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**UV irradiation during fluorescence measurements affects the
structures and fluorescence properties of isolated lignins in solution**

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UV irradiation during fluorescence measurements affects the structures and fluorescence properties of isolated lignins in solution

Abstract: There are no reports on the stability of autofluorescence, although lignins and their solutions are known to exhibit autofluorescence. An aim of this study is to investigate spectral change of lignin autofluorescence under prolonged excitation with ultraviolet (UV). Both fluorescence spectra of softwood milled wood lignin (MWL) and kraft lignin (SKL) in propylene glycol significantly changed upon an UV irradiation at 280 nm under O₂ and Ar atmospheres even for 10 min. By the further irradiation, the autofluorescence intensities were remarkably changed together with the emission wavelengths. However, no tendency for the autofluorescence change was observed. The change varied between lignin preparations and gas atmosphere. These phenomena should be caused by autoxidation upon UV irradiation, and the reaction resulted in not only photodegradation but also photopolymerization.

Keywords: milled wood lignin; kraft lignin; solution; autofluorescence; ultraviolet; photodegradation; photopolymerization.

INTRODUCTION

Fluorescence spectroscopy is more sensitive than ultraviolet–visible (UV–vis) spectroscopy, enabling the measurement of solution concentrations that are five orders of magnitude lower than those detectable by UV–vis spectroscopy.^[1] Therefore, fluorescence spectroscopy has been used for determining lignin concentrations in solutions based on autofluorescence.^[2, 3] This analysis is also useful for examining the environment of solute fluorophores, including factors such as solvent polarity and pH, as well as the interactions between different solutes and fluorophores through Förster resonance energy transfer (FRET).^[4–7]

In addition to the fluorescence measurement of lignin solutions,^[8, 9] fluorescence microscopy is often used to observe lignin distribution in wood.^[10–14] The resolution can be markedly improved through FRET by incorporating Rhodamine B into the wood specimen.^[15] Furthermore, researchers have developed functional materials by leveraging lignin autofluorescence.^[4] However, there are no reports on the autofluorescence stability of lignin in solution under continuous excitation in the UV region. When lignin in wood or isolated lignin in solution was irradiated with UV light, the absorption spectra changed in a time-dependent manner, indicating that the lignins underwent photodegradation.^[16–18] The presence of O₂ in air is involved in this photodegradation reaction.^[19] Thus, photodegradation upon UV irradiation is easily

anticipated to affect lignin autofluorescence. However, there are no reports that examined the autofluorescence change upon prolonged UV irradiation, i.e., long-term excitation with UV light in the fluorescence measurement.

This study aims to trace changes in autofluorescence properties in solution state of lignins under continuous UV irradiation under O₂ atmosphere and Ar atmosphere as a control condition. As samples, softwood milled wood lignin (MWL) and softwood kraft lignin (SKL) were used as isolated lignin preparations. These lignins were then dissolved in propylene glycol, which has a high boiling point (188 °C), enabling stable fluorescence measurements over an extended period. First, to collect the information between excitation and emission (fingerprint fluorescence) in the fluorescence measurement, the emission spectra of both lignin solutions in propylene glycol were obtained in air within the excitation wavelength (Ex) range of 200–750 nm (with a 10 nm interval) to determine the Ex that offers the maximum emission intensity. Next, the fluorescence spectra were collected every 10 min for 12 h under UV irradiation at the determined Ex range in both O₂ and Ar atmospheres to investigate the stability of autofluorescence of lignins.

