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Simultaneous dyeing and enzyme processing of fabrics in a non-ionic surfactant reverse micellar system

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Abstract

Simultaneous dyeing and enzyme processing of wool fabrics in a non-ionic surfactant reverse micellar system has been investigated using Polyoxyethylene Sorbitan Trioleate (Tween-85) as a surfactant. Solubilization of enzymes in the Tween-85 reverse micellar system was evaluated by small angle X-ray scattering method. Small angle X-ray scattering analyses showed that the Tween-85 reverse micellar system had an ability to incorporate enzymes and dyes in the interior of reverse micelles without changing their structure. The acid dye and the reactive dye solubilized in the Tween-85 reverse micellar system were effectively adsorbed on wool fabrics. Activities of enzymes in the same system were different depending on the used enzyme and took negative effects from dyes in the system.

Keywords: Reverse micelle; Protease; Small angle X-ray scattering; Dyeing; Non-ionic surfactant

1. Introduction

Reverse micelles have the property of enclosing small amounts of water in their interior so providing a stable aqueous microenvironment, the so-called water-pool, in non-aqueous media [1]. Reverse micelles have attracted considerable interest, because of this characteristic solubilization property and the high potential for experimental and industrial application. For example, reverse micelles have been widely applied in the field of chemical reactions of solubilized substances such as artificial photosynthesis [2,3] and for the synthesis of fine-grains [4–6]. Catalysis and carrier function of reverse micelles have also become a major field in recent years [7–9].

In a series of our previous study, we have also investigated an application of ionic surfactant (Aerosol-OT, AOT) reverse micellar systems for dyeing media [10–12] and for enzyme processing of fabrics [13–16]. Those investigations indicated that conventional water-soluble dyes were

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apparently solubilized in the AOT reverse micellar system even in organic media. Cotton, wool and silk fabrics were dyed in deep shade with this system under mild dyeing condition. Further, cotton–polyester blends were also dyed in this system when reactive dyes and disperse dyes were present at the same time. We have also found that representative enzymes are solubilized in the AOT reverse micellar system in a similar manner as dyes. However, enzymes in that system took remarkable influence of ionic head groups of surfactant molecules [17] and showed lower activity compared to that in an aqueous system. In fact, the previous study indicated that the polarity of interface regions of the water-pool in an ionic surfactant reverse micelle was abnormally high compared to that in core regions [18]. At this time, lower activity of enzymes in an anionic surfactant reverse micellar system cannot be explained clearly, but it may be closely related to the uneven polar environments of the water-pool. In order to evaluate this hypothesis, our previous study examined an application of the non-ionic surfactant reverse micellar system that can ignore an influence of ionic head groups of surfactant molecules [19,20]. The protease in the non-ionic surfactant reverse micellar system was found to be very active and showed remarkable ability to hydrolyze wool fibers without losing fiber strength. Surrounding environments for enzymes such as micellar structure and micellar size might be suitable for enzyme reactions. However, solubilization behaviors of enzymes into the micelle in that system are still unclear. If characteristics of non-ionic surfactant reverse micelles that contain enzymes can be clarified, the enzyme reaction in this system would become easy to understand. In addition, if enzymes are stably maintained in the micelle together with dyes and keep their peculiar roles against fabrics, this system may be applied to the simultaneous one step dyeing and textile processing without requiring multiple treatment step and high energy.

The purpose of the present study is an accumulation of fundamental knowledge about one step dyeing and enzyme processing of fabrics at the same bath. In order to achieve this purpose, experiments were carried out using conventional acid dyes, reactive dyes and two kinds of proteases. In the first half of this paper, solubilization of enzymes into the non-ionic surfactant (Tween-85) reverse micellar system was discussed by small angle X-ray scattering analysis. In the latter half of this paper, the results of simultaneous dyeing and enzyme processing of wool fabrics in the same system were discussed.

