Decoloration of Anthraquinone Dye Using the Enzyme Bleaching Agent System

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The decoloration of Alizarin Red S and Reactive Blue 2 with HRP-H_2O_2 system was examined. The decoloration rate of dyes and the pH dependence was improved when the activator was added. The decoloration and the migration prevention to the non-dyed nylon cloth at the washing condition became possible.

Key words : anthraquinone dye
peroxidase
bleaching agents
decoloration
dye transfer

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1. Introduction

The basic research of dye decoloration reactions using horseradish peroxidase (HRP), which is the enzyme which makes hydrogen peroxide (H_2O_2) to be a substrate, is carried out in order to develop the bleaching agent for preventing the migration in the washing. Using the HRP bleaching system under the mild condition (room temperature, pH9) for not giving the damage to the fiber, the migration protective effect of Orange II to the white cloth is high. In the other, the tone of color of the cloth can be retained, since dyed fabric is not bleached using HRP bleaching system[1, 2]. Like this, it becomes clear that the HRP bleaching system demonstrates the performance, which is higher than the conventional bleaching agent such as sodium percarbonate (PC) is. The decoloration rate of Orange II increases, when the activator is added. In addition, using the reaction that couples the glucose oxidase system with the HRP system, the method for stably supplying H_2O_2, which is a substrate of the HRP, has already been established[3]. In actual cleaning system, it is rare that the dye independently exists. Therefore, it is necessary to assume the solution in which the dye of the manifold structure mixed for the practical application of bleaching agent. When 3 kinds of azo dye (Orange I , Orange II , and OrangeG) were also mixed, the decoloration reactions of dye solution progressed[4]. Similarly, it is necessary to also examine the dye except for the azo dye. The anthraquinone dye is contained for natural colourant such as the madder even in the inside, and in addition, the anthraquinone dye of various types is synthesized and is utilized in the dye industry. This study carried out the examination for the purpose of applying HRP-H_2O_2 reaction system for decoloration and migration prevention of anthraquinone dye.

2. Experimental

2.1 Reagent and Apparatus

Two of anthraquinone dyes and one of azo dye were used in this work, Alizarin Red S and Reactive Blue 2, and Orange II (CI Acid Orange 7). HRP, which is the enzyme bleaching agent, came from Sigma Chemical Co. Molar concentration (mol dm^{-3}=M) of HRP was determined spectrophotometrically, for which the following extinction value was used[5]: $\varepsilon_{403}=1.02\times10^5 M^{-1}$. A working solution of H_2O_2, which is the substrate of HRP, was made daily with Tris-HCl buffer solution (pH 9.0). As an activator, p-iodo phenol was used. The solution of PC was prepared by adding sodium carbonate solution to H_2O_2 solution (mole rate=2 : 3) just before use. All of dyes and other chemicals were guaranteed reagents and used without further purification. Water was purified using Millipore. Non-dyed nylon cloth (plain weave) was purified with ethanol: water=1 : 1 (v/v) mixed solution for remove to water-soluble component, before used to migration prevention experiment. The structures of three dyes and the activator were shown in Fig. 1. Using Shimazu MPS-2000 made all of the absorbance measurements, and Minoruta Camera Color Meter CR-300 used the measurements of the color difference.

2.2 Method

HRP Procedure: Tris-HCl buffer solution (pH9.0) 2.55ml containing 5.0×10^{-3}M of H_2O_2 and 4.0×10^{-7}M of HRP was put in the cell. The reaction was started when 0.45ml of aqueous solution containing 3.3×10^{-4}M p-iodophenol and 3.3×10^{-4}M dye was injected to the cell. The variation of absorbance of dye was monitoring at each of λ_{max}. All of the reactions were carried out used stirrer and thermostat cell holder at 20℃.

