22 Bioremediation of Treated Wood with Bacteria

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22.1 INTRODUCTION

Bioremediation is the process of exploiting microorganisms to degrade or remove hazardous components of a waste from the environment. This may be accomplished through bioleaching, which is the solubilization or dissolution of components from a material through the actions of microorganisms.[1] The key to bioremediation of treated wood is controlling specific microorganisms under specific environmental conditions to achieve removal or transformation of a contaminant.[2] Primary conditions required to sustain a bioremediation process are one or more bacterial species capable of removing or degrading the contaminant and controlled nutrients, moisture, pH and temperature. Without optimizing these conditions, the process will be limited or result in lack of contaminant remediation. Bioremediation may also be limited by the recalcitrance of the target pollutants as a result of their chemical stability, or by the toxicity of such compounds.[3]

Bacteria are often able to tolerate wood preservatives at retention levels usually employed to prevent fungal attack.[4] For example, members of *Bacillus* spp. and *Pseudomonas* spp. have the ability to attack wood treated with oilborne preservatives,

such as copper naphthenate and pentachlorophenol, and both genera of bacteria are more resistant to copper than are decay fungi.[5] *Pseudomonas* sp., *Bacillus polymyxa* and *Clostridium* sp. produce cellulases and pectinases that alter wood permeability by opening up the crystalline structure of cellulose microfibrils.[6] Because these bacteria are more resistant to preservatives, their enzyme systems allow them to derive nutrients present in the wood cell wall while simultaneously opening the crystalline structure for advance diffusion of cellulolytic enzymes to pave the way for invasion by fungi. Nonspore-forming *Pseudomonas* spp. are commonly replaced with spore-forming *Bacillus* spp. in the biological succession of species in wood. Increasing quantities of preservative hardly change the overall bacterial flora. Rather, under those circumstances, the flora is naturally supplemented with an increased number of cellulolytic bacteria. High loadings of preservatives also indirectly enhance the bacterial population by excluding fungal competitors.[4]

Bacterial associations with preserved wood products have been recognized since the 1950s but have received little attention because preservation research has always emphasized extending the service life of treated wood against a few chemically resistant soft-rot fungi and the wood-decaying *Basidiomycotina*.[5] Until recently, relatively few papers have been devoted to the bacterial decomposition of wood preserved by creosote, pentachlorophenol or chromated copper arsenate (CCA).[7-9] These studies identified bacteria responsible for decomposition rather than emphasizing purposeful isolation of preservative-tolerant organisms with the intent of utilizing them in bioremediation processes. There are potential benefits of using bacteria to bioremediate treated wood. Unlike fungi, bacteria cause no structural damage to wood fiber so that a bacterial process that separates the preservative chemicals from the wood allows for recycling the preservative as well as reuse of the cleaned wood fiber in a secondary application.

This chapter reviews prior research in the field of bacterial bioremediation for wood treated with oilborne and inorganic preservatives. Current state of the art is summarized along with potential benefits and pitfalls of a pilot-scale bioremediation process for CCA-treated waste wood.

22.2 OILBORNE PRESERVATIVES

In 1996, 1.3×10^6 m^3 of creosote-treated railroad ties and 2×10^6 m^3 of pentachlorophenol- (PCP) and creosote-treated utility poles were available for recycling annually in the $U.S_{10}$ Both creosote- and PCP-treated wood are mainly used in rail sleepers and power transmission poles, ensuring that waste wood from these applications can he readily collected and that the material is sizable and uniform, which is a desirable characteristic for remediation. Despite an average service life of 50 years, treated utility poles often retain PCP at concentrations close to original treatment retention when they are removed from service.[11] Environmental regulations on the release of chlorinated organics in pulp mill effluents eliminated pulping as a means of disposing of PCP-treated wood.[12] Giveaway programs were once a popular outlet for used railroad ties and utility poles, providing little incentive to develop bioremediation methods for spent creosote-treated wood, but this practice has since become restricted.[10,13] Limited capacity of landfill sites and problems with high-temperature

incineration creating dioxins in PCP-treated wood have often resulted in utility companies storing treated utility poles until acceptable disposal methods could be developed.[12]

