Relationship Between Cellulase Treatment and the Dyeability with a Direct Dye for Various Kinds of Cellulosic Fibers

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 051, Japan

SYNOPSIS

The cellulosic fibers were dyed to equilibrium with Congo Red before and after cellulase treatment. The fibers examined were rayon, polynozic, cupra, flax, and cotton. It was found that the volume term for dyeing (V) was associated with weight loss (WL) caused by the cellulase treatment, for the original fibers. Apparent affinity for dyeing (AF) for the cellulase-treated fibers was calculated using a constant value of V obtained for each kind of the original fibers. The results led to the assumption that there would be two kinds of the regions that could be accessible to dye. One would be the region that was readily digested by the enzymatic hydrolysis. The other would be the region that was additionally developed by the attack of cellulase. The previously dyed fibers were hydrolyzed by cellulase. It was found that the physical bondings that formed between cellulose and Congo Red molecules would block the hydrolysis by cellulase for all the fibers examined. It was also assumed that there would be a region that could be accessible to cellulase but not entirely to Congo Red.

INTRODUCTION

This study was carried out to obtain fundamental data with respect to the finishing of the cellulosic fabrics. The dyeability with Congo Red of the cotton fabric, which was previously hydrolyzed by cellulase, has been reported.[1] It was observed that the volume term for dyeing decreased at the earlier stage of the hydrolysis.[1] This would be because of the selective digestion of the amorphous region. It has been also reported[1] that cotton fabric previously dyed with several direct dyes was hydrolyzed with more difficulty than was the undyed original cotton.

In this study, various kinds of cellulosic fibers were dyed with a direct dye of Congo Red before and after cellulase treatment. Cellulosic fibers examined were flax, rayon, polynozic, and cupra. The results in terms of dyeability and weight loss were compared to those for cotton[1] in connection with the structure of the fibers.

EXPERIMENTAL

Samples

Sample fabrics were previously purified with 2 g/L aqueous solution of nonionic surfactant. The characteristics of the fabrics used are shown in Table I. Congo Red (C.I. Direct Red 28) used was special reagent grade.

Commercial cellulase (Meicelase) was kindly given by Meiji Seika Co. This is from *Trichoderma viride* and was used without further purification. This was the same as used in the previous paper.[1]

Cellulase Treatment

The procedures were the same as in the previous paper.[1] The treatment was carried out under the

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 48, 1223–1227 (1993)
 CCC 0021-8995/93/071223-05

Table I The Characteristics of the Sample Fabrics Used

Fabrics	Weave	Thickness (cm)	WL (g/m^2)	Count (tex)		Thread per cm	
				Warp	Weft	Ends	Picks
Polynozic	Plain	0.026	90	15	14	32	28
Rayon	Plain	0.017	85	13	14	40	24
Cupra	Plain	0.013	68	7	9	50	37
Flax	Plain	0.032	144	26	27	23	26
Cotton	Plain	0.021	108	19	18	32	27

conditions of pH 4.5, 40°C, bath ratio of 1 : 100, and enzyme concentration of 0.2% for a given time. The enzyme was then inactivated by treating in boiling water.

Dyeing Method

The fabrics, 0.2 g were dyed to equilibrium with Congo Red at 80°C. This was performed under the conditions of a liquor-to-sample of 1 : 100 and 0.05*N* of NaCl. Dyeing residue was colorinated to obtain dye uptake of the fibers.

Calculation of Affinity of Dyeing

The same eq. (1) as used in the previous paper[1] was available here also:

$$-\Delta\mu^0 = RT \ln \frac{[D]\phi[\mathrm{Na}]\phi^z}{[D]\sigma[\mathrm{Na}]\sigma^z V^{z+1}} \quad (1)$$

where $-\Delta\mu^\circ$ is the affinity for dyeing of the fiber; $[\mathrm{D}]\phi$, the dye uptake of the fiber; $[\mathrm{Na}]^z\phi$, the Na^+ concentration in the fiber; $[\mathrm{D}]\sigma$, the dye concentration in the residual solution; $[\mathrm{Na}]\sigma$, the Na^+ concentration in the residual solution; z, the number of molecules of Na in a dye molecule; V, the effective volume term of dyeing for the fiber; R, the gas constant; and T, the absolute temperature.

