LACCASE ACTIVITY ENHANCERS

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ABSTRACT

New mediators for use in laccase-mediator systems (LMS) are disclosed. The mediators enhance the activity of enzymes exhibiting laccase activity and can be used, for example, to bleach a dye in solution, to oxidize an appropriate compound, or to initiate vinyl or phenol polymerizations. A composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent (also referred to as a mediator or an activating agent) is also disclosed. A process for oxidizing a substrate that comprises treating the substrate with a composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent is also disclosed.
Bleaching of DB1 with Aspergillus sp. Laccase and ABTS (pH 7.0)

FIGURE 1
LACCASE ACTIVITY ENHANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/318,291, filed on Sep. 10, 2001.

FIELD OF THE INVENTION

The present invention relates to the enhancement of enzyme activity and the activation of enzymes. More specifically, the present invention relates to mediators, enhancing agents, or activating agents that are useful in enhancing the activity of enzymes having laccase activity.

BACKGROUND OF THE INVENTION

Laccases are copper-containing enzymes that are known to be good oxidizing agents in the presence of oxygen. Laccases are found in microbes, fungi, and higher organisms. Laccase enzymes are used for many applications, including pulp bleaching, treatment of pulp waste water, de-inking, industrial color removal, bleaching laundry detergents, oral care teeth whiteners, and as catalysts or facilitators for polymerization and oxidation reactions. Commercial enzymes are often produced from fungal sources.

For many applications, the oxidizing efficiency of a laccase can be improved through the use of a mediator, also known as an enhancing agent. Systems that include a laccase and a mediator are known in the art as laccase-mediator systems (LMS). The same compounds can also be used to activate or initiate the action of laccase.

There are several known mediators for use in a laccase-mediator system. These include HBT (1-hydroxybenzotriazole), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), NHA (N-hydroxyacetanilide), NHAA (N-acetyl-N-phenylhydroxylamine), HBTBO (3-hydroxy-1,2,3-benzoazirin-4(3H)-one), and VIO (violuric acid). In addition, there are several compounds containing NH—OH or N—O that have been found to be useful as mediators.

Functional groups and substituents have large effects on mediator efficiency. Even within the same class of compounds, a substituent can change the laccase specificity towards a substrate, thereby increasing or decreasing mediator efficiency greatly. In addition, a mediator may be effective for one particular application but unsuitable for another application. Thus, there is a need to discover efficient mediators for specific applications. One such application is the bleaching of pulp, wherein it is also important that the mediators are not unduly expensive or hazardous. Other applications of the laccase-mediator system are given below.

Thus, there is a need to identify additional mediators that activate laccase, and/or enhance the activity of enzymes that exhibit laccase activity.

SUMMARY OF THE INVENTION

The present invention provides mediators for use in laccase-mediator systems (LMS). The mediators of the invention enhance the activity of enzymes exhibiting laccase activity and can be used, for example, to bleach a dye in solution, to oxidize an appropriate compound, or to initiate vinyl or phenol polymerizations.

[0009] The invention provides a composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent (also referred to as a mediator or an activating agent). The enzyme enhancing or activating agent is selected from:

[0010] (i) a monomer, dimer, or oligomer of at least one of:

[0011] wherein:

[0012] R₁ is OCH₃, H, or another moiety;
[0013] R₂ is OCH₃, H, or another moiety;
[0014] R₃ is OH, sulfonate, or another moiety;
[0015] R₄ is an OH or methylene that connects to another monomer; and
[0016] R₅ is SO₃H or H;

[0017] (ii) N-benzylidene benzylamine (NBBA) or a substituted NBBA having the structure:

[0018] wherein:

[0019] R is H, OCH₃, NH(CH₂)₂, Cl, Br, an aliphatic group, or an aromatic group; and
[0020] R₁ is H, OCH₃, NH(CH₂)₂, Cl, Br, an aliphatic group, or an aromatic group;

[0021] (iii) a derivative of cinnamic acid having the structure:

[0022] wherein:

[0023] R₁ is OCH₃, NH₂, Cl, NH(CH₂)₂; and
[0024] R₂ is OH or H;

[0025] (iv) salicylic acid;
(v) epigallocatechin gallate;
(vi) melamine;
(vii) 3,4-dihydroxybenzyl alcohol (DHBA) or a substituted DHBA;
(viii) a hardwood black liquor;
(ix) a hardwood black liquor that comprises the monomer, dimer, or oligomer of group (i);
(x) a softwood black liquor; and
(xi) a softwood black liquor that comprises monomer, dimer, or oligomer under group (i).

