Preliminary study on preparing carboxymethyl cellulose–keratin biofilm

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Abstract. Keratin is a structural protein. Keratin can be obtained from chicken feathers. At present, chicken feathers are one of the great potentials of waste because of its abundance and cheap price. CMC has a similar structure to cellulose. Cellulose is very abundant in nature and its existence as waste and CMC is a derivative of natural cellulose polymers that are easily degraded. This paper reports our preliminary work on developing novel composite biofilm from both natural biopolymers to provide novel environmentally friendly biomaterials. Keratin solution was prepared from chicken feathers by the acid method. Variations in the number of CMCs were used to see differences in biofilm rigidity. The size of soluble keratin was measured using PSA, surface topography of biofilms was observed using SEM and FTIR was used to observe the presence of keratin embedded in biofilms. Based on the results of the PSA analysis, the size of soluble keratin was 1570.8 ± 26.30437 nm. FTIR analysis of CMC-keratin biofilm content showed an absorption peak in the area of 1240 cm⁻¹ - 1546 cm⁻¹ which the signature of an amide band which confirms the presence of keratin (protein) in the biofilm. SEM showed the surface structure of CMC-keratin biofilms forming a fibrous pattern. In this study, we concluded that CMC-keratin biofilm was generated, and it potentially can be developed further to generate environmentally friendly biomaterials.

1. Introduction

The chicken slaughterhouse industry produces large amounts of chicken feather waste. Based on the data from the Indonesian Central Bureau of Statistics in 2018, 2,144,013 tonnes of broiler meat were produced in Indonesia. The 10% of chicken weight is chicken feather, thus 214,401.3 tonnes of chicken feathers were produced [1]. Chicken feathers contain 90% keratin [2]. Keratin is a specific protein in chicken feathers, which as natural polymer, it has a great potential as a biodegradable material [3].

Recently, synthetic polymers have been widely used. Synthetic polymers lead to some major problems. First, synthetic polymers are derived from non-renewable petroleum sources. Secondly, synthetic polymers are non-biodegradable which causes accumulated waste. Recycling cannot fix this problem maximally because plastic consumption increased in environment. An alternative solution to fix this problem is the use natural polymer.

Keratin from chicken feathers can be used in various forms for biotechnological applications such as film and fibre constituents either as a single material or mixture with other natural or synthetic materials [4]. Besides, dissolved keratin is more often used as a source of biomaterials because it has biocompatibility, biodegradability and gas barrier properties which are better than polysaccharides and
liphids [5]. Keratin has \(\alpha\) - helix (\(\alpha\) - keratin) and \(\beta\) - sheet (\(\beta\) - keratin) structure which is stabilized by the presence of hydrogen bonds, disulphide bonds, and hydrophobic interactions which make it strong and difficult to dissolve in water [6,7,8,9]. The natural structure of keratin fibres can be changed so it became soluble in water. Soluble keratin has the potential as a composite biomaterial in the manufacture of biofilms.

Extraction of keratin has been done in various methods, such as chemical, enzymatic, and ionic solutions. The enzymatic method has the advantage of being environmentally friendly, but it takes a long time. Using acids for chemical method can reduce production time. Degradation of chicken feathers using the enzymatic method produced keratin fibre powder as a by-product [10]. It had a large size of keratin and was difficult to dissolve in water. This research is a preliminary study to develop a method for biofilm synthesis which latter could be applied in the development of keratin-based bioplastic.

Keratin has a strong protein structure but as a single material has brittle properties in forming film. Therefore, blending it with other natural polymer was needed to improve the mechanical properties of biofilms. Cellulose derivatives showed bioplastic forming characteristics with excellent mechanical properties such as transparent, flexible, resistant to oil and fat, and easily soluble in water solvents. Carboxymethyl cellulose/CMC is a derivative of natural cellulose polymers which abundant in nature [11]. The aim of this study was to generate CMC-keratin biofilm. The new generated biofilm is expected to improve the mechanical properties of keratin-based biofilms.

