Abstract

A defining feature of Earth is the continuous cycle of organic carbon as it traverses through the atmosphere, oceans, continents, and mantle reservoirs. Between these reservoirs and pathways is the chemical exchange of stored carbon and the production of various reduced carbon materials. This ultimately leads to substantial organic carbon accumulation within the primary reservoirs, including the atmosphere, terrestrial biosphere, and ocean. Vascular plants serve as a predominant source of organic matter and a significant source of terrestrial organic carbon in the sea. When the on-land-derived organic matter enters the ocean, it meets remarkably rapid remineralization. Still, the mechanism by which these vast amounts of organic materials are rapidly oxidized is mainly unknown. Therefore, we have derived a simple phenolic model system to study the oxidative degradation of lignin, the second most abundant biopolymer on land and used Bobbitt’s salt to explore the pathways of degradation of lignin that occurs in the environment. Ultimately, the insight of these chemical processes provides transformations of land-derived organic matter that appear in the ocean, which plays a crucial role in the global carbon cycle.

Introduction

Large vascular plants, with their high concentration of carbon-rich biomacromolecules such as lignin, cellulose, tannin, suberin, and cutin, represent a significant fraction of the total organic carbon transported into the ocean from land. This organic matter dissolves into the vast ocean waters, emerging as an important contributor to global marine carbon fluxes and sinks.\(^1\) The organic carbon introduced into the ocean's reservoir contains a total amount of carbon similar to atmospheric carbon dioxide. Therefore, understanding the movement and transformation of carbon during the carbon cycle is crucial to quantifying the amount of dissolved organic carbon. Lignin is a phenolic biopolymer that accounts for approximately 25% of the dry weight of cellular carbon stored in the biosphere.\(^2\) Consequently, understanding lignin metabolism is paramount in the earth's carbon cycle. However, the concentration of dissolved lignin in marine environments is not proportional to its abundance in soil.\(^3\) Though the complete degradation of lignin to CO\(_2\) and H\(_2\)O could account for the loss of organic material, kinetic investigations into the degradation of lignin in rivers cannot account for the complete mineralization of lignin into CO\(_2\) and H\(_2\)O.\(^4\) Understanding the mineralization of lignin poses a significant challenge due to the limited knowledge in this area, which particularly impacts the determination of lignin concentrations in the ocean through phenolic biomarkers. However, oxidative degradation of lignin into compounds unrecognizable by this method could lead to a lower perceived concentration of lignin in marine environments. It is firmly understood that lignin degradation occurs through natural oxidative processes such as photooxidation.
and Fenton reaction. However, these processes face limitations in laboratory applications, primarily due to the influence of spin-active species (such as radicals and metals) on spectroscopic techniques like EPR, NMR, and mass spectrometry. Bobbitt’s salt, a popular organic oxidant known for its diverse and selective oxidative transformations, can replicate oxidations observed in Fenton reactions in a controlled manner. Therefore, this work aims to investigate the oxidative degradation of lignin by examining the development of a new, operationally simple phenol system using a dibenzodioxin model to simulate rapid remineralization and investigate oxidative degradation in the environment. The findings from our investigation into the oxidative degradation of lignin have implications for carbon cycling, offering valuable understanding regarding the influx of terrestrial carbon into the world's rivers, estuaries, and oceans.

