REMOVAL OF PHENOL FROM INDUSTRIAL WASTEWATERS
BY HORSERADISH (Cochlearia armoracia L) PEROXIDASE

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Abstract. This work presents a process for phenol removal comprising a reaction step
in which phenol is polymerized in the presence of an enzyme horseradish peroxidase. A
crude preparation from horseradish roots was used as a low purity source of the
enzyme. The technical feasibility of the process was studied using 1 to 10 mM synthetic
phenol solutions. Experimental results showed the potential of the proposed technique.
A phenol conversion higher than 90% was observed at the polymerization process.

Key Words: Wastewaters, Phenol Removal, Horseradish Peroxidase

1. INTRODUCTION
Phenolic contaminants are found in wastewaters of various industries such as petro-
leum refining, coal conversion, plastics, textiles, iron and steel manufacturing as well as
pulp and paper manufacturing [1]. Typical phenol concentrations in these wastewaters
range from 1.0 to 10.0 mM (100 to 1000 mg/L). It is very important to remove phenols
and aromatic compounds from contaminated water before discharge into any natural wa-
ter because of their toxicity to aquatic organisms. The majority of phenols are toxic sub-
stances, some have been classified as hazardous wastes and some are known or suspected
carcinogens [3].

Conventional processes for removal of phenols from industrial wastewaters include
extraction, adsorption on activated carbon, bacterial and chemical oxidation, electro-
chemical techniques, irradiation, etc. All of these methods suffer from serious shortcom-
ings such as high costs, incompleteness of purification, formation of hazardous by-prod-
ucts, low efficiency and applicability to a limited concentration range (these methods are
not suitable for treating moderate to high concentrations of phenols).
As a result, in the 1980s, research projects started to study an alternative treatment method to remove phenols from wastewater [2]. Because of its capacity to catalyse the oxidation of a wide variety of aromatic compounds, enzyme peroxidase isolated from the roots of horseradish (Cochlearia armoracia L.) has been studied as an alternative method to substitute conventional methods [4].

One-electron oxidation of aromatic substrates (AH2) catalyzed by peroxidases is depicted as the following:

\[ \text{E} + \text{H}_2\text{O}_2 \rightarrow \text{E}_i + \text{H}_2\text{O} \quad (1) \]

\[ \text{E}_i + \text{AH}_2 \rightarrow \text{E}_{ii} + \text{AH}^\bullet \quad (2) \]

\[ \text{E}_{ii} + \text{AH}_2 \rightarrow \text{E} + \text{AH}^\bullet + \text{H}_2\text{O} \quad (3) \]

The native enzyme (E) is oxidized by peroxide (H2O2) to an active intermediate enzymatic form called compound I (Ei). Compound I accepts an aromatic compound (AH2) into its active site and carries out its oxidation. A free radical (AH•) is produced and released into solution leaving the enzyme in the compound II (Eii) state. Compound II oxidizes a second aromatic molecule, releasing another free radical product and returning the enzyme to its native state, thereby completing the cycle. The overall peroxidase reaction consists of the reactions described by Equations (1), (2) and (3). Free radicals formed during the cycle diffuse from the enzyme into the bulk solution where they react to form polyaromatic products. These polymers are water-insoluble and may be removed by solid-liquid operations.

The present study focuses on the evaluation of parameters leading to phenol polymerization using crude enzyme preparation from horseradish roots over the phenol concentration range of 1 to 10 mM (0.1 to 1 g/L - typical in wastewaters). Reactions were conducted under the action of PEG, an enzyme protector additive and H2O2 was added from time to time in a semi-batch manner [5].

2. METHODS OF EXPERIMENTAL RESEARCH

Purified catalase from bovine liver (EC 1. 11. 1. 6., 1 5 000 000 U/ml), phenol (M. W. 94.11 g/mol, purity 99%) and 4-aminoantipyrine (98%) were purchased from MERCK, Germany. Polyethylene glycol (M. W. 4 000 g/mol) was purchased from Zdravlje, Leskovac. Hydrogen peroxide (30%) was purchased from Zorka, Šabac. All other chemical used were of analytical grade. Low purity horseradish peroxidase (HRP) was obtained by passing washed horseradish roots (40 g) through a commercial juicer. The obtained juice was extracted by phosphate buffer (pH 7.4) and it was diluted with buffer to 200 ml. Enzyme solution was stored at 4°C and warmed to room temperature immediately prior to use. Colorimetric assays were monitored using UV-VIS spectrophotometer PERKIN ELMER CAMDA 20.
3. ENZYME ACTIVITY ASSAY

Peroxidase activity in enzyme solution was measured before use at 25°C using 4 – AAP and H₂O₂ as substrates. 1 ml enzyme solution was diluted by buffer to 100 ml. The assay mixture contained 0.40 ml enzyme solution, 1.5 ml of 50 mM phenol, 0.75 ml of 24 mM 4 – AAP, 1.5 ml of 1 mM H₂O₂ and 1.85 ml buffer. The volume of this mixture was 6 mL. The HRP active concentration is proportional to the color development rate measured at 510 nm, during a period of time in which the substrate concentration is not significantly reduced. The color development rate during this period was converted to activity using an extinction coefficient of 6.280 M⁻¹cm⁻¹ based on hydrogen peroxide. One unit of enzymatic activity is defined as the amount of enzyme which transforms 1.0 µmol of hydrogen peroxide per minute at 25°C and pH 7.4.

