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Singlet oxygen degradation of lignin

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Abstract

Lignin obtained by steam explosion of straw, beech (a hardwood plant), and pine (a softwood plant) was isolated and characterized by using standard methods. The irradiation of lignins with visible light in the presence of oxygen and of a singlet oxygen sensitizer (Rose Bengal) induced several modifications in the lignin structures that were monitored by using gel permeation chromatography (GPC) and UV spectroscopy. We observed a remarkable reduction of the mass distribution by GPC when monitoring at 280 nm and the shift of the residual chromatograms to mass values lower than the native lignins. These data are in agreement with a depolymerization reaction of lignins. The UV spectra showed that all the chemical species of lignins undergo several modifications during the irradiation reaction. Thus, the E_1 band reduced its absorbance and underwent a hypsochromic shift. The E_2 and the B band were greatly reduced during the irradiation. © 1998 Elsevier Science S.A.

Keywords: Lignin; Singlet oxygen; Gel permeation chromatography; UV spectroscopy

1. Introduction

Lignin is a three-dimensional phenyl propanoid polymer mainly linked by ether bonds between monomeric phenylpropane units most of which are not readily hydrolyzable. The treatment of lignin is an actual environmental problem in the pulping and bleaching processes [1–8].

In a project devoted to studying the use of photochemical methodology in the setting up of low environmental-impact processes, we are studying the use of singlet oxygen in the photodegradation of lignin.

Recently, we found that some lignin models can undergo several modifications when treated with singlet oxygen [9,10]. In particular, we could see that most of model compounds gave a β-C–O cleavage reaction. This type of reaction was described as occurring by using direct irradiation of lignin models with UV light at λ 300–350 nm [11–17]. Furthermore, this reaction occurs also in the absence of the carbonyl group in the molecule and when the number of the substituents on the phenoxy part of the molecule decreases the reactivity of the models towards singlet oxygen, cleavage of the phenoxy part of the molecule was observed.

On the basis of these preliminary results, we decided to test the capability of singlet oxygen to induce degradation reactions on lignins obtained via steam explosion.

2. Materials and methods

The material used as the source of lignin (straw, beech, and pine) was mechanically reduced into pieces of about 1 cm in length and water was added to raise its initial content to 50%. Steam explosion runs were carried in a 10-l batch reactor, loading about 0.5 kg of material each cycle. Treatment conditions ranged from 188° to 230°C and from 3 to 10 min. The treatment condition together with the corresponding severity parameter, R_0, is reported in Table 1 [18]. The raw material (100 g) was extracted two times with hot water (250–300 ml, 65 ± 5°C) in order to eliminate sugars and hemicelluloses. The extraction of lignin from exploded materials was carried out using 1.5% sodium hydroxide solution (250 ml) at 90°C for 15 min two times. Lignin was precipitated at pH 2 with 20% H_2SO_4 when the solution was still warm, filtered, washed, and dried at 105°C.

Elemental analyses were obtained with a Carlo Erba Elemental Analyzer 1106. ^{13}C NMR spectra were recorded with

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Table 1
Lignins from steam explosion

Sample	Source	Time (min)	log R_0	Lignin (g)	Lignin (%)	Soluble lignin (g/l)	Soluble lignin (%)
1	straw	10	3.94	3.531	12.17	0.66	2.49
2	straw	3	3.93	7.153	27.85	0.37	1.45
3	beech	3	3.93	2.598	12.13	2.24	8.38
4	pine	3	3.93	3.189	14.95	5.65	18.56

$R_0 = t\cdot\exp(T-100)/14.75$.

a Bruker 300 AM instrument. All the spectra were recorded in DMSO-d_6. Gel permeation chromatography (GPC) analyses were performed on a Varian HPLC by using H-P Plgel 5 micro column. The lignin samples were acetylated (acetic anhydride and pyridine) before the use. Spectrophotometric grade THF was used as mobile phase and distilled (oven, $LiAlH_4$) before use. The chromatograms were obtained using an UV detector at 280 nm. The conversion from elution time to molecular weight was performed by using a calibration obtained using polystyrene samples [19]. Cary 2300 spectrophotometer was used for the UV spectra with spectrophotometric grade DMF as solvent. In some cases the 1:1 CH_3CN–EtOH mixture was used as solvent.