Oxoammonium salts, e.g., Bobbitt’s salt (2), are versatile oxidants widely used in organic synthesis (Figure 1). These oxidants can selectively oxidize alcohols to aldehydes or ketones, and can, facilitate the oxidation of aryl ethers. Within this class of organic oxidants are nitroxides, the precursors to oxoammonium salts. The Bobbitt’s salt precursor, 4-NHAc-TEMPO (ACT, 1), has also been applicable as an oxidant. Besides the fact that Bobbitt’s salt is commonly used to oxidize alcohols to either aldehydes, ketones, or carboxylic acid, this reagent can also be employed in several other transformations involving nitriles, lactones, esters, and C-C bond functionalizations. Its applications extend to coupling reactions of phenols and benzyl ether cleavage, which is pertinent to lignin degradation studies. The oxidative cleavage of benzyl ethers with Bobbitt’s salt yields aromatic aldehydes and alcohols in high yields under mild conditions. Furthermore, primary and secondary alcohols can be further oxidized to carboxylic acids or ketones. Bobbitt’s salt also facilitates biaryl coupling reactions when combined with phenolic components in the presence of potassium hydrogen carbonate. These oxidative transformations are significant for lignin oxidation due to their applicability. Bobbitt’s salt is favored for “green” oxidation reactions in organic chemistry due to its non-toxic, easy-to-handle nature, and the ability to convert its precursor, ACT (1), to Bobbitt’s salt (2) using household bleach. It can be safely handled and stored under ambient conditions, making it suitable for various oxidation reactions without extensive precautions. Given these factors and our research group's proficiency with these reagents and their analogous reactivity to Fenton oxidation, Bobbitt’s salt was chosen as the oxidative reagent for lignin degradation studies.

Lignin is an aromatic biopolymer found in the cell walls of plants composed of 35% wood by mass, with softwood containing about 30% and hardwood around 20% of its dry weight. Its intricate native structure is formed through the connection of phenylpropanoid units through various bonds. Within plants, lignin is synthesized through oxidative (radical-mediated) coupling between a monolignol and the growing oligomer or polymer of coniferyl, sinapyl, and p-coumaryl alcohols, resulting in diverse...
interunit linkages: \( \beta-O-4' \) (most abundant), \( \alpha-O-4' \), \( \beta-5' \), \( \beta-\beta',\beta-1' \), \( 4-O-5',5-5' \), and dibenzodioxins.\(^{11}\) Delignification involves cleaving the \( \beta \)-aryl ether, softwood lignin’s most abundant structural unit, constituting about 50% of the \( \beta-O-4' \) structure from the involvement of phenylpropanoid units.\(^9\) However, using or extracting meaningful information from lignin from its complex structure with various connections between its units isn’t easy. Hence, a model compound has been developed, mainly representing the \( \beta-O-4' \) bond. The dibenzodioxocin motif, a significant branching point in lignin structure, accounts for 5-7% of connections in softwood lignin.\(^{12}\) Despite its importance, the reactivity of this motif has not been extensively explored, and achieving selective catalytic cleavage of the dibenzodioxocin motif under mild reaction conditions is challenging. Therefore, to comprehend the reactivity of lignin, a dibenzodioxins model (3) mimicking the motifs present in lignin (Figure 2) was synthesized through the oxide-catalyzed radical coupling of biphenyl compound and coniferyl alcohol. It is hypothesized that treatment of the model compound with Bobbitt’s salt (2) under mild oxidation conditions would lead to a benzylic C-O bond cleavage within the \( \alpha-O-4' \) bond of the lignin model compound via hydride abstraction. Therefore, this proposed reaction pathway offers insights into lignin’s structural complexities and its reactivity under controlled conditions, and by investigating the oxidative cleavage of the dibenzodioxins compound, aimed to gain deeper insight into lignin chemistry.

\( \text{Figure 2: The anticipated outcome of the dibenzodioxocin model being subjected to Bobbitt’s Salt} \)

\( \text{Discussion} \)