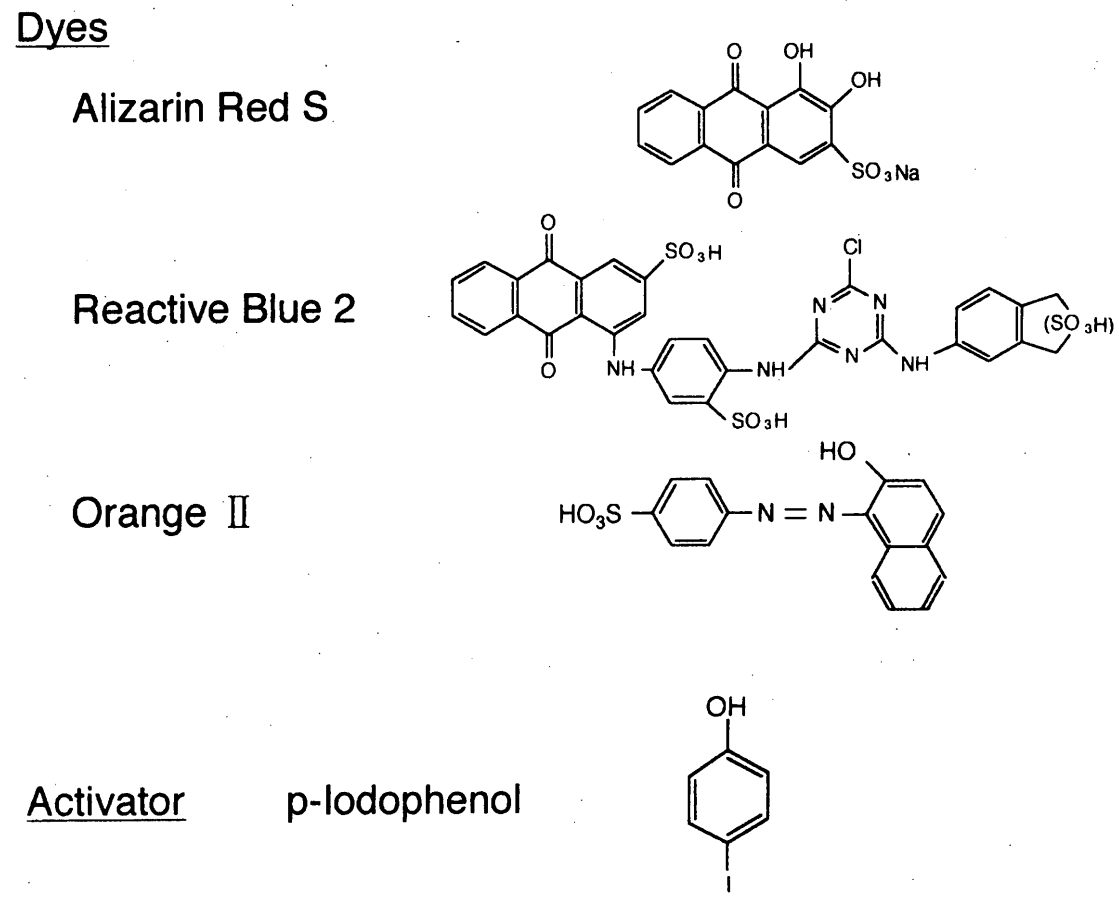


Fig. 1　Structures of the dyes and the activator

PC Procedure: Carmody buffer solution (pH 11.5) containing 0.24M of PC was prepared and 2.5ml of them was put in the cell. The reaction was started when 0.5ml of 3.0×10^{-3} M Reactive Blue 2 solution was injected to the cell. The reaction was carried out used stirrer at 60℃.

Migration Prevention Procedure: Tris-HCl buffer solution (pH9.0) 9.5ml containing 7.9×10^{-4}M of H_2O_2 and 3.6×10^{-7}M of HRP was put on the vial with 0.2g of the non-dyed nylon cloth. The reaction was started when 0.5ml of aqueous solution containing 1.0×10^{-3}M p-iodophenol and 1.0×10^{-3}M dye was injected to the vial. After the 30 minutes reaction, the cloth was picked up and rinsed and air drying. Then the color difference of cloth was measured. All of the reactions were carried out used stirrer and water bath at 20℃.