As long ago as 1966, Drisko and ONeill[14] identified a bacterium that decomposed creosote, *Pseudomonas creosotensis.* Petrenko[9] was able to readily isolate and identify a number of species of Pseudomonas and Bacillus in wood treated with anthracene oil, PCP and copper naphthenate. Schmidt et al.[15] investigated the biodegradability of creosote by bacteria and reported that *Aeromonas hydrophila, Flavobacterium* sp. and three species of *Pseudomonas* were able to grow in the presence of creosote. Yet, no literature exists demonstrating the utilization of one or more of these bacteria to remediate creosote-treated wood.

The bacterial degradation of PCP has been described in many studies,[16-20] and the bacteria responsible for degrading PCP have been isolated from contaminated soil and water.[21-24] Parameters such as pH, temperature, availability of nutrients and need for additional carbon sources have been resolved and tested for the degradation of PCP in water and oil.[25,26] Valo et al.[26] suggested that biological degradation of PCP to harmless inorganic end products is a viable alternative, but bioremediation of PCP-treated wood products was not explored until the early 1990s.[27,28]

Since that time, research on the mineralization of PCP from spent PCP-treated timber has primarily involved two bacteria, *Rhodococcus chlorophenolicus* and *Flavobacterium* sp.;[29] certain species of Flavobacterium can utilize PCP as their sole carbon source.[30] These organisms can degrade more than 99% of the PCP extracted as a water-soluble salt from sawdust or wood chips.[12] However, solid substrate fermentation of PCP-treated wood would not be suitable for bacterial inoculants. The fate of preservative elements released from the mineralization of PCP-treated wood remains a cause for environmental concern.[31]

22.3 INORGANIC COMPOUNDS

22.3.1 Laboratory-Scale Research

Beyond reports of tunneling and erosion bacteria deteriorating in-service preserved wood, bacteria have received little attention by researchers studying disposal methods for wood treated with copper inorganics, especially because CCA-treated wood is considered a nonhazardous material that can he disposed of in landfills. With 4×10^8 m^3 of CCA-treated wood entering service since the 1970s,[1] alternative disposal methods to landfilling became a research focus in the early 1990s in anticipation of diverting this waste material from landfills through an alternative recycling program. Unlike creosote- or PCP-treated products, CCA-treated dimension lumber and round wood have been used in numerous residential and commercial applications and are often mixed with other types of treated or finished wood products, making separation and identification of waste from mixed wood debris a complex problem.

Bacillus spp. and *Pseudomonas* spp. have been implicated in the deterioration of in-service CCA-treated horticultural posts[32,33] and poles.[34,35] Eaton and Hale[36] and Eaton[37] identified tunneling bacteria as the source of decay of ammoniacal-copper-quaternary (ACQ)-treated wood in water cooling towers. It has been theorized that

bacterial capsules and slime layers complex with elements such as copper to "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.[38] Daniel and Nilsson[39] and Daniel et al.[40] found copper accumulation as dense particles within the nuclear region of tunneling bacteria in CCA-treated radiata pine. whereas a majority of chromium and arsenic remained in extracellular secretions.

Environmental sampling demonstrated that bacteria could readily be isolated from research test sites where treated stakes were being evaluated for long-term efficacy of wood preservatives.[41,42] Isolates representing 13 species from eight different genera of soil-inhabiting bacteria were obtained by sampling eight such environments with presumed elevated levels of copper (Cu), chromium (Cr) and arsenic (As)[42] *Acinetobacter calcoaceticus, Aureobacterium esteroaromticum* and *Klebsiella oxytoca* were able to remove 98% chromium from CCA-treated wood. *Bacillus lichenifomis* removed the highest percentage of copper, 93%, from treated wood, whereas both *Bacillus licheni-fomis* and *Acinetobacter calcoaceticus* released over 40% of the arsenic from CCA-treated wood. The effectiveness of an organism to remove metals was determined by exposing 1 g of CCA-treated wood ground to pass a 20 mesh (0.84 1-mm sieve openings; nominal preservative retention of 6.4 kg/m^3) to 50 ml of nutrient broth inoculated with the test organism, and incubated at 30°C for 7 d and 15.7 rads (150 rpm.). Following incubation, sawdust was collected, oven-dried, digested and analyzed for Cu, Cr and As content by inductively coupled plasma (ICP) emission spectrometry according to American Wood Preservers' Association standard A-21-00.[43] Ideally, one would create a consortium with several isolates that could collectively remove Cu, Cr and As from treated wood. Attempts to develop such a consortium with these isolates failed due to a rapid replication time for *Bacillus lichenifomis* (21 min), leading to domination by this isolate in cultures of mixed bacteria.

