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Mycoremediation of Waste Wood Treated with CCA and ACQ

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ABSTRACT: Chromated copper arsenate (CCA) is no longer the wood preservative of choice for most residential uses, but large amounts of CCA-treated wood have been stockpiled or remain in use and will require waste management when removed from service. Appropriate disposal will also be needed for spent wood treated with copper-based preservatives, such as ammoniacal copper quaternary (ACQ), which have replaced CCA. Mycoremediation was evaluated as a waste management approach for CCA and ACQ-treated wood waste using wood decay fungi that are major degraders of lignocellulose. Preservative tolerant fungal strains were isolated and assayed for the capacity to degrade treated wood by an ASTM standard method. Two strains of *Meruliporia incrassata* and two strains of *Antrodia radiculosa*, exhibiting the highest tolerance to CCA and highest capacity to degrade CCA wood, were used to develop a mycoremediation technology that includes the preparation of a specially formulated fungal inoculum along with wood treatment conditions. While the discovery stage of the technology can continue to develop better fungal strains, the technology is ready for on-site scale-up treatment of waste wood.

INTRODUCTION

Chemically treated wood waste is a growing problem as pressure-treated residential decks are discarded, aged railroad ties and utility poles are removed, and buildings are demolished to make way for new. Chromated copper arsenate (CCA) is no longer the wood preservative of choice for most residential uses such as playsets and decks, but large amounts of CCA-treated wood have been stockpiled or remain in use and will require waste management when removed from service. Appropriate disposal will be needed for spent wood treated with the copper-based preservatives, such as ammoniacal copper quaternary (ACQ), which have replaced CCA.

Mycoremediation is alternative to stock piling or depositing CCA and ACQ wood waste in landfills. Fungi are the major degraders of the lignocellulose and hemicellulose components of woody biomass on the forest floor and wood structures. Wood decay fungi secrete enzymes and metabolites that degrade environmental contaminants. Rare strains of Basidiomycota and Ascomycota have been shown to be tolerant to organopollutants and toxic metals. Early work on fungal remediation of wood preservatives focused on wood treatment sites, with special emphasis on soil remediation (Lamar et al. 1994).

We have developed technologies for fungal bioremediation of waste wood treated with oilborne or metal-based preservatives. The technologies are based on specially formulated inoculum of wood decay fungi, obtained through strain selection to obtain preservative tolerant fungi. In this review paper we describe research on the bioremediation of CCA-treated wood waste with brown-rot fungi (Illman and Yang, 2000; Illman and Yang, 2006; Yang and Illman, 1999).

The objectives of the research were to isolate and characterize CCA-tolerant fungi, define economical materials and methods to prepare and package viable inoculum of metal-tolerant

decay fungi, establish treatment procedures for the remediation and degradation process, and conduct a laboratory scale-up to evaluate the method on solid preservative treated lumber.

MATERIALS AND METHODS

Fungi were collected from field test sites in Gulfport, Mississippi, and Madison, Wisconsin, or selected from the extensive fungal library at the Center for Forest Mycology Research, Forest Products Laboratory (FPL), Madison, Wisconsin. Fungi were cultured on 2% malt extract agar (DifcoBacto) or a modified Taylor's medium at 27° C and 70% relative humidity (RH). Mycelium was stored on 2% malt extract agar test tube slants or in Petri dishes at 4 ^{o} C. Fungal tolerance to CCA was determined by two bioassays, a growth response to CCA or ACQ treated wood and fungal growth in culture media amended with Cu, Cr, or As.

Optimum Growth Conditions. Temperature for optimum growth was determined by an in vitro assay. Four disks (9 mm) of freshly grown fungal cultures were removed from 2% MEA plates and inoculated into 125 ml Erlenmeyer flasks containing 25 ml of 2% malt extract liquid medium (DifcoBacto). Flasks were placed in an incubator at 20°C, 27°C, 32°C, or 37°C at 70% RH in the dark for 12 days. Mycelium was harvested by straining the liquid culture through previously weighed Whatman No. 1 filter paper, allowing the mycelia to air dry on the paper, reweighing and calculating biomass dry weight. The effect of oxygen and chemically defined media on fungal growth was determined for liquid cultures. Four disks (9 mm) of freshly grown fungal cultures were removed from 2% MEA plates and inoculated into 125 ml Erlenmeyer flasks containing 25 ml of Bailey's medium (Bailey et al. 1969) or BIII medium (Kirk et al. 1986). Flasks were kept stationary in an incubator at 27° C and 70% RH in the dark for 21 days, with or without an exposure to a 20 second oxygen flush on alternate days. Mycelia were harvested and biomass dry weight determined as above with temperature and light.

