

Article

Preliminary Exploration of the Red Pigment from *Scytalidium cuboideum* as a Cellulosic Pulp Colorant

Derek W. Stone ¹, Sarath M. Vega Gutierrez ^{2,*}, Zielle M. Walsh ³ and Seri C. Robinson ²

¹ Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR 97331, USA; stonede@oregonstate.edu

² Department of Wood Science & Engineering, Oregon State University, Corvallis, OR 97331, USA; seri.robinson@oregonstate.edu

³ Biology—Genetics, Oregon State University, Corvallis, OR 97331, USA; walshzi@oregonstate.edu

* Correspondence: sarath.vega@oregonstate.edu

Abstract: Pollution from the international dye industry continues to be a global problem. Biotechnology offers new options, including a closer look at select wood decay fungi to replace inorganic dyes. The pigments produced by a small group of soft rotting fungi are generally naphthoquinonic and remarkably stable. From this group, the dramada crystals, produced by *Scytalidium cuboideum*, are of particular interest. To test the application of this pigment as a natural colorant of cellulosic pulps, four different bleached pulps were selected (one hardwood, three softwood), in three different mediums (acetone, ethanol, and DI water). The pigment generated a significant change of color, but there was no significant difference in color intensity based on the solvent carrier. These preliminary results are promising as they open the door for further exploration of applications of fungal pigments in the paper industry. Once these pigments can be reliably grown, they will offer a sustainable organic alternative to polluting inorganic dyestuffs and help reduce the toxic effluent released into the soil and waterways.



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1. Introduction

Cellulose is a natural polymer of glucose and is produced by all plants, making it the most abundant and continually renewable polysaccharide on Earth [1]. Utilized by humans for thousands of years [2], it remains vital to the economy, with approximately 400 million tons of paper and associated products produced globally each year [3]. Cellulosic pulps are the product of delignification of wood chips, part of the papermaking process [4,5] where the undesired hemicelluloses, lignin, and other associated biopolymers of wood are mostly removed to create a purified cellulose product, also referred to as pulp [4,6–9].

Common delignification agents, such as hydrogen peroxide, whiten pulps by bleaching [5,9]. Environmental concerns regarding these processes have resulted in a shift away from sodium hypochlorite, the traditional bleach, and towards more chlorine-free bleaching options. Unfortunately, these options can degrade cellulose quality [10–12]. The final paper product, created by depositing the water-dissolved pulp slurry over a mesh and removing the water [13], may be additionally treated with pigments for desired appearance and/or esthetics.

For the application of coloring in pulps, the industry uses fillers such as calcium carbonate, clay, titanium dioxide, and talc to increase brightness and opacity. Water-soluble dyes (basic dyes, acid dyes, direct dyes) are used to add specified colors [5]. Basic dyes are cationic, contain amine groups, are fixed to fibers with inorganic anions, and have a high affinity for lignin but not bleached fibers. Acid dyes are anionic, contain sulfonate groups, and are fixed to fibers with organic cations such as alum (a common mordant). Direct dyes are similar to acid dyes, but direct dyes have higher molecular weights and

affinity for cellulose [9,14,15]. While the chemicals and processes used to color paper vary, these steps, along with pulping processes, are the most energy- and water-intensive aspects of papermaking.

The effluents, due to bleaching and coloration, are also highly polluting, with chemicals such as chlorinated phenolics and dioxins damaging aquatic ecosystems and challenging downstream water treatment processes [16]. Additionally, the most commonly used synthetic dyes are derived from nonrenewable mineral or petroleum sources [17], something industries will not be able to sustain in the future without a renewable alternative.

Recently, interest in using fungal pigments, especially red pigments, has increased across food, wood, and textile industries due to consumers' desires for natural colorants [18,19]. There is a number of emerging options; however, fungal pigments typically have antimicrobial properties, are less allergenic, and are more stable in use than their synthetic counterparts [20].