2.1. *Reactions with singlet oxygen*

A solution of the lignin (10 mg) in a 1:1 mixture of acetonitrile–ethanol (10 ml) containing 5×10^{-4} M Rose Bengal was irradiated in a Pyrex tube surrounded by a Pyrex water jacket connected to a Haake D9-G thermostat to maintain the temperature at 13.0±0.1°C. The Pyrex tube was dipped into a 1% (w/v) solution of $NaNO_2$ (previously saturated with bubbling oxygen for 1 h) in order to cut off the irradiation at 400 nm. The irradiation was performed by using a 50 W tungsten–halogen lamp.

3. Characterization of lignins from steam explosion

The results of elemental analyses are collected in Table 2. The samples were analysed for the presence of carbon and hydrogen in order to characterize the lignin, and also for the presence of both nitrogen and sulphur as a marker of the presence of protein materials in the lignin and of sulphonated lignins respectively. Sulphur was not detected in our samples. The elemental analysis allows us to give the molecular weight of the lignins expressed in phenylpropanoid (C9) units. The data are collected in Table 2. All the samples showed a molecular weight in the range 172–186.

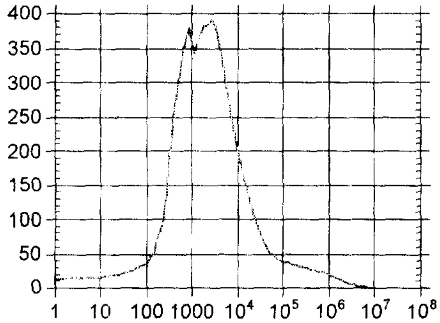


Fig. 1. MW distribution of lignin from straw at log R_0 = 3.94.

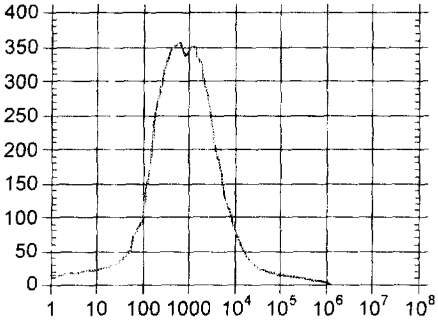


Fig. 2. MW distribution of lignin from straw at log R_0 = 3.93.

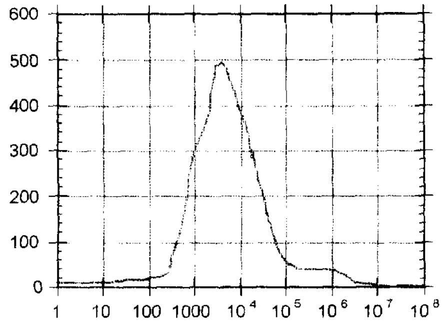


Fig. 3. MW distribution of lignin from beech at log R_0 = 3.93.

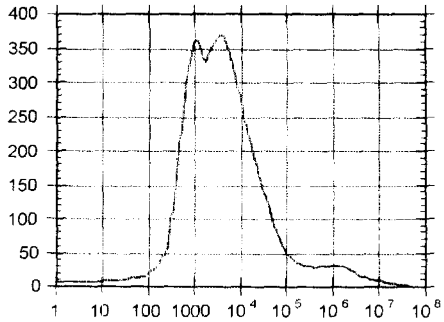


Fig. 4. MW distribution of lignin from pine at log R_0 = 3.93.

Table 2
Elemental analysis, empirical formulas, and molecular weights (in C9 units) of lignins from steam explosion

Sample	Log R_0	Elemental analysis				Empirical formula			MW
		N	C	H	O	C	H	O	
1	3.94	1.26	62.13	5.88	30.73	9	10.22	3.34	172
2	3.93	1.18	57.31	5.56	35.95	9	10.47	4.24	186
3	3.93	–	62.21	5.83	31.96	9	10.13	3.47	174
4	3.93	–	61.02	6.08	32.90	9	10.75	3.64	177