3. Result and Discussion

3.1 The effect of the activator on dye decoloration by the HRP-H_2O_2 system

The dye decoloration reactions scheme under the activator coexistence of the HRP system is considered like the following (Fig. 2). The numeral in the parenthesis of the figure shows acid value number of the active site. To begin with, native HRP (+3) becomes Compound I (+5) by oxidizing by H_2O_2. Compound I react with the activator which it is easy to reduce further than the dye, Compound I is reduced and becomes Compound II (+4). Compound II is reduced more and more by the activator, and it returns to native HRP (+3) of the origin. The activator that it lost the electron and became a radical receives the electron from the dye, and it returns to original condition again. In the meantime, it is considered that decoloration and decomposition of the dye (radical) advance afterwards, because radi-

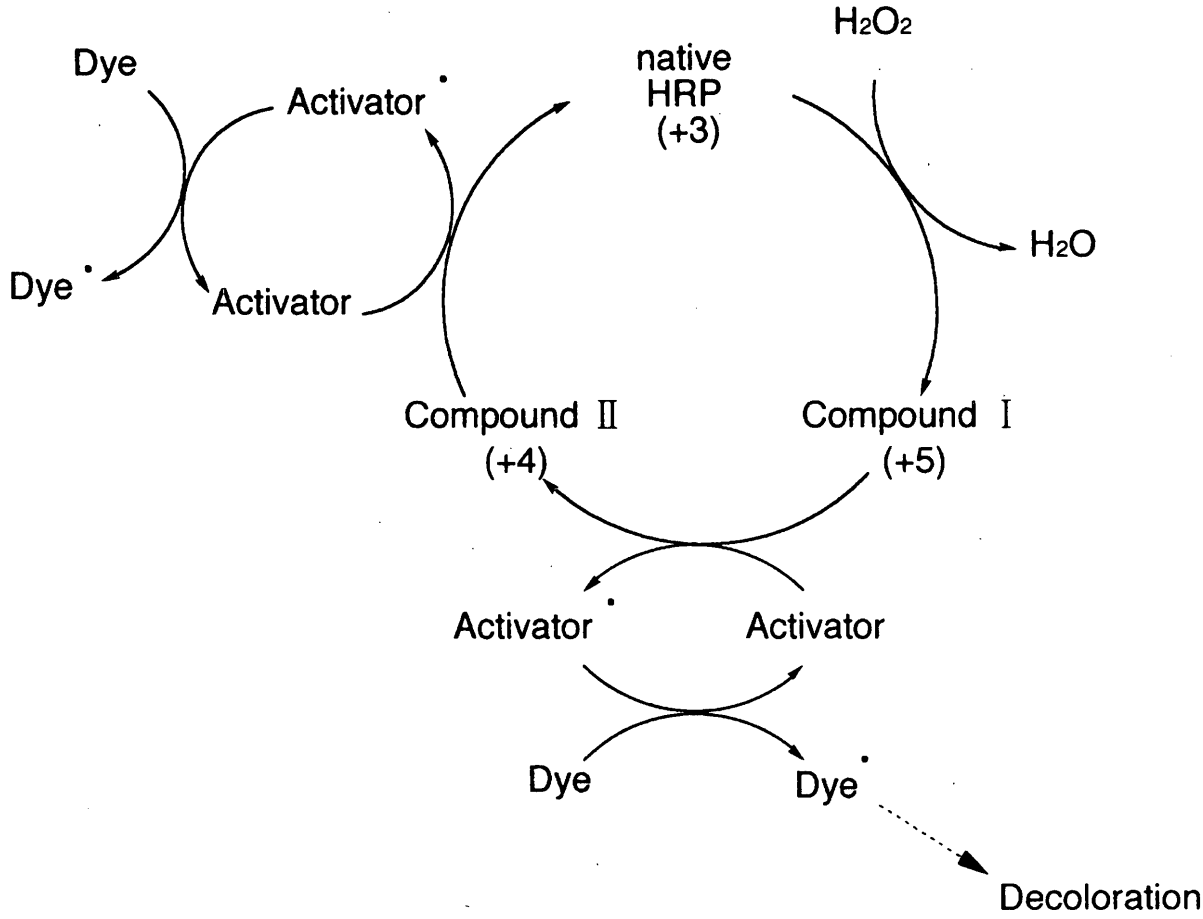


Fig. 2 The dye decoloration reactions scheme of the HRP system.

calized dye becomes unstable by oxidizing[6].

At the beginning, the effect of the activator on the dye decoloration was examined. The decoloration curves of Orange II is shown in Fig. 3. In this study, the examination was carried out under optimal condition (room temperature and pH 9.0) of HRP system of reaction. Needing time is the about 60 seconds, when the activator is not used, so that Orange II may decolorate at 80% (broken