Use of Chitosan for Removal of Naphthols through Tyrosinase-Catalyzed Quinone Oxidation

Yuji Kimura,[1] Ayumi Kashiwada,[1] Kiyomi Matsuda,[2] Kazunori Yamada[1]

[1]*Department of Applied Molecular Chemistry, College of Industrial Technology, Nihon University, Narashino, Chiba 275-8575, Japan*
[2]*Department of Sustainable Engineering, College of Industrial Technology, Nihon University, Narashino, Chiba 275-8575, Japan*

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ABSTRACT: In this study, the combined use of chitosan and mushroom tyrosinase was applied to remove 1-naphthol and 2-naphthol from aqueous solutions. In particular, the process parameters, such as the pH value, temperature, and enzyme dose, were discussed for tyrosinase-catalyzed quinone oxidation of 1-naphthol. The optimum conditions of enzymatic quinone oxidation of 1-naphthol were determined to be pH 8.0 and 40°C. Under the optimum conditions, quinone oxidation of 1-naphthol increased with an increase in the enzyme dose. Quinone derivatives enzymatically generated were chemisorbed on chitosan bead sand. The initial velocity of enzymatic quinone oxidation increased with an increase in the amount of added chitosan beads, since unfavorable interactions between quinone derivatives and tyrosinase in the solutions were restrained by quinone adsorption. 1-Naphthol was completely removed for 8 h by quinone adsorption on chitosan beads (0.10 cm^3/cm^3) at 20 U/cm^3. The removal time was shortened by increasing the enzyme dose or the amount of added chitosan beads. 2-Naphthol was also completely removed by increasing the enzyme concentration and reaction time, since enzymatic quinone oxidation of 2-naphthol was much slower than that of 1-naphthol. The above results reveal that the procedure constructed in this study was an effective technique to remove naphthols. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: biopolymers; adsorption; enzymes; UV–vis spectroscopy; surfaces

INTRODUCTION

Contamination of surface water and groundwater with aromatic compounds is one of the most serious environmental problems that we have faced recently. Of them, removal of naphthols, $C_{10}H_7OH$, should be placed at the top priority of tasks, because toxicity of naphthols is relatively high and their biodegradation is low. Naphthols were often discharged from industries related with medicine, dyestuff, photograph, and agrochemicals.[1,2] In addition, 1-naphthol is a major component of the pesticide napropamide and a principal reaction product in the hydrolysis of the pesticide carbaryl.

Many articles have been published on removal of naphthols by chemical procedures such as adsorption on polymeric materials,[3–5] biochar,[6] bentonite,[7] radiolysis,[8] photolysis,[9–11] and biodegradation.[12] However, these conventional procedures suffer from high cost, low efficiency, formation or remaining of hazardous by-products, and applicability to a limit concentration range. Therefore, enzymatic treatments have been more frequently used as an alternative procedure. In fact, oxidoreductases such as peroxidase[13,14] and tyrosinase[15,16] have been used to catalyze detoxication or transformation of bisphenol A (BPA) and its derivatives[13,14,16] as well as alkylphenols and chlorophenols.[15,17] Tyrosinase has the catalysis to convert phenolic compounds into the corresponding quinone derivatives. Since quinone derivatives can react with amino group-containing polymers such as chitosan and polyethyleneimine, water-insoluble aggregates were formed and settled down.[18]

We have focused attention on enzymatic quinone oxidation in the presence of porous chitosan beads. Various phenolic compounds were removed in short reaction times through nonenzymatic quinone adsorption on chitosan beads.[15–17] As described above, many studies have been reported on removal of various phenol compounds.[13–17] In addition, some studies were also reported on enzymatic treatment of naphthols.[1,2,19,20] But, little was reported on tyrosinase-catalyzed quinone oxidation of naphthols and their removal.

In this study, we studied removal of 1-naphthol through the tyrosinase-catalyzed quinone oxidation and subsequent quinone adsorption on chitosan

Correspondence to: K. Yamada (yamada.kazunori@nihon-u.ac.jp).

beads. First, the effects of the pH value, temperature, and enzyme dose on tyrosinase-catalyzed quinone oxidation were investigated to determine the optimum conditions. In addition, removal behavior of 1-naphthol through quinone adsorption on chitosan beads was estimated in comparison with that with chitosan solutions in the homogeneous system and chitosan powder in the heterogeneous system. Finally, the procedure constructed in this study was applied to removal of 2-naphthol as a regioisomer of 1-naphthol.

EXPERIMENTAL

Materials

Mushroom tyrosinase (EC 1.14.18.1) was purchased from Sigma (St. Louis, MO).The specific activity of tyrosinase used was 3960 U/mg solid. A chitosan bead (particle size, 70–210 μm; specific surface area, 70–100 m^2/g; water content, 92.5%) was purchased from Fuji Spinning (Tokyo, Japan) and had the density of amino groups at 0.25 $mmol/cm^3$ resin in water. These values were obtained from the manufacturer. The chitosan beads were stored in phosphate buffers at pH 6.0–10.0 before use. Chitosan powder (diameter, 74–100 μm) was supplied from Dainichiseika Color & Chemicals Mfg. Co. (Tokyo, Japan). 1-Naphthol and 2-naphthol were purchased from Wako Chemical Industry (Tokyo, Japan).

