SENSITIZED PHOTODEGRADATION OF CELLULOSE AND CELLULOSIC WASTES

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Abstract—The photodegradation of cellulose and cellulose-containing waste sensitized by a variety of dyes was measured by means of viscosity, tensile strength, and scanning electron microscopy. Anthraquinone-2-sulfonate and proflavin dihydrochloride were both more effective than either rose bengal or methylene blue for degradation. Samples degraded by these dyes were similar in appearance to enzyme-degraded ones, but were less susceptible to further degradative action by enzymes.

INTRODUCTION
Growing concern over cellulosic wastes from industrial and agricultural sources has revitalized efforts to degrade or otherwise utilize these wastes (USDA, 1971; Chemurgic Council, 1972). Agricultural waste, in particular, has demanded increased attention because large quantities of material are generated annually. From 60 to 80 per cent of solid animal waste is composed of undigested polysaccharide, protein and lignin (Burroughs et al., 1960). These materials contribute to the high chemical oxygen demand of waste and to the accumulation of residue in feedlots and sludge in sewage lagoons (Wadleigh, 1969). Since carbohydrates constitute a high percentage of both agricultural and industrial solid waste, major efforts have been expended on the degradation of these substances. A number of methods have been partially successful. These include: acid and enzymic hydrolysis (Wohl and Blumrich, 1921; Ghose and Kostick, 1970; Dunlap and Callihan, 1969; Sharkov, 1963), high energy irradiation (Millett et al., 1970; Luethy, 1971), and photodegradation (Williams, 1968; Anon., 1971).

It is in the area of dye-sensitized photodegradation of cellulose that we have attempted to make our contribution. Preliminary results have indicated the effectiveness of anthraquinone dyes as sensitizers for the degradation of pure cellulose (Haller and Wyszewianski, 1936; Baugh et al., 1969). Other photosensitizing dyes have been used to modify a variety of biological molecules (Eskins, 1972; Grams et al., 1972; Eskins et al., 1972). Therefore, we examined several dyes capable of generating both free radicals and singlet oxygen for their effectiveness in degrading pure cellulose. We extended our examination to a less pure carbohydrate fraction isolated from animal feedlot waste (FLW) and, finally, to whole FLW itself.

Our effort is part of a larger program that seeks to utilize the synergistic effects of chemical, photochemical, and microbial methods to degrade or recycle waste for animal feeds and industrial products.

EXPERIMENTAL METHODS
Materials. The following dyes were used without further purification: rose bengal (RB) from Eastman*; methylene blue (MB) from Matheson, Coleman and Bell; proflavin dihydrochloride (P2HCl) from Mann Research; and anthraquinone-2-sulfonate (AQS) from Aldrich. Cellulose strips for degradative studies were made from Whatman chromatography paper, 3 mm; basic weight, 185 g/m²; thickness, 0.33 mm. In addition, cellulose was isolated from the fiber fraction (Jones et al., 1972) of cattle FLW by repeated treatments with acidic solutions of NaClO₂ followed by suspension in previously deaerated solutions of 5% NaOH (Wise et al., 1946). The same procedure was adopted to isolate degraded cellulose from photooxidized FLW. Such cellulose when analyzed by GLC gave 82.53% glucose, 7.92% xylose, 1.58% mannose and 0.47% galactose.

Light sources. Photolysis of cellulose paper strips was by means of a 200 W high-pressure mercury arc lamp (Hanovia 654A-36) encased in a

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.
Pyrex glass filter. Unless otherwise stated, all photolyses were for 20 h.

Photolysis of FLW samples was in direct bright sunlight during the month of April at Peoria, Illinois. Photolysis was from early morning (8:00 a.m.) to early afternoon (2:00 p.m.) for a 6-h sample. A portion of it was photo-oxidized an additional 9 h the following day for a 15-h sample.

