

## BIOMASS PHOTOCHEMISTRY X: ANALYSIS OF STRUCTURAL MODIFICATIONS IN LIGNIN UNDER UV IRRADIATION†

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### Summary

The structural modifications of commercial lignin and lignin-dioxane caused by photochemical reactions were studied. The differential scanning calorimetry technique gave a reasonable correlation of enthalpy changes with fluence of irradiation and radical formation. The chemiluminescence method gave results which indicated that at higher irradiation fluence a greater number of cross-linking processes occur. The absorption and fluorescence studies indicate that pre-irradiated lignin chromophores are similar to quinonic and carboxylic groups conjugated to aromatic rings. All the parameters studied suggested that photochemical pre-treatment should be a good method for the biological degradation of lignin. Actually the photochemical pre-treatment of lignin at  $\lambda > 300$  nm increased the efficiency of single-cell protein production 2.2-fold compared with the unirradiated sample. This analytical methodology is important in understanding the biomass behaviour and is a good new approach for the study of the industrial and economical viabilities of all of the processes.

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### 1. Introduction

Lignin represents approximately 25% of the dry weight of wood. Its structure is based on phenylpropane units, is totally amorphous and the lignin may only be obtained from natural sources. Figure 1 shows an approximate formula which represents a portion of the lignin macromolecule.

Owing to its irregular structure, lignin is a strong barrier against attack by reagents or enzymes on the components of the cellulose.

Any modification of the lignin structure which increases the efficiency of chemical or enzymatic attack on cellulose is of great interest from an industrial point of view. This is most relevant if we consider that the biomass

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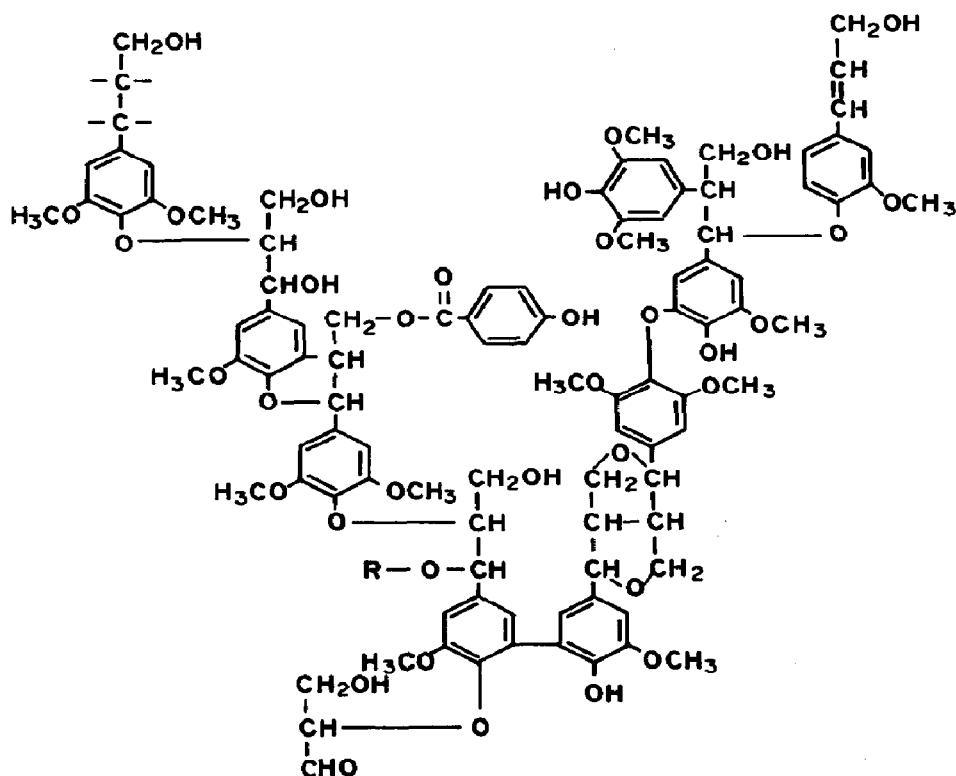


Fig. 1. A typical lignin structural unit (after ref. 1).

conversion processes currently used need dilute acids and high temperatures or concentrated acids and low temperatures [2], introducing serious technological difficulties and high energy costs.

