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Valorization of Chicken Feather Keratin as an Eco-Friendly Coagulant for Ochre Dye Removal: A Sustainable Approach

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total solid.

ABSTRACT. This study examines the extraction of keratin from chicken feather waste and its potential use as a coagulant in ochre dye solution treatment. The extraction procedure was investigated for the effect of some parameters, such as temperature (60 – 80 °C), sodium hydroxide concentration (1 – 2 N), and extraction duration (30 – 120 minutes). The maximum keratin yield of 85.64% was obtained using 1.75 N NaOH at 70 °C for 90 minutes. Fourier Transform Infrared spectroscopy confirmed the presence of characteristic protein functional groups in the extracted keratin. Keratin showed modest efficiency as a coagulant in treating the ochre dye solution, with a maximum total solid removal of 47.13% when using a 0.4% keratin dosage-coagulant, pH 10, and 180 minutes of settling time. The study shows that chicken feather waste can be valorized while addressing environmental issues. Nevertheless, additional modification of keratin extraction is required to increase coagulation efficiency, such as considering pH conditions and using an appropriate solvent to minimize keratin degradation.

INTRODUCTION

Feathers from poultry are seen as a growing and bothersome waste. Many countries treat them as environmental waste, which strains landfills and slows decomposition. As the demand for poultry grows, there is a significant release of feather waste into the environment. They are the primary by-product waste dumped about several million tons each year. Feather waste management requires more thought and calls for an effective, appropriate, and efficient technique (da Silva, 2018). Sadly, the most common disposal treatment is burning, which leads to the emission of carbon dioxide and sulfur oxides (Wang *et al*., 2020).

Keratin proteins are fibrous, with complex polypeptide chains and cross-linking fibers (Murray *et al*., 2017). Keratin is characterized by its high concentration of cysteine amino acids, which contain sulfhydryl groups (–SH). These groups facilitate the formation of robust covalent disulfide bridges, effectively cross-linking both the polypeptide chains within keratin molecules and the surrounding matrix proteins (McKittrick *et al*., 2012). Despite its structural stability, keratin retains chemical reactivity due to cysteine, which can undergo reduction, oxidation, and hydrolysis processes (Wang and Cao, 2012). Keratin can be obtained by disrupting the disulfide bonds in cystine, a process achieved through hydrolysis, which breaks this primary structure (Wrześniewska-Tosik *et al*., 2007; Shavandi *et al*., 2017).

Dyes are compounds for material colorization that are widely used in industries, such as printing, leather, rubber, plastics, cosmetics, and many more. The textile industry employs the most significant amount of dyes (Reisch, 1996). Dyes can be derived from their source: animal-based (from mollusks or shells), minerals (ochre and ferrous sulfate), and plant-based (saffron and indigo). The dying process consequently causes large quantities of waste and may affect the water body as a pollutant (Crini, 2006). Ochre has been used as a color pigment for hundreds of thousands of years (McBrearty and Brooks, 2000). Red ochre and white and black paints are among the oldest archaeologically persistent media humans use to transmit and store knowledge through material symbolism (Wreschner *et al*., 1980). People worldwide took advantage of ochre's essential

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features of vivid, bold color; the capacity to tint and color a range of substrates. In many cases, the color remains undiminished at roughly the same intensity over thousands of years (Popelka-Filcoff and Zipkin, 2022).

Based on the study, the weight content percentages of yellow ochre by gravimetric chemical analysis are iron (44.98%), silicon (3.25%), and aluminum (14.48%) (Singh *et al*., 1978). Ochres contain various proportions of octahedral iron oxides, such as hematite (Fe_2O_3) and goethite (FeOOH), as well as white pigments such as kaolinite or illite, quartz, and calcium compounds like calcite, anhydrite, gypsum, or dolomite. Hematite is the primary iron oxide, which gives a red color. However, when goethite dominates, a yellow color is formed. The colors in both oxides are generated by the ion Fe^{3+} and its ligands, O^{2-} or OH^-, which transfer charge (Elias *et al*., 2006). Iron ochres are common gelatinous sludges formed as ferrous ions in water. They can be found in various aquatic habitats, such as rivers, mining waste streams, home water systems, and field drainage. In some cases, they might cause drainage system blockages (Vaughan and Ord, 1994).

