

Production of Environmentally Bioplastics As Packaging Materials For Teaching Factory Products at State Polytechnic of Jember

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Production of Environmentally Bioplastics As Packaging Materials For Teaching Factory Products at State Polytechnic of Jember

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Abstract. Plastic waste ranks second in the environmental pollution in 35 major cities in Indonesia. Currently, one of the policies that are the main focus of research is green technology (go green) and green economy, with an emphasis on low carbon accompanied by indicators of pollution reduction as the performance target of activities. Following up on this policy, research was conducted to replace plastics with bioplastics using the main raw material of rice straw which had been dignified with 30% ethanol for 2 hours at 75°C which was then added with glycerol, CMC, and chitosan with certain formulations as well as a fermentation process using *L. plantarum*. The determination of product characterization is using SEM, XRD Spectra, tensile test, and biodegradation. The research results will be used as packaging material for Polije' s teaching factory products. Preliminary research results indicate that the greater the concentration of chitosan used, the smaller the swelling percentage and can inhibit bioplastic damage, but the resulting product becomes stiff. Products made with formulations determined based on observations using SEM can produce a fairly even morphology of bioplastics with a starch:cellulose ratio of 1:1.5 resulting in a tensile test of 3.47 Mpa and a strain of 4.8%. The product has been tested to be degraded by 95% within 11 days. Products made with formulations determined based on observations using SEM can produce a fairly even morphology of bioplastics with a starch:cellulose ratio of 1:1.5 resulting in a tensile test of 3.47 Mpa and a strain of 4.8%. The product has been tested to be degraded by 95% within 11 days. Products made with formulations determined based on observations using SEM can produce a fairly even morphology of bioplastics with a starch:cellulose ratio of 1:1.5 resulting in a tensile test of 3.47 Mpa and a strain of 4.8%. The product has been tested to be degraded by 95% within 11 days.

1. Introduction

River pollution due to plastic waste is a national and urgent issue, a clear example of which can be seen in the Bedadung River in Jember. As a result of the pandemic, there was an increase in the use of plastic by 17% compared to last year, including an increase in online sales turnover. The latest data shows that plastic waste in 35 big cities is ranked second in environmental pollution with an estimated waste of 5.4 million tons per year. This is very concerning, considering that the existence of plastic in nature is a material that is not easily biodegradable [1]. On the other hand, the main focus of current research policies is green technology (go green) and green economy, with an emphasis on low carbon with indicators of pollution growth from all aspects being significantly reduced.

Many pieces of research related to plastic substitutes, namely go green packaging, have been carried out, including those initiated from previous research by the team leader, by examining the use of cellulose from straw as waste, for recycled paper materials. The results of the research involve the innovative use of *L. plantarum* with the treatment of active compounds from soursop to produce paper packaging from cellulose waste that is environmentally friendly but termite-resistant [2]. The use of cellulose has been studied as a potential bioplastic material. After all, it is thermoplastic with a



potential source being straw because it contains 37.71% cellulose [3]. Jenggawah Village, located in Jember Regency, has an area of 3,593 ha of rice fields, with a by-product of 7.5 to 8 tons of straw per ha per planting season (3 months). The total available straw in Jenggawah Village is about 28,744 tons (per 3 months) or 86,232 tons per year and only 45% is absorbed for mulch (fertilizer) or fodder, while the rest is usually burned [2]. Thus, the potential for bioplastic development in Jember is very open and feasible.

The process of making bioplastics generally uses cross-linking engineering, acetylation, and increasing amylose content with sodium acetate [4]. The technique used to modify thermoplastic starch to improve mechanical properties and sensitivity to water is a modification with ascorbic acid and citric acid [5]. Until now, it is still very rare for the process of making bioplastics that involves bacteria in the fermentation process, almost all methods are using plasticizers.

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2. Materials and Methods

2.1 Materials and tools

16 The tools used in this activity are a fermenter (local design in Tefa Rotografur), measuring cup (800 mL, 500 mL, 250 mL, 200 mL, 100 mL, 50 mL, and 10 mL), spatula, thermometer, digital pH-meter, digital balance, desiccator, petri dish, filter paper, glass pipette, drying oven, porcelain crucible, blender, magnetic stirrer, sieve (sieve) size 63 μ m, mold, zip bag lock, ultrasonic water-bath.

