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Microbial-assisted remediation of creosote- and pentachlorophenol-treated wood products

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A recycling process designed to recover wood fiber from discarded utility poles and cross ties was tested. Laboratory and field studies were conducted using a combined physical, chemical and microbiological protocol designed for the removal of creosote and pentachlorophenol wood preservatives from wood fiber. Woodchips produced in an industrial type wood chipper were batch extracted in methanol. The extractions successfully removed more than 95% of eight major creosote compounds contained within the woodchips. An initial combined concentration of 29262 ppm during the extraction phase was reduced to 95 ppm in the laboratory study and to 1364 ppm in the field study. Biopolishing with a microbial consortium containing adapted strains from the genera *Pseudomonas*, *Flavobacterium* and *Acinetobacter* further reduced the preservative concentration to 8 ppm and 200 ppm, respectively, with anthracene being the most recalcitrant compound in both studies. Pentachlorophenol-treated wood with an initial concentration of 1190 ppm, when subjected to the recycling process, yielded end product wood containing less than 2 ppm of the preservative. The solvent/preservative mixture (miscella) produced during the extraction process yielded a pure methanol fraction and a still bottom mixture when subjected to flash distillation. Fractional (vacuum) distillation of the still bottom mixture produced methanol, creosote, pentachlorophenol, and coal tar fractions.

Keywords: recycle; creosote; pentachlorophenol; extraction; biopolish; flash distillation; fractional distillation

Introduction

Creosote and pentachlorophenol (PCP) are major wood preservatives registered by the Federal Government as pesticides which have been used for many years to preserve wood for exterior applications [4]. Creosote, the oldest known wood preservative, consists of petroleum products produced by the fractional distillation of crude coal tars [1]. The composition of creosotes varies depending upon the temperature used during coal tar production and the source of the coal used [6]. The petroleum products in creosote are mixtures of several complex compounds, many of which are polycyclic aromatic hydrocarbons (PAHs). Most creosotes contain as many as 200 different compounds, many of which are known or suspected carcinogens [9]. Of the hundreds of compounds present in creosote, only a small fraction are designated as major components [12]. Benz[a]anthracene, a major component in many creosote mixtures, is mutagenic towards *Salmonella typhimurium* [2]. Many case reports describe the development of cancers in humans exposed to creosotes [10].

The USA produces approximately 15000 tons of pentachlorophenol annually, 80% of which is used to protect structural wood and utility poles [13]. Current estimates for world production of PCP are at 30 million tons. PCP is produced either by chlorination of phenol or by alkaline hydrolysis of hexachlorobenzene in methanol [7]. PCP is rapidly absorbed through human skin and has been shown to penetrate mucous membranes. Exposure to PCP can lead to immune system abnormalities, blood chemistry imbalances, and lung, liver and/or kidney failure [3,11].

Concern over methods of acceptable environmental disposal of treated wood products along with an increasing shortage of available landfill space has spurred interest in alternative methods of handling discarded treated wood products. A recycling process using alcohol extraction in batch and continuous mode followed by addition of an adapted microbial consortium was tested. End product wood fiber contained greatly reduced levels of each type of wood preservative. Flash and fractional distillations were employed to recover the solvent and to separate the extracted creosote and pentachlorophenol preservatives contained in the solvent/preservative miscella. Data reported in this paper were generated in tests performed on creosote- and pentachlorophenol-treated pine woodchips using batch mode methanol extraction followed by biopolishing with adapted bacterial strains. Data generated from additional studies testing other alcoholic solvents, commercial continuous extractors, and other species of softwoods and hardwoods are not reported here.