Spalting, or the interior coloration of wood by decay fungi, is a process that affects all wood species, oftentimes enhancing its appearance [21,22]. A commonly used spalting fungus, *Scytalidium cuboideum* (Sacc. & Ellis) (syn. *Arthrographis cuboidea* (Sacc. & Ellis)), is a pigmented soft-rot fungus notable for its ability to produce pink and blue coloration in wood [23–25]. Today, there is increasing interest and research towards the use of this fungus both for its ability to pigment woody substrates [26,27] and for pigment extraction from these substrates [10,28,29]. The extracted pigment has shown promise for use as textile dyes [29–31] and remains an area of interest due to its potential for replacing (toxic) woodworker's traditional aniline dyes [32]. So far, little research [33] has been conducted regarding the affinity of pigments from any spalting fungi for colorants in cellulosic pulps.

The pigment from *S. cuboideum* specifically has been observed to have affinity to a wide array of materials, largely due to hydrophobic qualities that enable the pigment to persist on substrates even under elemental exposure [34]. These attributes make this pigment a promising agent for paper and pulp coloration. The red pigment, dubbed draconin red, has a naphthoquinone organic crystal structure and presents many desirable coloration traits that other natural pigments lack, such as high colorfastness, lightfastness, and stability in UV light [17]. The pigment was isolated and characterized in 2018 [35,36]. This study, designed as an exploratory, precursory study, sought to determine if pigments extracted from *S. cuboideum* showed promise as coloring agents for paper by examining the coloration produced in the paper manufactured from pigment-treated cellulosic pulps. This is primarily exploratory research to examine how fruitful additional studies in this area could be and is not intended to be a complete analysis of the pigment–pulp interaction or the potential of *S. cuboideum* as a factor in paper production.

2. Materials and Methods

2.1. Fungal Growth and Pigment Extraction

Scytalidium cuboideum (Sacc. & Ellis), strain UAMH 11517 isolated from *Quercus* sp. in Memphis, TN, USA, was cultivated on amended malt agar plates as described in [21]. Briefly, 2% malt Petri dishes were amended with sterilized white rotted wood chips, sterilized, and had *S. cuboideum* inoculated onto them. Once colonization of the dishes was complete (as measured by complete surface coverage), the Petri dishes were placed in a fume hood to dry for 48 h.

Pigment extraction was performed following the protocol in [27]. Briefly, the dishes were dried, blended, and the pigment was extracted with Merck 95% HPLC grade acetone.

2.2. Crystallization

The filtered solution was reduced to a fifth of its volume utilizing a Büchi rotovap, with a water bath at 30 °C. Once the solution was concentrated, it was poured into a 500 mL Erlenmeyer flask. Then, 150 mL of liquid nitrogen were added, and the flask was manually swirled for 30 s. The acetone freezing point (−95 °C) allowed the precipitation of draconin crystals. Once the precipitate was visible, the solution and the crystals were filtered using

a 15 cm 415 VWR filter paper and left to dry overnight. The crystals obtained with this method (Vega Gutierrez et al. (2018)) have high purity [36].

2.3. Pigment Preparation

Solutions of 0.73 mM of dramada (99% purity) were prepared in 100 mL of distilled water (the pigment formed a suspension and is referred to within the industry as a pigment dye), 100 mL of ethanol, and 100 mL of acetone. Each solution was placed in a stir plate (VWR) at 250 rpm for 30 min. This step achieved an even dispersion of the crystals in DI water, as well as a complete dissolution in ethanol and acetone.

2.4. Pulp Preparation

Four different pulps, designed in accordance with the Technical Association of the Pulp and Paper Industry (TAPPI) standards, were tested and characterized as follows: softwood pulp from *Pinus contorta* ssp. *murrayana* (Watson et al., 1880), *Pinus contorta* ssp. *latifolia* (Watson et al., 1871), *Picea galuca* (Deutsch, 1907), and *Picea mariana* (Britton et al., 1888) (Grand Prairie NBSK, Weyerhaeuser, Seattle, WA, USA). The pulps were provided dry. The average pulp fiber length was 2.3 mm, with a density of 0.79 g/cm³ and a calculated moisture content of 4.89%.

