# Reactive Dye Dyeability of Cellulose Fibers with Cellulase Treatment

REIKO MORI,[1] TOSHIO HAGA,[1]* and TORU TAKAGISHI[2]

[1]Faculty of Education, Hirosaki University, Hirosaki-City, Aomori 036, Japan; [2]Faculty of Engineering, University of Osaka Prefecture, Sakai-City, Osaka 593, Japan

**SYNOPSIS**

Reactive dye dyeing was performed on cellulose fibers in combination with cellulase treatment. First, polynosic and cupra fibers were selected to discuss the saturation dye uptake of the previously cellulase-treated fibers. Cupra fiber exhibited a similar saturation dye-uptake dependence on weight loss, irrespective of dye species and the substantivity of the dyes. In the polynosic fiber, the saturation dye-uptake dependence on weight loss exhibited a minimum using a lower substantive dye and a maximum using a higher substantive dye. Comparative discussions of the saturation dye uptake based on X-ray and infrared measurements led to the assumption that a region dyeable with the higher substantive dye is created by the cellulase treatment of the polynosic fiber. This region is assumed to have some degree of order as shown by infrared spectroscopy. Next, cotton fiber, which is important from a practical viewpoint, is dyed and then hydrolyzed. It was found that the hydrolysis of cotton fiber dyed with a reactive monofunctional dye was retarded almost in the same manner as that dyed with Congo Red. The hydrolysis of cotton fiber dyed with dyes of higher substantivity and more bifunctional property was probably retarded to a greater extent compared to dyes of lower substantivity and less bifunctional property.


## INTRODUCTION

Reactive dyes have been extensively used for dyeing cellulose fibers. Cellulase-catalyzed hydrolysis, which obeys the $C_1$-$C_x$ theory,[1] is expected to be utilized in textile finishing. This study deals with the reactive dye dyeability of cellulose fibers in combination with cellulase treatment.

The $C_1$-$C_x$ theory states that the relaxation of the cellulose chain by a $C_1$ enzyme is followed by its hydrolysis by a $C_x$ enzyme.[1] The molecular aggregate in the amorphous region is assumed to influence the extent of hydrolysis by cellulase associated with the relaxation by the $C_1$ enzyme.[2]

Direct dye dyeability of the cellulase-treated fibers was studied[2,3] and it was found[2] that the relationship between apparent affinity (AF) for dyeing and weight loss (WL) for polynosic fiber was completely opposite to that between AF and WL for cupra fiber. The AF for polynosic fiber increased at the initial stage of cellulase treatment,[2] which was assumed to be caused by the relaxation of cellulose molecules.[2] On the other hand, cupra fiber showed a decreased AF at the initial stage of hydrolysis.[2] The ready digestion of the amorphous region by the $C_x$ enzyme would effectively contribute to this decrease. In this report, polynosic and cupra fibers were selected for studies on the reactive dye dyeability of cellulase-treated fibers.

Cotton fiber is very important from a practical viewpoint; thus, it is used here to study the effects of hydrolysis of the dyed fiber. Physical bonding between cellulose and direct dye molecules was found to retard the hydrolysis by cellulase.[2,3] It is of interest to study the enzymatic hydrolysis of the cellulose fibers dyed with the reactive dyes in terms of the functionality and the substantivity of the dyes.

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 59, 1263–1269 (1996)
 CCC 0021-8995/96/081263-07

## EXPERIMENTAL

### Samples

Sample fabrics were previously purified with 2 g/L of an aqueous solution of a nonionic surfactant. Details of fabric construction were shown in a previous article.[3] Commercially available reactive dyes were used without further purification. The properties of the dyes used are shown in Table I. C. I. Reactive Blue 19 (Blue 19) was a monofunctional vinylsulfone dye. Dyes A and B were recently developed dyes with excellent fastness and reproducibility[4] and were kind gifts from the Sumitomo Chemical Industries Co. Each of these dyes has a chromophore bonded to the vinylsulfone group via a monochlorotriazine group. C. I. Reactive Blue 4 (Blue 4) was a bifunctional dichlorotriazine dye and was used only for studying the cellulase hydrolysis of previously dyed cotton fiber. The chemical structure of Blue 4 was similar to that of Blue 19 except for the presence of reactive groups in the former. Commercial cellulase (Meicelase) derived from Trichoderma viride was a kind gift from the Meiji Seika Co. and was used without further purification. This cellulase was the same as that used in the previous studies.[2,3]

