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Improving the two-step remediation process for CCA-treated wood: Part II. Evaluating bacterial nutrient sources*

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Abstract

Remediation processes for recovery and reuse of chromated-copper-arsenate- (CCA) treated wood are not gaining wide acceptance because they are more expensive than landfill disposal. One reason is the high cost of the nutrient medium used to culture the metal-tolerant bacterium, *Bacillus licheniformis*, which removes 70–100% of the copper, chromium, and arsenic from CCA-treated southern yellow pine (CCA-SYP) in a two-step process involving oxalic acid extraction and bacterial culture. To reduce this cost, the nutrient concentration in the culture medium and the ratio of wood to nutrient medium were optimized. Maximum metal removal occurred when *B. licheniformis* was cultured in 1.0% nutrient medium and at a wood to nutrient medium ratio of 1:10. Also, malted barley, an abundant by-product of brewing, was evaluated as an alternative nutrient medium. Tests were done to determine absorption of metals by barley, and the results indicate that the barley acted as a biosorbent, removing heavy metals from the liquid culture after their release from CCA to SYP. For comparison, tests were also performed with no nutrient medium. Following bacterial remediation, 17% copper and 15% arsenic were removed from an aqueous slurry of CCA-SYP (no medium). When oxalic acid extraction preceded the aqueous bacterial culture, 21% copper, 54% chromium, and 63% arsenic were removed. The two-step process (oxalic acid extraction and bacterial culture with nutrient medium) appears to be an effective, yet costly, way to remove metals.
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1. Introduction

Research on remediation methods for chromated-copper-arsenate- (CCA) treated wood has increased during the past decade as a result of concern about disposal of chromium and arsenic in landfills. CCA-treated wood has been restricted or phased out in a number of European and Asian countries and will be banned for residential use in the United States by 2004. Currently, CCA-treated wood is considered nonhazardous by the US Environmental Protection Agency, so waste material and material coming out of service are typically disposed in landfills. The increase in demand for CCA-treated wood products since the preservative gained popularity in the early 1970s has resulted in an annual production of this product of about 14 million m^3 (Micklewright, 1994). Based on an expected average service life of 20 to 50 years, Cooper (1994) estimated that by 2020, 16 million m^3 of CCA-treated wood will be removed from service in the United States annually. In the immediate future, alternatives to CCA will consist of copper-based organics, such as the ammoniacal copper quaternary compounds. These preservatives could face similar public concerns, specifically about the vulnerability of aquatic environments to copper leaching.

A two-step remediation process, involving a combination of oxalic acid extraction and bacterial culture with the metal-tolerant *Bacillus licheniformis* CC01, 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). Oxalic acid extraction serves to partially solubilize the insoluble metal compounds fixed in the wood so that the bacterium can more

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effectively release metals from the wood to the surrounding culture medium. Improvements in the oxalic acid leaching step are considered in Clausen (2003, this issue). Alone, the bacterium cannot remove chromium, but it has been shown to be effective at removing copper and arsenic from wood treated with a number of copper-based preservatives (Crawford and Clausen, 1999). Neither acid extraction nor bacterial culture alone is as effective at metal removal from CCA-treated wood as the combination of acid extraction and bacterial culture.

As the amount of CCA-treated wood waste increases, decreased landfill space and accompanying disposal fees will make remediation methods more economically attractive. Remediated wood fiber has been reassembled into particleboard panels (Clausen et al., 2001). But these panels are costly compared with manufacturing particleboard from virgin southern pine stock, mostly due to the cost of the nutrient medium required to support bacterial growth. Eliminating or reducing this cost handicap would substantially decrease the processing costs for the two-step remediation method. Several ways to accomplish this would be to (1) reduce the concentration of nutrient medium necessary for the bacterium to achieve satisfactory performance (70% removal of copper and arsenic), (2) optimize the ratio of wood to nutrient medium to accomplish maximum copper and arsenic removal, or (3) partially or completely replace the nutrient medium with an industrial waste by-product. Removal of copper and arsenic beyond 70% would be considered an improvement compared with the metal removal rates of the two-step remediation process of Clausen (2000). Spent malted barley from breweries is regionally plentiful and is considered a waste product that is donated 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 then was to evaluate these three methods of improving the economic feasibility of the two-step remediation process for CCA-treated wood.