Meanwhile, lignin by itself constitutes a very rich source of organic compounds, and this must be explored as there is tremendous potential for industrial applications [3, 4]. With this in mind we have decided to study the photochemical behaviour of different types of lignin.

Previously it has been found that the fluorescence at 480 nm ( $\lambda_{ex} = 330$  nm) is correlated with the carbonyl groups formed during irradiation [5]. The chemiluminescence spectra in basic media show that the maxima in the aromatic carbonyl region (400 - 500 nm), the quinone region (500 - 600 nm) and the singlet oxygen region (longer than 650 nm) constitute the total photon emission [6, 7]. The system peroxidase-H<sub>2</sub>O<sub>2</sub>-O<sub>2</sub> appeared to be an excellent ligninase model [1] and a good indicator for lignin modification [8]. Using this method we found that a convenient fluence for both lignin modification and easy degradation by peroxidase was 2000 - 4000 kJ m<sup>-2</sup> [5].

In this paper we report a study of the structural modifications obtained through photochemical reaction of a commercial lignin of *Eucalyptus paniculata* and from lignin extracted from rice hull using dioxane. These modifications were followed by spectroscopy, chemiluminescence and differential scanning calorimetry (DSC) methods.

## 2. Material and methods

Lignin from *E. paniculata* was extracted by sulphuric acid hydrolysis from wood (Engineering School, Lorena, Brazil). Its approximate composition was 72% lignin, 19.8% cellulose and 8.8% ash. The phenolic hydroxyl content was 0.9% [9], and the carbonyl content was 0.0029 as coniferyl aldehyde groups by  $\text{OCH}_3$  (by mole) and 0.034 as  $\alpha$ -carbonyl groups by  $\text{OCH}_3$  [10]. Dioxane-lignin from rice hull was obtained by extraction with 90vol.%dioxane-10vol.% $\text{H}_2\text{O}$  [11]. The phenolic hydroxyl content was 1.6%, and the carbonyl content was 0.012 as coniferyl aldehyde groups by  $\text{OCH}_3$  and 0.045 as  $\alpha$ -carbonyl groups by  $\text{OCH}_3$ .

Lignin irradiations in the solid state were carried out at different fluences with a mercury vapour HQL Osram lamp at fluence rates of  $108 \text{ W m}^{-2}$  ( $\lambda > 254 \text{ nm}$ ) and  $68.4 \text{ W m}^{-2}$  ( $\lambda > 300 \text{ nm}$ ) (using glass plates as the filter).

Ferulic acid, vanillic acid, vanillin, vanilpyruvic acid and 3,5-dimethoxy-1,4-benzoquinone were from Sigma. Diguaiacylstilbene was a gift from Dr. K. Lundquist of Göteborg University, Sweden. DSC measurements were carried out using a Perkin-Elmer model DSC-2 over a temperature range of 320 - 500 K at a heating rate of  $40 \text{ K min}^{-1}$ , using indium as the standard. Fluorescence and absorption spectra were measured using a Perkin-Elmer MPF-44B spectrofluorometer and a Zeiss DMR-21 spectrophotometer respectively. Chemiluminescence was measured in a Beckmann LS-7000 liquid scintillation counter.

The following procedure was adopted in the experiments with  $\text{NaOH-O}_2$ -lignin: to 3 ml lignin solution ( $150 \text{ mg l}^{-1}$ ) in 50vol.%dioxane-50vol.%-water,  $40 \mu\text{l}$  5 N NaOH were added and then counted every 0.1 min in the liquid scintillation counter.

*Neurospore sitophila* (TFB strain) was isolated [12] and cultured [13] as described previously.

