# Bacterial Biodegradation of CCA-Treated Waste Wood

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**Abstract**

As wood treated with chromated copper arsenate (CCA) is removed from service, methods for eliminating toxic metals from the wood are needed. CCA-tolerant bacteria were evaluated for their ability to modify CCA-treated wood. Aerobic bacteria were isolated from a 20-year-old test plot of CCA-treated stakes. Bacteria selected for their ability to grow on nutrient agar containing CCA components, were identified as *Pseudomonas putida, Bacillus licheniformis,* or *Bacillus coagulans.* X-ray analysis using scanning electron microscopy revealed intracellular uptake of copper, chromium, or arsenic from nutrient agar containing 0.12 percent CCA by one or more bacterial isolates. Atomic absorption analysis of the sawdust exposed to these bacteria for 3 weeks showed a reduction of 22 percent to 46 percent for copper, 0 percent to 9 percent for chromium, and 0 percent to 8 percent for arsenic by weight compared with the unexposed sawdust. Bacterial exposure of waste wood chips shows potential as an effective means of modifying CCA-treated wood, ideally for recycling into value-added products, such as wood fiber composites.

**Introduction**

Chromated copper arsenate (CCA) is a common wood preservative. Approximately $212 \times 10^6$ $m^3$ ($600 \times 10^6$ $ft.^3$) of CCA-treated lumber are produced annually in the United States and production is increasing. With an expected service life of 40 years, disposal of CCA-treated waste wood will pose a major environmental concern in the future when it is removed from service. Incineration of the treated wood concentrates the toxic metals contained in the preservative. Continually filling limited landfill space with CCA-treated waste wood is not an environmentally sound solution; it only postpones dealing with the problem. In this study, CCA-tolerant bacteria were evaluated as an effective means of modifying CCA-treated wood, prior to recycling into value-added products.

Bacteria are frequently isolated from preservative-treated wood. They appear to be tolerant to a large range of preservatives and to concentrations that norreally inhibit the development of fungi (1,6). Wood-inhabiting bacteria are associated with wood decay and may have an indirect influence on the decay process. Bacteria can affect wood permeability, attack wood structure, or work together with other bacteria and soft-rot fungi to predispose wood to fungal attack. Greaves (4) and Henningsson (7) found that bacteria can accelerate fungal decay in treated wood. Hochman (8) and Rossell et al. (9) suggested that bacteria reduce the toxicity of the preservative, hence facilitating fungal attack.

The objectives of this study were to:

- isolate and identify bacteria that would grow on CCA Type III incorporated in a nutrient agar

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- assess the ability of bacterial inhabitants of CCA-treated wood to sequester CCA components.

## Materials and methods

### Isolation of bacterial cultures

Aerobic bacteria were isolated from CCA-treated stakes that were located in a 20-year-old test plot in Madison, Wisconsin. The interface of the stake and soil was swabbed and then streaked on standard nutrient agar plates. Approximately 40 samples were taken from spruce, pine, or Douglas-fir stakes that were either incised 2 by 4s, unincised 2 by 4s (actual 37 by 86 mm), or plywood.

Agar plates were incubated for 24 hours at 25°C. Two colonies from each plate were randomly chosen, isolated, and restreaked to obtain purified cultures.

### CCA tolerance and viability

Purified cultures were each streaked on nutrient agar containing 0.08 percent or 0.12 percent CCA and their growth was monitored for 21 days at 25°C. *Pseudomonas putida* ATCC 11172 was included in all tests as a control organism. The percentage of CCA in the medium was calculated from an initial solution of CCA containing 23.75 percent $CrO_3$, 9.25 percent CuO, and 17.00 percent $As_2O_5$. Twelve cultures showing significant growth on the CCA agar were further tested for their ability to remove insoluble preservative elements fixed to wood.

Viability was determined by transferring each culture to fresh nutrient agar after 3 weeks of growth on 0.12 percent CCA agar.

### X-ray analysis of CCA uptake

Bacterial lawns of 12 isolates were grown on nutrient agar or on 0.12 percent CCA-nutient agar for 10 days. Ceils were washed off the agar surface with deionized $H_2O$ and collected on 0.22-µm filters. Cells from CCA agar were additionally washed with water and centrifuged for 20 minutes at 3000 xg to wash the cell surface before cells were collected on 0.22-µm filters. After air drying, bacterial cells, CCA-agar controls, and falter controls were examined in a JEOL scanning electron microscope with a Tracer Northern energy dispersive X-ray analysis attachment (EDAX). This instrument allows structural studies combined with qualitative analyses. The electron beam of the scanning electron microscope excites the specimen and X-rays are produced. The qualitative analysis is provided by a spectrometer, which detects the X-rays based on their intensities. As the electron beam penetrates the specimen, there is a dispersal of energy, and as the X-rays are emitted, energy is absorbed in passing through the specimen to the detector(6), allowing detection of intracellular accumulation of one or more elemental components of CCA.