Table 3
UV spectra of lignins from steam explosion

Sample	λ_1 (nm)	D (l g^{-1} cm^{-1})	λ_2 (nm)	D (l g^{-1} cm^{-1})	λ_3 (nm)	D (l g^{-1} cm^{-1})	Type I (meq g^{-1})	Type II (meq g^{-1})	Type IV (meq g^{-1})
1	321	16.80					0.43		0.12
2	316	17.60	278	21.60			0.47		0.13
3	306	10.00	277	16.80	340	6.40	1.28	0.13	0.11
4	308	9.60	273	15.60	340	6.60	1.95		0.12

Table 4
^{13}C NMR spectra of lignins from steam explosion, δ (ppm)

Sample 1	Sample 2	Sample 3	Sample 4
173	173		
153	153	153	153
148	148	148	148
145	145		
		138	138
136	136	136	136
	135.5	135.5	135.5
135	135	135	135
133			
130	131		131
	126		
	121		
120		119	119
115	115	115	115
112	112		
111	111		
	106		
104.5	104.5	104.5	104.5
87	87	87	87
72	72	72	72
60		60	60
55.5	55.5	55.5	55.5
39.5		40	40
28		28	28

The distribution of acetylated lignins considering their molecular weights was obtained by using GPC. The results are depicted in Figs. 1–4. Most of the molecules of our samples show a molecular distribution in the range 100–100000.

The UV spectra of our samples were recorded in DMF. We recorded also the differential spectra obtained by carrying out the spectrum of the samples in 1 M NaOH vs. the standard solution in DMF. These data allowed us to give the amount in meq g^{-1} of some structural features in the lignin samples. In this case we were able to give the amounts of syringyl and guaiacyl phenols (Type I), the amounts of phenols containing conjugated double bonds (i.e. HO–Ar–CH=CH–CH$_2$OH, Type II), and the amounts of stilbenic phenols (Type IV). The results are reported in Table 3 [20].

Finally, the characterization of the lignins used in this work was completed by using ^{13}C NMR spectroscopy. The signals observed with our samples are collected in Table 4 [21,22]. The presence of guaiacyl and syringyl structures were observed from the data. These structures are both β-O-4 etherified and nonetherified. The presence of both threo and erythro structures were noted and also the presence of structures of the type cinnamaldehyde and stilbenes. The ^{13}C NMR spectrum of lignin from steam exploded beech is reported in Fig. 5. All these data are critical in order to estimate the modification that occurred in the lignin structure after the irradiation.

4. Photochemical behaviour

The irradiation of the lignin from steam explosion was carried out in a 1:1 acetonitrile–ethanol mixture. The solution was saturated with oxygen and then irradiated by using visible light in the presence of a singlet oxygen sensitizer. Rose Bengal and tetraphenylporphyrin were used but only Rose Bengal gave interesting results. The reason for this behaviour can be understood considering that we used crude lignin samples which were not transparent in the range 600–650 nm.

The irradiation of lignins was performed in the presence of oxygen without the sensitizer. It could be seen that when direct absorption at 300–350 nm was avoided no reaction occurred. These experiments allowed us to establish that all

