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Yongqiang Wang
University of Windsor

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OXYGEN KINETICS IN THE LACCASE-CATALYZED REMOVAL OF CRESOLS AND PHENOL FROM WATER

By

Yongqiang Wang

A thesis submitted to the Faculty of Graduate Studies and Research through Civil and Environmental Engineering in partial fulfillment of the requirements for the Degree of Master of Applied Science at the University of Windsor

Windsor, Ontario, Canada
March, 2001

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ABSTRACT

Cresols and phenol are used widely in industry. They are toxic, colorless solids or liquids which can be absorbed through skin, eyes and respiratory mucosa and attack the central nervous system, respiratory system, liver, kidneys, skin and eyes. It is necessary to treat wastewater which contains cresols and phenol.

Laccase, a biochemical catalyst, catalyzes the oxidation of cresols and phenol to produce polymers which are easily removed from water. The previous research shows that almost 90% of cresols and phenol can be removed from water after 3 hours of treatment.

The aim of this study was to observe the kinetic behavior of the co-substrate oxygen in the laccase-catalyzed reaction of cresols and phenol. Under optimum pH, enzyme concentration, and with different concentrations of oxygen, cresol and phenol, the consumption rates of oxygen were recorded at fixed temperature and studied to find out the effects of oxygen in the reaction. Two kinds of laccase were tested: laccase SP850 and laccase SP504, both developmental preparations of an enzyme producer.

It was found that, with increasing cresol and phenol concentrations, initial oxygen consumption velocities increased for both laccase SP850- and laccase SP504-catalyzed reactions. However, oxygen consumption velocities did not always keep increasing with increasing oxygen concentrations for both laccase SP850 and laccase SP504. For laccase SP850, different cresols had different behaviors with varying oxygen concentrations. The initial oxygen consumption velocities were optimal at oxygen concentrations below 8.0 mg/L for m- and p-cresol. But for o-cresol, the initial oxygen consumption kept increasing with increase in oxygen concentration. The reaction between phenol and
oxygen was slow compared to the reaction between cresols and oxygen. The initial oxygen consumption velocities at different concentrations of phenol and oxygen were almost the same.

With laccase SP504, a bell-shaped curve was observed in all plots between initial oxygen consumption velocity and oxygen concentration. The maximum velocities occurred when the oxygen concentration was between 3.5 mg/L and 6 mg/L. The enzyme activity was inhibited when oxygen concentration was above 6 mg/L in the reaction between both cresols and oxygen and phenol and oxygen.
DEDICATION

I dedicate this thesis to my parents, Tinghua Wang and Gengfu Wang, for the support they have always given to me.
ACKNOWLEDGEMENTS

Sincere thanks to my advisors and committee members, Dr. J.K. Bewtra, Dr. K.E. Taylor, and Dr. N. Biswas for their guidance, support and constructive suggestions throughout the course of this research. I am grateful for the time they have taken to actively participate in my efforts. The careful and meticulous reading that the thesis received is very much appreciated.

My thanks are extended to my fellow graduate students, Amy B. Vermette and Ram Mantha, for their direction and suggestions in the laboratory. Thanks also go to Mr. Bill Henderson for his technical help in the laboratory.
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<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARP</td>
<td><em>Arthromyces ramosus</em> peroxidase</td>
</tr>
<tr>
<td>CMP</td>
<td><em>Coprinus macrorhizus</em> peroxidase</td>
</tr>
<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
</tr>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency of United States</td>
</tr>
<tr>
<td>$h$</td>
<td>Hill constant</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>$K_M$</td>
<td>Michaelis constant, $[S]<em>o$ at which $v_o = V</em>{max}/2$</td>
</tr>
<tr>
<td>$K_{mA}$</td>
<td>Michaelis constants for substrate A</td>
</tr>
<tr>
<td>$K_{mB}$</td>
<td>Michaelis constants for substrate B</td>
</tr>
<tr>
<td>$K_{iA}$</td>
<td>equilibrium dissociation constant of the EA complex</td>
</tr>
<tr>
<td>$K^{app}_{m}$</td>
<td>apparent Michaelis constant</td>
</tr>
<tr>
<td>NSC</td>
<td>National Safety Council</td>
</tr>
<tr>
<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>SBP</td>
<td>soybean peroxidase</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet standard test</td>
</tr>
<tr>
<td>$v_o$</td>
<td>initial reaction rate</td>
</tr>
<tr>
<td>$V^{app}_{max}$</td>
<td>apparent limiting initial velocity or maximum velocity at saturation</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>limiting initial velocity or maximum velocity at saturation</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>$[S]_o$</td>
<td>initial concentration of substrate</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Cresols and phenol are very harmful contaminants in industrial wastewaters. They are aromatic compounds which are used for insecticides, plastics and medical products. In 1999, 2.07 million tons of phenol was produced in the U.S and it is estimated that the phenol production would reach 2.41 million tons in 2003 (NSC, 2000). Since they are toxic substances, their removal from industrial wastewaters is necessary to reach the effluent standards established by different government agencies. Traditional treatment methods normally have high cost and low removal efficiencies. Therefore, research is being conducted in different research institutions to find better treatment methods.

Enzyme-based technology, among others, is being investigated to develop a new approach for cresols and phenol removal from wastewater.

1.1 CRESOLS AND PHENOL

Cresols are widely used in industrial production. They are isomeric phenols with a methyl substituent at the ortho-, meta-, or para- position relative to the hydroxyl group. Cresols are by-products of crude oil and coal tars and in coal gasification (Vermette, 2000). They are highly caustic, colorless solids or liquids with a sweet tarry odor. Repeated or prolonged exposure to low concentrations of cresols can produce chronic systemic poisoning. Symptoms of poisoning include vomiting, difficulty in swallowing, diarrhea, loss of appetite, headache, fainting, dizziness, mental disturbance and skin rash.

Phenol is used as a slimicide (a chemical that kills bacteria and fungi found in watery slimes) and a disinfectant as well as for medical and plastic products. The exposure to phenol has serious detrimental effects which increase as both the level and length of
exposure increase. For example, repeated exposure to low levels of phenol in drinking water has been linked with diarrhea and mouth sores in humans and eating very large amounts of phenol has resulted in death (EPA, 2000). The acceptable levels for cresol and phenols in effluent discharges are 22 mg/m$^3$ and 20 mg/m$^3$ respectively, based on their toxicity (EPA, 1999).

1.2 ENZYMES

A catalyst is a molecule which increases the rate of a reaction but is not the reactant or product of that reaction. An enzyme is a protein molecule which acts as a catalyst in biochemical reactions. It is a selective catalyst with very high molecular mass. Enzymes are proteins made up of amino acid units joined in series. They have affinity for the substrate in a transition state geometry, thereby distorting substrate into a conformation which will lead to the breakdown into products. The part of the enzyme that carries out reaction is called the active site where the catalyzed reaction occurs. Enzymes have wide applications in biochemical production, DNA detection and environmental engineering (Malmstrom, 1970).

Laccases are a class of enzymes that show promise for wastewater treatment, thus warranting further investigation, which is the focus of this study. Laccases catalyze the reaction between selected aromatic compounds, such as cresols and phenol, and oxygen. The selected aromatic compounds are oxidized and polymerized. These polymers then can be removed from wastewater.

1.3 APPLICATION OF ENZYMES IN WASTEWATER TREATMENT
Because of their high capacity as catalysts, enzymes have been applied in many different areas. In recent years, several studies have been conducted on wastewater treatment by using different enzymes. Enzymes are used widely in the treatment of aromatic compounds (Claus, et al., 1990), activated sludge digestion, underground water pollution control (Bollag, et al., 1980), dye industry wastewater treatment (Davis et al., 1992) and so on.

Finding suitable, low-cost, high efficiency enzymes for treating specific kinds of industrial wastewater or other wastes is of great interest to environmental scientists and engineers and was the reason for conducting this research.

1.4 PREVIOUS RESEARCH WORK

Since 1980, horseradish peroxidase (HRP), Coprinus macrorhizus peroxidase (CMP), Arthromyces ramosus peroxidase (ARP) and soybean peroxidase (SBP) have been studied to find out the effectiveness in the treatment of wastewater containing phenolic compounds (Klibanov et al., 1980, 1981; Al-Kassim et al., 1993, 1994; McEldoon et al., 1995; Taylor et al., 1996, 1998). In recent years, laccase has been used for removal of cresol and phenol from wastewater. In this University, Vermette (2000) and Zhao (2000) have shown that laccase SP850 and laccase SP504 can achieve more than 90% removal of cresols and phenol after 3 hours of batch reaction. The present study is the continuation of their research.

1.5 OBJECTIVE

The objectives of this study were to:
(i) determine the behavior and rate kinetics for oxygen in the laccase-catalyzed reaction with different cresols and phenol, and
(ii) to establish relationships between the concentrations of oxygen, cresol and phenol, laccase and time at room temperature.

1.6 SCOPE

The scope of this study, conducted at room temperature, included:

- M-cresol, o-cresol, p-cresol and phenol as aromatic compounds.
- Different concentrations of aromatic compounds.
- Laccase SP850 and laccase SP504 as enzymes.
- Varying concentrations of oxygen, both above and below saturation levels.
- Varying Laccase activities (ie.-concentrations).
2. LITERATURE REVIEW

2.1 CRESOLS AND PHENOL AS POLLUTANTS

Cresol is a highly caustic, colorless solid or liquid with a sweet tarry odor which is used mainly as a disinfectant. Phenol is a colorless or white solid when it is pure. It has a strong odor that is sickeningly sweet and irritating. It evaporates more slowly than water and dissolves fairly well in water. Phenol is flammable (EPA, 1999).

2.1.1 PROPERTIES OF CRESOLS AND PHENOL

There are three forms of cresols that are only slightly different in their chemical structure: ortho-cresol (o-cresol), meta-cresol (m-cresol), and para-cresol (p-cresol). The chemical formula for cresol is C₆H₄(OH)CH₃ and that for phenol is C₆H₅(OH).

Table 2.1 shows some physical and chemical properties of cresols and phenol.

<table>
<thead>
<tr>
<th>Property</th>
<th>Phenol</th>
<th>o-cresol</th>
<th>m-cresol</th>
<th>p-cresol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass (g/mol)</td>
<td>94.1</td>
<td>108</td>
<td>108</td>
<td>108</td>
</tr>
<tr>
<td>Air odor threshold (ppm)</td>
<td>0.04</td>
<td>1.4</td>
<td>0.007</td>
<td>0.004</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>43.0</td>
<td>30.9</td>
<td>12.2</td>
<td>34.7</td>
</tr>
<tr>
<td>Boiling point at 1 atm (°C)</td>
<td>181.7</td>
<td>191.0</td>
<td>202.3</td>
<td>201.9</td>
</tr>
<tr>
<td>Vapor pressure at 25°C (mmHg)</td>
<td>0.41</td>
<td>0.31</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Relative density at 25°C (kg/m³)</td>
<td>1070</td>
<td>1135</td>
<td>1030</td>
<td>1154</td>
</tr>
<tr>
<td>Solubility in water at 25°C (g/L)</td>
<td>27.1</td>
<td>25.95</td>
<td>22.70</td>
<td>21.52</td>
</tr>
<tr>
<td>pKₐ (25°C)</td>
<td>10.35</td>
<td>10.29</td>
<td>10.09</td>
<td>10.26</td>
</tr>
<tr>
<td>Saturation concentration in air at 20°C (g/m³)</td>
<td>0.18</td>
<td>1.2</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>
2.1.2 USES AND SOURCES OF THE SELECTED COMPOUNDS

Cresols are found in many foods and in wood and tobacco smoke, crude oil, coal tar, and in brown mixtures such as creosote and cresylic acids, which are wood preservatives. Organisms in soil and water produce cresols when they break down materials in the environment (EPA, 1999). Phenol is mainly a man-made chemical, although it is found in nature in animal wastes and organic material (EPA, 1999).

Uses of cresols are (EPA, 1999):

- as solvents for other chemicals,
- as disinfectants and deodorizers,
- in synthesis of plastics, resins and pesticides, and
- as pharmaceutical raw materials.

Uses of phenol are (EPA, 1999):

- production of phenolic resins, which are used in the plywood, construction, metal casting, automotive, and appliance industries,
- production of caprolactam and bisphenol A, which are intermediates in the manufacture of nylon and epoxy resins, respectively, and
- as a biocide, a disinfectant, and in medicinal products such as ear and nose drops, throat lozenges, and mouthwashes.

2.1.3 LEVEL OF EXPOSURE

The following adverse health effects of cresols and phenol present in the environment have been reported in the literature (EPA, 1999):
• Breathing high levels of cresols for a short time results in irritation of the nose and throat. Aside from these effects, very little is known about the effects of breathing cresols, for example, at lower levels over longer times.

• Ingesting high levels of cresols in drinking water or food results in kidney problems, mouth and throat burns, abdominal pain, vomiting, and effects on the blood and nervous system.

• Skin contact with high levels of cresols can burn the skin and damage the kidneys, liver, blood, brain, and lungs.

• Small amounts of phenol put on the skin of animals for short times can cause blisters and burns on the exposed area.

• Both cresols and phenol are suspected as carcinogenic substances in long term exposure no matter whether present in water or in air.

• The discharged industrial wastewaters which contain cresols and phenol can cause ecological disaster.

The Occupational Safety and Health Administration (OSHA) has set an exposure limit of 22 milligrams per cubic meter (22 mg/m³) for cresols in the workplace air for an 8-hour workday, 40-hour workweek. OSHA advises avoiding eye and skin contact because this may be a route of significant exposure. Similarly, OSHA has set an air exposure limit of 60 mg/m³ in 15 min. and 20 mg/m³ in 8 hours for phenol. The Ministry of Environment, Ontario, required phenols in the wastewater to be treated to as low as 20 μg/L before its discharge (Environment Ontario, 1988). The provincial water quality objectives of Ontario for surface water requires phenols not to exceed 1 μg/L in order to protect against tainting of edible fish flesh (Ministry of Environment, Ontario, 1984).
2.2 CONVENTIONAL AND ADVANCED TREATMENTS OF WASTE-WATER CONTAINING AROMATIC COMPOUNDS

Water can be used to dilute and to transport unwanted materials from the location at which they are generated to some distant place where their adverse effects will not be so unpleasantly evident. However, with industrial development and global population increase, it is not possible to discharge wastewaters without treatment. Aromatic compounds are commonly present in many different industrial wastewaters since these compounds are usually used as raw materials in different industrial operations.

Conventional treatments of aromatic compounds are:

a. Biological treatment (Geldreich et al, 1999)

Biological treatment is used most widely for its low cost and stable operation. It uses bacteria or other micro-organisms to degrade bio-degradable organic compounds.

b. Adsorption (Snoeyink et al, 1999)

Adsorption is generally used to describe the accumulation of a particular component at an interface between phases. There are different interfaces, for example solid/gas or solid/liquid interfaces. Commonly, activated carbon is chosen as adsorbent for its excellent adsorbing capacity.

c. Chemical reaction (Singer et al, 1999)

Through different chemical reactions, aromatic compounds can be transformed to bio-degradable compounds or completely destroyed. For example, incineration can completely transform aromatic compounds into carbon dioxide. Air-oxidation can break the cyclic structure of aromatic compounds and make it easy for further treatment.

However, all these conventional treatments have certain shortcomings when they are applied for treating wastewater which contains aromatic compounds. The basic
structure of aromatic chemicals is a cyclic ring of six carbon atoms. This structure is very stable in chemical reaction, especially, cresols and phenol are very toxic. The toxicity of cresols and phenol can make biological treatment less efficient. Cresols and phenol do not show good behavior in adsorption treatment. Furthermore, activated carbon adsorption can be quite expensive. Air-oxidation requires a lot of energy for high reaction temperature. Cresols and phenol do not react easily with oxygen under normal circumstances.

These disadvantages have resulted in attempts to explore new technology to treat these pollutants. In recent years, the following advanced technologies have been studied for removing aromatic contaminants:

- Removal of chlorinated phenols from aqueous solutions by adsorption on alumina pillared clays and mesoporous alumina aluminum phosphates (Danis et al., 1998).
- Development of a novel process for recovery of phenol from alkaline wastewater (Dutta et al., 1998).
- Anaerobic treatment of high COD loaded Split Flow Wastewater (Minke, et al., 1999).

2.3 ENZYMATIC TREATMENT OF WASTEWATER

The principle of enzymatic treatment of wastewater is to use isolated enzymes, as distinct from micro-organisms, to catalyze the conversion of target substances to removable products. For proper application, this reaction should be rapid, consume low energy and enzyme should remain stable under these reactions.
Compared to traditional treatment approaches, enzyme technology has many advantages (Vermette, 2000):

- operation under milder, less corrosive conditions,
- effective at both low or very high concentration levels,
- reduced consumption of oxidants,
- reduced amounts of adsorbent material required for disposal,
- action on, or in the presence of, many substrates which are toxic to microbes,
- no shock loading effects, and
- better defined systems with simpler process control.