### Cellulase Treatment

The procedures were the same as those described in previous articles.[2,3] Treatment was carried out under the conditions of pH 4.5, 40°C, a liquor-to-sample ratio of 1:100, and an enzyme concentration of 0.2% for a given period. Weight loss (WL) was determined by taking the dry weights before and after the cellulase treatment.

### Dyeing Method

A fabric (0.2 g) was dyed under the conditions of a liquor-to-sample ratio of 1:100 and sodium sulfate concentration of 50 g/L for 20 min, followed by fixation with 20 g/L sodium carbonate for 60 min at the same temperature. Treatment temperatures were 50°C for Blue 19, Dye A, and Dye B and 40°C for Blue 4. After fixation, the fabric was thoroughly washed with boiling water to remove unfixed dye. Saturation dye uptake was determined for studying the dyeing of previously cellulase-treated fibers. On the other hand, to study the effect of cellulase treatment on previously dyed fiber, the fibers are dyed with various concentrations of dye to obtain various values of dye uptake. The dyed fabric was dissolved in 70% (vol) aqueous sulfuric acid and saturation dye uptake (or dye uptake) was determined by measuring the optical density.

**Table I** Chemical Structure and molecular weight MW of dyes used.

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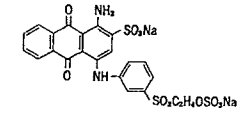
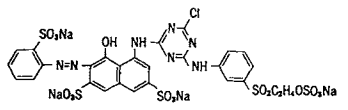
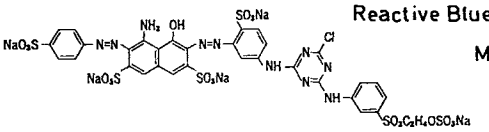
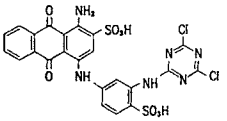
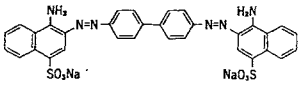
|---|---|
|  | C.I. Reactive Blue 19<br>MW=604 |
|  | Reactive Red Dye A<br>MW=984 |
|  | Reactive Blue Dye B<br>MW=1206 |
|  | C.I. Reactive Blue 4<br>MW=637 |
|  | C.I. Direct Red 28<br>(Congo Red)<br>MW=697 |

### X-ray Diffraction Measurement

An X-ray diffractometer (SRA M18XHF, MAC Science Co.) was used to obtain the crystallinity $X_c$ and line broadening $\theta_{1/2}$. Measurements were taken using pellets made of cut fiber segments. An Ni-filtered Cu$\alpha$ radiation source was used under the conditions of 45 kV and 200 mA. The $2\theta$ range for obtaining $X_c$ was from 8 to 40°. The background due to air scattering was eliminated from the diffractogram prior to the estimation of $X_c$ and $\theta_{1/2}$.

$X_c$ was represented by the ratio of integrated intensities of crystalline scattering to total scattering. The amorphous background was obtained by drawing a base line in the form of a curve to link the minima. $\theta_{1/2}$ was obtained by measuring the full-width at half-maximum intensity of the (002) reflection peak of cellulose II. The X-ray diffraction spectra of cupra and polynosic fibers exhibited peaks

corresponding to structures typical of cellulose II even after cellulase treatment.