Quinone oxidation and removal of 1-naphthol

Phosphate buffers (ionic strength: 0.01*M*) were prepared with $NaOH/KH_2PO_4$ at pH 6.0–10.0. Stock solutions of 1-naphthol at 0.40 m*M* and mushroom tyrosinase at 100 U/cm^3 were prepared in the buffers at pH 6.0–10.0. The enzymatic reaction was initiated by adding tyrosinase to a 1-naphthol solution (30 cm^3) in a 50 cm^3 Erlenmeyer flask at 20–60°C. The initial concentration of each component in the solutions was [1-naphthol] = 0.30 m*M* and [tyrosinase] = 20 U/cm^3 unless otherwise noted. The solutions were continuously stirred during the enzymatic reaction and the absorbance was measured at predetermined time intervals on a Shimadzu UV–visible recording spectrophotometer UV-260. For removal experiments, a given amount of chitosan beads stored in a buffer were added to 1-naphthol solutions with a bullet for titration, and then the enzymatic reaction was initiated by adding tyrosinase. Alternatively, chitosan powder was dispersed in a 1-naphthol solutions containing tyrosinase. In addition, removal of 2-naphthol was estimated by combined use of chitosan beads and tyrosinase under the optimum conditions determined for 1-naphthol.

Quantitative assay of naphthols

The concentration of naphthols in the solutions was determined by a Hitachi L-7000 HPLC system equipped with a UV spectrophotometer and an integrator. A reverse phase column, Inertsil ODS-3 (5 μm, 4.6 mm i.d. × 15 cm), was used.[15–17] The aliquots (0.3 cm^3) taken from the reaction solutions were immersed in hot water at 80–85°C for 5 min to deactivate tyrosinase and centrifuged at 4000 rpm for 10 min. Then, 20 mm^3 of solutions were injected to the HPLC system and an aqueous acetonitrile solution of 45 vol % was flowed at 1.0 cm^3/min as a mobile phase. The absorption spectra were measured at 295 and 274 nm and the retention times were 8.5 and 7.5 min for 1-naphthol and 2-naphthol, respectively. Since a good linear relationship was obtained between the concentration and the peak intensity for both 1-naphthol and 2-naphthol, the conversion % values were calculated from the peak areas before and after enzymatic treatment.

Instrumental analysis of chitosan/quinone reaction

A chitosan solution (ca. 1 w/v%) was prepared by the same procedure described in our previous article.[16,17] Chitosan solution of 5.0 g was pipetted into a Petri dish of 3.2 cm in diameter and the solution was allowed to dry at 50°C. The chitosan films (average thickness: 24 μm) were thoroughly washed with 1*M* NaOH and pure water to neutralize the amino groups on the surfaces, and then dried under reduced pressure.[16,17,21]

Chitosan films were incubated in 1-naphthol solutions (0.30 m*M*) containing tyrosinase (5 and 10 U/cm^3) at pH 8.0 and 40°C for 24 h. After incubation, the chitosan films were washed with water and dried under reduced pressure. The incubated chitosan films were fixed in the chamber of the spectrophotometer such that the light perpendicularly passed through the films and the UV–visible spectra were recorded at 200–600 nm.

Homogeneous system with chitosan solution

As a control experiment, removal of 1-naphthol was also estimated in a homogeneous system. Chitosan solutions were added to 1-naphthol solutions at pH 6.0 containing tyrosinase (20 U/cm^3) so as to reach the final amino group concentration from 0.015–0.9 m*M* ([—NH_2]/[1-naphthol] = 0.05–3.0), and the solutions were moderately stirred at 40°C for 24 h. Here, the pH value of the solutions was adjusted to 6.0. After stirring, the solutions were filtered with a 5C filter paper to remove aggregates generated, and then the UV–visible spectra of the filtrates obtained were measured.[17,21]

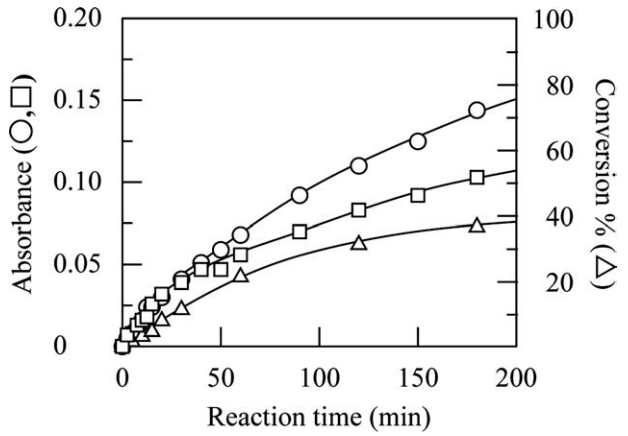

Figure 1 The time course of the absorbances at 520 nm before (○) and after (□) centrifugation and conversion % value (△) for tyrosinase-catalyzed (5 U/cm^3) quinone oxidation of 1-naphthol at pH 8.0 and 40°C.

