IRG/WP 02-50184

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 5 Environmental aspects

Semi-Solid State Bioremediation of CCA-Treated Wood Using Malted Barley as a Nutrient Source

Carol A. Clausen

USDA Forest Service
Forest Products Laboratory
One Gifford Pinchot Drive
Madison, Wisconsin 53705 USA

Paper prepared for the 33rd Annual Meeting
Cardiff, United Kingdom
12-17 May, 2002

IRG Secretariat
SE-100 44 Stockholm
Sweden

Semi-Solid State Bioremediation of CCA-Treated Wood Using Malted Barley as a Nutrient Source

Carol A. Clausen

USDA Forest Service, Forest Products Laboratory, Madison, WI. 53705-2398

Abstract

Bioremediation processes for recovery and reuse of CCA-treated wood invariably increase the cost of any secondary products manufactured from the remediated fiber. Microbial remediation using either bacteria or fungi has been shown to remove heavy metals from CCA-treated southern yellow pine (SYP). In a two-step remediation process utilizing oxalic acid extraction and the metal-tolerant bacterium *Bacillus licheniformis*, 70-100% of the copper, chromium and arsenic can be removed from CCA-treated SYP, but the liquid culture medium used to support the bacterial growth renders this process costly. Processing costs could be partially offset if the culture medium were replaced with an industrial by-product. In this study, an abundant by-product of the brewing industry, malted barley, was evaluated as a replacement for commercial nutrient broth in the bioremediation process for CCA-treated SYP. Malted barley's high moisture and nutrient content should support bacterial growth. When malted barley was substituted as a growth substrate for nutrient broth, it was discovered that either the culture inoculum or wood itself provided sufficient nutrients for the growth of *B. licheniformis*. Seventeen percent of the copper and 15% of the arsenic was removed from an aqueous slurry of CCA-treated SYP following bacterial remediation with *B. licheniformis*. When oxalic acid extraction preceded the aqueous bacterial culture of CCA-treated SYP, 21% Cu, 54% Cr and 63% As were removed. Incidentally, malted barley acted as a biosorbent, removing heavy metals from the liquid culture upon their release from CCA-treated SYP.

Keywords: bioremediation, CCA, *Bacillus licheniformis*, malted barley

Introduction

Research on remediation of CCA-treated wood has increased during the past decade as a result of public concern over disposal of chromium and arsenic in landfills. CCA-treated wood is banned for use in a number of European and Asian countries and very recently, a partial phase-out of CCA-production has been proposed for the U.S over the next 2-3 years (USA Today 2002). Currently, CCA-treated wood is considered nonhazardous, so waste material and material coming out of service is typically landfilled. The amount of CCA-treated material placed in service in the United States since the early 1970's, is estimated to be 1.4×10^7 m^3 (6×10^9 board feet) (Micklewright 1994). Based on an expected average service life of 20-50 yr, Cooper (1994) estimated that 1.6×10^7 m^3 of CCA-treated wood will be removed from service in the US annually by 2020. A more recent study by McQueen *et al.* (1998) showed that the actual average service life of CCA-treated wood in residential decks, which is the largest use of this material, is

in fact, 9 years. Survey responses listed aesthetics 43% of the time as a typical reason for early deck removal. This implies that Cooper's estimates will be reached significantly earlier than projected and increases the urgency for the development of alternative disposal methods for CCA-treated waste wood.

A two-step remediation process, which utilizes a combination of oxalic acid extraction and bacterial culture with a metal-tolerant bacterium, substantially reduces the amounts of copper (78%), chromium (97%), and arsenic (93%) in CCA-treated wood (Clausen 1997, 2000; Cole and Clausen 1997; Clausen and Smith 1998; Crawford and Clausen 1999). The remediated fiber has been reassembled into medium density particleboard panels (Clausen *et al.* 2001). Particleboard panel production from wood fiber remediated in this manner is prohibitively expensive compared to manufacturing particleboard from virgin southern pine stock, mostly due to the cost of the nutrient culture medium.

As volumes of waste CCA increase, decreased landfill space and accompanying disposal fees will make remediation methods more economically and environmentally attractive. Eliminating the cost of nutrient medium for bacterial culture would substantially decrease the processing costs for the two-step remediation method. One way to accomplish this would be to replace the nutrient medium with a plentiful industrial waste by-product. Spent malted barley from breweries, is regionally plentiful and is considered a waste product which is given away as cattle feed. Malted barley has a high protein and moisture content, making it an ideal nutritional source for microbial growth. The objective of this study was to evaluate malted barley for it's ability to support the growth of *Bacillus licheniformis* CC01 during bioremediation of CCA-treated wood.

Materials and methods

Bacterial culture

Bacillus licheniformis CC01 was maintained at 27^0C on nutrient agar (Difco Laboratories, Detroit, MI) supplemented with 0.01% CCA type C.