The materials used were straw, Sodium Hydroxide (NaOH) 40%, Hydrogen Peroxide (H₂O₂) 6%, Aquades, Sorghum Starch, Cellulose 10%, Glycerol 25%, and chemicals for analysis [11] as well as *L. plantarum* culture which was propagated first, previously used the Wibisono method [2].

2.2 Research Design

a. Material Preparation

The material in the form of straw was cleaned and dignified with ethanol (30% v/v) and heated at 80oC for 1 hour. The lignin removal process was carried out by 23xing it with a 40% concentration of NaOH solution (10% of the raw material) and extracted using a hot plate at a temperature of 100oC for 3 hours while stirring. Next, the extraction results were filtered. The residue obtained was then washed with water to pH 11 [7].

b. Cellulose Extract

The results of the fermentation were then added with a 6% concentrat2n of H₂O₂ solution and left at room temperature for 3 hours. Furthermore, filtering was carried out and the r20due obtained was washed with water until the pH of the washing water was 7. The residue obtained was then dried in an oven at 105oC until a constant weight was obtained. After the residue is dry, then milling is carried out, then sieved with a sieve size of 60 mesh [6].

c. Bioplastic Synthesis

This procedure uses a synthesis technique with modifications [9]. The above material was made a solution by sonication for 40 minutes, then added glycerol according to the treatment (ie 3%, 4%, and 5%) in the solution and sonicated for 60 minutes. Bioplastics are made by soaking cellulose in 10% acetic acid for 1 hour (temperature 80oC) and drying it in an oven. The final product was added with starch (1:2 and 1:1.5) and sorbitol as a plasticizer, as much as 20% [10]. Before being heated for 10 minutes (temperature 85oC), a fermentation process was carried out with *L. plantarum* for 8 hours for the production of nisin. After that, the bioplastic solution was removed and heated at a temperature and speed of T gelatinization = 95 0C. After 35 minutes, the solution is poured into a mold th10 has been coated with wax, then placed in a drying oven to be dried at a temperature of 600C for 8 hours. After drying in the oven, the mold was removed and put into a desiccator (conditioned for 24 hours). These steps were repeated for variations according to treatment, namely the mass ratio of CMC to chitosan, namely 6: 4; and 7 : 3

2. Testing

a. Tensile Strength and Elongation at Break

The mechanical properties of tensile strength and elongation at break were tested with ASTM (American Standard Testing and Material) [14] and carried out in Surabaya

b. Mechanical Properties Testing

This test shows different values of tensile strength, percent elongation, and Young's modulus that are different for each bioplastic. Plastic film samples were tested using an autograph tool. The test was carried out at a temperature of 23°C, the humidity of 50%, and a cross-head speed of 20 mm.min⁻¹ [8]

c. Density test

The mass of the sample to be tested is weighed using a digital balance. Then the 10 ml measuring cup was filled with water up to 5 ml and the plastic sample was put in a measuring cup filled with water. After 15 minutes, the new water volume (v) is recorded to calculate the actual plastic volume by calculating the difference between the final volume of water and the initial volume of water [9]

d. Electron Microscopy Scanning Test

The sample is cut in size 1 x 1 cm to be attached to the holder (sample container to be tested), then inserted into the chamber to be fired electrons later, but before that the situation in the chamber must first be vacuumed. Finally, the sample is fired electrons to determine the morphology of the bioplastic film. XRD test was conducted to determine the crystallinity of straw before and after treatment.

e. Microbial Analysis

Selected colonies were taken from the medium for MRS and put in a 1.5 ml tube while mashed and added distilled water (DW). Centrifugation was done at a speed of 12,000 rpm and discarded the supernatant. 200 µl InstaGene Matrix was added to the tube containing pellets, then incubated at 56°C for 30 minutes, vortex at high speed for 10 seconds, incubated again at 100°C for 10 minutes, vortex back for 10 seconds, and lastly centrifuged with a speed of 12,000 rpm for 3 minutes. The next step is as follows:

1. A total of 560 µl of the AVL buffer containing the carrier RNA was piped into a 1.5 ml microcentrifuge tube.
2. 140 µl supernatant was added to the microcentrifuge tube above and vortex for 15 seconds. Homogenization is necessary for lysis efficiency.
3. Incubation at a temperature of 15-25°C for 10 minutes. The process of lysis of viral particles will be completed in about 10 minutes if given a longer time and will not affect the results or quality of RNA. Virulent agents carrying the infection will be activated by administering an AVL buffer.
4. A total of 560 µl of ethanol (96 – 100%) was added to the sample and vortexed for 15 seconds.
5. Carefully, 630 µl of a solution is added from the previous step to the QIAamp Mini Spin column (in 2 ml of collection tube) without wetting the surrounding edges. The tube is closed and centrifuged at 6000xg (8000 rpm) for 1 minute.
6. QIAamp mini spin columns are opened and added with 500 µl of the AW1 buffer. The plug is closed and centrifuged at 6000xg (8000 rpm) for 1 minute. The QIAamp Mini Spin column is placed on a 2 ml collection tube, and the filtrate contained in the tube is discarded. The use of AW1 buffer does not need to increase the amount of volume if the volume of the solution (sample) is greater, in contrast to the AVL buffer whose volume must be given proportional to the solution (sample) given.
7. The QIAamp Mini Spin column is opened and added with 500 µl of the AW2 buffer. The plug is closed and centrifuged at full speed 20000xg (14000 rpm) for 3 minutes.

8. The QiAamp Mini Spin column is placed on a 1.5 ml micro centrifuge tube. Tubes containing filtrate are discarded and carefully open the QIAamp Spin column to be added with 60 l of the AVE buffer at room temperature conditions.

3. Result and Discussion

3.1. Bacterial Propagation Results

L. plantarum bacteria have been propagated by the Wibisono method [2] and have been re-sequenced by PCR following the Intron Biotechnology procedure (catalog no. 25027) and produced 820 sequences (using Primer 283F 5' – GAG AGT TTG ATC CTG GCT CAG GAC – 3' and Primary 261R 5' – AAA GGA GGT GAT CCA GCC GC – 3') which is shown in Figure 1. After blasting at NCBI, the bacteria confirmed that the *L. plantarum* 15420 strain that had been studied could produce heat-resistant nisin as anti-bacterial. *L. plantarum* 15420 have similar properties with *L. casei* which encoded for the genes LC2W_0247, LC2W_0909, LC2W_0925, LC2W_2007, LC2W_2678, and 174 base pairs up to 75% similarity with *L. jensenii* encoded with the genes LACJE0001_1464 and HMPREF0886_1035.

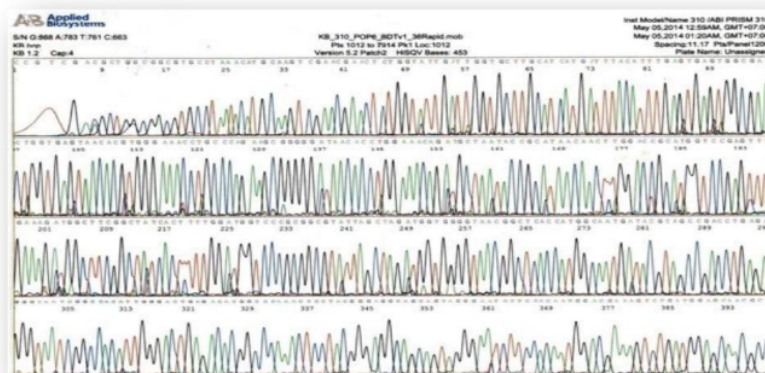


Figure 1. Electropherogram of *L. plantarum* 15420

3.2 Delignification

The results of the delignification showed that the process carried out increased the cellulose content to 47.18% (up 34.28%) and decreased lignin to 12.74% (down 18.75%) using 30% ethanol so that finally lignin can be easily separated from the fiber. Ethanol of more than 15% is known to damage the lignin structure of lignocellulosic as well as encourage the change of lignocellulose into a fiber with high cellulose and the results of this study following the results of previous research [11].

Preliminary research conducted by the team (unpublished) stated that the 35% ethanol used in the initial study reduced the prospect of delignification, presumably due to lignin precipitation, so the main study used 30% ethanol. The use of ethanol below 35% is often used as a reference by several studies, due to the role of water content in addition to ethanol which is dissolving and initiating lignin cleavage. The results of the SEM photos from the micrographs of delignification with 30% ethanol are presented below.

