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Colorfastness of Extracted Wood-staining Fungal Pigments on Fabrics: a new potential for textile dyes

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ABSTRACT

*In this study, pigments from three species of pigmenting fungi, Chlorociboria aeruginosa (xylindein, green), Scytalidium cuboideum (draconin red, red), and Scytalidium ganodermophthorum (yellow), were used to dye multi-fabric test strips. Tests for color stability and tests for colorfastness to washing with and without bleach, and to perspiration, were conducted. Color readings were taken using a colorimeter, and color differences were statistically determined based on overall color change as calculated using the CIE L*a*b* color space. The results indicated that all three pigments were stable over time, indicating that these pigments can be used for fabric dyeing, eliminating the need for additional chemicals or heat as is required for traditional fabric dyes. Xylindein and draconin red exhibited good colorfastness to washing, and xylindein good colorfastness to perspiration. These results indicate that xylindein shows good potential as a dye for garment fabrics, and draconin red shows good potential as a dye for second-layer garment fabrics.*

Keywords: fungi, pigments, textiles, dyeing

Introduction

Natural dyes have been used to color textiles for hundreds of years. However, since the advent of the Industrial Revolution and the invention of synthetic dyes, commercial dye houses have switched almost entirely to synthetics. This was due to the relative decreased expense of manufacturing(Slater, 2003). Recently, however, there has been growing consumer demand for more eco-friendly, sustainable, and natural coloration (Kumar & Sinha, 2004; Shahid & Mohammad, 2013). New forms of natural dyes are being investigated, and in the last twelve years there has been a focus on developing methods to use fungal pigments to color textiles (De Santis et al., 2005; Räisänen, Nousiainen, & Hynninen, 2001; Velmurugan et al., 2009; Weber et al., 2014). Of particular interest is using these

methods to achieve colorfastness to washing and perspiration without increasing energy consumption or using additional chemicals.

The most common methods for commercial dyeing with fungal pigments are heated dye bath, high temperature dyeing, and immersion dyeing. All of these methods require significant volumes of water. Both natural fibers and manufactured fibers, namely polyamide and polyester, have been tested, with and without mordants. For heated dye baths (30 - 100° C) without mordants, on both wool and silk, colorfastness to perspiration ranged from good to very good (Atalla et al., 2011), and wash fastness ranged from good to excellent (Atalla et al., 2011; Nagia & El-Mohamedy, 2007; Sharma et al., 2012), with a moderate result from the deep brown pigment extracted from *Penicillium chrysogenum* (Atalla et al., 2011). The combined results for the above pigment sources for heated dye baths with mordants on wool, silk, and cotton are shown in Table 1. Two of these studies (Sharma et al., 2012) (Velmurugan et al., 2009) presented results on a rating scale of 1 (poor) to 5 (excellent). The third study (De Santis et al., 2005) presented graphic results of ΔE^*, but the results were converted to a rating scale for comparative purposes. Importantly, these results illustrate that mordants do not necessarily improve colorfastness for fungal pigments as they do for other natural dyes such as those derived from plants, insects, etc.

Table 1. Results of colorfastness testing for heated dye bath method using mordants.(De Santis et al., 2005; Sharma et al., 2012; Velmurugan et al., 2009)

Fabric/Mordant	Perspiration	Wash
Wool/Alum	Fair	Fair
Wool/Stannic chloride	Excellent	Excellent
Wool/Copper sulfate and ferrous sulfate	-	Good to Excellent
Silk/Copper sulfate and ferrous sulfate	-	Good to Excellent
Cotton/Ferrous sulfate	-	Good to Very Good

For high temperature dyeing (101 - 130° C) using fungal pigments without mordants on wool, polyamide, and polyester, the best results were achieved with polyester (high temperature disperse dyeing). The results for wash colorfastness were very good to excellent (Räisänen, 2009; Räisänen et al., 2001). R. Räisänen noted only that there was a color change after washing but did not give any quantitative data (2009). As seen with the heated dye bath method, mordanting did very little to improve colorfastness.

Perumal obtained excellent colorfastness results for washing and steam using alum, copper sulfate, potassium dichromate, and stannous chloride, separately as mordants on cotton and silk(2009). The results of Velmurugan's tests on cotton, with alum and ferrous sulfate as mordants, on five different species of fungi are shown in Table 2. These results were presented on the same rating scale described above. Even mordanted, the results vary widely. This indicates the need for a method that produces more predictable colorfastness qualities. At this time, there are no study results for fungal pigment immersion dyeing without mordants, which presents an opportunity for further research.

