CHARACTERIZATION AND BIOREMEDIATION OF BIRCH CONDENSATE

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(Received September 1998)

ABSTRACT

Birch (*Betula papyrifera Marsh*) condensate collected from a veneer plant in Michigan was analyzed for its major chemical constituents. This condensate contained carbohydrates and lipids. In the lipid fractions, triglycerides were the most abundant component (35–40%), followed by phenolic compounds (30%) and waxes (25–30%). Gas Chromatography-Mass Spectrometry (GC-MS) and Fast Atom Bombardment (FAB) MS were used to identify 14 lipid compounds. A white rot fungus, *Phanerochaete chrysosporium* [Burdsall-lombard, 5176, HHB-6251], was tested as a means for the bioremediation of the condensate. *P. chrysosporium* reduced the total organic content (TOC) of the condensate from 350 ppm to 22 ppm and the color intensity from 0.614 to 0.355 absorbance units, after 2 weeks incubation in a liquid medium containing yeast and peptone at pH 5.

Keywords: Birch (*Betula papyrifera* Marsh), condensate, extractives, fungi treatment, *Phanerochaete chrysosporium*, GC-MS, FAB-MS, lipids, triglycerides.

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Wood and Fiber Science, 31(4), 1999, pp. 370–375

INTRODUCTION

The forest products industry is currently confronting several environmental issues. Legal mandates emanating from the 1990 amendments to the "Clean Air and Water Act" and other legislation focus not only on air quality but also on condensate emissions from wood processing operations (Rice et al. 1994). It is imperative to find alternative ways to dispose of condensates emanating from wood processing operations other than disposal in land fills, which is a common current practice. The availability of landfill space is rapidly diminishing and the cost is increasing at an exponential rate (Stalker 1993).

In this study, birch condensate is used as a model because birch is one of the Michigan hardwood species widely used for veneer production. To facilitate peeling of veneer from logs, green birch logs are preconditioned by treatment with live steam (170°F to 200°F) or heating with hot water in vats for 12 to 36 h depending upon log species, size, and the harvesting season. Consequently, the resulting condensate or leachate is a complex mixture of hot-water extractable material. The condensate presents potential disposal problems because of its bio-toxicity, high biological oxygen demand, high total organic carbon, and low pH (Servizi et al. 1986; Leach and Thakore 1975). A significant fraction of birch condensate is composed of carbohydrates and lipids (Edhborg 1956) and carbohydrates are used as food source by microorganisms (Theander et al. 1993; Ballard et al. 1982). In this study, we focused on the chemical identification of the lipophilic compounds, which are considered as one of the main causes of the pollution. Bioremediation of birch condensates was evaluated by inoculating condensates with the ligninolytic fungus, *P. chrysosporium,* which is reported to be capable of degrading a wide variety of aromatic compounds that are reported as environmental pollutants, including certain polycyclic aromatic hydrocarbons (PAHs) (Hammel et al. 1992).

MATERIALS AND METHODS

Birch condensate

Two liters of birch condensate, provided by a veneer company based in Escanaba, Michigan, were stored at 4°C in a laboratory refrigerator. Aliquots were pipetted into 10-ml glass vials with rubber stoppers and lyophilized. Lyophilized samples were kept in a freezer at −10°C until further use.

The pH of condensates was measured with a Brinkmann Instruments 716 DMS Titrino pH meter. The pH meter was calibrated using buffers with pHs 3, 7, and 10 before measuring the pH of condensate in triplicate.

Elemental analysis/(carbon/hydrogen/nitrogen) of birch condensates

Lyophilized birch condensate was analyzed for carbon, hydrogen, and nitrogen content using a C/H/N 2400 Elemental Analyzer (Perkin Elmer). Lyophilized samples were dried in an oven at 105°C for 2 h to remove any residual water before the elemental analysis. In order to get the total organic carbon content, 10% phosphoric acid was used to acidify samples, followed by purging with N_2 to remove inorganic carbon by conversion of carbonate ion CO_3^{2-} to gaseous CO_2.

UV-Vis spectra and color measurements of birch condensate

Ultraviolet and visible spectrophotometric analysis was performed on a Beckman DU 640B spectrometer using a quartz cuvet with light path of 10 mm. The sample solutions exhibited a maximum peak absorbance at 465 nm, and this peak was used for measuring color intensity (Marton et al. 1969). All solutions were prefiltered through a 0.45-μm filter millipore to minimize the particulate interference before the UV measurements.