2. Materials and Method

2.1. Preparing CMC-keratin biofilm

Chicken feathers were dissolved in 1 M \(\text{H}_2\text{SO}_4\). The hydrolysis process of chicken feathers was done at 75°C for ± 6 days. Then the filtrate of a keratin solution was neutralized by adding \(\text{NaOH}\) 3M. CMC was dissolved in distilled water and stirred at 400 rpm at 60°C for 30 minutes. The solution was cooled at room temperature. Keratin solutions were dialyzed and stirred at 100 rpm for 4 hours prior to mix with CMC solution. Soluble keratin size was measured using Particle size analyser (PSA). Two keratin solution samples were analysed using Particle Size Analyzer (PSA) and it was named as LKS1 and LKS2. Keratin solution was then mixed with CMC at keratin/CMC ratio of 19.412/0; 19.412/75; 19.412/150; 19.412/225; 19.412/300; and 0/300 (w/w). For simplicity, the films were named as biofilm 1, 2, 3, 4, 5, and 6 for those biofilms, respectively. The mixture solution was homogenized by stirring it at 400 rpm at room temperature for 30 minutes. The mixture was then poured in a Petri dish and dried at 75°C for 15 hours.

2.2. Initial characterization of CMC-keratin biofilm

Fourier Transform Infrared Spectroscopy (FTIR) was used to analyse the functional groups in keratin film. The FTIR was performed in wavenumber range of 400 cm\(^{-1}\) to 4000 cm\(^{-1}\). Biofilm absorption spectrum was compared with reference to find out keratin and CMC in biofilm. Scanning Electron Microscopy (SEM) was done to obtain surface image of biofilm topography to picture the profile of protrusions, curves, and holes on the surface of the biofilm.

| Sample | Chicken Feathers Degradation (day) | Dissolved Mass (g) | Total Volume (mL) | Degraded feathers (mg/mL) | Protein Concentration (mg/mL) | Yield (%) |
|--------|------------------------------------|--------------------|-------------------|---------------------------|-----------------------------|-----------|
| LKS1*  | 6                                  | 9.330              | 292               | 31.952                    | 0.9704                      | 93.300    |
| LKS2   | 6                                  | 9.419              | 307               | 30.681                    | 0.7660                      | 94.190    |
| Average|                                    |                    |                   |                           |                             | 93.745    |
3. Results and Discussion

3.1. Biofilm production

The keratin solution was successfully produced from degraded chicken feather using sulfuric acid with 2 repetitions. Our trial showed the best result when ten grams of chicken feathers was dissolved in 200 mL of 1M H$_2$SO$_4$ for 6 days with an average yield percentage of 93.745% (Table 1).

Figure 1 shows that soluble keratin concentration increased as seen from colourless solution to dark brown during degradation process and some residue which remained undegraded was part of calamus feather. Keratin solution was neutralized at pH ± 7 with 3M NaOH to stop degradation process.

![Figure 1. Color Change in LKS1 Day 1 (a) and Day 6 (b)](image)

CMC-keratin biofilm was produced using LKS1 because it had the greatest protein content at 0.9706 mg/mL and concentration of chicken feathers at 14.297 mg/mL. Keratin solution must be dialysed to eliminate the salt content because as shown in Figure 2, salts crystallised on CMC-keratin biofilm’s surface.

![Figure 2. Keratin (a) Biofilm produced without dialysis, (b) Biofilm produced using dialysis process](image)

As shown in Figure 3 CMC-keratin biofilm had a yellowish-brown colour. Biofilm without keratin had white colour. The increased yellowness of the biofilm indicated in increased keratin content in biofilms [4, 5, 12, 13]. Biofilms are more resistant to high temperatures with the addition of CMC volume. This can be seen from cracking in biofilm surface. Biofilm 3 has more cracking surface than biofilm 5. Addition of CMC increased the thickness of biofilm. Blending keratin with CMC makes CMC-keratin based biofilms more flexible and less brittle [5]. Addition of cellulose and its derivatives in keratin forms strong interaction between hydrophilic group of amino acid residues and hydrophilic...
surface of cellulose thereby increasing tensile strength and mechanical properties of the biofilms [12, 14, 15]. Biofilm 1 was not formed because the protein concentration is too low so keratin cannot interact and bind to each other. Composite concentration is required to make good biofilm properties.

