S-S-) in keratin polymers. Based on their catalytic mechanisms, most keratinases are classified in the subfamily of subtilisin-like or chymotrypsin-like (trypsin-like) proteases, belonging to serine protease (S8 family) [45,49]. A majority of keratinase are monomeric enzymes with a diverse range of molecular weights of 14-240 kDa [45-48,50]. Genetically, keratinases are identified and most well-characterized by sequencing of KerA from *B. licheniformis* PWD-1 [51].

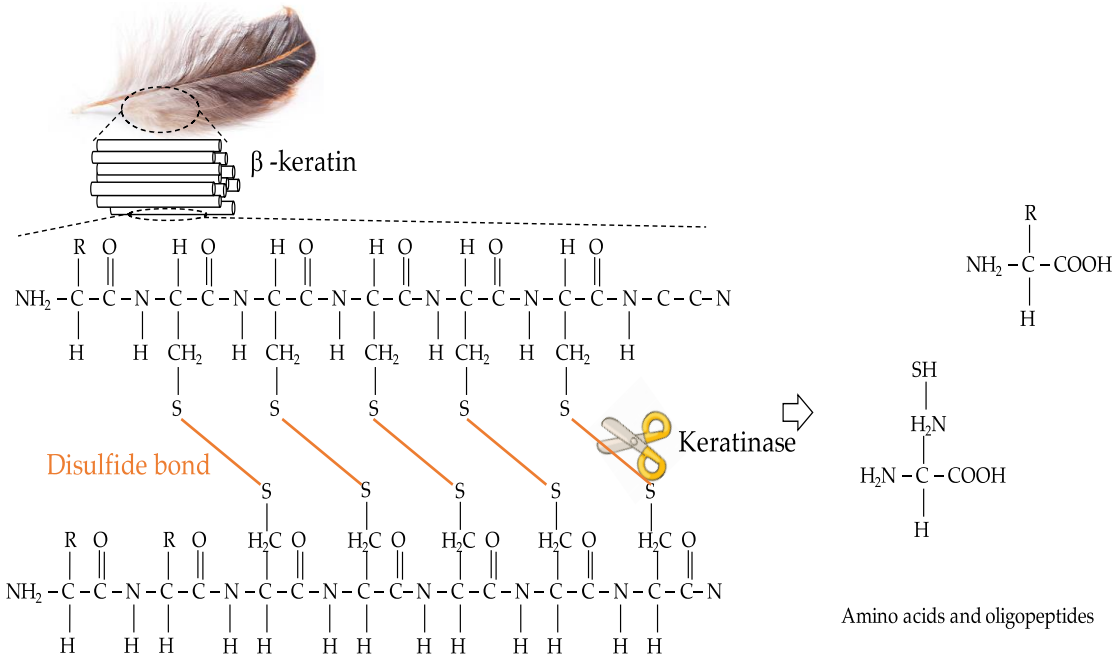

Figure 1. Mechanistic diagram of feather β-keratin hydrolysis using keratinase to produce soluble amino acids and oligopeptides. Keratinase breaks down disulfide bonds in insoluble macromolecules, which is difficult for other proteases