EXPERIMENTAL

Materials

Ezo spruce (*Picea jezoensis*) wood meal (passed through a 42-mesh sieve with particles retained on a 100-mesh sieve) was extracted using 1:2 ethanol/benzene (v/v) in a Soxhlet apparatus. MWL was prepared from the extracted wood meal and purified according to a traditional method established by Björkman.^[20, 21] SKL was prepared from black liquor obtained from softwood kraft pulp (Nippon Paper Industries Co., Ltd., Tokyo, Japan). This process involved acidification, filtration, and washing with distilled water, followed by freeze-drying according to a previously reported method.^[22] The frequency of β -O-4' linkages in these lignin samples were estimated using derivatization followed by reductive cleavage (DFRC) analysis.^[23]

Propylene glycol, docosane (GC–MS grade), *N,N*-dimethylformamide (DMF, HPLC grade), and LiBr were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Acetyl bromide was purchased from FUJIFILM Wako Pure Chemical Co. Ltd. (Osaka, Japan). All chemicals and solvents were used as received.

Fingerprint fluorescence (3D fluorescence spectra) of lignin preparations in solution

Lignin samples (1.5 g) were added to propylene glycol (50 mL). The mixture was stirred at 2,000 rpm for 1 h using a planetary centrifugal mixer (AR-100, THINKY Corp., Tokyo, Japan) to obtain a 30 mg/L solution. Next, 2.5 mL of this solution was transferred to a quartz cuvette for the fluorescence measurements. The fingerprint fluorescence of the lignin solution was recorded using a fluorescence spectrometer (F-7000; Hitachi High-Tech Corp., Tokyo, Japan). A 150 W Xe lamp with a slit width of 20 nm was used as the light source. Emission spectra were collected in the range of 200 to 750 nm at 10 nm intervals, using an Ex from 200 to 750 nm. The scan speed was set at 240 nm/min. Each measurement took 2 h. Immediately after the first measurement, a second measurement was conducted using the same sample solution.

Fluorescence spectroscopy of lignins in solution

MWL and SKL solutions (30 mg/L) in propylene glycol were prepared in the same manner as described above. Each solution was transferred to a quartz cuvette and bubbled with O₂ or Ar for 5 min. The cuvette was then placed in a vacuum desiccator and degassed for 15 min. Subsequently, O₂ or Ar was introduced into the desiccator. This procedure was repeated three times to replace the gas atmosphere in the solution with the desired gas. The fluorescence spectra of the lignin solutions were recorded at intervals of 10 min using a fluorescence spectrometer (F-7000, Hitachi High-Tech Corp., Tokyo, Japan) while O₂ or Ar gas was flown into the chamber at a rate of 0.2 L/min via a tube. A 150 W Xe lamp with a slit width of 20 nm was used as the light source. Measurements were conducted for 12 h under continuous UV irradiation at a wavelength of 280 nm. The measured fluorescence wavelengths ranged between 290–750 nm with a scan speed of 240 nm/min. Each measurement was performed in triplicate.

UV-vis absorption spectroscopy of lignins in solution

The MWL and SKL solutions were irradiated with UV light at 280 nm under O₂ and Ar atmospheres using a fluorescence spectrometer and then subjected to UV-vis spectroscopy. The absorption spectra were recorded in the wavelength range of 200–800 nm using a UV-vis spectrometer (U-2910, Hitachi High-Tech Corp., Tokyo, Japan). A D2 lamp was used as the light source for wavelengths below 340 nm, and a WI lamp was used for wavelengths above 340 nm. The powers of the D2 and WI lamps were 30 W and 50 W, respectively. The UV-vis absorptions of unirradiated lignin solutions (30 mg/L) stored under an Ar atmosphere and those irradiated for 12 h were also measured.

Structural characterization of UV-irradiated lignins

The lignin solutions were irradiated with UV light at 280 nm for 2 and 12 h under O₂ and Ar atmospheres, and this process was repeated in five separate batches. The resultant solutions (total 12.5 mL) were combined and subjected to a partially modified “cryovap” method to remove propylene glycol.^[24] The solution was first transferred to a 25 mL recovery flask, which was then placed in an aluminum block heater. The flask was connected to a U-shaped distillation adapter, to which a receiver with a three-way valve was connected. The joints were greased carefully to ensure proper sealing. The system was first vacuumed, and the receiver was immersed in liquid nitrogen while the sample solution was stirred at 50 °C. The evaporation process was continued for 4 h.

