BIOMASS PHOTOCHEMISTRY IX: PHOTOCHEMICAL PRETREATMENT OF CELLULOSE AND ITS EFFECT ON CELLULASE EFFICIENCY†

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Summary

Cellulose derivatives were irradiated by UV light at $\lambda > 254$ nm and $\lambda > 300$ nm. The degradation of cellulose derivatives was followed by fluorescence, viscosimetry, weight loss and thermogravimetry measurements. An attempt was made to correlate the changes in degree of polymerization and weight of the irradiated materials with the hydrolysis of cellulosic materials to glucose by cellulase under different conditions. The crystallinity and probably the specific surface area are the most important parameters for the enzymatic hydrolysis of cellulose. These results and the relative quantum yields showed that $\lambda > 300$ nm is excellent for the pretreatment of cellulose.

1. Introduction

Cellulose is one of the most studied substances in biomass photochemistry. From as early as 1916 [1] many studies have been published which deal mainly with the characterization of the photolysis products in cellulose degradation. In general, light brings about a decrease in the degree of polymerization and strength of the cellulose and an increase in alkali solubility and Cooper number, and is accompanied by yellowing and production of carbonyl and carboxyl groups along the cellulose chain [2, 3]. Systematic investigations, whose aim was to gain insight into the actual mechanism of the photochemical processes involved, were initiated a long time ago [4, 5]. Various factors such as the presence of moisture [6], oxygen [7] and atmospheric contaminants [8] greatly influence the degradation to varying degrees. UV light has a significant photochemical effect on cellulose degradation. The most commonly used light sources include the mercury vapor lamp, the xenon lamp and the carbon arc lamp [2].

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The bleaching action of sunlight on wood has been reported [9] and this has been explained as resulting from the oxidation of cellulosic materials; it was believed that the degradation was a surface effect only [2], catalyzed by moisture [10]. The pronounced effect of short wavelength radiation and some indications of the antagonistic action of long wavelength radiation have been reported [11]. It is believed that primary photochemical dissociation occurs in cellulose on irradiation with light at 185 nm regardless of the presence or absence of oxygen [12], while with light of wavelength 254 nm oxygen participation in the photochemical degradation is essential. These processes are presumably through activated oxygen species. This view is supported by the action of sensitizers in the photo-oxidation of cellulose [13, 14].

Increasing the temperature at which the cellulose is irradiated has been shown to have a marked effect [15]. Using electron paramagnetic resonance spectroscopy, Kleinert [16] demonstrated the generation of free radicals in purified wood cellulose irradiated with UV light in air, vacuum, nitrogen and oxygen. Phillips *et al.* [17] and Hon [15] have studied radical decay in cellulose. More recently, many papers have been published which deal with radical detection in irradiated cellulose [6, 7, 15, 18, 19].

As we know from previous photochemical studies on cellulose it is now well established that the heterogeneous chemical reactions of cellulose are controlled largely by the highly ordered molecular packing of its crystalline regions. With current technology, we should be able to devise a practical chemical or physical treatment to modify the carbohydrates of these biomass materials for a broad range of chemical, microbiological or enzymatic conversions. The basic goal of such efforts would be to modify the fine structure of cellulose and to disrupt the lignin–cellulose complex. While many of these procedures lead only to moderate changes, photochemical methods are capable of profound structural transformations and their application is currently in progress or under investigation [20 - 22].

The effect of light on biomass and its influence in subsequent degradation by micro-organisms have been studied since 1947 [23]. Thus, the Pressley index of cotton fibers at 40 °C was reduced by 26% on exposure of the fibers to UV light. Exposure of cotton fibers to UV light increases their resistance to subsequent attack by *Metarrhizicum glutinosum* and this increase in resistance to attack was not influenced by the oxygen concentration or the humidity. In contrast, some metals catalyzed the degradation of cotton cellulose by UV light. Microbial growth by both fungi and bacteria was not efficient with recalcitrant polymer down to shorter length units or the monomeric building blocks [24]. Studies of the modification of straw [25], of waste cellulosic materials [26] or of cellulose derivatives [27], with a corresponding increase in microbiological response, have been published.