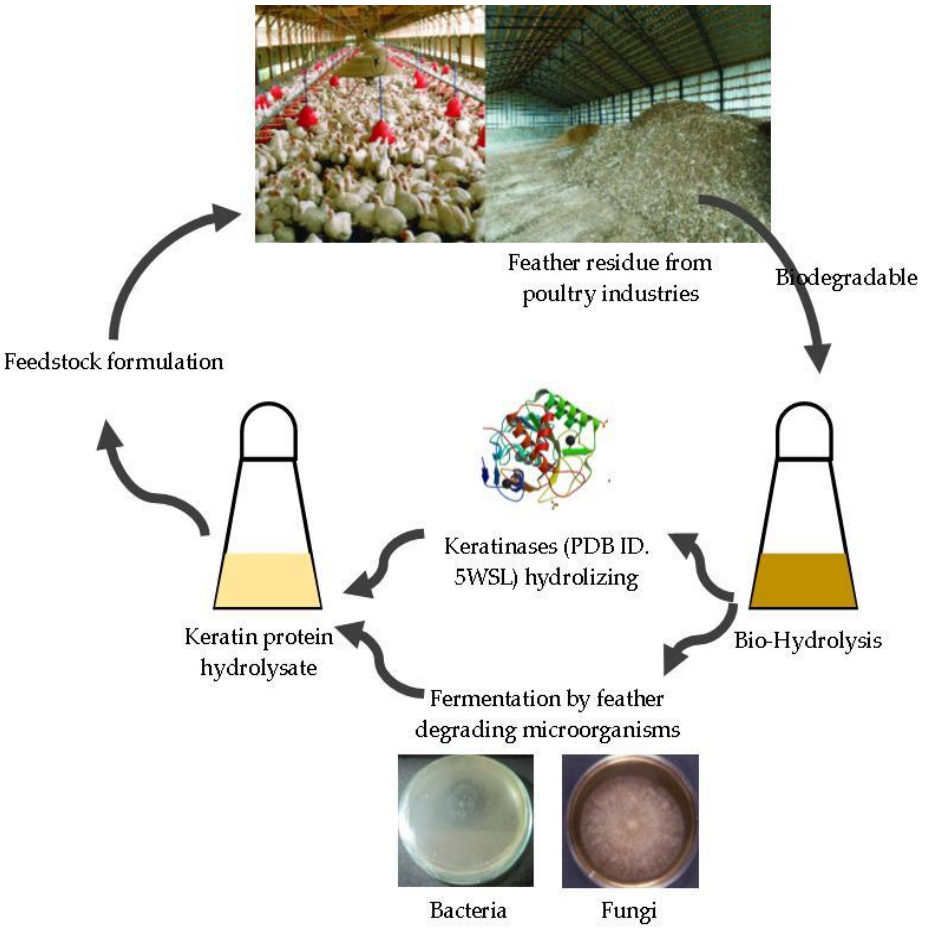

Figure 2. Schematic diagram for biodegradation modes of feather wastes from poultry industries using microbial fermentation (*Bacillus* spp. and *Aspergillus* spp.) and enzymatic (keratinase) reaction. Once keratin is degraded by keratinolytic enzymes, it is possible to use the crude hydrolysates as feedstock formulations

They are produced as extracellular proteases by various microorganisms only in presence of keratin containing substrates [50,52,53] with the keratin serving as an inducer [21]. Therefore, the best way to achieve high levels of keratinolytic microorganisms with high keratinase activities is through isolating them from keratin-rich environments such as poultry feathers, slaughterhouses and wool wastes [35,38,54]. Keratin-degrading abilities have extensively been studied for commercial purposes, especially in animal feed industries and most of the microbial groups belong to Gram-positive bacteria, including *Bacillus* and *Microbacterium* sp. [9,14,19,21,22].

Biochemical studies of keratinases have shown broad substrate specificities and actives against soluble and insoluble proteinaceous substrates [52,55]. Keratinases possess abilities to hydrolyze casein, gelatin, bovine serum albumin and hemoglobin, which are soluble proteins [55]. Therefore, keratinases include abilities to hydrolyze insoluble proteinaceous materials such as feathers, wools, silks, collagens, elastins, horns, hairs, azokeratins and nails [16,20,56]. Regarding their keratinolytic activities, they include much variations in their biochemical characteristics and are generally active in alkaline conditions at pH 7-8 [16,38,39,56]. However, keratinases from unique microorganism habitats have been reported to include optimum ranges of 9-10 [57-59]. Their temperature reaction conditions vary 37-100 °C [14,20,48,59,60]. Keratinase inhibitors have been reported to include metal-chelating agents such as ethylenediaminetetraacetic acid (EDTA), organic ligands (e.g., 1,10-phenanthroline) and metals such as Co^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and Fe^{2+} [9,16,20,61,62]. As a member of serine–family, keratinase is also inhibited by phenylmethanesulfonyl fluride (PMSF) and diiodo propyl fluorophosphates (DFP) [63,64]. Furthermore, activation and stabilization of keratinases are stimulated by the presence of various organic solvents, including, DMSO, methanol and isopropanol as well as surfactants such as Triton X-100, Tween 20 and Tween 80, non-ionic surfactants [20,47,65] and reducing agents such as dithiothreitol (DTT), β-mercaptoethanol, cysteine, and sodium sulfite [64,66]. These biochemical characteristics show potentials in industries.

