The 2014 World Congress on
Advances in Civil, Environmental, and Materials Research (ACEM14)
Busan, Korea, August 24-28, 2014

Antimicrobial Property and Biodegradability of Lignin Nanofibers

*Eunsil Lee[1)], Youjung Song[1)], Seungsin Lee[2)]

[1),2)]Department of Clothing and Textiles, Yonsei University, Seoul 120-749, Korea
[2)]sl158@yonsei.ac.kr

ABSTRACT

Lignin is one of the most abundant natural polymers in the biosphere, second to cellulose; yet, it is under-utilized and seen just as a waste. Previously, we successfully fabricated lignin nanofiberswithelectrospinning and investigated lignin as a new fiber material. In this study, we examine an insolubilization method for lignin nanofibers and evaluate if the inherent functionalities of lignin such as its antimicrobial property remain after being transformed into nanofibers. The biodegradability of lignin nanofibers was also investigated. The insolubilization was achieved by combining several mechanical cross-linking techniques. For the antimicrobial test, lignin nanofiber webs containing 85 wt% lignin with a 3.0 g/m^{2}web area density exhibited different antimicrobial properties depending on the type of bacteria; a 99.9% bacterial reduction against *Staphylococcusaureus*, but no reduction for *Escherichia coli*. We examined the enzymatic degradation of lignin nanofibers with laccase over ten days. The average weight loss ratios of lignin nanofibers containing 85 wt% lignin and 50 wt% lignin were 36.3% and 35%, respectively, whereas that of the 100 wt% PVA samples was 0%.

1. INTRODUCTION

Environmental concerns along with the depletion of fossil fuels have motivated numerous investigations into the potential of biomass as an alternative to petroleum resources. Natural polymers, such as carbohydrate polymers and animal protein-based polymers, are well-known examples of renewable biomass-based polymeric materials. Second to cellulose,lignin is one of the most abundant natural polymers in the biosphere and it possesses antibacterial, antioxidant, and biodegradable properties; yet, it is currently under-utilized and considered only as a byproduct in biorefinery operations. Recently, however, lignin has received new attention as a renewable resource, and considerable efforts are being made to develop more valuable applications of waste lignin (Thakur 2014). In a previous study, we successfully fabricated lignin nanofibers through electrospinning and investigated lignin as a new textile material (Lee 2014). However, when considering the properties of the final product,lignin nanofibers made with water soluble polymers such as alkali lignin with low sulfone content and PVA require a certain treatment in order to increase their

---

[1)]Graduate student
[2)]Professor

stability in the presence of water. A few mechanical and chemical insolubilization methods for lignin and PVA, respectively, have been reported, but few existing studieshave reported those for lignin/PVA nanofibers. In this study, we report a novel approach to insolubilizeelectrospun lignin nanofibers by combining several mechanical cross-linking treatments, with a view to investigating further applications of lignin nanofibers. We also evaluate the antimicrobial property and biodegradability thatare retainedafter the transformationof the lignin into nanofibers, asthese functionalities of lignin have not been exploited in the fiber and textile fields although they are being utilized in the food and cosmetic industries.

2. EXPERIMENTAL

Lignin (low sulfonate content, Mw=~10,000, Sigma Aldrich Co., USA) was used as received and poly(vinyl alcohol) (PVA) (>99% hydrolyzed, Mw=89,000-98,000, Sigma Aldrich Co., USA) was used as a carrier polymer to facilitate the fiber formation of lignin. Distilled water was used as a solvent.All the procedures for preparing lignin and PVA hybrid solutions as well asfor the electrospinning of lignin nanofibers have been described in our previous study (Lee 2014). The concentration of all PVA precursor solutions in this study was fixed at 5 wt%. The ratios of lignin to PVA were set at 85:15 for the insolubilizationand antimicrobial test, and at 85:15,50:50, and 0:100 (pristine PVA) forthe biodegradability test. The prepared solutions were kept at room temperature for 24 hours and were then electrospun on substrate material (polyester nonwoven)in the optimum processing conditions determined by our previous study (Lee 2014), i.e., with a voltage of 25kV at a working distance of 15 cm and a feed rate of 0.4 ml/hr through a 25 gauge needle.