Materials and methods

Recycling process

Bench scale and field pilot studies were conducted to test the efficacy of a recycling process designed to remove creosote and pentachlorophenol preservatives from wood matrices. Various methodologies for removal of the preservatives were tested in laboratory experiments. The most efficient laboratory process was scaled up in a field pilot to determine commercial feasibility. The combined process (Figure 1) involved five distinct phases: (a) bacterial culture

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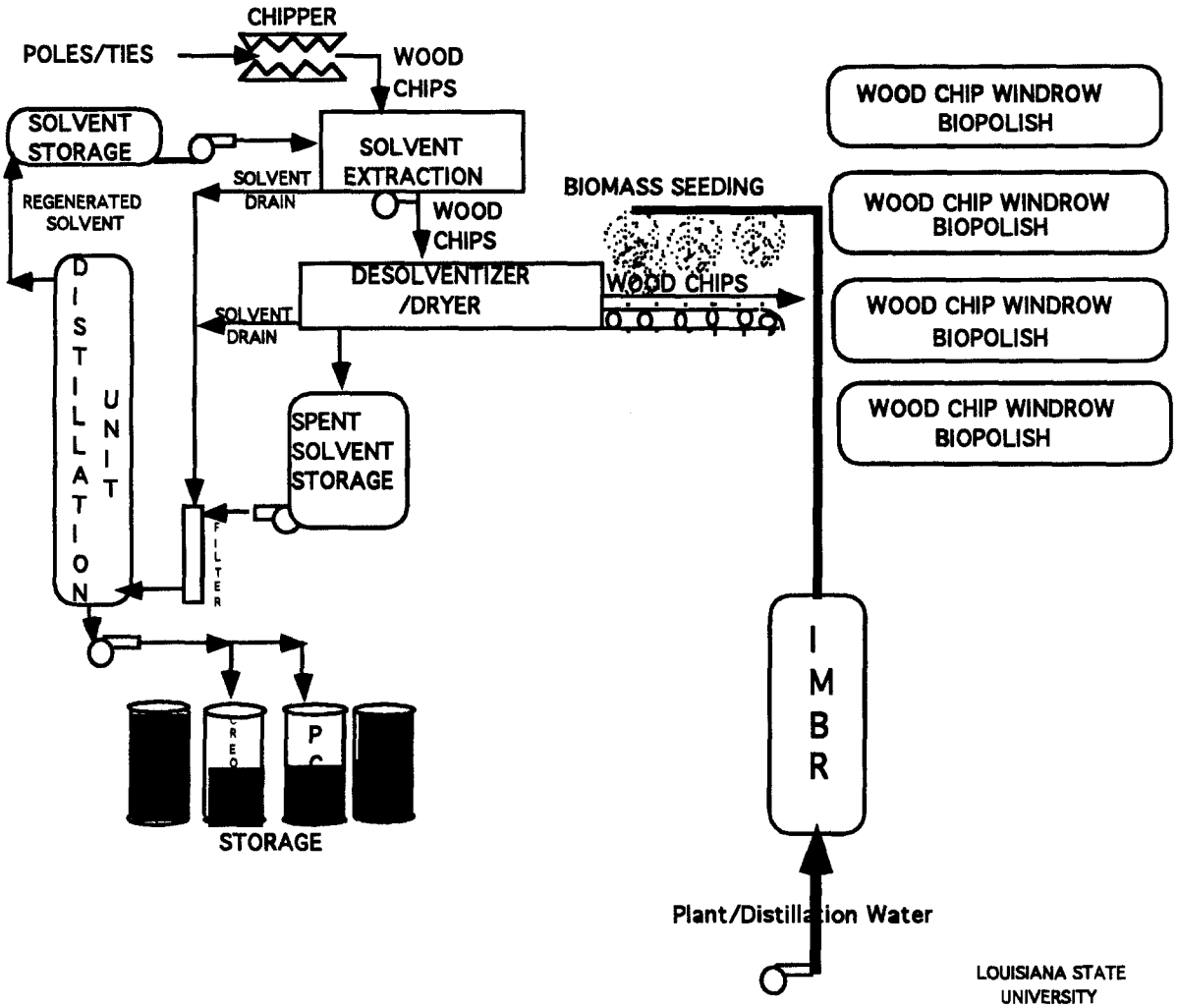

Figure 1 Process schematic for field pilot facility.

development; (b) wood preparation; (c) extraction with alcohol; (d) biological polishing; and (e) flash fractional distillation.