RESULTS AND DISCUSSION

Tyrosinase-catalyzed treatment of 1-naphthol

When tyrosinase was added to a 1-naphthol solution, the solution turned blue and a peak emerged at 520 nm. Figure 1 shows the time course of the absorbance and conversion % value of tyrosinase-catalyzed (5 U/cm^3) quinone oxidation of 1-naphthol (0.30 m*M*) at pH 8.0 and 40°C. Both absorbance and conversion % value increased over the reaction time and the conversion % value attained 45.7% at 5 h. During the enzymatic reaction, a small amount of blue precipitates were generated during the enzymatic reaction. Therefore, the aliquots taken from the reaction solution were centrifuged and the absorbances of the supernatants obtained were measured at 520 nm. For the first 20 min after the enzymatic reaction was initiated, no difference in the absorbances was observed before and after the centrifugation. This indicates that the precipitates were little observed in the solutions. Thereafter, the difference in both absorbances gradually increased due to the formation of precipitates. Other articles have reported that the oligomers was considered to be generated through self-polymerization, or coupling reaction, of quinone derivatives.[22–24] The formation of quinone oligomers is considered to depend strongly on the experimental conditions such as the chemical structure of the starting materials, rate of formation of quinone derivatives, solubility of quinone derivatives generated in water, concentration of enzyme, and so on. In our previous articles on quinone oxidation of alkylphenols,[15,17] chlorophenols,[17] and BPA[16] by mushroom[17] or *Aspergillus oryzae* tyrosinase,[15,16] no water-insoluble oligomers were generated during the enzymatic reaction. The enzyme concentration dependence on the generation of water-insoluble oligomers was discussed in more detail in the following section.

Determination of optimum conditions

The effects of the pH value and temperature on tyrosinase-catalyzed treatment of 1-naphthol were estimated at10 U/cm^3. First, 1-naphthol was quinone-oxidized by tyrosinase at 40°C in buffers of different pH values. The initial velocity of tyrosinase-catalyzed quinone oxidation was calculated from the slope of the straight line of the concentration of remaining 1-naphthol determined by the HPLC measurements against the reaction time in the initial stage. Figure 2 shows the changes in the initial velocity and the absorbance at 520 nm and conversion % value at the reaction time of 5 h with the pH value at 40°C. At pH 6.0 and 7.0, the conversion % values became almost constant after the enzymatic reaction for 2 h. On the other hand, the conversion % value increased over the reaction time in the range of the pH values from 8.0 to 10.0. Since the initial velocity of quinone oxidation and conversion % value had the maximum at pH 8.0, this value was determined to be as the optimum pH value.

Subsequently, the effect of the temperature on tyrosinase-catalyzed quinone oxidation of 1-naphthol was investigated at the optimum pH value of 8.0. Figure 3 shows the changes in the initial velocity and the absorbance and conversion % value at the reaction time of 5 h with the temperature. Tyrosinase-catalyzed quinone oxidation of 1-naphthol increased with an increase in the temperature and had the maximum value at 40°C. At temperatures higher than 40°C, tyrosinase was gradually deactivated probably due to thermal denaturation. A decrease in the enzymatic activity at temperatures higher than the optimum temperatures was observed for quinone oxidation of *p*-cresol and BPA by mushroom or *Aspergillus oryzae* tyrosinases.[15–17] Therefore, the optimum temperature was determined to be 40°C. It was concluded from the above results that the optimum conditions were pH 8.0 and 40°C.

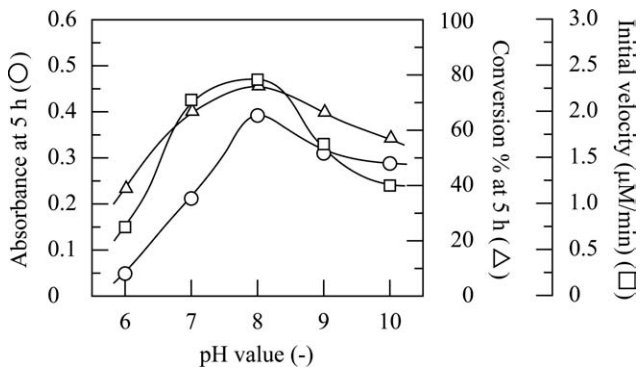

Figure 2 The effect of pH value on tyrosinase-catalyzed (10 U/cm^3) quinone oxidation of 1-naphthol at 40°C. The enzymatic reaction time was 5 h.