**Photolysis procedure.** After samples for photolysis were soaked in dye solutions ranging from 1·0−× 10−4 to 2·0× 10−3 M, they were air-dried at room temperature and 50 per cent relative humidity. Dried cellulose paper strips were wrapped around a water-cooled jacket surrounding the light source at a distance of 2·0 cm and photolyzed. A stream of air was fan-driven past the paper surface during photolysis. Samples for direct sunlight photolysis were made up as a paste combining a dye solution (5·0× 10−3 M) and FLW fiber fraction Wiley-milled to pass a 40-mesh screen. The paste was applied in a thin layer (0·5–1·0 mm) to glass plates and exposed to sunlight. After photolysis, the cellulose was isolated as previously described.

**Viscosity measurements.** Samples were dissolved in cadoxen containing 0·3 N NaOH (Henley, 1962) and then diluted 1:1 with water. Measurements of flow times were made in Cannon Ostwald 75 viscometers at five concentrations from 0·1 to 0·04% w/v. Calculations of average degree of polymerization (DP) were made according to an equation given by Henley (1962): \[ \eta_p = 1·8 \times 10^{-2} \text{DP}^{0·77} \] Plots of relative viscosity \([\eta]_p\) against decreasing concentration for highly oxidized samples of cellulose exhibited a positive curvature. For these samples the cadoxen–water solvent also contained 0·125 M NaCl to suppress ion effects.

**Enzyme studies.** Samples of cellulose and photo-oxidized cellulose (100 mg) were assayed for relative filter paper activity (mg glucose released/100 mg cellulose/h) according to the procedure of Mandels and Weber (1969) using *Trichoderma viride* and *Aspergillus niger* cellulase preparations. Photo-oxidized samples treated with 1·0% NaOH at room temperature for 1 h were also tested for activity.

**Tensile strength.** Cellulose and photo-oxidized cellulose strips were tested on a Schopper tensile apparatus. Results are reported as direct average load in kilograms for strips 1×7 in.

**Scanning electron microscope (SEM).** Comparative photographs of oxidized and nonoxidized cellulose were taken by means of a Cambridge Mark 2A stereoscan SEM. Samples were affixed to aluminum plates with silver paint and covered with a gold-palladium layer before examination. Care was taken to select areas for photography which were representative of the total sample.

**RESULTS**

The degradation of cellulose chromatography paper as a result of dye-sensitized photo-oxidation is shown in Table 1. The decrease in both intrinsic viscosity \([\eta]\) and tensile strength (TS) of the paper strips is a function of the degree of degradation. From these data, it is apparent that triplet dyes which operate by free radical abstractions (AQS, P2HCl) are more efficient at degrading cellulose than those that are primarily singlet oxygen generators.

Photo-oxidation by direct sunlight of cellulose derived from FLW supports this order of dye efficiencies closely and reinforces the special effectiveness of AQS (Table 2).

**Table 1. Cellulose photodegradation; dye efficiency**

<table>
<thead>
<tr>
<th>Dye</th>
<th>([\eta]_p)</th>
<th>(E_t)</th>
<th>(DP_t)</th>
<th>([\eta]_p)</th>
<th>(DP_t)</th>
<th>Tensile strength$§$</th>
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<tr>
<td>Blank</td>
<td>7·38</td>
<td>2473</td>
<td>7·35</td>
<td>2460</td>
<td>13·4</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>6·55</td>
<td>2118</td>
<td>6·42</td>
<td>2063</td>
<td>11·2</td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>6·09</td>
<td>1927</td>
<td>5·97</td>
<td>1878</td>
<td>11·2</td>
<td></td>
</tr>
<tr>
<td>P2HCl</td>
<td>5·91</td>
<td>1853</td>
<td>4·30</td>
<td>1226</td>
<td>10·3</td>
<td></td>
</tr>
<tr>
<td>AQS</td>
<td>3·30−60</td>
<td>1·41</td>
<td>9·7</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

*All samples photo-oxidized 20 h with 200 W mercury arc lamp.
†Cellulose strips soaked in solution 1·0× 10−3 M.
‡Herkstroeter et al., 1964; Nickon and Mendelson, 1965.

