Bioprocessing for Sustainable production of COLoured textiles

WP3
Synthesis of Bio-dyes
M4 to M28

Interim Report Meeting - Bruxelles, February 13th, 2012
Acid Dyes

**Phenoxazine Dye - Curie 22**

3-amino-4-hydroxybenzenesulfonic acid

\[ \text{SO}_3\text{H} \]

\[ \text{NH}_2 \]

\[ \text{OH} \]

2

\[ \xrightarrow{\text{laccase oxidation}} \]

3-amino-4-oxo-3H-phenoxazine-8-sulfonic acid

\[ \text{HO}_3\text{S} \]

\[ \text{N} \]

\[ \text{NH}_2 \]

\[ \text{O} \]

---

<table>
<thead>
<tr>
<th>Product</th>
<th>Precursor I</th>
<th>Precursor II</th>
<th>Reaction conditions</th>
<th>Product’s solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>CURIE_22</td>
<td></td>
<td></td>
<td>Laccase ~ 1 U/ml</td>
<td>H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Precursor I (5 mM)</td>
<td>MeOH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1mM tartrate buffer pH. 5</td>
<td>DMSO</td>
</tr>
</tbody>
</table>

Chemical Formula: C₆H₇NO₄S  
Molecular Weight: 189.2  
3-amino-4-hydroxybenzenesulfonic acid
1H 1D NMR spectrum of: precursor (lower trace) and product (upper trace) in (CD$_3$)$_2$SO, $T=300\, \text{K}$

**Chromatographic Analysis**

HPLC analytical: determination of sample purity

LC/ESI/MS: characterization of compound via combination of MS spectroscopic and LC-cromatography

**Spectroscopic Techniques**

Spectrometr c MS: functional groups determination

**Spectrophotometric NMR:** $^{13}\text{C};$ TOCSY; HSQC; HMBC (HSQC long range)

**Spectrophotometric UV-Vis:** kinetic studies on single precursors in presence of laccase have been performed by UV-Vis to test the efficiency of biotransformation.
Red Dye

DABS

pH = 3
-1 mM Na-acetate buffer pH = 3
-Supported Laccase (WET)
-precursor concentration 10 mM
-reaction time 8 h

pH = 6
-1 mM Na-phosphate buffer pH 6.0
-Supported Laccase (WET)
-precursor concentration 10 mM
-reaction time 8 h
La figura mostra un grafico di assorbimento a differenti pH. La lunghezza d’onda massima (λ_max) è indicata per ogni pH:

<table>
<thead>
<tr>
<th>pH</th>
<th>λ_max (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>553</td>
</tr>
<tr>
<td>4</td>
<td>549</td>
</tr>
<tr>
<td>5</td>
<td>480</td>
</tr>
<tr>
<td>6</td>
<td>466</td>
</tr>
<tr>
<td>7</td>
<td>464</td>
</tr>
</tbody>
</table>
(a) pH 3

(b) pH 4

(c) pH 5

(d) pH 6
Michaelis-Menten Kinetics

pH 3

\[ y = 2.4554x - 173.14 \]
\[ R^2 = 0.9987 \]

pH 4

\[ y = 0.9552x + 643.56 \]
\[ R^2 = 0.9960 \]

pH 5

\[ y = 0.4021x + 556.5 \]
\[ R^2 = 0.9793 \]

pH 6

\[ y = 0.2093x + 427.47 \]
\[ R^2 = 0.9958 \]
» constant precursor concentration
» constant laccase activity
» different buffer

» constant precursor concentration
» constant laccase activity

» variable buffer ionic force

» fconstant buffer ionic force
» Constant laccase activity
» variable precursor concentration
NMR structural characterization

\[ \text{NMR structural characterization} \]

\[ \text{SO}_3\text{H} \]

\[ \text{NH}_2 \]

\[ \text{H}_2\text{N} \]

\[ ^{13}\text{C} \ 1\text{D NMR in } \text{D}_2\text{O} \]

\[ \text{1NMR 1D NMR in } \text{D}_2\text{O} \]
Mass Spectrometry

6,6'-{(diaze-1,2-diyl)bis(3-aminobenzenesulphonic acid)}

Diffusion test

<table>
<thead>
<tr>
<th></th>
<th>precursore</th>
<th>prodotto</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>19.0 x 10^{-11}$ m^2$/s</td>
<td>19.3 x 10^{-11}$ m^2$/s</td>
</tr>
</tbody>
</table>

Molecular weight of the molecule 370. Sulphonic groups deprotonated

pH = 3
Blue dye 1

3-methyl-2-benzothiazolinone

DMAB

Bioreactor Synthesis:
- aqueous solution pH 6.5 (1 L solution at 2.5% of EtOH)
- Supported Laccase (WET)
- precursor concentration 5 mM (1:1 molar ratio)
- reaction time 8 h
Blue dye

Kinetic conditions:
- 1 mM Na-acetate buffer pH 4.5
- Laccase solution (1 mM Na-acetate buffer pH 4.5; 0.9 micromolar)
- precursor concentration 0.03 mM (DMAB was dissolved in a buffer solution at 20% ETOH)
- reaction time 1 h (cycle time 120 s)
Bioreactor Synthesis:
- 1 mM Na-acetate buffer pH 3 (1 L of solution at 20% of EtOH)
- Supported Laccase (WET)
- precursor concentration 2.5 mM (ABTS/DMAB ratio 1:10)
- reaction time 5 h
Kinetic conditions:
- 1 mM Na-acetate buffer pH 3
- Laccase solution (1 mM Na-acetate buffer pH 3; 0.5 micromolar)
- precursor concentration 25 micromolar
- reaction time 1 h (cycle time 30 s)
Blue dye

Kinetic conditions:
- 1 mM Na-acetate buffer pH 3
- Laccase solution (1 mM Na-acetate buffer pH 3; 0.5 micromolar)
- precursor concentration 25 micromolar
- reaction time 1 h (cycle time 30 s)
Blue dye
Bioreactor Synthesis:
- 1 mM Na-acetate buffer pH 4.5 (1 L of solution at 30% of EtOH)
- Supported Laccase (WET)
- precursor concentration 10 mM
- reaction time 8 h
Red dye

Synthesis:
- 1 mM Na-acetate buffer pH 3
- Laccase (0.5 micromolar)
- precursors concentration 0.4 mM (buffer solution at 20% EtOH, ratio 1:1)
- reaction time 15 h
Red dye

Acetosyringone:Syringaldehyde (1:1)

Kinetic conditions:
- 1 mM Na-acetate buffer pH 3
- Laccase solution (1 mM Na-acetate buffer pH 3; 0.5 micromolar)
- precursor concentration 0.4 mM (buffer solution at 20% EtOH)
- reaction time 15 h (cycle time 900 s)
Synthesis:
- Acetyl acetate (400 mL)
- Supported laccase (WET)
- precursor concentration 10 mM
- the solution was gently shaken at 35°C for 24 h
Future Activities

Biocatalyzed oxidation of phenolic compounds by Laccase generates under suitable conditions, extensive polymerization

• Synthesis of polymeric dyes for cotton dyeing using natural precursors and also soluble lignosulphonate

• Synthesis in biphasic system to obtain disperse dyes

• Synthesis of reactive dyes introducing specific functional groups by laccase