The comparison of Coprinus cinereus peroxidase enzyme and TiO$_2$ catalyst for phenol removal

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The comparison of *Coprinus cinereus* peroxidase enzyme and TiO$_2$ catalyst for phenol removal

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This article investigates phenol removal from an aqueous solution by using enzymatic and photocatalytic methods and the efficiency of these methods has been compared. In enzymatic and photocatalytic methods, *Coprinus cinereus* peroxidase enzyme and commercial TiO$_2$ powders (Degussa P-25) in aqueous suspension were used, respectively, in ambient temperature. The effects of different operating parameters such as duration of process, catalyst dosage or enzyme concentration, pH of the solution, initial phenol concentration and H$_2$O$_2$ concentration on both processes were examined. In enzymatic method, efficiency of degradation reached 100% within 5 min, while in the photocatalytic method, the efficiency of degradation reached approximately 70% within 60 min. In photocatalytic method, there is an optimum concentration for catalyst dosage (near 2.0 g/L) to gain 80% efficiency, while in the enzymatic method, increasing the amount of enzyme could lead to an increase in the efficiency up to 100%. Moreover, the optimum pH in enzymatic and photocatalytic methods stood at 8.0 and 7.0, respectively. In both methods, the addition of different amounts of H$_2$O$_2$ increased the degradation efficiency to 100%.

**Keywords:** Phenol removal, enzymatic method, *Coprinus cinereus* peroxidase, photocatalytic method, TiO$_2$, H$_2$O$_2$.

**Introduction**

The phenolic compounds, present in industrial wastewater, are produced from processes such as oil refining, resin plastic processing and agriculture production, which cause severe environmental problems. Therefore, the removal of phenols from industrial aqueous effluents are of great practical significance.$^{[1-3]}$ Nowadays, several treatment methods such as solvent extraction, adsorption on activated carbon, chemical oxidation and biodegradation methods have been applied to these compounds.$^{[5]}$ These methods have been categorized in two majors classifications which are: a) separation methods of phenol from water solutions including steam distillation, extraction, adsorption, membrane pervaporation, membrane-based solvent extraction; and b) destruction of phenol in water solution by total oxidation of phenol (using air or oxygen), wet oxidation with chemical oxidants, electrochemical oxidation, photocatalytic oxidation, supercritical water gasification, application of electrical discharges to degradation of phenol, biochemical abatement, and combination of above-mentioned technique.$^{[3]}$

Due to the biocidic nature of phenolic compound, most phenols cannot be completely degraded during common wastewater treatment, and the presence of phenols in high concentrations may be detrimental to live microbial systems.$^{[10]}$ As a key to solve this problem, application of new methods such as enzymatic and photocatalytic treatment (both only generated within the past 30 yrs) has been recommended for treatment of streams containing hazardous or xenobiotic organic pollutants. Peroxidases are one of the enzyme groups that were proposed for enzymatic method in phenol treatment.

The principle of enzymatic treatment using peroxidase is that phenols in the presence of H$_2$O$_2$ are oxidized and reformed to the corresponding radicals, and spontaneously react to form insoluble polymers after a couple of minutes.$^{[1,2,4]}$ As a completion step of the treatment process, the insoluble polymer will be separated by some conventional separating methods. The idea of using peroxidase, laccase and/or tyrosinase enzymes for the removal of phenols from wastewater solution was first introduced in the 1980s and has been employed to date.$^{[1,2]}$

Microbial peroxidase, among other enzymes, are more absorbing because of their sources,$^{[5]}$ as most of the peroxidase such as HRP are sourced from plant materials.$^{[2]}$ The
Peroxidase enzyme and TiO$_2$ catalyst for phenol removal

