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Removal of CCA from treated wood by oxalic acid extraction, steam explosion, and bacterial fermentation

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Most preservative-treated wood produced and consumed in the United States is treated with toxic inorganic compounds containing copper, chromium, and arsenic. Because chromated copper arsenate (CCA) is fixed to the wood, CCA-treated wood has not been considered toxic or hazardous and it is currently disposed of in approved landfills. Growing public concern about environmental contamination from treated wood combined with the removal of greater quantities of CCA-treated wood from service have presented a disposal challenge for this fiber source. In this study, CCA-treated wood was processed by acid extraction, steam explosion, and bacterial fermentation and evaluated for removal of copper, chromium, and arsenic. Copper was the easiest to remove by these treatments and chromium the most resistant to removal. Exposing CCA-treated wood to steady-state bacterial growth by continuous culture with *Bacillus licheniformis* CC01 did not enhance removal of CCA components compared to standard mixed culture when acid extraction preceded bacterial fermentation. Nor did steam explosion, alone or in conjunction with acid extraction and bacterial fermentation, enhance removal of CCA components; the chromium and arsenic components resisted removal. Grinding CCA-treated wood chips into 20-mesh sawdust provided greater access to and removal of CCA components by all processes. However, grinding the chips was unnecessary if they were treated with acid prior to bacterial fermentation. Extraction with oxalic acid as a precursor to bacterial fermentation with *B. licheniformis* CC01 removed 90% copper (CuO), 80% chromium (CrO_3), and 100% arsenic (As_2O_5) from treated chips. The combination of acid extraction and bacterial fermentation removed 80–100% of these metals from CCA-treated wood.

Keywords: steam explosion; acid extraction; *Bacillus licheniformis* CC01; bacteria; chromated copper arsenate (CCA); preservative

Introduction

Most of the 212 million cubic meters of preservative-treated wood produced and consumed in the United States annually is treated with toxic inorganic compounds containing copper, chromium, and arsenic [15]. The demand for treated lumber increased 300% between 1980 and 1993 [19]. Wood treated with chromated copper arsenate (CCA) has an expected service-life of 20–50 years depending on the conditions of service and method of treatment [6,19]. Based on current levels of production and expected service-life, Cooper [6] predicted that the million cubic meters of CCA-treated wood being removed from service annually in the United States will increase to 16 million cubic meters by 2020.

Currently, all CCA-treated wood is disposed of in approved landfills. This wood is generally not considered toxic or hazardous waste because the chemical components are fixed to the wood. Moreover, certain fungi and bacteria can degrade CCA-treated wood [4,5,20]. Nevertheless, there is increasing public concern about environmental contamination from treated wood removed from service and placed in landfills [19]. Potential soil and groundwater contamination in the ever-dwindling landfills could be alleviated by developing methods for recycling this wood resource. When composite manufacturers were asked if they would consider using spent CCA-treated wood fiber in composite products, they indicated that their two greatest concerns were the health of mill workers and residual chemicals in the fiber [19]. They felt that the fiber would need to be certified free of all chemical additives and be approved by governmental agencies before it could be recycled into composite products.

Methods for removing CCA from treated waste wood need to be developed for both economical and environmental reasons. One possible method for recycling CCA-treated wood fiber would be to modify it by removing the heavy metal components so that both the wood fiber and the metals could be reclaimed. Thorough removal of all metal components is essential for acceptance of spent CCA-treated wood by manufacturers and the public.

Previous studies on the chemical leachability of CCA evaluated acid extractions using citric, acetic, formic, oxalic, nitric, or sulfuric acids [13,16,21]. These studies showed that the type of acid has an effect on leaching rates and final concentrations of CCA in the wood, but the initial CCA concentration does not affect leaching. Treated wood contains chromium in the largest proportion and it is chromium that has a strong affinity for wood lignin [23], making it the most resistant component of CCA to chemical extraction or microbial release [4,22].

Certain isolates of fungi can readily decay wood that has been treated with CCA at levels intended to inhibit decay

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fungi. In fact, some fungal isolates can decay treated wood as rapidly as untreated wood. Previous studies [11] suggested that oxalic acid production by decay fungi plays a critical role in initiation of the decay process. A recent study by Stephan *et al* [20] examined the leachability of chromium and copper from chromium copper-treated wood using oxalic acid. The results showed that 98% of the initial chromium could be leached at a low pH (0.69), but that leachability declined quickly with an increase in pH. One objective of our study was to evaluate the ability of oxalic acid, in conjunction with other mechanical and microbial processes, to extract all components of CCA from treated wood.