line). In the meantime, Orange II decolorate within 10 seconds, when the activator was used (solid line). In case of Alizarin Red S, it hardly faded, when the activator is not used. However, it decolorated under the activator coexistence over 50% in reaction start 30 seconds, and the decoloration proportion is constant afterwards (Fig. 4). When the activator is not used, Reactive Blue 2 completely did not decolorate (Fig. 5). However, Reactive Blue 2 also rapidly decolorates under the activator coexistence in reaction time 2 seconds, and it became that it is constant at decoloration proportion about 50%.

From the above result, it was proven that Alizarin Red S and Reactive Blue 2 were difficult to decolorate only in the H_2O_2 addition in the HRP system, but decoloration proportion reach 50% within 30 seconds by adding the activator further. Since the present condition of reaction is optimal condition of Orange II, the reason why Alizarin Red S and Reactive Blue 2 decoloration reactions does not progress in middle point is anticipated with that H_2O_2 and activator are insufficient.

3. 2 The effect of the activator to the pH dependence of the dye decoloration rate on the HRP system

Next, the effect of the activator on pH

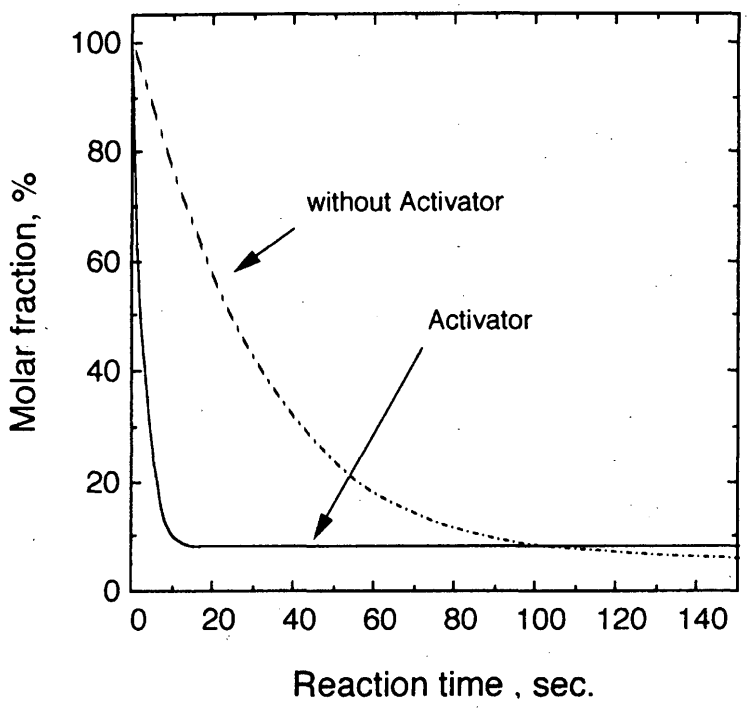


Fig. 3 The decoloration curves of Orange Ⅱ.
[dye]=5.0×10^{-5}M, [HRP]=3.1×10^{-7}M,
[H_2O_2]=7.5×10^{-4}M, [p-iodophenol]=5.0×10^{-5}M,
Tris-HCl buffer (pH 9.0)