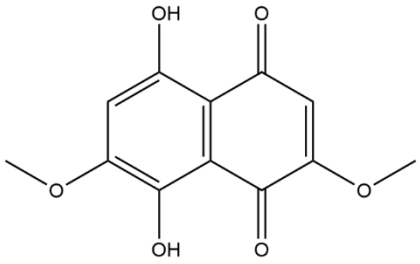
Table 2. Colorfastness for fungus/mordant combinations.(Velmurugan et al., 2010)

Fungus/Mordant	Wash	Perspiration
Monascus purpureus/Alum	Good	Moderate to Very Good
Isaria farinosa/Alum	Moderate to Good	Good to Very Good
Emericella nidulans/Alum	Moderate to Good	Moderate to Very Good
Fusarium verticillioides/Alum	Moderate to Good	Moderate to Very Good
Penicillium purpurogenum/Alum	Moderate to Good	Moderate to Very Good
Monascus purpureus/Ferrous sulfate	Good	Moderate to Very Good
Isaria farinosa/Ferrous sulfate	Moderate to Good	Good to Very Good
Emericella nidulans/Ferrous sulfate	Moderate to Good	Moderate to Very Good
Fusarium verticillioides/Ferrous sulfate	Good to Very Good	Good to Very Good
Penicillium purpurogenum /Ferrous sulfate	Moderate to Good	Moderate to Very Good

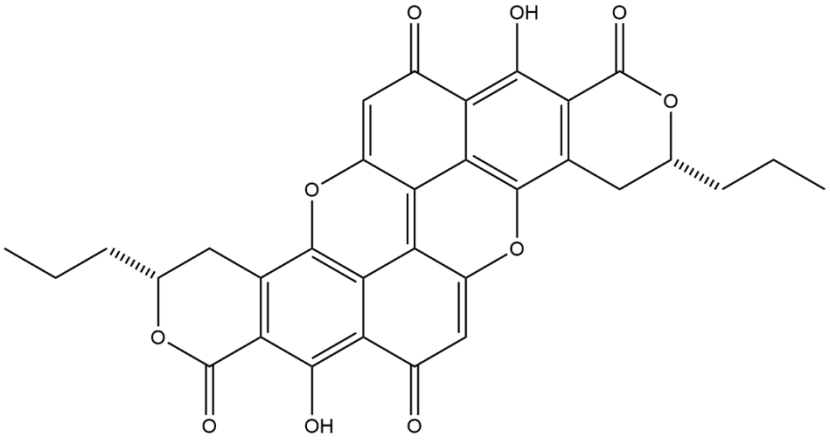
Wood pigmented with xylindein from *Chlorociboria* spp. has been utilized as far back as the 15th century for use in intarsia pieces (Blanchette, Wilmering, & Baumeister, 1992). However, the widespread use of wood pigmented by fungi died out around the mid-1800s. It is only recently that the use of fungal pigments, specifically extracted fungal pigments, to colorize wood has had a resurgence (Robinson, 2012; Robinson, Tudor, & Cooper, 2011). Using extracted pigments allows for the direct application of color to wooden objects in precise locations and patterns (Robinson, 2012). Only in the last couple of years has interest surfaced in using the extracted pigments from these fungi for the direct dyeing of textiles (Weber et al., 2014).

It has been observed during laboratory testing (Robinson, S. C. et al., 2014; Robinson, Sara C et al., 2014) that the pigments from *C. aeruginosa*, *S. cuboideum*, and *S. ganodermophthorum* adhere to nearly every surface with which they come in contact. This is not surprising as the pigments from these three fungi are not highly soluble in water and are meant to persist in nature. The free pigments from mold fungi traditionally used for dye extraction, such as those from *Monascus ruber* (De Santis et al., 2005), are not intended to persist in nature as these pigments are water soluble (Blanc et al., 1994). It is hypothesized that these pigments will also adhere to the fibers of various textiles, allowing the pigments to be used as dyestuffs. These pigments have been observed to be difficult to remove, either mechanically or chemically without the use of moderate to strong organic solvents. This strongly suggests that these pigments, when used as dyes for textiles, will exhibit superior colorfastness as compared to standard textile dyes, even without the use of mordants or additional chemicals.