The demand for clean water is escalating due to population growth and accelerated industrialization. Consequently, there is a commensurate increase in wastewater generation, leading to a progressive reduction in available clean water resources. The intensification of industrial activities has resulted in elevated concentrations of various contaminants, particularly heavy metals, in water bodies. This contamination poses significant threats to ecosystem integrity. Industries such as electroplating, mining, and battery manufacturing are primary contributors to heavy metal pollution, which harms the environment and ecological systems (McConnachie *et al*., 1999). For example, these industries result in various dissolved and suspended impurities, increasing the turbidity level in wastewater (Siddall, 2018).

Coagulation and flocculation stages in water treatment aim to remove suspended particles from water, resulting in clean and particle-free output. These processes typically occur during the initial phase of water or wastewater purification systems (Edzwald, 1993). The inorganic coagulants can provide high efficiencies; however, this type has a main problem, such as generating toxic sludge (Okoli *et al*., 2012). Using a natural coagulant is an alternative to this problem. It is way better than the chemical coagulants (environmentally friendly and biodegradable) (Dkhissi *et al*., 2018). Natural coagulation processes primarily operate through several mechanisms, such as adsorptive interactions, neutralization of electrical charges, formation of polymer bridges, precipitation-induced coagulation, and the creation of electrostatic patches (Kurniawan *et al*., 2020). Factors affecting the coagulation-flocculation process include the type of coagulants/flocculants, mixing process, and wastewater characteristics. The dosage of coagulants/flocculants and operational pH conditions must also be analyzed (Duan and Gregory, 2003).

The research on natural coagulants, such as chitosan, has been conducted. Momeni et al. (2018) studied the coagulation of melanoidin industrial dye, and their study shows that the maximum color removal was about 82.78% under conditions of a coagulant dose of 3 g/L, pH of 3, dye concentration of 1000 mg/L, and settling time of 78.93 minutes. Another study recorded that chitosan could remove the turbidity and color of anionic dye, with an initial concentration of about 5 mg/L, which was about 99.8% and 78%, respectively (Abdullah and Jaeel, 2019). The study of direct dye wastewater removal using chitosan powder was also shown by Elzahar and Bassyouni (2023). Their analysis shows that maximum capacities for dye removal were 94.4% using a 4.5 g/L chitosan dose.

Moreover, the research about keratin application on treating contaminated water was studied by Amin *et al*. (2024), who applied the extracted keratin from chicken feathers to reduce zinc and copper, resulting in 52% for zinc removal and 69% for copper from synthetic water with optimum pH of 5 and 4.5 for zinc and copper. While Wang *et al*. (2010) applied the feather keratin to treat potato starch wastewater, resulting in a chemical oxygen demand (COD) removal of about 75%, achieved using 0.4 g/L keratin and pH 8.

This study aimed to optimize the alkaline hydrolysis extraction of waste chicken feathers using sodium hydroxide, define the extract's functional groups, and assess its efficacy as a coagulant in ochre dye solutions under certain conditions. Furthermore, it aimed to convert low-value organic waste into environmentally beneficial waste processing products and investigate various applications of chicken feather waste extracts.

RESEARCH METHODS

Chicken feather waste was obtained from UD Dika Arendra Slaughterhouse in Kledokan, Sleman, Yogyakarta, Indonesia. Distilled water, NaOH 98%, and HCl 1 N are supplied by CV Progo Mulyo and ARD Chem, while yellow ochre dye is supplied by TB Arrafi Ringin Raya in Yogyakarta. The equipment used included an analytical balance (OHAUS PA323), a three-neck flask (PYREX), an allihn condenser, a magnetic

stirrer (IKA C-MAG HS 4), a thermometer, vacuum filtration, a centrifuge (Southwest SCL565-50), and an oven (Memert ULE 500) for drying the extract and gravimetric analysis. The instrument for analyzing keratin samples is an FTIR spectrophotometer, BRUKER Alpha II.

