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Preparation and characterization of lignin nanoparticles from rice straw after biosynthesis using *Lactobacillus bulgaricus*

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Abstract. Lignin is the most abundant aromatic natural polymer and comprises about 25% of straw biomass. Nanolignin biosynthetic production method is a simple method and safely better than chemical or physical methods. It has an interest in using lignin in more advanced applications. In particular, lignin-based nanoparticles could find potential application use in functional surface coatings, nano glue, drug delivery, microfluidic devices, and food additive. This study aimed to optimize *Lactobacillus bulgaricus* in nano lignin synthesis, the effect of the incubation period and freeze-drying on the quality of the nanolignin. Lignin particles were biosynthesized using rice straw and *Lactobacillus bulgaricus* in a dark place with a temperature of 37°C for 24 hours, 48 hours, and 72 hours. Lignin nanoparticle was characterized using Fourier Transformer Infrared Spectroscopy (FTIR), Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX). The results indicated that nano lignin has a spherical and amorphous shape. The average size of particles is 101.6 nm with an incubation period of 24 hours, 57.2 nm with an incubation period of 48 hours, and 276.9 nm with an incubation period of 72 hours. The incubation periods affect the size and shape of nanolignin and also show that the lignin chemical structure is within the nanoparticle formation process. Samples using freeze-drying enable have natural antibacterial compounds and have phenolic fragments containing recommended for nano preservatives.

1. Introduction

The development of nanotechnology is growing rapidly in various fields including environment, agriculture, beverages, and health. In nanotechnology, particles of matter which have sizes between 1-100 nm were defined as Nano. This particle has several chemicals, biological and physical properties that are superior to larger material [1].

Indonesia is the agricultural country with a rice farming area of 1,102,863.00 Ha located in East Java Province resulting in abundant rice straw waste and still not yet fully utilized optimally. Based on the government data, the amount of rice straw waste reaches around 20 million tons per year and the production of paddy fields can reach 12-15 tons of straw per harvest per hectare. Straw can be used as a natural preservative with the content of lignin in the straw through isolation and synthesis into lignin nanoparticle. So far, nano lignin is obtained from the process of isolation and used commercially for carbon fiber, adhesives, polyurethanes, polyesters, bioplastics, and bio-oil for petroleum blends from fossils [2]. However, this process is relatively more expensive, requires a long period and not safe for



health. Therefore it requires a cheap and safe method for nano lignin synthesis which is safe for human health and the environment.

In this study, the synthesis method used based on the microorganisms approach. These microorganisms, *Lactobacillus bulgaricus*, are probiotic bacteria and non-pathogenic, more safely in the environment. Also, *Lactobacillus bulgaricus* has a fast and optimal growth period at 12-18 hours that increases time efficiency lignin nanoparticle production [3]. In other studies, lignin nanoparticle as a natural preservative can inhibit bacterial growth [4].

Rice straw is one of the lignin organic resources obtained from agricultural residues. We consider rice straws a good source of lignin. It can be an important source of natural antibacterial compounds and can use as additive food. Thus, it is important to change macro-sized particles to a nano-sized particle as innovation in this study.

This study was done based on a different period of incubation between substrate and bacteria. The characterizations were done by using Fourier Transformer Infrared Spectroscopy (FTIR), Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX).

2. Material and methods

2.1. Lignin source and biosynthetic agent

A total of 7,5 g lignin was obtained from Malang, East Java, Indonesia as the natural resource of lignin nanoparticle. It sterilized using autoclave and mixed with *Lactobacillus bulgaricus*. The results were analyzed using FTIR, PSA, SEM, and EDX. The observations conducted in the Laboratory of biology and Halal Central Islamic of University Malang, Institut Biosains Brawijaya University, Laboratory of molecular biology and Chemistry Brawijaya University and Maulana Malik Ibrahim Islamic State University Malang.