Table 1. Parameter of enzymatic research on phenols by use of peroxidases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CIP (this research)</th>
<th>HRP$^{[7,8]}$</th>
<th>SBP$^{[9,10]}$</th>
<th>CMP$^{[11]}$</th>
<th>ARP$^{[12]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Enzyme concentration (U/mL)</td>
<td>1.07–5.36</td>
<td>5.36</td>
<td>2.1–18.8</td>
<td>—</td>
<td>0.01–1</td>
</tr>
<tr>
<td>H$_2$O$_2$ Conc. (mM)</td>
<td>0.55–2.2</td>
<td>1/1</td>
<td>—</td>
<td>1/1</td>
<td>0.1–1.5</td>
</tr>
<tr>
<td>Phenol initial Conc. (mg/L)</td>
<td>100–500</td>
<td>500 till 940</td>
<td>188</td>
<td>9.4–940</td>
<td>—</td>
</tr>
</tbody>
</table>

purification, crystallization and structural characterization of *Coprinus cinereus* peroxidase (CIP) as microbial was first reported by Morita et al.$^{[1,5]}$ There are only a few researches conducted on the application of CIP removal, among which the works of Kauffmann et al.$^{[1]}$ and Medusa et al.$^{[2]}$ can be mentioned. However, mentioned researches showed good potential for the use of this method as an industrial method.$^{[6]}$ The results of some researches in reaction parameters by use of peroxidase are illustrated in Table 1.

Another method that has been of interest for around 30 yrs is chemical treatment of photocatalytic. Many researchers investigated and improved this method for industrial applications of photocatalytic treatment. The commercial Degussa TiO$_2$ P-25 powder (containing both types of anatase and rutile particles, 80% and 20%, respectively) has been used as standard TiO$_2$ material in the studies of photocatalytic reactions. Researchers have concluded that there is synergetic effect between contacting anatase and rutile particles. The studies also illustrated that TiO$_2$ powders with both anatase and rutile structures have higher concentration in photocatalytic reactions.$^{[13]}$

In many researches, the operational parameters such as initial concentrations of organic pollutants, pH, and UV light intensity have been widely investigated.$^{[1,2,14,15]}$ The main mechanism of this method is the generation of hydroxyl radicals which can remove approximately all compounds except some principle, simple and refractory compounds like acetic acid, maleic acid, oxalic acid, acetone, as well as some chlorinated compounds such as chloroform.

In this article, the ability of both enzymatic and photocatalytic methods on phenol removal were investigated, the operational parameters of each method were studied and the results were finally compared.

Materials and methods

**Chemicals**

Polypepton and yeast extract were products of Sigma Co. (USA) and Wako Pure Chemical Co. (Japan), respectively. Phenol, 4-aminoantipyrine and Triton X-100 were purchased from Merck Chemicals Co. (Germany). All other reagents were of analytical grade available for commercial use. For photocatalytic process, the chemicals like H$_2$O$_2$, NaOH, HCl, etc. were purchased from Merck Chemicals Co. (Germany). Titanium dioxide (P-25, ca. 70% anatase and 30% rutile), with a BET surface area of 55.0 m$^2$.g$^{-1}$ and an average particle size of 30.0 nm was obtained from Degussa Co. (Germany).

**Peroxidase preparation**

The peroxidase was produced using *Coprinus cinereus*, which was extracted from rice stems (infected by Urea). The spore suspension was prepared by adding 10 mL of medium into the slant culture (7 mL) and vibrating it for 30 s, and then was inoculated into a 500-mL baffled Erlenmeyer flask containing 100 mL of liquid medium (18 g/L glucose, 5 g/L polypepton, 3 g/L yeast extract, pH 7.0). The cultivation was then performed with rotational shaking (150 rpm) under aerobic conditions at 35°C for 7 days. The supernatant from the broth was mainly used as the enzyme solution in this study. It was confirmed that the purified enzyme had a specific concentration of 350 U/mg.

**Phenol polymerizing reaction catalyzed by *Coprinus cinereus* peroxidase (CIP)**

Phenol polymerizing reactions with CIP and hydrogen peroxide were carried out in 100 mL of 100 mM phosphate buffer (pH 4–11). First, a solution containing the desired concentration of phenol (1.06–5.3 mM) and CIP (1.07–5.36 U/mL) was incubated at room temperature for 2–5 min. Second, the reaction was initiated by adding hydrogen peroxide to the solution. Finally, the reaction mixture was stirred continuously with a magnetic stirrer. Periodically 1.0 mL aliquot was withdrawn and the reaction was halted by adding 33.3 mL of 3.1 M sodium azide.
Photocatalytic degradation experiments

