Photochemical and microbial degradation of dissolved lignin phenols: Implications for the fate of terrigenous dissolved organic matter in marine environments

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Molecular level characterizations of dissolved lignin were conducted in Mississippi River plume waters to study the impact of various removal mechanisms (photooxidation, microbial degradation, and flocculation) on dissolved organic material (DOM) concentrations and compositions. Prior to analysis, dissolved (<0.2-μm pore size) samples were size fractionated by ultrafiltration into high molecular weight (HMW; >1 kDalton) and low molecular weight (LMW; <1 kDalton) components. At salinities <25 psu, flocculation and microbial degradation were the primary factors affecting lignin concentrations. At salinities >25 psu, photooxidation was a dominant factor influencing lignin compositions and concentrations. Diagnostic indicators of photooxidation include a sharp decrease in the percentage of lignin in the HMW size fraction, changes in ratios of syringyl to vanillyl phenols, and increases in LMW acid:aldehyde ratios for both vanillic and syringal phenols. A 10-day incubation experiment with plume water indicated rates of microbial degradation of dissolved lignin that were ~30% of photooxidation rates in surface waters. These results highlight the importance of microbial as well as photochemical processes in the cycling of terrigenous DOM in coastal waters. Neither flocculation nor microbial degradation significantly altered lignin composition, suggesting that composition is primarily determined by source and photochemical transformation. Overall, high removal rates indicate the potential importance of terrigenous DOM as a carbon and nutrient source in the coastal ocean. Strong correlations between absorption coefficients at 350 nm and dissolved lignin demonstrate the potential for using absorption to trace terrigenous DOM in coastal environments with significant riverine input.

INDEX TERMS: 1055 Geochemistry: Organic geochemistry; 4219 Oceanography: General: Continental shelf processes; 4805 Oceanography: Biological and Chemical: Biogeochemical cycles (1615); 4847 Oceanography: Biological and Chemical: Optics; 4852 Oceanography: Biological and Chemical: Photochemistry; KEYWORDS: Mississippi River Plume, photooxidation, terrigenous organic matter, lignin, microbial degradation, CDOM


1. Introduction

[2] Annual global discharge of terrigenous dissolved organic matter (DOM) via rivers (0.25 Pg) to the ocean is quite large, yet by all accounts terrigenous DOM comprises only a small fraction of oceanic DOM [Meyers-Schulte and Hedges, 1986; Hedges et al., 1997; Opsahl and Benner, 1997]. The fate of terrigenous DOM has increasingly come under focus as our understanding of its importance in ocean optics and the marine carbon cycle grows. Critical gaps in our knowledge include the molecular composition of terrigenous DOM, the relative importance of varying diagenetic processes, the kinetics of these processes, and the environments in which these processes occur.

[3] As an unambiguous tracer of vascular plant sources, lignin has proven to be a valuable tool for studying terrigenous DOM cycling and diagenesis. Lignin analyses of ultrafiltered high molecular weight (HMW; >1 kDalton) DOM indicate that the terrigenous component of DOM is ≤1% in the Pacific Ocean and ≤3% in the Atlantic Ocean [Opsahl and Benner, 1997]. Average cycling times of dissolved lignin in the ocean appear to be on the order of ~90 years [Opsahl and Benner, 1997; Hernes and Benner, 2002], demonstrating the importance of understanding removal processes. Our understanding of microbial degradation of dissolved lignin is limited, but its importance can...
be inferred from the 24% loss of lignin-rich dissolved humic substances during a 7-week incubation experiment [Moran and Hodson, 1994]. Recent work has identified photooxidation as a potentially critical mechanism for dissolved lignin removal, capable of removing 75% of dissolved lignin from river water in four weeks [Opsahl and Benner, 1998; Benner and Opsahl, 2001].

The recent development of a solid phase extraction (SPE) method for measuring dissolved lignin [Louchouarn et al., 2000] has now made it possible in combination with ultrafiltration to determine lignin concentrations and compositions in high molecular weight (HMW, >1 kDalton) and low molecular weight (LMW; <1 kDalton) components of DOM. The size distribution of dissolved lignin could be a critical parameter for identifying DOM that has undergone photooxidation because LMW lignin has been shown to be a photodegradation product of HMW lignin in river water [Opsahl and Benner, 1998]. About 80–90% of total dissolved lignin in rivers is in the HMW fraction [Opsahl and Benner, 1998; Louchouarn et al., 2000], whereas in the Pacific Ocean ~50% of total dissolved lignin is in the HMW fraction [Hernes and Benner, 2002]. Photooxidation imparts several molecular level compositional changes in lignin, and river plumes are likely systems in which these phototransformations occur. Some phototransformations were evident in previous work on HMW lignin in the Mississippi River plume [Benner and Opsahl, 2001], but the past inability to measure lignin in the LMW fraction in the ocean has left many questions about the cycling of terrigenous DOM unanswered.