## 3. Results and discussion

The DSC technique gave useful information regarding the changes in energy associated with the structural transformations that take place during the heating of the sample. The area under the curve is a direct measure of the enthalpy of the thermal transitions that occur in the substrate [14]. The DSC curve for unirradiated sulphuric acid lignin shows that there is a transition temperature at 370 K. For irradiation at different fluences, a transition temperature shift to 380 K was found. These values are in the region already reported for lignin sulphonates [15]. It is believed that in the case of the sulphuric acid lignin the transition occurs in three stages [16]. Condensation and splitting of the side chain occur at 220 - 250 K. Active pyrolysis leads to formation of free radicals between 300 and 400 K and finally a series of degradation and condensation reactions occur simultaneously at above 500 K. The high thermal stability of sulphuric acid lignin is due to the ther-

mally stable bonds formed by intramolecular and intermolecular condensation during lignin preparation.

Figure 2 shows a typical DSC curve for sulphuric acid lignin from *E. paniculata* irradiated in the solid state at  $\lambda > 254$  nm. The transition enthalpy  $\Delta H_t$  was measured relative to the  $\Delta H$  value of  $6.79 \text{ cal g}^{-1}$  for indium and Fig. 3 shows a reasonably clear relationship between fluence and  $\Delta H_t$ . This result may also be correlated with free-radical formation in the irradiated lignin in the solid state. Presumably these facts indicate that for

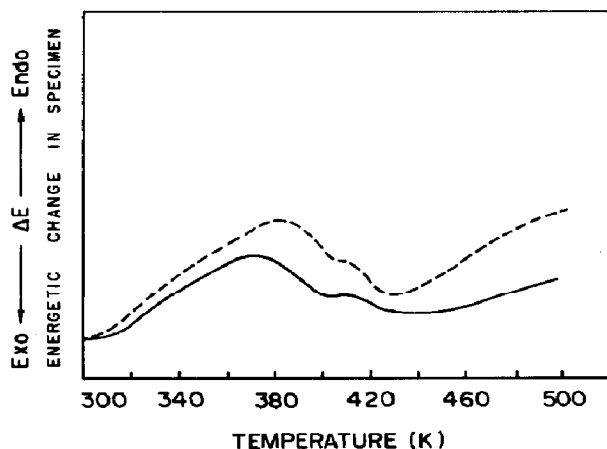


Fig. 2. DSC curve of unirradiated sulphuric acid lignin (—) and the lignin irradiated at  $\lambda > 254$  nm (fluence,  $400 \text{ kJ m}^{-2}$ ) (---). Heating rate,  $40 \text{ K min}^{-1}$ ; chart speed,  $20 \text{ mm min}^{-1}$ ; range,  $10 \text{ mcal s}^{-1}$ .

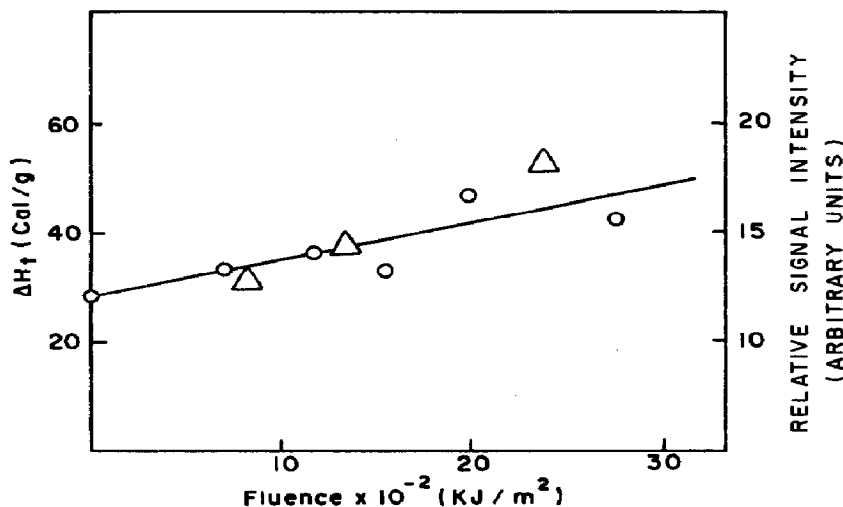


Fig. 3.  $\Delta H_t$  for sulphuric acid lignin irradiated at  $\lambda > 254$  nm at different fluences (○) and relative rate of free-radical formation by irradiation at room temperature (△) (taken from data in ref. 17).