Very recently, the photochemical behavior of lignin from *Eucaliptus paniculata* [28] or of dioxane–lignin from rice straw [29] has been studied in order to assess the modifications which are required for fungi degradation

[30]. This study was carried out at different wavelengths and fluences in order to correlate the wavelength effect with structural modifications and the effectiveness of fungi growth. The pre-irradiated lignocellulosic materials were used as the only carbon sources of the micro-organism [31]. In order to understand the lignin modifications, the role of oxygen radicals and carbon-centered radicals in lignocellulosic degradations were studied [32, 33].

In this paper we report a study of the photochemical pretreatment of cellulose and its effect on cellulase action.

2. Materials and methods

α-Cellulose, glucose oxidase, *o*-dianisidine, glucose, horseradish peroxidase type I and Triton X-100 were obtained from Sigma. Sodium carboxymethyl cellulose was from British Celanese (type F4) and methyl cellulose (Methocell MD) was from Dow Chem. Co. Whatman N. 1 filter paper was used. Citric acid monohydrate was from Merck. Cellulase (Mexazyme CLH) was from Gist-Brocades (Holland).

The irradiation was carried out with a 125 W HQL Osram mercury lamp without the glass protection. For irradiation at $\lambda > 254$ nm no filter was used. For irradiation at $\lambda > 300$ nm chemical filters were used [34]. Filter paper and cellulose were irradiated in the solid state. The cellulose derivatives were irradiated in a 0.5 wt.% aqueous solution. Tests of cellulase activity on a 50 mg filter paper were carried out (in triplicate) in 0.5 ml (1.5 mg ml^{-1} cellulase stock solution) and it was incubated in 1 ml citrate buffer (pH 4.6) for 1 h at 45 °C. Glucose was estimated (in triplicate) by the TGO method [35].

The fluence rate of the irradiation was measured using a Yellow Springs Instruments radiometer (model YSI 65 A). Thermogravimetric measurements were carried out on 1 mg samples at a heating rate of 10 K min^{-1} between 0 and 600 °C using a Perkin–Elmer model DSC-2. Viscosities for methyl cellulose were measured at 25.00 ± 0.05 °C in an Oswald viscosimeter with a drainage over 120 s in order to avoid kinetic energy loss. The pure solvent drainage was 275 s and the drainage for cellulosic solutions was between 400 and 600 s (for cellulose the temperature was 30.00 ± 0.05 °C). All the measurements were carried out at least in triplicate.

The absorption and fluorescence spectra were recorded using a Zeiss DMR-21 spectrophotometer and a Perkin–Elmer MPF-44B spectrofluorometer respectively.

For solubilizing the cellulose the method of Zhi-Li *et al.* [36] was slightly modified. Previously distilled dimethyl sulfoxide (DMSO) was stirred for 2 days at room temperature over silica gel (Merck type E) which had previously been activated for 2 h at 130 °C, and then the DMSO was distilled under vacuum and received over molecular sieves (and stored in a refrigerator with a $CaCl_2$ dryer tube). This purification procedure must

be repeated after the bottle has been opened three or four times. Before starting the reaction all the reagents must be totally dry. The best yield of solubilization of cellulose was when cellulose was heated at 120 °C with paraformaldehyde for 20 min.

3. Results and discussion

Figure 1 shows the fluorescence changes of filter paper after different lengths of irradiation at $\lambda > 254$ nm. In order to estimate the behavior of cellulose in solution we selected carboxymethyl cellulose and methyl cellulose, which are soluble in water, for the same group of experiments. The inset to Fig. 1 shows that both carboxymethyl cellulose and methyl cellulose behave similarly to cellulose in the solid state, although the effects were observed to be less pronounced in these cases. The fluorescence decrease is probably due to the cleavage of the chromophoric moiety, which is believed to be a cellulose–metal complex [37], through the formation of carbonyl and/or carboxylic groups [3]. From Fig. 1 we are able to observe a similarity between cellulose and the soluble cellulose derivatives. Thus, in order to understand the behavior of cellulose under irradiation conditions, the variation in the intrinsic viscosity $[\eta]$ of these derivatives was studied.