Some typical applications of using different enzymes for different waste removal and listed below:

- Horseradish peroxidase was applied to dephenolize coal-conversion wastewater. A typical coal-gasification wastewater was investigated. The removal efficiency was 97% (Klibanov et al., 1983).
- Immobilised laccase is being studied on a research scale for the removal of phenolic compounds from must and wine (Brenna et al., 1994).
- After being covalently immobilised to activated carbon, laccase has been used to remove color from industrial effluents (Davis et al., 1992).

Therefore, treating wastewater by enzymes is a promising technology and can provide a flexible treatment method. It can be applied in different areas, can be effective over a large concentration range and can lead to recovery of some wastes into products with a low operational cost. Since it is a new technology, further studies have to be carried out to develop possible applications and to determine the necessary operation parameters.
2.4 ENZYME KINETICS

2.4.1 THE MICHAELIS-MENTEN EQUATION

In 1913, Michaelis and Menten proposed the following equation for this mechanism (Cornish-Bowden, 1979):

\[ E + S \rightleftharpoons ES \rightarrow E + P \quad \text{Eq 2.1} \]

where S, P, E mean substrate, product and enzyme, respectively.

By controlling pH, temperature and measuring initial rates, they derived the following relationship:

\[ v_o = \frac{V_{\text{max}} [S]_0}{K_M + [S]_0} \quad \text{Eq. 2.2} \]

Where \( v_o \) = initial reaction rate

\( V_{\text{max}} \) = limiting initial velocity or maximum velocity at saturation

\( [S]_0 \) = initial concentration of substrate

\( K_M \) = Michaelis constant, \([S]_0\) at which \( v_o = \frac{V_{\text{max}}}{2} \)

Initial rates are used in this characterization to avoid any complications due to a constantly diminishing substrate concentration during a reaction and to avoid any influence of accumulating product.

The Michaelis-Menten equation is a fundamental tool for studying enzyme kinetics. Today, this equation is also derived using the steady-state assumption instead of equilibrium assumption, which was originally used by Michaelis and Menten. The present study of oxygen kinetics is based on approaches which are defined by the Michaelis-Menten equation.
The curve defining Eq. 2.2 is shown in Figure 2.4.2.1. It is a rectangular hyperbola through the origin. With the increase in substrate concentration, the reaction velocity increases very rapidly at the beginning. As the concentration of substrate increases, the rate of change in velocity slows down and \( v_o \) approaches \( V_{\text{max}} \) asymptotically, which is called the saturation situation. It is desirable to conduct the assay of catalytic activity under this condition. In this case, consumption of substrate or accumulation of product is linear in time (zero-order in all substrates) and first-order in enzyme concentration. The Y-coordinate represents reaction rate and X-coordinate represents the initial concentration of substrate. According to this equation, \( K_m \) can be read on X-coordinate when \( v_o = \frac{1}{2} V_{\text{max}} \).

Figure 2.4.2.1 Curve defining Michaelis-Menten equation
2.4.2 MICHAELIS-MENTEN EQUATION APPLIED TO TWO- SUBSTRATE REACTIONS

Normally, there are two kinds of mechanisms in enzyme-catalyzed two-substrate reactions: (1) Double-displacement or ping pong mechanism, in which the first product is produced and released from the enzyme before the second substrate is bound, (2) Single-displacement or ternary-complex mechanisms, in which the reaction passes through a state with both substrates bound simultaneously to the enzyme (Cornish-Bowden, 1979).

The following different forms of the Michaelis-Menten equations have been proposed for these two mechanisms without considering inhibition (Cornish-Bowden, 1979):

Ping pong mechanism:

\[
\nu_o = \frac{V_{\text{max}} [A] [B]}{K_{mB}[A] + K_{mA} [B] + [A] [B]} \quad \text{Eq. 2.3}
\]

where: \(K_{mB}, K_{mA}\) = Michaelis constants for substrates A and B respectively

\(\nu_o = \text{initial reaction rate}\)

\(V_{\text{max}} = \text{limiting initial velocity or maximum velocity at saturation.}\)

For ternary-complex mechanism:

\[
\nu_o = \frac{V_{\text{max}} [A] [B]}{K_{mB} + K_{mA} [B] + [A] [B]} \quad \text{Eq. 2.4}
\]

Where: \(K_{mB}, K_{mA}\) = Michaelis constants for substrates A and B respectively

\(\nu_o = \text{initial reaction rate}\)
\[ V_{\text{max}} = \text{limiting initial velocity or maximum velocity at saturation} \]

\[ K_{iA} = \text{equilibrium dissociation constant of the EA complex} \]

If the concentration of one substrate is held constant at a given pH and temperature, the above equations become similar to the one substrate Michaelis-Menten equation:

\[ v_o = \frac{V_{\text{app max}}^\text{max} \cdot [S]_o}{K_{\text{app m}} + [S]_o} \]

Eq.2.5

The \( V_{\text{app max}}^\text{app} \) and \( K_{\text{app m}}^\text{app} \) values are called apparent values as long as the concentration of one substrate is held constant. Subsequently, by changing the concentration of the fixed substrate, a matrix of apparent values of \( V_{\text{app max}}^\text{app} \) and \( K_{\text{app m}}^\text{app} \) can be obtained. The procedure is as follows: First, by plotting \([A]_v / [A] \) vs \([A] \) at fixed \([B] \), \( 1/V_{\text{app}} \) is obtained from the slope for a number of \([B] \), and then from plots of \([B] / V_{\text{app}} \) vs \([B] \), the necessary parameters of Eq.2.3 and Eq.2.4 are calculated. These plots also show the nature of the mechanism followed by the enzyme catalyzed reaction: ping-pong or ternary-complex mechanism.

Figures 2.2 to 2.4 show the nature of plots used for deriving these parameters.
Fig. 2.2: Primary plots for a ternary-complex mechanism

Fig. 2.3: Primary plots for the substituted-enzyme mechanism

Fig. 2.4: Secondary plots for ternary-complex mechanisms. It also applies to substituted-enzyme mechanisms
2.4.3 SIGMOID KINETICS

Certain enzyme-catalyzed reactions do not agree well with the Michaelis-Menten equation. Figure 2.4.5.1 shows a curve which is quite different from the Michaelis-Menten curve. This type of curve shows that enzymes obey co-operativity kinetics if no experimental errors exist. Co-operativity is a phenomenon reflecting the equilibrium binding of substrates where the binding of one molecule of a substrate to an enzyme can either facilitate (positive co-operativity) or hinder (negative co-operativity) the binding of subsequent molecules of the same substrate. (Eisenthal and Danson, 1992). Most co-operative enzymes also exhibit allosteric behaviour.

In the case of a co-operative enzyme reaction, the corresponding initial velocity equation can be written as (Cornish-Bowden, 1979):

\[
v_o = \frac{V_{\text{max}} [S]_o^h}{K_M + [S]^h}
\]

Eq.2.5

Where \(v_o\) = reaction rate

\(V_{\text{max}}\) = limiting initial velocity or maximum velocity at saturation

\([S]_o\) = initial concentration of substrate

\(K_M\) = Michaelis constant

\(h\) = Hill constant.

The Hill constant can be used to check the co-operativity of the enzyme catalyzed reaction:

if \(h>1\) it is a positive co-operativity;

if \(h=1\) there no co-operativity and the equation is Michaelis-Menten equation;

and if \(h<1\) it is a negative co-operativity.
2.5 ENZYME STABILITY

Many enzymes will lose activity if the temperature is over 40°C. Therefore, enzymes should be stored in a refrigerator if not in use. Most enzymes have their optimum pH range between 5 and 9 for catalyzing reaction. Heavy metals, cyanide and chlorine are toxic to many enzymes. Enzyme will also be changed irreversibly under high concentrations of certain organic solvents such as ethanol or acetone (Eisenthal and Danson, 1992). All these factors should be taken into consideration while storing and using enzymes.

2.6 LACCASE AS CATALYST

Before laccase was studied as a catalyst for wastewater treatment, several other enzymes were tested with different aromatic compounds. Kilbanov et al. (1980) first proposed a treatment method by using horseradish peroxidase (HRP) for removal of over 30 different phenols and aromatic amines from water with the highest removal efficiency
reaching 99%. Other peroxidases, such as *Coprinus macrohizus* peroxidase (CMP), *Arthromyces ramosus* peroxidase (ARP) and soybean peroxidase (SBP) have been proven to be very effective in removing several different phenolic compounds from water. (Al-Kassim *et al.*, 1993, 1994; Wu, 1993; McEldoon *et al.*, 1995; Taylor *et al.*, 1996, 1998; Ibrahim, 1998; Biswas, 1999).

Recently, laccase has been investigated because of its cheapness and effectiveness. Laccases belong to the group of copper-containing oxidases which are widely distributed in plants and microorganisms; normally it is present in the fruit bodies of many fungi. The molecular mass of laccase is from 64,000 to 140,000 (Malmstrom, 1970).

Laccase has more than 1 copper center which are involved in catalysis. The detailed mechanism of laccase catalyzed removal of cresols and phenol is unknown. It is believed that aromatic compounds are oxidized by laccase to the corresponding phenoxy radicals. Then these radicals couple with each other to form various dimers and subsequently form insoluble polymers and can be removed from the wastewater stream. The assumed reaction strategy is as follows (Dec *et al.*, 1994; Taylor *et al.*, 1998; Bollag *et al.*, 1979):

\[
\text{Phenol (or cresols) + Laccase + O}_2 \rightarrow \text{Phenol O}^- (\text{or Cresol O}^-) + \text{H}_2\text{O}
\]

\[
\text{Phenol O}^- (\text{or Cresol O}^-) + \text{Phenol O}^- (\text{or Cresol O}^-) \rightarrow \text{Dimers}
\]

\[
\text{Dimers + Laccase + O}_2 \rightarrow \text{Higher oligomers}
\]

Dec and Bollag (1994, 1995) showed that laccase from the fungus *Rhizoctonia praticola* and horseradish peroxidase were effective in polymerizing several phenols producing a mixture of oligomers with average molecular masses of up to 800 for the fraction soluble in dioxane. Vermette (2000) has shown that laccase SP850 could remove
90% of cresols. Zhao (2000) observed that laccase SP504 could reach 95% removal of cresols and phenol with a much lower concentration than laccase SP850. Laccase may prove to be the most useful of the oxidoreductases because they produce very reactive radicals and because, unlike peroxidases, they do not require the presence of hydrogen peroxide (Vermette, 2000).

Until now, most of the research on enzyme-catalyzed transformation of pollutants had focused on the disappearance of the pollutant from solution (Taylor et al., 1996, 1998). As such, the process design or performance criteria were developed based on the removal efficiency. The present research focuses on understanding the kinetic behavior of one of the reactants, oxygen. Through the study of oxygen consumption rate, the process design or performance criteria can be developed.
3. MATERIAL AND METHODS

3.1 MATERIALS

Both laccase SP 850 (Batch No. US Enz-142) and laccase SP504 (EC 1.10.3.2US-1999-00091) were provided by Novo Nordisk, Franklinton, North Carolina. The activity of SP504 was 200 LACU/mL and that of SP 850 was 1200 LAMU/mL. A laccase unit of activity (LACU for laccase SP504 and LAMU for laccase SP850) catalyzes the conversion of 1 µmol of syringaldazine per minute in a 19 µM solution at pH 7.5 for laccase SP850 and pH 5.5 for laccase SP504 at a temperature of 30 °C. Specific detailed information is given in appendix A.

Polyethylene glycol (average molecular mass of 3350 g/mol) was purchased from Sigma Chemical Co., St. Louis, Missouri. Hydrogen peroxide (30% mass/volume) was purchased from BDH Inc., Toronto, Ontario. A 50mM hydrogen peroxide stock solution was prepared and stored at 4 °C. The buffers were prepared according to the method of Gomori (1955). Two kinds of buffer solution were used in study: acetate buffer and phosphate buffer. The pH range of acetate buffer is from 4.4 to 5.6 and the pH range of phosphate buffer is from 5.7 to 8.0.

Phenol and m-, o-, p-cresols were purchased from Aldrich Chemical Inc., Milwaukee, Wisconsin. 1mM stock solutions of phenol and cresols were prepared and stored at room temperature. 1 mM cresols is equivalent to 108 mg/L and 1 mM phenol is equivalent to 94 mg/L.

3.2 EQUIPMENT
The pH of the samples was measured by using an Expandable Ion Analyzer, Model EA 940, manufactured by Orion Research, Ohio. The standard buffer solutions of pH 4.0, 7.0 and 10.0 were provided by BDH Inc. Toronto, Ontario. Dissolved oxygen in the reactors was measured in Biological Oxygen Monitor, YSI Model 5300, purchased from YSI Co. Inc., Yellow Springs, Ohio. The oxygen cylinder was provided by Praxair Products Inc., Mississauga, Ontario.

The concentrations of m-, o-cresols and phenol were determined by colorimetric absorbance measured with a Hewlett Packard, Model 8452A, Diode Array Spectrophotometer, obtained from Hewlett Packard Co., Mississauga, Ontario. The wavelength range was from 190 to 820 nm with a 2 nm resolution. The spectrophotometer was controlled by a Hewlett Packard Vectra ES/12 computer. Polystyrene disposable semi-micro cuvettes were provided by Bio-Rad Laboratories, Hercules, California. The UV method was used to measure the concentration of p-cresol by using quartz cuvettes which were provided by Bio-Rad Laboratories, Hercules, California.

3.3 EXPERIMENTAL PROCEDURE

3.3.1 pH AND TEMPERATURE CONTROL

According to the results from previous research, the optimum pH value for laccase SP850 catalyzed reaction was 6.0-8.0 and that for laccase SP504 catalyzed reaction was 5.0-6.0 (Vermette, 2000, Zhao, 2000). All reactions were carried out within these pH ranges and at room temperature controlled within 22 ± 2 °C.
3.3.2 LACCASE CONCENTRATION

Laccase concentration is represented in units of catalytic activity per mL (U/mL). All the reactions were carried out in 15 mL volume reactors. The laccase concentration used was the minimum concentration required to reach optimum oxygen consumption rate. The laccase provider, Novo Nordisk, also provided the protocols for laccase activity test at 30°C. In this study, all laccase concentrations, except in laccase activity test, were calculated through:

Laccase concentration = nominal laccase activity at 30°C/ dilution factors

Table 3.1 shows the concentrations added for different substrates and enzymes. These are based on previous studies conducted with these substrates (Vermette, 2000; Zhao, 2000).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>m-cresol</th>
<th>p-cresol</th>
<th>o-cresol</th>
<th>phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase</td>
<td>SP850</td>
<td>16</td>
<td>2</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Laccase</td>
<td>SP504</td>
<td>0.0067</td>
<td>0.0067</td>
<td>0.0067</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

* LAMU/mL for SP 850 and LACU/mL for SP 504

3.3.3 INITIAL DISSOLVED OXYGEN CONCENTRATIONS

The initial rate of oxygen consumption was determined at different oxygen concentrations. The range of dissolved oxygen was varied from 1 mg/L to 30 mg/L. The following approaches were tried to obtain a range of dissolved oxygen concentration:
a. For DO concentrations less than the air-saturated DO concentration, 8.8 mg/L at 20 °C, sodium sulfite (Na₂SO₃), in the presence of cobalt catalyst, was used to reduce the DO according to the following reaction formula:

\[
\text{CoCl}_2 + \text{Na}_2\text{SO}_3 + \text{O}_2 \rightarrow \text{Na}_2\text{SO}_4
\]

The amount of CoCl₂ catalyst added was 1% based on mass of Na₂SO₃.

b. For DO concentrations greater than air-saturated DO concentration, two different approaches were tried. First, hydrogen peroxide was added. However, H₂O₂ reacted with laccase and produced many tiny bubbles which upset the reaction system. Next, pure oxygen was bubbled into water for several hours until the desired DO was obtained. The highest DO concentration reached was 40 mg/L.