2.2. *Lactobacillus bulgaricus* culture methods

Lactobacillus bulgaricus obtained from Laboratory of microbiology Airlangga University, Surabaya. It was cultured in Instant Nutrient Agar (NA) 20 g/L aqua dest and Instant Nutrient Broth (NB) 8 g/L aqua dest with aseptic technique. It is dissolved in distilled water and sterilized by autoclave at 121°C, 1 atm, for 15 minutes. Bacteria were cultured in Instant NA and incubated for 24 hours at room temperature then they were cultured in Instant NB and incubated for 48 hours in water bath shaker.

2.3. Rejuvenation *lactobacillus bulgaricus* isolates

Rejuvenation was done by transferring the bacteria into a petri dish containing instant NA and then incubated at room temperature for 18 hours. The bacteria were grown in the instant NB and inoculated to the Erlenmeyer flask which contained sterile instant NB with added lactose. Erlenmeyer flask was sealed with plastic wrap and incubated at 37°C for 24 hours in a shaking incubator at a speed of 150 rpm. The separation of bacterias and media were using centrifugation at 37°C, 8000 rpm for 10 minutes. The pellet was washed with sterile distilled water twice using centrifugation at 37°C, 8000 rpm for 5 minutes to remove the residual media.

2.4. Lignin nanoparticle biosynthesis

Lignin nanoparticle biosynthesis as reported on Rahmawati, *et al* [5]. A total of 1.5 grams of rice straw was sterilized by autoclave at 121°C, 1 atm for 15 minutes. Then it was mixed with 5 g *Lactobacillus bulgaricus* in culture bottle covered by aluminum foil, then kept it in the water bath shaker at 37°C for 24 hours, 48 hours, and 72 hours. After the incubation, the suspension was filtered. The liquid substrate was dried by freeze drier and analyzed by PSA, FTIR, SEM, and EDX.

3. Result and discussion

The particle size distribution of lignin particle mentioned as following:

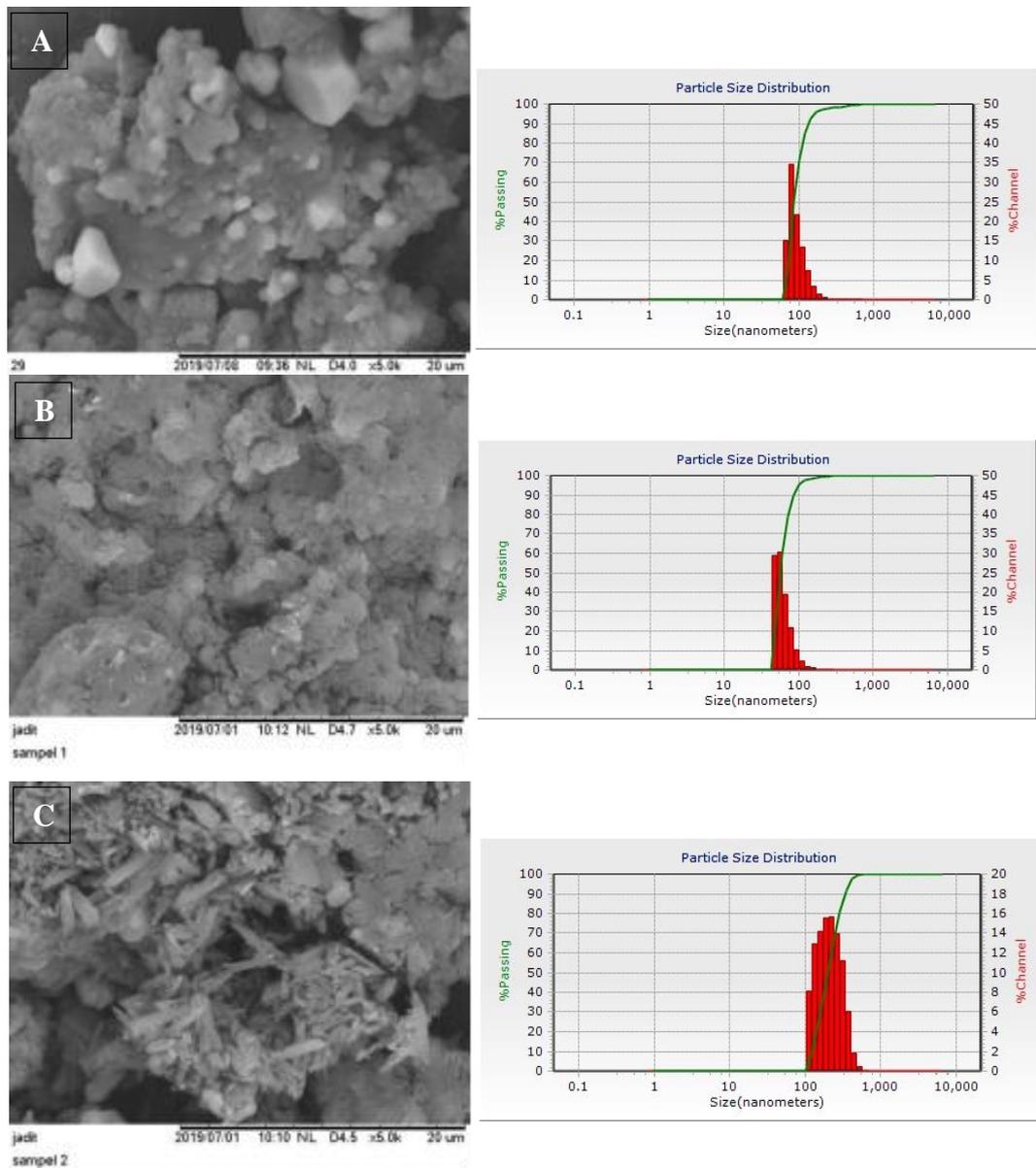


Figure 1. SEM image (left side) and PSA Graphic (right side) of the freeze-drying sample at time incubation of (a) 24 h (b) 48 h and (c) 72 h.

Figure 1 demonstrates the particle size distribution of lignin nanoparticles, as a function of the nanoparticles percentage. The lignin nanoparticle size at 24, 48, 72 hours length incubated was estimated to be between 72.3 - 6540 nm, 51.1 - 486 nm, 121.5 - 687 nm, respectively. Azimvand, *et al.*[6] estimated the average diameter of lignin nanoparticles to be 52.7 nm. To study morphology and approximate particle size of lignin nanoparticle, a scanning electron microscope was used. Figure 1 illustrates lignin nanoparticle morphologies also within incubation. Lignin nanoparticle at 24 hours length incubated had homogeneous and interconnected particles, relatively uniform and flat. Lignin nanoparticle, on the other hand, had a highly porous surface.