irradiation in the solid state more radicals are produced and a greater number of cross-linking processes occur in the macromolecule. In contrast, the radicals formed in lignin irradiated in solution have very short lifetimes [17]. This suggests that the behaviour of the lignin depends on whether it is irradiated in the solid or in solution. For the further study of these characteristics we selected lignin-dioxane extracted from rice hull.

Lignin-dioxane, from rice hull, irradiated in solution produced chemiluminescence in basic media. On the contrary, lignin-dioxane irradiated in the solid does not produce any chemiluminescence. This is probably due to the cross-linking reaction, as the DSC technique shows that there is an increase in  $\Delta H_t$  that is directly proportional to the fluence of irradiation. Figure 4 shows the integrated emission over 2 min for lignin-dioxane irradiated at  $\lambda > 254$  nm in basic solution. At around  $800 \text{ kJ m}^{-2}$  an emission maximum was observed. However, in the case of irradiation of the solid only a decrease in the emission was found, the emission being completely inhibited at around  $300 \text{ kJ m}^{-2}$ .

The fluorescence spectra showed that different chromophores are formed in the two processes. In this study, when the lignin was irradiated in solution a shift to higher wavelengths was observed. However, in the solid state the inverse effect was observed (not shown).

Several studies have been carried out on the spectroscopic properties of the lignin chromophore. The fluorescence spectra of different lignins indicate that the polymer behaves like a single chromophore [18 - 20]. To explain this observation it was assumed that energy is transferred from different excited structural elements in the lignin by a radiationless process to a specific structure which acts as an energy sink from which the fluorescence finally appears. In the photochemical degradation of lignin and lignin model

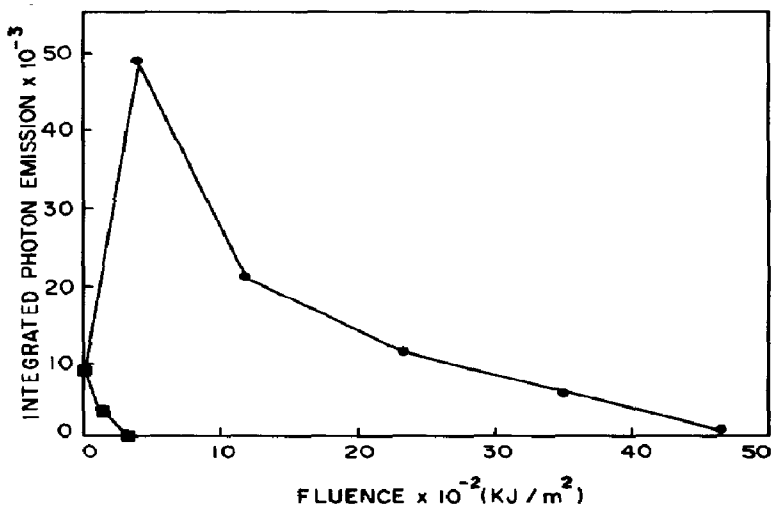


Fig. 4. Integrated photon emission (over 2 min) of dioxane-lignin oxidation in a basic medium after irradiation at different fluences at  $\lambda > 254$  nm in solution ( $\bullet$ ) and in the solid state ( $\blacksquare$ ).

compounds the formation of *para*- and *ortho*-quinones besides aldehyde and carboxylic acids occurs [21, 22]. In a previous study [5] there was found to be a correlation between the increase in fluorescence at 480 nm and the appearance of the carbonyl group at different fluences of irradiation. On comparing the fluorescence spectra of lignin-dioxane from rice hull with those of different lignin model compounds (Fig. 5), it was found that irradiated lignin shows a fluorescence maximum shifted by approximately 30 nm. Comparison of the spectra of model compounds with the spectrum of lignin indicates that after irradiation the chromophore is changed to a quinonic derivative. The unirradiated lignin has the chromophore characteristic of a stilbene-type compound with carbonyl groups conjugated to an aromatic ring.