Softwood pulp from *Tsuga heterophylla* (Sargent, 1898), *Pinus contorta* (Doug. ex Loud., 1842), and *Picea glauca* (Hort. Ex Beissn., 1891) (Howe Sound 100, Howe Sound P&P), provided dry, was used. The average pulp fiber length was 2.64 mm, with a density of 0.667 g/cm³ and a calculated moisture content of 4.76%. A hardwood pulp from *Alnus* species (Mill., 1754) (Longview Hardwood, Weyerhaeuser) was provided in the wet lap form. The average hardwood pulp fiber length was 0.9 mm, with a density of 0.80 g/cm³ and a calculated moisture content of 35.4%. Finally, a softwood pulp from *Pseudotsuga menziesii* (Mirb., 1950) (Longview FIR-85, Weyerhaeuser) was provided in the wet lap form. The average pulp fiber length was 2.4 mm, with a density of 0.70 g/cm³ and a calculated moisture content of 43.8%. Wet lap refers to a form of pulp produced from residual mill and whole log chips that must be kept in moist conditions.

The moisture content was determined for each pulp by measuring an initial mass, drying it for 24 h in a DZF-6020 450 W vacuum drying box at 103 °C, and measuring the final mass. All the other characteristics were as the manufacturer described.

After allowing each oven-dried sample to sit out for 48 h and reclaim atmospheric moisture, the mass of each pulp (corresponding to 10 g of its oven-dried mass) was left to soak for 96 h in 100 mL of water; 900 mL of water were then added to each of these pulp suspensions, which were then placed in a Herman Manufacturing Co. 390 W 1720 rpm induction motor for a minute and a half.

The resulting 1 L liquid–pulp suspensions, one for each pulp type, were further divided into four 250 mL samples corresponding to each treatment type: pigment–acetone solution, pigment–ethanol solution, pigment–water suspension, and control.

2.5. Pulp Treatment

After that, 0.075 mL of each pigment preparation (ethanol, acetone, and pigment dye) were added to three of the four 250 mL beakers of each of the four pulp types, respectively. Then, each preparation was mixed for 30 min using magnetic stir bars and plates (with the exception of the pigment–water suspension which was mixed shortly before paper pressing due to pigment hydrophobicity—this is dubbed as the “pigment dye” method).

Each of the sixteen samples was then placed in a British sheet mold, draining the liquid to form paper-like sheets, which were then placed in a TAPPI standard Essex paper press (Adirondack Machine Corporation, Hudson Falls, NY, USA) at 345 kPa for five minutes. The resulting paper sheets were separated and left for 72 h to dry under ambient room conditions (21 °C).

2.6. Color Reading

The samples were color-read on a Konica Minolta Chroma Meter CR-5 using the reflectance setting. This machine returns three values in the CIE L*a*b* color space. The calculation for color change, ΔE^* , was performed utilizing the CIEDE2000 formula:

$$\Delta E_{00}^* = \sqrt{\left(\left(\frac{\Delta L'}{k_L S_L} \right)^2 + \left(\frac{\Delta C'}{k_C S_C} \right)^2 + \left(\frac{\Delta H'}{k_H S_H} \right)^2 + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H} \right)}$$

The results of these readings are shown in Supplementary Material Table S1.

2.7. Statistical Methods

A two-way ANOVA test followed by Tukey's HSD (honest significant difference) were run with SAS v9.4 on the pigment-treated pulps using treatment types, pulp types, and their interaction as the independent variables and the ΔE^* color change value as the dependent variable.

3. Results

Statistical analysis was performed with the ΔE^* values using two-way ANOVA and Tukey's analysis at $\alpha = 0.05$. The ANOVA showed that there was no statistical significance between treatments and pulps, but that there was a significant (p -value < 0.05) color change overall (Figure 1).