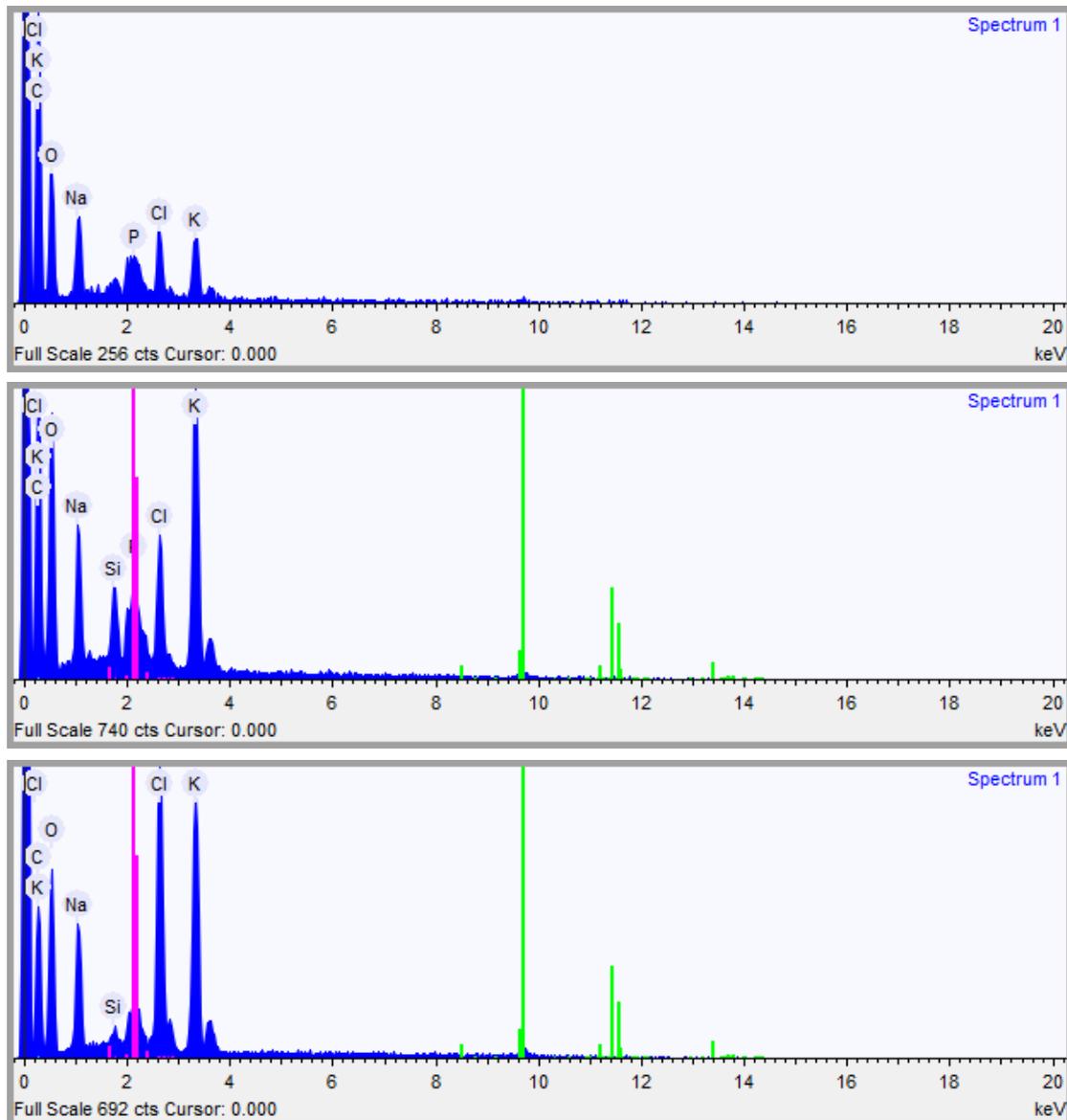


Figure 2. EDX measurement of freeze-drying sample in incubated time (a) 24 h : (b) 48 h : (c) 72 h.

Energy-dispersive X-ray spectrometer (EDX) data provide definite information regarding the elements present in the samples. Figure 2 shows the EDX pattern of lignin nanoparticle, respectively. The chemical composition of lignin nanoparticle is the same as indicated by the presence of similar signals (C and O) in their EDX spectra, respectively. The peak due to Si indicates the presence of Si along with lignin nanoparticle in incubated 48 h and 72 h. Among the three treatments, the sample is incubated 48 h of the elements formed was more than the others. The FTIR spectra and EDX analysis clearly showed that biosynthesis treatment leads to adsorption of the elements C, O, Cl, K, Na, Si, P as indicated by the observation of corresponding peaks in the spectra/graphs.