Pre-Treatment of Chicken Feather

Chicken feathers were cleaned and washed with water. The clean feathers were then dried in the sun and oven at 40 °C for about four hours. The dried feathers were cut into small pieces, approximately 5 mm in size. The treated feathers were stored at room temperature and sealed in a container for future use.

Extraction of Keratin From Chicken Feather Using Sodium Hydroxide

Twenty-five grams of treated feathers were extracted with different concentrations of sodium hydroxide solutions and heated at various temperatures based on Mengistu *et al.* (2024). The mixture was stirred for different lengths of time, then filtered and centrifuged. The resulting liquid was neutralized with hydrochloric acid (HCl), filtered again, and dried. The keratin extract was analyzed using an FTIR spectrophotometer to determine the various groups and characteristics inside the sample. The spectra of the sample were recorded in the range of 4000 – 500 cm^{-1}. The yield of keratin was determined by weighing the product and comparing it to the feather weight. The quantification of protein was analyzed using the Kjeldahl method. The yield was calculated by Equation 1.

$$\text{Yield}(\%) = \frac{\text{weight of extracted protein (gram)}}{\text{weight of chicken feather (gram)}} \text{x}100\% \quad (1)$$

Coagulation of Ochre Dye Using Keratin Extract

Four grams of yellow ochre dye were dissolved in 1000 mL of distilled water. After the solution was ready, it was stored in a bottle. Ochre dye solution (300 mL) was moved into the beaker glass and added with sodium hydroxide 1 N dropwise until the pH turned into some variations (8, 9, and 10). After that, the solution was added with a variation of the keratin powder dose while it stirred slowly for about ten minutes. Then, the sample was left behind for various times (30, 60, 90, 120, and 180 minutes).

Determination of Total Solid By the Gravimetric Method

The empty, dried porcelain was weighed as (A). After a given amount of time (30, 60, 90, 120, and 180 minutes) of settling, the solution above the sediment was extracted with a volumetric pipette and transferred to the porcelain. The solution was dried in an oven at 105 ℃ until the weight remained constant (B). The total solid (mg/L) was estimated using Equation 2. This step was also carried out on the ochre dye solution that had not been treated with keratin powder, where the total solid value was used as the initial value for total solid (TS).

$$\text{Total Solid (TS)} = \frac{[(\text{B}-\text{A})\text{gram}]\times 1000}{\text{volume of sample(liter)}} \quad (2)$$

where B is the weight of porcelain and dried sample (gram), and A is the weight of dried porcelain (gram). The total solid removal (%) was calculated by Equation 3.

$$\%\text{Total Solid Removal} = \frac{(\text{TS initial}-\text{TS final})}{\text{TS initial}} \times 100\% \quad (3)$$

where TS initial is the total solid value of the sample before coagulation treatment (mg/L) and TS final is the total solid value of the sample after coagulation treatment (mg/L).

RESULTS AND DISCUSSION

Characterization of Keratin Powder by Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis revealed distinctive spectral features that helped identify the keratin samples' structure. The absorption spectrum, presented in Figure 1, showcases several key bands. A prominent, narrow peak at 3274.75 cm^{-1} suggests the presence of N–H stretch. The band at 2850.51 cm^{-1} indicates C–H stretching, while the strong peak, 1651.70 cm^{-1}, corresponds to the carbonyl group in amides (C=O stretch). Bands in the 1543.04 cm^{-1} range represent N–H bending. The presence of N–H, amide, and various C–H bonds in the sample confirms its keratin content. The interpretation of FTIR spectroscopy is based on a previous report (Nandiyanto *et al.*, 2019). The FTIR spectrum of this sample was benchmarked against the keratin reference spectrum from (Mengistu *et al.*, 2024) (Figure S1).