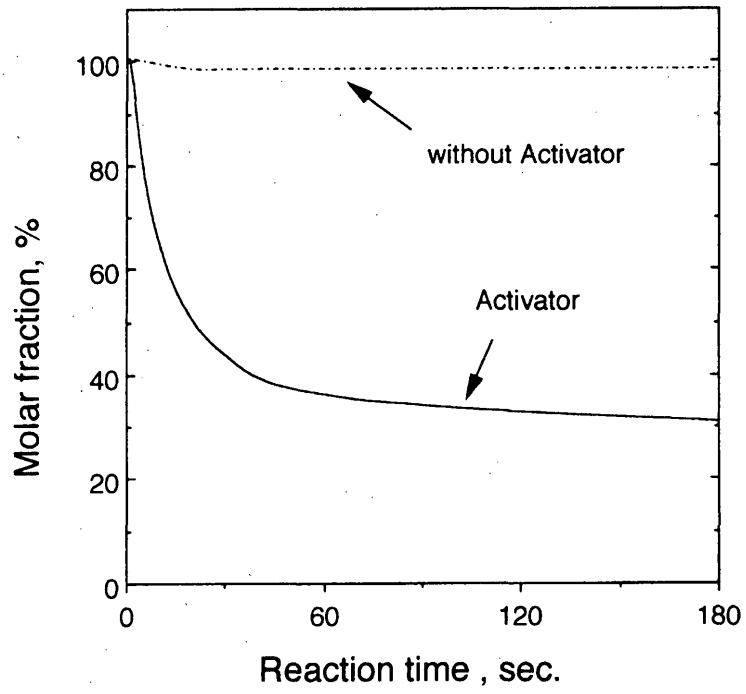


Fig. 4 The decoloration curves of Alizarin Red S.
[dye]=5.0×10^{-5}M, [HRP]=3.4×10^{-7}M,
[H_2O_2]=7.5×10^{-4}M, [p-iodophenol]=5.0×10^{-5}M,
Tris-HCl buffer(pH 9.0).