Malted Barley

Malted barley was provided by Capitol Brewery, Middleton, WI. The average moisture content (n=3) was 73%. Throughout this study, the barley supplement (w:v) was based on the dry weight equivalent of the barley.

Culture variables and growth conditions

Twenty-gram mixtures of malted barley and CCA-treated southern yellow pine (CCA-SYP) particles, 6-16 mesh (1.2-3.4 mm) were placed in 100 x 80mm Pyrex culture dishes with 80 mls of either tap water, deionized (DI) water, or nutrient broth prepared according to manufacturer's directions (Difco, Detroit, MI) as a moistening agent. Ratios of barley:CCA-SYP tested were 1:1, 1:1.5, 1:2, 1:2.5, and CCA-SYP without barley. Dishes were autoclaved at 121^0C and 103.4

kPa (15 psi) for 15 min., cooled and inoculated with 3 mls of an 18 hr nutrient broth culture of *Bacillus licheniformis* CC01. Tests were conducted in triplicate and compared to uninoculated 20 g samples of each mixture of barley:CCA-SYP moistened with DI water. Dishes were incubated for 8 d at 27^0C, barley:CCA-SYP mixtures were collected over a cheesecloth-covered screen, rinsed thoroughly in DI water and oven-dried at 60^0C for 24 hr.

Elemental Analysis

Oven-dried samples were ground to pass a US Standard 20-mesh (850 μm) screen and analyzed for copper, chromium and arsenic content by inductively coupled plasma emission spectrometry (ICP) according to AWPA standard A-21-00.

Acid Extraction

Five-gram samples of CCA-treated SYP particles were pretreated with 0.8% oxalic acid (Sigma Chemical, St. Louis, MO) (100 ml/sample) for 18 hr at 27^oC. Oxalic acid was collected by aspiration through Whatman filter paper No. 1, analyzed for Cu, Cr, and As, and retained for absorption experimentation. Acid-extracted CCA-SYP particles were rinsed thoroughly with distilled water, and analyzed for Cu, Cr, and As content.

Absorptive capacity of malted barley

Samples of barley (5, 10, 15, and 20 g) were added to 100 ml aliquots of the acid-extracted filtrate containing known amounts of copper, chromium and arsenic (described above). Barley and filtrate mixtures were held at 27^0C. Samples of filtrate were removed after 2, 4, 6, 12, and 24 hr and analyzed for Cu, Cr, and As by ICP.

2-Step Remediation without barley or nutrient broth

Twenty-gram samples of oxalic acid-extracted CCA-SYP particles plus 80 ml tap water were inoculated with 3 ml each of an 18-hr culture of *B. licheniformis* CC01, and incubated at 27^0C for 10 d at 90 rpm. Controls consisted of uninoculated acid-extracted CCA-SYP particles. Following incubation, all samples were collected over a cheesecloth-covered screen, rinsed thoroughly in DI water and oven-dried at 60^0C for 24 hr. Oven-dried samples were analyzed by ICP for copper, chromium and arsenic content.

Results

Culture variables and growth conditions

Results on the effect of the moistening agent and barley:CCA-SYP ratio on the removal of Cu, Cr, and As by *B. licheniformis* CC01 are shown in Table 1. Elemental analysis of 1:1 mixtures of barley:CCA-SYP exposed to *Bacillus licheniformis* revealed no loss in copper, chromium or arsenic with varied moistening agents. Ratios of 1:1.5, 1:2.0 and 1:2.5 barley:CCA-SYP showed little or no loss in metals except for small amounts of copper and arsenic for mixtures

cultured in nutrient broth. Losses for chromium were uniformly zero for all combinations of barley:CCA-SYP and moistening agents. Decreasing the ratio of barley to CCA-SYP clearly increased the amount of copper and arsenic removed, suggesting that malted barley may be absorbing or adsorbing metals from the culture supernatant as they are released from CCA-SYP by the bacterium. A slurry of 20g CCA-SYP exposed to *B. licheniformis* CC01 in tap water minus the barley supplement removed 17% Cu and 15% As.

Absorption of metals by malted barley

Varying amounts of barley were mixed with acid-extracted filtrate that contained 302, 40, and 107 mg/L of copper, chromium and arsenic. Samples taken after 2, 4, 6, 24, and 48 h were analyzed for copper, chromium, and arsenic. Figure 1 shows that increasing amounts of barley absorbed increasing amounts of Cu, Cr, and As (up to 45%, 63%, and 69%, respectively). Likewise, increasing the time the metal solution was exposed to the barley also increased the percent of metals removed from a solution containing known amounts of Cu, Cr, and As.