The collected lignin powder (ca. 0.4 mg) was dissolved in 100 µL of LiBr /DMF (0.5%, w/v) and filtered through a 0.45 µm poly(tetrafluoroethylene) syringe filter. Size-exclusion chromatography (SEC) was performed using an L-7000 series HPLC system (Hitachi High-Tech Corp., Tokyo, Japan) equipped with a photodiode array detector (detection wavelength: 280 nm). The eluent was 0.5% (w/v) LiBr/DMF, and the flow rate was set at 0.6 mL/min. The injection volume was 20 µL. A guard column (GPC KD-G) and two columns (GPC KD-803, ShodexTM, RESONAC Co. Ltd., Tokyo, Japan) were connected in series and used at 50 °C. Twelve authentic polystyrene standards with molar masses ranging from 580 to 995,600 g/mol were used for molar mass calibration.

Fourier-transform infrared (FT-IR) spectra of the unirradiated and UV-irradiated (12 h) lignin powder samples were recorded using an FT/IR-4100 spectrometer equipped with an attenuated total reflection (ATR) unit (Jasco Co. Ltd., Tokyo, Japan). Notably, the lignin powder samples irradiated with UV light for 2 h were not recovered in sufficient quantities for FT-IR spectroscopy using the “cryovap” method. The FT-IR spectra were measured in a wavenumber range of 650–4,000 cm⁻¹. The measurement was performed with 128 scans and resolution of 4 cm⁻¹. The spectra were normalized at 1455 cm⁻¹, which corresponds to the asymmetric C–H bending from methoxy groups of lignin.^[25]

RESULTS AND DISCUSSION

Fingerprint fluorescence of MWL in solution

The fingerprint fluorescence (3D fluorescence spectra) of the MWL solution in propylene glycol (30 mg/L) was measured to investigate the relationship between Ex, the emission wavelength (Em), and the corresponding fluorescence intensity (FI). In the first measurement, the MWL solution emitted intense fluorescence in the Em range of 290–560 nm when exposed to UV irradiation in the Ex range of 200–400 nm, as shown in **Fig. 1-a**. A fluorescence maximum with

an FI of 580 was observed at an Em of 360 nm and an Ex of 280 nm. When fingerprint fluorescence was obtained immediately after the first measurement, a new fluorescence maximum was observed at an Em of 350 nm and an Ex of 280 nm, with an increased FI of 670 (**Fig. 1-b**). Thus, the fluorescence maximum was observed at the same Ex as that of the first measurement, but both the FI and Em increased. Furthermore, during the second measurement, the MWL solution emitted intense fluorescence in the Em range of 290–590 nm and Ex range of 200–430 nm. Both the Ex and Em ranges increased compared with the initial measurements (**Fig. 1**). Thus, the fingerprint fluorescence of the MWL solution altered considerably after the first measurement for 2 h. This indicates that photoirradiation causes structural changes in MWL.

The same experiment was performed using the SKL solution (**Fig. S1**). In the first measurement, the fluorescence maximum of the SKL solution appeared at an Em of 370 nm and an Ex of 250 nm, with an FI of 528. In the second measurement, the fluorescence maximum appeared at an Em of 370 nm and an Ex of 300 nm with an increased FI of 807. Interestingly, in the case of SKL, the Em of the fluorescence maximum remained the same, even though Ex was different. Thus, the fluorescence spectrum of SKL was also influenced by photoirradiation; however, its behavior differed from that of MWL.

To examine the spectral changes occurring over extended periods of UV irradiation, the fluorescence spectra of the MWL and SKL solutions were monitored every 10 min at an Ex of 280 nm, which corresponds to the maximum emission of MWL.