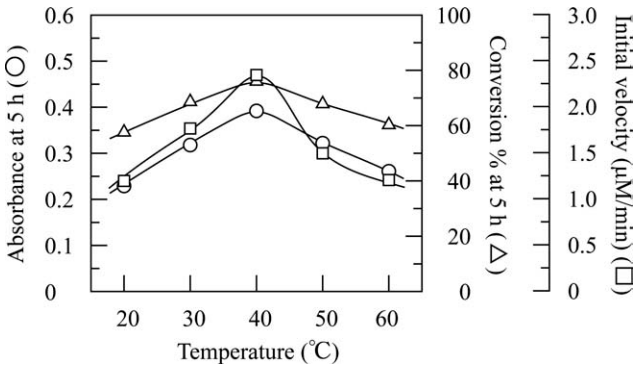

Figure 3 The effect of temperature on tyrosinase-catalyzed (10 U/cm^3) quinone oxidation of 1-naphthol at pH 8.0. The enzymatic reaction time was 5 h.

Effect of enzyme dose

Quinone oxidation of 1-naphthol was initiated by varying tyrosinase concentration at pH 8.0 and 40°C as the optimum conditions determined above. The absorbance and conversion % value sharply increased in shorter reaction times at higher enzyme concentrations. Figure 4(a) shows the changes in the absorbance and conversion % value with the tyrosinase concentration at pH 8.0 and 40°C. The conversion % value increased with an increase in the tyrosinase concentration and 1-naphthol was completely treated by tyrosinase for 8 h at 25 U/cm^3. When the enzyme concentration was increased to 30 U/cm^3, the treatment time was shortened to 5 h.

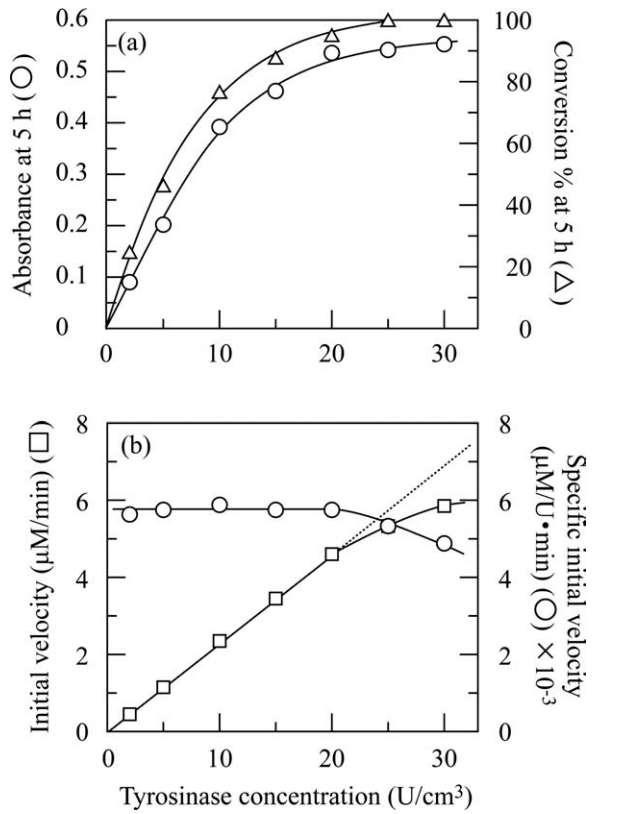

Figure 4 The effect of tyrosinase concentration on quinone oxidation of 1-naphthol at pH 8.0 and 40°C. The enzymatic reaction time was 5 h. (a) Changes in the absorbance and conversion % value with the tyrosinase concentration. (b) Changes in the initial velocity and specific initial velocity of tyrosinase-catalyzed quinone oxidation with the tyrosinase concentration.

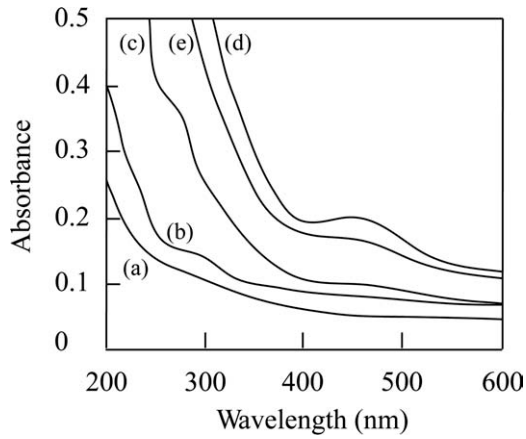

Figure 5 UV–visible spectra of chitosan films incubated in a 1-naphthol solution containing tyrosinase and in various control solutions for 24 h. Sample: (a) unreacted chitosan film, (b) cellulose film in 1-naphthol containing tyrosinase, (c) chitosan film in 1-naphthol, (d) and (e) chitosan films 1-naphthol containing tyrosinase. Tyrosinase concentration (U/cm^3): (d) 5 and (b) and (e) 10.