3.3.4 EXPERIMENTAL PROCEDURE

This study was designed to observe the oxygen kinetics under different concentrations of substrates while maintaining optimum pH and laccase concentration. The research was conducted as batch reactions in sealed 15 mL reactors. The reactor was stirred continuously with a magnetic stirrer. Cresols or phenol and buffers were mixed with deionized water and laccase was added last with a syringe. The reaction lasted from 5 min. to 27 min. depending on DO concentration. Table 3.2 shows the reaction conditions for laccase SP850 catalyzed reaction and Table 3.3 shows the reaction conditions for laccase SP504 catalyzed reaction.
### Table 3.2 Conditions for laccase SP850 catalyzed reaction

<table>
<thead>
<tr>
<th>Item</th>
<th>Range of values</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-, o-, p-Cresol</td>
<td>1 mM, 2 mM, 3 mM, 5 mM, 10 mM, 20 mM</td>
<td>All other parameters were kept constant when the concentration of cresol was changed</td>
</tr>
<tr>
<td>Phenol</td>
<td>Concentration: 2 mM</td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>1, 2, 2.7, 4, 5, 6, 8.8, 12, 15, 20, 25, 30, 40 mg/L</td>
<td>All other parameters were kept constant when the concentration of DO was changed</td>
</tr>
<tr>
<td>Laccase SP850</td>
<td>Constant</td>
<td>Table 3.1 give value for each substrate</td>
</tr>
<tr>
<td>Phosphate Buffer</td>
<td>2250 uL</td>
<td>pH value was controlled between 6.0 ~ 8.0</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td>Room temperature, 22±2°C</td>
</tr>
<tr>
<td>pH</td>
<td>6.0 ~ 8.0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3.3 Conditions for laccase SP504 catalyzed reaction

<table>
<thead>
<tr>
<th>Item</th>
<th>Range of Value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-, o-, p-Cresol</td>
<td>2 mM</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>2 mM</td>
<td></td>
</tr>
<tr>
<td>Laccase SP504</td>
<td>Constant</td>
<td>Table 3.1 give value for each substrate</td>
</tr>
<tr>
<td>Oxygen</td>
<td>1.0, 2.0, 2.7, 4.0, 5.0, 6.0, 8.8, 12.0, 15.0, 20.0, 25.0, 30.0, 40.0 mg/L</td>
<td>All other parameters were kept constant when the concentration of DO was changed</td>
</tr>
<tr>
<td>Actate and Phosphate Buffers</td>
<td>2250 uL</td>
<td>pH value was controlled between 5.0 ~ 6.0</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td>Room temperature 22 ± 2°C</td>
</tr>
<tr>
<td>pH</td>
<td>5.0 ~ 6.0</td>
<td></td>
</tr>
</tbody>
</table>
3.4 ANALYTICAL METHODS

3.4.1 ASSAY OF LACCASE ACTIVITY

Laccase activity assay was carried out to confirm the laccase activity assay results from Novo Nordisk. It was determined by testing the capacity of laccase to catalyze the oxidation of syringaldazine to corresponding quinone. The assay was carried out in a cuvette containing 1 mL solution. The mixture of solution consisted of:

- 850 µL Tris buffer with pH = 7.5 or 850 µL MES buffer with pH = 5.5
- 50 µL 0.38 mM syringaldazine solution
- 100 µL enzyme solution

The products of reaction produced a purple colored solution. The absorbance at a peak wavelength of 530 nm was proportional to the amount of products. The detailed procedure of this method is given in Appendix A.

3.4.2 CHEMICAL TITRATION OF DISSOLVED OXYGEN CONCENTRATION

Chemical titration for dissolved oxygen was carried out both for probe calibration and confirmation of experimental results. Standard methods, Azide Modification (Standard Methods, 1995), was used in this research. Under strong alkaline conditions, dissolved oxygen can oxidize divalent manganous hydroxide rapidly and produce hydroxides of higher valency manganese. In the presence of iodide ions and upon acidification, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content in the sample. The iodine is then titrated with a standard solution of thiosulfate. The detailed procedure of the method is given in Appendix B.

The procedure for calibration of the probe was as follows:
• Bubbled deionized water with compressed air for over 48 hours to reach saturation level.

• Stopped bubbling and kept deionized water open to the air for 24 hours to achieve steady-state DO.

• Titrated 250 mL of the water by using the Azide Modification Method. This DO concentration was used to calibrate the probe of monitor by setting the value of probe equal to 100%. This meant that the 100% value of probe equaled to the saturated DO concentration at that temperature.

The following procedure was used to check the accuracy of experimental results.

• A series of laccase-catalyzed cresols and phenol reactions with oxygen were carried out and the probe was used to determine the remaining DO at different times.

• At a certain time, reaction was stopped by adding strong alkali.

• The DO concentration was analyzed by the Azide Modification Method and compared with the probe values.

• This procedure was repeated several times for different reactions at different DO values.

Figure 3.4.2 shows the comparison of the two sets of values. The line of best fit clearly shows that the probe was responding properly and correctly to the change in DO concentration during these experiments.
3.4.3 CRESOLS AND PHENOL CONCENTRATION ASSAY

The m-, o-cresol and phenol concentrations were determined by a colorimetric method and p-cresol concentration was determined by a UV method. The colorimetric method used ferricyanide and 4-aminoantipyrine (AAP) reagents to react with cresols or phenol to produce color with absorbance at a peak wavelength of 510 nm. The concentration of p-cresol was tested by direct spectrophotometric method which was based on the absorbance of ultraviolet (UV) light by p-cresol. The assay mixture was placed in a quartz cuvette and the absorbance was measured at 276 nm. The detailed procedures of the two methods are given in Appendices C.
3.5 SOURCES OF ERROR

All experiments were repeated to avoid random errors. The calibration curves were prepared at least at three different times. However, some systematic errors may have occurred due to instrument, analytical methods and personal errors. Other possible sources of error were:

- chemical reagent contamination,
- loss of laccase activity due to improper storage, and
- Lack of probe response to the concentration of DO on time.

In order to minimize these errors, following efforts were made:

- Checked chemical reagent purity before its using.
- Stored enzyme properly and checked laccase activity before its using.
- Changed membrane every two weeks and calibrated probe after changing membrane.
4. RESULTS AND DISCUSSION

4.1 OXYGEN KINETICS FOR LACCASE SP850-CATALYZED REMOVAL OF m,o,p-CRESOL AND PHENOL

The behavior of oxygen consumption during laccase SP850 catalyzed removal of m, o, p-cresol appeared relatively more complex. The reactions were carried out with concentrations of oxygen around 2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L, 12 mg/L, 16 mg/L, and 20 mg/L while cresols concentrations were kept at 1 mM, 2 mM, 3 mM, 5 mM, 10 mM, and 20 mM. The reactions with different concentrations of cresols and oxygen formed a matrix. Under the optimized conditions for pH and enzyme concentration, generally the initial oxygen consumption velocity was not the maximum velocity. Also, for different types of cresols, oxygen consumption velocities were different as discussed below.

4.1.1 OXYGEN KINETIC BEHAVIOR OF REACTION BETWEEN m-CRESOL AND OXYGEN UNDER CATALYSIS BY LACCASE SP850

Figures 4.1.1.1 to 4.1.1.6 show oxygen reactions with m-cresol at different times under different conditions. The curves of best fit were drawn by using the polynomial equations given in the legends of these figures. The initial oxygen consumption velocity was determined from the first derivative of this equation at time zero. Figure 4.1.1.7 shows the change in initial oxygen consumption velocity with the concentration of m-cresol, whereas Figure 4.1.1.8 shows initial oxygen consumption velocity changing with the initial concentration of oxygen. It is observed that when the concentration of oxygen was below saturation, 8.8 mg/L at 20 °C, the oxygen consumption rate either remained
Figure 4.1.1.1 Progress curves of 1mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = 9.0 \times 10^{-4} x^2 - 0.20x + 2.1 \] (O₂=2.1 mg/L; (●));
\[ y = 4.0 \times 10^{-4} x^2 - 0.16x + 3.5 \] (O₂=3.5 mg/L; (■));
\[ y = 2.0 \times 10^{-4} x^2 - 0.16x + 5.3 \] (O₂=5.3 mg/L; (▲));
\[ y = -1.1 \times 10^{-3} x^2 - 0.058x + 7.3 \] (O₂=7.3 mg/L; (×));
\[ y = 2.0 \times 10^{-4} x^2 - 0.057x + 12 \] (O₂=11.9 mg/L; (★));
\[ y = 1.2 \times 10^{-3} x^2 - 0.028x + 19 \] (O₂=18.5 mg/L; (●)).
Figure 4.1.1.2 Progress curves of 2 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various $O_2$ concentrations. Lines shown are second-order polynomial fits of the data between $y = O_2$ concentration and $x =$ time and the equations are:

- $y = -5.3 \times 10^{-3} x^2 - 0.25x + 2.1 \ (O_2=2.1 \text{ mg/L}; (\text{♦}))$;
- $y = -2.1 \times 10^{-3} x^2 - 0.24x + 3.8 \ (O_2=3.8 \text{ mg/L}; (\square))$;
- $y = -2.1 \times 10^{-3} x^2 - 0.23x + 6.1 \ (O_2=6.1 \text{ mg/L}; (\triangle))$;
- $y = -2.3 \times 10^{-3} x^2 - 0.11x + 7.6 \ (O_2=7.5 \text{ mg/L}; (\times))$;
- $y = -1.5 \times 10^{-3} x^2 - 0.079x + 12 \ (O_2=12.0 \text{ mg/L}; (\ast))$;
- $y = -1.4 \times 10^{-3} x^2 - 0.076x + 20 \ (O_2=20.1 \text{ mg/L}; (\bullet))$. 

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Figure 4.1.1.3 Progress curves of 3 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between \( y = O₂ \) concentration and \( x = \) time and the equations are:

\[
\begin{align*}
  y &= -6.3 \times 10^{-3} x^2 - 0.37x + 2.0 \ (O₂=2.0 \text{ mg/L}; \ (\bullet)); \\
  y &= -1.7 \times 10^{-3} x^2 - 0.41x + 3.1 \ (O₂=3.1 \text{ mg/L}; \ (■)); \\
  y &= -1.5 \times 10^{-3} x^2 - 0.63x + 6.0 \ (O₂=6.2 \text{ mg/L}; \ (×)); \\
  y &= -1.4 \times 10^{-3} x^2 - 0.15x + 12 \ (O₂=12.0 \text{ mg/L}; \ (∗)); \\
  y &= -1.4 \times 10^{-3} x^2 - 0.076x + 20 \ (O₂=20.2 \text{ mg/L}; \ (●))
\end{align*}
\]
Figure 4.1.1.4a Progress curves of 5mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -5.6 \times 10^3 x^2 - 0.92x + 2.3 \quad (O_2 = 2.2 \text{ mg/L}; \quad \bullet) ; \]

\[ y = -3.3 \times 10^3 x^2 - 0.43x + 3.9 \quad (O_2 = 3.9 \text{ mg/L}; \quad \square) ; \]

\[ y = -4.6 \times 10^3 x^2 - 0.69x + 6.5 \quad (O_2 = 6.3 \text{ mg/L}; \quad \times) . \]
Figure 4.1.1.4.b Progress curves of 5mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
  y &= -2.0 \times 10^{-3} x^2 - 0.17x + 11 \quad (O_2=11.4 \text{ mg/L}; \text{(*)}); \\
  y &= -4.3 \times 10^{-3} x^2 - 0.15x + 20 \quad (O_2=19.5 \text{ mg/L}; \text{(*)}).
\end{align*}
\]
Figure 4.1.1.5a Progress curves of 10 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O$_2$ concentrations. Lines shown are second-order polynomial fits of the data between $y = O_2$ concentration and $x =$ time and the equations are:

$$y = -7.0 \times 10^{-1} x^2 - 2.4x + 2.0 \ (O_2=2.0 \text{ mg/L}; (\bullet));$$

$$y = -2.5 \times 10^{-1} x^2 - 2.7x + 4.2 \ (O_2=3.9 \text{ mg/L}; (\blacksquare));$$
Figure 4.1.1.5b Progress curves of 10 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
y = -1.0 \times 10^{-1} x^2 + 1.4x + 6.9 \quad (O₂ = 6.6 \text{ mg/L}; \, (*));
\]
\[
y = -3.5 \times 10^{-2} x^2 - 1.2x + 8.9 \quad (O₂ = 8.8 \text{ mg/L}; \, (\diamondsuit));
\]
\[
y = -1.2 \times 10^{-1} x^2 - 0.97x + 12 \quad (O₂ = 11.5 \text{ mg/L}; \, (\bullet)).
\]
Figure 4.1.1.6 Progress curves of 20 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O$_2$ concentrations. Lines shown are second-order polynomial fits of the data between y = O$_2$ concentration and x = time and the equations are:

\[ y = 2.4 \times 10^{-1} x^2 - 2.1x + 3.1 \quad (O_2=3.0 \text{ mg/L}; (\star)) ; \]
\[ y = 1.1 \times 10^{-1} x^2 - 2.0x + 5.5 \quad (O_2=5.5 \text{ mg/L}; (\bullet)) ; \]
\[ y = 9.5 \times 10^{-2} x^2 - 2.3x + 6.3 \quad (O_2=6.3 \text{ mg/L}; (\bullet)) ; \]
\[ y = 2.8 \times 10^{-2} x^2 - 1.8x + 7.4 \quad (O_2=7.3 \text{ mg/L}; (\blacktriangle)) ; \]
\[ y = 3.4 \times 10^{-2} x^2 - 1.2x + 9.0 \quad (O_2=8.8 \text{ mg/L}; (\blacktriangledown)) \]
\[ y = 8.6 \times 10^{-2} x^2 - 0.57x + 16 \quad (O_2=15.8 \text{ mg/L}; (\triangle)) \]
Figure 4.1.1.7 Relationship between the concentration of m-cresol and initial oxygen consumption velocity at different initial oxygen concentrations.

Figure 4.1.1.8 Relationship between oxygen concentration and initial oxygen consumption velocity at different concentrations of m-cresol.
constant or decreased with time. Generally, the initial oxygen consumption velocity decreased when the initial oxygen concentration was increased; however this decrease became quite significant when the initial oxygen was above saturation.

Initial oxygen consumption velocity kept increasing with an increase in initial m-cresol concentration, when the initial oxygen concentration was kept constant. The experimental results show that m-cresol did not inhibit the activity of laccase SP850 since the reaction velocity always increased with an increase in m-cresol concentration. However, laccase SP850 activity was inhibited significantly by oxygen when the concentration of oxygen was increased above 6.5 mg/L.
4.1.2 OXYGEN KINETIC BEHAVIOR OF REACTION BETWEEN o-CRESOL AND OXYGEN UNDER THE CATALYSIS BY LACCASE SP850

Figures 4.1.2.1 to 4.1.2.6 show the change in oxygen concentration with time for different o-cresol concentrations when started at different initial oxygen concentrations. Figure 4.1.2.7 shows the changes in initial oxygen consumption velocity with the concentration of o-cresol, whereas Figure 4.1.2.8 shows the change in initial oxygen consumption velocity with the concentration of oxygen. It is observed that the oxygen consumption velocity had always increased with time at all o-cresol and oxygen concentrations. Also, the initial oxygen consumption velocity always speeded up with an increase in initial o-cresol and oxygen concentrations.