### Utilization of CCA bound to wood

To determine the ability of the isolate to utilize CCA bound to wood, sawdust (20 mesh) from a CCA-treated 2 by 4 (6.4 kg/$m^3$ [0.4 lb./$ft.^3$] retention) was exposed to individual and mixed cultures of selected bacterial isolates. Five bacterial isolates, which exhibited significant growth on 0.12 percent CCA agar, were inoculated individually and in combination into flasks of nutrient broth (50 ml) with 0.5 g of CCA sawdust and incubated on an orbital shaker at 27°C for 3 weeks. An uninoculated control was included to compare the concentration of CCA before and after exposure with the various bacteria.

### Isolate identification

A gram stain divided the isolates into either gram-negative or gram-positive organisms. Identification tests were performed to determine fermentative, oxidative, and thermal differentiation of five isolates demonstrating potential to accumulate CCA from agar or utilize CCA bound to wood. Isolate numbers designate the stake of origin, for purposes of identify ing wood species, and type of stake.

## Results

### CCA tolerance and viability

Twelve isolates grew on the 0.12 percent CCA agar. Those isolates that showed excellent or good growth, based on a scale of 0 to 4 where 0 equals no growth and 4 equals excellent growth, were *P. putida* ATCC 11172,665,690,351, and 1. Those showing moderate growth were 137A and 137B. Poor growth was exhibited by 114,352, 123A, 123B, and 655. All 12 cultures retained viability after 3 weeks of growth on 0.12 percent CCA agar as determined by restreaking each isolate on fresh nutrient agar.

### X-ray analysis of CCA uptake

**The scanning** electron microscope analysis with the energy dispersive X-ray analysis (EDAX) revealed that six isolates accumulated one or more of the CCA components intracellularly. These were *P. putida,* 665, 351, 1, 114, and 352. Uptake values for copper, chromium, and arsenic compared with an agar control are shown in Table 1. Copper uptake was prominent (80 to 100% of the control) in 114, *P. putida,* 665, 1, and 351. Isolate 690 showed moderate uptake of copper and 352 demonstrated a small amount of copper

uptake relative to the control. Chromium uptake was less than 8 percent of the control value for six of the seven isolates. Arsenic uptake was uniformly zero.

### Utilization of CCA bound to wood

**The** atomic absorption analysis (Table 2) demonstrated the percentage weight reduction of each component for each isolate relative to an uninoculated sawdust control. All cultures displayed 22 to 46 percent reduction of copper compared with the control. Isolates 351 and 690 showed no weight reduction of chromium, and *P. putida,* 1, 665, and the culture containing a combination of the five bacteria ranged from 2 to 9 percent reduction of chromium by weight. No absorption of arsenic was seen for *P. putida,* 351, 690, and 665. Approximately 1 percent and 8 percent reduction was found in the combined culture and isolate 1, respectively.

### Isolate identification

Isolates indicating intracellular uptake of one or more components of CCA, as determined by EDAX, were identified. Isolate 114, a gram-negative rod, was presumptively identified as *P. putida.* The remaining four isolates, gram-positive spore-forming rods, were identified as members of the genus *Bacillus;* 351 and 1 were identified as *B. licheniformis* 665 and 352 were identified as *B. coagulans.*

## Discussion

Bacterial isolates were identified that could tolerate CCA and retain viability while intracellularly accumulating one or more of the CCA components. EDAX analysis revealed prominent uptake of copper, the least toxic component of CCA. Minimal uptake of chromium was noted in six of seven isolates. Arsenic uptake was believed to be uniformly negative, although it should be noted that the arsenic peak was masked by a magnesium reading in nearly the same place on the EDAX spectra.

Five isolates were also shown to scavenge CCA components away from pressure-treated wood upon 3 weeks of exposure. Isolate 1, presumptively identified as *B. licheniformis,* reduced the copper content of CCA-treated sawdust by 46 percent, chromium by 9 percent, and antic by 8 percent compared with the uninoculated control. Other isolates from this study reduced copper and chromium to a lesser degree and were unable to reduce arsenic, the most toxic component of CCA. Continued surveys for other CCA tolerant bacteria, possibly even anaerobes, may lead to a bacterial consortium with the ability to rapidly and efficiently remove CCA from chipped wood.