Confirmation of presence of reducing sugars in birch condensates

The presence of reducing sugars in birch condensates was determined by using the pro-

tocol of Morrow and Sandstrom (1935). After the addition of a solution of copper sulfate and alkaline tartrate, the condensate—a precipitate of orange cuprous oxide in test solution, confirmed the presence of reducing sugars.

The colorimetric method to quantify sugars (Dubois et al. 1956) was also used to estimate the content of glucose equivalents in the birch condensate using a standard curve made with D-glucose at concentrations of 0, 5, 10, 15, 20, 25, and 30 mg/ml and UV-Vis absorbance reading at 490 nm. Although this method is simple, rapid, and relatively inexpensive, the presence of colored substances such as quinones can interfere with the analysis.

Determination of gross lipophilic contents of birch condensate

About 100 mg of freeze-dried lyophilized birch condensate sample was dissolved in 5 ml $CHCl_3$, filtered through anhydrous sodium sulfate cartridge column to remove trace amounts of water and other solid particulate and was then washed twice with 5 ml of $CHCl_3$. The eluant was collected in a clean, preweighed glass vial and then evaporated to dryness under a nitrogen purge, and the glass vial was then reweighed to determine the total lipid content.

Solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS)

Different classes of lipids in the birch condensate were fractionated by SPE using a protocol developed by Chen et al. (1994). An SPE cartridge containing 200 mg of aminopropyl silica (Isolute International Sorbent Technology) was loaded with 15–20 mg of total lipid extract from the lyophilized condensate sample. The fractionation scheme using various elution solvents was the same as reported by Chen et al. (1994). Each fraction was collected in a prepared clean bottle. After elution, the fractions were dried under a stream of N_2 and weighed accurately using an analytical balance. The weight percentage of each class was then calculated. Using this technique, we isolated and quantified the following fractions: (1) fatty and resin acids; (2) steryl esters and waxes; (3) triglycerides; (4) sterols, fatty alcohols, and diglycerides; (5) monoglycerides; and (6) others.

An autosystem Gas Chromatograph (Perkin Elmer) interfaced to a Series 800 ion trap mass spectrometer (Finnigan) equipped with a 30-m × 0.25-mm DB-5 fused-silica capillary column (J & W scientific) was used to analyze the different fractions. The following temperature program was used: 160°C for 2 min, ramp at 7°C/min to 310°C and hold for 3.5 minutes. The m/z range scanned was from 0 to 650. Fatty and resin acids were methylated with fresh diazomethane in ether before GC injection (Chen et al. 1994). Triglycerides, steryl esters, and waxes were saponified according to the procedure of Saranpaa and Nyberg (1987). These fractions were heated with 0.5M KOH in 90% ethanol for 3 hours at 70°C (water bath). The solutions were then diluted with one volume distilled water and acidified to pH 2.0 with 1M HCl. The solutions were subsequently extracted with hexane-diethyl ether (1:1, v/v), dried under a stream of nitrogen, and methylated with ethereal diazomethane. The derivatization and GC analysis of other fractions were performed as reported by Chen et al. (1994).

Fast Atom Bombardment Mass Spectrometry (FAB-MS)

FAB analysis was performed on a JEOL HX-110 double-focusing mass spectrometer operating in the positive mode with ions produced by bombardment with a beam of Xe atoms (6 keV). The accelerating voltage was 10 kV, and the resolution was set at 1,000. Aliquots of the sample solution were mixed with the glycerol matrix on the end of the direct insertion probe. The matrix-sample solution was then introduced into the mass spectrometer via a vacuum interlock. The instrument was scanned from m/z 0 to 1,500, and data were collected in the range 50 to 1,500.

Total organic carbon (TOC) content in birch condensate

Total organic carbon content of aqueous samples was determined by direct aqueous injection using model 700 TOC Analyzer (O-I Analytical). Only prefiltered solutions of birch condensate were used for TOC analysis.