The parameters of the Michaelis-Menten equation for initial oxygen consumption velocity, Eq.2.3, were obtained through the following steps:

- Primary plot:

Rewritten Michaelis-Menten, Eq. 2.5 is rearranged, to allow the results to be plotted on a straight line:

\[
[A]_o / v_o = K_{\text{app}}/V_{\text{app}} + \frac{1}{V_{\text{app}}} [A]_o
\]

Eq. 4.1

Plotted \([A]_o / v_o\) against \([A]_o\) at a certain \([B]_o\) in Figure 4.1.2.9, where \([A]_o\) is the initial concentration of oxygen, \(v_o\) is initial oxygen consumption velocity and \([B]_o\) is the initial concentration of o-cresol. At different values of \([B]_o\), a set of best-fit lines was drawn as shown in the figure. The slopes of these lines is the inverse value of \(V_{\text{app}}\) at different values of \([B]_o\). It also shows that all lines intersect at a common point on the \([A]_o / v_o\) axis. This means
Figure 4.1.2.1 Progress curves of 1 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -5.4 \times 10^{-2} x^2 - 0.18x + 2.6 \ (O_2=2.6 \text{ mg/L}; \ (\bullet)) ; \]
\[ y = -3.7 \times 10^{-2} x^2 - 0.19x + 3.7 \ (O_2=3.7 \text{ mg/L}; \ (■)) ; \]
\[ y = -2.4 \times 10^{-2} x^2 - 0.31x + 5.2 \ (O_2=5.1 \text{ mg/L}; \ (▲)) ; \]
\[ y = -5.3 \times 10^{-3} x^2 - 0.12x + 7.6 \ (O_2=7.5 \text{ mg/L}; \ (×)) ; \]
\[ y = -1.8 \times 10^{-3} x^2 - 0.17x + 12.0 \ (O_2=12.1 \text{ mg/L}; \ (★)) ; \]
\[ y = -5.2 \times 10^{-3} x^2 - 0.28x + 17.0 \ (O_2=16.4 \text{ mg/L}; \ (●)). \]
Figure 4.1.2.2 Progress curves of 2 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -1.4 \times 10^{-1} x^2 + 0.13x + 1.9 \text{ (O₂=1.9 mg/L; (♦))}; \]
\[ y = -1.4 \times 10^{-1} x^2 - 0.23x + 3.6 \text{ (O₂=3.6 mg/L; (□))}; \]
\[ y = -3.8 \times 10^{-2} x^2 - 0.47x + 5.2 \text{ (O₂=5.0 mg/L; (△))}; \]
\[ y = -9.8 \times 10^{-3} x^2 - 0.18x + 8.5 \text{ (O₂=8.3 mg/L; (×))}; \]
\[ y = -1.7 \times 10^{-2} x^2 + 0.42x + 12.0 \text{ (O₂=11.3 mg/L; (★))}; \]
\[ y = -4.5 \times 10^{-3} x^2 - 0.47x + 16.0 \text{ (O₂=15.2 mg/L; (●))}. \]
Figure 4.1.2.3 Progress curves of 3 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between $y = O₂$ concentration and $x =$ time and the equations are:

\[
\begin{align*}
  y &= -6.7 \times 10^{-2} x^2 - 0.38x + 2.6 \ (O₂=2.5 \text{ mg/L}; \ (\bullet)); \\
  y &= -6.3 \times 10^{-2} x^2 - 0.64x + 3.5 \ (O₂=3.4 \text{ mg/L}; \ (■)); \\
  y &= -6.8 \times 10^{-2} x^2 - 0.70x + 6.5 \ (O₂=6.4 \text{ mg/L}; \ (\times)); \\
  y &= -4.4 \times 10^{-2} x^2 - 0.51x + 11 \ (O₂=11.3 \text{ mg/L}; \ (\ast)); \\
  y &= -1.5 \times 10^{-2} x^2 - 0.28x + 24 \ (O₂=23.9 \text{ mg/L}; \ (●)).
\end{align*}
\]
Figure 4.1.2.4a Progress curves of 5 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
y &= -5.2 \times 10^{-2} x^2 - 0.46x + 2.4 \text{ (O₂=2.4 mg/L; (○))}; \\
y &= -6.2 \times 10^{-2} x^2 - 0.68x + 4.3 \text{ (O₂=4.3 mg/L; (■))}; \\
y &= -1.4 \times 10^{-1} x^2 - 0.32x + 5.7 \text{ (O₂=5.7 mg/L; (×))}.
\end{align*}
\]
Figure 4.1.2.4.b Progress curves of 5 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -1.0 \times 10^{-4} x^2 - 0.60x + 12 \text{ (O}_2 = 11.8 \text{ mg/L; (⋆))}; \]
\[ y = -4.3 \times 10^{-2} x^2 - 0.66x + 23 \text{ (O}_2 = 22.7 \text{ mg/L; (●))}. \]
Figure 4.1.2.5 Progress curves of 10 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
  y &= -1.5 \times 10^{-1} x^2 - 0.45x + 2.8 \quad (O₂=2.8 \text{ mg/L}; \; (\Diamond)); \\
  y &= -3.1 \times 10^{-1} x^2 - 0.50x + 4.5 \quad (O₂=4.4 \text{ mg/L}; \; (\blacksquare)); \\
  y &= -1.7 \times 10^{-1} x^2 - 0.83x + 6.2 \quad (O₂=6.1 \text{ mg/L}; \; (\triangle)); \\
  y &= -2.9 \times 10^{-1} x^2 - 0.92x + 8.6 \quad (O₂=8.7 \text{ mg/L}; \; (\times)); \\
  y &= -3.5 \times 10^{-1} x^2 - 0.70x + 13 \quad (O₂=12.4 \text{ mg/L}; \; (\star)); \\
  y &= -1.2 \times 10^{-1} x^2 - 0.96x + 24 \quad (O₂=23.1 \text{ mg/L}; \; (\bullet)).
\end{align*}
\]
Figure 4.1.2.6 Progress curves of 20 mM o-cresol reacting with 4 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations

Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -5.7 \times 10^{-1} x^2 - 0.46x + 1.7 \quad (O_2=1.7 \text{ mg/L}; (⋆)) \]
\[ y = -5.5 \times 10^{-1} x^2 - 0.77x + 4.1 \quad (O_2=4.0 \text{ mg/L}; (○)) \]
\[ y = -6.0 \times 10^{-1} x^2 - 0.83x + 6.0 \quad (O_2=5.9 \text{ mg/L}; (△)) \]
\[ y = -3.1 \times 10^{-1} x^2 - 0.83x + 8.5 \quad (O_2=8.3 \text{ mg/L}; (□)) \]
\[ y = -5.9 \times 10^{-1} x^2 - 1.5x + 13 \quad (O_2=12.6 \text{ mg/L}; (×)) \]
\[ y = -5.8 \times 10^{-1} x^2 - 1.62x + 20 \quad (O_2=19.9 \text{ mg/L}; (+)) \]
Figure 4.1.2.7 Relationship between the concentration of o-cresol and oxygen consumption velocity at different initial oxygen concentrations

Figure 4.1.2.8 Relationship between oxygen consumption velocity and concentration of oxygen at different concentration of o-cresol
Figure 4.1.2.9 Relationship between oxygen consumption velocity and concentration of oxygen at different concentration of o-cresol.

Lines shown are linear fits of the data between $y = \text{O}_2$ concentration/initial $\text{O}_2$ consumption velocity and $x = \text{O}_2$ shown equations are:

\[
\begin{align*}
  y &= 102.54x + 4.63 \ (\text{o-cresol}=1 \ \text{mM}; (\bigstar)) \\
  y &= 63.90 \ x + 3.57 \ (\text{o-cresol}=2 \ \text{mM}; (\blacksquare)) \\
  y &= 51.05 \ x + 4.15 \ (\text{o-cresol}=3 \ \text{mM}; (\Delta)) \\
  y &= 44.14 \ x + 3.57 \ (\text{o-cresol}=5 \ \text{mM}; (\times)) \\
  y &= 32.34 \ x + 3.80 \ (\text{o-cresol}=10 \ \text{mM}; (\ast)) \\
  y &= 19.43 \ x + 3.12 \ (\text{o-cresol}=20 \ \text{mM}; (\bigcirc)).
\end{align*}
\]
Figure 4.1.2.10 Secondary plot for ping-pong mechanisms of o-cresol. Line shown is a linear fit of the data between $y = O_2$ concentration/$V_{app}$ and $x = o$-cresol concentration and the equation is:

$$y = 18.65x + 107.50$$
that the mechanism of the reaction between o-cresol and oxygen obeyed Ping-Pong mechanism.

- Plotted secondary plot:

  When $[A]_o$ is constant, eq.2.3 is rewritten as follows:

  \[
  \frac{[B]_o}{V^{app}_{max}} = \frac{K_{mB}}{V_{max}} + \frac{V_{max}}{[B]_o} \quad \text{Eq.4.2}
  \]

  \[
  K^{app}_{max} = K_{mA} [B]_o (K_{mB}^{+} [B]_o) \quad \text{Eq.4.3}
  \]

  \[
  \frac{V^{app}_{max}}{K^{app}_{max}} = \frac{V_{max}}{K_{mA}} \quad \text{Eq.4.4}
  \]

  Plotted $[B]/V^{app}_{max}$ against $[B]$ in Figure 4.1.2.10, and the values for $K_{mB}$, $V_{max}$ and $K_{mA}$ were obtained through Eq. 4.2, Eq. 4.3 and Eq.4.4. From these equations and plots, it is estimated that $V_{max} = 0.054 \pm 0.016 \text{ mM/min}$, $K_{mA} = 0.025 \pm 0.0075 \text{ mM}$ for oxygen and $K_{mB} = 5.77 \pm 1.33 \text{ mM}$ for o-cresol, $R^2 = 0.950$. 

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4.1.3 OXYGEN KINETIC BEHAVIOR OF REACTION BETWEEN p-CRESOL AND OXYGEN UNDER THE CATALYSIS BY LACCASE SP850

Figures 4.1.3.1 to 4.1.3.6 show the change in oxygen concentration with time for different p-cresol concentrations when started at different initial oxygen concentrations. Figure 4.1.3.7 shows the change in initial oxygen consumption velocity with the concentration of p-cresol, whereas Figure 4.1.3.8 shows the change in initial oxygen consumption velocity with the initial concentration of oxygen. It is observed that the oxygen consumption velocity had always speeded up with time no matter at what p-cresol and oxygen concentrations the reaction was carried out. Also, as in other case, initial oxygen consumption velocity had increased with an increase in p-cresol concentrations. However, the change with the increase in the concentration of oxygen was random. So the reaction between p-cresol and oxygen under catalysis by laccase SP850 did not follow any kinetic model and no related kinetic parameters could be derived.
Figure 4.1.3.1 Progress curves of 1 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
y = -1.6 \times 10^{1} x^2 - 0.098x + 2.2 \quad (O₂ = 2.2 \text{ mg/L}; \, (●));
\]

\[
y = -8.5 \times 10^{-2} x^2 - 0.30x + 3.7 \quad (O₂ = 3.6 \text{ mg/L}; \, (■));
\]

\[
y = -1.1 \times 10^{-1} x^2 - 0.19x + 5.4 \quad (O₂ = 5.4 \text{ mg/L}; \, (▲));
\]

\[
y = -2.2 \times 10^{-2} x^2 - 0.11x + 7.7 \quad (O₂ = 7.7 \text{ mg/L}; \, (×));
\]

\[
y = -4.4 \times 10^{-2} x^2 - 0.25x + 11 \quad (O₂ = 11.1 \text{ mg/L}; \, (★));
\]

\[
y = -4.7 \times 10^{-2} x^2 - 0.039x + 15 \quad (O₂ = 14.5 \text{ mg/L}; \, (+));
\]

\[
y = -6.6 \times 10^{-2} x^2 - 0.17x + 22 \quad (O₂ = 21.9 \text{ mg/L}; \, (●)).
\]
Figure 4.1.3.2 Progress curves of 2 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -4.3 \times 10^{-2} x^2 - 0.40x + 2.3 \ (O_2=2.2 \text{ mg/L}; \ (\Diamond)) ; \]
\[ y = -8.2 \times 10^{-2} x^2 - 0.48x + 4.4 \ (O_2=4.2 \text{ mg/L}; \ (■)) ; \]
\[ y = -2.6 \times 10^{-1} x^2 - 0.32x + 5.5 \ (O_2=5.5 \text{ mg/L}; \ (△)) ; \]
\[ y = -1.7 \times 10^{-2} x^2 - 0.35x + 7.6 \ (O_2=7.4 \text{ mg/L}; \ (×)) ; \]
\[ y = -6.3 \times 10^{-2} x^2 - 0.27x + 11.0 \ (O_2=11.2 \text{ mg/L}; \ (★)) ; \]
\[ y = -7.8 \times 10^{-2} x^2 - 0.18x + 15.0 \ (O_2=15.3 \text{ mg/L}; \ (+)) ; \]
\[ y = -8.3 \times 10^{-2} x^2 - 0.41x + 22.0 \ (O_2=20.5 \text{ mg/L}; \ (●)). \]
Figure 4.1.3.3 Progress curves of 3 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

- \( y = -1.3 \times 10^{-2} x^2 - 0.027x + 2.3 \) (O₂ = 2.3 mg/L; (+));
- \( y = -3.9 \times 10^{-1} x^2 - 0.17x + 3.8 \) (O₂ = 3.8 mg/L; (△));
- \( y = -9.8 \times 10^{-2} x^2 - 0.62x + 5.5 \) (O₂ = 5.4 mg/L; (●));
- \( y = -3.0 \times 10^{-2} x^2 - 0.51x + 9.7 \) (O₂ = 9.2 mg/L; (■));
- \( y = -7.3 \times 10^{-2} x^2 - 0.33x + 12.5 \) (O₂ = 12.2 mg/L; (×));
- \( y = -8.5 \times 10^{-2} x^2 - 0.19x + 17.0 \) (O₂ = 17.0 mg/L; (★));
- \( y = -8.8 \times 10^{-2} x^2 - 0.41x + 22.0 \) (O₂ = 20.5 mg/L; (●)).
Figure 4.1.3.4 Progress curves of 5 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
    y &= -1.4 \times 10^{-1} x^2 - 0.43x + 1.8 \ (O_2=1.7 \text{ mg/L}; \ (+)) ; \\
    y &= -2.7 \times 10^{-1} x^2 - 0.39x + 4.0 \ (O_2=3.9 \text{ mg/L}; \ (\bullet)) ; \\
    y &= -2.6 \times 10^{-1} x^2 - 0.80x + 5.8 \ (O_2=5.7 \text{ mg/L}; \ (\times)) ; \\
    y &= -1.5 \times 10^{-1} x^2 - 0.024x + 8.6 \ (O_2=8.6 \text{ mg/L}; \ (\triangle)) ; \\
    y &= -1.5 \times 10^{-1} x^2 - 0.34x + 12.5 \ (O_2=12.4 \text{ mg/L}; \ (\times)) ; \\
    y &= -5.8 \times 10^{-2} x^2 - 0.87x + 17.0 \ (O_2=16.7 \text{ mg/L}; \ (\blacklozenge)) ; \\
    y &= -2.6 \times 10^{-2} x^2 - 0.42x + 20.0 \ (O_2=20.4 \text{ mg/L}; \ (\blacklozenge)).
\end{align*}
\]
Figure 4.1.3.5 Progress curves of 10 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations.

Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = -2.4 \times 10^{-1} x^2 - 0.52 \times 2.1 \ (O_2=2.1 \text{ mg/L}; \ (\bullet)); \]
\[ y = -1.1 \times 10^{-1} x^2 - 1.2 \times 3.5 \ (O_2=3.4 \text{ mg/L}; \ (\ast)); \]
\[ y = -2.9 \times 10^{-1} x^2 - 1.2 \times 6.3 \ (O_2=6.1 \text{ mg/L}; \ (\times)); \]
\[ y = -2.9 \times 10^{-1} x^2 - 0.68 \times 12.4 \ (O_2=12.3 \text{ mg/L}; \ (\triangle)); \]
\[ y = -5.8 \times 10^{-1} x^2 - 0.60 \times 15.7 \ (O_2=15.6 \text{ mg/L}; \ (\square)); \]
\[ y = -6.5 \times 10^{-2} x^2 - 0.088x + 20.3 \ (O_2=20.3 \text{ mg/L}; \ (\Diamond)). \]
Figure 4.1.3.6 Progress curves of 20 mM p-cresol reacting with 2 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
y &= -3.5 \times 10^{-1} x^2 - 0.70x + 1.9 \quad (O_2 = 1.9 \text{ mg/L}; (*)); \\
y &= -1.3 x^2 - 0.66x + 4.4 \quad (O_2 = 4.4 \text{ mg/L}; (\bullet)); \\
y &= -7.5 \times 10^{-1} x^2 - 1.3 x + 5.9 \quad (O_2 = 5.8 \text{ mg/L}; (\diamond)); \\
y &= -3.3 \times 10^{-1} x^2 - 0.74x + 8.5 \quad (O_2 = 8.8 \text{ mg/L}; (\mathbf{m})); \\
y &= -5.8 \times 10^{-1} x^2 - 0.60x + 15.7 \quad (O_2 = 15.7 \text{ mg/L}; (\times)); \\
y &= -8.5 \times 10^{-2} x^2 - 0.72x + 17.9 \quad (O_2 = 17.9 \text{ mg/L}; (\triangle)).
\end{align*}
\]
Figure 4.1.3.7 The relationship between the concentration of p-cresol and initial oxygen consumption velocity
Figure 4.1.3.8 The relationship between initial oxygen consumption velocity and concentrations of oxygen at different concentrations of p-cresol
4.1.4 OXYGEN KINETIC BEHAVIOR OF REACTION BETWEEN PHENOL CRESOL AND OXYGEN UNDER THE CATALYSIS BY LACCASE SP850

The concentration of phenol used in these experiments was 2 mM. Figure 4.1.4.1 shows the change in oxygen concentration with time under different initial concentrations of oxygen. Figure 4.1.4.2 shows initial oxygen consumption velocity at different initial concentration of oxygen. These figures show that Laccase SP850 did not work well in the reaction between phenol and oxygen and initial oxygen concentrations had no effect on the reaction rate. Also, the oxygen consumption velocity was very low as compared to the velocities for cresols, even when 2-8 times more enzyme was used for phenol than for cresols. So the kinetic analysis could not be carried out because no relationship could be set up among initial oxygen consumption velocity, oxygen concentration and enzyme concentration.
Figure 4.1.4.1 Progress curves of 2 mM phenol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
y &= 4.0 \times 10^{-4} x^2 - 0.046x + 2.9 \quad (O_2 = 3.0 \text{ mg/L}; (■)); \\
y &= 2.0 \times 10^{-4} x^2 - 0.045x + 4.0 \quad (O_2 = 4.1 \text{ mg/L}; ( ● )); \\
y &= 3.0 \times 10^{-4} x^2 - 0.038x + 4.8 \quad (O_2 = 4.8 \text{ mg/L}; ( × )); \\
y &= 7.0 \times 10^{-5} x^2 - 0.036x + 6.3 \quad (O_2 = 6.3 \text{ mg/L}; ( ▲ )); \\
y &= 3.0 \times 10^{-4} x^2 - 0.042x + 7.3 \quad (O_2 = 7.4 \text{ mg/L}; ( ● )); \\
y &= 2.0 \times 10^{-4} x^2 - 0.050x + 8.1 \quad (O_2 = 8.1 \text{ mg/L}; ( * ));
\end{align*}
\]
Figure 4.1.4.2 The relationship between initial oxygen consumption velocity and initial concentration of oxygen at 2mM concentration of o-cresol
4.1.5 GENERAL DISCUSSION OF REACTIONS BETWEEN CRESOLS AND OXYGEN UNDER CATALYSIS BY LACCASE SP850

Generally, the initial reaction velocity either remained constant or decreased with time. However, in several reactions between cresols and oxygen, the reaction velocity kept increasing. The location of the methyl functional group (CH$_3$) on the benzene ring and the concentration of oxygen showed significant effects on this phenomenon. In the reactions between m-cresol and oxygen, such increase was not observed at low oxygen concentrations and even at high oxygen concentrations, above 8.8 mg/L, acceleration was not significant. However, in the reactions between o- and p-cresols and oxygen, the acceleration was very obvious, especially when the oxygen concentration was below the aqueous saturation level. The following factors were considered to be responsible for this behavior:

(a) Slow detector response

A lag phase may have resulted if the initial response of the detection system was too slow. In order to check it, chemical titration was done simultaneously. After comparing the two sets of results, it was concluded that this was not the cause.