### IR Spectroscopic Measurement

The absorptivity ratio of 1372–2900 $cm^{-1}$ was obtained as a crystallinity index $I_c$ (Ref. 5) using an infrared (IR) spectrophotometer (FTS-30, Bio-Rad Co.). Measurement was done by means of a diffuse reflection method[6] using a mixture of cut fiber segments and potassium bromide. The bands at 1372 and 2900 $cm^{-1}$ were assigned to C-H bending and C-H stretching, respectively.[5] The absorptivity ratio was estimated according to the method described in the literature.[5]

## RESULTS AND DISCUSSION

### Primary Hypotheses on Dyeing of Cellulase-treated Fibers

Blue 19, Dye A, and Dye B were used for studies on dyeing of previously cellulase-treated fibers. Reactive dye dyeing of the cellulose fibers must be discussed in connection with both the substantivity and reactivity of the dyes, in addition to the structure of the fibers. The primary hypotheses on dyeing of cellulase-treated fibers are as follows:

1. The substantivity increases in the order of Blue 19, Dye A, and Dye B, based on the molecular weight and the chemical structure of these dyes. This hypothesis was derived from the following discussion:

   Blue 19 is a vinylsulfone dye. This dye has the lowest molecular weight among the dyes examined and seems to have lower substantivity than have Dye A and Dye B.

   Dyes A and B have the same monochlorotriazine functional group. The introduction of the monochlorotriazine group to a dye molecule is known to enhance the substantivity of the reactive dye.[4] This is accounted for by the fact that the more dipolar the heterocycles, such as the triazine ring, are the higher the affinity of the dye.[7] Although Dyes A and B have similar chemical structures, the molecular weight of Dye B is markedly higher than that of Dye A. This suggests that the substantivity of Dye B would be higher than that of Dye A.
2. Blue 19 is monofunctional. On the other hand, Dye A and Dye B are apparently bifunctional, because these have two monofunctional groups: a vinylsulfone group and a monochlorotriazine group. However, the reactivity of the vinylsulfone group is assumed to be higher than that of the monochlorotriazine group for Dyes A and B. Therefore, the functionality of the three reactive dyes would not cause a significant difference in terms of dyeability.

   The assumption that the main reactive group of Dyes A and B is the vinylsulfone group is based on the literature[4] and supported by the results in this report. It is ascertained that the bifunctional property of Dyes A and B would be less effective in dyeing cellulose fiber than that of Blue 4. Blue 4 probably has a greater ability to form crosslinkages with cellulose molecules due to its bifunctionality.
3. There are two regions where dyeing occurs in the fibers.[2,3] One is the region which is readily digested by the $C_x$ enzyme and the other is the region which is newly developed due to relaxation by the $C_1$ enzyme.

   The decrease in AF was observed at the initial stage of hydrolysis for cupra and cotton fibers.[2] The decrease in AF was considered to correspond to a decrease in the effective volume term of dyeing,[2] and the decrease in AF was assumed to be due to a ready digestion of the disordered region.[2] Furthermore, the increase in AF with increasing WL was observed for all the fibers[2] within the limits of measurements. This suggested that the number of regions accessible to the dye is further increased by relaxation by the $C_1$ enzyme.
4. In the case of polynosic fiber, cellulase treatment degrades the microcrystallites and converts them into some ordered molecular aggregate. This newly developed region is assumed to give the peak of $I_c$, as will be explained in detail below.

The change in $X_c$ with WL for polynosic and cupra fibers is shown in Figure 1. $X_c$ showed a peak for cupra fiber. In contrast, a decrease in $X_c$ with WL to a minimum was observed for polynosic fiber. In Figure 2, the $I_c$ vs. WL plots exhibit a maximum for both cupra and polynosic fibers. Therefore, the crystallinity obtained by the IR method showed a tendency different from that obtained by the X-ray method, particularly for polynosic fiber.

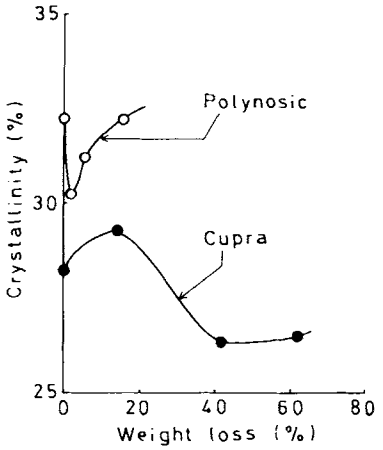



**Figure 1** Crystallinity $X_c$ obtained by X-ray method vs. WL due to cellulase treatment for cupra and polynosic fibers.