Steam explosion converts wood chips into a brown fibrous mass through a process that involves saturating the chips with steam for a specified time at a specified pressure, followed by rapid decompression [14]. Physical defibering occurs during the rapid decompression, and chemical autohydrolysis occurs during the steam treatment [19]. Some volatile degradation products may be produced, such as acetic acid, furfural, methanol, and hydroxymethyl furfural. Steam-exploded material is slightly acidic (pH 3.5–4.0) as a result of water-soluble lignin and acetic acid, but in general, all wood components are recoverable in total following this process. Steam explosion has been studied as a means of opening the chemical structure for accessibility of carbohydrates [2]. Thus, the second objective of our study was to evaluate the ability of steam explosion to open the chemical structure of CCA-treated wood, thereby providing accessibility to copper, chromium, and arsenic during subsequent oxalic acid extraction or bacterial fermentation.

Gram-positive spore-forming bacteria from the genus *Bacillus* are commonly isolated from preserved wood, possibly due to the resistance of bacterial spores to harsh conditions. These bacteria are tolerant of copper levels normally utilized to inhibit basidiomycetous fungi, the group of fungi targeted by wood preservatives [5,17]. Fungal and bacterial tolerance of CCA varies with the amount of preservative present and the service condition [18]. Singh *et al* [18] presented microscopic evidence of erosion bacteria causing degradation in failed CCA-treated timbers. Attack by erosion bacteria occurred throughout the diameter of the pole, while attack by soft-rot fungi was confined to the outer areas of the pole. Daniel and others [7,8] documented tunneling bacteria in degraded CCA-treated *Radiata* pine using energy-dispersive x-ray analysis–transmission electron microscopy (EDAX–TEM).

Cole and Clausen [5] surveyed an experimental test plot containing CCA-treated Southern Pine 38×89 mm (2 by 4) lumber in Madison, Wisconsin, for bacteria that could tolerate CCA. One isolate, identified as *Bacillus licheniformis* CC01, released copper, chromium, and arsenic from CCA-treated sawdust when the sawdust was exposed to the bacterium in a mixed liquid culture for 3 weeks. Intracellular accumulations of copper and chromium were also detected by EDAX. The final objectives of our study were to extend these observations by: (1) assessing whether continuous culture with *B. licheniformis* CC01 enhances removal of the metal components of CCA-treated wood in a shorter incubation time than does standard mixed culture, which generally results in nutrient-limiting culture conditions; and (2) evaluating the bacterial fermentation of acid-extracted or steam-exploded CCA-treated wood.

The objectives of the present work were to: (1) evaluate the ability of oxalic acid in conjunction with other mechanical and microbial processes to extract metals from CCA-treated wood; (2) evaluate the ability of steam explosion to open the chemical structure of CCA-treated wood, thereby providing accessibility to copper, chromium, and arsenic during subsequent oxalic acid extraction or bacterial fermentation; (3) assess whether continuous culture with *B. licheniformis* CC01 enhances removal of metals from CCA-treated wood in less incubation time compared to a standard mixed culture.

Materials and methods

Wood source

A 3-year-old residential deck was chipped, homogeneously mixed, and stored in a watertight container until further use. This material had been originally treated to 6.4 kg m^{-3} retention with chromium, copper, and arsenic formulation (CCA). In some experiments, chips were ground to 20 mesh in a Wiley mill (Figure 1). Unprocessed chips or sawdust served as controls.

Steam explosion

Wood chips were sealed in a batch steam exploder with 0.025-m^3 capacity and processed at the Virginia Tech Brooks Forest Products Center in Blacksburg, Virginia. Chips were processed for 10 min at 205°C and 2.4 GPa followed by instantaneous release of pressure. Steam-exploded chips were either: (1) tested for release of CCA chemicals (control); (2) washed with water for 4 h and tested for remaining chemicals; (3) extracted with oxalic acid; (4) treated by exposure to *B. licheniformis* CC01; or (5) treated with a combination of acid and bacterial fermentation.

Acid extraction

Oxalic acid (1%, pH 2.0) was tested for extraction of CCA chemicals. Treated unprocessed wood chips, sawdust, and steam-exploded chips were extracted for 24 h on a rotating platform. Wood samples were separated from the acid solution and either tested for removal of CCA chemicals or further treated with *B. licheniformis* CC01.