Bacillus lichenifomis has been the only bacterium extensively studied for bioremediating CCA-treated wood since 1996, when it was first isolated at the Valley View Experimental Exposure Site of the U.S. Department of Agriculture Forest Service, Forest Products Laboratory, near Madison, WI.[41] In laboratory studies, growth conditions for *B. lichenformis* to optimally remove copper and arsenic from treated wood particles or chips were determined to range from 25 to 28°C and from 15.7 to 20.9 rads (150 to 200 rpm) for 7 to 10 d.[44,45] Under these conditions, the bacterium was able to consistently remove 93% of Cu and 45% of As, but was unable to effectively remove significant quantities of chromium. Not surprisingly, B. lichenifomis could more effectively remediate particulate wood than chipped material, due to its increased surface area.

Acid extraction was evaluated as a precursor to bacterial fermentation in an effort to enhance metal removal; the rationale for combining acid extraction with bacterial fermentation was threefold. First, previous studies indicated that oxalic acid production plays a critical role in initiation of the decay process by brown-rot fungi.[46] Second, copper induces rapid oxalic acid production by copper-tolerant brown-rot fungi and is believed to be instrumental in the ability of these organisms to decay wood treated with copper organics.[47] Oxalic acid precipitates copper into the insoluble form of the oxalate, rendering the copper metabolite inert. Third, oxalic acid extraction as a means of chemically leaching copper, chromium and arsenic

from treated wood has been shown to be effective.[48,49] Clausen and Smith[45] determined that oxalic acid extraction as a precursor to bacterial fermentation removed 90% of Cu, 80% of Cr and 100% As from treated chips. Oxalic acid extraction was optimized at 0.8% acid for an 18-h exposure time at 25°C and a wood-to-acid ratio of 1:100 (w/v).[50] A two-step remediation sequence in which acid extraction preceded bacterial fermentation was shown to be more effective than either oxalic acid extraction or bacterial fermentation alone. Reversing the sequence (bacterial fermentation before acid extraction) was also not as effective at removing Cu, Cr and As from treated wood. Further, it was determined that oxalic acid could be reused up to three times at a 1:100 ratio of wood to extract solution to repeatedly extract CCA-treated particles.[50]

22.3.2 Pilot-Scale Remediation of Wood Treated with Chromated Copper Arsenate

Once conditions were optimized on a laboratory scale, the two-step bioremediation process was scaled up to pilot scale (approximate 10-fold increase) into a 150-1 stainless steel recirculating tank.[51] On a pilot scale, 12-kg batches of CCA-C treated Southern yellow pine were evaluated with this bioremediation process. Three configurations of wood treated to a nominal retention of 6.4 kg/m^3 were evaluated: particles (3 × 8 mm), flakes (0.5 mm thick × 11 cm long × varying width), and chips (3 × 2 × 0.3 cm). Particulate wood was placed in a woven polypropylene filter fabric bag within the stainless steel recirculating tank, while flakes and chips were processed in a polypropylene mesh bag within the tank? Wood was exposed to 125 1, 0.8% oxalic acid, which was recirculated at 50 l/min and 27°C for 18 h before exchanging the acid solution with 125 1 of sterile nutrient broth. The pH was adjusted to 5.5 to 5.6 with NaOH before adding 1 1 of nutrient broth inoculum containing 6 × 10^7 colony forming units of *B. lichenifomis* per milliliter. The inoculated broth was recirculated at 50 l/min at 27°C for 7 d. Samples of filtrate and bioremediated wood were analyzed for copper, chromium and arsenic.[51]