This study focuses on addressing critical questions about photochemical and microbial removal of dissolved lignin in coastal waters. Field and experimental data were collected during a cruise in the northern Gulf of Mexico and Mississippi River plume to quantify rates and extent of dissolved lignin removal and accompanying compositional transformations. In addition, absorption measurements were conducted to investigate the correlation between dissolved lignin and terrigenous DOM and the potential for using the two in tandem for large-scale studies of terrigenous DOM cycling in ocean margins.

2. Methods

All samples were collected in May 2000 aboard the R/V Longhorn. Mississippi River samples were collected from Head of Passes in the Mississippi delta. Additional samples were collected along a salinity gradient in the Mississippi River plume, which generally flowed south to southeast from the mouth of SW Pass at the time of sampling. Water was collected from varying depths with Niskin bottles mounted on a rosette with a CTD. At each station, ~150 L of surface water (1–3 m depth) was collected with two separate casts and composited. A portion of this composite (100 L) was filtered through a 0.2-μm pore size cartridge filter (Nucleopore) and subsampled for DOC measurements. The high molecular weight (HMW) dissolved fraction (1–200 nm) was isolated aboard ship using an Amicon DC10L ultrafiltration system with polysulfone membranes (S10N1; 1,000 Dalton cutoff) as previously described [Benner, 1991; Benner et al., 1997]. After concentration to a volume of ~1 L, the sample was desalted by diafiltration with 18 L of Milli-Q water. All low molecular weight (LMW) filtrate (<1 nm) from ultrafiltration was collected for lignin and DOC sampling. The HMW concentrate was transferred to a polycarbonate bottle and frozen. In addition to the ultrafiltration samples, surface water samples were collected along a low-salinity transect (0.9–11.4 psu) with a clean plastic bucket for lignin extraction.

Dissolved lignin was isolated from LMW filtrate and from a filtered (<0.2-μm pore size) subsample (3–30 L) of the composite water samples by solid phase extraction (SPE) on Varian Mega Bond Elut C18 cartridges according to the protocol of Louchouarn et al. [2000]. Briefly, filtered water samples were acidified to pH 2.5 with HCl and then pumped through C18 cartridges with peristaltic pumps at a flow rate of ~75 mL min⁻¹. Immediately prior to extracting the sample, the C18 cartridges were first cleaned and primed with 100 mL of methanol, followed by 50 mL of Milli-Q water acidified to pH 2.5. Approximately 30 L samples were extracted for LMW filtrate, whereas 0.2-μm filtered water volumes ranged from 3–30 L depending on salinity. Prior to lignin analysis of C18 samples, the cartridges were eluted with 50 mL of methanol, and the sample evaporated to dryness with a Savant SpeedVac system. HMW concentrates were prepared for lignin analysis by drying subsamples with the SpeedVac system.

A 10-day photooxidation experiment was conducted aboard ship with 8.5 psu plume waters. After filtration (0.2-μm pore size) to remove particulates, ~10 L of water was incubated in quartz tubes (light) and in aluminum foil-covered Pyrex tubes (dark control). The quartz tubes were sealed with silicon stoppers. All tubes were placed on racks in an ondeck water tank with flowing seawater for temperature control (27°C ± 1°C). At the end of ten days, water from the quartz tubes was composited and diluted to 20 L with Milli-Q water. The dark control was treated similarly, and both samples were separated into HMW and LMW fractions by ultrafiltration. HMW samples were diafiltered with 10 liters of Milli-Q water, concentrated to ~1 L, and stored frozen for later analysis. Dissolved lignin was isolated from LMW samples (ultrafiltration permeate plus diafiltrate) by SPE. The HMW concentrates were prepared for lignin analysis by drying subsamples with the SpeedVac system.

A long-term incubation experiment was conducted with Mississippi River water in order to evaluate lignin compositional changes associated with microbial degradation. River water (10 L) was filtered (0.2-μm pore size), inoculated with a small volume of unfiltered river water (~1:1000) and stored in fluorinated carboys that were kept in the dark at room temperature. DOC and lignin subsamples were collected and analyzed at the initiation of the experiment and after 1.7 yr. Both initial and final lignin samples (3.6 L) were collected by SPE and measured in duplicate.