Microorganism

Bacillus licheniformis CC01, a Gram-positive spore-forming rod, was utilized for all bacterial fermentations [5]. This facultative anaerobe forms smooth, opaque colonies at 30°C. It grows at temperatures ranging from 10°C to 55°C and at pH 3–10 in nutrient broth. *B. licheniformis* CC01 is non-fastidious and demonstrates luxurious growth with minimal nutrients (0.5% peptone). The organism was cultured and maintained on nutrient agar (Difco, Detroit, MI, USA) at 25°C throughout the study.

Bacterial culture

For the standard mixed culture, flasks (300-ml) containing 100 ml nutrient broth and 0.5 g CCA-treated chips or saw-

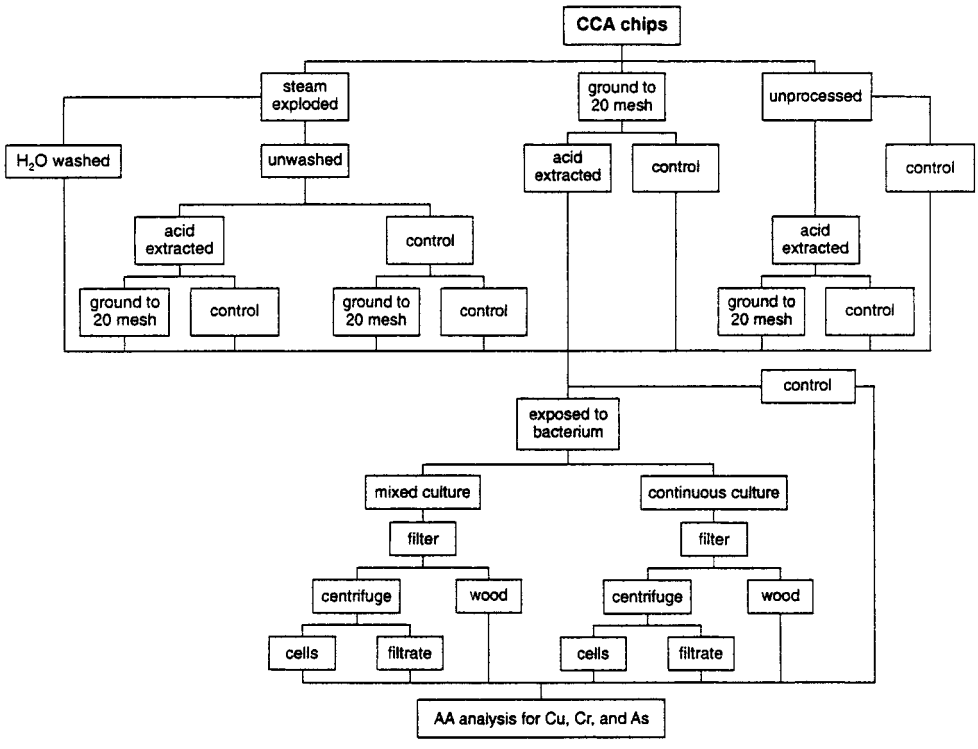

Figure 1 Schematic diagram of study plan—treatment methods and processing combinations.

dust were inoculated with 1 ml of an 18-h nutrient broth culture of *B. licheniformis* CC01. Cultures were incubated at 25°C on a rotating table at 200 rpm for 10 days. Controls consisted of uninoculated flasks of nutrient broth with sawdust that was added before autoclaving.

For continuous culture, a chemostat was devised from a series of flasks that delivered fresh nutrient broth drop by drop to a 2-L culture of *B. licheniformis* CC01 containing 30 g CCA-treated wood. The culture flask was inoculated with 10 ml of an 18-h broth culture of *B. licheniformis* CC01 and mixed at 200 rpm. Spent culture medium was collected in a third flask for analysis. A total of 9 L of nutrient broth was added to the actively growing culture of *B. licheniformis* CC01 at 25°C over a 7-day incubation period.

Sample collection

CCA-treated wood (sawdust or chipped) was separated from oxalic acid or culture filtrate by aspiration through Whatman filter paper. Wood samples were rinsed briefly with deionized water and dried at 60°C for 24 h.