**Table 2. Photodegradation of isolated feedlot waste (FLW) cellulose**

<table>
<thead>
<tr>
<th>Dye</th>
<th>([\eta_t]_p)</th>
<th>(DP_t)</th>
<th>([\eta_t]_p)</th>
<th>(DP_t)</th>
</tr>
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<tr>
<td>Blank</td>
<td>4·32</td>
<td>1234</td>
<td>3·82</td>
<td>1051</td>
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<tr>
<td>MB</td>
<td>4·09</td>
<td>1149</td>
<td>3·41</td>
<td>907</td>
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<tr>
<td>RB</td>
<td>3·92</td>
<td>1087</td>
<td>3·37</td>
<td>893</td>
</tr>
<tr>
<td>P2HCl</td>
<td>3·30</td>
<td>835</td>
<td>3·08</td>
<td>795</td>
</tr>
<tr>
<td>AQS</td>
<td>1·26$§$</td>
<td>1·00$§$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All samples treated with 5·0× 10−3 M dye solution.
†6 h of direct sunlight photo-oxidation.
‡16 h of direct sunlight photo-oxidation.
§Viscosities determined in cadoxen–water containing 0·150 M NaOH and 0·125 M NaCl.
The concentration of dye solution as a factor in degradation of cellulose is shown graphically in Fig. 1 for two of these dyes (AQS and P2HCl). For maximum degradation to occur, a dye solution of approximately $5 \times 10^{-3} M$ in AQS is necessary. No further decrease in intrinsic viscosity resulted by increasing the dye concentration. The action of P2HCl approximates that of AQS, but it is on a lower level of efficiency. Also, increases in dye concentration P2HCl past $5 \times 10^{-3} M$ allow some further degradation though slight.

A plot of intrinsic viscosity vs. time of photolysis for AQS-sensitized ($5 \times 10^{-3} M$) photodegradation of cellulose is shown in Fig. 2. The rate of reaction drops rapidly in the first few hours of photo-oxidation, levels off after 5–6 h, and is essentially complete in 12 h. No further degradation is apparent even after extended periods of photolysis.

Photographic evidence of the degree and manner of this degradation is reproduced in Fig. 3. Scanning electron micrographs taken at enlargements of 500× of blank chromatography paper (Fig. 3a) and chromatography paper photo-oxidized ($5 \times 10^{-3} M$ AQS) for 20 h (b) show the extensive breakdown of small connective fibers. Comparison of 200× enlargement of the blank (3c) with fibers that have been photo-oxidized (3d) or treated with T. viride cellulase (3e) show the similarity of photo-oxidized and enzyme-treated samples.

The decreased susceptibility of photo-oxidized samples to further degradation by enzyme treatment can be seen from Table 3. Treating photo-oxidized samples before enzyme assay with 1-0% NaOH partially restores this susceptibility.

Finally, the effectiveness of dyes to photodegrade cellulose in intact FLW is shown in Table 4. Lower viscosities probably reflect that FLW was ground in a Wiley mill to provide maximum surface area for photolysis.

### Table 3. Activity of cellulolytic enzymes toward photo-oxidized cellulose filter paper activity (FPA)

<table>
<thead>
<tr>
<th>Dye</th>
<th>Conc (M)</th>
<th>Time (h)</th>
<th>FPA* (Trichoderma viride)</th>
<th>FPA* (Aspergillus niger)</th>
<th>NaOH-treated (T. viride)</th>
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<tr>
<td>Blank</td>
<td></td>
<td>20</td>
<td>7.19</td>
<td>2.4</td>
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</tr>
<tr>
<td>MB</td>
<td>$5 \times 10^{-3}$</td>
<td>20</td>
<td>5.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>$5 \times 10^{-3}$</td>
<td>20</td>
<td>3.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2HCl</td>
<td>$5 \times 10^{-3}$</td>
<td>20</td>
<td>3.94</td>
<td></td>
<td></td>
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<tr>
<td>AQS</td>
<td>$5 \times 10^{-3}$</td>
<td>20</td>
<td>3.33</td>
<td>1.26</td>
<td>6.50</td>
</tr>
<tr>
<td>AQS</td>
<td>$5 \times 10^{-3}$</td>
<td>3</td>
<td>5.88</td>
<td>4.67</td>
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<tr>
<td>AQS</td>
<td>$5 \times 10^{-4}$</td>
<td>6</td>
<td>3.87</td>
<td>1.89</td>
<td>1.51</td>
</tr>
<tr>
<td>AQS</td>
<td>$1 \times 10^{-4}$</td>
<td>20</td>
<td>1.89</td>
<td></td>
<td></td>
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</tbody>
</table>