4. Feather protein hydrolysate

Feather decomposition process through keratinolytic microorganisms is an excellent strategy to improve nutritional values of feed proteins. Moreover, using keratin degrading microorganisms or keratinolytic enzymes to produce amino acids and peptides includes ability to act as supplement proteins in feed formulations [14,27]. Then, feathers are converted to animal feeds with rich sources of proteins in large quantities. Feather hydrolysates have been reported to be rich in serine, leucine, alanine and glutamine residues as well as minor quantities of histidine and methionine [4,11]. Table 1 shows several types of amino acids in feather meals with specific functional groups. However, the nutritional contents of feather meals can be varied depending on the origin of keratin sources; therefore, their uses vary as well. For example, pharmaceutical and cosmetic industries need more positively charges of amino acids [5,67,68] than that feedstuffs do [14].

Table 1. Compositional values of amino acids in feather meals [19,22,43,69]

Functional Groups	Amino acid
Positively charged	Arginine
Negatively charged	Aspartic acid
	Glutamine
Hygroscopic	Threonine
	Serine
	Tyrosine
Hydrophobic	Leucine
	Isoleucine
	Valine
	Cysteine
	Alanine
	Phenylalanine
	Methionine
Nonpolar	Proline
	Asparagine

5. Keratinolytic bacterial fermentation

Fermentation process is mostly used by keratinolytic microorganisms to biodegrade whole chicken feathers. Bacteria isolated from various sources are presented in Table 2. These bacteria have been investigated for active degraders of feather keratinous wastes due to their potentials as fermentation enablers in feather degrading activities. There are two ways to modify keratin-based materials, including fermentation by whole microbes and keratinolytic enzymes. Submerged fermentation of poultry wastes by keratinase producing microorganisms helps convert non-soluble keratin such as feathers into soluble proteins or polypeptides [22,69]. This is majorly based on the use of free-flowing liquid substrates in the system to secrete bioactive compounds into the fermentation media. This method is beneficial due to its ability to utilize substrates quite rapidly and is best suited for the bacteria that need high moisture contents [70]. The best strains of bacteria are selected using common methods such as screening and isolating the most pronounced clearing zones on feather supplemented semi-solid media to improve effectiveness of hydrolyzing feather biodegradation [37-39]. Screened and isolated colonies are analyzed to show their abilities to degrade feather polymers directly in the same media used for fermentation while monitoring their degradation in the media and the culture with the highest rate is selected for the fermentation process.

Table 2. Origin of feather degrading bacterial isolates

Bacterial isolates	Origin	Main medium	Reference
Bacillus sp.	Singkidang creater Indonesia	Chicken Feather	[14]
Bacillus megaterium F7-1,	Poultry farm	Chicken Feather	[19]
Fervidobacterium islandicum AW-1	Chicken Poultry	Chicken Feather	[39]
Caldicoprobacter algeriensis strain TH7C1(T)	Hydrothermal hot spring in Algeria	Chicken Feather	[46]
Streptomyces spp.	North East Indian Himayan Region	Chicken Feather	[48]
Stenotrophomonas maltophilia BBE11–1	Poultry farm	Wool waste	[54]
Bacillus amyloliquefaciens strain S13	Marine brown algae *Zonaria tournefortii*	Chicken Feather	[64]
Bacillus spp.	Poultry industry	Feather Meal and Whole Feather	[72]

Selection of keratinolytic bacterial property was also reported important for the improvement of fermentation process. Various strains have shown various levels of degradation as indicated by the feather weight loss and nutritional values such as soluble peptide and amino acid productions [57]. It is practically possible to use feather biodegradation as a single or cocktail microbe towards increasing degradation levels [71].