To insolubilize the lignin nanofibers made from water-soluble polymers such asalkali lignin with low sulfone content and PVA, crystallization was induced by combining several mechanical cross-linking techniques. Water vapor treatment, photo-crosslinking, and heat treatment were selected on the basis of previous research (Min 2006; Giebel 2012; Peresin 2012). Those methods were applied with different combinations of sequences and conditions. In order to investigate whether the insolubilization stabilized the lignin nanofibers against dissolution in water, the lignin nanofiber webs that had been treated with different combinations of insolubilization techniques wereimmersed in water at 18°C for 1 hr.The fiber morphology was then examined using a field-emission scanning electron microscope (FE-SEM)(JSM 6701-F, JEOL Ltd., Japan). In addition, the lignin nanofibers were observed using an X-ray diffraction (XRD)(Ultima IV, RIGAKU, Japan) in order to investigate the change in crystallinity before and after the insolubilization.The presence of lignin in the nanofiber web was confirmed by locating sulfone using an energy dispersive X-ray spectroscopy (EDX).

The antimicrobial properties of the lignin nanofibers were examined quantitatively in accordance with the ASTM E 2149-10 (Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions) against two representative microorganisms, *Staphylococcusaureus* (ATCC 6538, Gram-positive bacterium) and *Escherichia coli* (ATCC 25922, Gram-negative

bacterium). Lignin nanofibersof a 3.0 g/m^2 web area density were prepared to evaluate the antimicrobial activities, and the insolubilization treatment was conducted before the evaluation. The specimenswere immersed into an inoculated buffer solution in a flask and agitated using a shaker. The reduction owing to the specimenwas then calculated with the equation below:

$$\mathrm{R}(\%) = \frac{\mathrm{B} - \mathrm{A}}{\mathrm{B}} \times 100 \quad (1)$$

where R is the reduction rate in the number of colonies, A is the number of bacterial colonies after exposure to the test specimen, and B is the number of initial bacterial colonies in the flask.

The biodegrability of the lignin nanofibers wasexamined through an enzymatic hydrolysis with laccase, one of the ligninolytic enzymes. 10 mg specimens were placed in closed vials containing 500 mg/ml laccase (laccase from Trametesversicolor, Sigma Aldrich Co., USA) in 10 ml acetate buffer solution (acetate buffer solution pH 4.65, Sigma Aldrich Co., USA), and then incubated at 25°C for a certain amount of time. At specified time intervals, the specimens were filtered out and vacuum-dried to a constant weight. Afterwards, the specimens were weighed and the degree of disintegration was calculated using the following formula:

$$\text{Weight loss}\ (\%) = \frac{\mathrm{A}-\mathrm{R}}{\mathrm{A}} \times 100 \quad (2)$$

where A is the weight before the enzymatic hydrolysis, and R is the weight after the enzymatic hydrolysis.

3. RESULTS AND DISCUSSION

3.1. INSOLUBILIZATION OF LIGNIN NANOFIBERS

The optimum insolubilization conditions and sequence for lignin nanofibers were found as follows: water vapor treatment at 80°C for 180 min, photo-crosslinking for 30 min (irradiation of visible light with a distance of 17 cm), and heat treatment at 200°C for 60 min. The SEM image showed that the insolubilized lignin nanofiber web maintained its structure after exposure to an aqueous environment whereas the untreated lignin nanofiber web lost its fibrous structure following immersion into water. The results demonstrated that the insolubilization method we had determined was effective in stabilizing the lignin nanofibersagainst dissolution in water.

The XRD spectrum of the lignin nanofibers before and after the insolubilization confirmed that the stabilization methods we had determined were effective to increase the crystallinity of the lignin nanofibers. The XRD spectrum of the untreated lignin nanofibers revealed one very weak and broad peak at 2θ = 21-22.2 deg, indicating that the specimen was amorphous. After insolubilization, however, two new peaks at 2θ = 20 deg, and 2θ = 31.5 deg, were observed, indicating that crystalline regions had been

formed through the insolubilization.
The EDX analyzer detected sulfone in the nanofiber webs containing lignin at every experimental stage, i.e., afterelectrospinning, afterinsolubilization, and after immersion into water, but not in the pristine PVA nanofiber web. These observations confirm that lignin is not damaged by external forces through the fabrication process, and is well-preserved after being transformedinto nanofibers.

3.2. ANTIMICROBIAL PROPERTIES OF LIGNIN NANOFIBERS

The lignin nanofiber webs containing 85 wt% lignin at a 3.0 g/m^{2}web area density exhibited a 99.9 % reduction rate against *Staphylococcus aureus*, the representative of gram-positive organisms. However, for *Escherichia coli*, the representative of gram-negative organisms, the same system exhibited no reduction rate. Previous researchers who examined the antimicrobial activity of lignin reached consistent results in that lignin showed antimicrobial properties against different types of bacteria, depending on the origin and isolation methods of the lignin.Our result corresponds to a previous study (Dong 2011) in which the antimicrobial activity of lignosulfonate was evaluated. The lignin was isolated using the same method as had been employed in this study.