Lignin was analyzed using the CuO oxidation method of Hedges and Ertel [1982] with modifications described by Opsahl and Benner [1997], Louchouarn et al. [2000], and Hodson and Benner [2002]. Sample vessels (i.e., minibombs) and solvents were sparged with argon prior to oxidation. Glucose (10–15 mg) was included in all
Quantification was achieved using selected ion monitoring with a Hewlett Packard 5972 mass selective detector. 0.25-mm inner diameter, J&W Scientific) and equipped chromatograph with a DB5-MS capillary column (30 m, 0.25 mm ID) to separate lignin phenols. Separation of lignin from the eluted C18 cartridges and from HMW concentrates was achieved using a Hewlett Packard 5890A gas chromatograph with a UV-1601 Shimadzu spectrophotometer with 5 cm path length cells and Milli-Q water as a blank. Values are reported as absorption coefficients using the relation,

\[ A_{350} = 2.303A_{350}/r \]

where A is the optical density measured across the path r in meters.

3. Results

3.1. Mass Balance

[11] Mississippi River plume concentrations of dissolved lignin phenols for HMW and LMW dissolved fractions as well as for total dissolved lignin phenols in 0.2-μm filtered water (by SPE) are presented in Table 1. For mass balance purposes, the sum of HMW and LMW lignin phenol concentrations should equal total dissolved concentrations. In this study, HMW + LMW lignin phenol concentrations were 10–20% higher than total concentrations (Table 2), indicating extraction efficiencies of 80–90%. It is noteworthy that the wide ranges in salinity and lignin phenol concentrations did not affect lignin recovery or mass balances. Extraction efficiency was measured directly on the second cartridge recovered <10% of the primary cartridge in series with the primary cartridge. In each case, the second cartridge recovered <10% of the primary cartridge (Table 2) and accounted for differences relative to HMW + LMW values.

An important factor to evaluate in SPE techniques is differential extraction efficiencies among the compounds of lignin phenols for HMW and LMW dissolved fractions as well as for total dissolved lignin phenols in 0.2-μm filtered water. Extraction efficiencies are calculated by the formula (SPE measurement × 100%/HMW + LMW measurement)) and assumes that HMW + LMW represents the actual total concentrations. In Table 2, the sum of HMW and LMW lignin phenol concentrations did not affect lignin recovery or mass balances. Extraction efficiency was measured directly on the second cartridge in series with the primary cartridge. In each case, the second cartridge recovered <10% of the primary cartridge (Table 2) and accounted for differences relative to HMW + LMW values.

### Table 1. Concentrations of Dissolved Organic Carbon and Dissolved Lignin Phenols in Water Samples From the Mississippi Plume

<table>
<thead>
<tr>
<th>Salinity, psu</th>
<th>DOC, μM</th>
<th>VAL, μM L⁻¹</th>
<th>VON, μM L⁻¹</th>
<th>VAD, μM L⁻¹</th>
<th>SAL, μM L⁻¹</th>
<th>SON, μM L⁻¹</th>
<th>SAD, μM L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMW</td>
<td>LMW</td>
<td>SPE Total</td>
<td>HMW</td>
<td>LMW</td>
<td>HMW + LMW</td>
<td>SPE Total</td>
</tr>
<tr>
<td>0.2</td>
<td>81</td>
<td>1.42</td>
<td>0.805</td>
<td>1.52</td>
<td>1.24</td>
<td>1.04</td>
<td>1.48</td>
</tr>
<tr>
<td>8.5</td>
<td>60</td>
<td>1.10</td>
<td>0.569</td>
<td>0.927</td>
<td>0.982</td>
<td>0.906</td>
<td>0.591</td>
</tr>
<tr>
<td>15.3</td>
<td>55</td>
<td>0.745</td>
<td>0.389</td>
<td>0.659</td>
<td>0.608</td>
<td>0.407</td>
<td>0.391</td>
</tr>
<tr>
<td>23.1</td>
<td>35</td>
<td>0.296</td>
<td>0.196</td>
<td>0.296</td>
<td>0.269</td>
<td>0.171</td>
<td>0.175</td>
</tr>
<tr>
<td>27.8</td>
<td>34</td>
<td>0.215</td>
<td>0.097</td>
<td>0.188</td>
<td>0.207</td>
<td>0.108</td>
<td>0.114</td>
</tr>
<tr>
<td>31.4</td>
<td>23</td>
<td>0.088</td>
<td>0.042</td>
<td>0.103</td>
<td>0.084</td>
<td>0.050</td>
<td>0.070</td>
</tr>
<tr>
<td>33.5</td>
<td>20</td>
<td>0.034</td>
<td>0.118</td>
<td>0.049</td>
<td>0.037</td>
<td>0.128</td>
<td>0.018</td>
</tr>
<tr>
<td>35.4</td>
<td>12</td>
<td>0.015</td>
<td>0.040</td>
<td>0.004</td>
<td>0.002</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Abbreviations: DOC, dissolved organic carbon; VAL, vanillic acid; VON, acetovanillone; VAD, vanillin; SAL, syringaldehyde; SON, Acetosyringone; SAD, syringic acid; HMW, high molecular weight dissolved organic matter; and LMW, low molecular weight dissolved organic matter.