2. Materials and methods

2.1. Treated wood

CCA-treated southern yellow pine (SYP) lumber (6.4 kg/m^3 retention) obtained from Brunsell Lumber (Madison, WI, USA) was used throughout this study. The treated lumber was hammer-milled and sorted to approximately 1–3-mm(6–16-mesh)particle size.

2.2. Bacterial culture

Bacillus licheniformis CC01 (Cole and Clausen, 1997) was maintained at 27 °C on nutrient agar (Difco Laboratories, Detroit, MI, USA) supplemented with 0.01% CCA type C.

2.3. Elemental analysis

Ovendried test samples were ground to pass a US Standard 20-mesh (850-μm) screen, digested, and analyzed for copper, chromium, or arsenic content by inductively coupled plasma (ICP) emission spectrometry according to American Wood Preservers' Association (AWPA) standard A-21-00 (AWPA, 2001).

2.4. Minimal nutrient medium requirements

First, 5-g samples of CCA-SYP particles were placed in 300-mL Erlenmeyer flasks containing 100 ml of varying concentrations of nutrient medium (Difco, Detroit, MI, USA) (0%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%) and sterilized by autoclaving for 15 min at 121 °C and 103.4 kPa (15 lb/in^2). Cooled flasks (n=3) were inoculated with 1 ml of an 18-h nutrient medium culture of *B. licheniformis* and incubated at 27 °C for 10 d at 90 rpm on an orbital shaker. After 10 days, samples were collected over a cheesecloth-covered screen, rinsed thoroughly in deionized (DI) water, and ovendried at 60 °C for 24 h. Ovendried samples were analyzed by ICP for copper and arsenic content.

Next, various ratios of particles to nutrient medium (1:10, 1:5, 1:3.3, and 1:2.5) (g/ml) were evaluated for the ability to be loaded with cultures of *B. licheniformis* and still allow effective removal of copper and arsenic. CCA-treated wood particles in 10-, 20-, 30-, or 40-g amounts were added to 300-m1 Erlenmeyer flasks containing 100 ml 0.8% nutrient medium and autoclaved for 15 min at 121 °C and 103.4 kPa (15 lb/in^2). The manufacturer's recommended concentration for bacterial growth is 0.8% nutrient medium, and this concentration has been previously shown to support growth of the bacterium and removal of more than 70% copper and arsenic (Clausen, 2000). Cooled flasks were inoculated with 1 ml of an 18-h nutrient medium culture of *B. licheniformis* and incubated at 27 °C for 10 days at 90 rpm. Tests were conducted in triplicate and compared with uninoculated controls. Samples were collected over a cheesecloth-covered screen, rinsed thoroughly in deionized (DI) water, and ovendried at 60 °C for 24 h. Ovendried samples were analyzed by ICP for copper and arsenic content.

2.5. Replacing nutrient medium with malted barley

Malted barley was provided by Capitol Brewery (Middleton, WI, USA). For cultures containing a barley supplement (w/v), the barley weight (73% average moisture content) was based on the dry weight equivalent. Twenty-gram mixtures of malted barley and CCA-SYP

particles were placed in 100- by 80-mm Pyrex culture dishes with 80 m1 of either tap water, DI water, or 0.8% nutrient medium as a moistening agent. Ratios of barley/CCA-SYP tested were 1:1, 1:1.5, 1:2, and 1:2.5. CCA-SYP without barley was also tested. Dishes were autoclaved at 121 °C and 103.4 kPa (15 lb/in^2) for 15 min, cooled and inoculated with 3 ml of an 18-h nutrient medium culture of *B. licheniformis* CC01. Tests were conducted in triplicate and compared with uninoculated 20-g samples of each mixture of barley/CCA-SYP moistened with DI water. Dishes were incubated for 8 d at 27 °C. Barley/CCA-SYP mixtures were collected over a cheesecloth-covered screen, rinsed thoroughly in DI water, and ovendried at 60 °C for 24 h. Elemental analyses as described in Section 2.3 were conducted on each sample and results were expressed as weight percent/element in wood. Percentage of copper and arsenic removed were calculated based on values for uninoculated controls.