The antimicrobial properties of lignin have been attributed to the nature of phenolic compounds. The polyphenol compounds of lignin are known to damage the cell membranes of microorganism and to cause lysis of the bacteria, followed by release of the cell content (Sabu 2011). Our finding indicates that this inherent antimicrobial property of lignin was retainedfollowing its transformationinto nanofibers. This finding could be useful for the further applications of lignin nanofibers to end products such as medical textiles.

3.3. BIODEGRADABILITY OF LIGNIN NANOFIBERS

The degradation of lignin by laccase is known to occur through the one-electron oxidation of phenolic compounds. The phenolic nucleus is oxidized and generates phenoxy freeradical products, leading a polymer cleavage. Laccase is one of the major families of enzymes involved in ligninolysis, and it catalyzes the degradation process while acting as a low-molecular-weight mediator (Perez 2002).

To observe the effect of lignin concentration on degradability, three kinds of specimens of varyinglignin-to-PVA ratios were prepared; 85:15, 50:50, and 0:100 (pristine PVA). As a result of the enzymatic degradation experiment with laccase for ten days, the average weight loss ratio of the specimens containing 85 wt% lignin was 36.3 %, whereas that of the specimens containing 50 wt% lignin was 35 %, and that of the 100 wt% PVA specimens was 0 %. As evidenced by the results, the weight loss onlytook place in the specimens containing lignin, indicatingthat the inherent biodegradability of lignin is retained after its transformationinto nanofibers.

The difference in lignin concentration, however, did notmake asignificant impacton the degradation efficiency. According to a previous study (Im 2002) which studied the parameters of the degradation of polymer compounds, the degradation of polymer compounds is not only affected by chemical composition, but also by crystallinity, the

molecular weight, morphological structure, and so on. It has been reported that the initial degradation is more influenced by crystallinity, while the long-term degradation is largely controlled by the chemical composition of the polymer compounds. Therefore, the ten days of our experimental period might have only represented the initial stage of biodegradation and might not have been sufficient for the entire degradation of the lignin nanofibers, whose crystallinity had been heightened artificially through the insolubilization process.

4. CONCLUSIONS

Our findings reveal the potential for lignin to be developed as an advanced fiber material allowing us to achieve not only ecological efficiency through the transformation of a waste product into a renewable resource, but also economic efficiency by skipping the post-processing stage designed to impart functionalities in conventional textile production.

REFERENCES

Dong, X., Telysheva, G., Jurkjane, V. and Viesturs, U. (2004).“Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production”, *Ind. Crop. Prod.*, **34**(3), 1629-1634.

Giebel, E. and Greiner, A. (2012).“Water-stable nonwovens composed of electrospun fibers from aqueous dispersions by photo-cross-linking”, *Macromol.Mater. Eng.*, **297**(6), 532-539.

Im, S.S. (2002). “Structural and morphological effects on the biodegradation of aliphatic polyesters”, *J. Korean Ind. Eng. Chem.*, **13**(5), 389-402.

Lee, E. and Lee, S. (2014), “Fabrication of lignin nanofibersusing electrospinning”, *J. Korean Soc. Cloth.Text.*,**38**(3), 372-385.

Min, B.M., Jeong, L., Lee, K.Y. and Park, W.H. (2006), “Regenerated silk fibroin nanofibers: water vapor-induced structural changes and their effects on the behavior of normal human cells”, *Macromol.Biosci.*,**6**(4), 285-292.

Peresin, M.S., Kammiovirta, K., Setälä, H. and Tammelin, T. (2012), “Structural features and water interactions of etherified xylan thin films”, *J. Polym.Environ.*,**20**(4), 895-904.

Perez, J., Munoz-Dorado, J., de la Rubia, T. and Martinez, J. (2002), “Biodegradation and biological treatment of cellulose, hemicellulose and lignin: an overview”, *Int.Microbiol.*, **5**(2), 53-63.

Sabu, T., Visakh, P.M. and Mathew, A.P. (2011), “Advances in natural polymers: composites and nanocomposites”. Springer, Dordrecht.

Thakur, V.K., Thakur, M.K., Raghavan, P. and Kessler, M.R. (2014), “Progress in green polymer composites from lignin for multifunctional applications: a review”, *ACS Sustainable Chem. Eng.*, **2**(5), 1072-1092.