2. Experimental

2.1. Chemicals

The surfactant used was Tween-85 (Polyoxyethylene Sorbitan Trioleate) and was obtained from Nacalai Tesque Co., Ltd. Initial water content of the Tween-85 molecule was found through Karl Fisher titration to be 0.4% (w/w). Enzymes used in this study were subtilisin ('Nagarse', Type XXVII) and bioprase 30L (*Bacillus subtilis*). These were obtained from Sigma Chemical Co., Ltd and Nagase Biochemical Co., Ltd, respectively. These enzymes were used without further purification. Dyes used were the reactive dye (C.I. Reactive Red 2) and the acid dye (C.I. Acid Orange 7). These dyes were obtained from Nippon Kayaku Co., Ltd. and Tokyo Kasei Kogyo Co., Ltd., respectively. The fabrics used were wool muslin and were obtained from Shikisen-sha Co. Ltd. The fabric specimens, 0.5 g (5 cm × 5 cm), were prepared for each experiment by boiling in water for 1 h before use. All other chemicals used were of reagent grade and were obtained from Nacalai Tesque Co., Ltd.

2.2. Procedure

The Tween-85 reverse micellar solution was prepared by an injection of aqueous stock solutions to Tween-85 in isopropyl alcohol/*n*-hexane mixture. Mass fraction of Tween-85, isopropyl alcohol and *n*-hexane was adjusted to be 1:1:5. After an injection of aqueous stock solutions, the reverse micellar solution was vigorously stirred. In this experimental condition, transparent reverse micellar solution was obtained immediately after vigorous shaking. The quantity of solubilized water in the reverse micellar system was shown by the

molar ratio of injected water to the Tween-85, that is, $w = [H_2O]/[\text{Tween-85}]$. Maximal w value without phase separation of the Tween-85 reverse micellar system attained in this study was ca. 70.

2.3. Small angle X-ray scattering

Small angle X-ray scattering measurements were taken with SAXES (BL10C) installed in High Energy Accelerator Research Organization, Japan. An incident X-ray from synchrotron radiation was monochromatized to $\lambda = 1.5$ Å with the double-crystal monochrometer, and then focused to the focal point with a bent focusing mirror. The scattered X-ray from the sample cuvette was recorded by PSPC (Position Sensitive Proportional Counter) of an effective length 160 mm (Rigaku Denki Co.). The exact camera distance was calibrated by using the diffraction peaks of collagen fiber at the 6th, 9th and 11th orders. The temperature of samples was controlled to 298 K within ± 0.01 K. The scattered X-ray from the sample was analyzed [21] with Guinier approximation using Eq. (1).

$$\ln I(q) = \ln I(0) - \frac{1}{3}\mathrm{Rg}^2 q^2 \tag{1}$$

where q is the scattering vector $= (4\pi/\lambda)\sin(\theta/2)$ with λ and θ being the wavelength of the incident beam and the scattering angle, respectively. Eq. (1) is known to be a good approximate equation to the spherical scatterers when $q \to 0$. Radius of gyration (Rg) of scatterers was calculated schematically from the initial plots in scattering intensity $I(q)$ versus q^2. Since Rg is a good measure that displays spatial extent of the scatterers, approximate size of the scatterers can be estimated with Rg values regardless of the shape.

2.4. Simultaneous dyeing and enzyme processing

Subtilisin or bioprase 30L was previously dissolved as aqueous buffer solution (0.1 M tris hydroxymethyl aminomethane/0.1 M HCl). Unless otherwise noted, the pH of buffer solution was adjusted at 8 for subtilisin and 7 for bioprase 30L. Preliminary experiments confirmed that pH range examined in this study did not cause any degradation of the wool fiber [20]. These aqueous stock solutions were cooled with ice during the experiment. Reactive dyes or acid dyes were also dissolved in the prescribed amount of aqueous solutions previously. These aqueous solutions (aqueous enzyme solution and aqueous dye solution) were injected in Tween-85 in isopropyl alcohol/*n*-hexane mixture and dissolved completely at 40 °C. The wool fabric specimen was immersed in the reverse micellar solution and then simultaneous dyeing and enzyme processing were initiated at the same temperature. Subsequent fixation of the adsorbed dye on fibers was attained by an immersion of fabrics into 28% NH_4OH solution, with reactive dyes. After fixing step, unfixed reactive dyes on wool fabrics were completely removed by washing with 25% aqueous pyridine solution. The color depth of dyed wool fabrics was estimated from the reflectance of the dyed fiber mass measured with the Minolta CM-1000 spectrophotometer. Effect of enzymatic treatment of wool fabrics was evaluated by weighing absolute dry weight before and after the treatment at 383 K using a high temperature electric balance (Chyo Balance Co., MC-30MB). Before recording the dry weight of fabrics, fabrics were kept at 383 K for 10 min. Preliminarily experiments confirmed that experimental error in the weight measurement was less than 0.2%.

