Fungal Pretreatment Method Optimization for Small Wood Samples Degraded by *Ceriporiopsis Subvermispora*

Charles Warren Edmunds[1], Perry Peralta[2], and Ilona Peszlen[2], Richard Giles[3], and Vincent Chiang[2]

[1] Graduate Student , [2] Associate Professor, Department of Forest Biomaterials, North Carolina State University, Campus box 8005, Raleigh, NC 27695-8005, U.S.A.; [3] Department of Biology, University of North Carolina a t Charlotte, 9201 University City Blvd., Charlotte, NC 28223

INTRODUCTION

Environmental concerns and finite fossil fuel reserves drives research for the production of bio-energy and bio-products. Fungal pretreatment has been explored as a low-cost and environmentally friendly method to increase the reactivity of lignocellulosic biomass prior to biofuel/biochemical or pulping processes (Akhtar et al., 1997; Wan and Li, 2012). Testing of genetically modified wood specimens is increasingly becoming popular due to advances in the field and the potential to greatly enhance the wood's chemical and physical properties. One drawback of such research is the frequent requirement of testing young small greenhouse-grown wood specimens. Well established standards exist for testing woods' resistance to fungal degradation such as the U.S. standard "soil block test" (AWPA "E10-09", 2010) and the European standard "agar block test" (BS 113:2004/A1, 2004); however, these methods are not well suited for small young cylindrical wood samples with the goal of pretreating wood to increase reactivity.

This project explores the utilization of a white-rot wood degrading fungus (*Ceriporiopsis subvermispora*) as a means to increase biomass reactivity. The objective is to test several variables in the inoculation and incubation methods of small wood specimens to find which method results in a sufficient amount of biomass degradation (measured by weight loss) and low variation between replicates. The variables tested include: inoculation medium, wood particle size, and incubation container.

MATERIALS AND METHODS

Wood Samples
- Sweetgum wood (dowels 0.5 in dia.) were obtain from Capital City Lumber (Raleigh, NC)
- All samples dried at 103 °C in oven and weighed
- Water was added to condition wood to moisture content of 50 or 60% (wet basis)
- Samples were sterilized in autoclave at 121 °C for 20 min

Fungal Culture
- *Ceriporiopsis subvermispora* lignin selective white-rot fungus
- Cultured on 5% malt extract agar (MEA) plate for 7 days, then a fungal plug was used to inoculate 1) a liquid culture (2.5% malt extract) incubated at 27 °C for 30 days; or 2) 140 mm petri dish containing MEA
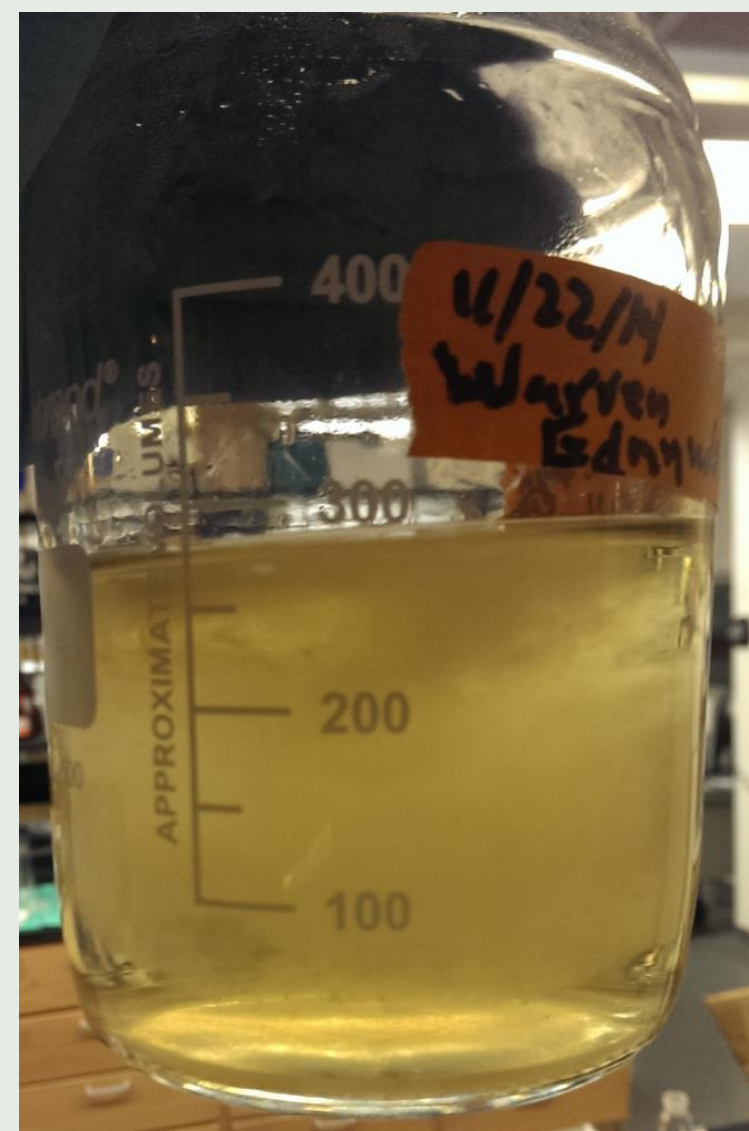
Statistics
- SAS (Cary, N.C.) software was used to perform ANOVA and mean separation