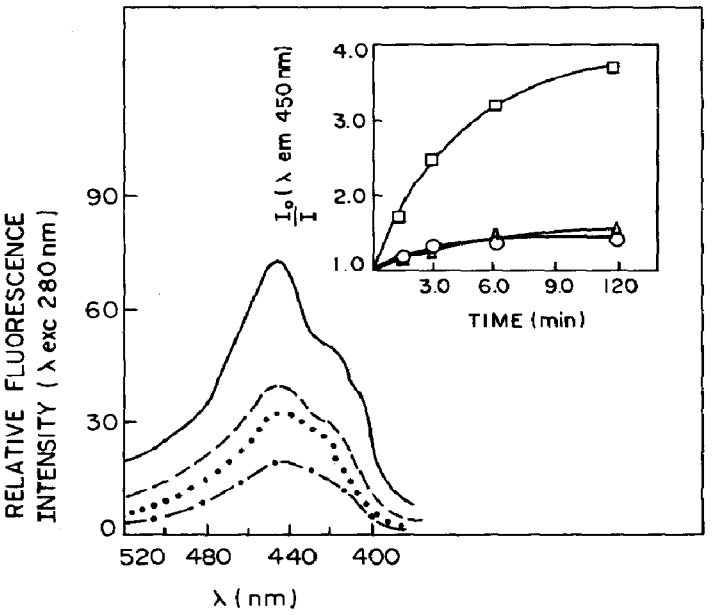


Fig. 1. Fluorescence spectra at $\lambda_{exc} = 280$ nm of filter paper after irradiation at $\lambda > 254$ nm (fluence rate, 108 kJ m^{-2}): ——, 0.0 min; — — —, after 15 min; ·····, after 30 min; — · —, after 120 min. (Inset, fluorescence spectra at $\lambda_{em} = 450$ nm ($\lambda_{exc} = 280$ nm), at different times, of filter paper (—□—), carboxymethyl cellulose (0.1 mM) (—△—) and methyl cellulose (0.1 mM) (—○—) given as I_0/I (relative intensity before irradiation divided by relative intensity after irradiation).)

Figure 2 shows the $[\eta]$ variation under different conditions. It can be observed that the effect on $[\eta]$ is greater for irradiation in solution than in the solid state. This is probably due to the greater migration or higher reactivity of the free radicals formed in solution than those in the solid state. For comparison, the $[\eta]$ variation for α-cellulose (as a cellulose–paraformaldehyde–DMSO solution) after irradiation was also plotted in Fig. 2. During the first 5 min the $[\eta]$ for cellulose and methyl cellulose irradiated in the solid state were observed to decrease. By 14 min $[\eta]$ was found to increase, except for the cellulose–paraformaldehyde–DMSO experiment, in which there was an obvious decrease in $[\eta]$. These results are indicative of different conformational changes at the different fluences [38].

Another method for measuring the degree of polymerization has recently been reported [36]. This consists of the reaction of cellulose with paraformaldehyde in the presence of DMSO as the solvent. According to Johnson *et al.* [39] and Zhi-Li *et al.* [36] the mechanism of dissolution of cellulose in paraformaldehyde–DMSO consists in the reaction of the hydroxyl group at the 6-position with formaldehyde liberated by the thermal degradation of paraformaldehyde, producing a formated methylol cellulose which dissolves almost simultaneously in DMSO. The degree DP of polymerization of cellulose is related to $[\eta]$ as shown in eqn. (1) [36]:

$$[\eta] = 2.78 \times 10^{-2}\, DP^{0.81} \tag{1}$$

When this slightly modified method was used (see Section 2) with the irradiated cellulose the $[\eta]$ value was obtained by extrapolation to an infinite dilution of the specific viscosity η_{sp}/C:

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{C} \tag{2}$$

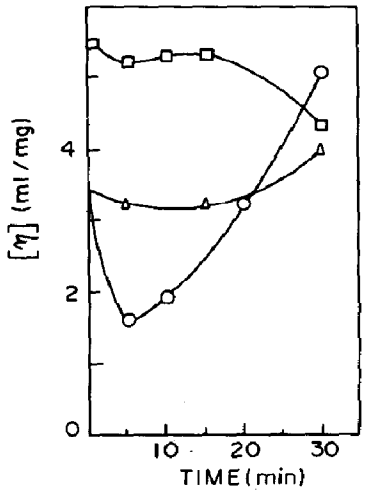


Fig. 2. Variation in intrinsic viscosity after different lengths of irradiation ($\lambda > 254$ nm): —□—, cellulose in the solid state; —△—, methyl cellulose in the solid state; —○—, methyl cellulose in 0.5 wt.% aqueous solution.