The existence of protein polymer and some functional groups, such as hydroxyl and carboxyl, formed the biocoagulant and bioflocculant (Ang and Mohammad, 2020). The hydroxyl group contributes to the coagulation process, followed by amine, carboxyl groups, and protein. During the coagulation, these groups were released and form positively charged biocoagulant particles that depend on pH and coagulant dose (Johnson *et al*., 2019). Regarding protein characteristics, it will provide positively charged functional groups or bridge the particles (Miyashiro *et al*., 2021).

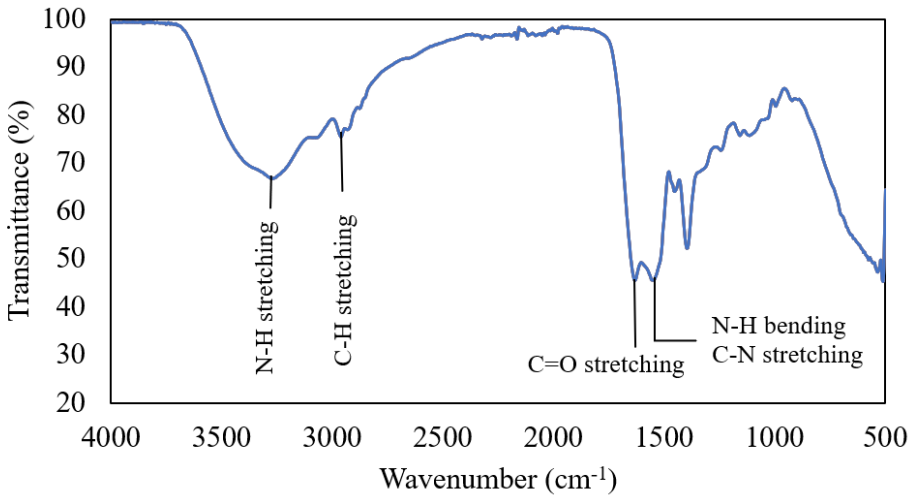

Figure 1. The FTIR spectrum for keratin extract.

As summarized in Table 1 and Figure S1, the FTIR spectra from this study show significant alignment with (Mengistu *et al*., 2024). This comparative analysis serves to validate our findings through three key observations: (1) the characteristic keratin protein backbone is confirmed by Amide I/II band correspondence, (2) the absence of S-S stretching vibrations in this study suggests further hydrolysis compared to (Mengistu *et al*., 2024), and possibly also because of the limitation of FTIR analysis to read the disulfide bonds, and (3) the relative intensity of CH_2 stretching indicate potential differences in hydrophobic side chain exposure post-hydrolysis.

Table 1. Comparison of FTIR spectral peaks between extracted and standard keratin reference (Mengistu *et al*., 2024).

Functional Group	Wavenumber (cm^{-1})	
	This Study	Reference Keratin (Mengistu *et al*., 2024)
N–H stretching	3274.75	3275.27
C–H stretching	2850.51	Not detected
CH_2 stretching	Not detected	2918.42
C=O stretching	1651.70	1651.14
N–H bending and C–N stretching	1543.04	1543.12
S–S stretching	Not detected	547.81
CH_2/CH_3 bending	Not detected	1458.25

Effect of The Variables on Yield

In this study, we investigated the influence of several key variables on the yield of keratin extraction, explicitly focusing on extraction time, sodium hydroxide concentration, and temperature. Understanding the effects of these parameters is crucial for optimizing the extraction process and achieving maximum yield.