Photo-degradation experiments were conducted in a batch reactor as shown in Figure 1. This small-scale system contained a cylindrical Plexy-glass cell with 2.0 L capacity (80 mm i.d. and 400 mm height) and a 15 W UV-C lamp was placed in the center of the reactor. The reactor was filled with 1.0 L of phenol solution (0.53–1.06 mM), with Degussa TiO\textsubscript{2} P-25 powders (0–4.0 g/L) and/or H\textsubscript{2}O\textsubscript{2} (2.32–69.6 mM). The pH value of aqueous solution was adjusted by adding a small amount of 0.1M NaOH or HCl. The suspended solution was stirred by a sparger system to maintain an aerobic environment (the compressed air was purged into the solution through bubbles).

All experiments, with or without H\textsubscript{2}O\textsubscript{2}, were performed under similar conditions with a phenol concentration of 1.06 mM and solutions were irradiated by UV–Vis light. Photocatalytic degradation was conducted up to 180 min and the liquid samples (10.0 mL) were withdrawn every 30 min. For TiO\textsubscript{2} powders present in the system, the samples were stirred with a centrifuge (5000 rpm) in 60 min to precipice the TiO\textsubscript{2}, then the remaining particles were removed before the phenol concentration was measured.

Analytical methods

Peroxidase concentration

The enzyme concentration was assayed under the following conditions. A reaction mixture containing 65 mM phosphate buffer (pH 7.0), 0.63 mM 4-aminoantipyrine (4-AAP), 10 mM phenol, 3.1 mM hydrogen peroxide and 0.97 g/L Triton X-100 with total volume of 3.1 mL was incubated at 37°C for 10 min. The reaction was started by addition of 0.1 mL of diluted enzyme solution. The initial increase in absorbance of spectrophotometric (Spectronic Co., Germany) was monitored at 500 nm during 1 min. One unit of peroxidase concentration was defined as the amount of the enzyme consuming 1 mM of hydrogen peroxide per min under the assay conditions.

Phenol concentration

Preparation of two solutions was necessary prior to analyzing the concentration of residual phenol. Solution A containing 90 mg of 4-aminoantipyrine was solved in 200 mL of carbonate-bicarbonate buffer and solution B including 2.6 g of boric acid and 3.8 g of potassium ferrocianide.
Results and discussion

Effect of enzyme concentration and TiO$_2$ dosage on phenol degradation

Figure 2 illustrates the enzyme concentration and its influence on phenol removal at 25°C, neutral pH of 7.0, initial phenol concentration of 1.06 mM and H$_2$O$_2$ concentration of 1.1 mM. As shown, when the enzyme concentration increased from 0.35 U/mL to 1.65 U/mL, the removal percentage also increased almost by a linear trend from 41% to 95%; an increase to 2.14 U/mL and more increased the removal percentage to 100%. This result showed that the sufficient amount of enzyme for this system was 1.9 U/mL, approximately.

In photocatalytic method, catalyst dosage is an important parameter in suspended system of photocatalytic degradation.[16] As shown in Figure 3, various amounts of P-25 TiO$_2$ (0–4.0 g/L) were used to mix with 1.06 mM of phenol solution at pH 7.0 within 180 min. It was observed that when TiO$_2$ dosage increased from 0.2 to 2 g/L, the phenol photo-degradation increased from 58% to 75%. Conversely, more increase in TiO$_2$ dosage resulted in a decrease in the phenol removal to 60%.