Fungal bioremediation treatment of birch condensate

A strain of *P. chrysosporium* [Burdsall-lombard, 5176, HHB-6251] was plated on a 2% malt-agar media and incubated at 25°C for 2 weeks. About 0.1% peptone hydrolysate I from Sigma Chemicals Co. and 0.5% yeast extract from Difco Chemical Co. were added to 200 ml of birch condensate in a 500-ml Erlenmeyer flask. A parallel set of controls was carried without peptone or yeast to determine their effects. The pH of the solutions was adjusted to 5, 7, or 10 using sodium acetate and/or sodium hydroxide. With a sterile hollow tube, five discs (5-mm diameter) were punched out of the periphery of the mycelia of *P. chrysosporium* growing on the 2% malt-agar plate and added aseptically to each flask. Six replicas were made for each condition. The flasks were inoculated and kept at 25°C and 90% relative humidity without shaking for 2 weeks. The temperature level used was suggested by the American Type Culture Collection (1991). At the end of the incubation period, the pH, the absorbance at 465 nm, and the TOC of the solutions were measured.

RESULTS AND DISCUSSION

Chemical characterization of birch condensates

Color and pH.—The birch condensate has a brown color and a pH of 3.90. When it was left exposed to air at room temperature for a minimum of 3 days, the color became darker and the pH increased to about 5. The low pH of condensate is attributed to formic, acetic, and other organic acids produced during the hot-water or steam treatment of green wood (Edhborg 1956). The color of the condensate may be due to the presence of hot-water-soluble lignin and breakdown of some phenolic glycosides. At lower pH (3.9–6), the color of birch condensate was lighter than at higher pH (7–14).

TABLE 1. *Types of lipids and their relative proportions.*

Classes of chemical compounds	Proportion of the lipid content
Free fatty and resin acid	1.6%
Steryl esters and waxes	27%
Triglycerides	38%
Sterols, fatty alcohols and diglycerides	1.6%
Monoglycerides	1.6%
Others (mainly Phenolic compounds)	30%

Elemental analysis and sugar content.—The lyophilized birch condensate contains 50.2% carbon, 6.05% hydrogen, and 2.37% nitrogen by weight, corresponding to the mole ratio of C/H/N to be 4.18/6.05/0.17 (average of 10 samples). Samples pretreated by the phosphoric purge method gave the same TOC results as those that were not pretreated. This indicates that the carbon was entirely in organic form. A carbon to hydrogen molar ratio of 4 to 6 was an indication of the presence of unsaturated hydrocarbons.

The colorimetric method measurements indicated that the sugar (D-glucose equivalent) content of the birch condensate was about 8.9 mg/ml.

Gross lipid content of lyophilized birch condensate.—Gross lipid content of lyophilized birch condensate was 9.1%. This is equivalent to 91 mg per gram of lyophilized birch condensate. Lipids include fatty and resin acids, sterols, waxes, triglycerides, phenolic compounds, etc. Those different fractions were separated and analyzed by solid phase extraction and GC-MS.

Separation and identification of chemicals in lipophilic fractions.—Solid phase extraction (SPE) was used to separate the different classes of lipid in the birch condensate. The type of lipid and relative amount are listed in Table 1. The quantitatively important components

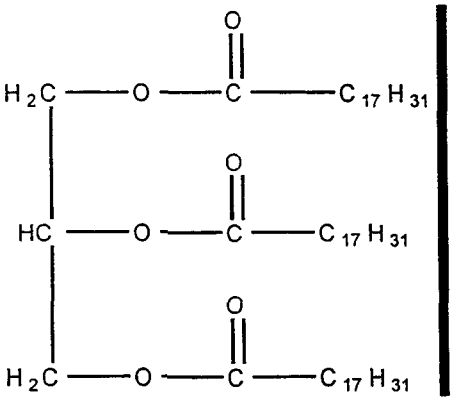
TABLE 2. *Compounds identified using GC-MS.*