Figure 6(A) shows the absorption spectra of unirradiated lignin. Figure 6(B) shows the change at  $\lambda = 280$  nm and  $\lambda = 315$  nm after irradiation with different fluences. This figure indicates that on irradiation in solution lignin undergoes a rapid decrease in absorbance at both wavelengths. When the lignin was irradiated in the solid state or in the rice hull, different behaviour was observed. In the latter the effect was larger than the irradiation effect in the lignin previously extracted. Janshekar *et al.* [23] explained that the 280 nm maximum is due to the substituted aromatic ring and that the carbonyl group and double bond conjugated to the benzenic ring are re-

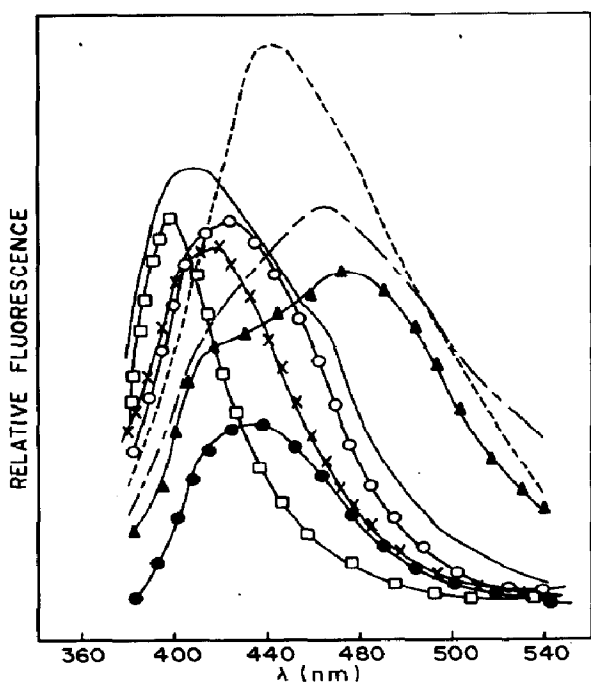


Fig. 5. Fluorescence spectra ( $\lambda_{exc} = 360$  nm) of dioxane-lignin ( $150 \text{ mg l}^{-1}$ ) and lignin model compounds ( $10 \text{ mM}$ ): unirradiated (—) and irradiated (---) dioxane-lignin at  $\lambda > 254$  nm (fluence of  $400 \text{ kJ m}^{-2}$ ) (●, ferulic acid; ▲, vanillic acid; ×, vanillin; ○, vanilpyruvic acid; ---, 3,5-dimethoxy-1,4-benzoquinone; □, diguaiacylstilbene).