In catalytic methods, the number of active sites, which is highly related to the adsorption of reactant (organic pollutants) on catalyst surface, is very important; in photocatalytic method, the catalyst dosage is strongly affected by the treatment efficiency.[17] Moreover, there are two phenomena of electron/hole pairs generation rate and the light penetration effecting photocatalytic treatment. Increasing the catalyst amount results in raising the generation rate of electron/hole pairs which consequently results in the enhancement of the formation of OH radicals. Nevertheless, this trend does not prove stable, as by the addition of more catalyst, due to saturation of solution, the photo rays will be reflected by excess catalyst and will not have efficient performance; therefore, an optimum amount of catalyst in each reactor proves necessary.[18]

Effect of H$_2$O$_2$ concentration on phenol degradation

The effect of hydrogen peroxide concentration was examined at 25°C and pH 7.0 during the initial phenol concentration of 1.06 mM. As shown in Figure 4, the efficiency of degradation was increased by increasing the concentration of phenol removal at 25°C, pH 7.0, phenol initial conc. 1.06 mM, H$_2$O$_2$ conc. 1.1 mM, CIP conc. 1.07–5.36 U/mL) (color figure available online).
Fig. 5. Effect of the H$_2$O$_2$ concentration on phenol degradation by photocatalytic method at 25°C using H$_2$O$_2$/UV process (phenol initial conc. 1.06 mM, pH 7.0) (color figure available online).

The effect of H$_2$O$_2$ and the efficiency almost reached 100% at 2.2 mM of concentration.

In photocatalytic method, the combination of H$_2$O$_2$ with UV irradiation has been most exhaustively investigated and the applied technology has been concerns as an advanced oxidation process (AOPs). Adding H$_2$O$_2$ causes an increased reaction speed since H$_2$O$_2$ prevents the recombination of h$^+$ and e$^-$ and also produces more hydroxyl radicals. On the other hand, H$_2$O$_2$ prevents the regrowth of microbial components in sterilization effluent. In contrast, adding too much H$_2$O$_2$ reduces the penetrative function of H$_2$O$_2$ and decreases the total speed of reaction. Figure 5 shows the effect of H$_2$O$_2$ concentrations in the range of 2.32–69.6 mM on phenol photodegradation at pH 7.0 using H$_2$O$_2$/UV process.

It is evident that the degradation increased with addition of H$_2$O$_2$ concentration. The addition of H$_2$O$_2$ from 2.32 mM to 9.28 mM leads to an increase in the removal efficiency from 45 to 55% within 3 h. However, phenol is completely degraded within 2–3 h when H$_2$O$_2$ concentration is raised up to 46.4 and 69.6 mM, respectively.

Time course of phenol removal by enzymatic and photocatalytic process

Figure 6 shows the time courses of phenol removal under constant initial conditions (the initial concentrations of phenol, hydrogen peroxide and CIP were 1.06 mM, 1.1 mM and 1.07 U/mL, respectively) in enzymatic process. As shown in this figure at min 5, the phenol removal reached the maximum value (~80%) and then the degradation remained almost constant up to min 55. These results could be compared with the similar phenomenon found by Masuda et al. Although 80% phenol removal is an acceptable amount in this field of study, but it could be enhanced with addition of excess enzymes (CIP) and hydrogen peroxide to obtain complete phenol removal.

Peroxidase enzyme can be inactivated by several mechanisms, for instance, (I) switching of hydrogen peroxidase reactions to intermediates of the enzyme’s catalytic cycle, (II) irreversible reactions between the enzyme and phenol or phenoxy radicals formed by one-electron oxidation of phenolic substrates during the catalytic cycle and (III) inaccessibility of pollutant to enzyme active site due to covering enzyme by final production of degradation process. Therefore, to obtain an optimized percentage of phenol removal, the optimization of corresponding parameters is necessary.

Figure 7 shows the effect of time on phenol removal in photocatalytic process in initial conditions (phenol concentration of 1.06 mM, Lamp: 15-W UV-C). In Direct photolysis with 15-W UV-C alone, the phenol removal percentage was 38%. After 180 min, by adding 9.28 mM H$_2$O$_2$, the degradation increased to 54%. The efficiency of 72% was obtained when 2 g/L of TiO$_2$ was added to the solution and treated under 15-W UV-C irradiation. Finally the combination of the last process (9.28 mM H$_2$O$_2$, 2.0 g/L TiO$_2$, 15-W UV-C system) improved phenol removal up to 85%. Comparing Figure 6 and Figure 7 on phenol removal using enzymatic and photocatalytic methods, it is observed that the required time for enzymatic method (5 min) is much less than photocatalytic process (180 min).