Bacterial cells were collected from the continuous-culture spent-medium reservoir by centrifugation at $16\,000 \times g$ for 30 min. Pelleted cells were resuspended in 40 ml water and autoclaved to kill remaining viable cells. A 40-ml sample of pooled culture filtrate was removed for analysis by atomic absorption (AA) spectroscopy and autoclaved. Any remaining culture medium was removed from the sawdust by aspiration through Whatman filter paper. The sawdust sample was rinsed briefly with deionized water and dried at 60°C for 24 h. The continuous culture control consisted of CCA-treated sawdust that was not exposed to culture medium or bacterium.

Sample analysis

For all experiments, 0.5-g samples of dried 20-mesh sawdust were analyzed by AA spectroscopy according to American Wood-Preservers' Association (AWPA) methods [1] to determine copper, chromium, and arsenic levels. For acid extractions and mixed culture bacterial fermentation experiments, 20-ml samples (acid and culture filtrate, respectively) were submitted to AA analysis. For the continuous culture experiment, 20-ml samples of cells and culture filtrate and 0.5-g samples of treated and untreated CCA sawdust were analyzed by AA spectroscopy.

Results

Processes for treating unprocessed, ground, and steam-exploded CCA-treated wood chips prior to acid extraction or bacterial fermentation are shown schematically in Figure 1. Results of AA analyses for copper, chromium, and arsenic following acid extraction, bacterial fermentation, and steam explosion of CCA-treated sawdust and chips are shown in Table 1. Exposure to *B. licheniformis* CC01 removed 14% more copper and 45% more arsenic but 10% less chromium from sawdust than from non-processed chips (91% *vs* 77% reduction in copper, expressed as CuO, 45% *vs* 0% reduction in arsenic, expressed as As_2O_5, and 15% *vs* 25% reduction in chromium, expressed as CrO_3) (Table 1). Likewise, exposure to the bacterium removed 22% more copper and 18% more arsenic from sawdust than from steam-exploded chips (91% *vs* 69% Cu reduction and 45% *vs* 27% As reduction). However, bacterial exposure removed more chromium (35%) from steam-exploded chips than from either unprocessed chips or sawdust (increase of 10% and 20%, respectively).

Table 1 Atomic absorption analysis of metals in CCA-treated sawdust and chips following acid extraction, bacterial fermentation, and/or steam explosion[a]

Treatment	CuO (ppm)	CrO_3 (ppm)	As_2O_5 (ppm)
Sawdust			
unprocessed control	2700 ± 77	8100 ± 396	4700 ± 104
bacteria/mixed culture	250 ± 19	6923 ± 193	2608 ± 476
acid extract	500 ± 67	3100 ± 143	500 ± 274
acid extract/bacteria/mixed culture	25 ± 0	1730 ± 85	0
acid extract/bacteria/continuous culture	50 ± 13	1730 ± 65	0
Unprocessed chips			
unprocessed control	2500 ± 185	7800 ± 215	3300 ± 266
bacteria/mixed culture	575 ± 57	5827 ± 245	3676 ± 104
acid extract	2100 ± 103	6700 ± 82	1900 ± 122
acid extract/bacteria/mixed culture	250 ± 51	1538 ± 76	0
acid extract/20-mesh grind/bacteria	250 ± 59	6154 ± 195	2454 ± 179
Steam-exploded chips			
unprocessed control	2200 ± 220	9900 ± 484	3500 ± 425
bacteria/mixed culture	676 ± 48	6423 ± 152	2562 ± 206
acid extract	600 ± 25	9800 ± 175	2200 ± 131
acid extract/bacteria/mixed culture	125 ± 62	8077 ± 448	2607 ± 110
acid extract/20-mesh grind/bacteria	250 ± 74	7692 ± 102	2454 ± 104
water wash	1727 ± 101	7981 ± 123	4571 ± 151
water wash/bacteria	476 ± 83	7519 ± 78	2991 ± 82

[a]$n = 3$.

Oxalic acid extraction removed 65% more copper, 48% more chromium, and 47% more arsenic from sawdust than from unprocessed chips (81% *vs* 16% Cu reduction, 62% *vs* 14% Cr reduction, and 89% *vs* 42% As reduction) (Table 1). Likewise, acid extraction removed more copper, chromium, and arsenic from sawdust than from steam-exploded chips (8%, 61%, and 52%, respectively).