In addition, the specific initial velocity was determined by dividing the initial velocity by the tyrosinase dose. Figure 4(b) shows the changes in the initial velocity and specific initial velocity of quinone oxidation with the tyrosinase concentration. The initial velocity of quinone oxidation was directly proportional to the enzyme concentration and a constant specific initial velocity of 5.75 μ*M*/U·min was obtained in the enzyme concentration range lower than 20 U/cm^3. However, the initial velocity was negatively deviated from the straight line at further increased enzyme concentrations. The water-insoluble quinone oligomers were also generated in addition to water-soluble quinone derivatives irrespective of enzyme concentration. When the enzyme concentration increased, the reaction time to start generating quinone oligomers was shortened from 60 min at 2 U/cm^3 to 5 min at 10 U/cm^3. At more than 20 U/cm^3, quinone oligomers were generated simultaneously when tyrosinase was added to a 1-naphthol solution.

Reaction of quinone with chitosan

Figure 5 shows the UV–visible spectra of chitosan films incubated in 1-naphthol solutions containing tyrosinase at 5 and 10 U/cm^3. A peak emerged at 450 nm when the chitosan film was incubated under

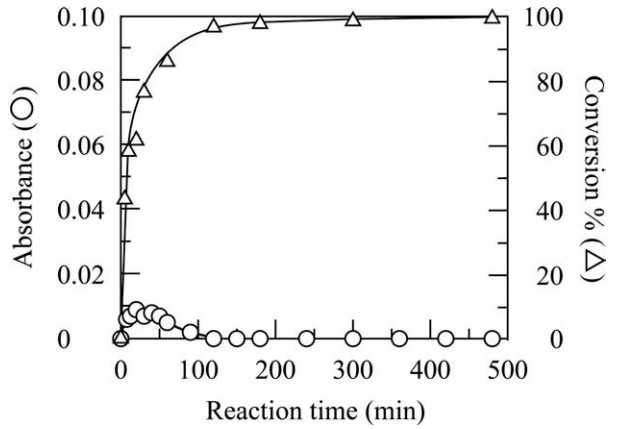

Figure 6 The time course of the absorbance (○) and conversion % value (△) for tyrosinase-catalyzed (20 U/cm^3) quinone oxidation of 1-naphthol at pH 8.0 and 40°C in the presence of chitosan beads at 0.10 cm^3/cm^3.

these conditions. This peak was not observed for a chitosan film incubated in a 1-naphthol solution without tyrosinase and a cellulose film incubated in a 1-naphthol solution containing tyrosinase. Cellulose has a —OH group in the C3 position in place of a —NH_2 group of chitosan.[15–17] Therefore, this difference suggested that an amino group is involved in the reaction of quinone derivatives enzymatically generated from 1-naphthol with chitosan, although the reaction of quinone derivatives to amino groups on chitosan competes with their oligomerization, or generation of water-insoluble oligomers.[16,17,25,26] The filtrates remained colored even after filtration to remove the quinone oligomers from the reaction solutions in the absence of chitosan. This means water-soluble quinone derivatives were present in the reaction solutions. Since it was found from the above results that amino groups on chitosan react with quinone derivatives from 1-naphthol, the use of chitosan was considered to be effective in removing 1-naphthol. Therefore, removal of 1-naphthol was investigated by combined use of tyrosinase and porous chitosan beads.

Removal of 1-naphthol through quinone adsorption on chitosan beads

Tyrosinase at different doses was added to 1-naphthol solutions in the presence of chitosan beads at 0.10 cm^3/cm^3. Figure 6 shows the time course of the absorbance and conversion % value at 20 U/cm^3 as a typical result. An increase in the absorbance at 520 nm considerably depressed through chemical adsorption of quinone derivatives enzymatically generated on chitosan beads in addition to formation of oligomers. The absorbance passed through the maximum value, and finally disappeared at 2 h. However, a small amount of unreacted 1-naphthol was left in the solution at 5 h (conversion = 98.5%). When the reaction time was prolonged to 8 h, unreacted 1-naphthol disappeared and completely removal was attained. As a control experiment, only chitosan beads (0.10 cm^3/cm^3) were dispersed in a 1-naphthol solution without tyrosinase at pH 8.0 and 40°C. The concentration of 1-naphthol slightly decreased by physical adsorption on chitosan beads. Even when the reaction time was prolonged to 24 h, the removal % value was limited to be only 13.1%. 1-Naphthol was removed through tyrosinase-catalyzed quinone oxidation and subsequent nonenzymatic quinone adsorption on chitosan beads and oligomer formation.

Figure 7 shows the changes in the conversion % value and removal time with the tyrosinase concentration in the presence of chitosan beads (0.10 cm^3/cm^3) at pH 8.0 and 40°C. The reaction time of 8 h was required to completely convert 1-naphthol by tyrosinase at 25 U/cm^3 in the absence of chitosan beads as shown in Figure 4. On the other hand, 1-naphthol was completely removed at more than 20 U/cm^3 in the presence of chitosan beads. When the tyrosinase concentration was further increased, the removal time was shortened from 8 h at 20 U/cm^3 to 3 h at 30 U/cm^3. However, below 15 U/cm^3, 1-naphthol was present in solutions even after the solutions were constantly stirred for 8 h. The concentration of remaining 1-naphthol increased with a decrease in the enzyme concentration and the portion of 30.9% of 1-naphthol was left unreacted at 2 U/cm^3.