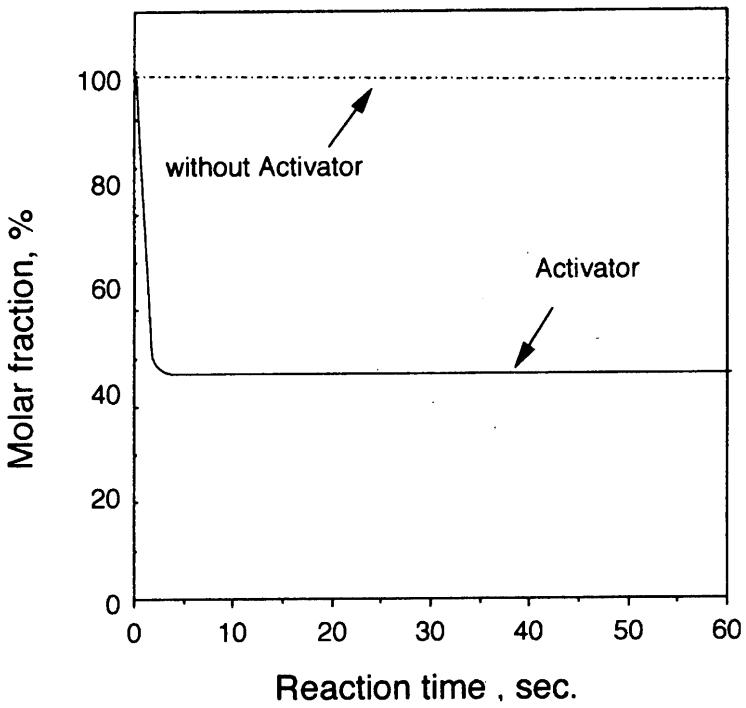


Fig. 5 The decoloration curves of Reactive Blue 2.
[dye]=5.0×10^{-5}M, [HRP]=3.4×10^{-7}M,
[H_2O_2]=7.5×10^{-4}M, [p-iodophenol]=5.0×10^{-5}M,
Tris-HCl buffer (pH 9.0)

dependence of the reaction rate of the dye decoloration was examined. The result of Alizarin Red S is shown in Fig. 6. The decoloration rate became a maximum without activator at pH 3, and the decoloration rate decreased with approaching the neutrality, and it did not decolorate over pH 7 almost. In the meantime, when the activator was added, the decoloration rate drastically increased from pH 3 to 6 acid areas, and the dye decoloration became possible even in alkali area, which was the cleaning optimal condition. From this result, it was proven that the pH dependence of the decoloration of Alizarin Red S was improved by the addition of the activator. In Reactive Blue 2 cases (Fig. 7), the decoloration rate became a maximum under the activator absence at pH 4, and the decoloration reactions did not progress over pH 7. However, the decoloration rate drastically increased over pH 4 under the activator coexistence. Especially, in the region (pH 10 from pH 6) which did not decolorate almost, the increase of rate was remarkable, and in pH 9 the decoloration rate constant surpassed 20. From this result, it was proven that the pH dependence of the decoloration of Reactive Blue 2 was also drastically improved by the addition of the activator.

3. 3 The comparison with PC

For the comparison with the HRP system using PC which is bleaching agent generally used at present, the decoloration experiments of Reactive Blue 2 were carried out. PC was reacted at 60℃, since it is not activated in room temperature, and pH conditions were done at pH 9 (the HRP system) and pH 11.5 (the optimal condition of PC). In the case of either condition, from the result which Reactive Blue 2 did not decolorate in reaction time 3 minutes almost, it was proven that this dye is very difficult to decolorate with PC (Fig. 8). When the activator is added in the HRP system, because Reactive Blue 2 deco-

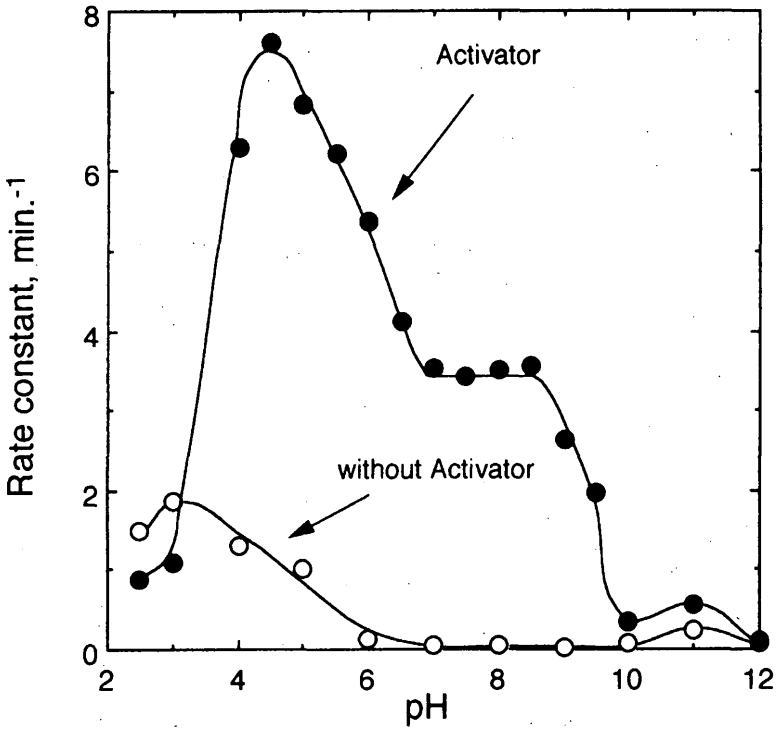


Fig. 6　The effect of the activator on pH dependence of the dye decoloration (Alizarin Red S).
[dye]=5.0×10^{-5}M, [HRP]=3.4×10^{-7}M,
[H_2O_2]=7.5×10^{-4}M, [p-iodophenol]=5.0×10^{-5}M, Carmody buffer.