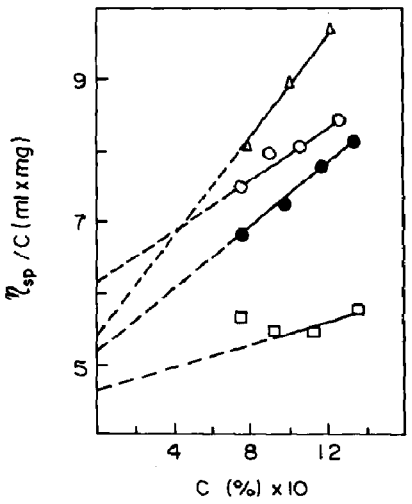
where

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \frac{\eta}{\eta_0} - 1 \qquad (3)$$

where η is the viscosity of a solution of concentration C and η_0 is the viscosity of the pure solvent.

Figure 3 shows the plot of specific viscosity *versus* cellulose concentration for irradiation at $\lambda > 254$ nm. The irradiation affects both the slope and the intercept of the curve, the latter indicating the intrinsic viscosity. The concentration was observed to have a large influence on the viscosity when the sample was irradiated for 30 min. In this case a larger interaction with the viscosimeter's walls was found with a solution of low concentration. Figure 4 shows the specific viscosity of the cellulose solution when irradiated at $\lambda > 300$ nm. In this case no effect of the viscosimeter walls was observed. All of these results are plotted in Fig. 5, in which it is observed that there are two maxima for both wavelengths of irradiation, but with $\lambda > 300$ nm the maxima appear later than with $\lambda > 254$ nm. In other words, similar viscosity changes were produced at both wavelengths.

There is a linear relationship between the loss of weight of the samples during the irradiation and the length of irradiation (Fig. 6). This weight loss is due to gas evolution during the irradiation [2]. Irradiation at $\lambda > 254$ nm is fourfold more effective than at $\lambda > 300$ nm.



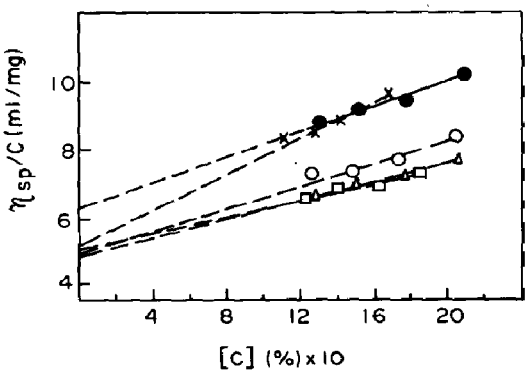


Fig. 3. Specific viscosity of cellulose–paraformaldehyde–DMSO after irradiation of the cellulose in the solid state at $\lambda > 254$ nm for 1 min (—○—), 5 min (—●—), 10 min (—△—) and 30 min (—□—).

Fig. 4. Specific viscosity of cellulose–paraformaldehyde–DMSO after irradiation of the cellulose at $\lambda > 300$ nm for 1 min (—○—), 5 min (—●—), 10 min (—△—), 15 min (—×—) and 30 min (—□—).

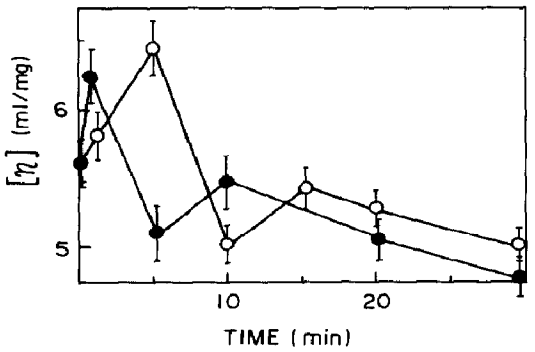


Fig. 5. Change in intrinsic viscosity at different times of irradiation at $\lambda > 254$ nm (—●—) and at $\lambda > 300$ nm (—○—).