The invention also provides a process for oxidizing a substrate that comprises treating the substrate with a composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent.

The enzyme enhancing agents of the invention are selected from:

(i) a monomer, dimer, or oligomer of at least one of:

R is an OH or methylene that connects to another monomer; and
R is SOH or H;

(ii) N-benzylidene benzylamine (NBBA) or a substituted NBBA having the structure:

R is OCH, H, or another moiety; R is OCH, H, or another moiety; R is OH, sulfonate, or another moiety;

(iii) a derivative of cinnamic acid having the structure:

R is H, OCH, NH(CH), Cl, Br, an aliphatic group, or an aromatic group; and
R is H, OCH, NH(CH), Cl, Br, an aliphatic group, or an aromatic group;

(iv) salicylic acid;
(v) epigallocatechin gallate;
(vi) melamine;
(vii) 3,4-dihydroxybenzyl alcohol (DHBA) or a substituted DHBA;

The enzyme enhancing agents of the invention are Selected from:

(i) a monomer, dimer, or oligomer of at least one of:

R is OCH_, H, or another moiety;
R is OCH_, H, or another moiety;
R is OH, sulfonate, or another moiety;
R is an OH or methylene that connects to another monomer; and
R is SOH or H;

(ii) N-benzylidene benzylamine (NBBA) or a substituted NBBA having the structure:

R is H, OCH, NH(CH), Cl, Br, an aliphatic group, or an aromatic group; and
R is H, OCH, NH(CH), Cl, Br, an aliphatic group, or an aromatic group;

(iii) a derivative of cinnamic acid having the structure:

R is OCH_, H, NH(CH), Cl, Br, an aliphatic group, or an aromatic group; and
R is OH or H;

(iv) salicylic acid;
(v) epigallocatechin gallate;
(vi) melamine;
(vii) 3,4-dihydroxybenzyl alcohol (DHBA) or a substituted DHBA;
(viii) a hardwood black liquor;
(ix) a hardwood black liquor that comprises the monomer, dimer, or oligomer of group (i);
(x) a softwood black liquor; and
(xi) a softwood black liquor that comprises monomer, dimer, or oligomer under group (i).

Without limiting the scope of the invention, the enzyme enhancing agents of the invention can be classified into four categories:

I. Lignin-like Materials

a. Organic soluble fraction of lignin (lignin organosolv)
b. Lignin sulfonate
c. Hardwood black liquor and softwood black liquor

Each of these three materials is a mixture comprising monomers, dimers, and oligomers with the following structures.

In these structures, R₁ and R₂ are OCH₃, H, or other moieties; R₃ is OH, sulfonate, or other moieties; R₄ is either an OH group or a methylene that connects to another monomer; R₅ is SO₃H or H.

II. N-benzylidene benzylamine (NBBA) and substituted NBBA.

In this structure, R₆ and R₇ are H or OCH₃, NH(CH₃)₂, Cl, Br, aliphatic or aromatic groups, substituted at the 2 and 4 positions.

III. Derivatives of Cinnamic Acid

a. 4-(dimethylamino)cinnamic acid
b. 3-hydroxy-4-methoxyxycinnamic acid
c. 4-aminocinnamic acid
d. 4-chlorocinnamic acid

In this structure, R₈ is OCH₃, NH₂, Cl, NH(CH₃)₂, and R₉ is OH or H.

IV. Other Aromatic Compounds

a. salicylic acid
b. epigallocatechin gallate
c. melamine
d. 3,4-dihydroxybenzyl alcohol (DHBA), and substituted DHBA.