No	Sample name	Retention time	Molecular weight	m/z value of major peaks (relative intensity, %)
1	Pentadecanoic acid, 13-methyl†	7′54″	256	55(51), 74(100), 88(62), 129(15), 143 (21), 171(8), 199(11), 227(11), 270(29)
2	7,10-Octadecadienoic acid†	10′14″	280	55(88), 67(100), 81(73), 110(23), 149(18), 220(10), 262(20), 294(13)
3	8,11-Octadecadienoic acid†	9′36″	280	67(39), 81(100), 95(71), 163(26), 245(53), 263(69), 294(55)
4	9,15-Octadecadienoic acid†	11′14″	280	28(100), 41(7), 55(9), 67(10), 81(9), 95(5), 109(3), 123(3), 294(2)
5	Heptadecanoic acid†	9′52″	284	55(55), 74(100), 88(83), 129(10), 143(20), 185(8), 199(16), 255(22), 299(98)
6	Eicosanoic acid†	8′	312	55(37), 74(100), 88(68), 143(23), 185(4), 199(11), 255(9), 283(19), 326(60)
7	Nonadecanoic acid, 10-methyl†	14′6″	312	28(100), 43(24), 74(23), 87(20), 143(7), 283(5), 326(16)
8	Nonadecanol	9′28″	284	55(100), 57(57), 69(71), 83(46), 97(47), 111(33), 125(13)
9	2-propenal, 2-methyl-3-phenyl	5′37″	146	28(37), 39(20), 55(10), 65(41), 91(100), 103(10), 115(43), 117(86), 145(54)
10	Bezenepropanol, 4-hydroxy, alpha-methyl(R-)	4′23″	166	51 (7), 77(21), 91(18), 107(100), 133(42), 148(14), 166(13)
11	Phenol, 2,6-Bis(1,1-Dimethylethyl)-4-methyl	4′	220	57(28), 91(6), 105(8), 145(12), 177(17), 205(100), 220(40)
12	Phenol, 3,5-dimethoxy-	4′32″	154	55(1), 81(2), 95(2), 111(4), 125(3), 139(2), 154(100)
13	Benzene, 1-methyl-3-phenoxy-	6′40″	184	53(7), 69(17), 141(25), 169(16), 185(100)

† The fatty acids were analyzed as their corresponding methyl esters.

are triglycerides (38%), phenolic compounds (30%), and waxes (27%). Other components such as free fatty and resin acids, monoglycerides, diglycerides, sterols, and fatty alcohols add up to 4.8% of the total weight.

Table 2 lists the major chemical compounds identified by GC-MS. A total of 14 compounds was identified by comparing sample spectra with those in the literature and spectra of standards. The mass to charge ratio (m/z) values and the relative intensity of the pronounced peaks are provided in Table 2. All the fatty acids listed were analyzed as their corresponding methyl esters. The GC-MS analysis of the triglyceride fraction following saponification showed that the dominant fatty acid was octadecadienoic acid ($C_{18}H_{32}O_2$). This was confirmed by FAB-MS of the intact triglyceride fraction. In the FAB-MS spectra of the triglyceride fraction, a weak molecular ion peak, MH^+ at m/z 879.7 was observed for the triglyceride $C_{57}H_{98}O_6$:



The molecular weight is theoretically calculated to be 879.4, using accurate atomic weight for C:12.01115, H:1.00797 and O: 15.9994 (Silverstein et al. 1981).

Bioremediation of birch condensate

Flasks containing birch condensate with pH adjusted at 3.9, 5.0, 7.0, and 10.0 were inoc-

ulated with *P. chrysosporium.* No evidence of fungal growth was noticed. Another set of experiments was carried out using the same conditions as before except adding 0.1% peptone and 0.5% yeast to each of the flasks. This time the flasks with pH 3.9, 7.0, 10.0 showed no change, but vigorous growth of fungus and color change were observed in the flask with pH 5.0. The color appeared to be much lighter after inoculation; UV-Vis measurements showed color intensity decreased from 0.614 to 0.355 after the *P. chrysosporium* treatment. TOC measurements also showed that the total organic carbon content of the solution was reduced from 350 ppm to 22 ppm after the treatment. This corresponds to a 42% reduction in color intensity and 94% reduction in total organic carbon content.

CONCLUSIONS

The birch condensate consists primarily of reducing sugars and lipids. In the lipid extract, triglycerides, waxes, and phenolics were the major compounds. The dominant fatty acid residue of triglycerides detected was octadecadienoic acid ($C_{18}H_{32}O_2$).

The lab-scale bioremediation technology employed is simple and effective, the optimum pH for bioremediation treatment is near 5, and the first stage nutrient (peptone and yeast) is needed to accelerate the fungus growth. Further work is needed to determine the best experimental parameters, such as temperature, fungus strain, fungus to solution ratio, incubation duration.

ACKNOWLEDGMENTS

We are grateful to Ismail Jusoh and Joseph Nguyen for their contribution. We wish to acknowledge the NIEHS Basic Superfund Research Center, Analytical Chemistry Core Grant P42ES04911, the MSU Mass Spectrometry Facility grant (DRR-00480) from the NIH, and the Michigan State University-Eastern Hardwood Utilization Program USDA/CSRS grant #95-34158-1304 for providing funds.

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