Bioremediation and Degradation of CCA-Treated Wood Waste

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ABSTRACT

Bioprocessing CCA wood waste is an efficient and economical alternative to depositing the waste in landfills, especially if landfill restrictions on CCA waste are imposed nation wide. We have developed bioremediation and degradation technologies for microbial processing of CCA waste. The technologies are based on specially formulated inoculum of wood decay fungi, obtained through strain selection to obtain metal tolerant fungi. Two strains of Meruliporia incrassata and two strains of Antrodia radiculosa were selected for capacity to degrade CCA wood, thereby reducing the waste volume. Formulation of the fungal inoculum requires a lignocellulose substrate and a nutrient supplement to optimize fungal establishment and growth on the waste wood. The inoculum can be prepared in standard sterile containers or small to intermediate scale bioreactors, applied to the waste wood and maintained in an aerated and hydrated environment having temperature conditions sufficient to allow fungal growth. Oxidation states of chromium, copper and arsenic remained constant during wood processing as determined earlier by synchrotron-based technologies. The criteria for selecting fungal strains, nutrients, lignocellulose substrate, optimum growth conditions and wood analysis provided the basis for developing a fungal processing system to degrade CCA-treated wood waste while removing the metals.

Keywords: Bioremediation, CCA, wood waste, *Meruliporia incrassata*

INTRODUCTION

Wood treated with chromated copper arsenate (CCA) in service in the United States was estimated to be over 85 million metric tons in 1997, with 8 million metric tons being produced each year [1]. CCA-treated wood removed from service is primarily sent to landfills. Large volumes of CCA-treated wood waste are expected be added to the solid waste stream in the next 20 years, causing potential problems for landfills [1,2,3]. Historically, economic, regulatory and environmental pressures stimulate development of alternative disposal methods and lead to recovery of waste wood. Between 1990 and 1999, 42.3 million tons of recoverable wood waste dropped to 29.6 million tons, primarily due to diversion of the waste to new uses or new materials [4]. Alternative strategies are being developed to decrease the volume of CCA-treated wood waste from landfill disposal. Several strategies will be useful in meeting the demands of differing collection methods,

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use categories, current urban and rural waste management systems, and desired end products. An economically feasible method for reducing the volume of CCA-treated waste and recovering the metals will serve small-scale niche markets.

As a nonspecific preservative, CCA has been highly effective in protecting wood against decay for many years. Most species of decay fungi are not CCA-tolerant and do not degrade the metal-treated wood. With long-term selection pressures on a population, however, it is possible for a fungal strain to develop resistance to preservatives, including CCA. We have screened hundreds of fungi in search of these rare strains as they have putative roles in the clean up of toxic waste sites and in the disposal of preservative-treated wood waste.

The objective of this research was to develop a method for fungal remediation and degradation of CCA-treated wood waste by (1) isolating and characterizing CCA-tolerant fungi, (2) defining economical materials and methods to prepare and package viable inoculum of metal-tolerant decay fungi, (3) establishing treatment procedures for the remediation and degradation process, and (4) conducting a laboratory scale-up to evaluate the method on solid lumber treated with CCA. This paper presents new and summarizes past research [5,6,7].

MATERIALS AND METHODS

Wood Decay Fungi

Isolation and culture conditions

Fungi were collected from field test sites or selected from the extensive fungal library at the Center for Forest Mycology Research, Forest Products Laboratory (FPL), Madison, WI, USA. The FPL fungal library was screened for isolates (identified to species but not to strain) that were collected from preservative-treated wood products, such as decks, poles and test stakes that had visible signs of decay. Additional isolates were taken from 20-30 year-old preservative-treated wood stakes taken from the FPL field test plots in Gulfport, Mississippi and Picnic Point, Madison, WI. 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° C.

Metal Tolerance Assay

Fungal tolerance to CCA was determined by two methods, an *in vitro* bioassay described as a 'choice test' by Leithoff et al. [8] and a growth response bioassay of *in vitro* exposure to metals. Briefly, for the 'choice test' assay a 9 mm fungal disk from a freshly grown malt agar culture was placed in the center of a Petri dish (14 cm diameter) containing 12 ml of a 2% agar medium with a CCA-treated and a non-treated southern yellow pine wood sample (1.5 cm x .3 cm) placed at opposite edges of the plate. Fungi were kept in an incubator at 27°C and 70% relative humidity (RH) for 14 days, observed for growth response to the treated wood. Metal tolerance was rated as fungal growth toward and/or on the treated wood. For the metal exposure assay, copper, chromium or arsenic were incorporated into 2% MEA in

Figure 1. Collecting field stakes to isolate fungi.