### Table 2. Mass Balance for Lignin Phenol Concentrations in Water Samples From the Mississippi Plume

<table>
<thead>
<tr>
<th>Salinity, psu</th>
<th>Volume Pumped, L</th>
<th>SPE Total</th>
<th>HMW</th>
<th>LMW</th>
<th>HMW + LMW</th>
<th>Extraction Efficiencies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>3.6</td>
<td>8.34</td>
<td>7.73</td>
<td>1.99</td>
<td>3.72</td>
<td>86</td>
</tr>
<tr>
<td>8.5</td>
<td>8.3</td>
<td>5.73</td>
<td>4.77</td>
<td>1.58</td>
<td>3.63</td>
<td>90</td>
</tr>
<tr>
<td>15.3</td>
<td>10.0</td>
<td>3.97</td>
<td>3.20</td>
<td>1.32</td>
<td>4.52</td>
<td>88</td>
</tr>
<tr>
<td>23.1</td>
<td>15.5</td>
<td>2.07</td>
<td>1.37</td>
<td>0.853</td>
<td>2.22</td>
<td>93</td>
</tr>
<tr>
<td>23.1³</td>
<td>15.5</td>
<td>0.136</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>27.8</td>
<td>30</td>
<td>1.39</td>
<td>0.930</td>
<td>0.578</td>
<td>1.51</td>
<td>92</td>
</tr>
<tr>
<td>27.8³</td>
<td>30</td>
<td>0.118</td>
<td></td>
<td></td>
<td></td>
<td>100²</td>
</tr>
<tr>
<td>31.4</td>
<td>30</td>
<td>0.706</td>
<td>0.437</td>
<td>0.394</td>
<td>0.831</td>
<td>85</td>
</tr>
<tr>
<td>33.5</td>
<td>30</td>
<td>0.451</td>
<td>0.193</td>
<td>0.342</td>
<td>0.535</td>
<td>84</td>
</tr>
<tr>
<td>35.4</td>
<td>30</td>
<td>0.379</td>
<td>0.047</td>
<td>0.225</td>
<td>0.273</td>
<td>139</td>
</tr>
</tbody>
</table>

Abbreviations: ASPE Total indicates a measurement made on C18 extracted material from 0.2-μm filtered water. Extraction efficiencies are calculated by the formula (SPE measurement × 100%/HMW + LMW measurement) and assumes that HMW + LMW represents the actual total. A scheme outlined previously [Hernes and Benner, 2002].
interest. In the Mississippi River plume, oligomers and polymers that were rich in vanillyl and syringyl phenols had similar retentions. On the other hand, components rich in cinnamyl phenols appear to be much less efficiently extracted, as indicated by calculated extraction efficiencies as low as 34% (Table 2). This indicates that a significant fraction of the cinnamyl phenols is not associated with lignin, and should not be included in lignin phenol totals.

3.2. DOC Concentrations Across the Salinity Gradient

[15] DOC concentrations in the Mississippi River and plume ranged from 268 \(\mu M\) at Head of Passes to 84 \(\mu M\) at 35.4 psu. The river concentration was \(\sim20\%\) lower than was measured during cruises between July 1990 and July 1993 [Benner and Opsahl, 2001]. Riverine discharge for those four cruises averaged \(\sim19,000\) m\(^3\) s\(^{-1}\) as compared to \(\sim9,000\) m\(^3\) s\(^{-1}\) during the May 2000 cruise (U.S. Geological Survey streamflow data, http://water.usgs.gov/nwis/ discharge). Average riverine discharge during the month of May from 1990 to 1999 was \(\sim24,000\) m\(^3\) s\(^{-1}\), indicating that discharge was unusually low during the May 2000 cruise.

[16] HMW DOC concentrations ranged from 81 \(\mu M\) at Head of Passes to 12 \(\mu M\) at 35.4 psu (Table 1). Overall retention of HMW DOC by the ultrafiltration membranes ranged from 30% in the river to 14% at high salinity. This compares to a range of 49% to 24% measured during a Mississippi River plume cruise in May 1992 [Benner and Opsahl, 2001]. The lower values during the May 2000 cruise are primarily a reflection of changing retention characteristics of the ultrafiltration membranes. The retention of a standard compound, polyacrylic acid (MW 2000), was tested following the cruise, and a 47% reduction in retention was observed compared to when the membranes were new.