Effect of pH on phenol removal

To clarify the effect of pH on enzyme ability and to reveal the applicability of this method, 1.07 U/mL of enzyme concentration was employed. Phenol and hydrogen peroxide concentrations were fixed at 1.06 mM and 1.1 mM, respectively, and the reactions were performed at 25°C and in 5 min.

The relation between the removal efficiency and the pH is demonstrated in Figure 8(a). As shown, the optimum pH was around 9.0 under the reaction condition. This result

![Fig. 6. Effect of time on phenol removal by enzymatic method (Temp. 25°C, pH 7.0, phenol initial conc. 1.06 mM, H$_2$O$_2$ conc. 1.1 mM, CIP conc. 1.07 U/mL) (color figure available online).](image-url)
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Indicates that the enzyme shows its maximum concentration in alkaline pH, although other works have shown that by addition of more enzyme, the optimum pH will improve to a wider pH range.$^{[1,2]}$

Figure 8(b) shows the effect of solution pH on phenol photo-degradation (phenol initial concentration 1.06 mM) in the TiO$_2$/UV system. The pH of the solution is closely related to the surface charge of catalyst, flat band potential and dissociation of the solution to obtain the optimum pH for degradation of pollutants. In the case of Degussa P-25 TiO$_2$, the point of zero charge (pH$_{pzc}$) is between 6.25 and 6.60.$^{[22,23]}$ As shown in Figure 8(b), the optimum amount of pH in this process at above condition is about 7.0, with this result being close to the inherent characteristic of TiO$_2$ Degussa P-25.

**Effect of the initial phenol concentration on phenol degradation**

The range of phenol concentration was 1.06–5.3 mM and the enzyme concentration, pH, and H$_2$O$_2$ concentration...
were 1.07 U/mL, 7.0, and 1.1 mM, respectively. The time of reaction, according to the previous experiment, was set to 5.0 min. As shown in Figure 9(a), by increasing the phenol initial concentration from 1.06 mM to 5.3 mM, the efficiency of phenol removal decreases from 70 to 4%. This occurrence is due to the insufficient enzyme as mentioned above. By addition of phenol concentration, the enzyme concentration should be increased dramatically to reach the same efficiency.

In photocatalytic method, the photo-degradation of phenol at different initial concentrations (phenol initial conc. 0.53–1.06 mM) in the TiO2/UV system are compared in Figure 9(b). At a phenol concentration of 0.53 mM and after 180 min, about 70% of the initial phenol was degraded. By increasing the phenol concentration to 0.74 mM of phenol, the degradation efficiency reached to 65%, by increasing to 1.06 mM initial phenol, the degradation efficiency reached 43%. The results show that photocatalytic oxidation is appropriate for only low organic pollutant concentrations.

Conclusion

The present work deals with the enzymatic (by Coprinus cinereus peroxidase) and photocatalytic (using TiO2 particles) methods on phenol removal from aqueous solution and their operational parameters. There are some similarities on parameters such as the amount of enzyme (or catalyst) to increase the reaction speed and concentration, H2O2 as an oxidant agent and enhancer in the reaction, reaction time and pH. However, these parameters have different effects in each treatment method. In case of catalyst, as the results have been shown in photocatalytic method, there is an optimum amount which is 2.0 g/L of TiO2, while in enzymatic method by adding more enzymes up to 2.2 U/mL, the amount of phenol degradation increased to 100%.

As for the time of the process, the results show that at 5.0 min, in enzymatic method the degradation efficiency reached its maximum amount of almost 100% (pH 7.0, phenol initial conc. 1.06 mM, H2O2 conc. 1.1 mM, CIP conc. 2.2 U/mL), but in photocatalytic, it takes nearly 60 min to reach the 70% of degradation (phenol initial conc. 1.06 mM, pH 7.0, 2.0 g/L TiO2, 9.28 mM H2O2). The effect of H2O2 concentration indicates that in both methods the degradation occurred faster and with lower amount of enzyme concentration and photocatalyst amount. The optimum pH of reaction for both methods is in the range of 7.0–8.0. The results indicate that for a given phenol concentration, enzymatic method has higher degradation efficiency in benign reaction condition in comparison with photocatalytic method.

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