Two-step remediation without barley or nutrient broth

Bacillus licheniformis, in aqueous culture, removed 21%, 54% and 63% of the residual copper, chromium and arsenic from oxalic acid-extracted CCA-SYP. Oxalic acid pretreatment of CCA-SYP increased bioleaching capability for copper and arsenic and enabled *Bacillus licheniformis* to remove half the chromium from the test samples.

Discussion and conclusions

Economic feasibility is a critical aspect to the success of any bioremediation process. Remediation processes involving microbial growth incur the additional cost of growth medium. In this study, an abundant byproduct of the brewing industry, malted barley, was evaluated as a substitute for commercial nutrient broth as the source of microbial nutrition in the bioremediation process for CCA-treated wood. *Bacillus licheniformis* CC01 was grown on various ratios of malted barley:CCA-SYP with three different moistening agents to evaluate the bacteria's ability to remove copper, chromium, and arsenic from CCA-SYP particles (Table 1). CCA-SYP without a barley supplement showed the highest percentage of copper and arsenic (17% and 15%, respectively) was removed from cultures without nutrient broth. These results suggested that an alternative nutrient source for *Bacillus licheniformis* CC01 was not necessary and that *B. licheniformis* was able to remove copper and arsenic from CCA-treated SYP without an added nutrient source.

Most mixtures of barley:CCA-SYP tested showed little or no removal of metals, suggesting that malted barley was acting as an absorbent, such that metals removed from the treated wood remained in the barley:CCA-SYP mixture. Evaluation of malted barley for absorbing capacity showed that increasing amounts of copper, chromium, and arsenic were removed from a solution containing known amounts of each element. Both time of exposure and concentration of barley (w/v) influenced the absorption of elemental components of CCA from solution (Figure 1).

Previously, in the two-step remediation process, nutrient broth was utilized as the nutrient source for *B. licheniformis* (Clausen 2000). In this study, tap water replaced nutrient broth in a two-step remediation of CCA-SYP. Although removing nutrient broth from the process greatly reduced remediation costs, the amount of metals removed from a sample of CCA-SYP was greater when nutrient broth was used; 78% vs 21% copper, 97% vs 54% chromium, and 93% vs 63% arsenic. This study revealed that the need for a substitute nutrient source for *Bacillus licheniformis* CC01 was unnecessary; either the culture inoculum or the wood itself provided ample nutrients for the growth of and metal removal by *B. licheniformis*, although the total percent of each metal removed was lower without a nutrient broth supplement.

Acknowledgments

The author would like to thank Henry Spelter and James Howard for their economic analysis and input on processing costs, and Dan Foster for ICP analysis.

References

ASTM. 1996. D 1413-76 (1994). Standard test method for wood preservatives by laboratory soil-block cultures. P. 207-213. *In*: ASTM. Annual Book of Standards. Vol. 4.10 Wood. American Society for Testing and Materials, West Conshohocken, PA. 646 p.

AWPA. 2001. A21-00. Standard Method for the Analysis of Wood and Wood Treating Solutions by Inductively Coupled Plasma Emission Spectrometry. pp. 313-317. *In*: Book of Standards. American Wood Preservers' Association, Granbury, TX. 2001.

Clausen, C.A. 1997. Enhanced removal of CCA from treated wood by *Bacillus licheniformis* in continuous culture. IRG/WP 97-50083. Inter. Res. Group on Wood Preservation, Stockholm, Sweden. 8pp.

Clausen, C.A. 2000. CCA removal from treated wood using a dual remediation process. *Waste Management & Research* **18**: 485-488.

Clausen, C.A. Smith, R.L. 1998. Removal of CCA from treated wood by oxalic acid extraction, steam explosion, and bacterial fermentation. *Journal of Industrial Microbiology* **20**: 251-257.

Clausen, C.A., Kartal, S.N., Muehl, J. 2001. Particleboard made from remediated CCA-treated wood: Evaluation of panel properties. *Forest Products Journal* **51**(7/8):61-64.

Cole, F.A. and Clausen, C.A. 1997. Bacterial biodegradation of CCA-treated waste wood. *In:* Proc. Use of Recycled Wood and Paper in Building Applications. Forest Prod. Soc. Madison, WI. 201-204.

Cooper, P.A. 1994. Disposal of treated wood removed from service: The issues. *In:* Proc. Environmental Considerations in the Manufacture, Use, and Disposal of Preservative-Treated Wood. Forest Products Society, Madison, WI. pp. 85-90.

Crawford, D.M. and Clausen, C.A. 1999. Evaluation of wood treated with copper-based wood preservatives for Cu loss during exposure to heat and copper-tolerant *Bacillus licheniformis*. IRG/WP 99-20155. Inter. Res. Group on Wood Preservation, Stockholm, Sweden. 8pp.

Eisler, P. 2002. "Arsenic treated lumber to be cut." USA Today, January 31, 2002.