Fungal Inoculum. Nutrient supplements were tested for effects on fungal growth. Disks (9 mm) of freshly grown fungal cultures were removed from 2% MEA culture plates and inoculated into 125 ml Erlenmeyer flasks containing 25 ml of 2% malt extract liquid medium supplemented with or without 1% sterile corn steep liquor (CSL) from corn processing (ADM, Cedar Rapids, Iowa). Flasks were kept stationary in an incubator at 27°C and 70% RH for 3 weeks. Mycelium was separated from liquid culture by filtration through previously weighed Whatman No. 1 filter paper, allowed to air dry on the paper and reweighed. Dry weight of CSL fed mycelium was expressed as % dry weight of mycelium grown without CSL. Lignocellulose substrates (sawdust, wood chips, rice straw, corn stalks, and wheat straw) were evaluated for effectiveness as a long-term food source in the fungal inoculum and as a matrix for inoculum storage and handling. Similar methods apply to all. Sawdust and wood chips were steam-sterilized and cooled at room temperature prior to mixing with the fungal nutrient supplement mixture. Steam sterilization of the lignocellulose substrate is preferred as the steam provides both sterility and moisture content. Moisture from steam-sterilization enhances fungal growth.

Wood degradation. Blocks of southern yellow pine (1 x 1 x 0.3 inches) were treated with CCA to 6.4 kg/m^3 (.40-pounds/cubic foot) according to American Wood Preserver's Association standards (AWPA 1991). Treated blocks were inoculated with *Meruliporia incrassata*

(TFFH-294), *Antrodia radiculosa* (MJL-630), *Meruliporia incrassata* (Mad-563) or *Antrodia radiculosa* (FP-90848-T) according to the ASTM standard soil bottle decay test (ASTM 1991). Decay was expressed as per cent weight loss. A fungal inoculum amended with nutrients and lignocellulose supplements was prepared with minor modification of the method described in the section above. Fungal mycelium was transferred from stock culture to 10 ml 2% MEA in a glass bottle (2 x 2 x 5 inches) and incubated in the dark at 27°C and 70% RH for 2 weeks. The resulting mycelium was mixed in the bottle with 10 g of sterile sawdust, 20 ml sterile water, and 20 ml sterile 1% CSL or 0.25 g of a 50/50 mixture of sterile wheat-bran and cornmeal, incubated in the dark at 27°C and 70% RH for 6 weeks.

Laboratory Scale-Up. The inoculum preparation described in the sections above was modified to evaluate the effectiveness of the method on larger volumes of solid lumber and particulate, flaked, or chipped CCA-treated southern yellow pine. Sawdust (350 g) was sterilized in an aluminum tray (9 x 13 x 2.5”), cooled to room temperature, mixed with 700 ml 1% CSL. *Meruliporia incrassata* (TFFH-294) was cultured in seven Petri dishes (14 cm diameter) containing 2% MEA, incubated at 27° C and 70% RH for 2 weeks. The resultant culture was cut into pieces (approximately 1.5 in square), mixed with the solid substrate in the tray, incubated at 27°C, 70% in the dark for 8 weeks. If not used for processing right away, the inoculum should be stored at 4°C. The bioprocessing method was evaluated in a laboratory scale-up with CCA-treated and nontreated southern yellow pine lumber. Several large metal chambers (33 x 6 x 8 inches) with sliding covers were custom-made for the decay test on lumber. A 2-inch layer of moistened soil with a water content of 35% lined the bottom of the chamber. Test samples of CCA-treated and nontreated wood (2 x 4 x 12 inch) were placed on top of the soil and steam sterilized. After the chambers had cooled to room temperature, wood was completely covered with the *Meruliporia incrassata* TFFH-294 inoculum. The closed chamber was placed in the dark in an incubator at 27°C and 70% RH for 12 weeks.