Effect of Extraction Time on Yield

The relationship between extraction time and yield is illustrated in Figure 2, which indicates that optimal results were obtained at an extraction duration of 90 minutes, yielding approximately 85.64%. Otherwise, extending the extraction time to 120 minutes significantly reduced the yield to 45.40%. The optimal extraction duration allows sodium hydroxide to solubilize keratin effectively. However, it does not imply that prolonged extraction times will enhance yield. Extended exposure may lead to the degradation of specific amino acids due

to prolonged thermal conditions. According to Mengistu *et al.* (2024), their research demonstrated that an extended extraction time of 2 hours, conducted at a temperature of 70 °C with 0.85 N sodium hydroxide, resulted in a decrease in yield.

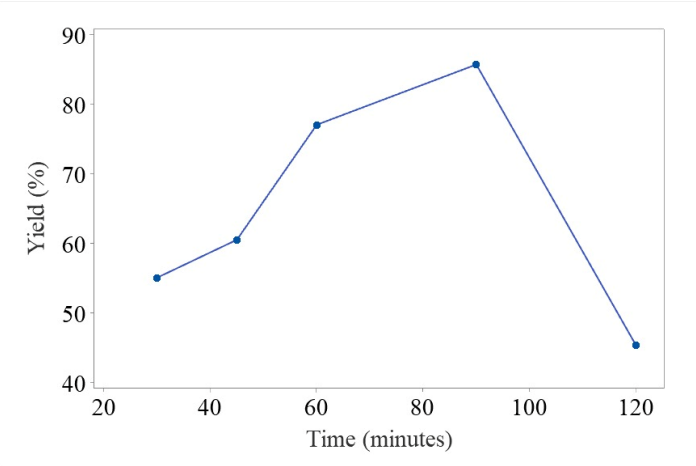

Figure 2. The curve of the relation between the time of extraction and the yield (%).

Effect of Sodium Hydroxide Concentration On Yield

The sodium hydroxide concentration was critical for maximizing keratin solubilization. The experiment revealed that applying 1.75 N of sodium hydroxide yielded the best yield of 85.64%. Figure 3 shows that the yield increases with greater sodium hydroxide concentrations up to 1.75 N. According to Wang *et al.* (2010), low sodium hydroxide concentrations reduce yield because chicken feathers are unable to dissolve properly. Meanwhile, the higher the solvent concentration, the more disulfide bonds will be reduced, resulting in smaller keratin particles. Meko *et al.* (2024) examined the influence of NaOH concentration on yield. They discovered that the greatest concentration (6 N) produced a lower yield of around 38%, while the highest yield was reached using 3 N sodium hydroxide for about 62%. Meanwhile, the lowest concentration (0.1 N) generated the lowest yield of approximately 3%.

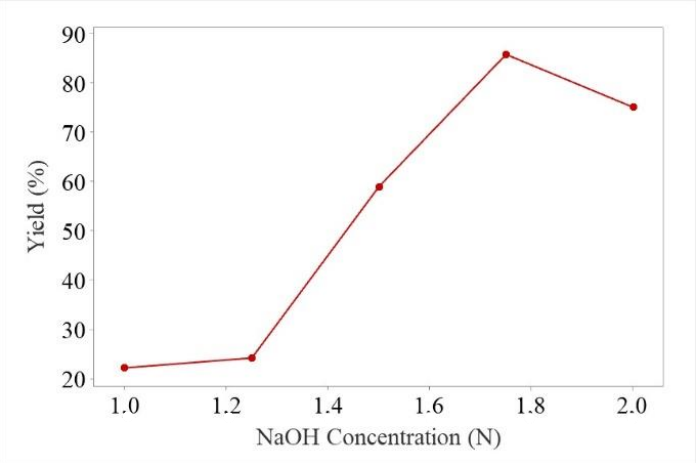

Figure 3. The curve that shows the relation between NaOH concentration and the yield (%).