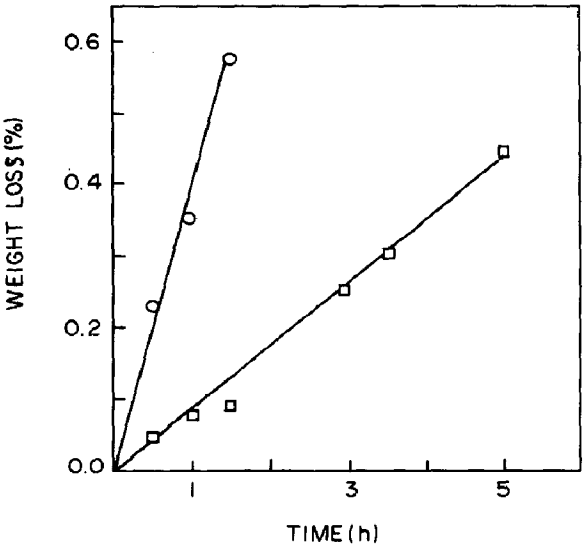


Fig. 6. Weight loss during the irradiation of cellulose at $\lambda > 254$ nm (—○—) and at $\lambda > 300$ nm (—□—).

It is essential to know the efficiency of these photochemical processes for cost estimation and for analyzing the economic viability of their applications. The quantum yield can be calculated by the equation

$$\frac{1}{P_n} = \frac{1}{P_0} + \frac{\phi}{n} D_\alpha \tag{4}$$

where P_0 is the average degree of polymerization before degradation, P_n is the average degree of polymerization after irradiation, n is the number of bonds originally present and D_α is the number of photons absorbed by the cellulose.

To a first approximation, P_n and P_0 can be calculated from the intrinsic viscosities, and n is considered equal to P_0. Thus, we can calculate the ϕ by

TABLE 1

Relative quantum yield of irradiated cellulose at different fluences

Time (min)	*254 nm*	*300 nm*
5	1.00 [a]	—
10	0.08	1.32
15	0.10	1.33
30	0.11	0.65

The quantum yields are relative to that for cellulose at $\lambda > 254$ nm for 5 min. Fluence rates: $\lambda > 254$ nm, 108 kJ m^{-2}; $\lambda > 300$ nm, 67 kJ m^{-2}.
[a]The quantum yield for cellulose in cadoxen at $\lambda > 254$ nm was 1.7×10^{-3} (20% error) [40]; this value is of the same order as that obtained in earlier investigations carried out with cellulose in the solid state [41 - 43].

measuring the number of photons that are absorbed by the sample. Table 1 gives the quantum yields relative to that for cellulose irradiated at $\lambda > 254$ nm for 5 min.

After these parameters were studied we analyzed the efficiency of cellulase activity on the photochemically pretreated cellulose. Figure 7 shows the amount of glucose formed after incubation of pre-irradiated cellulose with cellulase. In Fig. 7, at low fluence, a small increase in glucose formation was observed at each wavelength. Even though at $\lambda > 300$ nm and $\lambda > 320$ nm there were some variations, at these longer wavelengths an increase in glucose formation was observed. On the contrary, at $\lambda > 254$ nm a strong decrease in glucose formation was observed. Presumably, after irradiation for long times at this wavelength, the crystalline region dominates in the overall structure of the cellulose. The change in intrinsic viscosity (or molecular weight) (Fig. 2) does not correlate with the glucose produced