![Figure 3. CMC-keratin (a) biofilm 1; (b) biofilm 2; (c) biofilm 3; (d) biofilm 4; (e) biofilm 5; and CMC (f) biofilm 6](image)

3.2. **Determination of keratin particle size using particle size analyser (PSA)**

This study aimed to develop standard procedures in the manufacturing of CMC-keratin biofilm. In order to make biofilm from chicken feathers, keratin in chicken feathers should be degraded into smaller size. Table 2 shows the size of soluble keratin LKS1 and LKS2 samples were measured by reading data 5 times each sample and showed the average size at 1570.8 nm. Based on PSA data, degradation of chicken feathers using acid method can produce keratin in smaller size. That was indicated keratin content in biofilms has size at about 1.5 µm.

| Number measurement | LKS1 (nm) | LKS2 (nm) |
|--------------------|-----------|-----------|
| 1                   | 1648      | 1567      |
| 2                   | 1759      | 1763      |
| 3                   | 1529      | 1580      |
| 4                   | 1554      | 1373      |
| 5                   | 1457      | 1478      |
3.3. **FT-IR analysis of CMC-keratin biofilms**

FTIR was done to observed CMC and keratin in biofilms. Five biofilm samples (biofilm 2, 3, 4, 5, and 6) were tested and the FTIR spectrum is shown in Figure 4. Biofilm 6 containing CMC gives an absorption peak that widens in area 2841.5 cm\(^{-1}\)-3531.66 cm\(^{-1}\). The absorption peak in area 3200 cm\(^{-1}\)-3600 cm\(^{-1}\) indicates the presence of O-H vibration while the absorption peak in area 2800 cm\(^{-1}\)-2900 cm\(^{-1}\) shows the presence of C-H strain from the CH\(_2\) and CH\(_3\) groups in CMC structure [16][17]. Absorption peaks in area 1050 cm\(^{-1}\)-1300 cm\(^{-1}\) showed a primary strain of C-O alcohol [18]. Based on the results of FTIR biofilm 6 contained CMC because it showed absorption peaks of O-H, C-H, and C-O bonds in CMC structure.

![Figure 4. FTIR result from biofilm 2, 3, 4, 5, and 6](image)

3.4. **SEM analysis of CMC-keratin-based biofilms**

The difference in the surface structure of CMC-keratin biofilms with CMC biofilms can be seen in Figure 5. The presence of keratin makes a fibrous pattern on biofilm surface. The size of fibrous pattern was 1.165 µm. Heterogenous fibrous pattern in CMC-keratin biofilm is caused by low protein concentration. Besides, there are still CMC particles that have not completely dissolved with a size at 2.189 µm on CMC biofilm surface. Increasing the concentration of keratin protein in biofilm mixture can possibly make CMC-keratin biofilms with homogeneous fibrous pattern. Based on SEM data, the addition of keratin can increase the interaction between bio-composite materials to produce better biofilms.
4. Conclusions
In this study, CMC-keratin biofilms were successfully synthesized. Keratin solution was produced through the acid method. Degradation chicken feathers using acid method can produce micro-sized keratin. However, nano keratin has better properties, thus we need more efficient degradation process. Enzymatic methods can be tried to get smaller size and higher concentrations of keratin. The addition of CMC into keratin proved to increase rigidity and flexibility. This may occur because CMC as a matrix can glue and transfers stresses between keratin fibres in biofilms. Based on FTIR data, biofilms have polypeptide peak shows that keratin incorporated into CMC matrix. CMC-keratin biofilms have better transparency properties than CMC biofilm. Presence of CMC can increase rigidity, while keratin can improve film-forming ability of biofilm. Mechanical tests are needed to determine the mechanical properties of biofilms. Future studies will be done including the chemical modification,
different matrix materials, and addition of plasticizer to improve the mechanical properties of keratin-based biofilms.

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6. References
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