3.3. Lignin Phenol Concentrations Across the Salinity Gradient

[17] In open ocean samples, reported lignin phenol concentrations do not include the cinnamyl phenols, \(p\)-coumaric acid and ferulic acid, because of significant nonlignin sources of the former and unmeasurable levels of the latter [Opsahl and Benner, 1997; Hernes and Benner, 2002]. Mass balance analyses in this study also indicated nonlignin sources for cinnamyl phenols, so they are not included in the sum of the lignin phenols. Concentrations of the six vanillyl and syringyl phenols (vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone, syringic acid) in the HMW fraction ranged from 7.7 \(\mu G\) L\(^{-1}\) in the Mississippi River at Head of Passes to <0.1 \(\mu G\) L\(^{-1}\) at 35.4 psu (Table 2 and Figure 1). These data can be compared to previous measurements in the Mississippi plume (May 1992), which were similar at high salinity but higher (\(\sim15\) \(G\) L\(^{-1}\)) in the river [Benner and Opsahl, 2001]. The lower concentration in this study was due in part to the reduced retention of HMW DOC by the ultrafiltration membranes on this cruise. Corresponding LMW concentrations for these six phenols ranged from 2.0 \(\mu G\) L\(^{-1}\) in the river to 0.2 \(\mu G\) L\(^{-1}\) at 35.4 psu (Table 2 and Figure 1). Total dissolved lignin phenols (SPE) ranged from 8.3 \(\mu G\) L\(^{-1}\) to 0.4 \(\mu G\) L\(^{-1}\) (Table 2).

3.4. Low-Salinity Transect

[18] Lignin phenol concentrations (SPE) from the low-salinity (0.9–11.4 psu) transect are presented in Table 3 along with DOC measurements. Lignin phenol concentrations decreased from 7.1 to 4.8 \(\mu G\) L\(^{-1}\) with increasing salinity, whereas DOC initially increased slightly from 269 to 272 \(\mu M\) and then decreased to 227 \(\mu M\) (Figure 2). Lignin

![Figure 1](image1.png)

Figure 1. Concentrations of lignin phenols (\(\mu G\) L\(^{-1}\)) in LMW and HMW DOM across the Mississippi plume salinity gradient.

![Figure 2](image2.png)

Figure 2. Concentrations of lignin phenols (\(\mu G\) L\(^{-1}\)) and DOC (\(\mu M\)) along a low-salinity transect within the Mississippi plume. Conservative mixing lines are drawn with lignin phenol and DOC concentration end-members at 0.9 and 35.4 psu.
phenol and DOC concentrations ≤5.9 psu plot above a conservative mixing line (Figure 2), indicating a net source consistent with desorption from particulates. No significant lignin compositional changes were noted in the low salinity transect.

### 3.5. Lignin Parameters

[19] The percentage of lignin phenols in HMW DOM ranged from 79% in the river to 62% at 27.8 psu (Table 4 and Figure 3). The %HMW lignin decreased sharply from 62% to 17% between 27.8 and 35.4 psu (Table 4 and Figure 3). For comparison, the decrease in percent HMW DOC from 30% to 14% across the entire salinity gradient is fairly linear (Figure 3). The greatest changes in ionic strength occur at salinities <10 psu, and the absence of any abrupt changes in either HMW lignin or DOM at these salinities suggests minimal changes in the retention characteristics of the ultrafiltration membranes across the salinity gradient.

[20] Carbon-normalized yields of lignin phenols, $\Lambda_{L}$, are useful indicators of the relative contribution of terrigenous organic matter to marine systems [e.g., Hedges et al., 1988a; Opsahl and Benner, 1997]. In the Mississippi River plume, $\Lambda_{L}$ in HMW and LMW size fractions decreased across the salinity gradient (Table 4). At 0.2 psu HMW $\Lambda_{L}$ was an order of magnitude higher than LMW $\Lambda_{L}$, but only 50% higher at 35.4 psu. HMW $\Lambda_{L}$ at 35.4 psu (0.034 mg 100 mg OC$^{-1}$) was similar to HMW $\Lambda_{L}$ values measured during the cruise ~80 miles south of the plume in the northern Gulf of Mexico (0.036 mg 100 mg OC$^{-1}$) and compares with values at the Bermuda Atlantic Time Series (BATS) station that ranged from 0.013 to 0.029 mg 100 mg OC$^{-1}$ [Opsahl and Benner, 1997]. On the other hand, LMW $\Lambda_{L}$ at 35.4 psu (0.022 mg 100 mg OC$^{-1}$) was nearly double that of the northern Gulf of Mexico station (0.012 mg 100 mg OC$^{-1}$) and a factor of ten greater than BATS values (0.002–0.003 mg 100 mg OC$^{-1}$) (P. J. Hernes and R. Benner, manuscript in preparation, 2003).