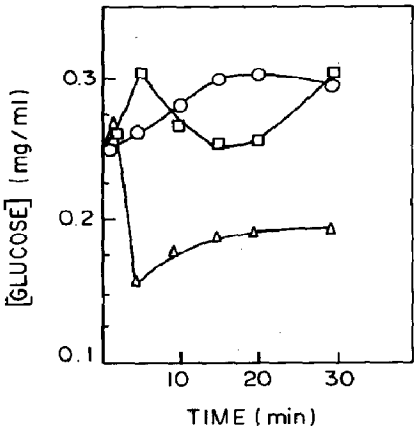


Fig. 7. Glucose production by cellulase (see Section 2) after pretreatment of cellulose by irradiation at $\lambda > 254$ nm (—△—), at $\lambda > 300$ nm (—○—) and at $\lambda > 320$ nm (—□—) (error, about 2% - 5%).

by the cellulase (see Fig. 7) or the total weight loss (Fig. 6) either at $\lambda > 254$ nm or $\lambda > 300$ nm. This indicates that the degree of polymerization is not very important for cellulase activity in pre-irradiated cellulose. Again, as was shown for chemical pretreatment [44], the crystallinity and specific surface area are important parameters for cellulase action. Preliminary results have shown that the cellulose paracrystallinity index is markedly different after irradiation, and this correlates well with the glucose formation (not shown).

A property that is, in part, related to the crystallinity of cellulose is its thermogravimetric behavior [45, 46].

Figure 8 is an example of the thermal behavior of filter paper under different conditions of irradiation. In this study the unirradiated filter paper possessed the lowest humidity (3%), which corresponds exactly to the water concentration at which cellulose is most crystalline and at which minor amounts of free radicals are produced in irradiated cellulose [6].

Cellulose irradiated for 5 min at $\lambda > 300$ nm starts to decompose at the same temperature as the unirradiated sample, but the final degradation is at a lower temperature than the control. In other words, the slopes $\Delta m/\Delta T$ were different for the two cases, indicating different activation energies for the stepwise propagation of cellulose pyrolysis. A similar effect was observed at $\lambda > 320$ nm. The activation energies of these processes are related to the degree of crystallinity of the cellulose [47 - 49]. An attempt to correlate this variation at $\lambda > 300$ nm and at $\lambda > 320$ nm is presented in Fig. 9, which shows the variation in weight (in per cent) at initial decomposition minus weight (in per cent) at the final stage at different wavelengths and different times of irradiation. Surprisingly, a good correlation was found with glucose production by enzymatic hydrolysis (Fig. 7). This is further proof that crystallinity and probably the specific

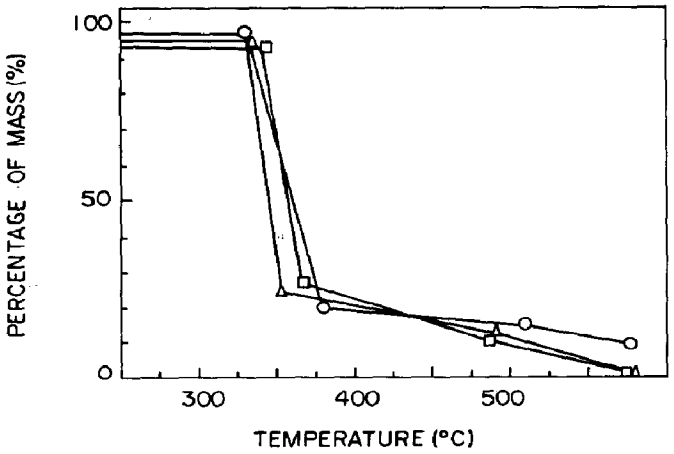


Fig. 8. Thermogravimetric analysis of unirradiated cellulose (—○—) and cellulose irradiated at $\lambda > 300$ nm (—△—) and at $\lambda > 320$ nm (—□—) for 5 min (error, about 2%).

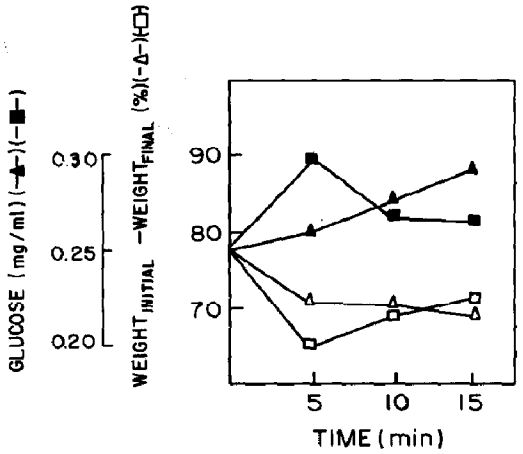


Fig. 9. Attempt to correlate the weight loss at $\lambda > 300$ nm (—△—) and at $\lambda > 320$ nm (—□—). Also shown is the glucose formation by cellulase at $\lambda > 300$ nm (—▲—) and at $\lambda > 320$ nm (—■—).

surface area are important in the enzymatic hydrolysis of cellulose by cellulase.