3. Results and discussion

3.1. Small angle X-ray scattering

Fig. 1 shows small angle X-ray scattering profiles for Tween-85/isopropyl alcohol/*n*-hexane/water system. As shown in Fig. 1, every scattering profile has linear relationship in the initial q^2 region. Moreover, initial slope of the scattering profile becomes steep with increasing w value, indicating that the scatterers grow with an addition of water. As is generally known, X-ray is scattered as a result of electron density fluctuations. Hydrocarbon tails of surfactant molecules dissolved in the organic media are, therefore, assumed to have similar electron density as the organic media and supposed not to contribute to the scattered intensity. As a result, characteristics

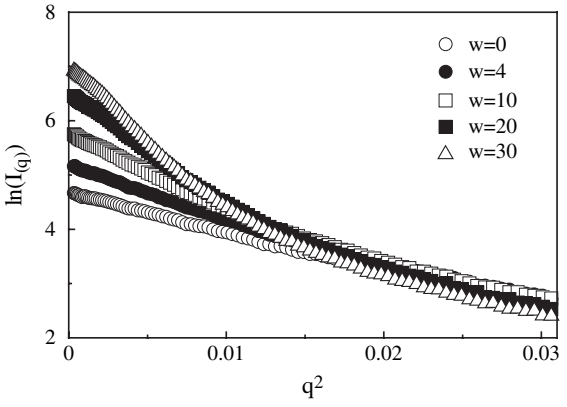

Fig. 1. Guinier plots of the Tween-85 reverse micellar system.

of scattering intensity observed from this system would be attributed to the water-pool that has higher electron density than the surrounding hydrocarbon solvent. Fig. 2 shows the variation of Rg value of Tween-85 reverse micelles with (system I) and without (system II) subtilisin. We confirmed that similar results were also obtained when bioprase 30L was used. As shown in Fig. 2, Rg values in system II linearly increase with increasing w value. These results obviously indicate that the water-pool of the Tween-85 reverse micelle is swollen by an addition of water. On the other hand, swelling behavior of the water-pool in system I is different from that in system II. Rg values in system I are nearly constant in the low w range ($w < 10$) and are higher than those in system II. These differences in apparent micellar

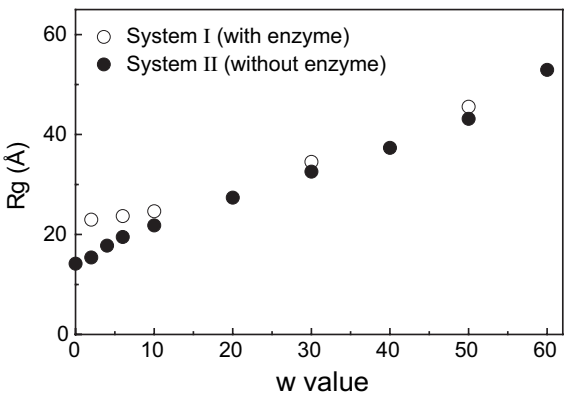

Fig. 2. Variation of Rg value of the Tween-85 reverse micelle as a function of water content.

size may be explained in terms of the solubilization of the enzyme. Wright et al. [22] reported that subtilisin (subtilisin BPN′) has an approximately spherical structure with a radius of about 21 Å. It is of interest to note that the size of the subtilisin roughly agree with the Rg value in system I at the low w range. From these results, it can be assumed that solubilization of subtilisin into the smaller water-pool results in the forcible swelling of the water-pool. Therefore, the size of the forcibly swollen water-pool would hardly be varied by further solubilization of water until the water-pool is densely packed with water. After the water-pool is packed with water in a similar manner to that without enzyme, swelling behavior of the water-pool in system I is similar to that in system II. In order to obtain further information about the structural change of the water-pool due to solubilization of subtilisin, further analysis of scattering profiles has been carried out. Fig. 3 shows corrected scattering profiles of the Tween-85 reverse micellar system. In Fig. 3, scattering intensities $I(q)$ were normalized to become 1 when $q \rightarrow 0$. The abscissa was also rescaled by $q \cdot \mathrm{Rg}$ in order to correct dimensional differences. Therefore, the differences in the shape of scatterers can be displayed as different scattering profiles. When the enzyme is not present in the system, every scattering profile fits on the identical curve (Fig. 3A). These results obviously demonstrate that the Tween-85 reverse micelle without enzyme has identical shape over the whole w range. Fig. 3B and C shows the results of similar investigation when the enzyme is present in the system. In both Figs. 3B ($w = 4$) and 3C ($w = 30$) scattering profiles completely agree with each other, indicating that solubilization of the enzyme into the water-pool also has no influence on the shape change of the Tween-85 reverse micelle.