The structure of xylindein was first described, briefly, in 1962 (Blackburn, Todd, & Neilson, 1962) and the definitive structure was elucidated by Edwards and Kale (Edwards & Kale, 1965) in 1965 (Figure 1). Draconin red, the pigment extracted from *Scytalidium cuboideum*, is composed of many components, only one of which has been characterized (Golinski, 1995) (Figure 1). The yellow pigment produced by *S. ganodermophthorum* has yet to be characterized. The precursors for xylindein and draconin red are quinones, or benzene rings in which two of the hydrogen atoms have been replaced by oxygen. Quinones are also precursors for the pigments found in many higher fungi (Velíšek & Cejpek, 2011).



5,8-dihydroxy-2,7-dimethoxy-1,4-naphthalenedione
The only known component of draconin red.



Xylindein

Figure 1. Structures of xylindein and the known component of draconin red.

The purpose of this preliminary research was to determine if extracted fungal pigments from *C. aeruginosa* (xylindein), *S. cuboideum* (draconin red), and *S. ganodermophthorum* (unknown yellow pigment) would be viable as textile dyes and would be colorfast to both home laundering and perspiration. The advantages of using fungal pigments as textile dyes include the lack of need for water in either the dyeing process or in after-treatment, such as reductive washing, and the relative short time (minutes as opposed to hours) it takes for the pigments to bond with the textiles. Additionally, fungal pigments are not fiber specific and should therefore be viable as textile dyes across a broad range of fiber types.

Experimental

Fungal Pigments

Pigments were extracted from laboratory-cultured strains of *C. aeruginosa* (strain UAMH 11657 isolated from a rotting hardwood log in Halliburton, Ontario, Canada), *S. cuboideum* (strain UAMH 11517 isolated from *Quercus* sp. in Memphis, TN), and *S. ganodermophthorum* (strain UAMH 10320 isolated from oak logs used for mushroom cultivation in Gyeonggi Province, Korea) via the method developed by Robinson, et al. (2014) The fungi were cultured separately on 2% malt agar media mixed with wood in sterile petri plates (Robinson et al., 2012) for two to three months. The plates were allowed to air dry under a fume hood for 24 hrs. before extraction. Once dry, the plates were broken

up into 1-2 cm sized pieces and placed into 250 mL round-bottom flasks containing magnetic stir bars (7.9 x 25.4 mm). 50 mL of dichloromethane (DCM) was added to the flasks and the flasks were stirred for 30 min. on a stir plate at 230 rpm. The resultant liquid was filtered through laboratory-grade Whatman No. 1002150 filter paper into 8 dram borosilicate glass vials. The DCM was then evaporated from the vials using a Büchi RE 111 Rotovapor at 25 psi with no heat. The extraction and evaporation process was repeated twice more on each culture. Prior to the third evaporation, color readings were taken on the solutions using a Konica Minolta Chroma Meter CR-5 utilizing the CIE L*a*b* color space. Because these pigments are naturally produced by the fungi, their hues can vary from plate to plate, making concentration via dry weight of the pigment an unacceptable method for determining concentration. Factors such as substrate, temperature, and pH can have an effect on pigment color, even from fungi within the same strain. One-hundred percent concentration was thus initially determined by calculating color saturation (ΔC*) based on color readings as described in Robinson, et al. (2014) The mean L*a*b* values of samples at full saturation were then taken as target color readings for solubilized pigments to be used in experiments. A standard deviation of ±2.00 was allowed for all values as a change of this magnitude shows no visible difference in color (Weber et al., 2014). Target color readings are listed in Table 3. The extracted pigments were resolubilized in 27 mL of DCM prior to use for dyeing.

Table 3- CIE L*a*b* targets for fungal pigments. Pigments are considered usable if all values fall ±2 of the listed values.