(b) Slow dissociation of a reversible inhibitor

Some enzymes may show significant time-dependence in their rates of dissociation of enzyme and inhibitor and thus may have caused acceleration in reaction.

(c) Pre-steady-state transient

The mechanism of cresols reacting with oxygen seems to be complex and it is not completely understood. It is possible that there was a transient state which did not disappear quickly as in normal situation.
(d) Activation by product

A progress curve of this nature may be observed if one of the products in the reaction behaved as an activator.

Further studies are necessary to understand the reason for this acceleration.

The Michaelis-Menten equation for two substrates by the ping-pong mechanism, eq.2.3, was applied as a kinetic model for o-cresol. The oxygen kinetic behavior in the reaction between oxygen and m-, p-cresol and phenol were irregular for setting up kinetic models.

4.2 OXYGEN KINETICS IN LACCASE SP504-CATALYZED REMOVAL OF m-, o-, p-CRESOL AND PHENOL

4.2.1 INHIBITION OF LACCASE SP504 ACTIVITY DUE TO DISSOLVED OXYGEN

Figure 4.2.1.1 to Figure 4.2.1.4 show the relationship between the concentration of oxygen and reaction velocity. All experiments were conducted in duplicate. In all the reactions between cresols and phenol with oxygen at different dissolved oxygen concentrations, there existed an optimum oxygen concentration range at which peak velocity was observed. Table 4.2.1.1 shows these optimum values and corresponding maximum oxygen consumption velocities corresponding to Figure 4.2.1.1 to Figure 4.2.1.4. Below the optimum range, the oxygen consumption velocity either decreased with the decrease in the concentration of cresols or phenol and oxygen or, in some instances, the oxygen consumption velocity remained constant (i.e. - no acceleration in a progress curve). However, in all reactions between cresols, phenol and oxygen, the
Figure 4.2.1.1 The relationship between initial oxygen consumption velocity and concentration of oxygen at concentration of 2 mM m-cresol under catalysis of 0.0067 LACU/mL laccase SP504

Figure 4.2.1.2 The relationship between initial oxygen consumption velocity and concentration of oxygen at concentration of 2 mM o-cresol under catalysis of 0.0067 LACU/mL laccase SP504
Figure 4.2.1.3 The relationship between initial oxygen consumption velocity and concentration of oxygen at concentration of 2 mM p-cresol under catalysis of 0.0067 LACU/mL laccase SP504
Figure 4.2.1.4 The relationship between initial oxygen consumption velocity and concentration of oxygen at concentration of 2 mM phenol under catalysis of 0.0067 LACU/mL laccase SP504
initial velocity slowed down significantly when the concentration of oxygen was increased above the optimum range. Obviously, the activity of laccase SP 504 was inhibited when the oxygen concentration was present beyond the optimum range. Based on these observations, all further experiments were conducted at oxygen concentrations up to optimum range in order to determine the nature of the kinetic equation. Direct computer analysis was applied to set up kinetic models for cresols and phenol. The data used in computer analysis was an average of experimental data.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>m-cresol</th>
<th>o-cresol</th>
<th>p-cresol</th>
<th>phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optimum of oxygen concentration</strong></td>
<td>4.0-5.0</td>
<td>3.5-5.0</td>
<td>4.0-5.0</td>
<td>5-6</td>
</tr>
<tr>
<td><strong>Maximum initial velocity</strong></td>
<td>2.5</td>
<td>0.80</td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Table 4.2.1.1 Optimum oxygen concentrations for the maximum initial oxygen consumption velocity with different substrates with laccase SP504.**

4.2.2 OXYGEN KINETICS IN LACCASE SP504-CATALYZED REMOVAL OF m-CRESOL

Figures 4.2.2.1a to 4.2.2.1b show oxygen reactions with m-cresol at different times when the concentration of m-cresol was 2 mM. The reaction between m-cresol and oxygen, catalyzed by laccase SP504 at low oxygen concentrations, showed cooperativity. This is clearly indicated in Figure 4.2.2.1c which shows that the reaction velocity increased with an increase in oxygen concentration. Instead of a hyperbolic curve, which obeys the Michaelis-Menten equation, the reaction between m-cresol and oxygen, catalyzed by laccase SP504 shows a sigmoid curve. The
Hill equation, Eq.2.4, was applied as a model which may explain cooperativity. Computer analysis was used to set up this model. Figure 4.2.2.2 shows the result of computer analysis. It shows $V_{\text{max}} = 0.080 \pm 0.008$ mM/min., $K_m = 0.090 \pm 0.004$ mM, $h = 2.7 \pm 0.29$, $R^2 = 0.951$. 
Figure 4.2.2.1a Progress curves of 2 mM m-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
\begin{align*}
    y &= 2.5 \times 10^{-2}x^2 - 0.30x + 0.81 \quad (O₂ = 0.8 \text{ mg/L}; (\_\_)); \\
    y &= 5.4 \times 10^{-2}x^2 - 0.30x + 1.3 \quad (O₂ = 1.3 \text{ mg/L}; (\_\_\_)); \\
    y &= 6.3 \times 10^{-2}x^2 - 0.36x + 2.0 \quad (O₂ = 2.0 \text{ mg/L}; (\_\_)); \\
    y &= 6.5 \times 10^{-2}x^2 - 0.99x + 2.7 \quad (O₂ = 2.7 \text{ mg/L}; (\_\_\_)); \\
    y &= 7.9 \times 10^{-2}x^2 - 1.13x + 3.0 \quad (O₂ = 3.0 \text{ mg/L}; (\_\_\_\_)); \\
    y &= 2.1 \times 10^{-1}x^2 - 1.6x + 3.3 \quad (O₂ = 3.2 \text{ mg/L}; (\_\_\_\_\_)); \\
    y &= 8.5 \times 10^{-2}x^2 - 2.1x + 3.6 \quad (O₂ = 3.6 \text{ mg/L}; (\_\_\_\_\_)); \\
    y &= 1.2 \times 10^{-2}x^2 - 2.4x + 3.8 \quad (O₂ = 3.8 \text{ mg/L}; (\_\_\_\_\_)).
\end{align*}
\]
Figure 4.2.2.1b Progress curves of 2 mM m-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[
y = 3.0 \times 10^{-2} x^2 - 0.31 x + 0.83 \quad (O₂ = 0.8 \text{ mg/L; (•)})
\]
\[
y = 5.1 \times 10^{-2} x^2 - 0.30 x + 1.4 \quad (O₂ = 1.4 \text{ mg/L; (+)})
\]
\[
y = 6.9 \times 10^{-3} x^2 - 0.37 x + 2.0 \quad (O₂ = 2.0 \text{ mg/L; (×)})
\]
\[
y = 1.0 \times 10^{-1} x^2 - 0.85 x + 2.7 \quad (O₂ = 2.6 \text{ mg/L; (★)})
\]
\[
y = 6.1 \times 10^{-2} x^2 - 1.2 x + 3.0 \quad (O₂ = 3.1 \text{ mg/L; (●)})
\]
\[
y = 2.3 \times 10^{-1} x^2 - 1.6 x + 3.3 \quad (O₂ = 3.2 \text{ mg/L; (▲)})
\]
\[
y = 1.6 \times 10^{-1} x^2 - 2.3 x + 3.8 \quad (O₂ = 3.7 \text{ mg/L; (■)})
\]
\[
y = 4.3 \times 10^{-2} x^2 - 2.4 x + 4.0 \quad (O₂ = 3.9 \text{ mg/L; (●)})
\]
Figure 4.2.2.1c The relationship between initial oxygen consumption velocity and low concentration of oxygen at concentration of 2 mM m-cresol
Figure 4.2.2.2. Hill plot of the data from Figure 4.2.2.1c for oxidation of m-cresol by laccase SP504 at pH 5.6. $V_{\text{max}} = 0.083 \pm 0.007 \text{ mM/min.}, K_m = 0.090 \pm 0.004 \text{ mM}, h = 2.7 \pm 0.29, R^2 = 0.949$
4.2.3 OXYGEN KINETICS IN LACCASE SP504-CATALYZED REMOVAL OF o-CRESOL

Figure 4.2.3.1a shows oxygen reactions with o-cresol at different initial oxygen concentrations at an o-cresol concentration of 2 mM. Figure 4.2.3.1, shows co-operativity behavior in the reaction between o-cresol and oxygen. However, the co-operativity is not as significant as in the reaction between m-cresol and oxygen. The Hill equation was applied as shown in Figure 4.2.3.1 to obtain the appropriate model.

The Hill coefficient for o-cresol is greater than 1 indicating a positive co-operativity. Also two kinetic models, Michaelis-Menten model and Hill model, were used to get kinetic equation by direct computer analysis. Figure 4.2.3.1 shows best fit Hill plot and Figure 4.2.3.2 shows best fit Michaelis-Menten plot. A comparison of the parameters, obtained from the two plots, shows considerably more deviation in parameter values for Michaelis-Menten model than for Hill model. Therefore, it is concluded that the Hill model is more suitable for oxygen kinetics in the removal of o-cresol. Figure 4.2.3.1 shows $V_{\text{max}} = 0.030 \pm 0.005$ mM/min., $K_m = 0.066 \pm 0.003$ mM, $h = 2.4 \pm 0.4$.

4.2.4 OXYGEN KINETICS IN LACCASE SP504-CATALYZED REMOVAL OF p-CRESOL

Figures 4.2.4.1a to 4.2.4.1.b show oxygen reactions with p-cresol at different times where the concentration of p-cresol equal to 2 mM. Figure 4.2.4.1c shows that the plot between initial oxygen consumption velocity and the concentration of oxygen during p-cresol removal is a hyperbolic curve. Thus, reaction between p-cresol and oxygen,
Figure 4.2.3.1a Progress curves of 2 mM o-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various $O_2$ concentrations. Lines shown are second-order polynomial fits of the data between $y = O_2$ concentration and $x =$ time and the equations are:

- $y = 1.9 \times 10^{-1} \, x^2 - 0.10 \, x + 0.90 \, (O_2 = 0.8 \, \text{mg/L}; \, (-))$
- $y = 2.0 \times 10^{-1} \, x^2 - 0.10 \, x + 0.99 \, (O_2 = 1.0 \, \text{mg/L}; \, (+))$
- $y = 4.8 \times 10^{-2} \, x^2 - 0.26 \, x + 1.4 \, (O_2 = 1.4 \, \text{mg/L}; \, (●))$
- $y = 3.4 \times 10^{-2} \, x^2 - 0.26 \, x + 1.5 \, (O_2 = 1.5 \, \text{mg/L}; \, (★))$
- $y = 8.7 \times 10^{-2} \, x^2 - 0.34 \, x + 1.9 \, (O_2 = 1.9 \, \text{mg/L}; \, (×))$
- $y = 6.4 \times 10^{-2} \, x^2 - 0.43 \, x + 2.3 \, (O_2 = 2.3 \, \text{mg/L}; \, (▲))$
- $y = 9.2 \times 10^{-2} \, x^2 - 0.68 \, x + 3.4 \, (O_2 = 3.4 \, \text{mg/L}; \, (■))$
- $y = 8.4 \times 10^{-2} \, x^2 - 0.68 \, x + 3.7 \, (O_2 = 3.7 \, \text{mg/L}; \, (♦))$
- $y = 7.1 \times 10^{-2} \, x^2 - 0.75 \, x + 4.1 \, (O_2 = 4.1 \, \text{mg/L}; \, (♦))$
- $y = 1.6 \times 10^{-1} \, x^2 - 0.65 \, x + 4.7 \, (O_2 = 4.8 \, \text{mg/L}; \, (==))$. 
Figure 4.2.3.1 Hill plot for oxidation of o-cresol by laccase SP504 at pH 5.6. $V_{max} = 0.030 \pm 0.003$ mM/min., $K_m = 0.066 \pm 0.0008$ mM, $h = 2.36 \pm 0.4$, $R^2 = 0.994$

Figure 4.2.3.2 Michaelis-Menten plot for oxidation of m-cresol by laccase SP504 at pH 5.6. $V_{max} = 0.18 \pm 0.34$ mM/min., $K_m = 0.95 \pm 1.98$ mM, $R^2 = 0.952$
Figure 4.2.4.1a Progress curves of 2 mM p-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations.

Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

- \( y = -8.8 \times 10^{-2} x^2 - 0.48 x + 0.81 \) (O₂ = 0.8 mg/L; (-));
- \( y = -9.6 \times 10^{-2} x^2 - 0.88 x + 1.4 \) (O₂ = 1.4 mg/L; (+));
- \( y = -8.5 \times 10^{-3} x^2 - 1.1 x + 1.7 \) (O₂ = 1.6 mg/L; (●));
- \( y = -5.6 \times 10^{-2} x^2 - 1.0 x + 2.5 \) (O₂ = 2.5 mg/L; (★));
- \( y = -3.2 \times 10^{-2} x^2 - 1.1 x + 2.7 \) (O₂ = 2.6 mg/L; (×));
- \( y = -9.0 \times 10^{-2} x^2 - 1.3 x + 3.7 \) (O₂ = 3.7 mg/L; (▲));
- \( y = -5.0 \times 10^{-2} x^2 - 1.4 x + 4.0 \) (O₂ = 4.0 mg/L; (■));
- \( y = -3.3 \times 10^{-1} x^2 - 1.7 x + 4.4 \) (O₂ = 4.3 mg/L; (●));
Figure 4.2.4.1b Progress curves of 2 mM p-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O$_2$ concentrations. Lines shown are second-order polynomial fits of the data between y = O$_2$ concentration and x = time and the equations are:

\[ y = -1.5 \times 10^{-2} x^2 - 0.63 x + 0.91 \text{ (O}_2 = 0.9 \text{ mg/L; (·));} \]
\[ y = -3.4 \times 10^{-3} x^2 - 0.86 x + 1.2 \text{ (O}_2 = 1.2 \text{ mg/L; (+));} \]
\[ y = 8.5 \times 10^{-3} x^2 - 1.1 x + 1.7 \text{ (O}_2 = 1.7 \text{ mg/L; (○));} \]
\[ y = 5.0 \times 10^{-2} x^2 - 1.1 x + 2.1 \text{ (O}_2 = 2.0 \text{ mg/L; (★)));} \]
\[ y = -3.1 \times 10^{-2} x^2 - 1.2 x + 2.8 \text{ (O}_2 = 2.8 \text{ mg/L; (×));} \]
\[ y = -8.1 \times 10^{-2} x^2 - 1.3 x + 3.7 \text{ (O}_2 = 3.6 \text{ mg/L; (▲));} \]
\[ y = -4.1 \times 10^{-1} x^2 - 1.6 x + 4.1 \text{ (O}_2 = 4.0 \text{ mg/L; (■)));} \]
\[ y = -3.4 \times 10^{-1} x^2 - 1.6 x + 4.4 \text{ (O}_2 = 4.4 \text{ mg/L; (◆));} \]
Figure 4.2.4.1c The relationship between initial oxygen consumption velocity and low concentrations of oxygen at p-cresol concentration of 2 mM
catalyzed by laccase SP504 followed Michaelis-Menten equation, Eq. 2.2. The parameters of the Michaelis-Menten equation were obtained directly through standard analysis. Figure 4.2.4.2 shows \( V_{\text{max}} = 0.061 \text{ mM/min.}, K_m = 0.044 \text{ mM} \) for oxygen.