2.6. Absorptive capacity of malted barley

Five-gram samples of CCA-SYP particles were treated in 0.8% oxalic acid (Sigma Chemical, St. Louis, MO, USA) (100 ml/sample) for 18 h at 27 °C. The filtrate was collected by aspiration through Whatman filter paper No. 1 (Hillsboro, OR, USA) and analyzed for copper, chromium, and arsenic. Samples of barley (5, 10, 15, and 20 g) were added to 100-m1 aliquots of the acid-extracted filtrate containing 302 mg/l copper, 40 mg/l chromium, and 107 mg/l arsenic. Barley and filtrate mixtures were held at 27 °C. Samples of filtrate were removed after 2, 4, 6, 12, and 24 h and analyzed for copper, chromium, and arsenic by ICP.

2.7. Two-step remediation without barley or nutrient broth

Twenty-gram samples of oxalic-acid-extracted CCA-SYP particles plus 80 m1 tap water were inoculated with 3 ml each of an 18-h culture of *B. licheniformis* CC01 and were incubated at 27 °C for 10 days 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 ovendried at 60 °C for 24 h. Ovendried samples were analyzed by ICP for copper, chromium, and arsenic content.

3. Results and discussion

3.1. Minimal nutrient medium requirements

Fig. 1 shows the effect of varying concentration of nutrient medium on the ability of *B. licheniformis* to remove copper and arsenic from CCA-treated wood. Chromium is resistant to bioleaching by the bacterium. As the concentration of nutrient medium decreased, so did the microbe's ability to effectively remove arsenic and to a lesser extent, copper. Maximum metal removal occurred in 1% nutrient medium and reached 93% and 74% for copper and arsenic, respectively.

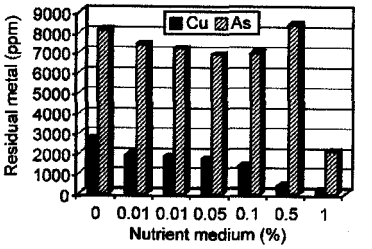

Fig. 1. Residual copper and arsenic in CCA-treated particles following exposure to *Bacillus licheniformis* in culture with varying concentrations of nutrient medium. *B. lichenformis* alone does not remove chromium from CCA-treated wood.

To efficiently treat a given amount of particles, the minimum amount of reagent that is still effective should be used. Fig. 2 shows that a 1:10 ratio of particles to culture medium removed the most copper. There was little change in arsenic removal (44-55%) as the ratio of particles to culture medium was decreased. However, copper removal declined from 56% to 25% as the particle to medium ratio decreased from 1:10 to 1:5. It has been shown that when CCA-treated wood particles are pretreated with oxalic acid, significant quantities of copper and arsenic are initially removed (Clausen, 2000). Acid pretreatment lowers the optimal ratio of particles to nutrient medium in the remediation process.

3.2. Replacing nutrient medium with malted barley

The effect of the moistening agent and barley/CCA-SYP ratio on the ability of *B. licheniformis* CC01 to remove copper and arsenic is shown in Table 1. Elemental analysis of 1: 1 mixtures of barley/CCA-SYP exposed to *B. lichenformis* revealed no loss in copper or arsenic

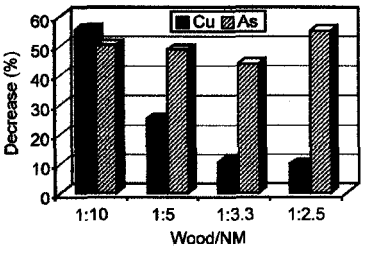

Fig. 2. Percentage decrease of copper and arsenic in CCA-treated particles following exposure to *B. licheniformis* in culture with varying ratios of wood to nutrient medium (NM). B. lichenformis alone does not remove chromium from CCA-treated wood.