(Figure 1)

Change in fluorescence spectra of MWL in solution upon continuous UV irradiation at 280 nm

The MWL solution was subjected to continuous UV irradiation at 280 nm under either an O₂ or Ar atmosphere, and fluorescence spectra were obtained every 10 min at an Ex of 280 nm. The changes in the spectra are depicted in **Fig. 2a–d**. Under an O₂ atmosphere, the fluorescence maximum (Em_{max}) (364 nm) hypsochromically shifted (blue-shifted) by 2.8 nm, and its FI decreased by 14% after 10 min. This phenomenon persisted until 2 h and 30 min of irradiation (**Fig. 2-a**). Subsequently, the Em_{max} bathochromically shifted (redshifted) and its FI increased. The final FI after 12 h of irradiation was twice that after 2 h and 30 min of irradiation (**Fig. 2-b**). However, under an Ar atmosphere, the initial Em_{max} (365 nm) of unirradiated MWL bathochromically shifted, accompanied by an increase in FI, until 3 h and 50 min of irradiation. Concomitantly, the FI of the shoulder at approximately 350 nm also increased until 3 h 50 min, reaching a clear peak (**Fig. 2-c**). At 4 h, the FI of the new peak was larger than that of the peak at 368 nm, resulting in the fluorescence maximum peak (**Fig. 2-d**).

The changes in Em and FI at the fluorescence maximum over time are summarized in **Fig. 2-e** and **f**, respectively. Under Ar atmosphere, the Em_{max} drastically shifted to a lower wavelength

at 4 h. This was due to the peak switch of the fluorescence maximum, as mentioned above. Therefore, in **Fig. 2-f**, the FI of the new peak after 4 h is depicted as blue dots, and the FI at 368 nm after 4 h is represented by orange dots. **Fig. 2-e and f** clearly suggest that the gas atmosphere significantly affected the fluorescence spectral change.

(Figure 2)

UV absorption spectra of MWL in solution after UV irradiation at 280 nm

To investigate the relationship between the fluorescence and UV absorption spectra upon UV irradiation, the UV spectra of the irradiated MWL solution were measured. The spectra at the end point of the hypsochromic shift, that is, 2 h 30 min under O₂ atmosphere and 4 h under Ar atmosphere, are displayed in **Fig. 3**. For comparison, **Fig. 3** also includes the spectra of the sample irradiated for 12 h and the unirradiated samples. The unirradiated MWL solution exhibited maximum absorption at 280 nm, which was easily deduced from the Ex of the fluorescence maximum in the fingerprint fluorescence (**Fig. 1**). Upon irradiation under O₂ atmosphere, the absorbance decreased by 40%, and the peak changed to a shoulder after 2 h and 30 min of irradiation (**Fig. 3-a**). Subsequently, the absorbance at 280 nm increased by 22% compared with that at 2 h and 30 min. In contrast, the absorbance in the longer wavelength range (300–400 nm) steadily decreased with increasing irradiation time.

Under Ar atmosphere, the absorbance at 280 nm decreased by 35% without a peak shift after 4 h of UV irradiation. After 12 h of irradiation, the peak at 280 nm shifted to 269 nm, and the absorbance at 280 nm increased remarkably compared to that of unirradiated MWL (**Fig. 3-b**). This change in absorbance at 280 nm differed from the change in FI at the fluorescence maximum, which increased and then decreased. Although both fluorescence and UV measurements clearly demonstrated structural changes in lignins upon UV irradiation, this study did not elucidate the correlation between the UV and fluorescence spectral changes. However, these results suggest that fluorescence and UV absorbance measurements for the characterization and concentration determination of lignins should be completed within a very short time, even when performed using inert gases.