Measurement of Weight Loss (WL)

The following equation was used to calculate WL:

$$\mathrm{WL}(\%) = \frac{W - W'}{W} \times 100 \quad (2)$$

where W and W' are the weights of the fibers before and after cellulase treatment, respectively.

Measurement of Water Sorption

Equilibrium sorption was done in an atmosphere of 20°C and 65% RH for more than 2 weeks.

RESULTS AND DISCUSSION

Volume Term for Dyeing and Weight Loss (WL) by Hydrolysis

The WL of the fabrics by cellulase treatment was measured and plotted against treating time t in Figure 1. The WL increases with increasing t for each kind of fiber including cotton, which was previously reported.[1] The WL observed for cupra is the largest at a given t. The magnitude of the WL at each of t is larger in the following order: cupra, flax, rayon, cotton, and polynozic.

The affinity of Congo Red to the original cotton fabric obtained was 8.33 kcal/mol. In this calcula-

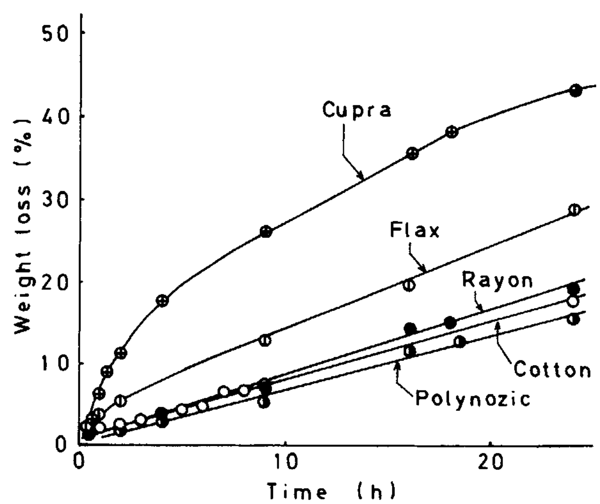

Figure 1 Plots of WL against treating time given by cellulase treatment for the fibers indicated.

tion, 0.22 L/kg[1] was taken as the V of the cotton fabric. V for all kinds of the original fabrics was obtained by numerical calculation from eq. (1), provided that the affinity of dyeing would be equally 8.33 kcal/mol for each fiber.

V of 0.55 and 0.42 L/kg was obtained for cupra and rayon, respectively. These values are remarkably close to the V values of 0.60 and 0.45 L/kg, which were reported[2] using a direct dye of Chlorazol Sky Blue FF for the respective fibers. The magnitude of V for regenerated fibers was larger in the order of cupra, rayon, and polynozic.

Plots of V against WL are shown in Figure 2. WL exhibited here was obtained by cellulase treatment for 24 h and was a suitable value for comparison with V in magnitude for each fiber. V increases with increasing WL in respective cases of natural and regenerated fibers.

It is probable that a cellulase molecule that is a polymer could first penetrate the disordered region of the fibers. Nisizawa[3] considered that the amorphous region in the cellulosic fiber is first attacked by cellulase, leading to an enrichment of the crystalline region. It has also been considered[4] that a dye molecule could penetrate the disordered region of the fibers. Figure 2 shows that the V obtained for the original fibers is closely connected with the WL obtained by cellulase treatment for 24 h and suggests that the amorphous region would play an important role in the enzymatic hydrolysis.