The mediators of the invention can be used, for example, for pulp delignification and bleaching. Laccase itself can bleach pulp only to a limited extent. The use of the mediators as disclosed herein enhances the activity of laccase in pulp bleaching through delignification. Other applications that may use the present invention include: polymerization of vinyl monomers or phenolic compounds; oxidation of materials containing hydroxy, amine, mercaptan, olefin, and aromatic moieties; microbial control in personal care products; bleaching of hair; treatment of waste water, particularly waste water in pulp mills; and bleaching in laundry detergents.

Another aspect of the invention provides a process for oxidizing a substrate that comprises treating the substrate with a composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent. The enzyme enhancing agent can be selected from one or the above described enzyme enhancing agents.

The enhancing agent may be present in concentrations of from about 0.01 micromolar to 1000 micromolar, more preferably from about 0.1 micromolar to about 250 micromolar and most preferably from about 0.5 to about 100 micromolar.

The enzyme is used in amounts of from 0.001 to 50 units (defined in the Examples using ABTS as substrate) in 1 ml of the reaction solution, preferably from about 0.01 to 20 units and even more preferably from 0.1 to 10 units and most preferably from 2 to ~3 units.

The process of the invention can further include the step of adding an oxidizing agent, such as at least one of air, oxygen, and hydrogen peroxide.

One aspect of the invention provides a process for bleaching a lignin-containing material that comprises treating the material with an enzyme exhibiting laccase activity and an enzyme enhancing agent. In this aspect of the invention, the enhancing agent may be present in an amount of from about 0.1% to about 15% based on the weight of the dry lignin containing material, more preferably from about 0.1% to about 10% and even more preferably from about 0.5% to about 5% and most preferably from about 1% to about 4%. One example of a lignin containing material is wood pulp. The process for bleaching a lignin-containing material can further include the step of adding an oxidizing agent, such as at least one of air, oxygen, and hydrogen peroxide.
The following examples are illustrative of the present invention, and are not intended to be construed in any way as limiting the scope of the invention.

The specific activity was determined using ABTS (0.5 mM) as substrate. One unit of activity is equal to the umol of the oxidized product from ABTS per min per mg protein at pH 6.0 at 23°C. The extinction coefficient of the oxidized ABTS is ε(max) at 420 nm=36,000 M⁻¹cm⁻¹.

Alternatively, the activity of laccase (NS51003) was determined using syringaldazine as substrate. In this case, one unit of activity is equal to the change of 0.001 UV absorbance at 530nm per minute per mg protein in 2 ml of 100 mM, pH 5.5 potassium phosphate buffer, and 0.5 ml of 0.25 mM syringaldazine in methanol at 23°C.

The assay results for three samples of laccase enzyme are shown in the following table. Samples NS51002 and NS51003 were obtained from Novozymes A/S (Denmark). Unless otherwise stated, the results given in the examples were based on the enzyme NS51003.

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Activity (umol/min. ml)</th>
<th>Protein con. (mg/ml)</th>
<th>Specific activity (umol/min. mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS51002</td>
<td>Aspergillus sp.</td>
<td>1.08</td>
<td>895</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Novozymes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS51003</td>
<td>Aspergillus sp.</td>
<td>1.04</td>
<td>750</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Novozymes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example 1

Use of the Chicago Blue assay.

In the following example, several compounds were tested to identify enzyme enhancing agents that enhance the activity of an enzyme that exhibits laccase activity. The Chicago Blue Dye, also known as Direct Blue 1 or DB 1, was used to identify these compounds. The Chicago Blue Dye is fully described by Schneider et al. in U.S. Pat. No. 5,885,304, which is hereby incorporated by reference, and has the following formula.