(Figure 3)

Characterization of irradiated MWL in Solution

The SEC and FT-IR spectra of the MWLs before and after 2 h of UV irradiation were measured to investigate the structural changes in lignin, as suggested in previous experiments. This time point corresponds to the end of the decrease and increase in the FI of the fluorescence maximum under O₂ and Ar atmospheres, respectively (**Fig. 2-f**). The size-exclusion chromatograms, including those of the MWLs irradiated for 12 h, are shown in **Fig. 4**, and the number-average molar mass (M_n), weight-average molar mass (M_w), and molar mass at the top of

the peak (M_p) are provided in **Table 1**. Under Ar atmosphere, all molar masses, M_n , M_w and M_p , simply decreased until 12 h of irradiation. Assuming that the inert gas had no effect on the lignin structure, the photodegradation of MWL stemmed solely from the effect of UV irradiation. In contrast, M_p significantly decreased under O₂ atmosphere after 2 h of irradiation, suggesting photodegradation due to cleavage of the β -O-4' linkage.^[26–28] However, after 12 h of irradiation, an increase in molar mass was observed, suggesting photopolymerization (**Fig. 4-a**). This phenomenon can be explained as follows: Oxygen molecules, specifically the bi-radical species, are responsible for the formation of MWL radicals upon exposure to UV irradiation. Subsequently, a one-electron reaction occurs, leading to degradation in the early stages. After 2 h of UV irradiation, photopolymerization occurred via radical coupling. Therefore, the change in molar mass under the Ar atmosphere was different from that under the O₂ atmosphere.

(Figure 4)

(Table 1)

Fig. 5 shows the FT-IR spectra of unirradiated MWL and MWL irradiated for 12 h under O₂ and Ar atmospheres in the fingerprint IR region and the full spectra in the 600–4000 cm⁻¹. Unirradiated MWL exhibited an absorption band at 1710 cm⁻¹, which was assigned to the carbonyl group, along with prominent bands at 1597 and 1510 cm⁻¹, which were assigned to the aromatic ring.^[25] UV irradiation under Ar atmosphere resulted in a decrease in the absorbance of the band at 1510 cm⁻¹, and a significant increase in the absorbance of the band at 1710 cm⁻¹ assigned to carbonyl groups. MWL irradiated under O₂ atmosphere displayed an intermediate absorbance at 1710 cm⁻¹ between the spectra of the unirradiated MWL and MWL irradiated under an Ar atmosphere. Sonntag reported that carbonyl groups could be generated from secondary alcohol by UV irradiation through homolytic cleavage.^[29] Müller et al. reported that carbonyl compounds could be formed from a β -O-4' model dimer by the irradiation of UV at > 280 nm.^[30] Therefore, the UV irradiation, particularly under Ar atmosphere, caused the generation of carbonyl groups.

(Figure 5)

Change in fluorescence spectra and UV absorption spectra of SKL in solution upon successive UV irradiation at 280 nm

As well as MWL, the fluorescence spectra of SKL, as a technical lignin, was monitored upon UV irradiation at 280 nm under both O₂ and Ar atmospheres (**Fig. 6**). Under O₂

atmosphere, the Em of the maximum FI hypsochromically shifted by 7.6 nm, and its FI was reduced by 34% after just 10 min of UV irradiation. These changes persisted for up to 6 h and 40 min after the irradiation (**Fig. 6-a**). Thereafter, the Em_{max} shifted bathochromically, and its FI increased until 12 h of irradiation (**Fig. 6-b**). These observations are similar to those observed for MWL under O₂ atmosphere.

Under Ar atmosphere, the FI at the fluorescence maximum increased with increasing irradiation time, although the Em_{max} was almost constant during the measurements (**Fig. 6-e, f**). These spectral changes in SKL upon UV irradiation under Ar atmosphere are different from those of SKL under O₂ and MWL under an Ar atmosphere.

The UV absorption spectra of the SKL samples irradiated for 6 h and 40 min in O₂ and 50 min in Ar are depicted in **Fig. 7**, including those of the unirradiated SKL samples and those irradiated for 12 h. Under O₂, the absorbance at 280 nm first decreased and then increased. This trend is similar to that observed for MWL. In contrast, the samples treated under Ar showed a slight and gradual decrease in peak absorbance, unlike the drastic changes observed for MWL. These results demonstrate that the changes in the fluorescence spectra were affected not only by the gas atmosphere but also by lignin preparation.