The extent of metal removal following acid extraction is shown in Figure 22.1. Figure 22.2 shows the total percentage of each metal removed from CCA-treated particles, flakes and chips following the two-step bioremediation process. Higher percentages of each metal were removed from all three wood geometries following the two-step process than following the acid extraction alone. Sixty-five percent to 83% of copper was removed following the two-step bioremediation, compared with 1 to 16% following acid extraction alone. Pilot-scale bioremediation of particulate material removed 79% of Cu, 70% of Cr, and 88% of As, which is similar to results seen in bench-scale studies for this method.[1,44-45] The bacterial fermentation step clearly increased the removal of all three CCA components and preferentially copper.

The cumulative proportion of Cu, Cr and As removed from flaked material following the pilot-scale bioremediation process was 83%, 86% and 95%, respectively. Chipped material showed the lowest removal of CCA components in the pilot-scale bioremediation process:65%, 64% and 81% for Cu, Cr and As, respectively. Factors that affected the efficiency of the scale-up included wood size and thickness,

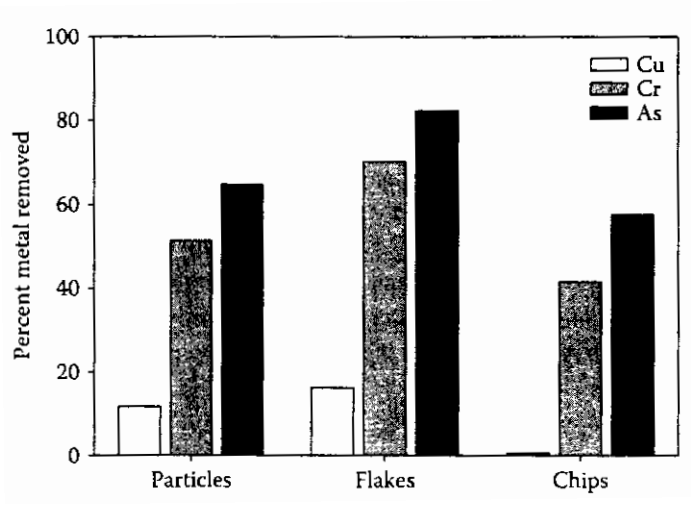

FIGURE 22.1 Metals removed by acid extraction of CCA-treated particles, flakes and chips. Percentage of copper removed following oxalic acid extraction was lowest of CCA-treated components, particularly for chipped wood. Flaked wood had highest percentage of Cr (70%) and As (82%) removed, presumably because of increased surface area exposed to the acid.

unencumbered recirculation of the acid and bacterial growth medium, maintenance of aseptic conditions and rapid growth of the bacterium. Low pH during the acid extraction and the presence of soluble metals in the filtrate discouraged growth of potential contaminants, such as mold fungi. Likewise, overwhelming the system with a bacterial inoculum with a short regeneration time provided an opportunity for the *Bacillus* to out-compete potential contaminants for nutrients, especially those susceptible to the toxic components of CCA.

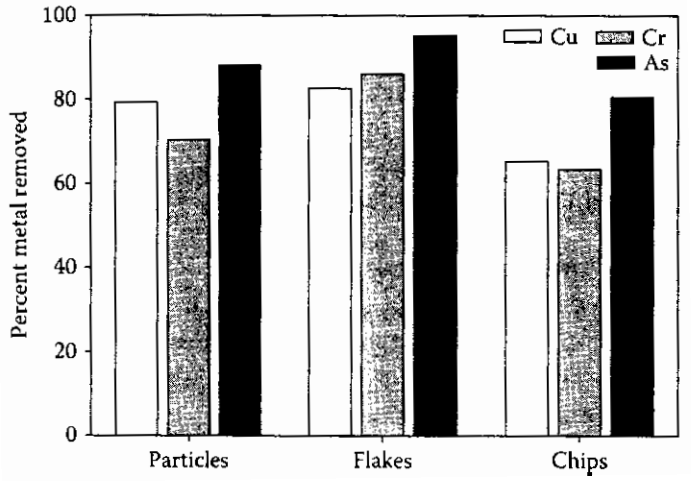

FIGURE 22.2 Percentage of Cu, Cr and As removed after 7 d in the two-step bioremediation with oxalic acid and *Bacillus licheniformis.*