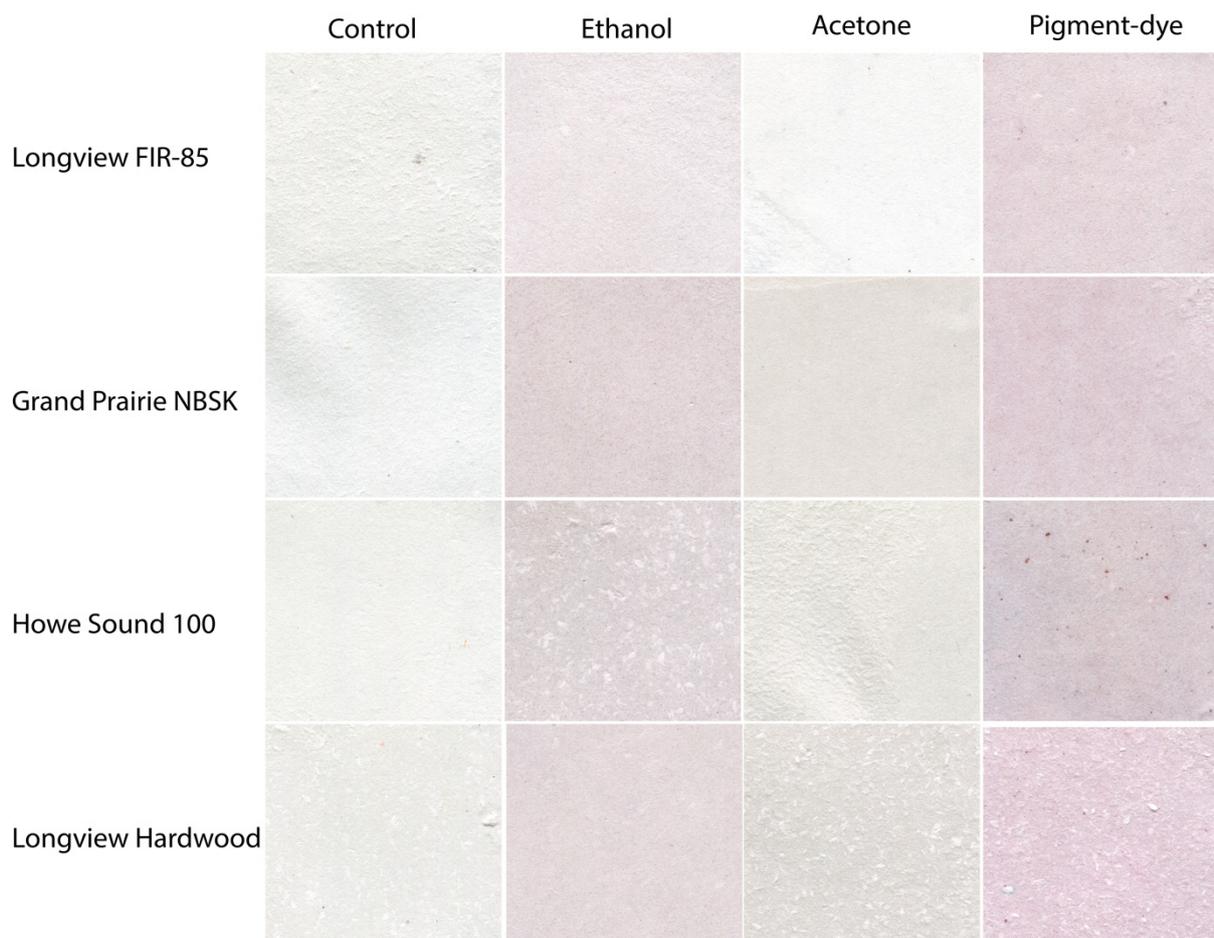


Figure 1. Scans of the final paper samples produced from the treated pulps by treatment type (vertical) and pulp type (horizontal).

Tukey's HSD analysis found that while the pigment dye (pigment suspended in DI water) treatment method on the Howe Sound 100 pulp produced the most coloration change and the acetone treatment method on the Howe Sound 100 pulp produced the least coloration change, no significant difference between these or any of the other samples occurred. Pigment dye had the highest mean color change among the treatment types. The Grand Prairie NBSK had the highest mean color change among the pulp types, but not significantly more so than all the other pulp and treatment types. These findings mirror qualitative results: cosmetically, each of the treated samples produced a noticeably more red/pink hue than that of their control pulp counterparts, but the differences in vibrancy among the treated samples were small. Pigments from *S. cuboideum*, regardless of how they were applied or to what type of pulp, were shown to produce color change in paper.