Table 1
Effect of barley/copper-chromated-arsenate-treated southern yellow pine (CCA-SYP) ratio and moistening agent on the removal of copper and arsenic from treated wood particles by B. licheniformis CC01[a]

Barley/CCA-SYP (wt/wt)	Moistening agent	Wt. percent (mg/g) ± S.D.		Percentage removed[b]	
		Cu	As	cu	As
1:1	Tap water	0.76±0.05	1.03±0.25	0	0
1:1	DI[c] water	0.75±0.03	1.15±0.10	0	0
1:1	Nutrient medium	0.72±0.04	1.22±0.13	0	0
Control[d]	DI water	0.65	1.02		
1:1.5	Tap water	0.79±0.05	1.44±0.08	13.2	2
1:1.5	DI water	0.82±0.04	1.36±0.17	9.9	7.5
1:1.5	Nutrient medium	0.78±0.04	1.23±0.03	14.3	16.3
Control	DI water	0.91	1.47		
1:2	Tap water	0.88±0.04	1.45±0.02	0	0
1:2	DI water	0.87±0.05	1.46±0.09	0	0
1:2	Nutrient medium	0.78±0.02	1.48±0.06	7.1	0
Control	DI water	0.84	1.34		
1:2.5	Tap water	0.97±0.03	1.61±0.04	0	0
1:2.5	DI water	0.92±0.10	1.59±0.16	5.2	0
1:2.5	Nutrient medium	0.80±0.06	1.42±0.12	17.5	4.1
Control	DI water	0.97	1.48		
CCA-SYP only	Tap water	1.24±0.07	2.14±0.08	17.3	15.1
CCA-SYP only	DI water	1.37±0.06	2.24±0.12	8.7	11.1
CCA-SYP only	Nutrient medium	1.22±0.05	2.21±0.29	18.7	12.3
Control	DI water	1.5	2.52		

[a] n = 3
[b] Zero may indicate a negative value that is within experimental error.
[c] DI, deionized.
[d] Control is uninoculated.

with varied moistening agents. On the contrary, many mixtures showed an increase in copper and arsenic compared with controls. This may reflect biological variability, experimental error, or trace amounts of copper or arsenic in the moistening agents. Increases are depicted as 0% removal in Table 1. 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 (not shown) were uniformly zero, as expected, for all combinations of barley/CCA-SYP and moistening agents; *B. licheniformis* CC01 alone does not remove chromium from CCA-treated wood. 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 20 g CCA-SYP exposed to *B. licheniformis* CC01 in tap water without the barley supplement removed 17% copper and 15% arsenic. This suggested that exposing particles to *B. licheniformis* in aqueous culture (with no nutrient medium) was as effective at removing copper and arsenic as nutrient medium. Additional testing showed that *B. licheniformis* in aqueous culture removed 21%, 54%, and 63% of the residual copper, chromium, and arsenic, respectively. If the particles were pretreated with oxalic acid, however, the bioleaching capability for copper and arsenic increased and *B. licheniformis* was then able to remove half the chromium from the test samples. Despite this finding, aqueous cultures of oxalic-acid-extracted particles with *B. licheniformis* were not as effective at metal removal as nutrient medium cultures of oxalic-acid-extracted particles with *B. licheniformis* (21% vs 78% copper removal, 54% vs 97% chromium removal, and 63% vs 93% arsenic removal). The two-step process of oxalic acid extraction and bacterial culture, even with an expensive nutrient medium, appears to be the most effective method of metal removal.