Petri plates and inoculated in the center with *Meruliporia incrassata* (TFFH-294) and *Meruliporia incrassata* (TFFH-295). Cupric sulfate, potassium dichromate or sodium arsenate (Aldrich) were added to MEA at levels of 0, 0.1, 1 10 and 100 mM of Cu, Cr, or As. Mycelium was measured from the center of the plate on day 14 to determine metal effect. Response was expressed as % of growth without metals in the medium.

Optimum Growth Conditions

Temperature: 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, 27, 32 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.

Light conditions: Liquid cultures and biomass dry weight were prepared as described above for temperature. Flasks were kept stationary in an incubator at 27° C and 70% RH for 12 days under one of the following light regimes: 24 hours of light, 12 hours of light with 12 hours of darkness, or 24 hours of darkness.

Oxygen and chemically defined medium: 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 [9] or BIII medium [10]. 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 [9]. Mycelia were harvested and biomass dry weight determined as above with temperature and light. There were three replicate flasks per medium per oxygen treatment.

Fungal Inoculum

Nutrient Supplement

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. Biomass dry weight of CSL fed mycelium was expressed as % dry weight of mycelium grown without CSL.

Lignocellulose substrate

The 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

Wood decay test

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 (AWPA) standards [11].

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 [12]. Briefly, CCA-treated blocks (2.5 by 2.5 by 0.9 cm) were inoculated with fungi in soil-bottles, incubated for 12 weeks at 27 C and 70% RH, and weight loss determined. Treated and nontreated control blocks were incubated without exposure to fungi. The test was replicated 5 times. Decay was expressed as per cent weight loss.

Effect of supplements on decay

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 wheatbran and cornmeal, incubated in the dark at 27 C and 70% RH for 6 weeks. Names of fungal isolates are listed in Table 6.

Laboratory Scale-up

Fungal Inoculum

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 $27\,^{o}$ C, 70% in the dark for 8 weeks. If not used for processing right away, the inoculum should be stored at $4\,^{o}$ C.

Bioprocessing

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 $27\,^{o}$ C and 70% RH for 12 weeks. Weight loss of wood was calculated as described for the decay test above.

RESULTS

Wood Decay Fungi

A total of 150 brown- and white-rot wood decay fungi were obtained from metal-treated wood. The isolates that exhibited CCA-tolerance in the ‘choice test’ are listed in Table 1. Most fungi grew toward nontreated wood with no growth toward CCA-treated wood. The 18 fungal isolates in Table 1 grew toward and/or on CCA-treated wood. 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 (Table 2).

The response of *M. incrassata* (TFFH-294) to temperature and light was the same as reported for

Table 1. Wood decay fungi tolerant to CCA[a]

Species	Isolate	Collection Site
Antrodia radiculosa	L-11659-sp	FPL-MC[b]
Gloeophyllum subferrugineum	FRI 417/R	FPL-MC
Polyporus sp.	FP134933	FPL-MC
Trichaptum byssogenum	FP105308-R	FPL-MC
Gloeophyllum trabeum	Boat 228	FPL-MC
--	TLH-1	FPL[c]
Antrodia radiculosa	MJL-630	FPL-MC
Neolentinus lepideus	HHB 1 3625	FPL-MC
Antrodia xantha	MB268	FPL-MC
--	CAC-1	FPL
--	P6G	FPL-pp[d]
--	P71H	FPL-pp
--	UpK	FPL
--	UpL	FPL
Meruliporia incrassata	FFH-294	FPL
Meruliporia incrassata	Mad-563	FPL
Antrodia radiculosa	FP-103272-sp	FPL-MC
Antrodia radiculosa	FP-90848-T	FPL-MC