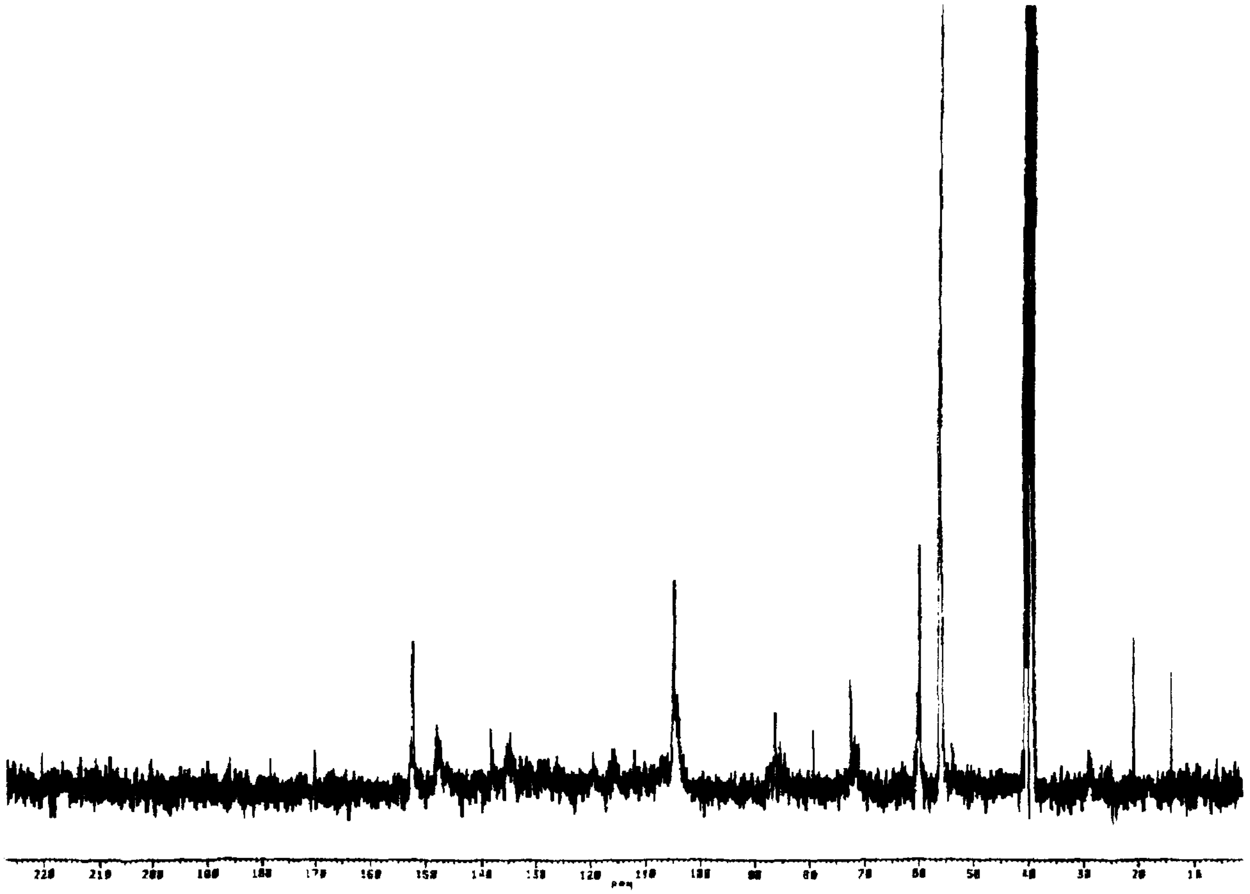


Fig. 5. ^{13}C NMR spectrum of beech lignin.

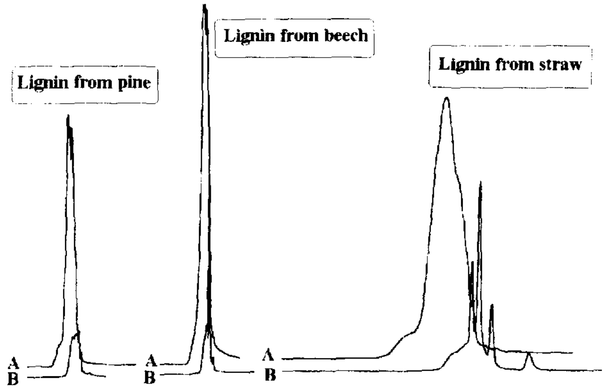


Fig. 6. Gel permeation chromatograms of lignins from steam explosion before and after irradiation with visible light in the presence of both oxygen and Rose Bengal. (A) Before irradiation; (B) after irradiation.

the reactions observed in the following experiments were due to the presence of singlet oxygen in the reaction mixture.

First, the lignin obtained from the steam explosion of straw was irradiated using sample 1 of Table 1. After 24 h irradiation the solvent was removed and the mixture was acetylated with acetic anhydride in pyridine. The sample thus prepared was analysed by using GPC and the result is shown in Fig. 6. An important reduction of the absorption at 280 nm and the shift of the residual signals at longer retention times were observed. These results were confirmed recording the UV spectrum on the photolysed solution after 24 h irradiation. The spectrum is shown in Fig. 7. A remarkable reduction of all the UV spectra was noted. In particular, the absorption at 278 nm (typical of guaiacyl and syringyl phenols) was almost completely reduced. Furthermore, Type IV structures (stilbenic phenols) are reduced. Nevertheless, the residual UV spectrum shows the same typical absorption bands of the lignins (a shoulder at 230 nm and a peak at 215 nm).