Exposure of sawdust to bacterial fermentation in continuous culture enhanced the removal of metals compared to removal of metals from the standard mixed culture (Table 2). Continuous culture with *B. licheniformis* CC01 removed more copper, chromium, and arsenic (13%, 6%, and 3%, respectively) from sawdust after 7 days of incubation than from the standard mixed culture after 10 days of incubation. As an individual method for processing sawdust, unprocessed chips, and steam-exploded chips (washed and unwashed), continuous culture showed no significant advantage over standard mixed culture (Table 3).

Reductions of CuO, CrO_3, and As_2O_5 for individual and combined processing methods are shown in Figures 2, 3, and 4 for sawdust, unprocessed chips, and steam-exploded chips, respectively. Acid extraction followed by bacterial fermentation removed 99% CuO, 79% CrO_3, and 100% As_2O_5 from sawdust in standard mixed culture. Identical results were observed for sawdust exposed to acid extraction and the bacterium in continuous culture. Removal of metals from unprocessed chips subjected to acid extraction prior to bacterial fermentation in mixed culture was similar to that from sawdust (90% CuO, 80% CrO_3, and 100% As_2O_5 reduction) (Table 1). Grinding chips to 20-mesh after acid extraction and prior to fermentation did not enhance removal of metals.

Acid extraction of steam-exploded chips followed by bacterial fermentation or by grinding and then fermentation did not enhance removal of metals (Table 1, Figure 4). Washing the steam-exploded chips with water to remove some acids produced during the steam explosion process did not enhance bacterial removal of metals.

Discussion

Wood chips or sawdust treated with CCA were subjected to chemical modification (acid extraction), mechanical modification (steam explosion), or microbial modification

Table 2 Atomic absorption analysis of metals in CCA-treated sawdust after exposure to *B. licheniformis* CC01 in standard mixed and steady-state continuous cultures[a]

Treatment	CuO		CrO_3		As_2O_5	
	(ppm)	(percent reduction)	(ppm)	(percent reduction)	(ppm)	(percent reduction)
Untreated	2280 ± 60	–	3800 ± 122	–	4210 ± 167	–
Mixed culture	460 ± 58	80	3850 ± 25	0	2410 ± 99	43
Continuous culture	170 ± 12	93	3580 ± 217	6	2320 ± 8	45

[a]$n = 3$.

Table 3 Atomic absorption analysis of metals in CCA-treated sawdust, unprocessed chips, steam-exploded chips, and washed steam-exploded chips after exposure to *B. licheniformis* CC01 in steady-state continuous culture[a]

Wood type	CuO		CrO_3		As_2O_5	
	(ppm)	(percent reduction)	(ppm)	(percent reduction)	(ppm)	(percent reduction)
Sawdust						
untreated	3004 ± 239	–	7885 ± 288	–	5829 ± 315	–
treated	250 ± 105	92	8269 ± 432	0	3528 ± 85	39
Chips						
untreated	2191 ± 221	–	5539 ± 70	–	4479 ± 340	–
treated	575 ± 13	74	5827 ± 0	0	3697 ± 427	17
Steam exploded						
untreated	2879 ± 298	–	9039 ± 106	–	5522 ± 468	–
treated	376 ± 150	87	9231 ± 544	0	3298 ± 368	40
Washed steam-exploded						
untreated	2128 ± 201	–	8269 ± 144	–	4142 ± 215	–
treated	375 ± 51	82	8846 ± 664	0	3321 ± 165	22

[a] $n = 2$.

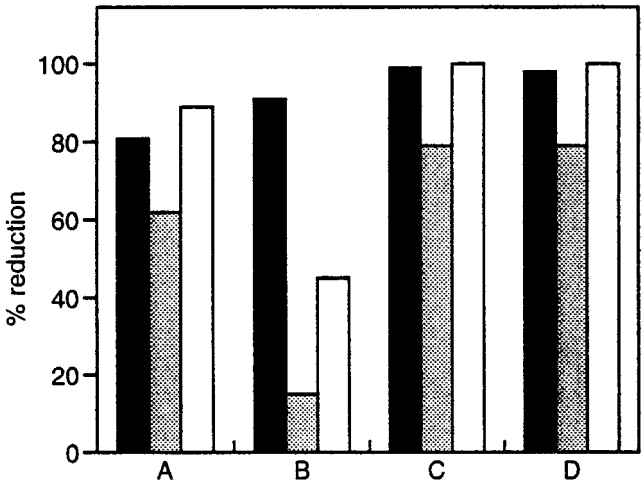

Figure 2 Reduction of CuO (■), CrO_3 (▨), and As_2O_5 (□) after treatment of CCA-treated sawdust with oxalic acid extraction (A), bacterial exposure in mixed culture (B), oxalic acid extraction followed by bacterial exposure in mixed culture (C), and oxalic acid extraction followed by bacterial exposure in continuous culture (D).