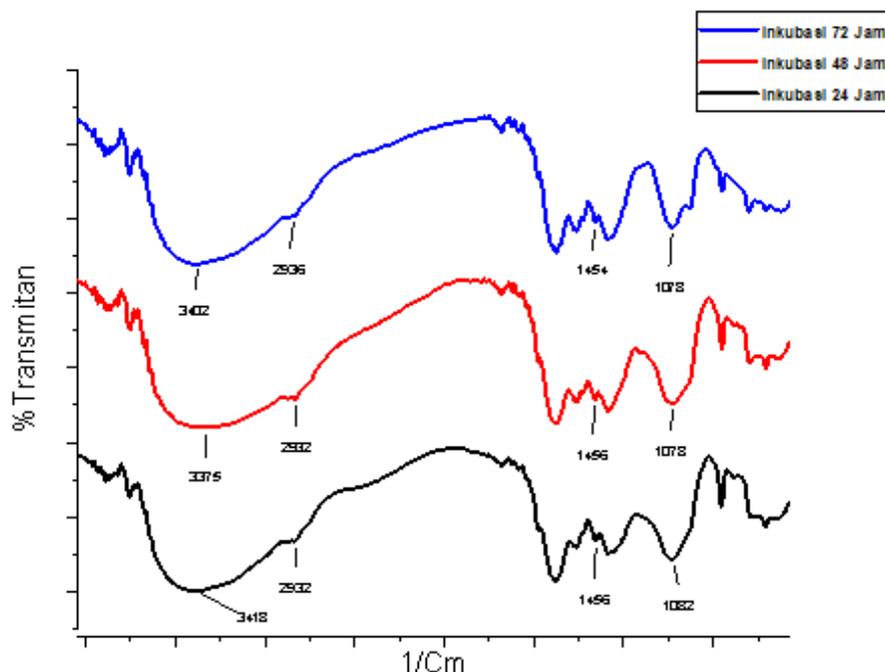


Figure 3. FTIR spectra of freeze-drying sample in incubated time (a) 24 h : (b) 48 h : (c) 72 h.

Figure 3 shows the FTIR spectra of the freeze-drying sample in incubated time (a) 24 h (b) 48 h (c) 72 h, showing that the lignin chemical structure within the nanoparticle reaction process. Suspension sample of rice straw and *Lactobacillus bulgaricus* that have been freeze-drying shows that the incubated sample along 24 hours, 48 hours, and 72 hours found aromatic and aliphatic parts, after being tested by FTIR. Large broadband at 3500–3100 cm^{-1} is assigned to OH stretching vibrations. This band is caused by the presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds [6], while lignin is shown a strong intense band at 2935–2931 cm^{-1} is associated with Alkana, aldehyd stretch [7]. Absorption bands caused by stretching of Aromatic ring are located in the 1615–1580 cm^{-1} , while lignin is shown a weak intense band at 1095–1075/1055–1020 cm^{-1} is associated with the asymmetric stretching vibration of organic siloxane bond (Si–O–Si) [8].

We found that Lignin Nanoparticles can be used as bactericidal in some studies. Lignin contains different phenolic monomer fragments and is an important source of natural antibacterial compounds. Phenolic fragments containing a double bond in α , β positions of the side chain and a methyl group in the γ position show, in general, the most inhibitory effect [9]. Lignin from different sources showed successful decontamination properties against various pathogenic microorganisms [10]. The polyphenolic compounds of lignin cause cell membrane damage and lysis of bacteria with subsequent release of cell content [9].

The antibacterial activity of LNPs incorporated in polymer films for food packaging was evaluated by Yang *et al.* [4][11]. Nanocomposites containing LNPs were evaluated against *Xanthomonas arboricola* and *Pectobacterium carotovorum* bacterial plant pathogens [11]. Both bacteria can damage fresh fruits and vegetables in open fields and after harvesting. The results of the investigation that All LNP-incorporated polymer blends showed lower colony-forming unit (CFU) concentrations within the first 3 h of immersion in the nutrient broth. After 24 h, the *Xanthomonas arboricola* PV. pruni and *Pseudomonas syringae* PV. tomato concentrations in the tests with incorporated LNPs were significantly lower compared to the neat polymers. However, increasing CFU concentrations were observed in each sample between 3 h and 24 h but remained at lower CFU concentrations compared to

the neat polymers [4][11]. There was possible that lignin nanoparticle can be used as additive food for example natural preservatives.

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