(**Figures 6 and 7**)

Characterization of Irradiated SKL in Solution

In addition to MWL, unirradiated SKLs and SKLs irradiated for 2 h and 12 h were characterized using SEC and FT-IR spectroscopy. The changes in the SEC profiles of SKL were quite different from those of MWL. The SEC peak of SKL irradiated for 2 h under O₂ atmosphere shifted towards a higher molar mass region, suggesting the occurrence of photopolymerization. After 12 h, this peak shifted to a lower molar mass region, but remained higher than the peak of the unirradiated sample. Furthermore, a new peak appeared in the profile at 2.1×10^3 g/mol. This was presumed to be a fragment resulting from photodegradation. The differences between the SEC profiles of MWL and SKL can be explained as follows: MWL contained a significant amount of β -O-4' linkages, which were predominantly cleaved when exposed to UV irradiation, leading to a decrease in its molar mass. In contrast, SKL underwent polymerization because of the coupling of the photogenerated radicals. The lower content of β -O-4' linkages in SKL (28 mmol/mg; yield 0.74%), which accounted for only 10% of that in MWL (254 mmol/mg; yield 6.7%), was confirmed by DFRC measurement.

The SEC profiles of SKL irradiated under Ar atmosphere exhibited a similar tendency to those of SKL irradiated under O₂ atmosphere. However, the change in molar mass was less

pronounced than that observed under O₂ atmosphere, and the subsequent shift towards smaller molar mass regions was also subtle. Consequently, the area of the newly emergent peak was traced. Therefore, the involvement of O₂ in photopolymerization and subsequent photodegradation is important.

(Figure 8)

The FT-IR spectra of SKL before and after 12 h of irradiation are shown in **Fig. 9**. The FT-IR spectrum of unirradiated SKL showed clear absorption bands at 1510, 1597, and 1710 cm⁻¹, similar to that of MWL. In the FT-IR spectrum of SKL irradiated under Ar atmosphere, the absorbance bands at 1510 and 1600 cm⁻¹ decreased, while that at 1710 cm⁻¹ increased. Under O₂ atmosphere, the absorbance bands at 1510 and 1597 cm⁻¹ decreased further, while the absorbance band at 1710 cm⁻¹ remarkably increased. Furthermore, a new band appeared at 1666 cm⁻¹ and was assigned to a conjugated carbonyl group. This result suggests the generation of a carbonyl group at the α -position in the lignin unit.^[25] In SKL, O₂ may have had a prominent effect on carbonyl group formation.^[31] The formation of a carbonyl group at the α -position in SKL was a unique phenomenon and was not observed in irradiated MWL.

Although further structural analyses are required, it is difficult conducting them because only limited quantities of sample can be obtained in the present cuvette-based system.

CONCLUSIONS

As anticipated, autofluorescence of isolated lignin solutions were easily altered by prolonged UV irradiation or long-term excitation. This is probably due to the autooxidation upon UV irradiation, resulting in photodegradation of MWL in O₂ and Ar atmospheres, and in photopolymerization of SKL in both gas atmospheres. Thus, these findings indicate that a brief exposure of lignin to UV irradiation provides structural alternation of the lignin and the change in autofluorescence behavior, even under an inert atmosphere. Although lignin analyses, such as lignin distribution and solution concentration, have been conducted on the basis of its autofluorescence, much attention must be paid to handle lignin samples in order to obtain accurate data.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All data generated or analyzed during this study are included in this published article.