3.3. Absorption of metals by malted barley

Varying amounts of barley were mixed with acid-extracted filtrate that contained known amounts of copper (302 mg/l), chromium (40 mg/l), and arsenic (107 mg/l). Samples taken after 2, 4, 6, 24, and 48 h were analyzed for copper, chromium, and arsenic. Fig. 3 shows that increasing amounts of barley absorbed increasing amounts of copper, chromium, and arsenic (up to 45%, 63%, and 69%, respectively). Likewise, increasing the time the metal solution was exposed to the barley also increased the percentage of metals

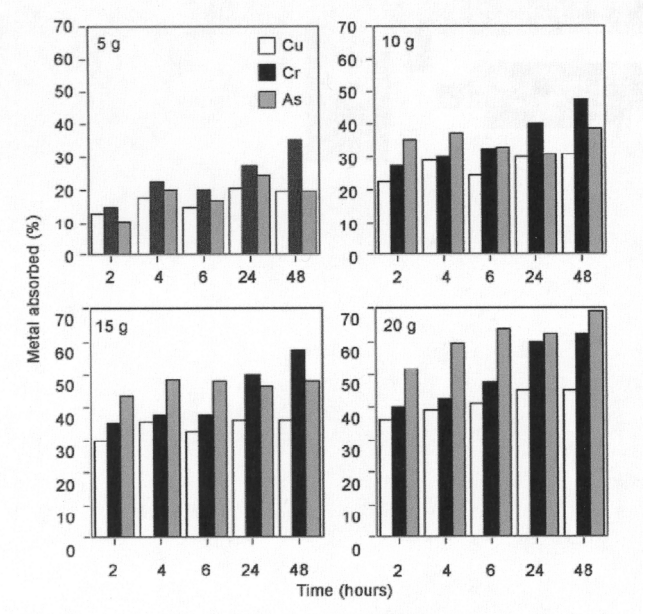

Fig. 3. Absorptive capacity of 5, 10, 15, and 20 g of barley exposed to a solution containing known amounts of copper, chromium, and arsenic for varying amounts of time.

removed from a solution containing known amounts of copper, chromium, and arsenic.

4. Conclusions

Treated wood is a voluminous material that will continue to present disposal problems in landfills unless economically feasible methods for disposal are developed. Remediation processes involving microbial growth incur the cost of growth medium. The growth medium for the two-step process accounts for 99% of the total chemical costs. In this study, decreasing concentrations of nutrient medium resulted in decreased copper, chromium, and arsenic removal from CCA-treated particles (Fig. 1). Concentrations of nutrient medium greater than 0.5% were needed to achieve greater than 70% removal of copper and arsenic.

Results suggested that an alternative nutrient source for *B. licheniformis* CC01 may not be necessary and that *B. licheniformis* was able to remove copper and arsenic from CCA-treated SYP when tap water was the moistening agent and without an added nutrient source. However, in the two-step remediation process, additional testing of CCA-SYP with tap water showed that the efficiency of metal removal without a nutrient source was considerably lower than that with nutrient medium supplementation.

Malted barley, an abundant byproduct of the brewing industry, was not effective as a substitute for commercial nutrient broth as the source of microbial nutrition in the bioremediation process for CCA-treated wood. Most mixtures of barley/CCA-SYP tested showed little or no removal of metals, suggesting that malted barley was acting as an absorbent. In other words, metals removed from the treated wood remained in the barley/CCA-SYP mixture. Evaluation of malted barley for absorbing capacity showed that with time, increasing amounts of barley in a solution containing known amounts of copper, chromium and arsenic removed increasing amounts of each element (Fig. 3). Both time of exposure and concentration of barley (w/v) influenced the likely absorption of elemental components of CCA from solution.

This remediation process results in the metals being separated from the wood fiber into a liquid medium where they can be recovered for reuse. The remediated wood fiber has been reassembled into particleboard panels (Clausen et al., 2001). The effectiveness of the two-step process as a function of wood chip size and configurations, for example, flaked or pulp-chipped material, needs to be evaluated as well as the effects of scaling-up the process beyond the laboratory scale.

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