[0098] Experiments were conducted at pH levels of 5.5 and 7.0. The results of the Chicago Blue Assay at pH 5.5 and pH 7.0 are provided in the following tables below. The last column gives the decrease in absorbance at 610 nm. The larger the decrease in absorbance at 610 nm, the better is the ability to discolor the dye.
TABLE 1

<table>
<thead>
<tr>
<th>Potential Mediator (0.1 micromole/ml)</th>
<th>Appearance in water</th>
<th>ΔmA610 (3 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>clear</td>
<td>0</td>
</tr>
<tr>
<td>4-(Dimethylamino)cinnamic acid</td>
<td>clear, yellow</td>
<td>519</td>
</tr>
<tr>
<td>3,5-Dimethoxy-4-hydroxycinnamic acid</td>
<td>clear</td>
<td>491</td>
</tr>
<tr>
<td>4-Hydroxy-3-methoxycinnamic acid (ferulic acid)</td>
<td>clear</td>
<td>441</td>
</tr>
<tr>
<td>Hardwood black liquor (150 ul, 12%)</td>
<td>yellow</td>
<td>425</td>
</tr>
<tr>
<td>Lignin, organosolv (20 ug/ml)</td>
<td>clear, brown</td>
<td>390</td>
</tr>
<tr>
<td>Melamine</td>
<td>cloudy</td>
<td>128</td>
</tr>
<tr>
<td>3-Hydroxy-4-methoxycinnamic acid</td>
<td>clear</td>
<td>94</td>
</tr>
<tr>
<td>4-Aminocinnamic acid</td>
<td>clear</td>
<td>43</td>
</tr>
<tr>
<td>4-Hydroxybenzaldehyde</td>
<td>clear</td>
<td>42</td>
</tr>
<tr>
<td>N-benzylidene benzylamine</td>
<td>clear</td>
<td>28</td>
</tr>
<tr>
<td>Lignosulfonic acid, (25 ug/ml)</td>
<td>Light brown</td>
<td>24</td>
</tr>
<tr>
<td>4-Chlorocinnamic acid</td>
<td>cloudy</td>
<td>13</td>
</tr>
<tr>
<td>Salicilic acid</td>
<td>clear</td>
<td>11</td>
</tr>
<tr>
<td>Softwood black liquor (150 ul, 12%)</td>
<td>yellow</td>
<td>10</td>
</tr>
<tr>
<td>2-Chlorocinnamic acid</td>
<td>cloudy</td>
<td>9</td>
</tr>
<tr>
<td>3-Chlorocinnamic acid</td>
<td>cloudy</td>
<td>8</td>
</tr>
<tr>
<td>3-Hydroxybenzaldehyde</td>
<td>clear</td>
<td>7</td>
</tr>
<tr>
<td>3-Methoxycinnamic acid</td>
<td>cloudy</td>
<td>6</td>
</tr>
<tr>
<td>Isatin</td>
<td>clear</td>
<td>6</td>
</tr>
<tr>
<td>2-Hydroxybenzaldehyde</td>
<td>clear</td>
<td>5</td>
</tr>
<tr>
<td>3,4,5-Trimethoxycinnamic acid</td>
<td>cloudy</td>
<td>5</td>
</tr>
<tr>
<td>3-Methoxycinnamic acid</td>
<td>clear</td>
<td>4</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>clear</td>
<td>3</td>
</tr>
<tr>
<td>2-Aminopyridine</td>
<td>light brown</td>
<td>3</td>
</tr>
<tr>
<td>Ventriyl alcohol</td>
<td>clear</td>
<td>3</td>
</tr>
<tr>
<td>Ethyl Salicylate</td>
<td>cloudy</td>
<td>2</td>
</tr>
<tr>
<td>3,4-Dimethoxycinnamic acid</td>
<td>clear</td>
<td>0</td>
</tr>
<tr>
<td>4-Nitrocinamic acid</td>
<td>cloudy</td>
<td>0</td>
</tr>
</tbody>
</table>

[0099]

TABLE 2

<table>
<thead>
<tr>
<th>Potential Mediator (0.1 micromole/ml)</th>
<th>Appearance in water</th>
<th>ΔmA610 (3 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>clear</td>
<td>0</td>
</tr>
<tr>
<td>Hardwood black liquor (150 ul, 12%)</td>
<td>yellow</td>
<td>358</td>
</tr>
<tr>
<td>Lignin, organosolv (20 ug/ml)</td>
<td>clear, yellow</td>
<td>218</td>
</tr>
<tr>
<td>Lignosulfonic acid, (25 ug/ml)</td>
<td>clear</td>
<td>18</td>
</tr>
<tr>
<td>2-Aminopyridine</td>
<td>light brown</td>
<td>2</td>
</tr>
<tr>
<td>N-Benzylidene benzylamine</td>
<td>clear</td>
<td>2</td>
</tr>
</tbody>
</table>

Example 3

Combination of Laccases and Xylanases using N-benzylidene benzylamine to Enhance the Bleaching of Chicago Blue at pH 7.0.