Natural fibers exhibit smaller V than do regenerated fibers at a fixed WL. This means that natural fibers have a larger amount of inner surface, which can be accessible to cellulase polymer but not to Congo Red. It is probable that the extent of the difference noticed between two groups of the fibers would depend on the high-order structure. It is well known that natural fibers have distinct and complicating features in the microstructure as observed by an electron microscope.[5]

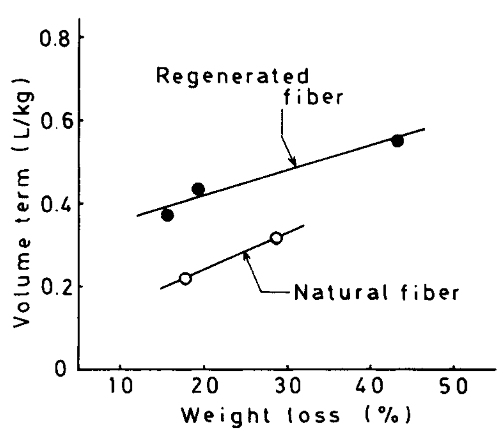

Figure 2 Relationship between volume term (V) for dyeing and WL given by cellulase treatment for the fibers.

Dyeability of the Previously Cellulase-treated Fibers

The fibers were previously treated with cellulase and then were dyed to equilibrium at a initial dye concentration of 4×10^4 g ion/L. Consequently, the apparent affinity of dyeing (AF) was obtained for cellulase-treated fibers. A constant V obtained in terms of each kind of the original fibers was used in this calculation. These values of V were mentioned in the preceding section.

AF was plotted against WL in Figure 3. The affinity for untreated fibers was taken as 8.33 kcal/mol regardless of the kind of the fiber except for cotton. Cotton was dyed at 90°C.[1] Therefore, the affinity of 8.15 kcal/mol was used for this fiber.

AF for polynozic, rayon, and flax increases initially and then decreases, with increasing WL. On the contrary, AF for cupra and cotton decreases initially and then increases with WL. Also, an additional peak appears at a higher WL of about 32% for cupra.

The change in AF with cellulase treatment as shown in Figure 3 would take place due to the following possible reasons:

(1) The decrease in V given by the selective hydrolysis of the amorphous region.
(2) The change in the microstructure of the fibers.
(3) The change in chemical structure of the fibers. Cellulase-catalyzed hydrolysis generates many reducing aldehyde groups at the end of fiber molecules.

(1) and (2) are based on the change in the physical structure, and (3), in the chemical structure. It is possible that the change regarding (2) and (3) would have influence on the dye adsorption mechanism. In a previous paper,[1] dye adsorption isotherm and enthalpy for dyeing were studied with respect to untreated and cellulase-treated cottons, using Congo Red. It was considered[1] that the dye adsorption mechanism for these cottons would be almost equal. It was, therefore, inferred here that the influence of (2) and (3) on the mechanism would be negligible for the same cellulosic fibers as it would be for cotton.[1]

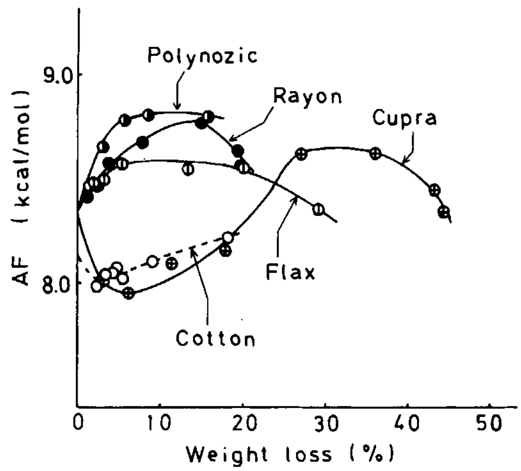

Figure 3 Plots of apparent affinity of dyeing (AF) against WL for the fibers indicated. WL was obtained by cellulase treatment before dyeing.

In general, V is certain to decrease with decreasing content of the amorphous region accessible to a dye molecule. The initial decrease in AF with increasing WL observed for cupra and cotton indicates that the content of the region accessible to the dye would decrease by the digestion. The increase in AF with increasing WL observed for all the fibers tested suggests that the region accessible to the dye would be additionally developed by the attack of cellulase. It is, therefore, considered that there would be two kinds of the regions to be dyed. One would be the region that was readily digested by the enzymatic hydrolysis and the other would be the region that was additionally developed by the attack of cellulase.