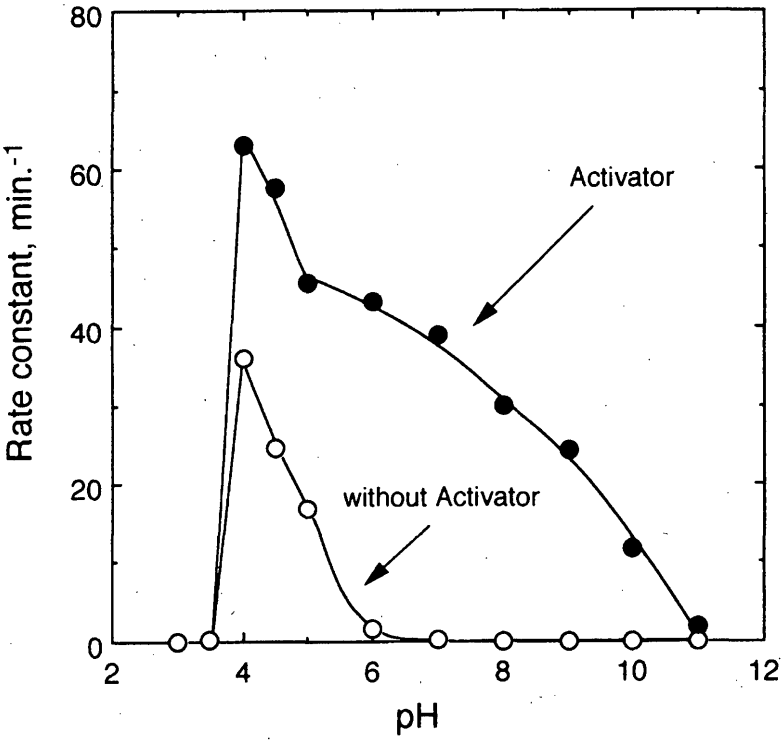


Fig. 7　The effect of the activator on pH dependence of the dye decoloration (Reactive Blue 2).
[dye]=5.0×10^{-5}M, [HRP]=3.4×10^{-7}M,
[H_2O_2]=7.5×10^{-4}M, [p-iodophenol]=5.0×10^{-5}M, Carmody buffer.