[0100] In the following example, the enzyme enhancing agent N-benzylidene benzylamine was used as a mediator for a laccase to enhance the bleaching of Chicago Blue at pH 7.0 in the presence of a laccase and a xylanase.

[0101] The activity of the xylanase (Pulpzyme HC, Novozymes A/S) is 1000 units per ml enzyme solution. One xylanase unit is defined as the amount of enzyme which, under standard conditions (pH 9.0, 50° C., 30 minutes of incubation), release one micromole of the dye from a dyed RBB xylan.

[0102] N-Benzylidene benzylamine was dissolved in ethanol and then mixed with a phosphate buffer and a Chicago Blue solution. A solution of laccase (NS51003, Novozymes A/S) and xylanase (Pulpzyme HC, Novozymes A/S) was added to make 1 ml of the final solution, containing 20 μM of the mediator, 20 mM buffer at pH 7.0 and Chicago Blue solution, with absorbance at A610 nm between 0.6 to 0.8. After the enzyme was added, the change in the absorbance was measured immediately using a UV spectrophotometer (UV-1201, Shimadzu Scientific Instruments). The decrease in absorbance was recorded after 3 minutes and was used to estimate the results of bleaching. The results are shown below.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Concentration (μM)</th>
<th>Laccase (%)</th>
<th>Xylanase (%)</th>
<th>ΔmA610 (3 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>71</td>
</tr>
<tr>
<td>N-Benzylidene benzylamine</td>
<td>20</td>
<td>0.5</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>N-Benzylidene benzylamine</td>
<td>20</td>
<td>0</td>
<td>0.5</td>
<td>112</td>
</tr>
<tr>
<td>N-Benzylidene benzylamine</td>
<td>20</td>
<td>0.5</td>
<td>0.5</td>
<td>163</td>
</tr>
</tbody>
</table>
[0103] It is to be understood that the above described embodiments are illustrative only and that modification throughout may occur to one skilled in the art. For example, a person of skill in the art will recognize that the mediators of the invention also include mediators which are functionally equivalent to the mediators specifically recited herein, such equivalents having minor structural variations such as the addition of a methyl or ethyl substituent or the formation of a methyl ester from a carboxylic acid. Accordingly, this invention is not to be regarded as limited to the embodiments disclosed herein.

What is claimed is:

1. A composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent Selected from:
   (i) a monomer, dimer, or oligomer of at least one of:

   ![Chemical Structure](image)

   wherein:
   - R₁ is OCH₃, H, or another moiety;
   - R₂ is OCH₃, H, or another moiety;
   - R₃ is OH, sulfonate, or another moiety;
   - R₄ is an OH or methylene that connects to another monomer; and
   - R₅ is SO₂H or H;

   (ii) N-benzylidene benzylamine or a substituted N-benzylidene benzylamine having the structure:

   ![Chemical Structure](image)

   wherein:
   - R₆ is H, OCH₃, NH(CH₃)₂, Cl, Br, an aliphatic group, or an aromatic group; and
   - R₇ is H, OCH₃, NH(CH₃)₂, Cl, Br, an aliphatic group, or an aromatic group;

   (iii) a derivative of cinnamic acid having the structure:

   ![Chemical Structure](image)

   wherein:
   - R₈ is OCH₃, NH₂, Cl, NH(CH₃)₂; and
   - R₉ is OH or H;
   (iv) salicylic acid;
   (v) epigallocatechin gallate;
   (vi) melamine; and
   (vii) 3,4-dihydroxybenzyl alcohol or a substituted 3,4-dihydroxybenzyl alcohol.