To initiate the study of the oxidation of lignin, a polymer-supported oxoammonium salt was synthesized by coupling the 4-amino group of nitroxide (4) with poly(ethylene-co-acrylic acid)- (5 wt% acrylic acid), obtaining a solid-support nitroxide (5) in a 62% yield. Oxidation of this intermediate with household bleach formed oxoammonium salt (6) in an 86% yield. This synthesis aimed to establish a straightforward method for investigating substrates like soil and sediment, where the oxidant can be easily removed via filtration. Filtration enables the separation of the spent oxidant from water or extraction from soil/sedimentary environments, allowing for the recycling of beads derived from poly(ethylene-co-acrylic acid). This polymer-based approach simplifies analytical analysis by facilitating the convenient filtration of spent oxidants from lignin oxidation reactions.
Moreover, due to the catalytic nature of the process, the recovered beads can be re-used for subsequent oxidation reactions, enhancing the method’s simplicity and environmental friendliness. However, the synthesis of the polymer-supported oxoammonium salt presented several challenges and required multiple attempts, particularly in accurately determining the stoichiometric quantities of reagents needed for successful product formation. One notable advantage of nitroxide and oxoammonium salt synthesis is their colorimetric nature, observable during the reaction progress. The transformation of the orange nitroxide to the yellow oxoammonium salt provides a visual indication of reaction completion. Additionally, the functional conversion of the co-acrylic acid within the polymer was assessed through oxidation with 4-methoxy benzyl alcohol, revealing a 62% conversion rate out of the available acid sites. Furthermore, the successful synthesis of the polymer-supported oxoammonium salt opens avenues for exploring other potential applications. For instance, ongoing research includes investigating electrochemical reactions in saline solutions to simulate oceanic environments, aiming to deepen our understanding of lignin degradation in marine and estuarine settings.

Figure 3: The synthesis of a polymer-supported oxoammonium salt (6)
intricacies of lignin degradation. To initiate this investigation, multiple reactions with benzyl alcohol (7) were performed to obtain ideal conditions. Several conditions, including solvent choice, electrolyte composition, electrolyte and oxidant (ACT) quantities, and electrode type, were systematically evaluated to achieve maximal percent conversion (%). Transitioning to graphite electrodes revealed heightened reactivity with benzyl alcohol (7), signaling a promising avenue for exploration. While some test conditions from Table 1, yielded unsatisfactory results, the introduction of NaCl exhibited an improvement in percent conversion rates. Notably, the inclusion of NaHCO₃ led to a markedly enhanced conversion rate. Therefore, the reactions conducted in the presence of NaCl and graphite electrodes demonstrated the best electrochemical oxidation, resulting in the conversion to benzaldehyde (8). The of electrolyte and electrode type was made to mimic seawater environments, aligning with our objective to simulate realistic conditions for lignin degradation studies.

Following this, additional phenolic compounds (9, 10, 11, and 12) underwent retesting with the NaCl electrolyte and graphite electrodes, and the results are demonstrated in Table 2. However, a persistent issue with the IKA Electrasyn arose as it failed to maintain a consistent positive current throughout the reaction, potentially leading to a reduction reaction in our phenolic compounds and even the benzyl alcohol. Furthermore, attempts were made to explore electrochemical reactions of the solid-support nitroxide in saline solutions alongside benzyl alcohol, albeit without success. The desired oxidized product remained elusive despite adjustments to the current, polarity, and aqueous saline concentration. This challenge likely stems from the solid-support nitroxide's insolubility issues in the aqueous saline solutions, leading it to float within the solution resulting in insufficient contact with the substrate. Nonetheless, are continuing to explore potential modifications and reactions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Electrolyte</th>
<th>Amount of Electrolyte</th>
<th>Equiv of ACT (1)</th>
<th>Electrodes</th>
<th>% Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>NaCl</td>
<td>99 mmol</td>
<td>50 mo%</td>
<td>Platinum Coated/ Glassy Carbon</td>
<td>0.4 %</td>
</tr>
<tr>
<td>MeCN</td>
<td>LiBF₄</td>
<td>28 mmol</td>
<td>5 mo%</td>
<td>Graphite</td>
<td>9 %</td>
</tr>
<tr>
<td>Water</td>
<td>LiBF₄</td>
<td>3 mmol</td>
<td>5 mo%</td>
<td>Graphite</td>
<td>9 %</td>
</tr>
<tr>
<td>Water</td>
<td>KCl</td>
<td>7 mmol</td>
<td>10 mo%</td>
<td>Graphite</td>
<td>9 %</td>
</tr>
<tr>
<td>Water</td>
<td>KBr</td>
<td>6 mmol</td>
<td>10 mo%</td>
<td>Graphite</td>
<td>9 %</td>
</tr>
<tr>
<td>Water</td>
<td>NaCl</td>
<td>20 mmol</td>
<td>10 mo%</td>
<td>Graphite</td>
<td>7 %</td>
</tr>
<tr>
<td>Water</td>
<td>NaHCO₃</td>
<td>9 mmol</td>
<td>10 mo%</td>
<td>Graphite</td>
<td>50 %</td>
</tr>
</tbody>
</table>