Crystallinity of cellulose was suggested to be the major structural feature that influences the hydrolytic degradation by enzymes.[8] Furthermore, crystallinity of cellulose was reported to increase during enzymatic hydrolysis.[9] This was because the amorphous region was more easily penetrated by cellulase than was the crystalline region.

These views are incompatible with the result showing the initial decrease in $X_c$ for polynosic fiber in Figure 1. The molecular aggregate relaxed by the $C_1$ enzyme in polynosic fiber should be noted. The opposite tendencies of $I_c$ and $X_c$ for polynosic fiber in the initial WL stage led to the assumption that the region hydrolyzed by the $C_x$ enzyme is not necessarily a fully relaxed disordered region. A considerable part of the relaxed region was detected to be crystalline by infrared spectroscopy and amorphous

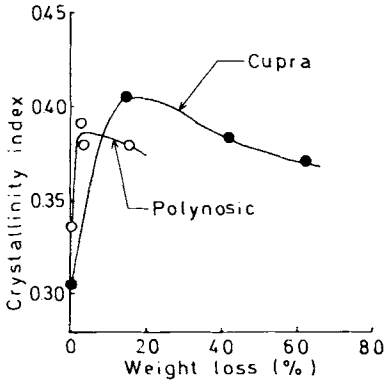



**Figure 2** Crystallinity index $I_c$ obtained by IR method vs. WL due to cellulase treatment for cupra and polynosic fibers.

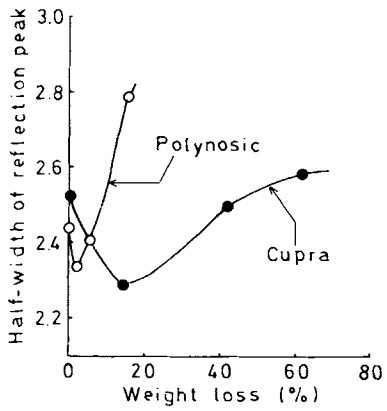



**Figure 3** Plot of full-width at half-maximum intensity of (002) X-ray reflection peak $\theta_{1/2}$ against WL due to cellulase treatment for cupra and polynosic fibers.

by the X-ray method. It was reported that $I_c$ had rather weak correlation with $X_c$.[5]

The plot of $\theta_{1/2}$ vs. WL is shown in Figure 3. A minimum is observed for both cupra and polynosic fibers. The decrease in $\theta_{1/2}$ is known to reflect the increase in crystal size or the reduction of crystal defects. The appearance of the $\theta_{1/2}$ minimum led us to the assumption that a large amount of microcrystallites having an imperfect molecular order would be lost due to enzymatic attack.

## Dyeing of the Cellulase-treated Fibers

The relationship between saturation dye uptake and WL for cupra fiber is shown in Figure 4. The AF of Congo Red is also shown in the figure for reference.

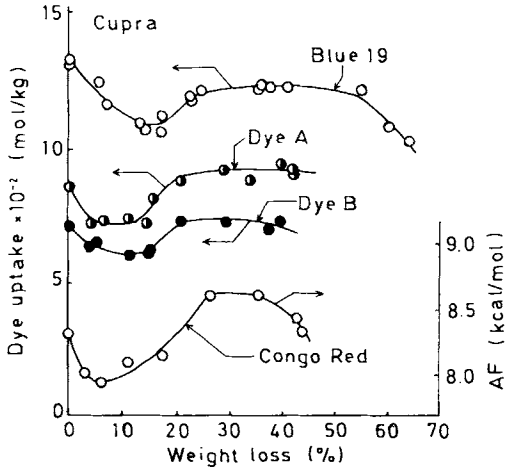



**Figure 4** Saturation dye uptake plotted against WL cellulase pretreatment for cupra fiber. The AF with Congo Red is added.