Fungus	Pigment	CIE L*a*b* Target
Chlorociboria aeruginosa	Xylindein	L*= 82.28, a*= -11.06, b*= -5.40
Scytalidium cuboideum	Draconin red	L*= 82.32, a*= 26.84, b*= 13.19
Scytalidium ganodermophthorum	Unknown	L*= 95.46, a*= -3.00, b*= -8.15

Dyeing of the Test Strips

AATCC approved multi-fabric test strips were used for all testing. The 120 mm x 75 mm multi-fabric test strips, purchased from Testfabrics, Inc., were composed of 120 mm x 10 mm strips of spun diacetate, bleached cotton, spun polyamide, spun polyester, spun polyacrylic, and worsted wool. The spun diacetate was excluded from testing as DCM melts the fabric. DCM does not appear to damage any of the other fabrics, and this observation is borne out by a concurrent study (unpublished data). Resolubilized pigment was applied to the test strips using the drip method as described by Weber, et al. (2014) The resolubilized pigment was dripped via pipette in the following manner: 10 drops evenly spaced on the upper third of the test strip, 10 drops evenly spaced on the middle third of the test strip, and 10 drops evenly spaced on the lower third of the test strip. The dyed test strips where then placed on glass plates and allowed to dry for 30 min. under a fume hood, after which they were placed in the dark in a drawer between successive color readings. Nine replicates for each of the three pigments were dyed.

Color Stability

Color stability was tested by taking color readings with a Konica Minolta Chroma Meter CR-5 utilizing the CIE L*a*b* color space. A 60 mm x 9 mm mask was used and the colorimeter was set to reflectance with an 8 mm aperture. Color readings were taken at 30 min, 24 hrs, and 1 week. Color variation was determined statistically by calculating Δ*E** from undyed control test strips and running a two-way ANOVA and Tukey HSD on the Δ*E** using SAS version 9.4.

Perspiration and Wash Testing

Colorfastness to perspiration was tested using AATCC Test Method 15-2009. Three dyed specimens from each pigment were used. After testing, the Perspiration Tester was removed from the oven and allowed to cool and air dry at 21° C for 48 hrs. The specimens were removed from the Perspiration Tester for evaluation.

Colorfastness to washing was tested using AATCC Test Method 61-2010 (Colorfastness to Laundering: Accelerated). Three dyed specimens from each pigment were used. After testing, the specimens were allowed to dry at 21° C for 48 hrs, then evaluated. Colorfastness to washing with bleach was also tested using AATCC Test Method 61-2010 and the method described above.

Color variation was determined in the same way as it was for color stability. A two-way ANOVA and Tukey HSD were run separately for each fabric on the Δ*E** using SAS version 9.4. The Tukey HSD results for treated multi-fabric test strips were compared to the results from the one-week color stability tests.

Results and Discussion

Color Stability

The two-way ANOVA on Δ*E** as a function of color and time indicated that time had no significant effect on color and there was no interaction between time and color for any of the five fabrics (P-values 0.2589 – 0.9727). The mean Δ*E** and results for the Tukey HSD for all three pigments are shown in Table 4. Xylindein exhibited a decrease in Δ*E** over time on every test fabric but wool, on which there was a slight increase in Δ*E** from 30 min to 24 hrs with a decrease to below the 30 min reading after one week. Draconin red exhibited a similar decrease in Δ*E** over time on wool, polyacrylic, and cotton, but an increase in Δ*E** on polyester and polyamide. The yellow pigment exhibited either an increase or no change in Δ*E** over time on all but cotton. None of these color changes was significantly different.

Table 4 – Mean ΔE* and Tukey HSD results (in parentheses) for time on all three pigments. Results with the same letter within a row are not significantly different at α = 0.05.

Fabric	Pigment	30 min.	24 hrs.	1 week
Wool	Xylindein	90.03 (A)	94.46 (A)	88.23 (A)
Polyacrylic	Xylindein	212.80 (A, B)	211.24 (A, B)	194.79 (A, B)
Polyester	Xylindein	252.12 (B)	244.91 (B)	221.15 (B)
Polyamide	Xylindein	113.08 (B)	104.29 (B)	91.53 (B)
Cotton	Xylindein	153.00 (C)	140.64 (C)	114.34 (C)
Wool	Draconin red	61.16 (A)	59.35 (A)	58.57 (A)
Polyacrylic	Draconin red	147.97 (A, B)	131.49 (B)	121.74 (B)
Polyester	Draconin red	884.17 (A)	876.49 (A)	889.78 (A)
Polyamide	Draconin red	84.38 (B)	82.24 (B)	92.88 (B)
Cotton	Draconin red	301.01 (A, B)	265.39 (B)	276.22 (A, B)
Wool	Yellow	89.36 (A)	88.81 (A)	90.13 (A)
Polyacrylic	Yellow	238.67 (A)	243.03 (A)	243.43 (A)
Polyester	Yellow	198.63 (B)	195.71 (B)	197.90 (B)
Polyamide	Yellow	195.50 (A)	197.91 (A)	202.10 (A)
Cotton	Yellow	382.95 (A)	377.94 (A)	347.63 (A, B)