From these results, enzymes used were satisfactorily solubilized in the interior of the Tween-85 reverse micelle without changing micellar structure. The detailed structure of the Tween-85 reverse micelle cannot be specified with the limited results obtained in this study, but it may be spherical like subtilisin molecule. In order to elucidate the solubilizing behavior of enzyme into the water-pool, further detailed investigation would be necessary.

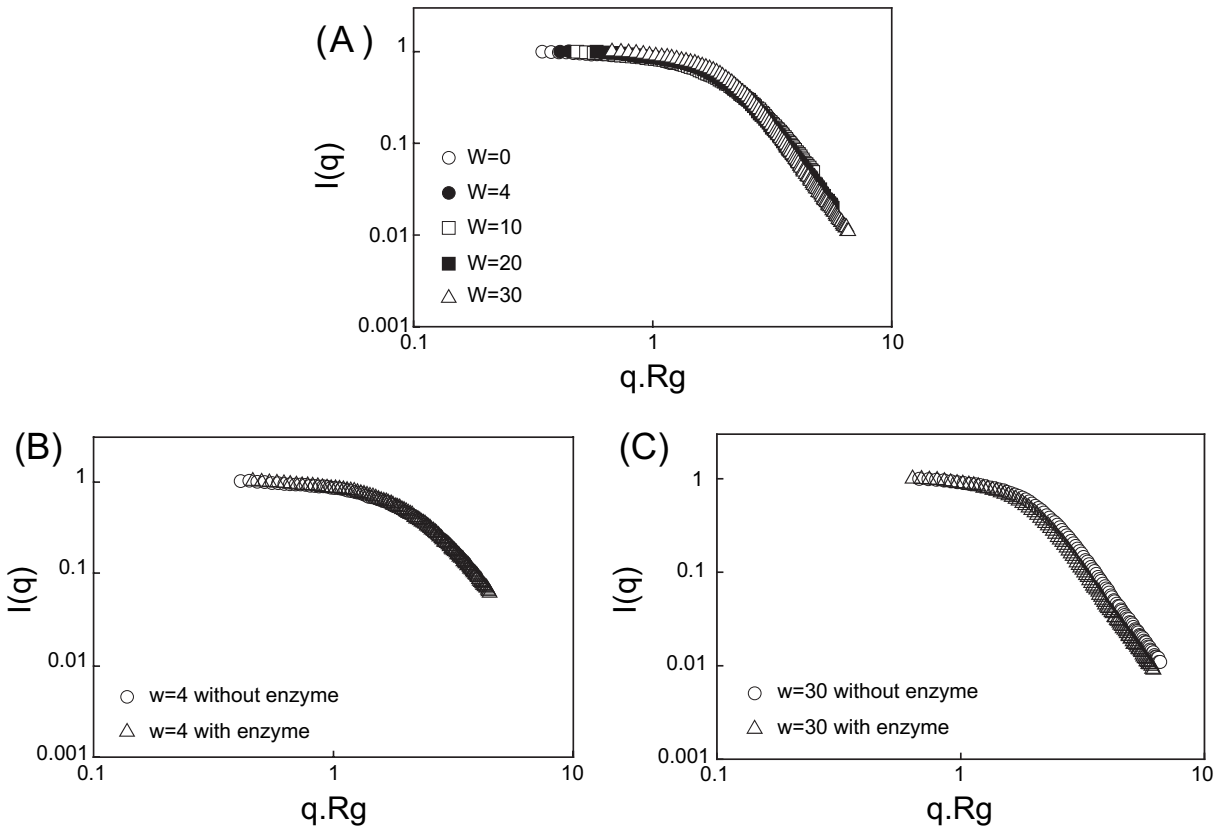

Fig. 3. Small angle X-ray scattering profiles from the Tween-85 reverse micellar solutions.