[21] Syringyl to vanillyl phenol ratios, S:V, can be used to distinguish between angiosperms and gymnosperms in fresh terrigenous organic matter because of the unique angiosperm source of syringyl phenols [Hedges and Mann, 1979]. In addition, S:V is sensitive to photooxidation [Opsahl and Benner, 1998] and decreases in S:V of HMW lignin phenols have been interpreted as evidence of photooxidation [Benner and Opsahl, 2001]. In the Mississippi River plume, S:V indicated differential behavior between HMW and LMW lignin phenols across the salinity gradient (Figure 4a). HMW S:V ranged from 0.78–1.06 at lower salinities before exhibiting a ~25% decrease to 0.58 at 35.4 psu. LMW S:V trends were the inverse of HMW S:V. LMW S:V varied from 0.72–0.91 at lower salinities and then increased ~50% to 1.08 at 35.4 psu. Mass balance indicates that approximately one third of the syringyl phenols lost from the HMW lignin fraction at high salinities was transferred to the LMW fraction. Total S:V remained virtually unchanged at ~0.8 before increasing abruptly to 1.3 at 35.4 psu (Figure 4d).

[22] Acid:aldehyde ratios of vanillyl and syringyl phenols, (Ad:Al)v and (Ad:Al)s, are a measure of relative oxidation of lignin. Higher acid:aldehyde ratios indicate greater oxidation, and acid:aldehyde ratios have been shown to increase with greater microbial degradation [Hedges et al., 1988b; Opsahl and Benner, 1997, 1998]. (Ad:Al)v and (Ad:Al)s in the Mississippi River plume showed nearly identical behavior across the salinity gradient (Figures 4b, 4c, and 4e). HMW (Ad:Al)v varied from 0.8 to 1.2 and HMW (Ad:Al)s from 0.5 to 1.0 (Figures 4b and 4c). In contrast to relatively minor changes in HMW acid:aldehyde ratios, LMW (Ad:Al)v and (Ad:Al)s increased dramatically at salinities >25 psu, i.e. >3 fold between 23.1 psu and 35.4 psu (Figures 4b and 4c). Total (Ad:Al)v and (Ad:Al)s
reflected the dominance of LMW lignin phenols at high salinities, also increasing >3 fold between 23.1 psu and 35.4 psu (Figures 4e and 4f).

3.6. Optical Measurements

[23] Photosynthetically Active Radiation (PAR) measurements taken ~1 m above the sampling depth indicate two zones of light penetration along the salinity gradient (Figure 5). At salinities <27, plume waters were turbid and less than 200 μE m⁻² s⁻¹ reached 1 m depth. However, at salinities >27, dilution with seawater along with the settling of riverine particulate material led to a substantial increase in light penetration with measurements up to 1200 μE m⁻² s⁻¹.

[24] Optical absorption coefficients at 350 nm ($a_{350}$) of 0.2-μm filtered water and ~1-nm filtered water (i.e. waters corresponding to total DOM and LMW DOM) exhibited similar trends to total dissolved lignin phenols and LMW lignin (Figure 6a). The $a_{350}$ of LMW DOM decreased steadily from 1.82 to 0.09 m⁻¹ s⁻¹ across the salinity gradient, exhibiting conservative behavior (Figure 6a). Total $a_{350}$ decreased nonconservatively from 8.90 to 0.09 m⁻¹ across the salinity gradient (Figure 6a). Absorption coefficients were linearly correlated with dissolved lignin phenols (Figure 6b), as demonstrated by $r$ values of 0.990 (P < 0.001) for 0.2-μm filtered water, and 0.957 (P < 0.001) for ~1-nm ultrafiltered water. Linear regression analysis of DOC concentrations and $a_{350}$ for 0.2-μm

![Figure 4. Changes in (a) syringyl:vanillyl (S:V), (b) vanillic acid:vanillin, (Ad:Al)v, and (c) syringic acid:syringaldehyde (Ad:Al)s in LMW and HMW lignin across the Mississippi plume salinity gradient and corresponding changes in (d) syringyl:vanillyl (S:V), (e) vanillic acid:vanillin, (Ad:Al)v, and (f) syringic acid:syringaldehyde (Ad:Al)s for SPE total dissolved lignin.](image_url)
filtered water gave an r value of 0.899 (P < 0.001). This demonstrates that specific compounds such as lignin that absorb light at 350 nm are better predictors of \(a_{350}\) than bulk DOC.