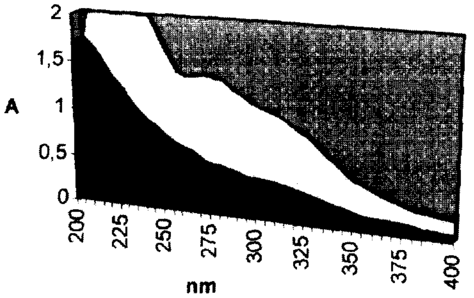


Fig. 7. UV spectra of lignin from straw (sample 1) before and after 24 h irradiation with visible light in the presence of both oxygen and Rose Bengal. Solvent: MeCN–EtOH 1:1.

The same trend was observed on the same lignin (Sample 2, Table 1) performing UV spectra at different irradiation times (Fig. 8). In the native lignin a peak at 278 nm and a

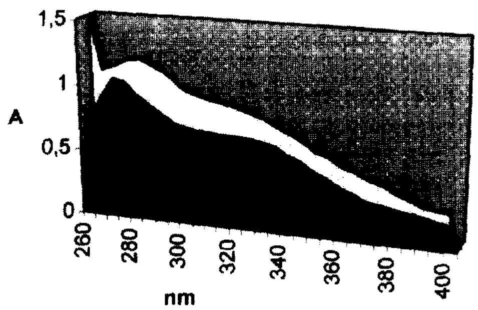


Fig. 8. UV spectra of lignin from straw (sample 2) before and after irradiation with visible light in the presence of both oxygen and Rose Bengal. In order from back: before irradiation, after 1 h irradiation, after 3 h irradiation. Solvent: DMF.

shoulder at 316 nm were observed. After 1 h the absorption at 278 nm was reduced and it was shifted at 272 nm. The absorption at 316 nm was reduced. After 3 h irradiation the peak at 278 nm was greatly reduced (the absorbance was reduced to one half) and the peak was observed only with difficulty resembling a plateau at 270 nm.

The irradiation of lignin obtained from the steam explosion of beech wood showed similar trends to those described above. Fig. 9 shows the photochemical behaviour of lignin in the presence of singlet oxygen from data collected through GPC. It can be seen that, also in this case, the irradiation leads to a reduction of the original peak and to a shift of the residual chromatogram towards lower molecular weights.

The UV spectra of this softwood lignin showed typical absorption peaks at 215 (E_1), 240 (shoulder; E_2), 274 nm (B). The UV spectrum registered after 1 h irradiation showed a reduction of the absorptions at 240 and 274 nm and an increase of the E_1 band. However, a shift of the maximum of this absorption band at 210 nm should be noted. After 6 h irradiation the absorptions at 240 and 274 nm were greatly reduced, being reduced to one half, while the E_1 band shifted at 206 nm underwent a relevant reduction in the absorbance.

The irradiation of the softwood lignin obtained from the steam explosion of pine does not show a particular modification of the described trend. GPC (Fig. 6) showed that the irradiation induces a behaviour similar to that described above. Also, in this case, a reduction of the signals on the chromatograms and the shift of the residual signals toward lower molecular weights were observed.

In the case of lignin from pine, the UV spectrum (Fig. 10) showed peaks at 210 (E_1), 242 (shoulder; E_2), and 274 nm (B). After 24 h irradiation in the presence of singlet oxygen, the spectrum showed significant modifications. In fact, the E_1 band was reduced and shifted at 206 nm. The E_2 band appeared as a shoulder with an absorbance reduced by 25%. The B band appeared only as a shoulder with an absorbance also in this case reduced of 25%.

In order to establish if singlet oxygen was involved in the above described reactions, all of them were repeated in the presence of one equivalent of DABCO, a singlet oxygen quencher [23]. Under these photochemical conditions all the reactions were quenched.