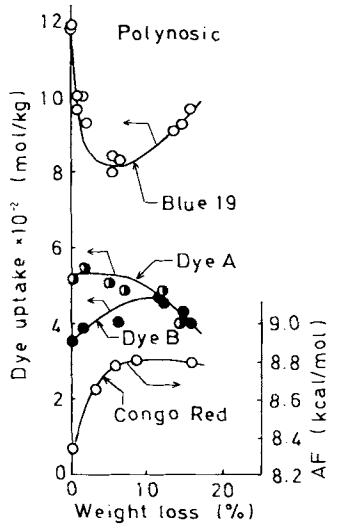



**Figure 5** Saturation dye uptake plotted against WL due to cellulase pretreatment for polynosic fiber. The AF with Congo Red is added.

A minimum at WL of about 10% and a maximum at WL of about 35% were observed for each of the dyes studied here.

In general, the properties of the dyes had less influence on the saturation dye uptake dependence on WL for cupra fiber. Since the reactive dyes studied exhibited dyeability dependence on WL similar to that of Congo Red, the substantivity which governs the dyeability with direct dyes is inferred to play an important role in reactive dye dyeing. The large fully relaxed region in the original cupra fiber might be closely related to the appearance of the minimum observed in Figure 4. This region is assumed to be readily digested by the $C_x$ enzyme and all the dyes used here are assumed to be substantive to this region. Also, the extensive digestion of this region is thought to cause the maximum in $X_c$ and $I_c$, in agreement with the results shown in Figures 1 and 2.

An increase in saturation dye uptake with increasing WL was observed after the minimum was attained for all the dyes tested. This suggests that the number of regions accessible to the dye is further increased by relaxation by the $C_1$ enzyme.

Polynosic fiber exhibits interesting features of its saturation dye uptake using Blue 19, Dye A, and Dye B, as illustrated in Figure 5. A minimum of the saturation dye uptake was observed with Blue 19. This tendency is opposite to that observed with Congo Red, which gave a maximum at the same WL of about 10%. The minimum disappeared in the case of Dye A. Finally, a maximum can be observed for Dye B, showing almost the same tendency as that for Congo Red. Accordingly, the saturation dye uptake–WL relationship is thought to change systematically in the order of Blue 19, Dye A, and Dye B, which is of the same order as the magnitude of the substantivity of the dyes. Thus, it is assumed that the substantivity of dyes plays an important role in dyeing.

The appearance of the maximum for Dye B implies that a region which has high affinity for Dye B is formed during the digestion. This region can be dyed with substantive dyes such as Dye B or Congo Red and is possibly associated with the region which is detected by $I_c$ to have some degree of order. The infrared absorptivity ratio is dependent upon the environment of individual molecules and reflects the freedom of vibration of atomic groups in the molecules, whereas the crystallinity measured by the X-ray method is dependent upon the quantity and size of larger units of the molecular structure.[5] The intermediate molecular order detected by $I_c$ is thought to be nearly the same as that detected by the saturation dye uptake in this study.

## Cellulase Treatment of Dyed Fiber

The effect of cellulase treatment on cotton fiber dyed with Blue 19 was compared with that dyed with Blue 4, as shown in Figure 6. The cotton fibers having various values of dye uptake are examined and saturation dye uptake is not necessarily used here. The

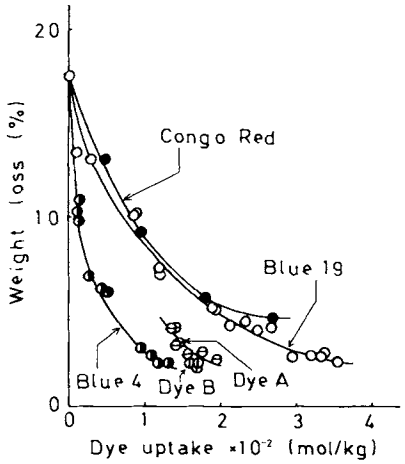



**Figure 6** WL due to cellulase treatment against dye uptake of cotton fiber. The fiber was previously dyed with reactive dyes and Congo Red and subsequently cellulase-treated.

results for Congo Red[3] are also shown. WL decreases with increasing dye uptake for all the dyes used, in a manner similar to that of direct dyes.[2,3] Physical bonding with Congo Red retards the hydrolysis by cellulase almost in the same manner as bonding with Blue 19. This is rather surprising since the affinity of Congo Red to cotton fiber, which is estimated to be about 8 kcal/mol,[3] is extremely lower than the bond energy for chemical bonding, which is nearly 100 kcal/mol. Consequently, a considerably large surface of the cellulose molecules is bonded with Congo Red having planar and linear configurations and this is thought to contribute fairly to the retardation of the digestion by cellulase.