2. The composition of claim 1 wherein said enzyme exhibiting laccase activity is selected from a laccase enzyme of enzyme classification EC 1.10.3.2, a catechol oxidase enzyme of enzyme classification EC 1.10.3.1, a monophenol monooxygenase enzyme of enzyme classification EC 1.14.99.1, a bilirubin oxidase enzyme of enzyme classification EC 1.3.3.5, and an ascorbate oxidase enzyme of enzyme classification EC 1.10.3.3.

3. The composition of claim 1 that further comprises a hydrolase.

4. The composition of claim 3 wherein said hydrolase is a xylanase.

5. The composition of claim 1 wherein said monomer, dimer, or oligomer is an organic soluble fraction of lignin or a lignin sulfonate.

6. The composition of claim 1 wherein said derivative of cinnamic acid is selected from:
   - 4-(dimethylamino)cinnamic acid;
   - 3-hydroxy-4-methoxyacinnamic acid;
   - 4-aminoacinnamic acid; and
   - 4-chlorocinnamic acid.

7. A process for oxidizing a substrate, comprising treating the substrate with a composition comprising an enzyme exhibiting laccase activity and an enzyme enhancing agent selected from:

   (i) a monomer, dimer, or oligomer of at least one of:

   ![Chemical Structure](image)

   wherein:
   - R₁ is OCH₃, H, or another moiety;
   - R₂ is OCH₃, H, or another moiety;
R₈ is OH, sulfonate, or another moiety;
R₉ is an OH or methylene that connects to another monomer; and
R₁₀ is SO₃H or H;

(ii) N-benzylidene benzylamine or a substituted N-benzylidene benzylamine having the structure:

\[ \text{wherein:} \]
R₁ is H, OCH₃, NH(CH₃)₂, Cl, Br, an aliphatic group, or an aromatic group; and
R₂ is H, OCH₃, NH(CH₃)₂, Cl, Br, an aliphatic group, or an aromatic group;

(iii) a derivative of cinnamic acid having the structure:

\[ \text{wherein:} \]
R₈ is OCH₃, NH₂, Cl, NH(CH₃)₂; and
R₉ is OH or H;

(vi) salicylic acid;
(vii) 3,4-dihydroxybenzyl alcohol or a substituted 3,4-dihydroxybenzyl alcohol.

8. The process of claim 7 wherein said enzyme exhibiting laccase activity is selected from a laccase enzyme of enzyme classification EC 1.10.3.2, a catechol oxidase enzyme of enzyme classification EC 1.10.3.1, a monophenol monoxygenase enzyme of enzyme classification EC 1.1.99.1, a bilirubin oxidase enzyme of enzyme classification EC 1.3.3.5, and an ascorbate oxidase enzyme of enzyme classification EC 1.10.3.3.

9. The process of claim 7 wherein said enzyme exhibits laccase activity.

10. The process of claim 9 wherein said enzyme is a xylanase.

11. The process of claim 7 wherein said monomer, dimer, or oligomer is an organic soluble fraction of lignin or a lignin sulfonate.

12. The process of claim 7 wherein said derivative of cinnamic acid is selected from: 4-(dimethylamino)cinnamic acid; 3-hydroxy-4-methoxy cinnamic acid; 4-aminocinnamic acid; and 4-chlorocinnamic acid.

13. The process of claim 7 that further comprises adding an oxidizing agent.

14. The process of claim 13 wherein said oxidizing agent is at least one of air, oxygen, and hydrogen peroxide.

15. A process for bleaching a lignin-containing material, comprising treating the material with an enzyme exhibiting laccase activity and an enzyme enhancing agent selected from:

(i) a monomer, dimer, or oligomer of at least one of:

\[ \text{wherein:} \]
R₁ is OCH₃, H, or another moiety;
R₂ is OCH₃, H, or another moiety;
R₇ is OH, sulfonate, or another moiety;
R₈ is an OH or methylene that connects to another monomer; and
R₉ is SO₃H or H;

(ii) N-benzylidene benzylamine or a substituted N-benzylidene benzylamine having the structure:

\[ \text{wherein:} \]
R₈ is OCH₃, NH₂, Cl, NH(CH₃)₂; and
R₉ is OH or H;

(vi) salicylic acid;
(vii) epigallocatechin gallate;
(vii) melamine; and
(vii) 3,4-dihydroxybenzyl alcohol or a substituted 3,4-dihydroxybenzyl alcohol.

