

Nanocellulose-based Antimicrobial Systems for Mitigating *E. coli* outbreaks in water bodies

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In Alabama, there has been a significant *E. coli* pollution problem in water sources due to an overabundant feral hog population. A study tested the impacts these feral hogs have on the water quality and found that in treatment watersheds, the values of *E. coli* were up to 40 times greater than the reference watershed due to fecal material (Bolds et al., 2021). This is an obvious threat to entire populations that have their water sources contaminated by hogs.

One solution to this problem may be the use of silver nanoparticles, which are well known for their antimicrobial properties. Silver ions liberated by them can enter the cells through pores in the membrane, damaging proteins and DNA, disrupting the functioning of the cell, and resulting in cell death (Liao et al., 2019). For silver nanoparticles to function, they first need to be anchored to other structures to control the release and long-term use of these silver ions. Nanocellulose-based hydrogels are an attractive alternative as they can be used as reactants for synthesis while serving as an anchoring structure.

Cellulose nanofibrils are attractive due to their morphology, high surface area, low density, and sustainable sourcing. Cellulose can form hydrogel structures which can be used as scaffolds for synthesizing silver nanoparticles. To form them, premade nanocellulose spheres are immersed into Tollens' reagent where the hydroxyl and carboxyl groups in cellulose fibers interact with the silver ammonium oxidizing to aldehyde groups. Silver interacts with these fibers, building silver seeds and, once heated, crystallizes to form silver nanoparticles (Wu et al., 2014).

The objectives of this project were to generate silver nanoparticles using nanocellulose spheres from different raw materials as scaffolds and test their efficiency at inhibiting *E. coli* growth. Two common raw materials, wood and soybean hulls, were chosen to determine which has the best-reducing capacity.

Characterization using Fourier-transform infrared spectroscopy (Figure 1) showed a change in the carbon hydrogen-bond due to aldehyde formation. There is also a decrease in carboxyl groups as they are used during the reaction.

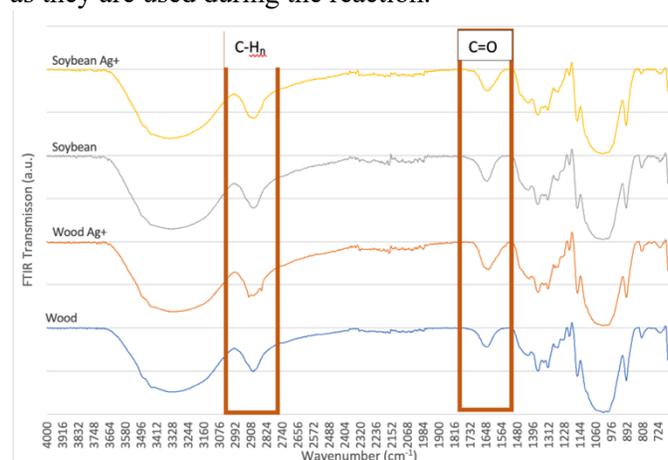


Figure 1. Fourier Transform Infrared Spectroscopy of Four Materials

Scanning electron microscopy (Figure 2-5) showed an increase in rugosity in the presence of silver nanoparticles, inferring that the higher presence in soybean-spheres might be caused by the additional components such as pectin.

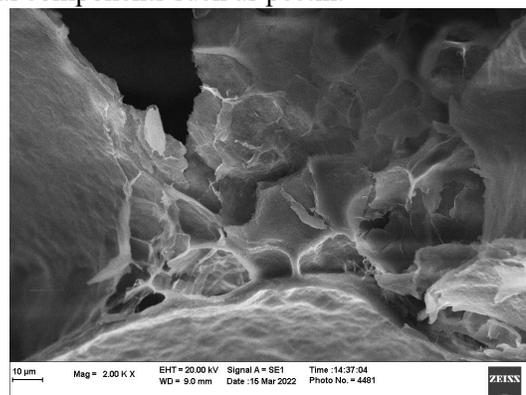


Figure 2. Scanning Electron Microscopy Wood with Silver

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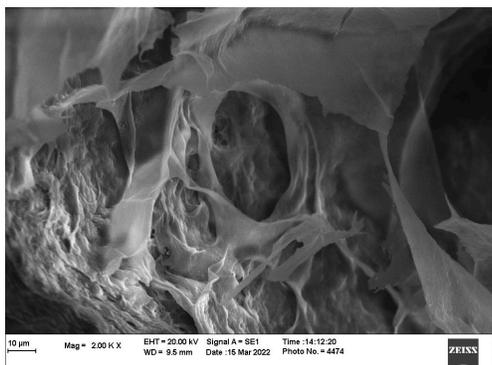


Figure 3. Scanning Electron Microscopy Wood without Silver

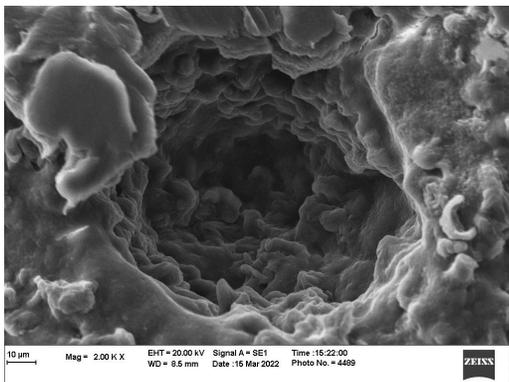


Figure 4. Scanning Electron Microscopy Soybean with Silver.