Table 1: Different conditions used for the benzyl alcohol reaction.

<table>
<thead>
<tr>
<th>Phenolic Compound</th>
<th>Amount of NaCl</th>
<th>Equiv of ACT (1)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillin (8)</td>
<td>1 mmol</td>
<td>5 mo%</td>
<td>After 3 h, NMR showed a product. Following column chromatography, NMR analysis of the spots revealed several complex products, and starting material.</td>
</tr>
<tr>
<td>2-Methoxy-4- methylphenol (10)</td>
<td>1 mmol</td>
<td>5 mo%</td>
<td>TLC was done after 2 h and 5 h and both showed consumption of starting material.</td>
</tr>
<tr>
<td>Eugenol (11)</td>
<td>0.5 mmol</td>
<td>10 mo%</td>
<td>A NMR was taken after running the reaction overnight and it showed no change</td>
</tr>
<tr>
<td>1,3-Dimethoxybenzene (12)</td>
<td>1 mmol</td>
<td>5 mo%</td>
<td>NMR was recorded after running the reaction overnight and the results showed title to no product.</td>
</tr>
</tbody>
</table>

Table 2: Different conditions of the electrochemical oxidation that were performed on various phenolic compounds
to surmount these obstacles, aiming to achieve successful oxidation and enrich our understanding of lignin degradation within marine ecosystems.

![Chemical structures](image)

**Figure 4**: Several oxidation performed on various phenolic compounds

To further explore the environmental oxidation of lignin, we investigated the reactivity of Bobbitt’s salt (2) with phenolic compounds. Vanillin, a phenolic compound, underwent oxidations with Bobbitt’s salt in methanol and water at room temperature. This method aimed to generate lignin monomers via oxidative cleavage of C-C bonds, diverging from typical C-O bond cleavage by Bobbitt’s salt or other TEMPO-based oxidants. Monitoring the reactions by TLC revealed new spots compared to the starting materials (9-11), accompanied by a color change in the reaction mixture from yellow to light brown. Subsequently, the reaction residue underwent column chromatography to isolate specific products and to elucidate the oxidative transformation observed. The anticipated outcome was the formation of a quinone product based on our oxidation of catechol (13), yielding 1,2 benzoquinone (14) quantitatively (Figure 5). However, isolating the reaction products proved challenging, yielding complex mixtures that hindered the identification of specific oxidative transforms. These findings suggest the presence of potential intermediate products or byproducts requiring further analysis and characterization. To delve deeper into these reactions and identify the unknown species, we plan to explore various reaction conditions, adjusting parameters such as solvent composition and temperature. Additionally, the use of advanced spectroscopic techniques such as 2-D NMR and high-resolution mass spectrometry are on-going to characterize the complex mixtures obtained, aiming to uncover valuable insights.

![NMR spectra](image)

**Figure 5**: Oxidation of catechol (13) with Bobbitt’s Salt to afford 1,2 benzoquinone (14)
into the reactivity of phenolic compounds with Bobbitt’s salt and the implications for lignin degradation in environmental settings.