4. PHENOL CONCENTRATION MEASUREMENT

Phenol concentration was measured using a colorimetric assay and the analytic range covers phenol concentrations from 0.03 to 0.12 mM. The assay mixture contained 2 ml of different phenol concentrations, 4 ml of 0.25 M sodium bicarbonate and 0.9 ml of 20.8 mM 4 – AAP. After vigorous mixing 0.9 ml of 83.4 mM potassium ferricyanide was added and mixed again. Samples absorbance was measured at 510 mM, 9 minutes after the ferricyanide addition and converted to concentration using calibration curve.

5. EXPERIMENTAL PROCEDURE

Experiments were carried out in 75 ml beakers at room temperature. Reaction medium was prepared by adding individually certain amounts of phenol, HRP, PEG and H₂O₂ into the phosphate buffer (pH 7.4). The operating conditions are listed in Table 1. The volume of assay mixture was 50 ml.

The reaction was initiated by H₂O₂ which was added discretely in one, two or three aliquots at 30 minutes to assure total phenol conversion. After this time 2 ml of sample was mixed with enzyme catalase to stop the reaction and then was measured phenol concentration.

<table>
<thead>
<tr>
<th>Test</th>
<th>C_integer (mM)</th>
<th>C_integer (mg/L)</th>
<th>Low purity HRP (U/mL)</th>
<th>PEG (g/L)</th>
<th>H₂O₂ (mM)</th>
<th>Aliquots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>94</td>
<td>0.06</td>
<td>0.08</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>188</td>
<td>0.07</td>
<td>0.14</td>
<td>2.2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>376</td>
<td>0.12</td>
<td>0.27</td>
<td>4.4</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>565</td>
<td>0.20</td>
<td>0.39</td>
<td>6.6</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>8.0</td>
<td>753</td>
<td>0.31</td>
<td>0.52</td>
<td>8.8</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>941</td>
<td>0.44</td>
<td>0.64</td>
<td>11.0</td>
<td>1</td>
</tr>
</tbody>
</table>
6. Results and Discussion

Experiments were designed to achieve conversion of at least 90% of phenol initially present in the synthetic wastewater. The results are summarized in Table 2.

Table 2. Phenol Conversion

<table>
<thead>
<tr>
<th>Test</th>
<th>C&lt;sub&gt;Ph&lt;/sub&gt; Initial (mM)</th>
<th>C&lt;sub&gt;Ph&lt;/sub&gt; Final (mg/L)</th>
<th>Phenol conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>0.011</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>0.003</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>0.005</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>0.002</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>8.0</td>
<td>0.012</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>0.009</td>
<td>99</td>
</tr>
</tbody>
</table>

Phenol conversion in all experimented conditions was greater than 90%. The high efficiency observed is in accordance with conditions optimized to guarantee 90% polymerization using purified HRP. Crude enzyme preparation is protected from inactivation due to the significant quantity of proteinaceous matter present.

The reactor residence time is one of the main parameters that determine the economics of an enzymatic process. A low enzyme concentration will decrease materials costs but will increase the reactor residence time needed to obtain the same level of phenol separation. This would lead to the use of larger reactors to treat the same wastewater flow rate. Clearly, there is a compromise between the reduction in variable costs by the use of less enzyme and the increase in capital investment at the time of building the treatment facility.

The composition of the wastewater leaving the enzymatic treatment unit must also be considered. The residual organic content of the effluent is of concern, especially since the process involves the addition of crude enzyme preparation and PEG, organic bearing compounds with high carbon contents. These compounds plus the organic reagents in excess, residual phenol and soluble byproducts of the reaction, can adversely impact the aquatic environment due to their high oxygen demand. However, all experiments showed that PEG used at the minimum dose is completely removed with the phenolic compound.

7. CONCLUSIONS

The water pollution is very important problem in recent years. There are aromatic compounds in wastewaters of various industries and that compounds must be removed before discharge of wastewaters in native waters. One way of their remove is using peroxidase enzyme. This enzyme can be isolate from horseradish roots and then use for polymerization of aromatic compounds. This way has significant advantages comparing to conventional methods such as broad substrate specificity, effectiveness over a wide range of operating conditions including pH and temperature. Due to experimental results can be seen that phenol conversion in synthetic wastewaters is almost complete.
REFERENCES


UKLANJANJE FENOLA IZ INDUSTRIJSKIH OTPADNIH VODA PEROKSIDAZOM IZ RENA (Cochlearia armoracia L)

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Ovaj rad prikazuje proces uklanjanja fenola iz sintetičkih otpadnih voda koji uključuje reakciju polimerizacije fenola u prisustvu enzima peroksidaze iz rena (Cochlearia armoracia L). Sirovi preparat dobijen iz korena rena je korišten kao izvor enzima niske čistoće. Tehničke mogućnosti ovog procesa su proučavane upotrebom sintetičkog rastvora fenola koncentracije 1-10 mM. Eksperimentalni rezultati pokazuju potencijal predložene tehnike. Konverzija fenola veća od 90% je dobijena u ovom procesu polimerizacije.

Ključne reči: Otpadne vode, uklanjanje fenola, peroksidaza iz rena