Bacterial culture development

A bacterial consortium of known pentachlorophenol- and creosote-degraders [14] was prepared from selected laboratory stock cultures including adapted strains from the genera *Pseudomonas*, *Flavobacterium* and *Acinetobacter*. All bacterial isolates used in the study had been in cold storage and were maintained on media containing creosote or pentachlorophenol (as a carbon source). Following acclimation to room temperature, the isolates were mixed and initially cultured in 250-ml Erlenmeyer flasks containing a mineral salts medium with sodium acetate added as a simple carbon source (Table 1). Incubation was controlled at 30°C for 48 h on a multi-tier Orbitmatik shaker (Lab-Line Bioengineering, Melrose Park, IL, USA). Final culture preparation for the bench-scale studies was carried out in a 19-L lab fermentor (Bioengineering model D407, Bioengineering AG, Wald, Switzerland). An 800-L immobilized bed fermentor [8] produced bacterial cultures for the field pilot study. Media formulations used for bacterial culture production were similar for each study. Weekly additions of ammonium nitrate, potassium phosphate and sodium acetate were used for culture maintenance.

Table 1 Mineral salts medium used for the culturing of creosote- and pentachlorophenol (PCP)-degrading bacteria

Nutrient	Concentration
Potassium phosphate (K_2HPO_4)	500 ppm
Ammonium nitrate (NH_4NO_3)	750 ppm
Sodium acetate ($NaC_2H_3O_2$)	150 ppm

pH = 6.5–7.0

Wood preparation

Discarded creosote- and pentachlorophenol-treated utility poles were manually cleaned of metal and debris. Mechanical chipping of the treated wood in a paper mill disc-type wood chipper produced standard paper mill-size chips measuring 4.45 cm long × 2.54 cm wide × 0.95 cm thick. The woodchips were screened for size then rechipped in a chipper/shredder to increase woodchip surface area. The resulting chips had average dimensions measuring 1.90 cm long × 0.64 cm wide × 0.64 cm thick. Woodchips were stored at ambient temperatures in a dry location.

Alcohol extraction

Batch mode methanol extractions performed in the bench scale studies were conducted in 80-L stainless steel pots for 30 min at approximately 64°C. Three consecutive extractions, using virgin alcohol for each extraction, were performed on each batch of wood chips. The resulting methanol/preservative extract mixture (miscella) was separated into methanol and preservative fractions by evaporation in a laboratory rotovapor (Büchi, R110, Brinkman Instruments, Westbury, NY, USA). The batch-extracted wood chips were allowed to air dry.

Batch mode methanol extractions performed in the field pilot were carried out in a 1500-L cone-bottom extraction chamber under conditions similar to those used in the bench study. The resulting methanolic miscella, when subjected to flash distillation, produced methanol and mixed preservative fractions. The batch-extracted wood chips were allowed to air dry.

Biological polishing

Wood chip windrows constructed with the extracted wood chips were inoculated with the microbial consortium of creosote- and pentachlorophenol-degraders. Weekly additions of microbial inoculum and nitrogen/phosphorous/potassium nutrients were made. Biopolishing was carried out for 28 days at temperatures ranging from 25°C to 32°C.

At the bench scale level, biopolishing was accomplished using 30.48 cm × 121.92 cm wood chip windrows constructed in open-top stainless steel trays. A flat layer of wood chips was inoculated with a 48-h culture of the mixed inoculum prior to windrow formation. Windrows were flattened and then re-rowed for the weekly additions of nutrients and inoculum.

Biological polishing at the field pilot level involved the construction of wood chip windrows housed on the concrete floor of a large metal building. Initial inoculation and nutrient amendment of the wood chips was achieved by wetting them with inoculum and nutrients prior to windrow formation. The windrows, measuring 3.04 m × 60.98 m long, were leveled then re-rowed for the weekly additions of nutrients and inoculum. Figure 1 depicts a process schematic for the field pilot facility. The immobilized bed bioreactor (IMBR), which was filled with a diatomaceous earth catalyst for enhancement of microbial growth [8], served as a fermentor for culture production in the field study.