The P-values for time and time/color interaction for the color stability tests, as well as the Tukey HSD results, show that the colors for all three pigments are stable on fabrics, at least up to one week. There was no significant color change on any fabric for any of the pigments during this time. The increase in ΔE^* for the yellow pigment indicates that the yellow color darkens slightly, but not significantly, over time. These results were expected based on the color stability of xylindein over centuries in both wood intarsia and marquetry (Blanchette et al., 1992; Otterstedt, 2001). More recent studies have also shown that fungal pigments exhibit superior resistance to fading under UV light when compared to other natural pigments (Robinson et al., 2013). Leaving the dyed samples out and exposed to ambient light may have produced different results and could be the subject for a follow-up study.

Washing and Perspiration

The two-way ANOVA on ΔE^* as a function of color and treatment indicates that there is an interaction between color and treatment for all fabrics (P-values <0.0001 – 0.0046). Tukey HSD results are given in Table 5. Xylindein exhibited the best resistance to color change for washing and perspiration. Draconin red exhibited resistance to color change for washing, but was less resistant to perspiration. The yellow pigment exhibited the least resistance to color change for washing, but performed relatively well for perspiration. Polyester exhibited the most resistance to color change, and cotton the least resistance.

The P-value for the interaction between color and treatment shows that there is an interaction between the two. As shown in Table 5, xylindein is the most resistant to treatment, followed by draconin red and the yellow pigment from *S. ganodermophthorum*. Xylindein demonstrated no significant color change for any treatment on any fabric with two exceptions: wool and polyacrylic. A surprising result here is that wool showed no significant color change to washing with bleach, but showed a significant color change to washing without bleach. Wool is highly susceptible to damage from oxidizing agents, such as bleach, so it would have been expected that there would have been a significant color change after washing with bleach. Looking at all the treatment results, this is the only case where the harsher treatment did not have an effect while the milder treatment did. However, it appears that the bleach completely removed the pigment and oxidized the wool, causing it to turn a medium brown color. As ΔE^* does not indicate where in the L*a*b* color space color changes have occurred, the shift from green to brown yielded a misleading statistical, but visually obvious, result. Polyacrylic, the synthetic fabric with the most wool-like properties (Kadolph, 2010), showed significant color change to washing with bleach, but not to washing without bleach. Although the former was to be expected, due to the color change exhibited by wool, it would have been expected that there would have been a significant color change to washing without bleach as well. Based on the overall results, xylindein has the potential to be used as a dye for fabrics for garments, with appropriate washing instruction for wool and polyacrylic. The unexpected results for the treatment of these two fabrics when dyed with xylindein warrants further research.

Table 5 –TukeyHSD results for treatments on all three pigments. Results with the same letter within a row are not significantly different at α = 0.05. Treatment results marked with a * are significantly different from the ΔE* of the one-week test.

Fabric	Pigment	1 Week	Washing with Bleach	Washing without Bleach	Perspiration
Wool	Xylindein	B, C	C, D	**D***	B, C, D
Polyacrylic	Xylindein	A, B	**C***	B, C	A, B
Polyester	Xylindein	C	C, D	C, D	C, D
Polyamide	Xylindein	C, D	D	D	C, D
Cotton	Xylindein	D, E, F	F	E, F	D, E, F
Wool	Draconin red	B, C, D	C, D	C, D	**A***
Polyacrylic	Draconin red	A, B, C	C	B, C	B, C
Polyester	Draconin red	A	A	A	**B***
Polyamide	Draconin red	B, C, D	D	D	**A***
Cotton	Draconin red	B, C	**E, F***	**A***	B, C, D
Wool	Yellow	B, C	**D***	**D***	B
Polyacrylic	Yellow	A	**C***	**C***	A, B
Polyester	Yellow	C, D	D	C, D	C, D
Polyamide	Yellow	A, B	**D***	**D***	A, B, C
Cotton	Yellow	A, B	**F***	**D, E, F***	**C, D, E***