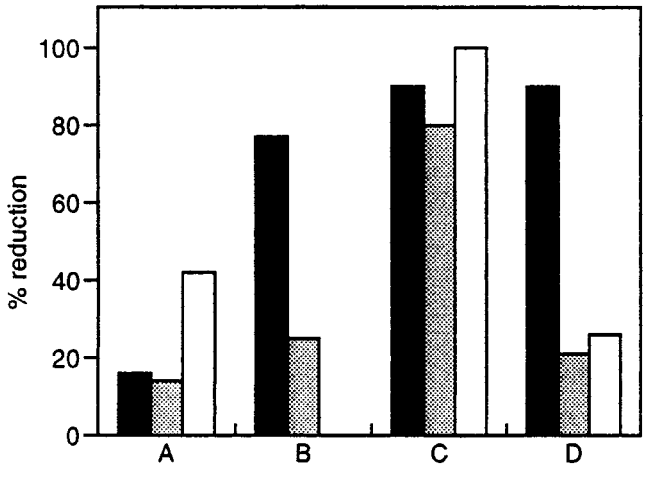

Figure 3 Reduction of CuO (■), CrO_3 (▨), and As_2O_5 (□) after treatment of CCA-treated chips with oxalic acid extraction (A), bacterial exposure in mixed culture (B), oxalic acid extraction followed by bacterial exposure in mixed culture (C), and oxalic acid extraction followed by grinding chips to 20 mesh and bacterial exposure in mixed culture (D).

(exposure to a bacterium) to evaluate these processes for metal removal from the wood fiber. The CCA-treated wood was processed by individual methods as well as combinations of two or more methods in an effort to remove more than 90% of the metals from the wood. Oxalic acid extraction followed by bacterial fermentation removed 90% CuO, 80% CrO_3 and 100% As_2O_5 of the initial concentration of these metals in CCA-treated wood chips.

Spent CCA-treated wood and treated waste wood present a unique recycling challenge. Copper chromated arsenate is fixed to the lignin component of wood by the reduction of Cr^{+6} to Cr^{+3} [12]. The strong affinity of chromium for lignin [22] aids in the retention of arsenic and copper, but the actual complexing of chromium appears to inhibit its removal in appreciable quantities when exposed to the CC01 isolate of *B. licheniformis* [4].

Exposure of CCA-treated wood to acid and heat may reverse the CCA fixation process [19]. High acidity may be the key to 'unfix' copper for further leaching by other processes [20]. Leachability tests with many different acids have shown some degree of success at removing various amounts of CCA metals from treated wood [13,16,20]. Oxalic acid, which is implicated in the wood decay process by brown-rot fungi [11], has recently been examined for leachability of chromium and copper from chromium copper-treated wood under high acidity conditions [20]. In the current study, oxalic acid extraction at pH 2.0 was the most successful single treatment of CCA sawdust for removing copper, chromium, and arsenic. Oxalic acid extraction followed by bacterial fermentation removed 80–100% of CCA chemicals from chipped wood. Oxalic acid extraction may act to partially reverse the fixation process. Similar results were seen by Smith and Shiau [19] in treating CCA sawdust

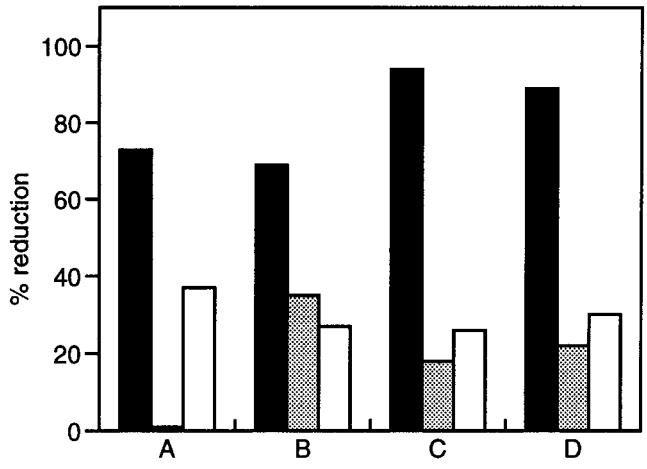

Figure 4 Reduction of CuO (■), CrO_3 (▨), and As_2O_5 (□) after treatment of steam-exploded CCA-treated chips with oxalic acid extraction (A), bacterial exposure in mixed culture (B), oxalic acid extraction followed by bacterial exposure in mixed culture (C), and oxalic acid extraction followed by grinding chips to 20 mesh and bacterial exposure in mixed culture (D).