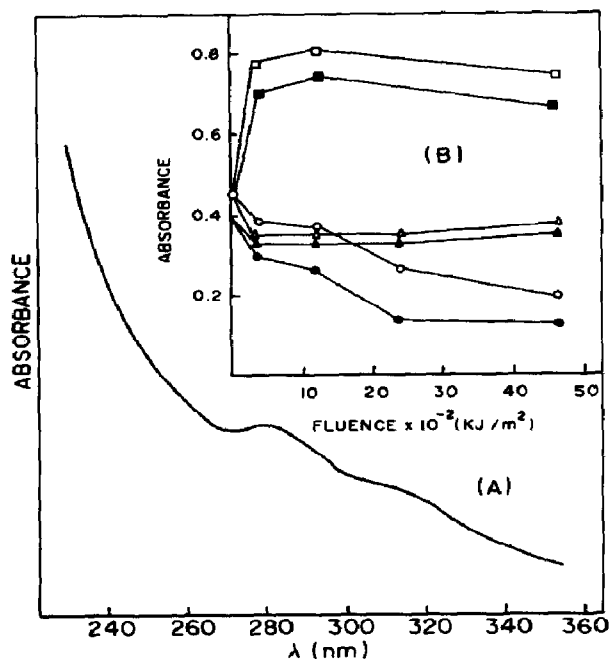


Fig. 6. (A) Absorption spectra of unirradiated dioxane-lignin ( $150 \text{ mg l}^{-1}$ ); (B) variation in absorbance at  $\lambda = 280 \text{ nm}$  (open symbols) and at  $\lambda = 315 \text{ nm}$  (full symbols) of extracted dioxane-lignin irradiated under different conditions. Irradiations were performed at  $\lambda > 254 \text{ nm}$  in solution ( $\circ$ ), in the solid state ( $\triangle$ ) or in the rice hull ( $\square$ ).

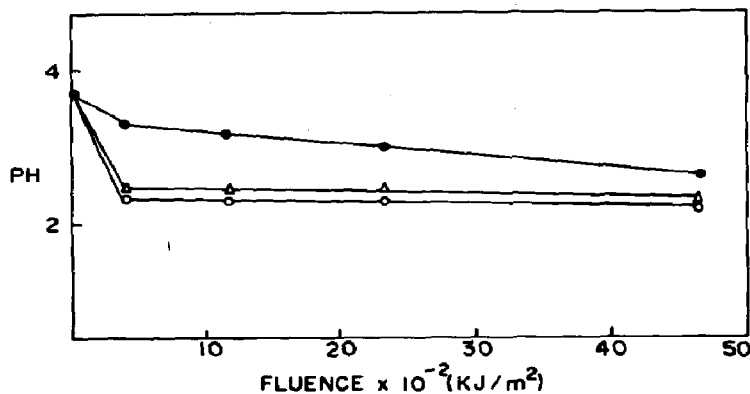


Fig. 7. Variation in pH after irradiation of dioxane-lignin ( $150 \text{ mg l}^{-1}$ ) at  $\lambda > 254 \text{ nm}$  under different conditions (irradiated in solution ( $\bullet$ ), in the solid state ( $\circ$ ) and in the rice hull and then extracted as dioxane-lignin ( $\triangle$ )).

sponsible for the 315 nm shoulder. These groups disappeared in lignin-dioxane when the fluence of the irradiation was increased.

Another quantity that changes during the irradiation of lignin under different conditions is the pH of the final solution. Figure 7 shows that the pH decreased on irradiation at  $\lambda > 254 \text{ nm}$ , the decrease being larger in the solid state than in solution. The lignin irradiated in the rice hull showed

behaviour similar to the isolated lignin irradiated in the solid. This effect can be explained by the formation of carboxylic acid as a product of lignin photo-oxidation [24].

The information gained from the photolysis of lignin formed the basis for an interesting study on fungal growth. Recently we have isolated the *N. sitophila* (TFB strain) fungus [12], which is very efficient in degrading several biomass substrates [13]. Table 1 shows that dioxane-lignin is degraded by this fungus, leading to an increase in total weight of the fungal growth, this being more efficient in the case of irradiation for 1 h at  $\lambda > 300$  nm than at  $\lambda > 254$  nm. These studies indicate that there are similarities between the photochemical and the enzymatic degradation, as did the experiments on the behaviour of lignin in the presence of  $H_2O_2$  with ligninase or peroxidase [25] after photochemical pretreatment. The fungus can thus differentiate between the photochemical transformations and could be used generally to increase the degree of degradation of lignocellulosic materials. The analytical techniques used in this work help in the understanding of the biomass behaviour and provide a good approach for the study of the industrial and economic viabilities of these processes.

TABLE 1

Total weight of fungal growth using *Neurospore sitophila* (TFB strain) acting over rice hull lignin irradiated at different wavelengths

Conditions <sup>a</sup>	Solid	Filtrate	Total weight (mg)
Unirradiated	38	6	44
$\lambda > 300$ nm	76	18	94
$\lambda > 254$ nm	82	12	89

<sup>a</sup>Irradiation for 1 h. The total weight was measured after 10 days of culture.

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