The conversion % values in the presence of chitosan beads were higher than that in the absence of chitosan beads irrespective of the enzyme concentration. Figure 8 shows the changes in the initial velocity and specific initial velocity of tyrosinase-catalyzed quinone oxidation with the tyrosinase concentration in

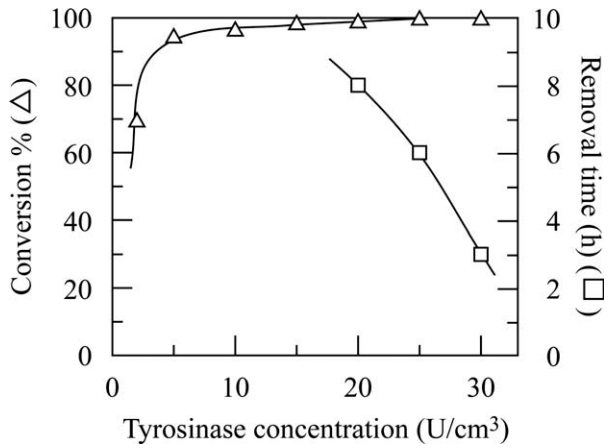

Figure 7 The effect of tyrosinase concentration on removal of 1-naphthol through quinone adsorption on chitosan beads (0.10 cm^3/cm^3) at pH 8.0 and 40°C.

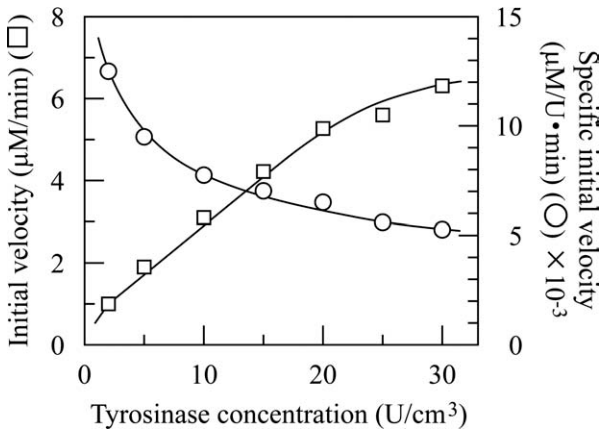

Figure 8 An increase in the initial velocity of tyrosinase-catalyzed quinone oxidation of 1-naphthol through quinone adsorption on chitosan beads (0.10 cm^3/cm^3) at pH 8.0 and 40°C.

the presence of chitosan beads at 0.10 cm^3/cm^3. This result supports that a decrease in the quinone concentration will depress unfavorable interactions between quinone derivatives and tyrosinase. The initial velocity of tyrosinase-catalyzed quinone oxidation increased with an increase in the enzyme concentration. The removal % values and removal times were summarized in Table I to discuss the effect of the addition of chitosan beads on removal of 1-naphthol at different tyrosinase concentrations. When the amount of chitosan beads was increased to 0.15 cm^3/cm^3 at 20 U/cm^3, the removal time was shortened to 5 h. At 15 U/cm^3, 1-naphthol was completely removed at 8 h by adding chitosan beads at 0.15 cm^3/cm^3 and the removal time was shortened to 6 h at 0.20 cm^3/cm^3.The results in Table I showed that an increase in the amount of added chitosan beads led to either a decrease in the removal time or the tyrosinase dose required for complete removal of 1-naphthol.

Removal of 1-naphthol with chitosan solutions in homogeneous system

Removal of 1-naphthol was estimated in the homogeneous system with chitosan in the form of solution at pH 6.0 and 40°C. Water-insoluble aggregates were generated through the binding of quinone derivatives to amino groups on chitosan chains and settled down. The formation of water-insoluble aggregates was also observed for the solutions of alkylphenols and BPA containing tyrosinase and chitosan.[16,18] After the aggregates generated were filtrated out, the UV–visible spectra of the filtrates were measured. The filtrates were transparent and colorless, and the absorbance at 520 nm was not observed. Figure 9 shows the change in the absorbance at 290 nm as the maximum absorption wavelength of 1-naphthol with the amino group concentration. A peak at 290 nm was observed irrespective of amino group concentration. These results mean that enzymatic quinone oxidation was slow under these conditions. After stirring for 24 h, the lowest absorbance of 1.179 was obtained at the amino group concentration of 0.075 m*M* ($[-NH_2]/[1\text{-naphthol}] = 0.25$). The concentration of 1-naphthol remaining in the solution was calculated to be 0.248 m*M* from this absorbance by the calibration curve previously prepared. This

TABLE I
Removal of 1-Naphthol and 2-Naphthol through Tyrosinase-Catalyzed Quinone Oxidation and Subsequent Quinone Adsorption on Chitosan Beads at pH 8.0 and 40°C