RESULTS AND DISCUSSION

Brown- and white-rot wood decay fungi (150) were isolated from metal-treated wood. Only 18 fungal isolates were CCA or ACQ tolerant. The brown-rot fungus, *M. incrassata* (TFFH-294) exhibited the most tolerance and was selected for the growth response studies. Two isolates of M. incrassata were tolerant to 1mM copper, chromium and arsenic. Four isolates, *Meruliporia incrassata* (TFFH-294), *Antrodia radiculosa* (MJL-630), *Meruliporia incrassata* (Mad-563) and *Antrodia radiculosa* (FP-90848-T), degraded the CCA-treated wood more than 20% of the original dry weight of the wood. Supplements enhanced degradation of CCA-treated. *M. incrassata* (TFFH-294) degraded CCA-treated lumber by 28%. The fungus produced 33% more biomass when incubated in the dark for 24 hr per day than for 24 hr per day in light. Biomass production was slightly higher on Bailey’s than on BIII medium and production was enhanced on both media when exposured to oxygen.

Fungal growth and biomass production were enhanced by the addition of 1% CLS to the growth medium (Table 1), but was adversely affected by higher concentrations. Lignocellulose provided a solid, organic matrix for the fungal inoculum.

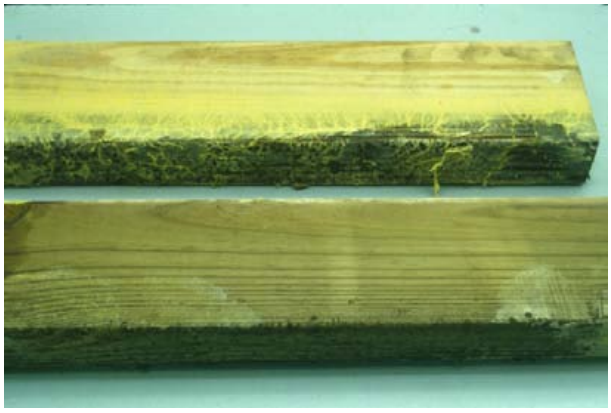

FIGURE 1. *Meruliporia incrassata* (TFFH 294) decay of CCA-treated wood. Top lumber is nontreated southern yellow pine and bottom is CCA-treated.

TABLE 1. Effect of corn steep liquor on biomass production of metal-tolerant *Meruliporia incrassata* (TFFH-294)

CSL concentration	% Dry weight[a]
0	100
1.0 %	321
2.5 %	256
5.0 %	196

[a]% dry weight of mycelium grown without CSL

The inoculum in this method has many advantages. It is cost effective, utilizing agricultural waste products and waste products from saw mills and urban chipping. The products provide a quick and low cost food source for the fungus, stimulate rapid and extensive fungal growth, and provide a readily storable and transportable solid matrix. Fungal growth is substantially enhanced with aeration and nutrients added to the culture medium. This method is designed for remediating and degrading solid pieces of lumber (Figure 1), not costly chipping or flaking that may require environmental oversight in the future with increased concerns about worker exposure to airborne particles; can be conducted on a small scale without costly transportation to larger, distant disposal sites; is designed for a wood use category that will not require extensive sorting, i.e., wood from docks, decks or landscaping can be kept separate more easily than mixed grades of wood from building demolition. The CCA-treated wood in this study was not wood waste; therefore we expect higher yields with spent wood taken out of service.

The mechanism(s) of preservative tolerance is not known. In parallel experiments, novel strains of *Antrodia radiculosa* and *Neolentinus lepideus* showed promise for remediating waste wood treated with the aromatic and chlorinated preservatives creosote and pentachlorophenol, respectively. This technology represents a broader use of fungi for bioremediation than the established soil bioremediation developed in our Institute at the Forest Products Laboratory.

CONCLUSIONS

An economical method was developed to reduce the volume of CCA and ACQ treated wood waste as an alternative to landfilling. While the discovery stage of the technology can continue to develop better fungal strains for mycotransformation or mycodetoxification of environmental pollutants and for degradation of lignocellulosics, the mycoremediation technology is ready for the scale-up stage. Our long-term goal is to work with partners to conduct on-site in situ mycoremediation of waste wood treated with CCA and other preservatives.

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