3.2. Simultaneous dyeing and enzyme processing

Based on the small angle X-ray scattering analysis described above, the following experiments were performed at the system with $w = 20$ which provides sufficient solubilizing space for enzymes. Fig. 4 shows the results of dyeing wool fabrics in the Tween-85 reverse micellar system. As shown in Fig. 4, both acid dyes and reactive dyes were effectively adsorbed on wool fabrics. These results completely agree with the previous results obtained in the AOT reverse micellar system [10–12]. Since water-soluble dyes do not dissolve in bulk organic media but solubilized in the small amount of water in the water-pool, very low bath ratio dyeing would be attained. Fig. 5 compares the effect of pH on the dyeability of acid dyes on wool fabrics. For comparison, the results of dyeing wool fabrics in an aqueous system were also shown. The color depth of wool fabrics dyed in an aqueous system gradually decreased with increasing pH and suddenly dropped at more than pH = 7. On the other hand, remarkable changes in the color depth of wool fabrics dyed in the Tween-85 reverse micellar system were not observed. The acid dye in the Tween-85 reverse micellar system seems to have high ability to adsorb on wool fabrics even in alkaline condition. From these results, dyeing of wool fabrics in this system seems to be performed at broad pH ranges compared to that in an aqueous system. However, the reason for the effective dyeing of wool fabrics at broad pH ranges in this system is not clear. In general, an inside of the water-pool of anionic surfactant reverse micellar systems is known to be in the acidic condition because of the ionization of aggregated surfactant

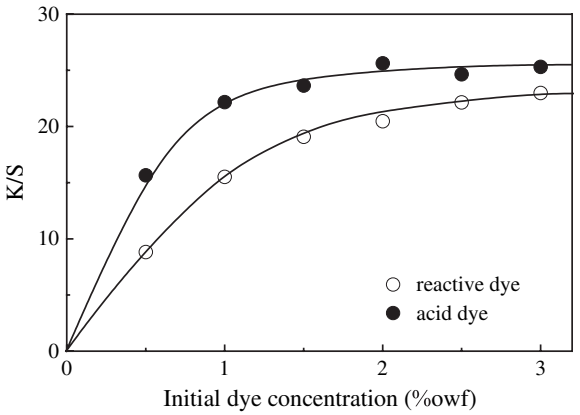

Fig. 4. Adsorption of acid dyes and reactive dyes on wool fabrics in the Tween-85 reverse micellar systems. Dyeing time: 3 h.

ionic head groups such as sulfonic acid group and carboxyl group. Therefore, the actual pH of water in the water-pool in anionic surfactant reverse micellar systems would be lower than that in bulk solution. Unfortunately, these concepts cannot be applied in this system because the Tween-85 molecule does not have any factor to reduce the pH of water in the water-pool. The pH of aqueous buffer solution before and after an injection would not be changed. One reasonable explanation for these results may be an influence of the outer solvent. Zhu and Schelly [23] reported that outer hydrocarbon solvent in non-ionic surfactant reverse micellar systems penetrated into the water-pool. Therefore, hydrophilic dyes and water in the water-pool may be stuck out to the interface of the micelle. As a result, the acid dye and water may be effectively transferred to fibers when micelles are in contact with wool fibers. Alternatively, effective adsorption of the acid dye on wool fibers may be related to the high concentrated dye solution in the system. As described above, the acid dye in the system does not dissolve in bulk organic media but solubilized in the water-pool as a high concentrated dye solution. Consequently, apparent high dyeability of dyes in this system may be attained even in comparatively high pH range. In order to clarify the relationships between dyeability of acid dyes and the pH in this system, further investigation would be necessary.