4.2.5 OXYGEN KINETICS IN LACCASE SP504-CATALYZED REMOVAL OF PHENOL

Figure 4.2.5.1a shows oxygen reactions with phenol at different times at a phenol concentration of 2 mM. The reaction between phenol and oxygen catalyzed by laccase SP504, was quite similar to the reaction observed for p-cresol. It also followed the Michaelis-Menten equation. Figure 4.2.5.1b shows the plot of the oxygen consumption velocity against the concentration of oxygen which is a hyperbolic curve if oxygen concentration is below 6 mg/L. Similarly, direct computer analysis of data, Figure 4.2.5.2, shows \( V_{\text{max}} = 0.061 \text{ mM/min.}, K_m = 0.044 \text{ mM} \) for oxygen.

4.2.6 GENERAL DISCUSSION OF OXYGEN KINETICS IN REMOVAL OF CREOSOLS AND PHENOL UNDER CATALYSIS BY LACCASE SP504

It was observed that m-, o-cresols followed Hill equation, Eq. 2.4, and p-cresol and phenol followed Michaelis-Menten equation, Eq. 2.2. It is possible that the location of the methyl function group (\( \text{CH}_3 \)) on the benzene ring had effects on oxygen kinetics.

Although different cresols and phenol did not follow a single kinetic model during catalysis by laccase SP504, there were still some similarities. They are:

- In all reaction with oxygen, cresols and phenol had optimum oxygen concentration range in which oxygen consumption velocity reached maximum.
Figure 4.2.4.2. Michaelis-Menten plot of the data from Figure 4.2.4.1.c for oxidation of p-cresol by laccase SP504 at pH 5.6. $V_{\text{max}}: 0.076 \pm 0.008$ mM/min., $K_m: 0.081 \pm 0.018$ mM, $R^2 = 0.940$
Figure 4.2.5.1a Progress curves of 2 mM phenol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x= time and the equations are:

\[
\begin{align*}
  y &= -1.2 \times 10^{-1} x^2 - 0.90 x + 1.0 \quad (O₂ = 1.0 \text{ mg/L}; (\triangle)); \\
  y &= -1.4 \times 10^{-1} x^2 - 0.99 x + 1.1 \quad (O₂ = 1.1 \text{ mg/L}; (\square)); \\
  y &= -2.7 \times 10^{-1} x^2 - 1.0 x + 1.8 \quad (O₂ = 1.7 \text{ mg/L}; (\blacklozenge)); \\
  y &= -2.2 \times 10^{-1} x^2 - 0.97 x + 1.7 \quad (O₂ = 1.7 \text{ mg/L}; (\blacklozenge)); \\
  y &= -2.6 \times 10^{-1} x^2 - 1.0 x + 2.1 \quad (O₂ = 2.0 \text{ mg/L}; (+)); \\
  y &= -2.7 \times 10^{-1} x^2 - 1.3 x + 2.5 \quad (O₂ = 2.4 \text{ mg/L}; (+)); \\
  y &= -1.6 \times 10^{-1} x^2 - 1.4 x + 2.9 \quad (O₂ = 2.8 \text{ mg/L}; (\bullet)); \\
  y &= -1.9 \times 10^{-1} x^2 - 1.5 x + 3.1 \quad (O₂ = 3.0 \text{ mg/L}; (\star)); \\
  y &= -2.0 \times 10^{-1} x^2 - 1.4 x + 4.1 \quad (O₂ = 4.0 \text{ mg/L}; (\times)); \\
  y &= -2.1 \times 10^{-1} x^2 - 1.4 x + 4.4 \quad (O₂ = 4.3 \text{ mg/L}; (\triangle)); \\
  y &= 7.7 \times 10^{-2} x^2 - 1.7 x + 5.7 \quad (O₂ = 5.4 \text{ mg/L}; (\blacklozenge)); \\
  y &= 7.6 \times 10^{-2} x^2 - 1.7 x + 5.6 \quad (O₂ = 5.5 \text{ mg/L}; (\blacklozenge)).
\end{align*}
\]
Figure 4.2.5.1b The relationship between initial oxygen consumption velocity and low concentration of oxygen at concentration of 2 mM phenol
Figure 4.2.5.2. Michaelis-Menten plot of the data from Figure 4.2.5.1b for oxidation of phenol by laccase SP504 at pH 5.6. \( V_{\text{max}} = 0.062 \pm 0.004 \text{ mM/min.} \), \( K_m = 0.045 \pm 0.01 \text{ mM} \), \( R^2 = 0.928 \)
- Observed $V_{\text{max}}$ and $K_m$ for oxygen with different cresols and phenol, which are two most important parameters for judging an enzyme's catalytic capacity, they were all in same magnitude as shown in Table 4.2.6.1. It can be interpreted that the laccase SP504 capacity for removal of different cresols and phenol is similar.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Applied model</th>
<th>$V_{\text{max}}$, mM/min.</th>
<th>$K_m$, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-cresol</td>
<td>Hill (h = 2.7)</td>
<td>0.083</td>
<td>0.090</td>
</tr>
<tr>
<td>o-cresol</td>
<td>Hill (h = 2.4)</td>
<td>0.030</td>
<td>0.066</td>
</tr>
<tr>
<td>p-cresol</td>
<td>Michaelis-Menten</td>
<td>0.076</td>
<td>0.081</td>
</tr>
<tr>
<td>phenol</td>
<td>Michaelis-Menten</td>
<td>0.062</td>
<td>0.044</td>
</tr>
</tbody>
</table>
4.3 LOSS IN LACCASE ACTIVITY DURING OXYGEN KINETIC REACTION

An assay was designed to determine the activities of laccase SP850 and laccase SP504 during the reactions started under different initial concentrations of oxygen. The steps followed were:

a) o-cresol was chosen as substrate and its concentration was kept at 2mM.

b) Experimental steps were the same as described in Chapter 3.3.4.

c) Samples were withdrawn for laccase activity test after 27 minutes of reaction time.

All experiments were conducted in duplicate. The activity of laccase SP850 before reaction was 0.0067 LACU/mL and the activity of laccase SP504 before reaction was 6.67 LAMU/mL. Table 4.3.5.1 shows the average results of assay.

Table 4.3.5.1 Changes in laccase activities during reactions under different concentrations of oxygen

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Concentration of oxygen before reaction, mg/L</th>
<th>Cresol concentration, mM</th>
<th>Enzyme activity, LAMU/mL or LACU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Laccase SP 504</td>
<td>25.3</td>
<td>22.3</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>5.90</td>
<td>0.1</td>
<td>2.00</td>
</tr>
<tr>
<td>Laccase SP 850</td>
<td>33.4</td>
<td>15.2</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>25.5</td>
<td>11.8</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>17.4</td>
<td>4.1</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>0.0</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 4.3.5.1 shows that laccase SP504 lost much of its activity (87%) when the concentration of oxygen was high, 25.3mg/L as compared to low oxygen concentration of 5.9 mg/L when only 7% of the activity was lost. It shows that laccase SP504 was inhibited
when the concentration of oxygen was high. On the other hand, laccase SP850 showed no difference in change in activity, no matter what was the value of initial oxygen concentration.

The other experiment was designed to check whether the inhibition of laccase SP850 by oxygen was reversible or irreversible:

a) m-cresol was chosen as substrate and its concentration was kept at 2mM. The initial oxygen concentration was 22.0 mg/L.

b) Experimental steps were the same as described in Chapter 3.3.4.

c) The reaction was continued 5 min. and the oxygen concentrations were recorded. Through polynomial curve fitting, the initial oxygen consumption velocity was derived. It was 0.070 mg/L.min. At this time, it was estimated that in the reactor there was 1.95 mM m-cresol.

d) Sodium sulfite was added to "kill" oxygen. In a couple of seconds, the oxygen concentration reached 4.5 mg/L.

e) The reaction was continued 5 min. and the oxygen concentrations were recorded. Through polynomial curve fitting, the initial oxygen consumption velocity was derived. It was 0.063 mg/L.min. Compared to the initial oxygen consumption velocity, 0.25 mg/L.min., at which the reaction began with the initial oxygen concentration of 4.2 mg/L, it could be concluded that the inhibition of laccase SP850 in the reaction between m-cresol and oxygen was irreversible. Figure 4.3.1 and Figure 4.3.2 Show the progress curves of the reaction.
Figure 4.3.1 Progress curves of 2 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = 8 \times 10^{-4}x^2 - 0.0698x + 22.0 \ (O_2=22.0 \ mg/L; \ (\bullet)); \]
\[ y = -1.4 \times 10^{-3}x^2 - 0.0633x + 4.86 \ (O_2=4.5 \ mg/L; \ (\square)). \]

Figure 4.3.2 Progress curves of 2 mM m-cresol reacting with 16 LAMU/mL laccase SP850 at pH = 6.7 and room temperature at various O₂ concentrations. Lines shown are second-order polynomial fits of the data between y = O₂ concentration and x = time and the equations are:

\[ y = 3.3 \times 10^{-2}x^2 - 0.254x + 4.30 \ (O_2=4.2 \ mg/L; \ (\bullet)); \]

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4.3 ERROR ANALYSIS

Experiments were conducted to determine the reliability of the results for oxygen consumption velocity obtained in this study. To test the reliability of the laboratory techniques used, the reaction between m-cresol and oxygen was repeated five times under identical conditions and the standard deviation was calculated. The batch reactions were carried out as follows: pH = 6.7, initial m-cresol concentration = 2mM, initial oxygen concentration = 6.0 mg/L, Laccase SP 850 enzyme activity = 16 LAMU/mL. Polynomial lines were used to fit the progress curves and derive initial oxygen consumption rate. The results are listed in Table 4.4.1

Table 4.4.1: Error analysis data obtained for oxygen consumption velocity

<table>
<thead>
<tr>
<th>No. of Sample</th>
<th>Oxygen Consumption Rate (mg/L·min)</th>
<th>Mean (mg/L·min)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
<td>±0.013</td>
</tr>
<tr>
<td>2</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These results indicate that the standard deviation was ±5.7% of the mean value, which is considered to be within the acceptable range for this type of study. Same experiments were also conducted to determine the reliability of the enzyme activity test. Five samples were withdrawn from a single reactor after the reaction was finished. The reaction condition and procedure were same as for the above experiment. The results are shown in Table 4.4.2
Table 4.4.2 Error analysis of laccase SP850 activity test

<table>
<thead>
<tr>
<th>No. of Sample</th>
<th>Enzyme activity (LAMU/mL)</th>
<th>Mean (LAMU/mL)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.52</td>
<td>16.13</td>
<td>±0.32</td>
</tr>
<tr>
<td>2</td>
<td>15.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16.36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The standard deviation in these results was ±2% of the mean value, which is acceptable. Compared to Table 4.3.5.1, the activities of laccase SP850 did not lose in the reaction between m-cresol and oxygen. The results in table 4.3.5.1 shows the activities of laccase SP850 lost 30-50% in the reaction between o-cresol and oxygen.

In summary, the error analyses carried out show that all deviations in major analyses were within an acceptable range. Therefore, the results obtained in this study are considered to be reliable.
5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The results of this study demonstrate the effectiveness of laccase catalyzed reactions between cresols or phenol and oxygen.

- Laccase SP504 is more efficient in the removal of all substrates than laccase SP850.
- High concentrations of oxygen inhibited the catalytic capacity of laccase SP 850 in the removal of m-cresol. Oxygen consumption rate decreased or remained constant with the reduction in oxygen concentration. Oxygen consumption rate always increased with an increase in m-cresol concentration.
- In laccase SP850 catalyzed reaction, oxygen consumption rate increased with the reduction in oxygen concentration and o-, p- cresol concentration in the removal of o-, p-cresols. The Michaelis-Menten equation for two substrates was applied to develop a relationship among oxygen consumption rate, concentration of o-cresol and concentration of oxygen.
- Laccase SP850 was not a good catalyst for the removal of phenol. All the reactions showed that the oxygen consumption rate was slow.
- Concentrations of oxygen above 6 mg/L inhibited laccase SP504 in all reactions with m-, o-, p-cresols and phenol. The reaction for removal of m- and o-cresol demonstrated co-operativity at low oxygen concentration. Hill equation was applied for developing an oxygen kinetic plot. However, in the removal of p-cresol and phenol catalyzed by laccase SP504 at low oxygen concentrations, the Michaelis-Menten equation was applicable as an oxygen kinetic model.
5.2 RECOMMENDATIONS

- All laccase catalyzed reactions, no matter whether using laccase SP850 or laccase SP504, did not follow Michaelis-Menten equation completely. Further study is necessary to obtain explanations for the kinetic behavior of laccase.

- Oxygen in excess is inhibitory and should be maintained at low concentrations in full industrial applications.

- Further studies should be carried out to investigate the influence of different concentration of cresols and phenol in laccase SP 504 catalyzed reaction.

- The studies on reversibility or irreversibility inhibition of laccase SP850 and laccase SP504 were incomplete. Further studies should be carried out in this area.

- Although laccase SP850 was found to be a poor enzyme for these substrates, it would still be interesting to study the reason for acceleration in the progress curve.

- Two different kinetic models were obtained for the removal of cresols and phenol under the catalyzed by laccase SP504. It was possible that the different location of function group, -CH₃, on aromatic cycle had caused different kinetic behavior. Further studies should be conducted to confirm it.

- A real industrial wastewater should be used to determine the effectiveness of laccase in a real situation. Cost analysis of the process should also be carried out to determine the economic feasibility.
REFERENCES


Environmental Protection Agency (1999, 2000) Cresol/phenol, EPA Report No.: 60014-89-017 Environmental Protection Agency, USA


Appendix A

ENZYME ACTIVITY ASSAY

(VERMETTE, 2000 as formulated by Novo Nordisk)
1. General

The purpose of enzyme activity assay is to determine the amount of active enzyme that is contained in a solution. Under saturating conditions of syringaldazine, the initial rate is measured by observing the rate of color formation in a solution. Laccase catalyzes the oxidation of syringaldazine to the corresponding quinone such that the products of the reaction form a purple-color solution that absorbs light at a peak wavelength of 530 nm.

2. Reagents

i) Tris Maleate buffer for laccase SP850
   (23 mM, pH 7.5±0.05)
   25 mL of 1.0 M Tris solution
   5 mL of 1.0 M maleic acid
   Distilled water to 1 L
   MES buffer for laccase SP504
   (23mM, pH 5.5±0.05)
   2.66 g of MES
   1.0 mL 2 M sodium hydroxide
distilled water to 1 L

ii) Syringaldazine solution (0.38 mM)
   6.8 mg syringaldazine in flask
   25 mL of 96% ethanol dissolved 1.5 hours
   Distilled water to 50 mL.
   Store in dark

3. Procedure

In a semi-micro cuvette, combine in the following order:
   850 µL Tris Maleate buffer
   50 µL Syringaldazine solution
   100 µL Laccase solution

The sample volume must be 1 mL and the rate of color formation must be measured before substrate depletion becomes significant. Immediately after the addition of the sample, shake the cuvette and then place it in the spectrophotometer to monitor the absorbance change with time at 530 nm. The change in absorbance should be measured at 15 s and 75 s.
4. Calculation

i) Calculation the activity in the cuvette:

\[
\text{Activity in the cuvette (LACU/mL or LAMU/mL)} = \frac{(\Delta A \times 1.0 \text{ mL} \times 10^{-3} \times D)}{(0.065 \times 0.1 \text{ mL})} = \Delta A \times 0.1538 \times D
\]

where, \( \Delta A \) : Change in absorbance per minute: \( \Delta A = (A_{75s} - A_{15s}) \)

The absorbance range should be 0.1 - 0.4 \( \Delta A / \text{min.} \)

1.0 : Total volume in cuvette (mL)

0.065 : Micromolar extinction coefficient (µM/L)

10^{-3} : LACU/L or LAMU/L converted to LACU/mL or LAMU/mL

D : Dilution factor

The activity is in terms of micromoles of syringaldazine converted per minute at 20°C and pH 7.5.

ii) Calculate the activity of sample:

\[
\text{Activity in the sample (LACU/mL or LAMU/mL)} = \frac{\text{Activity in the cuvette (LACU/mL or LAMU/mL) \times enzyme solution added to reactor (mL) \times reactor volume (mL)}}{\text{reactor volume (mL)}}
\]
Appendix-B

CHEMICAL TITRATION OF DO-AZIDE MODIFICATION
1. General

This test is based on the addition of divalent manganese solution, followed by strong alkali, to the water sample in a glass-stoppered bottle. DO present in the sample rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxide of higher valency state. In the presence of iodide ions and upon acidification, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content in the sample. The iodine is then titrated with a standard solution of thiosulfate.