22.3.3 Full-Scale Bioremediation Potential

Bioremedial processes involving metal–microbe interactions have been developed to pilot scale, with some processes reaching commercial operation.[52] Indeed, metal–microbe removal and transformation processes are intrinsic in systems such as water and sewage treatment. Biological processes that minimize environmental contaminants clearly have the potential for broader applications than ex situ processing of soil or wastewater.[53,54] For bioremedial processing of treated-waste wood, comminution is an essential first step. Providing a large wood surface area for microbial action increases the removal rate of metals. Except for flake production, which requires large uniform timbers for uniform flakes, most existing comminution technologies should be suitable for preserved wood.[10] Pilot-scale bioremediation results for CCA-treated wood indicate that full-scale bioremediation would be a matter of proportional scaling of raw materials and equipment availability. Both the acid extraction step and the bacterial fermentation step can be accomplished in a stainless-steel fermentor. Following the acid extraction, the acid would be drained into an adjacent fermentor that contains treated wood because the acid can be reused up to three times. Next, by adding hot nutrient growth medium to the wood in the fermentor, the medium would act as a surface sanitizer for the wood and the equipment. After cooling to the proper temperature, the medium would be inoculated with an actively growing culture of the bacterium and the wood-bacteria-liquid medium slurry would be mixed in the fermentor for a designated amount of time. Finally, the liquid from the fermentor, which now contains copper, chromium and arsenic, would be collected for recycling. The remediated wood would be rinsed thoroughly to remove any residual chemicals and dried before reuse.

22.3.4 Bioremediation of Wood Treated with Arsenic-Free Preservatives

Increasing environmental concerns led to discontinuation of arsenic-containing wood preservatives for residential use in the US. after 2003, thereby reducing by 70% the amount of CCA-treated wood being placed in service annually. CCA has been replaced in the residential market by a new generation of copper-organic preservatives systems that are arsenic and chromium free.[55] In the future, these preservatives could face similar scrutiny, particularly regarding the vulnerability of aquatic environments to copper leaching. Because *Bacillus licheniformis* preferentially removed copper from CCA-treated wood, this bacterium was evaluated for its ability to remove copper from the new generation of arsenic-free, copper-based formulations. Crawford and Clausen[56] reported that in samples of treated Southern pine, 85% of copper was removed from copper citrate- and ACQ-D-treated wood, and 94% of copper was removed from copper naphthenate-treated wood after 10 d of exposure to the bacterium. Bacterial bioremediation is an effective means of copper removal from wood treated with the new generation of copper organics.

22.4 SUMMARY

Research on bioremediation of treated wood with a bacteria has primarily focused on removing chemical components from CCA-treated wood with Bacillus licheniformis. This two-step bioremediation process liberated metal through the solubilization of CCA components with no mass loss to the wood fiber. The "cleaned" wood particles and flakes were reassemble into compsites.[57] Bioremediation enables CCA-treated waste wood to be recycled into secondary products, thereby diverting this waste material from landfills and dreducing the risk of metals leaching into soil or groundwater.

Sample thickness and surface area of CCA-treated particles, flakes and chips affected metal removal during the two-step bioremediation process. Flaked wood was most amenable to metal removal, due to its large surface area, followed by particulated wood (3 × 8 mm). Pretreating wood with oxalic acid served two purposes. First, it partially solubilized the components of CCA so that the bacteria could more readily remove the metals. Second, acid extraction also served to santize the wood and processing equipment to reduce contamination during the process. The rapid replication and robust nature of the bacterium also aided in preventing contamination. Similar to bench-scale studies, oxalic acid extraction removed a greater amount of chromium and arsenic, whereas the bacterium was most effective at removing copper. Scaling up the two-lstep bioremediation process to 12-kg batches of CCA-treated wood removed metals with an efficiency similar to that seen in 1-kg batches at the optimized 1:10 ratio (w/v) of solid to liquid.[51]

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