### 3.7. Photooxidation Experiment

Results from the photooxidation experiment are given in Table 5. Total dissolved lignin phenol concentrations in the dark control decreased by 21% over the course of ten days, while concentrations in the sample exposed to sunlight decreased by 72%. The corresponding removal rates are 0.46 \(\mu\)g L\(^{-1}\) d\(^{-1}\) in the exposed sample versus 0.14 \(\mu\)g L\(^{-1}\) d\(^{-1}\) in the dark control. The percentage of lignin phenols in the HMW fraction remained fairly constant in both treatments during the 10-day experiment. HMW \((\text{Ad:Al})_v\) more than doubled from 0.84 to 1.96 in the exposed sample, but increased only slightly in the dark control. Similarly, LMW \((\text{Ad:Al})_v\) nearly doubled from 0.74 to 1.38 in the exposed sample, with only a slight decrease in the dark control. \((\text{Ad:Al})_s\) showed the same pattern as \((\text{Ad:Al})_v\), increasing in the HMW (0.60 to 1.09) and LMW (0.68 to 0.89) fractions of the exposed sample but remaining essentially unchanged in the dark control. S:V ratios in the exposed sample changed little in the HMW fraction, while decreasing by a third from 0.86 to 0.57 in the LMW fraction. S:V in the dark control decreased slightly in both the HMW and LMW fractions.

Weighted averages for total dissolved lignin primarily reflected HMW lignin. \((\text{Ad:Al})_v\) doubled from 0.82 to 1.78 in the exposed sample, but only increased to 0.90 in the dark control. \((\text{Ad:Al})_s\) increased from 0.62 to 1.05 in the exposed sample, but remained unchanged at 0.62 in the dark control. S:V decreased slightly from 0.82 to 0.74 in the exposed sample and 0.72 in the dark control.

### 3.8. Long-Term Microbial Degradation Experiment

DOC and lignin phenol concentrations decreased by 37% and 52%, respectively, during the 1.7 yr decomposition experiment (Table 6), DOC concentrations decreased from 268 to 170 \(\mu\)M, while dissolved lignin phenol concentrations decreased from 8.3 to 4.0 \(\mu\)g L\(^{-1}\). Rates of degradation are not representative of in situ rates because of the manner in which the experiment was conducted. However, the extent of degradation is such that any lignin compositional changes associated with microbial processing should be readily apparent. Accordingly, carbon-normalized yields, \(\Lambda_6\), decreased from 259 to 195 \(\mu\)g 100 mg OC\(^{-1}\), while

![Figure 5](image-url)  
**Figure 5.** Light penetration at \(~1\) m depth across the salinity gradient in the Mississippi plume as measured by a photosynthetically active radiation (PAR) sensor.

![Figure 6](image-url)  
**Figure 6.** (a) Comparisons of absorption coefficients at 350 nm \(a_{350}\) and lignin phenol concentrations for total and LMW DOM across the Mississippi plume salinity gradient. (b) Linear correlations between \(a_{350}\) and the total and LMW DOM lignin phenol concentrations.

### Table 5. Photooxidation Effects on Lignin Phenol Concentrations (Sum of Vanillyl and Syringyl Phenols) and Composition of HMW and LMW DOM in Mississippi Plume Waters (8.5 psu) Relative to Initial Water and Dark Controls

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>10-Day Sunlight Exposure</th>
<th>10-Day Dark Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMW</td>
<td>LMW</td>
<td>HMW</td>
</tr>
<tr>
<td>Concentration, (\mu)g L(^{-1})</td>
<td>4.77</td>
<td>1.58</td>
<td>1.32</td>
</tr>
<tr>
<td>Removal rate, (\mu)g L(^{-1}) (\text{d}^{-1})</td>
<td>0.345</td>
<td>0.115</td>
<td>0.078</td>
</tr>
<tr>
<td>((\text{Ad:Al})_v)</td>
<td>0.84</td>
<td>0.74</td>
<td>1.96</td>
</tr>
<tr>
<td>((\text{Ad:Al})_s)</td>
<td>0.60</td>
<td>0.68</td>
<td>1.09</td>
</tr>
<tr>
<td>S:V</td>
<td>0.84</td>
<td>0.86</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*Abbreviations: HMW, high molecular weight, 1 – 200 nm; LMW, low molecular weight, <1 nm; \((\text{Ad:Al})_v\), ratio of vanillic acid to vanillin; \((\text{Ad:Al})_s\), ratio of syringic acid to syringaldehyde; and S:V, ratio of syringyl to vanillyl phenols.*
the lignin parameters, S:V, (Ad:Al)V, and (Ad:Al)S remained largely unchanged (Table 6).

4. Discussion

4.1. Diagenesis of Terrigenous DOM

[25] The lignin data in this study directly address two important questions about the fate of terrigenous DOM in marine systems: 1) What are the important removal processes? 2) What processes alter lignin composition? It is clear that removal processes above and below 25 psu in the plume are quite different. Nonconservative decreases in dissolved HMW lignin phenols at salinities <25 psu are likely due to flocculation and microbial degradation, whereas LMW dissolved lignin phenols mix conservatively. At salinities >25 psu, evidence for photooxidation is very strong. S:V and acid:aldehyde ratios in LMW and total dissolved lignin phenols are transformed dramatically when light penetration is no longer limited by turbidity. These transformations are generally consistent with results from a previous photooxidation experiment [Opsahl and Benner, 1998] and results from the photooxidation experiment in this study.