Distillation/recovery

Flash and fractional distillations were employed for solvent recovery and creosote/pentachlorophenol separation. Flash distillation of the mixed alcohol/preservative/water miscella at 70°C under atmospheric conditions produced a purified alcohol and a preservative/water still bottom mixture. The recycled methanol was re-used in the extraction process. An attempt to break the alcohol/water azeotrope was not made. Sequential fractionation of the preservative/water still bottom was carried out at 10 mm Hg in a HIVac C distillation unit. Creosote, pentachlorophenol, water and alcohol fractions were produced. The fractions, ranges, and percent recovery of each component resulting from fractional distillation are listed in Table 2.

Table 2 Fraction profile data obtained during the fractional (vacuum) distillation of a still bottom mixture remaining after the flash distillation of a mixed solvent/miscella mixture

Fraction	Dist range	Major component	% Yield
1	26–100°C	Methanol	5.14%
2	219–307°C	Creosote	15.78%
3	307–313°C	Pentachlorophenol	02.64%
4	313 + °C	Coal tar	73.70%

Analytical protocol

All wood chip samples were extracted in methylene chloride for 24 h in a soxhlet apparatus. The resulting extract was evaporated in a Brinkmann rotavapor R110 prior to concentration in a stream of N_2 gas. Gas chromatography/mass spectroscopy analysis of all extracts followed EPA method 8270 [5]. Sample analysis was performed on a Hewlett Packard 5890 gas chromatograph with attached 5970 series mass selective detector. Required surrogate and internal standards were used with all samples.

Preliminary analysis of the creosote-preserved wood indicated that eight polycyclic aromatic hydrocarbons (PAH) accounted for more than 99% of the total combined creosote compounds present. The concentrations of these eight major PAH compounds (Table 3) were monitored throughout the laboratory and field experiments on creosote-treated wood.

Results

Laboratory experiments—creosote removal

Removal efficiencies of close to 100% were obtained in the laboratory study for the eight major creosote compounds. An initial creosote concentration of 29262 ppm (the sum of the eight monitored creosote compounds) was reduced to 95 ppm in the extraction phase with acenaphthene and fluorene being reduced to analytically non-detectable limits. Biopolishing removed 91% of the remaining 95 ppm to leave a final combined creosote concentration of 8 ppm with anthracene as the major remaining contributor at

Table 3 Gas chromatography/mass spectroscopy data for the laboratory studies comparing concentrations of individual creosote compounds initially present in the woodchips (control) with those concentrations remaining in the woodchips after the extraction and biopolish steps

Compound	mg kg^{-1} (dry wt) in:		
	Control	Extraction	Biopolish
Acenaphthene	2400	non-detect[a]	non-detect
Fluorene	2830	non-detect	non-detect
Phenanthrene	9370	12	1
Anthracene	2610	31	4
Fluoranthene	5680	20	2
Pyrene	4320	15	1
Benzo[a]anthracene	940	6	non-detect
Chrysene	932	11	non-detect
Total (mg kg^{-1})	29262	95	8

[a]Refers to concentrations below the analytically detectable limits.

4 ppm. Phenanthrene, anthracene, fluoranthene, and pyrene were the only compounds detected after biopolish. Benz[a]anthracene and chrysene were reduced to an analytically non-detectable limit in the biopolish phase. Creosote-removal data generated in the laboratory experiments can be seen in Table 3.

Field pilot study–creosote removal

Field pilot extractions were effective in removing 95.3% of the eight monitored compounds, reducing the initial creosote concentration from 29 262 ppm to 1364 ppm. As in the laboratory study, acenapthene and fluorene were reduced to analytically non-detectable levels in this phase. Biopolishing removed 85% of the remaining creosote compounds leaving a final combined creosote concentration of 200 ppm. Phenanthrene and benz[a]anthracene were reduced to non-detectable limits in the biopolishing phase. Anthracene, fluoranthene, pyrene, and chrysene were the only remaining compounds after biopolish with anthracene being the most concentrated at 164 ppm. Creosote removal data generated in the field pilot experiments can be seen in Table 4.