Effect of Temperature on Yield

Figure 4 shows that the highest temperature leads to the lowest yield. The optimal yield occurred at 70 °C, resulting in approximately 80.28%. Another study by Meko *et al.* (2024) reported the extraction at lower temperatures (8 °C, 28 °C, and 60 °C), confirming that increasing the temperature decreases yield because the keratin structure can be easily destroyed. When Meko *et al.* (2024) applied the extraction at temperatures of 60 °C, 70 °C, and 80 °C for 1 hour extraction with 0.85 N of sodium hydroxide, the yield decreased to 75.82% (at 80 °C) because there was more amino acid breakdown. Compared to that result, this investigation reveals the

lowest yield attained at 80 °C, approximately 60.48%, possibly because they used a different process and condition to extract the keratin.

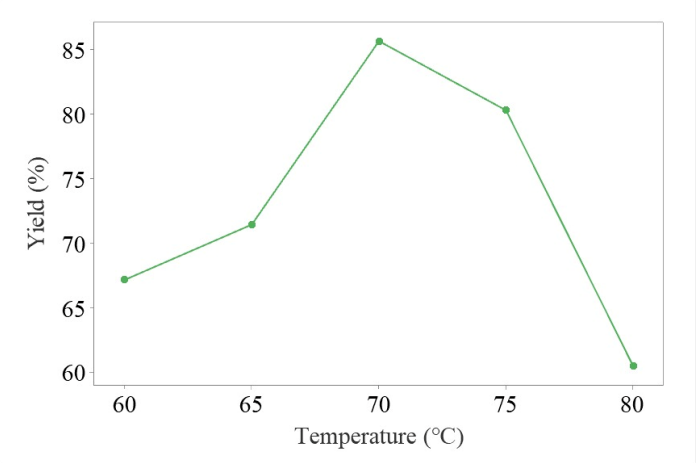

Figure 4. The curve that shows the relation between temperature and the yield (%).

Application of Keratin Extract as Coagulant to Ochre Dye

The results of the coagulation process in the ochre dye solution are shown in Figure 5. The initial color of the ochre dye solution was orange. After adding the NaOH 1 N, the solution turned into a lighter color with a chalky-textured solid. This experiment shows that the solubility of ochre dye was reduced due to the alkali addition, but it takes a longer time to settle down the solids. Keratin addition to the solution may help the settling process and act as a coagulant under the right conditions, such as dosage, settling time, and pH. Specifically, the sediment layer is clearly shown in Figure 6d.

(a) (b) (c) (d)

Figure 5. (a) Ochre dye solution before NaOH 1 N addition, (b) ochre dye solution after NaOH 1 N addition, (c) ochre dye after coagulation, and (d) top-view of ochre dye after coagulation.

Effect of Dose and Settling Time on Total Solid Removal

The parameter that was analyzed in the ochre dye is total solid removal. Total solid is the residue left after evaporating a sample and drying it in an oven at a certain temperature. Total solids refer to suspended and dissolved solids that are physically separated during filtration. Solid particles can be filtered as "suspended" or "dissolved.". The portion of a filter varies in its thickness, area, pore size, porosity, holder type, particle size, and amount of particles filtered (Baird *et al*., 2017).

The results of various doses and settling times were presented in Figure 6, with the highest total solid removal (47.01%) obtained by using 0.4% (keratin dosage) and 180 minutes (settling time). The lowest total solid removal (1.46%) was achieved using a 1% keratin dosage for about 30 minutes of settling time. Enhancing the coagulant dose leads to overdosing, which reduces the total solid removal. This condition stabilizes colloid particles in the ochre solution, preventing aggregation that increases turbidity. Additionally, a lower coagulant dose is inefficient for total solid removal, because insufficient coagulant promotes aggregation.