2. Reagents

a. Manganese sulfate solution: Dissolve 480 g MnSO₄·H₂O in distilled water, filter and dilute to 1 liter.

b. Alkali-iodide-azide reagent: Dissolve 500 g sodium hydroxide, NaOH, and 135 g sodium iodide, NaI, in distilled water and dilute to 1 liter. To this solution add 10 g sodium azide, NaN₃, dissolved in 40 mL distilled water.

c. Sulfuric acid, concentrated: The strength of this acid is about 36 N.

d. Starch: Add 5 g soluble starch to 800 mL boiling water while stirring. Dilute to 1 liter, allow to boil for a few minutes, and let it settle overnight. Use clear supernate.

e. Sodium thiosulfate stock solution, 0.10 N: Dissolve 24.82 g Na₂S₂O₃·5H₂O in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 1 g NaOH per liter.

f. Standard sodium thiosulfate, 0.0250 N: Dilute 250.0 mL sodium thiosulfate stock solution to 1,000 mL freshly boiled and cooled distilled water.
3. **Procedure**

   a. To the sample collected in a 250 mL bottle, add 2 mL manganese sulfate solution, followed by 2 mL alkali-iodide-azide reagent, well below the surface of the liquid; stopper with care to exclude air and mix by inverting the bottle at least 15 times. When the precipitate settles, leaving a clear supernate above the manganese hydroxide floc, shake again. After at least 2 min. period of settling has produced at least 100 mL of clear supernate, carefully remove the stopper and immediately add 2.0 mL concentrated H₂SO₄ by allowing the acid to run down the neck of the bottle, restopper, and mix by gentle inversion until dissolution is complete.

   b. Titrate with 0.0250 N thiosulfate solution to a pale straw color. Add 1-2 mL starch solution and continue the titration to the first disappearance of the blue color.

4. **Calculation**

   Because 1 mL 0.0250 N sodium thiosulfate titrant is equivalent to 0.200 mg DO, so:

   \[
   \text{Dissolved oxygen (mg/L)} = \frac{\text{titrant value (mL)}}{\text{sample volume (L)}} \times 0.2 \text{ (mg/mL)}
   \]
Appendix C

AROMATIC SUBSTRATE ASSAY

(VERMETTE, 2000)
1. **General**

This is a colorimetric assay used to measure the concentration of an aromatic substrate in an aqueous sample. The assay uses ferricyanide and 4-aminoantipyrine as color-generating substrates when combined with the aromatic sample. The limiting reagent is the amount of aromatic compound in the sample. Therefore, the absorbance of the color developed at a peak wavelength of the 510 nm is proportional to the aromatic concentration present in the sample.

2. **Reagents**

   i) Ferricyanide reagent (83.4 mM of K₃Fe(CN)₆ in 0.25 M NaHCO₃)
      
      2.75 g K₃Fe(CN)₆
      2.1 g NaHCO₃
      distilled water to 100 mL

   ii) 4-aminoantipyrine (AAP) reagent (20.8 mM of AAP in 0.25 M NaHCO₃)

      0.423 g AAP
      2.1 g NaHCO₃
      Distilled water to 100 mL

3. **Procedure**

   In a semi-micro cuvette, combine in the following order:

   800 μL deionized water + sample
   100 μL AAP reagent
   100 μL ferricyanide reagent

   The final assay sample volume should be 1 mL. After approximately 12 minutes, measure the absorbance at 510 nm against a reagent blank.
4. Calculation

Using the appropriate calibration curve covert the absorbance readings into desired concentration units.
APPENDIX D

EXPERIMENTAL RESULTS
Table 4.1.1.1 The experimental results of 1 mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>18.52</td>
<td>18.39</td>
<td>18.35</td>
<td>18.18</td>
<td>17.87</td>
<td>17.44</td>
<td>16.88</td>
<td>8.83</td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>11.90</td>
<td>11.27</td>
<td>11.06</td>
<td>10.89</td>
<td>10.97</td>
<td>10.76</td>
<td>10.04</td>
<td>9.40</td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>7.25</td>
<td>7.12</td>
<td>6.99</td>
<td>6.66</td>
<td>6.17</td>
<td>5.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>5.30</td>
<td>4.89</td>
<td>4.45</td>
<td>3.84</td>
<td>3.06</td>
<td>2.14</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.47</td>
<td>3.17</td>
<td>2.74</td>
<td>2.14</td>
<td>1.37</td>
<td>0.46</td>
<td>0.00</td>
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<tr>
<td>Reaction time, min.</td>
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<td>5</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>2.06</td>
<td>1.74</td>
<td>1.16</td>
<td>0.75</td>
<td>0.33</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1.1.2 The experimental results of 2 mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>20.13</td>
<td>19.87</td>
<td>19.69</td>
<td>19.35</td>
<td>18.74</td>
<td>18.00</td>
<td>17.05</td>
<td>8.67</td>
</tr>
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<td>Reaction time, min.</td>
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<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>11.99</td>
<td>11.27</td>
<td>11.10</td>
<td>10.89</td>
<td>10.37</td>
<td>9.57</td>
<td>8.58</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>7.53</td>
<td>7.32</td>
<td>6.98</td>
<td>6.39</td>
<td>5.50</td>
<td>4.32</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>6.09</td>
<td>5.73</td>
<td>5.01</td>
<td>3.90</td>
<td>2.48</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>2</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.78</td>
<td>3.26</td>
<td>3.02</td>
<td>2.45</td>
<td>1.87</td>
<td>1.27</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>2.05</td>
<td>1.79</td>
<td>1.53</td>
<td>1.26</td>
<td>0.97</td>
<td>0.67</td>
<td>0.37</td>
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</tbody>
</table>
Table 4.1.1.3 The experimental results of 3 mM m-cresol reacting with 16 LAM U/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
<th>16.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>20.26</td>
<td>19.91</td>
<td>19.65</td>
<td>19.09</td>
<td>18.35</td>
<td>17.36</td>
<td>16.06</td>
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<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>6.42</th>
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<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>12.03</td>
<td>11.14</td>
<td>10.46</td>
<td>10.08</td>
<td>9.52</td>
<td>8.16</td>
<td>6.42</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>5</th>
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<th>9</th>
<th>11</th>
<th>14</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>6.18</td>
<td>4.49</td>
<td>3.30</td>
<td>2.45</td>
<td>1.60</td>
<td>0.73</td>
<td>0.10</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>8</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.09</td>
<td>2.72</td>
<td>2.32</td>
<td>1.94</td>
<td>1.10</td>
<td>0.25</td>
<td>0.01</td>
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</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>5</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>1.99</td>
<td>1.74</td>
<td>1.33</td>
<td>0.87</td>
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</table>

Table 4.1.1.4 The experimental results of 5 mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>19.48</td>
<td>19.17</td>
<td>18.74</td>
<td>17.87</td>
<td>16.62</td>
<td>14.85</td>
<td>12.51</td>
<td>8.67</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>14</th>
<th>20</th>
<th>26</th>
<th>6.11</th>
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</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.41</td>
<td>10.89</td>
<td>10.44</td>
<td>9.75</td>
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<td>7.23</td>
<td>5.61</td>
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</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>6.31</td>
<td>5.81</td>
<td>5.17</td>
<td>4.45</td>
<td>2.85</td>
<td>1.17</td>
<td>0.01</td>
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</tr>
</tbody>
</table>

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<th>2</th>
<th>3</th>
<th>3</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.93</td>
<td>3.49</td>
<td>3.24</td>
<td>3.00</td>
<td>2.36</td>
<td>1.69</td>
<td>0.93</td>
<td>0.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.23</td>
<td>1.99</td>
<td>1.46</td>
<td>1.03</td>
<td>0.67</td>
<td>0.32</td>
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</tbody>
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Table 4.1.1.5 The experimental results of 10 mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>2</th>
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<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>18.00</td>
<td>17.36</td>
<td>15.93</td>
<td>13.59</td>
<td>9.82</td>
<td>5.50</td>
<td>0.04</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>11.51</td>
<td>10.77</td>
<td>10.33</td>
<td>9.73</td>
<td>7.32</td>
<td>5.48</td>
<td>3.54</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>8.78</td>
<td>8.27</td>
<td>7.83</td>
<td>6.57</td>
<td>5.09</td>
<td>3.32</td>
<td>1.53</td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>6.58</td>
<td>6.47</td>
<td>5.73</td>
<td>4.79</td>
<td>3.68</td>
<td>1.33</td>
<td>0.01</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1</td>
<td>1.5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.85</td>
<td>3.61</td>
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<td>0.40</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>2.00</td>
<td>1.12</td>
<td>0.28</td>
<td>0.11</td>
<td>0.07</td>
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</table>

Table 4.1.1.6 The experimental results of 20 mM m-cresol reacting with 16 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>15.75</td>
<td>14.41</td>
<td>10.69</td>
<td>7.70</td>
<td>3.72</td>
<td>0.61</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>8.78</td>
<td>7.83</td>
<td>6.57</td>
<td>5.09</td>
<td>3.32</td>
<td>1.53</td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>7.32</td>
<td>5.81</td>
<td>4.05</td>
<td>1.90</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>6.26</td>
<td>5.46</td>
<td>3.93</td>
<td>3.22</td>
<td>2.21</td>
<td>1.33</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>5.46</td>
<td>4.69</td>
<td>3.46</td>
<td>2.68</td>
<td>1.97</td>
<td>1.25</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.3</td>
<td>0.5</td>
<td>0.8</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.01</td>
<td>2.79</td>
<td>2.21</td>
<td>1.68</td>
<td>1.24</td>
<td>0.58</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.46</td>
<td>2.68</td>
<td>1.97</td>
<td>1.25</td>
<td>0.78</td>
<td>0.41</td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>0.3</td>
<td>0.5</td>
<td>0.8</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>2.68</td>
<td>1.97</td>
<td>1.25</td>
<td>0.78</td>
<td>0.41</td>
<td>0.32</td>
</tr>
</tbody>
</table>
Table 4.1.1.8 The relationship between concentration of oxygen and oxygen consumption velocity at different concentration of m-cresol under the catalysis by laccase SP850

The relation between concentration of oxygen and initial reaction velocity at concentration of m-cresol = 1 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.06</th>
<th>3.47</th>
<th>5.3</th>
<th>7.25</th>
<th>11.90</th>
<th>18.52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>0.20</td>
<td>0.16</td>
<td>0.16</td>
<td>0.058</td>
<td>0.044</td>
<td>0.028</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of m-cresol = 2 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.05</th>
<th>3.78</th>
<th>6.09</th>
<th>7.53</th>
<th>11.99</th>
<th>20.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>0.25</td>
<td>0.25</td>
<td>0.23</td>
<td>0.307</td>
<td>0.079</td>
<td>0.076</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of m-cresol = 3 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>1.99</th>
<th>3.09</th>
<th>6.18</th>
<th>8.50</th>
<th>12.03</th>
<th>20.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>0.37</td>
<td>0.41</td>
<td>0.63</td>
<td>0.401</td>
<td>0.1520</td>
<td>0.111</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of m-cresol = 5 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.23</th>
<th>3.93</th>
<th>6.31</th>
<th>7.695</th>
<th>11.41</th>
<th>19.48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>0.92</td>
<td>0.40</td>
<td>0.69</td>
<td>0.811</td>
<td>0.1670</td>
<td>0.145</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of m-cresol = 10 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.00</th>
<th>3.85</th>
<th>6.58</th>
<th>8.78</th>
<th>11.51</th>
<th>18.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>2.36</td>
<td>2.69</td>
<td>1.38</td>
<td>1.80</td>
<td>0.97</td>
<td>0.48</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of m-cresol = 20 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>3.01</th>
<th>5.46</th>
<th>6.26</th>
<th>7.32</th>
<th>14.92</th>
<th>15.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L·min.</td>
<td>2.05</td>
<td>2.00</td>
<td>2.25</td>
<td>1.74</td>
<td>1.66</td>
<td>0.78</td>
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</table>
Table 4.1.2.1 The experimental results of 1 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<th>14</th>
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<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>16.36</td>
<td>16.19</td>
<td>15.37</td>
<td>13.81</td>
<td>11.56</td>
<td>8.66</td>
<td>5.37</td>
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<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>12.13</td>
<td>11.98</td>
<td>11.48</td>
<td>10.60</td>
<td>9.42</td>
<td>7.92</td>
<td>6.57</td>
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</tbody>
</table>

<table>
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<th>Reaction time, min.</th>
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<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>7.46</td>
<td>7.34</td>
<td>7.00</td>
<td>6.18</td>
<td>4.79</td>
<td>2.85</td>
<td>0.54</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.70</td>
<td>3.56</td>
<td>3.26</td>
<td>2.83</td>
<td>1.77</td>
<td>0.59</td>
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</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.14</td>
<td>4.95</td>
<td>4.62</td>
<td>4.16</td>
<td>3.01</td>
<td>1.87</td>
<td>0.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.58</td>
<td>2.40</td>
<td>2.04</td>
<td>1.54</td>
<td>0.98</td>
<td>0.37</td>
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Table 4.1.2.2 The experimental results of 2 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>15.19</td>
<td>14.89</td>
<td>13.72</td>
<td>11.34</td>
<td>8.14</td>
<td>4.03</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.31</td>
<td>11.21</td>
<td>10.89</td>
<td>9.27</td>
<td>7.65</td>
<td>6.26</td>
<td>2.47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>8.28</td>
<td>8.16</td>
<td>7.57</td>
<td>5.98</td>
<td>3.84</td>
<td>1.02</td>
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</tbody>
</table>

<table>
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<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.02</td>
<td>4.76</td>
<td>4.20</td>
<td>3.45</td>
<td>1.59</td>
<td>0.08</td>
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</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.58</td>
<td>3.31</td>
<td>2.99</td>
<td>2.57</td>
<td>1.60</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>2.5</th>
<th>3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>1.91</td>
<td>1.70</td>
<td>1.42</td>
<td>1.07</td>
<td>0.67</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.1.2.3 The experimental results of 3 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<th>11</th>
<th>14</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>23.85</td>
<td>23.41</td>
<td>21.51</td>
<td>17.70</td>
<td>15.58</td>
<td>12.29</td>
<td>5.89</td>
</tr>
</tbody>
</table>

<table>
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<th>Reaction time, min.</th>
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<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.12</td>
<td>10.95</td>
<td>10.50</td>
<td>7.81</td>
<td>5.50</td>
<td>3.07</td>
<td>0.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>6.36</td>
<td>5.92</td>
<td>5.02</td>
<td>3.85</td>
<td>2.50</td>
<td>1.04</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.43</td>
<td>3.01</td>
<td>2.04</td>
<td>0.76</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Reaction time, min.</th>
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<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.52</td>
<td>2.20</td>
<td>1.87</td>
<td>1.52</td>
<td>0.71</td>
<td>0.01</td>
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Table 4.1.2.4 The experimental results of 5 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

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<th>Reaction time, min.</th>
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<th>7</th>
<th>9</th>
<th>11</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>22.88</td>
<td>21.94</td>
<td>19.00</td>
<td>16.23</td>
<td>13.37</td>
<td>10.26</td>
<td>5.58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.84</td>
<td>11.64</td>
<td>10.85</td>
<td>9.61</td>
<td>7.69</td>
<td>4.68</td>
<td>0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.66</td>
<td>5.35</td>
<td>4.55</td>
<td>3.42</td>
<td>2.04</td>
<td>0.59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>4.26</td>
<td>3.62</td>
<td>3.14</td>
<td>2.69</td>
<td>1.65</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.39</td>
<td>1.90</td>
<td>1.62</td>
<td>1.28</td>
<td>0.88</td>
<td>0.60</td>
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</tbody>
</table>
Table 4.1.2.5 The experimental results of 10 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>DO concentration, mg/L</th>
<th>23.11</th>
<th>22.51</th>
<th>21.47</th>
<th>19.87</th>
<th>15.49</th>
<th>10.21</th>
<th>4.54</th>
<th>1.79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time, min.</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>12.38</td>
<td>11.89</td>
<td>9.91</td>
<td>7.23</td>
<td>3.97</td>
<td>0.60</td>
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<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>8.67</td>
<td>7.25</td>
<td>5.75</td>
<td>3.29</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>3.5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>6.09</td>
<td>5.26</td>
<td>3.81</td>
<td>2.16</td>
<td>0.96</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>4.40</td>
<td>3.79</td>
<td>2.18</td>
<td>1.14</td>
<td>0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>2.83</td>
<td>2.26</td>
<td>1.78</td>
<td>1.39</td>
<td>0.18</td>
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</table>