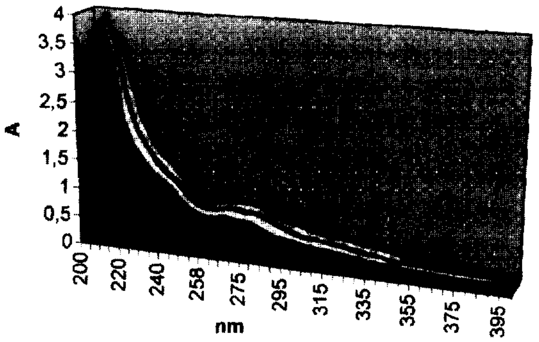


Fig. 9. UV spectra of lignins from beech before and after irradiation with visible light in the presence of both oxygen and Rose Bengal. In order from back: before irradiation, after 1 h irradiation, after 3 h irradiation, after 6 h irradiation. Solvent: MeCN–EtOH 1:1.

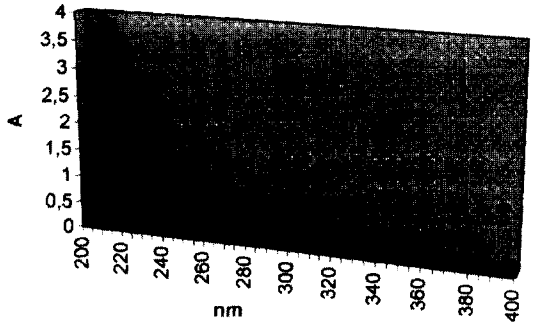
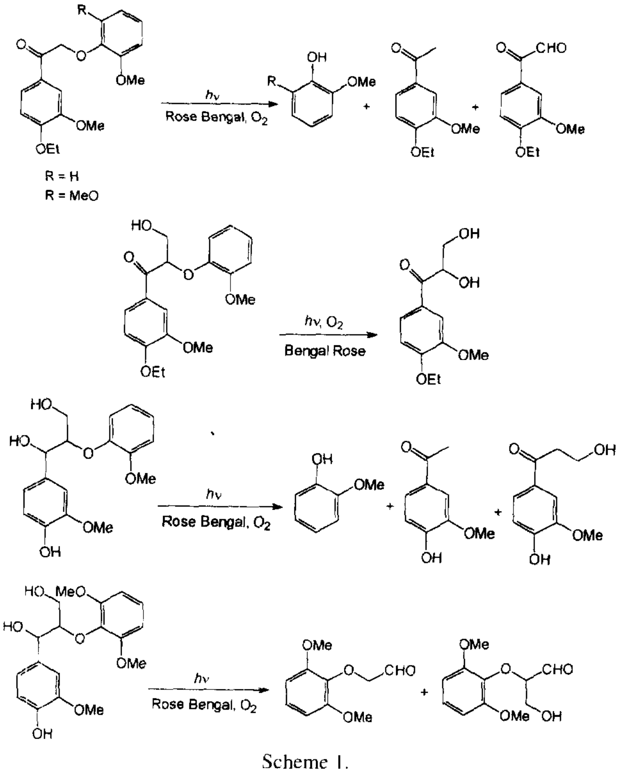


Fig. 10. UV spectra of lignin from pine before and after 24 h irradiation with visible light in the presence of both oxygen and Rose Bengal. Solvent: MeCN–EtOH 1:1.



Scheme 1.

5. Conclusion

Some years ago, discussing a photochemical oxidation of lignin and using as model compounds styryl derivatives, the authors reported the involvement of singlet oxygen in the process via the formation of the corresponding endoperoxide that then led to the products derived from a carbon–carbon bond cleavage [24]. Now, we showed that the oxidation with singlet oxygen of lignins obtained from steam explosion occurred. However, the structural modifications detected in both GPC and UV analyses can not be due only to the oxidation of the double bond. Clearly, all the structures usually involved in the lignin structures underwent oxidation reaction as reported in our previous papers in this field [9,10]. It is noteworthy that the most sensitive peak in the UV spectrum seems to be the B band at 270 nm. This band, usually attributed to phenols, is greatly reduced in the presence of singlet oxygen, in agreement with the well-known properties of this reactant to react with phenols [15–17].

GPC showed that an actual depolymerization occurs during the irradiation. These results can be explained considering the reactions reported in the Scheme 1 [9,10]. Also in this case, i.e., in the second and fourth reaction of this scheme, the singlet oxygen oxidation of lignin model induced an extensive destruction of the model compounds; other fragments of the substrates were not found.

We are studying the possible use of this oxidation in the bleaching process.

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