After 120 minutes, total solid removal slows down. However, greater removal can be achieved with longer settling time, allowing flocs to settle. This coagulation process is less efficient compared to extensively studied chitosan coagulants. As Abdullah and Jaeel (2019) reported, the coagulation results by chitosan on textile wastewater were significantly higher. Chitosan was able to reduce turbidity up to 99.78% in 15 minutes. The

acceleration of settling time also depends on floc size. The settling velocity will increase with the larger sediment grain size due to flocculation, and the residence time of the solution will decrease (Lee *et al.*, 2020). The coagulation efficiency over time improves due to the coagulant's ability to form larger flocs. Further studies on extracted keratin conditions, such as pH or modifications, can also analyze their impact on coagulation performance.

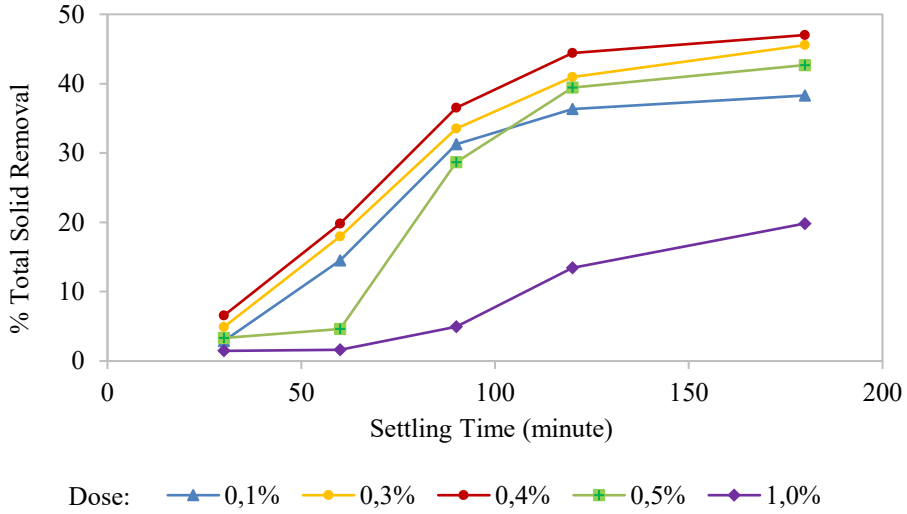

Figure 6. The curve that shows the relation between keratin dosages and settling times on total solid removal (%) in the ochre solution.

Effect of pH Condition on Total Solid Removal

The pH variations were performed to determine the optimal pH for coagulation. The pH solution condition has a very influential role in the coagulation performance. The highest total solid removal was obtained at pH 10 with the same keratin dose of about 0.4% and 180 minutes of settling time. The base condition helps the coagulation process. This condition affects the floc formation in the ochre solution because the solubility of the ochre solution decreases in a basic condition. From the data shown in Table 2, total solid removal between pH 9 and 10 was close. Unfortunately, the results show that keratin is still less effective for reducing the total solids in the ochre solution. However, surface modification of keratin can improve coagulation efficiency, as demonstrated by Zubair et al. (2022). Their study found that chicken feather keratin treated with graphene oxide can remove heavy metals from synthetic wastewater by up to 99%. Combining graphene oxide with keratin enhances its surface area and improves biosorption performance.

Table 2. The coagulation of ochre dye in a different pH condition using a keratin dosage of 0.4%.

pH	Initial Total Solid (mg/L)	Final Total Solid (mg/L)	% Total Solid Removal
8	3446	1980	44,63
9	3976	2106	47,01
10	4426	2340	47,13

Comparison of Keratin-Based Coagulant and Other Natural Coagulants

This study compares the efficacy of keratin with chitosan extracted from shrimp shells and commercially available chitosan for the reduction of synthetic pigments and textile wastewater, as illustrated in Table 3. This comparison shows that chitosan has greater coagulation ability, which might have been attributed to its chemical structure's effectiveness in binding waste particles. Nonetheless, chicken feather keratin may still have potential as an alternative coagulant, particularly in specific applications like total solids reduction. Clearly, optimization is required by considering other factors that may improve its effectiveness as a coagulant.

Table 3. Comparison of Keratin-Based Coagulant and Other Natural Coagulants for wastewater treatment.