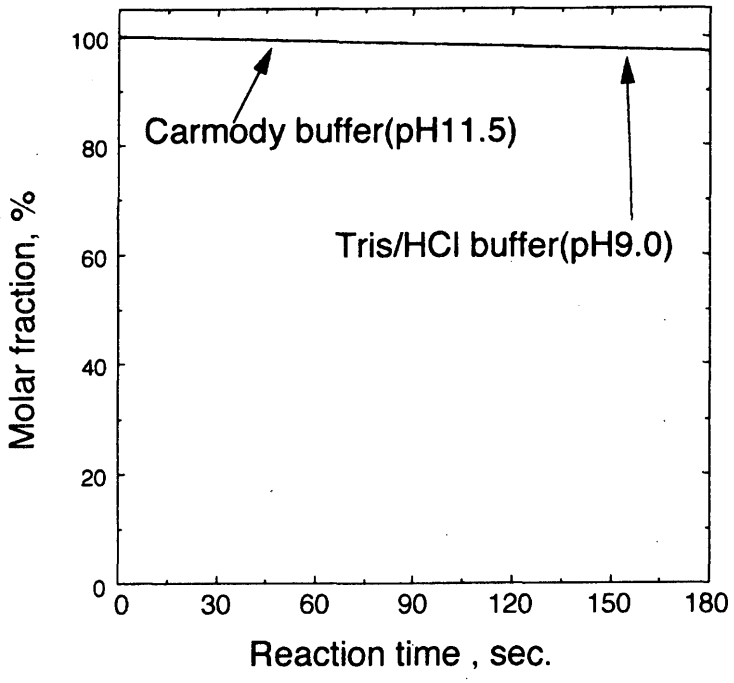


Fig. 8 The decoloration curves of Reactive Blue 2 with sodium percarbonate. [dye]=5.0×10^{-5}M, [percarbonate]=0.1M, temperature: 60℃.

lorate at 50% in seconds, the HRP system is very more effective than PC system for Reactive Blue 2.

3. 4 Migration protective effect to the non-dyed nylon cloth by the HRP system

The effect of the existence of the activator on the decoloration rate constant of each dye at pH 9 in the HRP system is shown at Table 1. It became clear that the decoloration rate constant also drastically increases by the addition of the activator in either case. In the research by the present, it has been proven that the migration to the cloth can be prevented, when the decoloration rate constant is over 2 under the coexistence of the non-dyed nylon cloth and Orange II. Whether the migration prevention is also possible on the cases of Alizarin Red S and Reactive Blue 2 under the similar condition actually, the experiment on the confirmation were carried out. The color difference of the nylon cloth after the treatment is shown at Table 2. In the condition for not putting the bleaching agent, the color difference of Orange II and Reactive Blue 2 consisted over 10, and the migration which could be clearly confirmed by the macroscope was generated. It lowered, as the color difference of Orange II (1.51) can not be judged in the macroscope, when the HRP system bleaching agent was used, and in addition, the color difference lowered by the activator addition, and the migration was able to be prevented sufficiently. In case of the HRP system, the color difference of Reactive Blue 2 rose with 7.46, and the migration was generated. It could be confirmed that the color difference lowered to 1.16 by the addition of the activator in the HRP system and that the migration prevention is possible. In the meantime, the color difference of Alizarin Red S lowered with 1.79 under the condition for not putting bleaching agent, and the migration could not be recognized. In present condition of reaction, the metal did not coexist. Therefore, it seemed that the migration did not generate, since Alizarin Red S is mordant dye. However, it was proven that the color difference surely lowered in each case with 0.95 (HRP system) and 0.34 (adding the activator to HRP system).

Table 1　The decoloration rate constant of each dye in the HRP system.

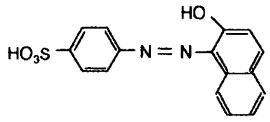
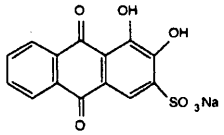
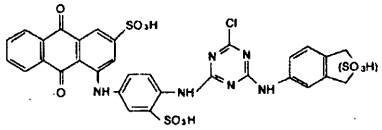
Dye	Construction	Rate constant /min.(pH9.0) Activator	
		Not added	Added
Orange II		2.00	13.1
Alizarin Red S		0.031	2.62
Reactive Blue 2		0.015	24.3

Table 2　The color difference of the nylon cloth after the treatment.

	Orange II	Reactive Blue 2	Alizarin Red S
Water	11.1	10.3	1.79
HRP	1.51	7.46	0.95
HRP with Activator	0.88	1.16	0.34

4. Conclusion

The decoloration of the anthraquinone dye by the reaction of HRP-hydrogen peroxide system was examined. By adding the activator in this reaction system of, the decoloration rate of Alizarin Red S and Reactive Blue 2 increased. Simultaneously, the pH dependence was also improved, and the decoloration at pH 7-10 became possible which was the washing condition. In addition, it became clear that the migration to the nylon white cloth could be also prevented.

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