Draconin red showed no significant color change after washing with or without bleach on any fabric with the exception of cotton. Many natural pigments are quinone derivatives, including xylindein, which is an extended quinone. Anthraquinones are often used as precursors for dyes (Bauer, Garbe, & Surburg, 1988), and naphthoquinones are precursors to anthraquinones. All of the quinone derivatives achieve their color through alternating carbon-carbon double bonds in conjugated cyclic systems. Wool, polyester, and polyamide all showed significant color change from perspiration, indicating that one or a combination of components of the perspiration solution (sodium chloride, lactic acid, disodium hydrogen phosphate, or histidine monohydrochloride) induces modification to the optical properties of the compound, resulting in visually significant color changes. Based on the overall results, draconin red has the potential to be used as a dye for top-layer (not next-to-skin) garments from wool, polyester, and polyamide. Although polyacrylic is not usually worn next to the skin, its resistance to color loss from perspiration when dyed with draconin red indicates it could be used as performance wear if desired.

The yellow pigment from *S. ganodermophthorum* was the pigment least resistant to treatment. Polyester was the only fabric dyed with yellow that did not show a significant color change after washing with or without bleach. Based on the overall results, polyester is the only fabric that has potential as garment material when dyed with the yellow pigment. Further studies on the effects of exposure to weather would need to be conducted to determine if the yellow pigment from *S. ganodermophthorum* would have potential as a dye for exterior fabrics.

In the context of the fabrics, polyester appears to have the most potential as a garment fabric dyed with fungal pigments. In order for a dye to "take" in a fabric, the dyeing process must swell the fabric fibers to allow the dye particles in, then allow the fibers to shrink back down to entrap the dye. If the dye remains only on the surface of the fibers, it will not be colorfast. The swelling and shrinking of fibers, especially synthetic fibers like polyester, is usually

achieved by the application and removal of heat. The assumption in the present research is that the DCM swells polyester fibers in a similar way, allowing the fungal pigments to enter the fibers. As the DCM evaporates, the fibers shrink and retain the pigment. Wool, polyacrylic, and polyamide all demonstrate approximately equal potential as garment fabrics when dyed with xylindein or draconin red. As the structure of the yellow pigment from *S. ganodermophthorum* is as yet unknown, it is difficult to hypothesize as to why this pigment is not as colorfast as xylindein or draconin red. Further examination of the yellow pigment is needed.

Cotton is the worst performer of the fabrics tested, although it appears colorfast when dyed with xylindein. The poor performance was unexpected as extracted xylindein and draconin red, and to some extent the yellow pigment, have each been shown to color other cellulosic substrates, specifically wood. Cotton is 91% cellulose and contains no lignin or hemicellulose. It may be that the extracted pigments bind preferentially to either hemicellulose or lignin, and only moderately to cellulose. Other cellulosic fibers containing some lignin, such as those from bast fibers like linen and hemp, would need to be tested to determine if it is indeed the lack of lignin in cotton fibers that leads to poor colorfastness with fungal pigments.

Although small amounts of pigment/DCM solution were used in this study, hypothetically the process could be easily scaled up by using a spray system, along the lines of an ink jet printer, to dye textiles in quantity. The system would need to be a closed system in order to recover the DCM as well as to protect textile workers from exposure to the organic solvent.

Conclusion

The extracted pigments from *C. aeruginosa* and *S. cuboideum*, xylindein and draconin red, respectively, when solubilized in DCM, demonstrate good potential as textile dyes. Draconin red's susceptibility to fading from perspiration limits its use to second layer garments or other non-skin contact applications. The yellow pigment from *S. ganodermophthorum* does not exhibit qualities conducive to the dyeing of garment fabrics, but may have other coloration applications. The structure of this yellow pigment needs to be thoroughly investigated to determine if there is a way to enhance its utility as a fabric dye. All three pigments are most effective on polyester, and moderately effective on wool, polyacrylic, and polyamide. The extracted fungal pigments were least effective on cotton, and further investigation would need to be conducted on other cellulosic fibers to determine if this is specific to cotton or if similar results are achieved across the spectrum of cellulosic fibers.

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