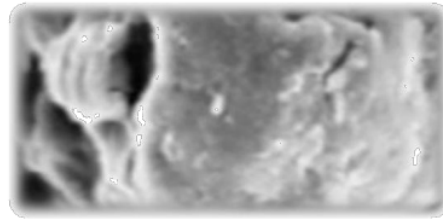


Figure 2. Morphology of Delignification Results with 30% Ethanol

The SEM results above show that the straw structure becomes coarser and breaks after the delignification process. The results of this research are similar to similar studies which state that the mechanism of ethanol damages the structure and dissolves lignin and hemicellulose, which increases cellulose. Further test results with XRD spectra in the following figure show that the highest peak after delignification is in the 2θ (39.60) area. Furthermore, for the calculation of percent critalin, the value of 58.23% is obtained which is the result of a decrease in lignin content and confirmed by experts as an amorphous cellulose component [6] [9].

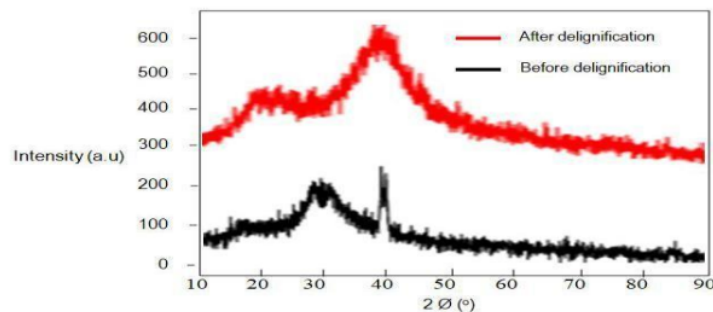


Figure 3. Results of XRD Spectra from Delignification with 15% Ethanol

3.3 Bioplastic Characteristics

In Table 1 below it can be seen that the highest stress value was obtained from the use of starch to cellulose ratio = 1:1.5; with the average stress value (based on 3 repetitions) of 3,470 MPa and the average percentage strain (based on 3 repetitions) of 4.8%. The cellulose content in the starch:cellulose ratio = 1:2 causes the bioplastic to be relatively more "brittle" compared to the starch:cellulose ratio = 1:1.5. The following is Table 1 for the final value of the tensile test

Table 1. Final Tensile Test Value

Starch Ratio: Cellulose	Voltage (Mpa)	Strain (%)
1: 2	2,148	3.6
1: 1.5	3,470	4.8

SEM results of bioplastics are shown in the following figure.



Figure 4. Bioplastic Morphology (5000x Magnification)

In Figure 4, it can be seen that the surface of the bioplastic is uneven and it can be seen that the fiber area is separated from the starch matrix. The assumption given from the results of the image above is that the fiber bond with the matrix is weak so the mechanical properties of bioplastics tend to be weak. This is due to the ratio of starch to cellulose which is prepared in a ratio of 1:1.5, it is suspected that it has not been able to homogenize at the molecular level between the two, resulting in a weakening of the interfacial bond.

The results of testing the level of degradation of bioplastics showed that the highest percentage of mass loss was recorded on day 11 with a degradation percentage of 95%, then there was a gradual decrease in overhaul from day 12 to day 4.20 (degradation of 99%). Different results were shown by other researchers [6] [9] which stated that the average maximum degradation produced on day 10 was 99%. This difference is thought to be due to the presence of nisin in the bioplastic produced by Polije (from the activity of *L. plantarum* bacteria) so that it can survive (and prevent protected products) from being damaged due to microbial activity during storage and when used as food packaging. But on the other hand, these advantages become one of the drawbacks, namely extending the time required for the degradation/decomposition of starch and cellulose polymers from microbial activity in the soil.

4. Conclusions

The bioplastic produced from the research has a stress of 3,470 Mpa and a strain of 4.8%, with delignification using 30% ethanol. Nisin produced by *L. plantarum* 15420 can maintain bioplastic damage due to microbial activity during storage and when used as food packaging, but on the other hand, it provides a drawback that it requires a degradation time of 95% on the 11th day and 99% on the 20th day of decomposition. In the future, regulations and policies are needed to ensure the use of bioplastics as a substitute for conventional plastics.

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