Maximum peak values of AF are smaller in the order of cupra, rayon, and polynozic for regenerated fibers, as shown in Figure 3. This order is opposite to that of magnitude of V calculated for each of the original fibers. It is possible that the content of the region that was readily digested would increase with increasing V of the original fibers. This fact might result in the observation that the AF peak heights decreased with increasing V of the original fibers.

Considering that a minimum AF value for cupra, which appeared at WL of 6.2%, reflects the content of the region that cellulase readily digested, the maximum AF at WL of about 32% was recalculated. V, to satisfy the affinity of 8.33 kcal/mol, was found to be 0.43 L/kg for the fiber having a WL of 6.2%. Using this value of V, 9.03 kcal/mol was obtained as the maximum at a WL of about 32%. This value was larger than 8.82 kcal/mol, which was the maximum AF value for polynozic. This suggests that the content of the region that was readily digested would be larger for cupra than for polynozic. The value of V for cupra is the largest in the original fibers tested. The appearance of the minimum AF in the initial step of the WL for cupra is presumably a result of the fact that a great extent of the region would be readily digested. It is reasonably assumed that the AF peak height observed for each of polynozic and rayon would decrease with increasing content of the region that was readily digested.

AF finally decreased with increasing WL after showing maxima for all regenerated fibers studied here. This indicates that an additionally developed accessible region would finally decrease by the hydrolysis.

The $(C_1 - C_x)$ hypothesis[6] to explain the mechanism of cellulase action could describe the behavior observed here to a considerable extent. In most cellulolytic organisms, several cellulase components form a cellulase complex.[6] These components have different functions from each other in the hydrolytic action.[6] The $(C_1 - C_x)$ hypothesis is that the first component C_1 deaggregates the cellulose in preparation for attack by the next hydrolytic component C_x of the cellulase complex.[6] The increase in AF observed suggests that the region accessible to a dye molecule would be newly developed by the action of the component C_1. The initial decrease in AF observed for cupra indicates that there might be a region that could be readily digested by the component of the C_x.

In the case of natural fibers, flax and cotton show clearly different behaviors from each other. Flax shows a maximum peak of AF, as shown in Figure

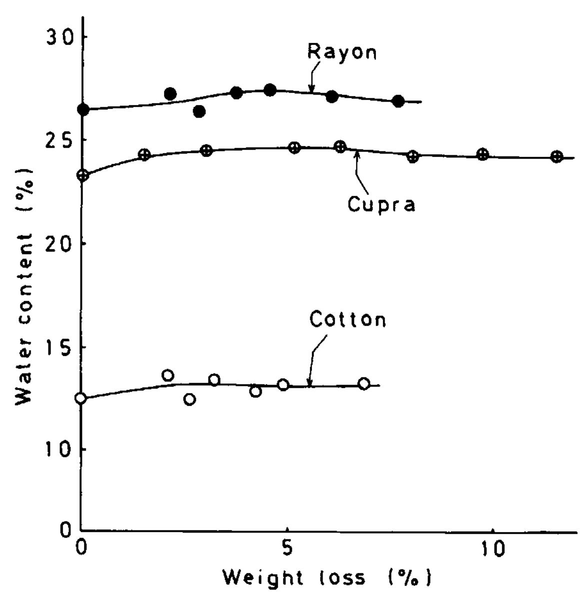

Figure 4 Plots of water sorption (S) against WL given by cellulase treatment for the fibers indicated.

3. On the contrary, cotton shows a minimum. The peak height for flax is lower than those for polynozic and rayon. This suggests that the content of the region that was readily digested and contributed to depress AF in the initial step of WL would be larger for flax than for these regenerated fibers.