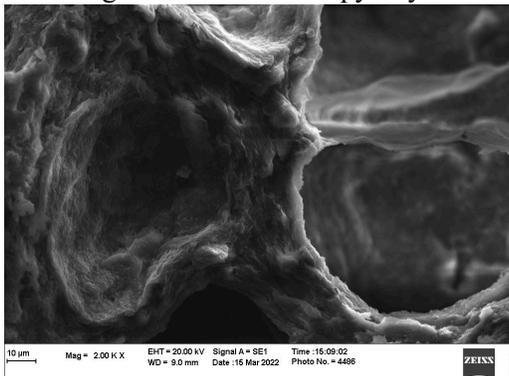


Figure 5. Scanning Electron Microscopy Soybean without Silver

A bacterial suspension was performed through serial dilutions to obtain bacterial concentrations similar to those found in watersheds. These bacterial concentrations include 10^{-8} , 10^{-7} , and 10^{-6} colony forming units. Ten milliliters of each concentration were obtained, and three milliliters of each concentration were run through tubes at volumes of 48.4-, 10.89-, and 16.64-millimeters of spheres. The last milliliter of the solution was collected and plated along with a control plate. Plates were placed in an incubator at 37 °C overnight, and the resulting colonies were counted and recorded. Figure 6 indicate the antimicrobial efficacy in wood and soybean spheres with and without silver nanoparticles. The 1mL, 2mL and 3mL on Figure 6 indicate the depths of spheres in the tube and the -8, -7, and -6 indicate the bacterial concentrations. Overall, we found that there is an increase in antimicrobial

efficacy with silver nanoparticles. Silver nanoparticles in the soybean spheres seem to have greater efficacy than wood.

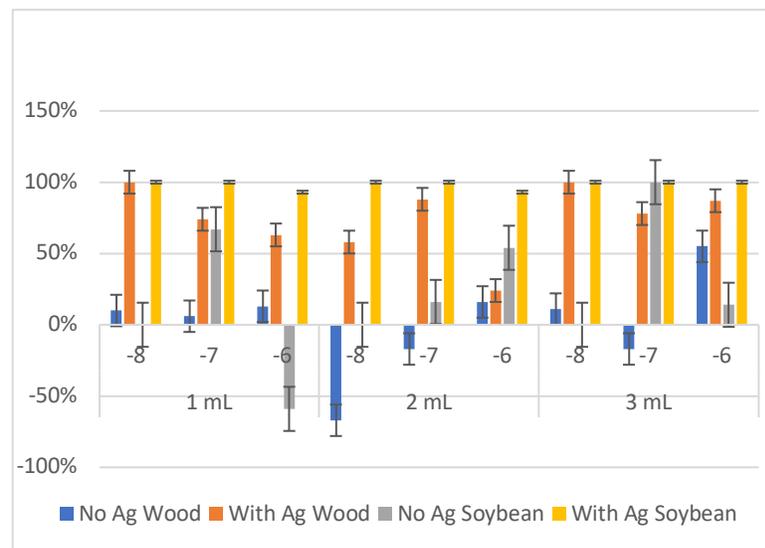


Figure 6. Comparison of Antimicrobial Efficacy in Wood and Soybean Spheres with and without Silver Nanoparticles

Statement of Research Advisor

During this project, Brienne has developed a method to produce nanocellulose-based hydrogel beads and decorated them with silver nanoparticles for reducing *E. coli* content in water. This work advances our understanding of this system so we can move forward with field trials that has great potential for alleviate environmental concerns related to the bacterial contamination in water bodies.

-Maria Soledad Peresin, College of Forestry and Wildlife Sciences

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Authors Biography



Brianne Dickson is a Senior pursuing a B.S. degree in Microbiology at Auburn University with plans to pursue PA school upon graduation. She has been working as an undergraduate research fellow in the Sustainable Bio-based Materials Laboratory.



Gabriel is a sophomore currently pursuing a degree in biophysics at Johns Hopkins University. At the Sustainable Bio-based Materials Laboratory, he helped develop antimicrobial and antigen assays as an NSF CAREER intern.



Diego Gomez-Maldonado is a Post-Doctoral Research Fellow in the Sustainable Bio-based Materials Laboratory at Auburn University. He is very active in helping undergraduate students expand their knowledge with research.



Brendan Higgins is an assistant professor in Biosystems Engineering at Auburn University. He studies biological processes that incorporate algae and bacteria for waste remediation, water quality, biofuels, and high-value nutraceuticals.



Maria Soledad Peresin is an assistant professor of forest biomaterials in the Auburn University College of Forestry and Wildlife Sciences. She is the principal investigator for the Sustainable Bio-based Materials Laboratory.