4. Discussion

One of the desired properties of pigment dyes is their ability to color pulps of different wood species with no significant color difference. This characteristic is essential because at the industrial level, coloration consistency is required across all products. With the results obtained from the test, the crystal pigment from *S. cuboideum* (dramada) can meet this requirement as there was no significant difference between the pigment delivery methods (DI water, acetone, and ethanol) and the pulps (softwood and hardwood). The color was also unaffected by the carrier (DI water, acetone, and ethanol). This is another advantage as different industrial processes require different pigment delivery methods.

Besides the coloration capacity, being able to add colorants in a water dispersion shows an advantage of dramada as a crystalline fungal pigment. The pigment can be incorporated in water-reliant processes such as the mat-forming stage in the paper manufacturing process [8], which allows for utilization in an array of industrial practices. Further testing will be required on paper quality after dyeing and the effects of the solvents, but previous research showed that the pigment generated by *S. cuboideum* can be carried in other types of solvents if necessary [37].

Another observation was that the coloration of the dry paper samples was even, thus showing that the different methods utilized did not cause stains of different tones on the final products. This will allow a larger-scale use of the pigment as it can be used in an identical manner to other pigment colorants in the paper industry [38]. More testing is required to evaluate the colorfastness of the fungal pigments in paper, but previous studies showed that the pigments are resistant to QUV light on textiles [30] and wood coatings [39].

An additional advantage of using spalting-derived pigments is that, generally, they possess low toxicity compared to inorganic pigment colorants [40]. Pulp and paper manufacturers are constantly pushing for more environmentally friendly processes as they were considered a high-pollution industry in the past [38], and the adoption of fungal pigments can be aligned with trends like biopulping [38,41,42].

There is great potential for the utilization of the crystalline pigment from *S. cuboideum* in paper manufacturing. However, more exhaustive testing is required to determine the interactions of the pigments with the mechanical properties of paper, colorfastness, optical properties of the dyed pulps, and their applications in an industrial setting.

5. Conclusions

The test showed that the crystal pigments of *Scytalidium cuboideum* carried in different carriers and applied to different pulps produced a significant change of color. However, no difference was found between the application methods (DI water, ethanol, and acetone) and the pulps (hardwood, softwood). These preliminary results have exciting implications for the future of the application of fungal pigments in the pulp and paper industry. Dramada has low environmental toxicity, especially compared to common inorganic dyes. This fungal pigment has the potential to replace red-based pulp dyes in the future, leading to a less problematic dye effluent in the soil and waterways.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/challe13010015/s1>. Table S1: The L*a*b* values for each pulp and treatment type.