with citric acid. Not only did the authors show that citric acid extraction of treated sawdust over 21 days could remove more than 80% of copper, chromium and arsenic, but also that pH has a dramatic effect on metal removal. In extractions with citric acid, a pH of 3.5 removed chromium nearly twice as effectively as a pH of 5.0. Further evaluation is necessary to assess whether oxalic acid alters the wood structure or fiber strength.

If combined acid extraction and bacterial fermentation could remove most metals from CCA-treated chipped wood, this would present a tremendous economical advantage over processing sawdust, as well as provide a safer process for mill workers. Chipping is a standard process during recycling of untreated wood for composite products. An economical and environmentally benign process or processes to remove significant quantities of CCA from treated wood may be readily incorporated into current manufacturing processes.

Members of both *Bacillus* and *Pseudomonas* genera are ubiquitous in soils and produce pectinolytic and cellulolytic enzyme systems that may assist in releasing copper and arsenic from wood. Members of both genera are more resistant to copper than are Basidiomycetes and have been isolated from preservative-treated wood [3,10]. Tolerance levels of CCA in artificial medium showed that concentrations as high as 0.675% cause growth of these bacteria to cease, but the organisms remain viable [10]. Energy-dispersive x-ray analysis suggests that the CC01 isolate of *B. licheniformis* accumulates copper and chromium intracellularly [5]. However, bacterial cells collected from spent medium accounted for a small fraction of total CCA by weight of the initial test material. Most of the released metals were found in the spent culture medium (data not shown). It has been suggested that bacterial capsules or slime layers complex with elements and then release them enzymatically in small quantities [9]. Daniel and others [7,8] examined degraded CCA-treated *Radiata* pine utilizing TEM–EDAX and found copper accumulations as dense particles within the nuclear region of tunneling bacteria. They also noted that a majority of the chromium and arsenic remained as extracellular secretions. Surely, if bacteria from CCA-treated wood that have adapted to release metals can be isolated, then public concern over potential soil and groundwater contamination from the landfilling of spent CCA-treated wood is a valid concern.

In our study, steam explosion as a means of opening the chemical structure of wood for accessibility to chemical components demonstrated limited success in releasing metals from treated wood. Steam-exploded chips exposed to the bacterium showed a 35% reduction in chromium, while chromium removal was nearly zero for the oxalic acid extraction alone. Further combinations of treatments following steam explosion (for example, acid extraction and bacterial fermentation) were successful only at removing greater quantities of copper. Smith and Shiau [19] showed that citric acid extraction for copper was similar for CCA-treated sawdust and steam-exploded pulp, but chromium and arsenic were more effectively removed from acid-extracted CCA-treated sawdust.

In this evaluation of the ability of physical, chemical, and microbial processes to remove chemicals from CCA-treated wood wastes, mechanical modification by steam explosion demonstrated limited success. Chemical fiber modification with oxalic acid was the single most successful treatment, removing 62–89% of copper, chromium, and arsenic from CCA-treated sawdust. Fermentation with *B. licheniformis* CC01 released 77% copper from unprocessed chips and 91% copper from sawdust, but the microorganism was unable to release more than 45% arsenic from sawdust or 35% chromium from steam-exploded chips. Ideally, a treatment or combination of treatments should remove more than 90% of all CCA components for the fiber to be acceptable for use in recycled composites. Overall, oxalic acid extraction was the most successful single treatment at removing significant quantities of metals from sawdust (81% CuO, 62% CrO_3, and 89% As_2O_5). Oxalic acid extraction plus fermentation by *B. licheniformis* CC01 was the only treatment combination able to remove significant quantities of CCA metals from chipped wood. Optimizing treatment conditions, such as exposure time to the acid and nutritional requirements of the bacterium, will be studied further to increase chromium release by these two processes. We will also evaluate the mechanical integrity of the wood structure after exposure to *B. licheniformis* CC01.

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