Factors affecting feather biodegradation are fascinatingly studied, especially in areas of kinetics, enzyme activities and nutritional values [19,22,43,69]. Therefore, ability of fermenters to degrade is affected by optimization of feather concentration, incubation time, pH and temperature [38,39,43,71]. Due to the role of keratin in microbial metabolism as sources of carbon, nitrogen, sulfur and energy, the initial concentration of feathers needs calculation properly to improve growth levels in culture media [37,8,53]. Raw feather concentration is usually in the range of 0.5-2% w v^{-1} during keratinase submerged fermentation [39,72]. Furthermore, appropriate incubation time includes effects on the production of nutritional values and microbial metabolites [58] and a long incubation time has been found to decrease amino acid concentration because it is used by the microbes [40].

Keratinase fermentation from thermophilic *Bacillus* spp. has been reported in recent studies to include optimum production of soluble proteins at 22.06 g l^{-1} in minimal media containing 10 g l^{-1} feather meals at room temperature and pH 7 for 24 h [14,69]. Previous reports by Fakhfakh et al. [22] have shown that *B. pumilus* strain A1 is able to achieve complete feather degradation in feather meal media of 50 g l^{-1} at 45 °C for 40 h at pH 10 to produce maximum amino acid and peptide productions of 42.4 g l^{-1}. Additionally, mesophilic keratinolytic bacteria are interesting agents for industrial uses of biotechnology due to their abilities to consume less energy [38]. This, therefore, means that *B. megaterium* F7-1 is a promising bacterial strain to biodegrade poultry and abattoir wastes [19].

6. Advanced updated fermentation strategies using keratinase

Use of keratinase in fermentation processes is a low-cost method of decreasing keratin-based wastes [14,60]. Therefore, several investigations have been carried out to improve keratinase production for industrial uses. Occasionally, those derived from microbes such as bacteria and fungi are produced using solid-state fermentation of raw feathers as the substrates [23]. However, in soli-state and submerged fermentations, composition of the media affects microbial cell growth to produce primary and secondary metabolites in term of concentration, yield and volumetric productivities [69,73]. The initial media moisture also affects the biosynthesis as well as the physiochemical attributes and stabilities of keratinase [22,26,42,54].

Effective feather degradation and keratinase production from various sources have been studied using various techniques [25,42,44,71]. The selected techniques for the enzymatic fermentation should be moderated to ensure that native conformation of the enzyme does not change (Table 3). This process usually starts with the fractionation of proteins based on solubility; after which, several chromatographic and electrophoretic methods are used for purification [19,22,53].

Bacterial keratinase mostly includes a lower activity than that the other microbial keratinases do in enzymatic fermentation. To improve their activities, a mild modification is needed. The best way to achieve this includes immobilization of keratinase to avoid conditions inhibiting its effectiveness such as high sensitivity to reducing agents as well as non-reusability [2,50,73]. The immobilized enzyme includes a higher stability, compared to that the free enzymes do.

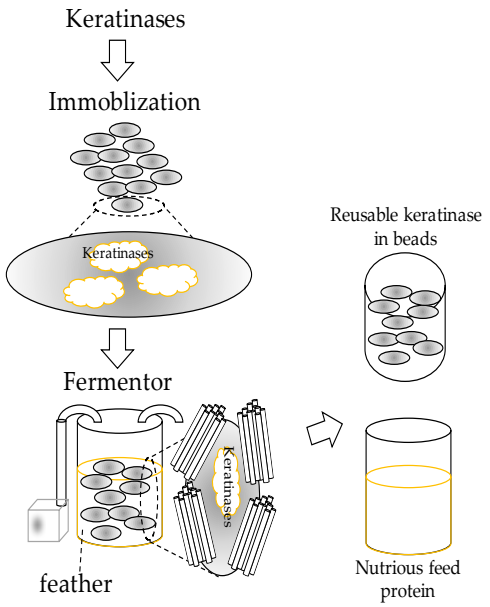

Figure 3. Immobilization of keratinase on chitosan beads [50] and its feather bioconversion use in fermenters to produce nutritious feed proteins

An example involving use of chitosan is shown in Figure 3 to develop a powerful tool for the simplification of keratinase reuses. One of the robust techniques is using covalent bonding to generate a stable binding between the carrier and the enzyme [2,50]. Immobilization is usually used to decrease production costs, increase recycle efficiencies and simplify process controls [73,74].