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References

1. Klemm, D.; Heublein, B.; Fink, H.-P.; Bohn, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Angew. Chem. Int. Ed.* **2005**, *44*, 3358–3393. [[CrossRef](#)] [[PubMed](#)]
2. Torén, K.; Blanc, P.D. The history of pulp and paper bleaching: Respiratory-health effects. *Lancet* **1997**, *349*, 1316–1318. [[CrossRef](#)]
3. Bajpai, P. *Recycling and Deinking of Recovered Paper*; Elsevier Science: Burlington, ON, Canada, 2013.
4. West, C.J. *Pulping Processes*; Institute of Paper Chemistry: Appleton, WI, USA, 1949.
5. Roberts, J.C. *The Chemistry of Paper*; Royal Society of Chemistry: Cambridge, UK, 1996.
6. Forest Products Laboratory. *Commercial Processes of Pulping Woods*; Forest Products Laboratory, U.S. Forest Service: Madison, WI, USA, 1953.
7. Roth, L. *Constitution and Pulping of Aspen and Poplar Woods*; Institute of Paper Chemistry: Appleton, WI, USA, 1964.
8. Biermann, C.J. *Essentials of Pulping and Papermaking*; Academic Press: San Diego, CA, USA, 1993.
9. Biermann, C.J. *Handbook of Pulping and Papermaking*, 2nd ed.; Elsevier Science: Burlington, ON, Canada, 1996.
10. Przybysz Buzala, K.; Przybysz, P.; Kalinowska, H.; Przybysz, K.; Kucner, M.; Dubowik, M. Evaluation of pine kraft cellulosic pulps and fines from papermaking as potential feedstocks for biofuel production. *Cellulose* **2016**, *23*, 649–659. [[CrossRef](#)]
11. Bajpai, P. *Bleach Plant Effluents from the Pulp and Paper Industry*; Springer: Heidelberg, Germany, 2013.
12. Bajpai, P. *Environmentally Friendly Production of Pulp and Paper*; Wiley: Hoboken, NJ, USA, 2011.
13. Wang, X.; Zhang, H.; Ni, Y.; Du, Q.; Zhang, X.; Chen, J. Kinetics of PCDD/Fs formation from non-wood pulp bleaching with chlorine. *Environ. Sci. Technol.* **2014**, *48*, 4361. [[CrossRef](#)] [[PubMed](#)]
14. John, G.R. Paper making in a low carbon economy. *AIMS Energy* **2018**, *6*, 187–202. [[CrossRef](#)]
15. Drnovšek, T.; Perdih, A. Selective staining as a tool for wood fibre characterization. *Dye. Pigment.* **2005**, *67*, 197–206. [[CrossRef](#)]
16. Sekar, N. *Acid Dyes-15*; Elsevier Ltd.: Alpharetta, GA, USA, 2011; pp. 486–514. [[CrossRef](#)]
17. Vega Gutiérrez, S.M. Spalting Fungi: Genetic Identification, Material Interactions and Microscopical Characteristics of Extracted Pigments. Ph.D. Thesis, Oregon State University, Corvallis, OR, USA, 2017.
18. Lebeau, J.; Venkatachalam, M.; Fouillaud, M.; Petit, T.; Vinale, F.; Dufossé, L.; Caro, Y. Production and New Extraction Method of Polyketide Red Pigments Produced by Ascomycetous Fungi from Terrestrial and Marine Habitats. *J. Fungi* **2017**, *3*, 34. [[CrossRef](#)]
19. Robinson, S.C.; Hinsch, E.; Weber, G.; Leipus, K.; Cerney, D. Wood Colorization through Pressure Treating: The Potential of Extracted Colorants from Spalting Fungi as a Replacement for Woodworkers' Aniline Dyes. *Materials* **2014**, *7*, 5427–5437. [[CrossRef](#)]
20. Akilandeswari, P.; Pradeep, B. Exploration of industrially important pigments from soil fungi. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1631–1643. [[CrossRef](#)]
21. Robinson, S.C.; Richter, D.L.; Laks, P.E. Colonization of sugar maple by spalting fungi. *For. Prod. J.* **2007**, *57*, 24–32.
22. Robinson, S.C.; Richter, D.L.; Laks, P.E. *Inducing and Stimulating Spalting in Sugar Maple*; International Research Group on Wood Protection: Stockholm, Sweden, 2008.
23. Schmidt, E.L.; Dietz, M.G. *Arthrographis cuboidea* causing pink stain of sodium pentachlorophenoxide-treated red oak. *Mycologia* **1985**, *77*, 316–318. [[CrossRef](#)]
24. Robinson, S.C.; Tudor, D.; Cooper, P.A. Utilizing pigment-producing fungi to add commercial value to American beech (*Fagus grandifolia*). *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1041–1048. [[CrossRef](#)] [[PubMed](#)]
25. Anagnost, S.E.; Worrall, J.J.; Wang, C.J.K. Diffuse cavity formation in soft rot of pine. *Wood Sci. Technol.* **1994**, *28*, 199–208. [[CrossRef](#)]