Table 4.1.2.6 The experimental results of 20 mM o-cresol reacting with 4 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3.5</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>19.87</td>
<td>18.39</td>
<td>16.75</td>
<td>14.33</td>
<td>12.08</td>
<td>9.95</td>
<td>7.36</td>
<td>4.55</td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>12.56</td>
<td>11.15</td>
<td>9.40</td>
<td>7.25</td>
<td>5.14</td>
<td>2.56</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>8.27</td>
<td>7.63</td>
<td>5.66</td>
<td>2.71</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>5.86</td>
<td>5.60</td>
<td>4.73</td>
<td>3.33</td>
<td>1.67</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>1</td>
<td>1.5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>3.99</td>
<td>3.76</td>
<td>2.68</td>
<td>1.58</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time, min.</td>
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<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO concentration, mg/L</td>
<td>1.65</td>
<td>1.61</td>
<td>1.39</td>
<td>0.99</td>
<td>0.60</td>
<td>0.30</td>
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</tbody>
</table>
Table 4.1.2.8 The relationship between initial oxygen consumption velocity and concentrations of oxygen at different concentrations of o-cresol under the catalysis by laccase SP850

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=1 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.6</th>
<th>3.7</th>
<th>5.14</th>
<th>7.46</th>
<th>12.13</th>
<th>16.36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.18</td>
<td>0.19</td>
<td>0.31</td>
<td>0.12</td>
<td>0.17</td>
<td>0.28</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=2 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>1.91</th>
<th>3.58</th>
<th>5.02</th>
<th>8.28</th>
<th>11.31</th>
<th>15.19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.13</td>
<td>0.23</td>
<td>0.47</td>
<td>0.18</td>
<td>0.42</td>
<td>0.47</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=3 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.52</th>
<th>3.43</th>
<th>6.36</th>
<th>7.03</th>
<th>11.12</th>
<th>23.85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.38</td>
<td>0.65</td>
<td>0.70</td>
<td>0.25</td>
<td>0.51</td>
<td>0.59</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=5 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.39</th>
<th>4.26</th>
<th>5.66</th>
<th>8.54</th>
<th>11.84</th>
<th>22.68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.46</td>
<td>0.68</td>
<td>0.33</td>
<td>0.56</td>
<td>0.61</td>
<td>0.66</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=10 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.83</th>
<th>4.40</th>
<th>6.10</th>
<th>8.67</th>
<th>12.38</th>
<th>23.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.45</td>
<td>0.50</td>
<td>0.83</td>
<td>0.92</td>
<td>0.70</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of o-cresol=20 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>1.65</th>
<th>3.99</th>
<th>5.86</th>
<th>8.54</th>
<th>12.56</th>
<th>19.87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L/min.</td>
<td>0.46</td>
<td>0.77</td>
<td>0.83</td>
<td>1.03</td>
<td>1.49</td>
<td>1.62</td>
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</tbody>
</table>
Table 4.1.3.1 The experimental results of 1 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>5</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>21.81</td>
<td>21.38</td>
<td>19.52</td>
<td>14.80</td>
<td>12.25</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>14.54</td>
<td>14.35</td>
<td>13.24</td>
<td>11.93</td>
<td>10.26</td>
<td>8.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.14</td>
<td>10.71</td>
<td>8.96</td>
<td>7.24</td>
<td>5.26</td>
<td>3.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>9</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>7.67</td>
<td>7.56</td>
<td>6.74</td>
<td>4.83</td>
<td>1.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.35</td>
<td>5.15</td>
<td>4.62</td>
<td>3.87</td>
<td>2.89</td>
<td>1.63</td>
<td>0.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.64</td>
<td>3.37</td>
<td>3.14</td>
<td>2.85</td>
<td>2.01</td>
<td>0.96</td>
<td>0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.22</td>
<td>2.15</td>
<td>1.97</td>
<td>1.72</td>
<td>1.39</td>
<td>1.01</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table 4.1.3.2 The experimental results of 2 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>21.47</td>
<td>20.69</td>
<td>17.49</td>
<td>10.34</td>
<td>6.67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>15.34</td>
<td>14.97</td>
<td>12.59</td>
<td>10.32</td>
<td>7.43</td>
<td>4.13</td>
</tr>
</tbody>
</table>

<table>
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<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>11.22</td>
<td>10.79</td>
<td>8.63</td>
<td>6.30</td>
<td>3.63</td>
<td>1.03</td>
</tr>
</tbody>
</table>

<table>
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<th>9</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>7.35</td>
<td>7.00</td>
<td>6.12</td>
<td>2.79</td>
<td>0.37</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.51</td>
<td>5.35</td>
<td>4.97</td>
<td>4.47</td>
<td>3.85</td>
<td>2.28</td>
<td>0.50</td>
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</tbody>
</table>

<table>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>4.23</td>
<td>3.93</td>
<td>3.58</td>
<td>3.16</td>
<td>2.10</td>
<td>0.87</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.19</td>
<td>2.12</td>
<td>1.93</td>
<td>1.65</td>
<td>1.30</td>
<td>0.50</td>
<td>0.08</td>
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</table>
Table 4.1.3.3 The experimental results of 3 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>5</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>20.30</td>
<td>19.52</td>
<td>15.54</td>
<td>6.79</td>
<td>2.42</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>16.97</td>
<td>16.44</td>
<td>14.16</td>
<td>11.49</td>
<td>8.27</td>
<td>4.86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>12.24</td>
<td>11.78</td>
<td>9.20</td>
<td>6.43</td>
<td>3.16</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>9.22</td>
<td>8.84</td>
<td>7.74</td>
<td>5.82</td>
<td>3.22</td>
<td>0.52</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.42</td>
<td>4.93</td>
<td>4.47</td>
<td>3.98</td>
<td>2.73</td>
<td>1.20</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.77</td>
<td>3.63</td>
<td>3.26</td>
<td>2.66</td>
<td>1.86</td>
<td>0.98</td>
<td>0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.25</td>
<td>2.17</td>
<td>1.76</td>
<td>1.14</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 4.1.3.4 The experimental results of 5 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>20.38</td>
<td>18.70</td>
<td>17.01</td>
<td>11.64</td>
<td>4.98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>16.69</td>
<td>15.52</td>
<td>12.20</td>
<td>8.04</td>
<td>3.00</td>
<td>1.49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>12.37</td>
<td>11.46</td>
<td>10.34</td>
<td>6.92</td>
<td>2.53</td>
<td>0.58</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>8.55</td>
<td>7.96</td>
<td>7.18</td>
<td>6.09</td>
<td>4.66</td>
<td>2.94</td>
<td>1.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.66</td>
<td>5.08</td>
<td>4.25</td>
<td>3.18</td>
<td>1.88</td>
<td>0.71</td>
<td>0.18</td>
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</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>3.94</td>
<td>3.39</td>
<td>2.82</td>
<td>2.08</td>
<td>1.25</td>
<td>0.48</td>
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</table>

<table>
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<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>1.73</td>
<td>1.58</td>
<td>1.21</td>
<td>0.74</td>
<td>0.40</td>
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</table>
Table 4.1.3.5 The experimental results of 10 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
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<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>20.26</td>
<td>19.61</td>
<td>17.49</td>
<td>14.02</td>
<td>9.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>15.59</td>
<td>14.41</td>
<td>12.87</td>
<td>10.73</td>
<td>8.04</td>
<td>4.96</td>
<td>1.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>12.20</td>
<td>11.57</td>
<td>9.69</td>
<td>7.72</td>
<td>5.00</td>
<td>1.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>6.14</td>
<td>5.87</td>
<td>5.07</td>
<td>3.91</td>
<td>2.68</td>
<td>1.36</td>
<td>0.41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>2.06</td>
<td>1.95</td>
<td>1.68</td>
<td>1.38</td>
<td>1.04</td>
<td>0.70</td>
<td>0.22</td>
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</table>

Table 4.1.3.6 The experimental results of 20 mM p-cresol reacting with 2 LAMU/mL laccase SP 850 at pH = 6.7 and room temperature at different initial DO concentrations

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>17.92</td>
<td>16.23</td>
<td>12.77</td>
<td>8.35</td>
<td>1.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>15.69</td>
<td>12.29</td>
<td>8.54</td>
<td>6.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>8.27</td>
<td>7.63</td>
<td>6.78</td>
<td>5.65</td>
<td>2.70</td>
<td>0.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>5.77</td>
<td>5.43</td>
<td>3.95</td>
<td>1.93</td>
<td>0.56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>4.37</td>
<td>3.96</td>
<td>3.31</td>
<td>2.33</td>
<td>1.37</td>
<td>0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction time, min.</th>
<th>0</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO concentration, mg/L</td>
<td>1.87</td>
<td>1.62</td>
<td>1.22</td>
<td>0.74</td>
<td>0.43</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Table 4.1.3.8 The relationship between initial oxygen consumption velocity and concentrations of oxygen at different concentrations of p-cresol under the catalysis by laccase SP850

The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 1 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.22</th>
<th>3.64</th>
<th>5.35</th>
<th>7.67</th>
<th>11.14</th>
<th>14.54</th>
<th>21.81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.13</td>
<td>0.19</td>
<td>0.12</td>
<td>0.26</td>
<td>0.25</td>
<td>0.15</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 2 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.19</th>
<th>4.28</th>
<th>5.51</th>
<th>7.35</th>
<th>11.22</th>
<th>15.34</th>
<th>21.47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.40</td>
<td>0.48</td>
<td>0.27</td>
<td>0.35</td>
<td>0.27</td>
<td>0.18</td>
<td>0.41</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 3 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.25</th>
<th>3.77</th>
<th>5.42</th>
<th>8.20</th>
<th>12.24</th>
<th>16.97</th>
<th>20.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.03</td>
<td>0.40</td>
<td>0.40</td>
<td>0.51</td>
<td>0.33</td>
<td>0.19</td>
<td>0.49</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 5 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>1.73</th>
<th>3.94</th>
<th>5.66</th>
<th>8.55</th>
<th>12.37</th>
<th>16.69</th>
<th>20.38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.43</td>
<td>0.39</td>
<td>0.80</td>
<td>0.64</td>
<td>0.34</td>
<td>0.68</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 10 mM

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>2.37</th>
<th>3.38</th>
<th>6.14</th>
<th>8.11</th>
<th>12.27</th>
<th>15.59</th>
<th>20.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.52</td>
<td>1.19</td>
<td>1.16</td>
<td>0.70</td>
<td>0.68</td>
<td>0.23</td>
<td>0.09</td>
</tr>
</tbody>
</table>
The relation between concentration of oxygen and reaction velocity at concentration of p-cresol = 20 mM

<table>
<thead>
<tr>
<th>Concentration of Oxygen, mg/L</th>
<th>1.87</th>
<th>4.37</th>
<th>5.77</th>
<th>8.27</th>
<th>12.20</th>
<th>15.69</th>
<th>17.92</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity (mg/L*min)</td>
<td>0.70</td>
<td>0.66</td>
<td>1.29</td>
<td>0.83</td>
<td>0.80</td>
<td>0.60</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Table 4.2.2.1 The relationship between initial oxygen consumption velocity and low concentration of oxygen at concentration of 2 mM m-cresol

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>0.8</th>
<th>1.35</th>
<th>2.02</th>
<th>2.61</th>
<th>3.05</th>
<th>3.19</th>
<th>3.66</th>
<th>3.86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L*min.</td>
<td>0.31</td>
<td>0.30</td>
<td>0.37</td>
<td>0.92</td>
<td>1.17</td>
<td>1.57</td>
<td>2.18</td>
<td>2.39</td>
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Table 4.2.4.1 The relationship between initial oxygen consumption velocity and low concentration of oxygen at concentration of 2 mM p-cresol

<table>
<thead>
<tr>
<th>Concentration of oxygen, mg/L</th>
<th>0.86</th>
<th>1.26</th>
<th>1.64</th>
<th>2.22</th>
<th>2.70</th>
<th>3.62</th>
<th>4.00</th>
<th>4.35</th>
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</thead>
<tbody>
<tr>
<td>Reaction velocity, mg/L*min.</td>
<td>0.56</td>
<td>0.87</td>
<td>1.08</td>
<td>1.11</td>
<td>1.08</td>
<td>1.35</td>
<td>1.52</td>
<td>1.65</td>
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Table 4.2.2.1 Progress curves of 2 mM m-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations.

<table>
<thead>
<tr>
<th>reaction time (min.)</th>
<th>0.0</th>
<th>0.5</th>
<th>0.8</th>
<th>1.0</th>
<th>1.3</th>
<th>1.5</th>
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<td>DO depletion concentration (mg/L)</td>
<td>3.78</td>
<td>2.92</td>
<td>2.18</td>
<td>1.32</td>
<td>0.72</td>
<td>0.52</td>
</tr>
<tr>
<td>reaction time (min.)</td>
<td>3.86</td>
<td>2.98</td>
<td>2.22</td>
<td>1.29</td>
<td>0.67</td>
<td>0.44</td>
</tr>
<tr>
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<td>3.62</td>
<td>2.97</td>
<td>2.32</td>
<td>1.70</td>
<td>1.15</td>
<td>0.65</td>
</tr>
<tr>
<td>reaction time (min.)</td>
<td>3.66</td>
<td>2.96</td>
<td>2.25</td>
<td>1.68</td>
<td>1.09</td>
<td>0.63</td>
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<td>0.85</td>
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<td>0.75</td>
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<td>1.5</td>
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<td>1.99</td>
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<td>1.09</td>
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<td>0.75</td>
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<td>1.5</td>
<td>2</td>
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<tr>
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<td>1.33</td>
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<td>2.66</td>
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<td>1.27</td>
<td>0.86</td>
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<tr>
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<td>1.5</td>
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<tr>
<td>reaction time (min.)</td>
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<td>1.84</td>
<td>1.70</td>
<td>1.56</td>
<td>1.43</td>
<td>1.30</td>
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<td>0.75</td>
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<td>1.5</td>
<td>2</td>
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<tr>
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<td>0.72</td>
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<td>1.25</td>
<td>1.5</td>
<td>2</td>
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<tr>
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<td>0.72</td>
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<td>0.38</td>
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Table 4.2.3.1 Progress curves of 2 mM o-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations.

<table>
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<th>reaction time(min.)</th>
<th>0</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
<th>2</th>
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<tr>
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<tr>
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<td>3.60</td>
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<td>1.25</td>
<td>1.5</td>
<td>2</td>
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<td>1.25</td>
<td>1.5</td>
<td>2</td>
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<td>concentration(mg/L)</td>
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<td>1.25</td>
<td>1.5</td>
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<td>1.89</td>
<td>1.75</td>
<td>1.66</td>
<td>1.42</td>
<td>1.15</td>
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<td>0.75</td>
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<td>1.25</td>
<td>1.5</td>
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<td>0.93</td>
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<tr>
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<td>0.75</td>
<td>1</td>
<td>1.25</td>
<td>1.5</td>
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<td>DO depletion</td>
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<td>0.92</td>
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<td>0.75</td>
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<td>1.25</td>
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<td>1.25</td>
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<td>2</td>
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<td>DO depletion</td>
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<td>0.75</td>
<td>1</td>
<td>1.25</td>
<td>1.5</td>
<td>2</td>
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<td>0.49</td>
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Table 4.2.4.1 Progress curves of 2 mM p-cresol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations.

<table>
<thead>
<tr>
<th>reaction time (min.)</th>
<th>0.00</th>
<th>0.50</th>
<th>0.75</th>
<th>1.00</th>
<th>1.25</th>
<th>1.50</th>
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<tbody>
<tr>
<td>DO depletion concn (mg/L)</td>
<td>4.38</td>
<td>3.76</td>
<td>2.83</td>
<td>2.45</td>
<td>1.79</td>
<td>1.25</td>
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<tr>
<td>reaction time (min.)</td>
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<td>0.75</td>
<td>1.00</td>
<td>1.25</td>
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<td>1.72</td>
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<td>0.75</td>
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<td>0.85</td>
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<td>0.75</td>
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<td>1.25</td>
<td>1.50</td>
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<td>0.75</td>
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<td>0.50</td>
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<td>0.75</td>
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<td>0.75</td>
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<td>1.50</td>
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<td>1.25</td>
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<td>0.65</td>
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<tr>
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<td>0.75</td>
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<tr>
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<td>0.75</td>
<td>1.00</td>
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<td>1.50</td>
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Table 4.2.5.1 Progress curves of 2 mM phenol reacting with 0.0067 LACU/mL laccase SP504 at pH = 5.6 and room temperature at various O₂ concentrations.

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<th>1.5</th>
<th>2</th>
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<td>DO depletion concentration(mg/L)</td>
<td>5.38</td>
<td>5.10</td>
<td>4.14</td>
<td>3.27</td>
<td>2.53</td>
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