Laboratory experiments—pentachlorophenol removal

During the combined extraction and biopolish phases of the recycling process, the pentachlorophenol concentration was reduced by 99.8% from an initial concentration of 1190 ppm to less than 2 ppm. An attempt to differentiate the effectiveness of the extraction and biopolish phases was not made.

Flash and fractional distillation

Consecutive flash and fractional distillations of the solvent/preservative miscella successfully separated the mixture into its component parts. Flash distillation of the miscella mixture at 70°C and atmospheric pressure produced methanol and still bottom fractions. The methanol fraction, accounting for 95% of the miscella mixture, was recycled and used for additional extractions. The still bottom fraction, which made up the remaining 5% of the miscella mixture, yielded four additional fractions when subjected to fractional distillation. Fraction 1, consisting mainly of methanol, was collected at atmospheric pressure. The remaining material required a 10-mm Hg vacuum for distillation and produced creosote, pentachlorophenol, and coal tar fractions. The distillation temperatures used for fractionation, major component of each fraction, and percent yield for each fraction are listed in Table 2.

Table 4 Gas chromatography/mass spectroscopy data for the field studies comparing individual creosote compound concentrations initially present in the woodchips (control) with those concentrations remaining in the woodchips after the extraction and biopolish steps

Compound	mg kg^{-1} dry weight of wood in:		
	Control	Extraction	Biopolish
Acenaphthene	2400	non-detect	non-detect
Fluorene	2830	non-detect	non-detect
Phenanthrene	9370	59	non-detect
Anthracene	2610	957	164
Fluoranthene	5860	40	11
Pyrene	4320	173	13
Benzo[a]anthracene	940	23	non-detect
Chrysene	932	112	12
Total (mg kg^{-1})	29262	1364	200

Discussion

Laboratory and field pilot studies undertaken to test the removal of creosote and pentachlorophenol preservatives by the combined extraction and biopolish process yielded encouraging results. Numerous experiments conducted over a 2-year period involved the use of both batch and continuous extractions followed by the biopolishing of both domestic and European species of various softwoods and hardwoods. Data presented in this paper are limited to those tests performed on domestic yellow pine species utilizing batch methanolic extractions followed by microbial biopolishing. Overall reproducibility of the data generated from all tests was good for each species tested with reductions in preservative concentrations generally exceeding 99%. Experimental controls for all studies consisted of wood chips that were not subjected to the extraction and biopolish phases of the recycling process. An attempt to assess abiotic loss resulting in concentration reductions was not made.

Miscella is a term used in the commercial extraction industry denoting a heterogeneous mixture of solvent and extracted materials. The data from distillations indicate that separation of the preservative and solvent components within the miscella is possible. An initial flash distillation of the miscella mixture produced pure methanol. The remaining still bottom fraction produced methanol, creosote, pentachlorophenol and coal tar fractions when subjected to fractional distillation with a vacuum. Separation of the pentachlorophenol and creosote fractions is necessary if the recycled creosote and pentachlorophenol are to be reused as blending agents in newly produced wood preservative formulations. The coal tar fraction, by far the largest of the four fractions obtained by the fractional distillation of the still bottom mixture, contributed almost 74% to the overall makeup of the mixture. Creosotes lose most of their lighter components shortly after impregnation into wood products [2]; therefore, a large coal tar fraction distilling at temperatures above 313°C (596°F) was expected.

Based on engineering calculations, data generated in the field pilot study and on projected tipping fees for treated wood at a commercial landfill or incinerator, the combined extraction and biopolish process appears to be a feasible alternative for discarded treated wood products. In addition, the wood fiber is not lost and can be recycled into usable end products.

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