*FPA reported as mg reducing sugar/ml.
Figure 3. Scanning electron micrographs at 500× of cellulose filter paper (a) and the same paper photooxidized (b); and at 200× of cellulose filter paper (c), photooxidized filter paper (d), and cellulose filter paper treated with *Trichoderma viride* cellulose (e).
Table 4. Photodegradation of cellulose in F.L.W*  

<table>
<thead>
<tr>
<th>Dye</th>
<th>[η]</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>3:20</td>
<td>835</td>
</tr>
<tr>
<td>MB</td>
<td>3:06</td>
<td>802</td>
</tr>
<tr>
<td>RB</td>
<td>3:00</td>
<td>792</td>
</tr>
<tr>
<td>P2HCl</td>
<td>2:92</td>
<td>742</td>
</tr>
<tr>
<td>AQS</td>
<td>2:38</td>
<td>569</td>
</tr>
</tbody>
</table>

*All samples treated with $5 \times 10^{-3} M$ dye solutions and photo-oxidized by direct sunlight for 15 h.

DISCUSSION

Our results indicate that all the dyes we tested are somewhat effective in degrading not only pure cellulose, but also the carbohydrate fraction isolated from F.L.W. Both decreased TS and lowered viscosities are evidence of this degradation. Moreover, there appears to be a direct correlation between the triplet energy ($E_T$) of the dye and the degree of cellulose degradation. This correlation indicates that the absorbed dye functions primarily by a free-radical hydrogen abstraction, since the higher energy triplet dyes are known to be more efficient hydrogen abstractors (Bourdon and Schnuriger, 1967). The free radical sites created on the cellulose chain react with oxygen and initiate degradation processes while the reduced dye is being regenerated. However, for at least two of these dyes (P2HCl and AQS), the amount of cellulose degraded is related to dye concentration. As seen in Fig. 1, the dye is a limiting factor below concentrations of $5 \times 10^{-3} M$. Evidently, regeneration of sensitizer is rate limiting at low dye concentrations and in a low moisture environment (Baugh et al., 1969). With AQS concentrations above $5 \times 10^{-3} M$, available cellulose becomes the limiting factor. This limited amount of degradation is reached after approximately 6–12 h of photo-oxidation with a mercury arc lamp or a similar amount of time with direct sunlight (Fig. 2 and Table 2).

Scanning electron micrographs (Fig. 3) show that when this limit is reached, many of the tiny connective and surface fibers have been degraded. This amorphous region of the cellulose is highly susceptible to both photo-oxidation and cellulase enzymes (Fig. 3), and the results of their action appear remarkably similar. In fact, highly oxidized cellulose samples resist further action of cellulases. However, RB, P2HCl and AQS are about equally effective in decreasing cellulase activity (Table 3) even though the amounts of dye-sensitized degradation of cellulose measured by viscosity differ significantly (Table 1). This difference is not necessarily inconsistent. Previously published results indicate that photo-oxidative changes at secondary carbons are as efficient in inhibiting cellulase activity as is degradation of amorphous areas of the cellulose matrix (Kaplan et al., 1970). Consequently, both oxidative degradation and oxidative modification of cellulose occur.

These degradative and modifying processes appear to function quite well on purified fractions of cellulose but less so on complex organic mixtures like those found in F.L.W. This result is not surprising since other organic ingredients dilute the degradative effects on cellulose by being degraded themselves or by acting as singlet oxygen (Grams and Eskins, 1972) or as free radical traps. It is obvious, though, that AQS is still an effective sensitizer despite these limiting factors. Also, the reduced susceptibility of photo-oxidized cellulose to enzyme degradation need not deter the use of photo-oxidation for F.L.W degradation. Klein et al. (1970) reported that lignin fractions exposed to UV radiation are quite easily utilized by microbes. Qualitatively, we have also observed an increased rate of growth of T. viride on photo-oxidized F.L.W over that of untreated F.L.W. We are currently expanding our observations in this area and in other methods of F.L.W disposal and utilization.

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REFERENCES