Substance	Source	Extraction Method	Application	Percentage of Removal	Reference
Chitosan	Shells of marine shrimp	Deprotonation using sodium hydroxide	To remove methylene blue and reactive red	91.54% (methylene blue) and 82.74% (reactive red)	(Theerakarunwong and Boontong, 2020)
Chitosan	Commercial	-	To remove TSS and color from textile effluent	95% (TSS), 85% (color removal), and	(Asif *et al*., 2016)
Keratin	Chicken feathers	Hydrolysis extraction using sodium hydroxide	To reduce the total solids from the ochre dye	47,13%	This study

Impact and Future Research

Future research on keratin application in wastewater should focus on optimizing its application and understanding the mechanisms that could lead to innovation for wastewater problem-solving. Once the most effective conditions and methods are identified, the next step toward industrial-scale implementation involves developing a pilot-scale wastewater treatment system that integrates keratin. Further studies should be conducted, including economic and environmental feasibility analyses, compliance with regulatory standards, and fostering collaboration with industries and researchers to ensure successful adoption and scalability. Table 4 summarizes several studies investigating the application of keratin from different sources to reduce other pollutants, besides ochre dye, demonstrating that keratin is widely utilized in reducing heavy metals.

Table 4. Several studies of keratin applications for wastewater treatment.

Substance	Source	Extraction Method	Application	Percentage of Removal	Reference
Keratin	Chicken feathers	Hydrolysis extraction using sodium sulphate	To remove copper and zinc from synthetic water	52% (zinc) and 69% (copper)	(Amin *et al*., 2024)
Keratin	Human hairs	Treatment using hydrogen peroxide	Removing metals from aqueous effluent	86% for Cd (II), 92% for Cu(II), 96% for Pb(II), and 98% for Cr(III)	(Zhang *et al*., 2020)
Keratin	Chicken feathers	Reduction using Na_2S and modified by grafting keratin with 4-nitro-aniline	To remove the Pb^{2+} ion and the Cu^{2+} ion	40% (copper)	(Almoukayed and Barhoum, 2023)
Keratin	Raw wool	Treatment using HCl	To remove Cu (II) and Zn(II)	95.9% for Zn(II) and 94% for Cu(II)	(Simonič *et al*., 2022)
	Waste wool	Treatment using deionized water		34.4% for Zn(II) and 60.4% for Cu(II)	

CONCLUSION

This study about the utilization of keratin extracted from chicken feather waste on ochre solution concluded that the highest yield of keratin extraction, about 85.64%, can be obtained by using 1.75 N NaOH at 70 °C and 90 minutes of extraction time, with the most significant parameter that influences the yield of keratin being sodium hydroxide concentration. Total solid removal on the ochre solution using keratin was highest at a dose of 0.4%, pH 10, and 180 minutes of settling time. The highest removal was shown at pH 9, about 47.01% by using the same dose and settling time, showing that the alkali condition plays an impactful role in the coagulation process of the ochre solution. This condition reduces ochre solubility, indicating that ochre dye coagulation using chicken feather keratin remains less efficient, and research in this area is still limited. Therefore, further studies are still required to explore the other factors in keratin modification for optimal applications. To enhance

the research, adjusting pH conditions during keratin extraction and using alternative solvents like ionic liquids (e.g., Bmim[Cl]) could minimize degradation. Additionally, future research could focus on developing a kinetic model for keratin extraction.

SUPPLEMENTARY INFORMATION

Figure S1 is available in Supplementary Information (SI) and accessible at https://jurnal.uns.ac.id/alchemy/article/view/96879/supp.info.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

TMS: Conceptualization and Methodology; AN and SPP: Data Analysis and Manuscript Drafting; SWM: Manuscript Review and Editing.

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DECLARATION OF GENERATIVE AI

During the preparation of this work, the authors used DeepSeek-V3.1 Terminus in order to improve readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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