Figure 1. *Ceriporiopsis subvermispora* in liquid culture

METHODS TESTED

Method 1

- 3 stems (0.5 in. dia) per 20 ml scintillation vial
- Fungal mycelium was filtered, and rinsed with DI water
- 2 ml of slurry was used to inoculate wood
- Mycelium slurry was homogenized by:
  - Shaking (15 samples)
  - Blending (15 samples)
- Sterile control (5 samples)
- Incubated for 40 days

Method 2

- ~1g of ground wood (40 mesh) per 20 ml scintillation vial
- Fungal mycelium was filtered, rinsed with DI water, and suspended in DI water
- 1ml of slurry used to inoculate wood
- Mycelium slurry was homogenized by:
  - Shaking (15 samples)
  - Blending (15 samples)
- Sterile control (5 samples)
- Incubated 40 days

Method 3

- 140 mm dia. Petri dish with agar-water
- 6 sets of three stems on glass rod supports
- Fungal mycelium was filtered, rinsed with DI water, and suspended in DI water
- Mycelium slurry was homogenized by shaking
- 2 ml of slurry used to inoculate wood
- Three fugal treatment plates (6 samples x 3 plates = 18 reps)
- 1 sterile control plate (1 plate x 6 samples = 6 reps)
- Incubated 40 days

Method 4

- 140 mm dia. Petri dish with malt extract agar
- 6 sets of three stems on glass rod supports
- Plate was pre-inoculated, and fungi covered surface before wood was added
- Three fugal treatment plates (6 samples x 3 plates = 18 reps)
- Incubated 40 days

Method 5

- 3 stems (0.5 in dia.) per 20 ml scintillation vial, cap loosened
- Fungal mycelium not filtered
- Liquid culture was shaken to homogenize and 2ml was directly added to inoculate wood
- 1 ml of slurry used to inoculate wood
- 5 fungal treated samples
- 3 sterile control samples
- Incubated 30 days