These results and the quantum yields (Table 1) for photolytic scission of C1–C2 bonds of the polymer show that $\lambda > 300$ nm is an excellent wavelength for pretreatment of cellulose for enzymatic hydrolysis, as is shown in Fig. 7.

As we are interested in the production of glucose by cellulase acting on cellulose and also in the efficiency of the photochemical pretreatment, we have defined a quantum yield of hydrolysis as

$$\phi_H = \frac{\text{Number of moles of glucose formed}}{\text{Number of photons absorbed by cellulose}}$$

ϕ_H was calculated for 30 min irradiation, and this relative quantum yield of cellulose hydrolysis by cellulase is 3.5-fold better after photochemical pretreatment at $\lambda > 300$ nm than at $\lambda > 254$ nm (Table 2).

TABLE 2

Relative quantum yield of cellulose hydrolysis on photochemical pretreatment by cellulase

Wavelength	$\lambda > 254$ nm	$\lambda > 300$ nm
Quantum yield	1.00	3.5

After 30 min irradiation (fluence rates, as in Table 1).

In summary, we have reported an efficient method to pretreat cellulose for enzymatic hydrolysis, without the problems associated with chemicals (impurities) or other physical pretreatments (high temperatures and

pressures) in cellulosic materials. The effect of the sensitizer on cellulosic materials is currently under study, in order to improve the quantum yield of the production of glucose by the photochemical pretreatment.

Acknowledgments

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References

1 C. F. Cross and E. J. Bevan, *J. Soc. Dyers Colour., 32* (1916) 135.
2 R. L. Desai, *Pulp. Pap. Mag. Can., 71* (1970) T305.
3 D. N.-S. Hon, *Develop. Polymer. Degrad., 3* (1981) 228.
4 S. Oguri, *J. Soc. Chem. Ind. Jpn., 38* (1935) 392.
5 G. A. Richter, *Ind. Eng. Chem., 27* (1935) 432.
6 D. N.-S. Hon, *J. Polym. Sci., Polym. Chem. Ed., 13* (1975) 955.
7 D. N.-S. Hon, *J. Polym. Sci., Polym. Chem. Ed., 17* (1979) 441.
8 V. A. Shenai and R. J. Rao, *J. Soc. Dyers Colour., 89* (1937) 364.
9 R. L. Desai and J. K. Shield, *Bi-Mon. Res. Notes, Can. For. Serv., 26* (1970) 3.
10 C. W. Mason and F. B. Rosevear, *J. Am. Chem. Soc., 61* (1939) 2995.
11 L. N. Guseva, Yu. A. Mikleev, L. S. Rogova and D. Ya. Toptygin, *Dokl. Akad. Nauk. SSSR., 226* (1976) 863.
12 C. Kujirai, *Sen'i Gekkaishi, 22* (1966) 20.
13 M. Kaneko, J. Motoyoshy and A. Yamada, *Nature, 285* (1980) 468.
14 R. Harold and J. P. Fouassier, *Angew. Makromol. Chem., 86* (1980) 123.
15 D. N.-S. Hon, *J. Polym. Sci., Polym. Chem. Ed., 13* (1975) 2363.
16 T. N. Kleinert, *Holzforschung, 18* (1964) 24.
17 G. O. Phillips, O. Hinojosa, J. C. Arthur and T. Mares, *Text. Res. J., 36* (1966) 822.
18 A. T. Nanassy, *J. Appl. Polym. Sci., 16* (1972) 2577.
19 H. Kashinwagi and S. Enomoto, *Chem. Pharm. Bull., 29* (1981) 913.
20 M. A. Millet, A. J. Baker and L. D. Satter, *Biotechnol. Bioeng. Symp., 5* (1975) 193.
21 L. T. Fan, Y.-H. Lee and M. M. Gharpuray, *Adv. Biochem. Eng., 23* (1982) 157.
22 Y. Nakamura, Y. Ogiwara and G. O. Phillips, *Polym. Photochem., 6* (1985) 135.
23 R. P. Wagner, H. H. Webber and R. G. H. Siu, *Arch. Biochem., 12* (1947) 35.
24 D. A. Klein, *Am. Chem. Soc., Div. Water Air. Waste Chem. Gen. Pap., 10* (1970) 187.
25 J. E. Park, R. C. Rockhill and D. A. Klein, *J. Environ. Qual., 1* (1972) 298.
26 C. J. Rogers, E. Coleman, D. F. Spino and T. C. Purcell, *Environ. Sci. Technol., 6* (1972) 715.
27 D. H. Davies and G. D. Dixon, *J. Appl. Polym. Sci., 16* (1972) 2449.
28 N. Durán, H. Mansilla and E. Gómez, *Actual. Fis. Quim. Org. (Brazil), 2* (1983) 152.
29 N. Durán, H. Mansilla, E. Gómez, J. L. Reyes and L. C. C. Leite, *Actual. Fis. Quim. Org. (Brazil), 3* (1984) 230.
30 N. Durán, E. Gómez and H. Mansilla, *2nd Informal Brazilian Meet. of Photochemistry and Photobiology, August 1983, San Paulo, Brazil*, University of San Paulo Press, p. 45.