[29] Microbial degradation in the dark controls from the photooxidation experiment removed 21% of the dissolved lignin phenols over the course of ten days. Similar losses of dissolved lignin phenols were noted in dark controls from an earlier 28 d experiment [Opsahl and Benner, 1998]. Total DOC losses were 10% from a similar 51 d experiment using Satilla River estuary waters [Moran et al., 2000]. Microbial degradation of dissolved lignin is clearly not as rapid as photooxidation in sunlit surface waters—photodegradation rates in the two incubation experiments were threefold to fivefold greater than microbial degradation rates. However, microbial degradation occurs around the clock and throughout the water column and is clearly an important removal mechanism in the coastal ocean. Degradation of terrigenous organic matter is a competitive process between microbes and sunlight, with the importance of the individual process dependent on light exposure. The light history of any parcel of water is not readily known, which makes it challenging to determine the relative importance of photochemical versus microbial degradation. Flocculation and microbial degradation in the plume environment do not substantially alter lignin compositions, suggesting that molecular alterations are largely determined by photooxidation. If so, the lignin compositional imprint left by photochemical transformation could allow us to better constrain the extent of light exposure for any given parcel of water.

4.2. Absorption Correlations

[32] Molecular level measurements of dissolved lignin phenols are highly informative for tracing the transformations and fates of terrigenous DOM in seawater. However, lignin measurements are also analytically intensive and therefore not possible for routine analyses. An alternative measurement that has been utilized for studying terrigenous DOM in coastal regions is the absorption of light at wavelengths ranging from 300 to 355 nm [e.g., Blough et al., 1993; Miller and Zepp, 1995; Vodecek et al., 1997; Andrews et al., 2000]. Aromatic compounds, such as lignin, are the primary components of DOM that absorb light. An absorption spectrum of solubilized birch lignin exhibits a local maximum at ~290 nm with a smaller inflection at ~350 nm, while absorption of marine DOM is greatest at wavelengths >400 nm [Fooken and Liebezeit, 2000]. In coastal areas where terrigenous materials can comprise a significant fraction of DOM, absorption coefficients at ~350 nm (\(a_{350}\)) have been shown to correlate with salinity [e.g., Blough et al., 1993], and by inference the riverine or terrigenous component of DOM. Dissolved lignin, however, is a sensitive and unambiguous tracer of terrigenous DOM at all salinities. Therefore comparisons of \(a_{350}\) with lignin phenol concentrations in the Mississippi River plume
provide a more robust test of the utility of \( a_{350} \) for tracing terrigenous DOM.

[33] Measurements of \( a_{350} \) on total and LMW DOM exhibit similar trends to total and LMW dissolved lignin phenol concentrations (Figure 6a). As with dissolved lignin phenols, the \( a_{350} \) of DOM also indicates nonconservative losses at low salinities. On the other hand, \( a_{350} \) of LMW DOM indicates conservative mixing, just as was observed with LMW lignin. The high correlation coefficients (\( r \)) between lignin phenol concentrations and \( a_{350} \) (0.984 and 0.957) highlight the potential for relating \( a_{350} \) to terrigenous DOM. The slopes of the lines for total and LMW DOM (1.01 and 0.85 \( \text{m}^{-1} \) [\( \mu g/L \text{ dissolved lignin phenols} \]) respectively) are within standard error of each other, indicating the absence of size dependence in the \( a_{350} \) response. The unvarying response across a salinity gradient and across size classes suggests that it could be possible to calibrate absorption coefficients for measuring terrigenous DOM in regional coastal systems. Previous work with satellite and aircraft imagery indicates that absorption can be determined remotely using measured fluorescence spectra or passive reflectance measurements [e.g., Green and Blough, 1994; Blough and Del Vecchio, 2002; Nelson and Siegel, 2002]. Properly calibrated, remote measurements could provide a powerful tool in studying the fate of terrigenous DOM in ocean margins.

[34] Acknowledgments. We dedicate this paper to the memory of John I. Hedges, the “father” of lignin geochemistry. His insightfulness and enthusiasm for organic geochemistry will be greatly missed. We thank the crew and captain of the R/V Longhorn for assistance in the collection of samples. We also thank Robert Bourgeois for DOC analyses, as well as the comments of the Biogeochemistry group at the University of South Carolina and two anonymous reviewers. This research was supported with funding from the NSF (OCE-0096102).

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