The cotton fiber dyed with Blue 4 was hydrolyzed with much more difficulty than that dyed with Blue 19. This proves that Blue 4 having the dichlorotriazine group retards the hydrolysis more extensively than does Blue 19. It is probable that cotton fiber dyed with a reactive dye having a dichlorotriazine group has cross-linkage between two cellulose molecules via bridging with the dye.[10,11] The cross-linkage between two different cellulose molecules via this type of dye was reported to occur most efficiently at pH 11.2 and in a cold state.[12] These conditions are similar to those in this study. The steric hindrance caused by the cross-linkage with Blue 4 would be one of the possible explanations for the retardation of hydrolysis, as well as the subtantivity increase due to the introduction of the chlorotriazine group to Blue 19. The solution velocity of cotton fiber dyed with Blue 4 in 50% sulfuric acid was clearly lower than that with Blue 19.

Plots for Dyes A and B are also shown in Figure 6. These dyes have two kinds of reactive monofunctional groups and seem to be bifunctional. The cotton fiber dyed with the direct dyes was reported[3] to be more difficultly digested by cellulase due to an increasing affinity of the dye to the fiber. This means that the substantivity of the dyes indeed contributes to the retardation of the digestion by cellulase. Consequently, because of the higher substantivity and the possibility of cross-linkage formation, the fibers dyed with Dyes A and B are more difficult to hydrolyze than are those dyed with Blue 19 at a given dye uptake, as shown in Figure 6.

Fiber dyed with Dye B seems to be slightly more difficult to hydrolyze than that dyed with Dye A at a given dye uptake. Since both dyes have the same functional groups, this difference could be attributed to the difference in substantivity based on molecular weight.

The WL plots for Dyes A and B are situated between those for Blue 4 and Blue 19 at a given dye uptake in Figure 6. The substantivity of Dye A (or Dye B) seems to be higher than that of Blue 4 based on molecular weight. Then, if the bifunctional reactivity of Dye A (or Dye B) is equivalent to that of Blue 4, it might be difficult to explain the fact that the WL plots for Dyes A and B are larger than those for Blue 4 at a given dye uptake. The bifunctional property of Dyes A and B is assumed to be weaker than that of Blue 4.

## CONCLUSION

Polynosic and cupra fibers previously treated with cellulase were dyed with several reactive dyes and saturation dye uptake was discussed in relation to weight loss due to the hydrolysis. The properties of the dyes had less influence on the dependence of the saturation dye uptake on the weight loss for cupra fiber. The large fully relaxed region in the original cupra fiber is assumed to play an important role in this behavior. In the case of polynosic fiber, the relationship between saturation dye uptake and weight loss exhibited a maximum using a higher substantive dye. A region having some degree of order and being dyed with higher substantive dyes is assumed to be created in polynosic fiber during cellulase treatment.

Next, the enzymatic hydrolysis of cotton fiber previously dyed with the reactive dyes was studied. The hydrolysis of the fiber dyed with a monofunctional vinylsulfone dye was retarded almost in the same manner as that dyed with a direct dye of Congo Red. The fiber dyed with a dye having a bifunctional dichlorotriazine group retarded the digestion more extensively at a given weight loss than that with a dye having a monofunctional group and dyes having two monofunctional groups. One of the possible reasons for the above findings is the formation of the crosslinkage between two cellulose molecules via bridging with the dye. The reactive dyes with higher substantivity and with more bifunctional property are assumed to retard the hydrolysis of the dyed fiber to a greater extent compared to the dyes of lower substantivity and less bifunctional property.

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*Received December 8, 1994*
*Accepted June 25, 1995*