31 N. Durán, E. Gómez and V. Campos, *1st Semin. on Enzymatic Hydrolysis of Biomass, October 1983, Maringa, Parana, Brazil*, University of Maringa Press, p. 17.
32 N. Durán, H. Mansilla and L. C. C. Leite, *Actual. Fis. Quim. Org. (Brazil)*, *4* (1985) 205.
33 N. Durán and H. Mansilla, *J. Macromol. Sci. Chem. A*, *21* (1984) 1467.
34 J. G. Calvert and J. N. Pitts, Jr., *Photochemistry*, Wiley, New York, 1966, p. 728.
35 A. Dahlqvist, *Anal. Biochem.*, *22* (1968) 99.
36 Y. Zhi-Li, W. Kuo-Ming, M. Chen-Fong and F. Han-De, *Cellul. Chem. Technol.*, *16* (1982) 651.
37 D. N.-S. Hon, *J. Appl. Polym. Sci.*, *19* (1975) 2789.
38 H. Morawetz, *High Polymers*, Vol. XXI, *Macromolecules in Solution*, Wiley, New York, 1975, 2nd edn., p. 326.
39 D. C. Johnson, P. M. Nicholson and F. C. Haigh, *Appl. Polym. Symp.*, *3* (1976) 931.
40 P. Williams, *Arkiv. Kem.*, *29* (1968) 485.
41 J. Jortner, *J. Polym. Sci.*, *27* (1959) 199.
42 H. F. Launer and W. K. Wilson, *J. Am. Chem. Soc.*, *71* (1943) 958.
43 J. H. Flynn, W. K. Wilson and W. L. Morrow, *J. Res. Natl. Bur. Stand.*, *60* (1958) 229.
44 F. Gharpuray, Y.-Y. Lee and L. T. Fan, *Biotechnol. Bioeng.*, *25* (1983) 157.
45 A. Basch and M. Lewin, *J. Polym. Sci., Chem. Ed.*, *11* (1973) 3095.
46 T. Nguyen, E. Savarin and E. M. Barrall, *J. Macromol. Sci., Rev. Macromol. Chem. C*, *20* (1981) 1.
47 M. Z. Sefain and F. El-Kalyoubi, *Thermochim. Acta*, *75* (1984) 107.
48 A. Basch and M. Lewin, *J. Polym. Sci., Polym. Chem. Ed.*, *11* (1973) 3071.
49 A. A. Hanna, A. Abal-El-Wahid and M. H. Abbas, *Cellul. Chem. Technol.*, *18* (1981) 11.