14. The process of claim 13 wherein said oxidizing agent is at least one of air, oxygen, and hydrogen peroxide.
(vii) 3,4-dihydroxybenzyl alcohol or a substituted 3,4-dihydroxybenzyl alcohol;
(viii) a hardwood black liquor;
(ix) a hardwood black liquor that comprises (i) said monomer, dimer, or oligomer;
(x) a softwood black liquor; and
(xi) a softwood black liquor that comprises (i) said monomer, dimer, or oligomer.

16. The process of claim 15 wherein said enzyme exhibiting laccase activity is selected from a laccase enzyme of enzyme classification EC 1.10.3.2, a catechol oxidase enzyme of enzyme classification EC 1.10.3.1, a monophenol monoxygenase enzyme of enzyme classification EC 1.14.99.1, a bilirubin oxidase enzyme of enzyme classification EC 1.3.3.5, and an ascorbate oxidase enzyme of enzyme classification EC 1.10.3.3.

17. The process of claim 15 wherein said enzyme exhibiting laccase activity is a xylanase.

18. The process of claim 17 wherein said enzyme exhibiting laccase activity is a xylanase.

19. The process of claim 15 wherein said monomer, dimer, or oligomer is an organic soluble fraction of lignin or a lignin sulfonate.

20. The process of claim 15 wherein said derivative of cinnamic acid is selected from: 4-(dimethylamino)cinnamic acid; 3-hydroxy-4-methoxycinnamic acid; 4-aminocinnamic acid; and 4-chlorocinnamic acid.

21. The process of claim 15 that further comprises adding an oxidizing agent.

22. The process of claim 21 wherein said oxidizing agent is at least one of air, oxygen, and hydrogen peroxide.

23. The process of claim 15 wherein said material is a wood pulp.

24. The process of claim 23 wherein said wood pulp is a raw material used to form a cellulose or a cellulose derivative.

25. A process for enhancing the activity of an enzyme exhibiting laccase activity, comprising adding an enzyme enhancing agent to said enzyme, wherein said enzyme enhancing agent is selected from:

(i) a monomer, dimer, or oligomer of at least one of:

![Chemical structure](image1)

wherein:

- \( R_1 \) is \( \text{OCH}_2 \), \( \text{H} \), or another moiety;
- \( R_2 \) is \( \text{OCH}_2 \), \( \text{H} \), or another moiety;
- \( R_3 \) is \( \text{OH} \), sulfonate, or another moiety;
- \( R_4 \) is an \( \text{OH} \) or methylene that connects to another monomer; and
- \( R_5 \) is \( \text{SO}_2 \text{H} \) or \( \text{H} \);

(ii) N-benzyldiene benzylamine or a substituted N-benzyldiene benzylamine having the structure:

![Chemical structure](image2)

wherein:

- \( R_6 \) is \( \text{H} \), \( \text{OCH}_2 \), \( \text{NH}(\text{CH})_2 \), \( \text{Cl}, \text{Br} \), an aliphatic group, or an aromatic group; and
- \( R_7 \) is \( \text{H} \), \( \text{OCH}_2 \), \( \text{NH}(\text{CH})_2 \), \( \text{Cl}, \text{Br} \), an aliphatic group, or an aromatic group;

(iii) a derivative of cinnamic acid having the structure:

![Chemical structure](image3)

wherein:

- \( R_8 \) is \( \text{OCH}_2 \), \( \text{NH}_2 \), \( \text{Cl}, \text{NH}(\text{CH})_2 \); and
- \( R_9 \) is \( \text{OH} \) or \( \text{H} \);

(vi) salicylic acid;

(v) epigallocatechin gallate;

(vi) melamine;

(vii) 3,4-dihydroxybenzyl alcohol or a substituted 3,4-dihydroxybenzyl alcohol;

(viii) a hardwood black liquor;

(ix) a hardwood black liquor that comprises (i) said monomer, dimer, or oligomer;

(x) a softwood black liquor; and

(xi) a softwood black liquor that comprises (i) said monomer, dimer, or oligomer.

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