RESULTS

Weight Loss

Figure 2. Weight loss results after fungal pretreatment. Error bars represent standard deviation, and different letters represent significantly different means in sampled degraded by fungus (Tukey's α=0.05)
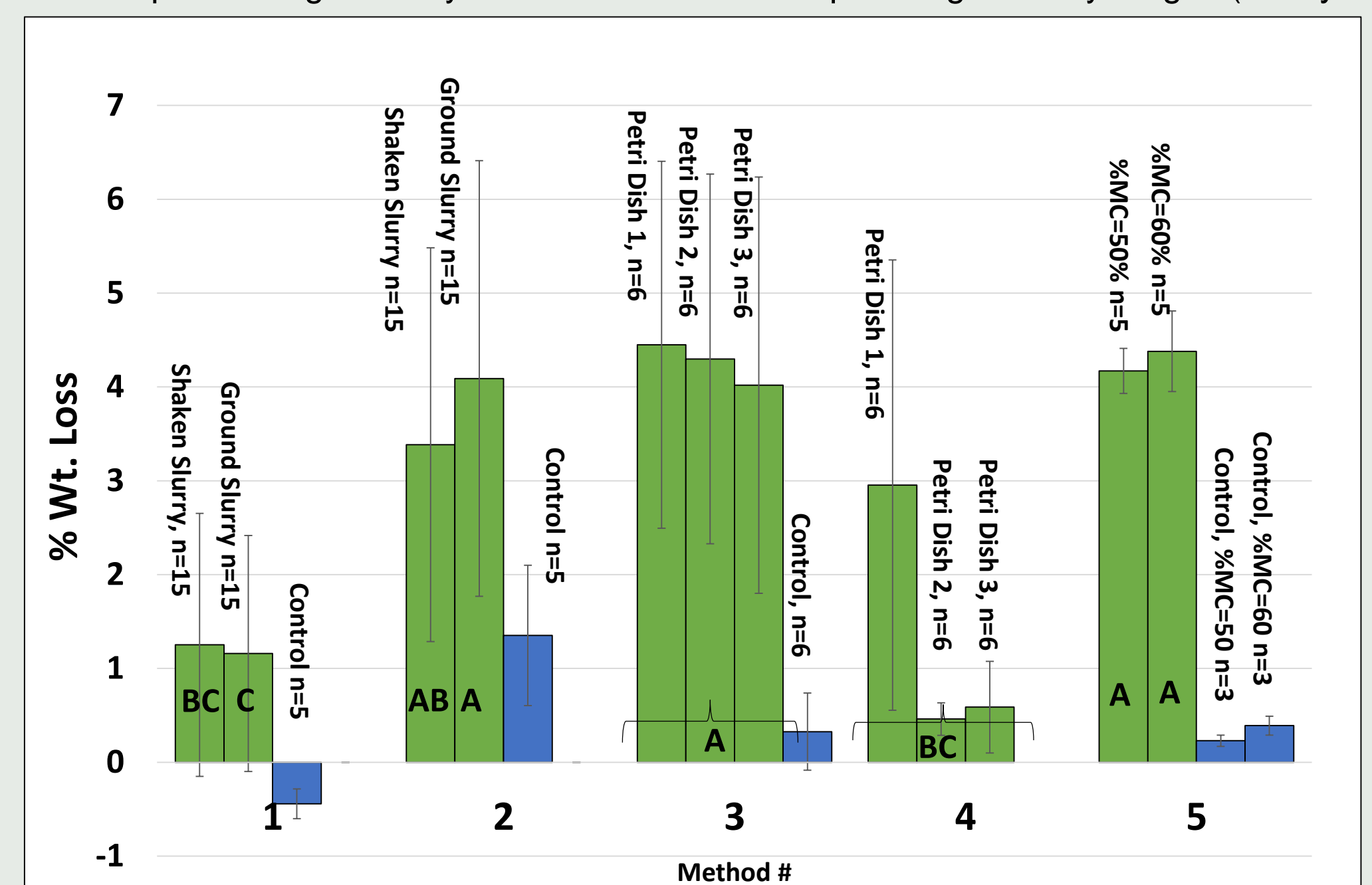
- **Methods 1 and 2** – Not much fungal growth
- **Method 2** – Higher weight loss in sterile control due to smaller particle size
  - No significant difference between shaken and blended
- **Method 3** – Greater weight loss, but standard deviation is still high
- **Method 4** – Too much MAE resulted in little wood degradation (fungi preferred malt extract)
- **Method 5** – Weight loss similar to Method 3
  - Variation between replicates is **much lower** than other methods

Figure 3. Images from Methods 5

CONCLUSIONS

- **No** significant difference **between shaking** and **blending** homogenization of fungal mycelium slurry
- **Filtering and rinsing** mycelium may have been responsible for **poor/inconsistent fungal growth**
  - Temperature shock, osmotic shock, and/or pH shock could be to blame
- Using **non-filtered** and **non-rinsed** mycelium for inoculation resulted in **more fungal growth**, **greater weight loss**, and **less variation** between samples as seen in Method 5
- Using **loose cap** instead of Parafilm© may allow **more gas exchange**
- **Method 5** was utilized for future fungal pretreatment experiments

REFERENCES

Akhtar, M., Blanchette, R. A., and Kirk, T. K. 1997. "Fungal Delignification and Biomechanical Pulping of Wood." In *Biotechnology in the Pulp and Paper Industry*, ed. Eriksson K. EL. Springer Verlag, 159–96.
AWPA. (2010). E10-09: Standard methods of testing wood preservatives by laboratory soil-block cultures. *AWPA Book of Standards*.
BS 113:2004/A1. (2004). BS EN 113/A1: Wood preservatives — Test method for determining the protective effectiveness against wood destroying basidiomycetes — Determination of the toxic values. *British Standard*, 3.
Wan, C., and Li, Y. 2012. "Fungal Pretreatment of Lignocellulosic Biomass." *Biotechnology advances* 30(6): 1447–57.

ACKNOWLEDGMENTS