7. Conclusion

Keratinases are widely used as proteolytic enzymes not only in chemical and medical industries, but also in green feedstock modifications. Several studies on keratinolytic microbes have verified the high activity of keratinase either as a whole cell or through enzyme fermentation. The enzymatic technologies have also discovered to provide safe methods to recycle recal-citrant compounds from poultry wastes with low production costs. These encouraging reviews in feather waste managements are suggested to continue and the use of keratinases needs further assessments in treating other types of wastes.

Table 3. Pretreatment methods and their associated advantages and disadvantages

Keratin Treatment method	Fermentation Process	Advantages	Disadvantages
Whole microbes	Submerged fermentation	Easy to scale up	Sophisticated in downstream process
	Solid-state fermentation	Easy method since the conditional of microbes' growth are similar to those natural inhibits	Sophisticated in downstream process
Keratinase	Submerged fermentation	Product may be incorporated directly to feedstocks	Non–reusable
	Solid-state fermentation	Produces a minimum amount of waste and liquid	The activity decrease
Immobilized keratinase	Submerged fermentation	Reuse of immobilization beads and facilitates the cell contacts with the substrate	Agitation is essential
	Solid-state fermentation	Reuse of immobilization beads and facilitates the cell contacts with the substrate	Keratinase has limited mobility and this causes loss of activity

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9. Conflict of Interest

The authors report no conflicts of interest.

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پیشرفت‌هایی در استفاده از کراتیناز حاصل از ضایعات پر به‌منظور اصلاح خوراک دام

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۲- گروه تحقیقات فناوری آنزیم و زیست‌مواد، آزمایشگاه میکروبیولوژی و تکنولوژی زیستی، دانشکده علوم و تجزیه و تحلیل داده‌ها، انستیتو تکنولوژی سپولاه نوپمبر (ITS)، ساختمان پردیس H ITS سوکولیلو کمپوتیح ، سورابایا - ۶۰۱۱۱ اندونزی.

چکیده

سابقه و هدف: اصلاح آنزیمی مواد برپایه پروتئین موضوع نوظهور سریعی به‌عنوان ابزاری امیدبخش برای کاتالیست-های شیمیایی براساس افزایش دانش در زمینه واکنش آنزیمی و دستیابی به سامانه‌های پایدار است. آنزیم‌ها به‌طور فعال در اصلاح پروتئینی شامل پروتئازها، به ویژه کراتیناز، مورد استفاده قرار گرفتند و اغلب ویژگی‌های جالب آنها شامل توانایی تجزیه کراتین به مولکول‌های کوچکتر می‌شود. این مقاله مروری راهبردهای[1] اصلاح کراتین با استفاده از کراتیناز به منظور افزایش و به‌روز رسانی خوراک دام فراسودمند بر پایه پروتئین را خلاصه می‌کند.

یافته‌ها و نتیجه‌گیری: کراتینازها ترکیبات ایمن و مفیدی برای اصلاح ضایعات پر در خوراک دام می‌باشد. اصلاح می‌تواند یا با استفاده از سلول‌های کامل میکروبی و یا فعالیت آنزیمی از طریق فرایندهای تخمیری با روش‌های سازگار با محیط زیست[2] و مقرون به‌صرفه[3] انجام شود. در این مطالعه، نتایج امیدبخش در مدیریت ضایعات پر به‌دست آمده است و از این رو مطالعات می‌توانند برای تیمار ضایعات سایر منابع ادامه یابند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

دریافت ۱۷ آپریل ۲۰۲۰
داوری ۲۶ می ۲۰۲۰
پذیرش ۵ آگوست ۲۰۲۰

واژگان کلیدی

- زیست‌فراهمی
- اصلاح آنزیمی
- خوراک دام
- کراتیناز
- پروتئین

***نویسنده مسئول**

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[1] Strategies
[2] Environmental-friendly
[3] Cost-effective