	Absence of chitosan beads		Presence of chitosan beads			
Concentration of tyrosinase (U/cm^3)	Conversion % at 5 h	Initial velocity (μ*M*/min)	Amount of added chitosan beads (cm^3/cm^3)	Removal % at 5 h	Removal time (h)	Initial velocity (μ*M*/min)
1-Naphthol						
2	24.1	0.45	0.10	69.1		1.00
5	45.7	1.15	0.10	94.0		1.90
10	76.0	2.35	0.10	96.3		3.10
15	87.2	3.45	0.10	97.9		4.22
			0.15	98.1	8	4.67
			0.20	99.2	6	4.92
20	91.1	4.60	0.10	98.5	8	5.27
			0.15	100	5	5.49
			0.20	100	4	5.72
25	98.8	5.33	0.10	98.9	7	5.60
30	98.9	5.85	0.10	100	3	6.31
2-Naphthol						
20	30.1	0.067	0.10	31.5		0.105
100	68.1	1.60	0.10	68.6		1.70
200	81.3	2.00	0.10	81.4	48	2.20
			0.20	82.8	42	2.50
300	89.2	2.35	0.10	92.3	30	2.55

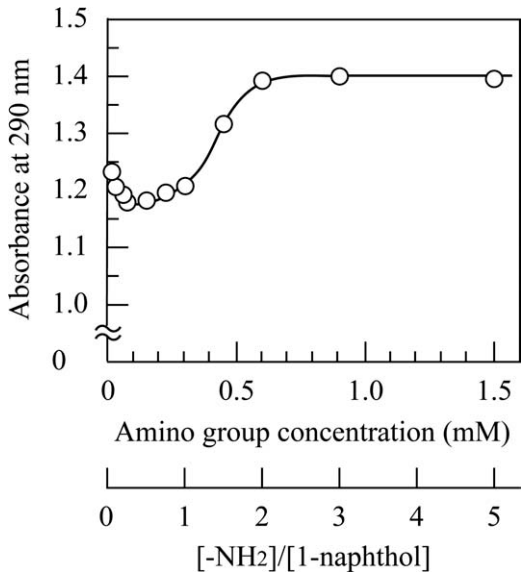

Figure 9 Removal of 1-naphthol through tyrosinase-catalyzed quinone oxidation and subsequent homogeneous quinone reaction with chitosan at pH 6.0 and 40°C.

concentration corresponded to the removal % value of 17.4%. The reason for a low value of removal % is that activity of tyrosinase is considerably low at pH 6.0 as shown in Figure 3. In other words, it was found from the comparison with the results obtained above that tyrosinase-catalyzed quinone oxidation at pH 8.0 and quinone adsorption on chitosan beads, or heterogeneous reaction, are an important factor for effective removal of 1-naphthol.

Removal of 1-naphthol with chitosan powder in heterogeneous system

After 0.30 g of chitosan powder, which corresponded to 4.0 cm^3 (0.10 cm^3/cm^3) of chitosan beads, was dispersed in a 1-naphthol solution, tyrosinase was added to the solution so as to reach the concentrations of 20 U/cm^3. Figure 10 shows the time course of the absorbance and conversion % value for 1-naphthol solution containing tyrosinase in the presence of chitosan powder. The conversion % value of 98.6 was obtained at the reaction time of 8 h at which 1-naphthol was completely removed in the presence of chitosan beads at 0.10 cm^3/cm^3. This value was a little lower than the conversion % in the presence of chitosan beads. When the enzymatic reaction was continued to 10 h, 1-naphthol was completely removed. On the supposition that the chitosan powder used is a perfect sphere with the diameter of 100 μm and chitosan has the density of 1.0 g/cm^3, the surface area of 0.30 g of the chitosan powder is calculated to be 180 cm^2, which corresponds to the specific surface area of 0.060 m^2/g. This value was much lower than the specific surface area of the chitosan beads used in this study. This comparison showed that porosity of the chitosan beads, or high-specific surface area, is also involved in an increase in removal of 1-naphthol through quinone adsorption.

Removal of 2-naphthol

An effect of the enzyme concentration on tyrosinase-catalyzed quinone oxidation of 2-naphthol was investigated at pH 8.0 and 40°C as the optimum conditions determined for 1-naphthol. A peak emerged at 500 nm through the generation of quinone derivatives. The conversion % value at 5 h for 2-naphthol was limited to 30.1% at 20 U/cm^3 as seen in Table I. At this reaction time, 1-naphthol was completely removed with chitosan beads at 0.10 cm^3/cm^3 as shown in Figure 6. This indicates that tyrosinase-catalyzed quinone oxidation of 2-naphthol was much slower than that of 1-naphthol. In addition, the optimum conditions for 2-naphthol were determined to be pH 8.0 and 40°C (not shown), which are the same as those of 1-naphthol. Therefore, the enzyme concentration was further increased. Although the conversion % value increased with an increase in the enzyme concentration and attained 89.2% at 300 U/cm^3, no water-insoluble oligomers were observed irrespective of enzyme concentration.