To further investigate lignin degradation with a more relatable environmental model, a dibenzodioxocin model (3) was synthesized (Figure 6). The construction of 3 was envisioned to occur through the hypoiodate oxidation of 4-methoxy-4-propylphenol (16), yielding a biaryl compound 17. Oxidative coupling with coniferyl alcohol (18) in the presence of horseradish peroxidase was envisioned to afford 3. The synthesis of the biaryl compound 17 was successful, obtaining a yield of 97%. The synthesis involving coniferyl alcohol (18), which required the esterification of ferulic acid (19) to produce methyl ferulate (20), followed by reduction to coniferyl alcohol using lithium aluminum hydride reagent, faced challenges in purification due to difficulties in column chromatography. However, coniferyl alcohol

\[
\begin{align*}
&\text{H}_3\text{CO} \quad \text{H}_2\text{SO}_4 \quad \text{MeOH} \quad \text{LiAlH}_4 \quad \text{THF} \\
19 &\rightarrow 20 & 17 &\rightarrow 18
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 &\quad \text{Na}_2\text{CO}_3 \quad \text{H}_2\text{O}_2 \quad \text{TBAI} \quad \text{EtOAc, 8 h} \\
16 &\rightarrow 17 & 17 &\rightarrow 18 & 18 &\rightarrow 3
\end{align*}
\]

Completed reaction to form the biaryl compound 16

Isolated biaryl compound 16

Reaction to form the dibenzodioxocin model 3

Extracted dibenzodioxocin model (3) compound in ethyl acetate

**Figure 6:** a) Synthesis of coniferyl alcohol (18) b) Synthesis of dibenzodioxocin model (3) c) Images of synthesis of a dibenzodioxocin model of lignin.
(18) was successfully obtained for use in the model system in low yield. Despite several attempts, the synthesis of the dibenzodioxocin model (3) encountered challenges. When the reaction mixture containing biaryl compound (17) and coniferyl alcohol was stirred in the presence of horseradish peroxidase, a color change from clear to yellow/gold was observed. However, attempts to isolate and purify the crude product of the model compound resulting from the enzymatic reaction were unsuccessful.

Progress was made with the development of a new synthetic route to address this challenge in producing model 3. This alternative approach involves synthesizing 3 through a silver-catalyzed radical coupling of biphenyl compound 17 and coniferyl alcohol 18 using silver oxide.11 Currently, this new synthetic route has been attempted and efforts are underway to purify the crude product (3) that was successfully prepared (Figure 7). As the work continues, the primary objective remains to complete the isolation of the model compound (3) for subsequent oxidation with Bobbitt’s salt versus Fenton oxidation. The goal is to investigate the oxidative transformations resulting from the degradation of the dibenzodioxocin moiety present in natural lignin. To achieve this, thorough characterization of the resulting compounds using NMR and HRMS techniques is being conducted in collaboration with the Hatcher group here at Old Dominion University.

**Figure 7:** a) Alternative synthesis route for dibenzodioxocin model of lignin (3) b) Images from alternative synthesis route of dibenzodioxocin model (3) c) $^1$H NMR spectra of crude dibenzodioxocin model (3).
Conclusion

In summary, the use of oxoammonium salts, particularly Bobbitt’s salt, presents a promising avenue for investigating environmental oxidation processes, particularly in lignin degradation studies. We have synthesized a model lignin substrate (3) and a solid-supported oxoammonium salt (6) to delve deeper into the oxidative degradation mechanisms and the resulting products from natural lignin decomposition. Moving forward, the oxidation of the prepared dibenzodioxocin model with Bobbitt’s salt will be completed. Additionally, we plan to explore the oxidation of the lignin model system using both Bobbitt’s salt and the polymer-supported oxoammonium salt. Furthermore, we intend to compare the oxidation pathways of the lignin model system with Fenton degradation of environmental lignin samples. This project seeks to enhance our understanding of the oxidative degradation pathways of terrestrial lignin and their environmental impacts, particularly on the world’s oceans.

Acknowledgments

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Literature Cited