Fig. 6 compares the weight loss of wool fabrics treated by enzymes in the aqueous and the Tween-85 reverse micellar systems. As shown in Fig. 6, the weight loss of bioprase 30L catalyzed wool fabrics in an aqueous system increased with increasing treatment time, indicating that bioprase 30L had an ability to hydrolyze wool fabrics. On the other hand, the weight loss of wool fabrics treated by subtilisin in both aqueous and the reverse micellar systems were little observed. Subtilisin seems to have little or no activity to wool fibers. Deviation of pH from the optimal one may be the major factor of the inactivity of subtilisin. A slight weight

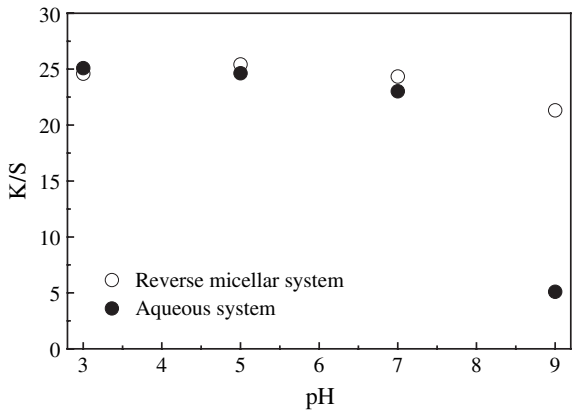

Fig. 5. Variations of the color depth of dyed wool fabrics as a function of pH of the solution. Dyeing time: 3 h, concentration of dyes: 2%owf.

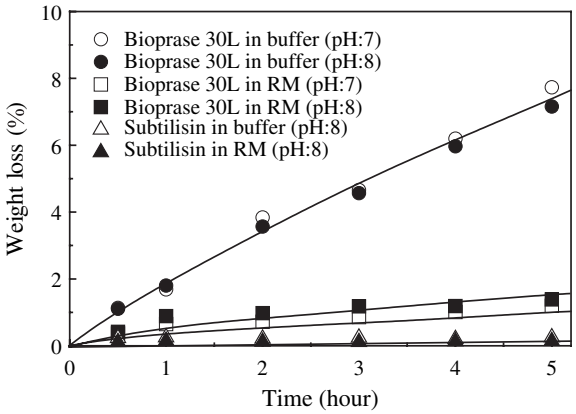

Fig. 6. The weight loss of wool fabrics treated by subtilisin and bioprase 30L in an aqueous and the Tween-85 reverse micellar (RM) systems. Concentration of enzymes: 3.5×10^{-7} M.

loss of wool fabrics would be attributed to the falling off of the yarn waste at the edge of fabrics. In contrast, bioprase 30L in the Tween-85 reverse micellar system showed their activity to wool fabrics, although the activity was quite lower than that in an aqueous system. These results do not agree with the previous study obtained in the similar system [19,20]. When dyes were not present in the system, bioprase 30L in the same system showed equivalent activity to that in an aqueous system [20]. The presence of dyes in the same system obviously gives negative effects to enzyme activity. The low activity of bioprase 30L in this system may be related to the adsorption of the acid dye on enzyme molecules. Since enzyme molecules also consist of polyamino acid, intermolecular interaction between dye molecules and amino acid residue may act in this experimental condition. Consequently, the acid dye adsorbed at the vicinity of active center of bioprase 30L may inhibit the activity of enzyme. This problem may be solved by simple pH adjustment. As shown in Fig. 6, an increase in pH from 7 to 8 of the Tween-85 reverse micellar system results in the improvement of enzymatic activity toward wool fabrics. An increase in pH would reduce opportunities of the adsorption of dyes on enzymes. If the optimal pH for both the dyeing and the enzyme processing are specified without causing the drop of fiber strength, the potential of this system for the simultaneous dyeing and enzyme processing of fabrics would be increased. Since the optimal pH range for dyeing wool fabrics in this system is broad compared to that for enzyme reaction, the dyeing would be carried out at the optimal pH for enzyme reaction. In addition, selection and application of other proteases that have an activity against wool fiber at more alkaline range may become good solutions to overcome some problems found in this study.

4. Conclusion

Solubilization of enzymes into the Tween-85 reverse micellar system was investigated using small angle X-ray scattering method. Tween-85 reverse micelles had an ability to incorporate enzymes at the inside of the water-pool without changing their structure. Simultaneous dyeing and enzyme processing of wool fabrics in the same system were also investigated. Wool fabrics in this system were effectively dyed in deep shade with conventional acid dyes at broad pH ranges. However, enzymes in the same system took negative effect from dyes and showed lower activity compared to that in an aqueous system. Specification of the optimal experimental condition for both dyeing and enzyme reaction at the same bath is necessary.

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