[a]CCA toleranc determined by 'choice test'
[b]FPL-MC Forest Products Lab Center for Forest Mycology Research
[c]FPL-RWU4502 Forest Products Lab Biodeterioration Unit
[d]FPL-PP Forest Products Lab Picnic Point

most wood decay fungi, known to be metal-sensitive [10]. The optimum temperature range for *M. incrassata* (TFFH-294) biomass production was between 27 ^{o} C. and 32 ^{o} C, with declining production at the higher and lower temperatures of 35 ^{o} C and 20 ^{o} C (Table 3). Light inhibited fungal growth (Table 4). The fungus produced 33% more biomass when incubated in the dark for 24 hr per day than for 24 hr per day in light. *M. incrassata* (TFFH-294) exhibited a similar growth response on chemically defined medium and exposure to oxygen as that reported for many brown-rot fungi and for the white-rot fungus, *Phanerochaete chrysosporium* [13,10]. Biomass production was slightly higher on Bailey's than on BIII medium and production was enhanced on both media when exposured to oxygen (Table 5).

Table 2. Growth response of Meruliporia incrassata TFFH-294 and MAD-563 on malt extract agar amended with copper, chromium or arsenic

	Inhibition of growth (%)									
	M. incrassata (TFFH-294)					M. incrassata (MAD-563)				
Metal (mM)	0	0.1	1.0	10	100	0	0.1	1.0	10	100
Copper	0	0	0	100	100	0	0	0	100	100
Chromium	0	0	0	100	100	0	0	0	100	100
Arsenic	0	0	13.3	80.3	100	0	0	0	100	100

Table 3. *Meruliporia incrassata* (TFFH 294) response to light

Light condition	weight (mg)
24 hours of light	66 ± 8*
12 hours of light	70 ± 6
24 hours of dark	88 ±13

* ± Standard error

Table 4. *Meruliporia incrassata* (TFFH-294) growth response to oxygen and culture medium

Medium	O_2	weight (mg)	pH
Bailey	+	23 ±1	2.73
	–	20 ±1	3.04
BIII	+	21 ±2	2.96
	–	18 ±2	3.61

Fungal inoculum

Fungal growth and biomass production were enhanced by the addition of 1% CLS to the growth medium, but was adversely affected by higher concentrations (Table 5). Lignocellulose provided a solid, organic matrix for the fungal inoculum.

Wood Decay

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 (Table 6).

Supplements enhanced degradation of CCA-treated wood (Table 7).

Laboratory Scale up

M. incrassata (TFFH-294) degraded CCA-treated lumber by 28%. Fungal growth is slower on treated vs. nontreated wood, but mycelia are clearly visible on the outer surface and interior of the wood (Figure 3).

Figure 3. *Meruliporia incrassata* (TFFH 294) decay of CCA-treated wood. Top lumber is nontreated southern yellow pine and bottom is CCA-treated.

Table 5. 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

Table 6. Fungal Degradation of Preservative-Treated Wood*

Fungal species	UNTREATED Weight (mg) Avg	(SD)	CCA Weight (mg) Avg	(SD)
Meruliporia incrassata (TFFH-294)	62.2	(2.9)	36.8	(2.7)
Antrodia radiculosa (MJL-630)	32.6	(4.8)	26.6	(2.9)
Meruliporia incrassata (Mad-563)	62.5	(2.5)	23.7	(3.2)
Antrodia radiculosa (FP-90848-T)	39.5	(4.1)	20.1	(7.7)
Antrodia radiculosa (FP-103272-sp)	24.6	(6.0)	6.5	(4.7)
Antrodia radiculosa (FP-105309-R)	27.2	(3.0)	2.3	(0.8)
Antrodia radiculosa (L-11659-sp)	23.1	(2.7)	1.3	(1.3)
Neolentinus lepideus (Mad-534)	38.8	(5.3)	0.7	(0.4)

* ASTM D-1413-76 Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures

Table 7. Effect of inoculum supplements on *Meruliporia incrassata* TFFH 294 degradation of CCA-treated wood

Fungal Species	Isolate	Inoculum Supplements: No supplement[a] weight (mg)	CMWB[b]	CSL[c]
Antrodia radiculosa	L-11659	32.0	150%	446%
Meruliporia incrassata	TFFH 294	34.0	32%	208%

[a]Inoculum with no CMWB or CSL
[b]Corn meal and wheat bran amended inoculum
[c]Corn starch liquor amended inoculum
[d]Weight of CCA-treated wood inoculated with no supplements
[e]Degradation expressed as % of wood weight loss with no supplements

DISCUSSION

This paper describes the development of a method for the fungal degradation and/or bioremediation of CCA-treated wood waste, providing a product with reduced waste volume and the capacity for reuse of waste metals. A market analysis confirmed the economic feasibility of the method (unpublished). An ICP/MS analysis of residual metals in the wood waste will be reported elsewhere. Early results indicate that residual metal concentrations decrease during the processing.