McQueen, J., Stevens, J. and Kamdem, D.P. 1998. Recycling of CCA treated wood in the U.S. *In:* 4th International Symposium on Wood Preservation IRG/WP 98-50101, Stockholm, Sweden, pp. 77-93.

Micklewright, J.T. 1994. Wood Preserving Statistics, 1993: A report to the Wood Preserving Industry in the United States. American Wood Preservers' Association, Granbury, TX.

Table 1. Effect of moistening agent and barley:CCA-SYP ratio on the removal of copper, chromium and arsenic by *Bacillus licheniformis* CC01.

Barley:CCA-SYP) (wt:wt)	Moistening Agent	Wt. % element in wood (mg/g)			Percent reduction		
		Cu	Cr	As	Cu	Cr	As
1:1	A	0.76±0.05	1.50±0.22	1.03±0.25	0	0	0
"	B	0.75±0.03	1.59±0.13	1.15±0.10	0	0	0
"	C	0.72±0.04	1.68±0.16	1.22±0.13	0	0	0
uninoculated	B	0.65	1.1	1.02			
1:1.5	A	0.79±0.05	1.84±0.07	1.44±0.08	13.2	0	2
"	B	0.82±0.04	1.78±0.20	1.36±0.17	9.9	0	7.5
"	C	0.78±0.04	1.70±0.03	1.23±0.03	14.3	0	16.3
uninoculated	B	0.91	1.59	1.47			
1:2	A	0.88±0.04	1.87±0.01	1.45±0.02	0	0	0
"	B	0.87±0.05	1.94±0.09	1.46±0.09	0	0	0
"	C	0.78±0.02	2.00±0.03	1.48±0.06	7.1	0	0
uninoculated	B	0.84	1.46	1.34			
1:2.5	A	0.97±0.03	2.07±0.05	1.61±0.04	0	0	0
"	B	0.92±0.10	2.10±0.19	1.59±0.16	5.2	0	0
"	C	0.80±0.06	1.97±0.16	1.42±0.12	17.5	0	4.1
uninoculated	B	0.97	1.68	1.48			
CCA-SYP	A	1.24±0.07	2.65±0.08	2.14±0.08	17.3	0	15.1
"	B	1.37±0.06	2.77±0.14	2.24±0.12	8.7	0	11.1
"	C	1.22±0.05	2.78±0.09	2.21±0.29	18.7	0	12.3
uninoculated	B	1.5	2.64	2.52			

A=Tap water
B=DI water
C=Nutrient broth
n=3

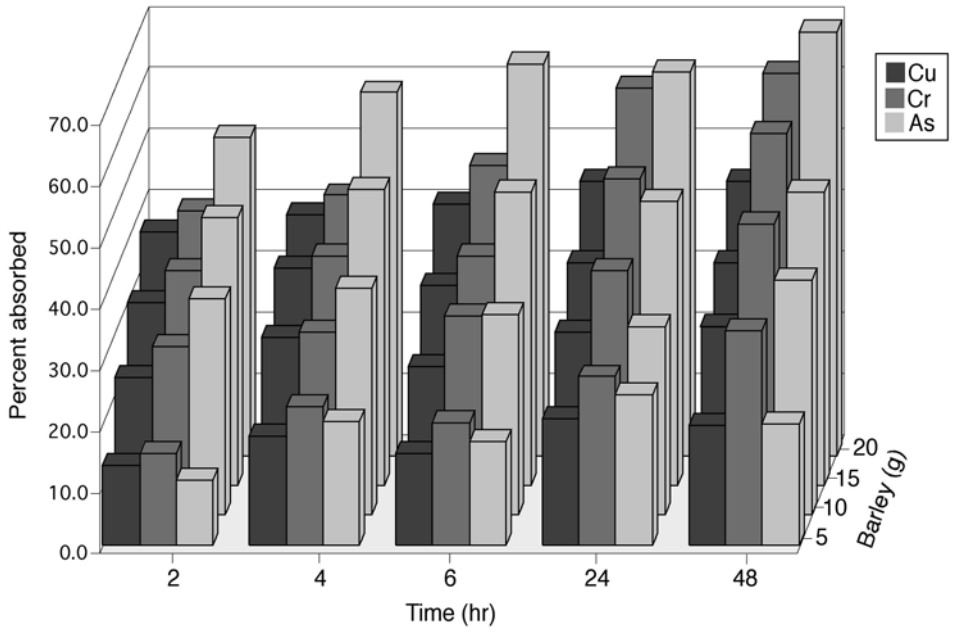

Figure 1. Ability of varied amounts of malted barley to absorb copper, chromium and arsenic over time from a solution of oxalic acid-extracted CCA-SYP which contained 302 mg/L copper, 40 mg/L chromium, and 107 mg/L arsenic.