Subsequently, tyrosinase at different concentrations was added to 2-naphthol solutions in the presence of chitosan beads at 0.10 cm^3/cm^3. The results of tyrosinase-catalyzed quinone oxidation and removal of 2-naphthol were also summarized in Table I. The conversion % value increased over the reaction time and reached 98.6% by stirring the solution for 48 h at 100 U/cm^3. At both 200 and 300 U/cm^3,

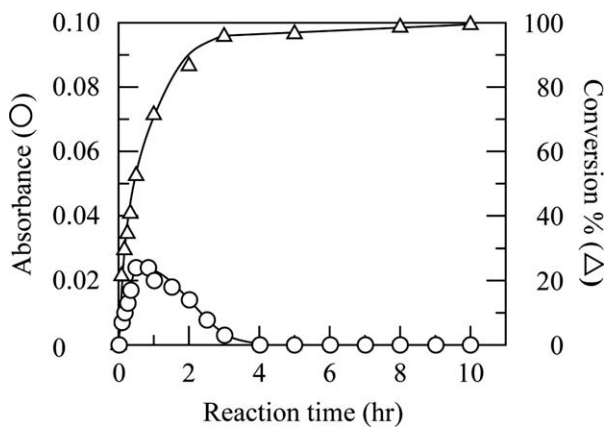

Figure 10 Removal of 1-naphthol through tyrosinase-catalyzed quinone oxidation and subsequent quinone adsorption on chitosan powder at pH 8.0 and 40°C.

the absorbance passed through the maximum value, and then gradually decreased. However, the value of the conversion % and the initial velocity in the presence of chitosan beads were almost the same as those in the absence of chitosan beads. This is mainly because tyrosinase-catalyzed quinone oxidation of 2-naphthol was slower and subsequent quinone derivatives generated were immediately adsorbed on chitosan beads. As a result, 2-naphthol underwent complete quinone oxidation by prolonging the reaction time to 48 h at 200 U/cm^3. When the amount of added chitosan beads was increased to 0.20 cm^3/cm^3, the removal time was shortened to 42 h. In the case where the enzyme concentration was increased to 300 U/cm^3 at the amount of chitosan beads of 0.10 cm^3/cm^3, the removal time was shortened to 30 h.

CONCLUSIONS

In this study, we studied removal of 1-naphthol and 2-naphthol through the tyrosinase-catalyzed quinone oxidation and subsequent quinone adsorption on chitosan beads. First, the effects of the process parameters, such as pH value, temperature, and enzyme dose were investigated for quinone oxidation of 1-naphthol. The optimum conditions for treatment of 1-naphthol by tyrosinase was determined to be pH 8.0 and 40°C and a small amount of water-insoluble quinone oligomers as well as soluble quinone derivatives were generated in the solutions. 1-Naphthol was completely removed by filtration of quinone oligomers and adsorption of quinone derivatives on chitosan beads. The removal time of 1-naphthol was 8 h at 20 U/cm^3 in the presence of chitosan beads at 0.10 cm^3/cm^3 and shortened by an increase in the enzyme concentration. Also, when the amount of dispersed chitosan beads was increased, removal of 1-naphthol was attained at lower enzyme doses. These procedures will depress unfavorable interactions between quinone derivatives and tyrosinase, which results in a decrease in the enzymatic activity or deactivation. In addition, the initial velocity of tyrosinase-catalyzed quinone oxidation was increased by addition of chitosan beads.

On the other hand, the pH value was adjusted to 6.0 in the experiment of the homogeneous system with chitosan and tyrosinase. Since this pH value was deviated from the optimum pH value, the activity of tyrosinase was considerably low and the portion of 82.6% of 1-naphthol was left unreacted in the solution, even when the solution was continuously stirred for 24 h at the amino group concentration of 0.075 m*M*, that is, [—NH_2]/[1-naphthol] = 0.25. As described above, 1-naphthol was completely removed at 3–8 h in the heterogeneous system with chitosan beads and tyrosinase. The comparison between these experimental results indicates that quinone adsorption on porous chitosan beads is an effective procedure in removing 1-naphthol from aqueous solutions. The removal efficiency a little decreased and consequently the removal time was prolonged due to a considerably low-specific area of chitosan powder used. So, porosity of chitosan beads, that is, a high-specific surface area, is also an important factor to effectively remove 1-naphthol through quinone adsorption. Since tyrosinase-catalyzed quinone oxidation of 2-naphthol was much slower than that of 1-naphthol, 2-naphthol was treated at further increased tyrosinase concentrations. As a result, 2-naphthol was completely removed at 200 U/cm^3 in the presence of chitosan beads at 0.10 cm^3/cm^3.

Our study on removal of naphthols provides the results comparable to or better than studies reported previously because alternative conventional techniques for removing naphthols would be operationally complex and would require capital investments. Although we focused on the technical feasibility in this study, the practical application of this procedure may require the immobilization of tyrosinase.

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