Unique, natural mutants of wood decay fungi were taken from FPL collections or isolated from field test samples, characterized and reintroduced into CCA-treated wood to determine if they degraded wood, testing Koch's postulates. Fungal isolates identified as CCA-tolerant and as degraders of CCA-treated wood include *Meruliporia incrassata* (TFFH-294), *Antrodia radiculosa*

(MJL-630), *Meruliporia incrassata* (Mad-563) and *Antrodia radiculosa* (FP-90848-T), *Antrodia radiculosa* (L-11659).

An advantage of the bioprocessing method is that the CCA-tolerant fungi do not require genetic alteration to grow in the presence of and degrade CCA-treated wood. Thus, the introduction of the fungi into the environment provides no new, non-naturally occurring organisms. However, the method is designed for contained waste management facilities where fungal viability and growth can be altered with temperature, water, oxygen, light, nutrients and other conditions. Metal-tolerant isolates such as *M.. incrassata* (TFFH 294) thrive only in optimum conditions. It is a slow growing isolate that would not be expected to compete in nature with faster growing microorganism such as molds, bacteria or the decay fungi *Postia placenta* and *Gleophyllum trabeum*. *M. incrassata* (TFFH 294) has the advantage over other microorganisms during the bioprocessing because it has extensive mycelia growth in the inoculum and because competing microorganisms do not tolerate CCA on the lumber.

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. These 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. The inoculum should limit the disadvantage experienced by various fungal strains, such as *M. incrassata* (TFFH-294), which are typically disadvantaged by a slow growth that limits their competition with other dominant fungi in nature. The metal-tolerant strains can be easily grown as indicated by the optimum growth and nutrient conditions identified in this study. Fungal growth is substantially enhanced with aeration and nutrients added to the culture medium.

Another advantage is that the inoculum and its method of use are particularly well suited for wood waste such as pressure-treated lumber from buildings, decks, utility poles and railroad ties. The solid matrix of the fungal inoculum provides a wood environment for fungal growth, which is similar to that of the wood waste. The fungal strain is, therefore, readily adapted to the waste wood upon inoculation and does not require a period of adjustment before degradation and bioremediation begins.

The bioprocessing method is designed for remediating and degrading solid pieces of lumber, 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; and . The CCA-treated wood in this study was not wood waste; therefore we expect higher yields with spent wood taken out of service.

A major component of the inoculum proved to be the lignocellulose substrate. Sawdust or wood chips are preferred for several reasons: (1) they provide a long-term food source for the fungus while providing fungal growth in a wood environment similar to the CCA-treated waste wood environment experienced during inoculation; (2) they permit the production of a large quantity of fungi in a single container; (3) they provide a substrate for easily storing and transporting the fungi; (4) they provide a matrix for convenient and even distribution of the fungus at the inoculation site; and (5) they provide a low cost use of a waste product from saw mills.

Several aspects of the procedure will need to be addressed for pilot level scale-up. Potential contamination, especially mold contamination at later stages of the procedure warrants an evaluation of specific mildewcides to be added with the inoculum and/or applied during the incubation.

Procedures for various sizes of lumber need to be identified and schedules developed for incubation on a pilot scale.

SUMMARY

An economical fungal bioprocessing method was developed in which a fungal inoculum is prepared by culturing a CCA-tolerant fungus on 2% MEA under aerobic conditions in the dark at a temperature range of 27 – 32° C and 70% RH for 2 weeks; combining the fungal culture with a heat-sterilized mixture of 1% nutrient supplement (CSL), a lignocellulose substrate (sawdust) and water at 2-3 volumes per volume substrate; incubating the mixture under aerobic conditions in the dark at a temperature range of 27 – 32 ^{o} C and 70% RH for 6 weeks. The inoculum can be transferred to bioprocessing containers or stored at 4° C. Large quantities of inoculum can be stored in trays or large durable plastic bags, loosely packed to allow aeration. Trays and bags can be transported easily to the field sites and applied to the wood waste. Inoculum can be prepared directly in a truck bed or in a truck loaded container.

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