Pseudomonades are a prominent part of the bacterial flora of preserved wood. They are nonspore forming, gram-negative organisms with the ability to degrade xenobiotic compounds. *P. putida* ATCC 11172, included in this study, is known to degrade phenol. Gram-positive spore-forming bacteria from the genus *Bacillus* are also commonly isolated from preserved wood, possibly because of the resistance of bacterial spores to harsh conditions. Members of both genera possess the ability to attack oil-based wood preservatives, such as copper naphthenate, and are more resistant to copper than Basidiomycetous fungi, the group of fugi targeted for inhibition by wood preservatives.

It has been theorized that bacterial capsules and slime layers complex with elements, such as copper, "lock up" the toxic metal when it is released in small quantities by bacterial enzymes. In this form, the copper would be nontoxic to wood decay fungi (5). Daniel and Nilsson (2) and Daniel et al., (3) found

**Table 1.**—Energy dispersive X-ray analysis (EDAX) of bacteria grown on CCA agar.

| Culture | EDAX | | |
|---|---|---|---|
| | Copper | Chromium | Arsenic |
| | (net counts) | | |
| Agar control | 332 | 4,051 | 337 |
| *P. putida* | 427 | 290 | 0 |
| 1 | 340 | 81 | 0 |
| 114 | 304 | 82 | 0 |
| 351 | 268 | 157 | 0 |
| 352 | 66 | 301 | 0 |
| 665 | 320 | 68 | 0 |
| 690 | 199 | 0 | 0 |

**Table 2.**—Atomic absorption analysis of CCA-treated sawdust after exposure to bacteria.

| Culture | Weight reduction | | |
|---|---|---|---|
| | Copper | Chromium | Arsenic |
| | (%) | | |
| Sawdust control | -- | -- | -- |
| *P. putida* | 25.21 | 3.94 | 0.00 |
| 1 | 46.22 | 9.06 | 7.64 |
| 351 | 21.85 | 0.00 | 0.00 |
| 665 | 38.66 | 4.59 | 0.00 |
| 690 | 22.69 | 0.00 | 0.00 |
| Combined culture | 26.05 | 2.10 | 0.90 |

copper accumulation as dense particles within the nuclear region of tunneling bacteria, while a majority of chromium and arsenic remained in extracellular secretions. This observation may account for the limited chromium and arsenic reduction observed in the study reported here. Although intracellular accumulation of copper was demonstrated together with the ability of bacteria to scavenge metals away from pressure-treated wood, it must be noted that there is no indication whether metals are being stored in the toxic form (e.g., Cr VI and As III) or altered to a less toxic form (e.g., Cr II or III and As V). Additional studies are needed to determine the fate of each metal component of CCA.

The 46 percent reduction of copper from CCA-treated sawdust by *B. licheniformis,* isolate 1, was the result of a 3-week incubation. As a result of the short generation time for bacteria, the incubation time needs to be studied to determine if a similar result will occur in a shorter time frame. Also, after a 3-week incubation, the culture was in stationary phase and the culture medium was spent. A chemostat, providing continuous culture, is the next step to investigate copper reduction beyond 46 percent.

## Concluding remarks

The United States urgently needs to reduce the amount of wood wastes being landfilled by developing alternative uses for recycled wood fiber. Recycling of CCA-treated wood is almost nonexistent, because it currently is only a small fraction of demolition wood or wood removed from service. However, an upward trend in the consumption of CCA-treated wood began in the 1980s and has continued to climb. With an expected service life of 40 years, CCA-treated waste wood disposal will become a prominent concern when it is removed from service in large quantities. Currently, treated demolition wood and wood removed from service is being placed in landfills. Centralized storage facilities do not exist for accumulation of CCA-treated waste wood, where it could be studied on a large scale for recycling into value-added products. If bacterial pretreatment of chipped waste wood could scavenge toxic metal components from CCA, the modilfied chips could be recycled into wood/fiber composites, wood/plastic composites, or wood/concrete composites, to be used to building applications.

Our preliminary study shows the potential of certain bacterial species to release one or more of the elemental components of CCA from pressure-treated wood and accumulate the elements intracellularly. Bacterial exposure of CCA-treated wood chips shows potential as an effective means of modifying treated wood so that it may be recycled into value-added products.

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