26. Robinson, S.C.; Tudor, D.; Cooper, P.A. Feasibility of using red pigment producing fungi to stain wood for decorative applications. *Can. J. For. Res.* **2011**, *41*, 1722–1728. [[CrossRef](#)]
27. Robinson, S.C.; Weber, G.; Hinsch, E.; Vega Gutierrez, S.M.; Pittis, L.; Freitas, S. Utilizing Extracted Fungal Pigments for Wood Spalting: A Comparison of Induced Fungal Pigmentation to Fungal Dyeing. *J. Coat.* **2014**, *2014*, 759073. [[CrossRef](#)]
28. Robinson, S.C.; Hinsch, E.; Weber, G.; Freitas, S. Method of extraction and resolubilisation of pigments from *Chlorociboria aeruginosa* and *Scytalidium cuboideum*, two prolific spalting fungi. *Coloration Technol.* **2014**, *130*, 221–225. [[CrossRef](#)]
29. Weber, G.; Chen, H.-L.; Hinsch, E.; Freitas, S.; Robinson, S. Pigments extracted from the wood-staining fungi *Chlorociboria aeruginosa*, *Scytalidium cuboideum*, and *S. ganodermophthorum* show potential for use as textile dyes. *Coloration Technol.* **2014**, *130*, 445–452. [[CrossRef](#)]
30. Hinsch, E.M.; Weber, G.; Chen, H.-L.; Robinson, S.C. Colorfastness of Extracted Wood-staining Fungal Pigments on Fabrics: A new potential for textile dyes. *J. Text. Appar. Technol. Manag.* **2015**, *9*, 3.
31. Hinsch, E.M.; Robinson, S.C. Mechanical Color Reading of Wood-Staining Fungal Pigment Textile Dyes: An Alternative Method for Determining Colorfastness. *Coatings* **2016**, *6*, 25. [[CrossRef](#)]
32. Young, J.A. Aniline. *J. Chem. Educ.* **2009**, *86*, 683. [[CrossRef](#)]
33. Robinson, S.C.; Michaelsen, H.; Robinson, J.C. *Spalted Wood. The History, Science and Art of a Unique Material*, 1st ed.; Schiffer Publishing, Ltd.: Atglen, PA, USA, 2016; p. 287.
34. Hinsch, E.M. A Comparative Analysis of Extracted Fungal Pigments and Commercially Available Dyes for Colorizing Textiles. Master's Thesis, Oregon State University, Corvallis, OR, USA, 2015.
35. Vega Gutierrez, S.M.; Hazell, K.K.; Simonsen, J.; Robinson, S.C. Description of a Naphthoquinonic Crystal Produced by the Fungus *Scytalidium cuboideum*. *Molecules* **2018**, *23*, 1905. [[CrossRef](#)] [[PubMed](#)]
36. Vega Gutierrez, S.; Van Court, R.C.; Stone, D.; Konkler, M.; Groth, E.; Robinson, S. Relationship between Molarity and Color in the Crystal ('Dramada') Produced by *Scytalidium cuboideum*, in Two Solvents. *Molecules* **2018**, *23*, 2581. [[CrossRef](#)] [[PubMed](#)]
37. Pittis, L.; Rodrigues de Oliveira, D.; Vega Gutierrez, S.M.; Robinson, S.C. Alternative Carrier Solvents for Pigments Extracted from Spalting Fungi. *Materials* **2018**, *11*, 897. [[CrossRef](#)] [[PubMed](#)]
38. Messner, K.; Srebotnik, E. Biopulping: An overview of developments in an environmentally safe paper-making technology. *FEMS Microbiol. Rev.* **1994**, *13*, 351–364. [[CrossRef](#)]
39. Vega Gutierrez, S.M.; Stone, D.W.; He, R.; Vega Gutierrez, P.T.; Walsh, Z.M.; Robinson, S.C. Potential Use of the Pigments from *Scytalidium cuboideum* and *Chlorociboria aeruginosa* to Prevent 'Greying' Decking and Other Outdoor Wood Products. *Coatings* **2021**, *11*, 511. [[CrossRef](#)]
40. Almurshidi, B.H.; Van Court, R.C.; Vega Gutierrez, S.M.; Harper, S.; Harper, B.; Robinson, S.C. Preliminary Examination of the Toxicity of Spalting Fungal Pigments: A Comparison between Extraction Methods. *J. Fungi* **2021**, *7*, 155. [[CrossRef](#)]
41. Kirk, T.K. *Biotechnology in Pulp and Paper Manufacture Applications and Fundamental Investigations*; Elsevier Science: Burlington, ON, Canada, 2013.
42. Singh, P.; Sulaiman, O.; Hashim, R.; Rupani, P.; Peng, L. Biopulping of lignocellulosic material using different fungal species: A review. *Rev. Environ. Sci. Biotechnol.* **2010**, *9*, 141–151. [[CrossRef](#)]