Water contents (S) for rayon, cupra, and cotton that were treated by cellulase were measured. Figure 4 represents the relation between S and WL. It is noteworthy that S might be almost constant regardless of WL for each fiber. Water sorption could take place mainly in the disordered region. Therefore, within the limits of the extent of the hydrolysis shown in Figure 4, the results support that both the digestion and the development of the accessible region would arise during the cellulase treatment.

Weight Loss (WL) of the Previously Dyed Fibers

The previously dyed fibers were hydrolyzed by cellulase for 24 h. The dyeing was carried out at the initial dye concentrations ranging from 1×10^{-4} to 8×10^{-4} g ion/L. The WL obtained are plotted against dye uptake in Figure 5. The WL of each fiber decreases initially with increasing dye uptake. The WL exhibits a constant value at higher dye uptake for each fiber tested.

It became clear that physical interactions such as hydrogen bonding that formed between cellulose and the Congo Red molecules would block cellulase-catalyzed hydrolysis for all the fibers studied. The behavior of cotton shown in Figure 5 was already reported[1] and reconciled with those observed for the other cellulosic fibers tested here.

WL was independent of dye uptake at higher dye uptake. This shows that there would be just a region that could be accessible to cellulase and not entirely to Congo Red. This would be still true for the fibers having considerably lower WL, such as polynozic.

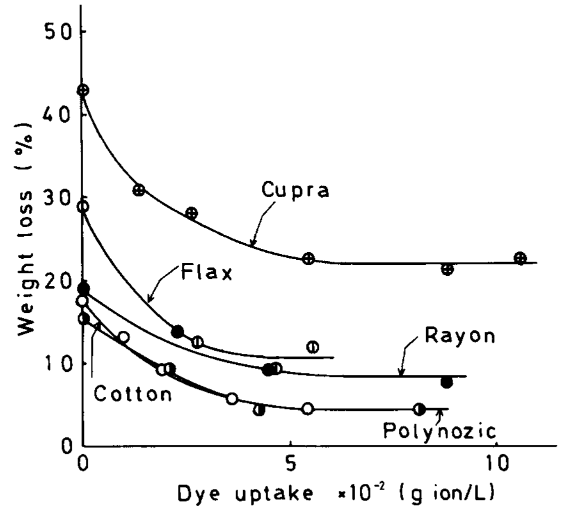

Figure 5 WL against dye uptake for the fibers indicated. The fibers were previously dyed and then hydrolyzed by cellulase.

CONCLUSION

Various kinds of cellulosic fibers were dyed to equilibrium with a direct dye of Congo Red before and after cellulase treatment. It was found that volume term for dyeing was associated with weight loss caused by the cellulase treatment for the original fibers.

The apparent affinity of dyeing was obtained for the cellulase-treated fibers having various values of WL, taking a constant value of V for each fiber. It was assumed that there would be two kinds of the regions that could be accessible to the dye. One would be the region that was readily digested by the enzymatic hydrolysis. The other would be the region that was additionally developed by the attack of cellulase.

It was found that there would be a region that could be accessible to cellulase but not entirely to Congo Red. It was considered that a high-order structure had great influence on the hydrolysis of the fibers.

REFERENCES

1. R. Mori, T. Haga, and T. Takagishi, *J. Appl. Polym. Sci.*, **45,** 1869 (1992).
2. T. Vickerstaff, *The Physical Chemistry of Dyeing*, Oliver & Boyd, London, 1937, p. 217.
3. K. Nisizawa, *J. Ferment. Technol.*, **51,** 267 (1973).
4. B. C. Burdett, *The Theory of Coloration of Textiles*, C. L. Bird and W. S. Boston, Eds., Dyers, Bradford, 1987, p. 111.
5. B. Rånby, *Adv. Chem. Ser.*, **95,** 134 (1969).
6. L. T. Fan, M. M. Gharpuray, and Y.-H. Lee, *Cellulose Hydrolysis*, Springer-Verlag, Berlin, 1987, p. 29.

Received April 10, 1992
Accepted August 8, 1992