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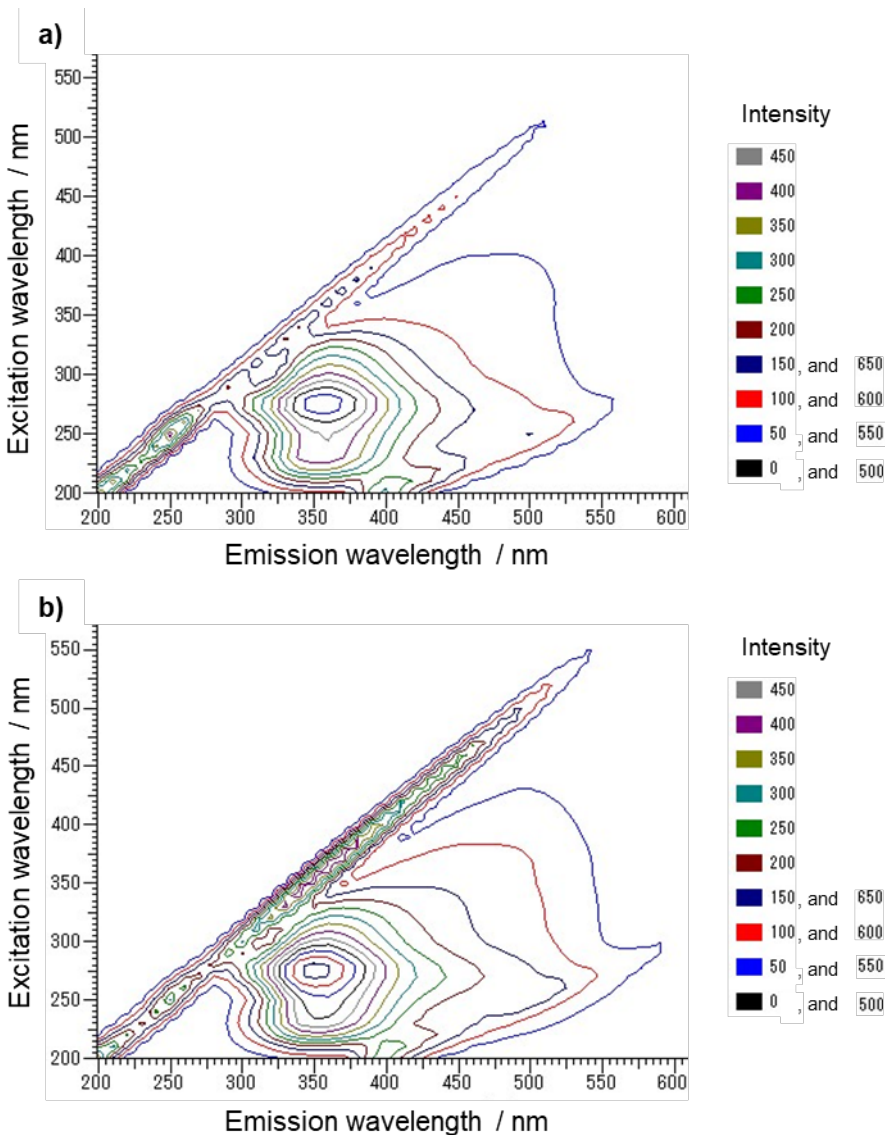
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Tables

Table 1. Molar mass distribution of unirradiated and irradiated lignins at 280 nm under O₂ and Ar atmospheres determined by SEC.

	Atmosphere gas	Irradiation time (h)	M_p ($\times 10^4$ g/mol)	M_n ($\times 10^4$ g/mol)	M_w ($\times 10^4$ g/mol)	DP
Unirradiated MWL			3.37	1.88	3.64	1.93
Irradiated MWL	O ₂	2	1.55	1.11	2.00	1.80
		12	2.13	1.38	2.36	1.70
	Ar	2	2.82	1.64	2.73	1.67
		12	1.00	1.18	1.78	1.51
Unirradiated SKL			1.24	7.79	1.71	2.20
Irradiated SKL	O ₂	2	2.61	14.1	2.79	1.96
		12	1.52	9.81	1.86	1.90
	Ar	2	1.56	12.4	3.01	2.43
		12	1.50	11.1	2.71	2.43



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427 **Figure 1.**

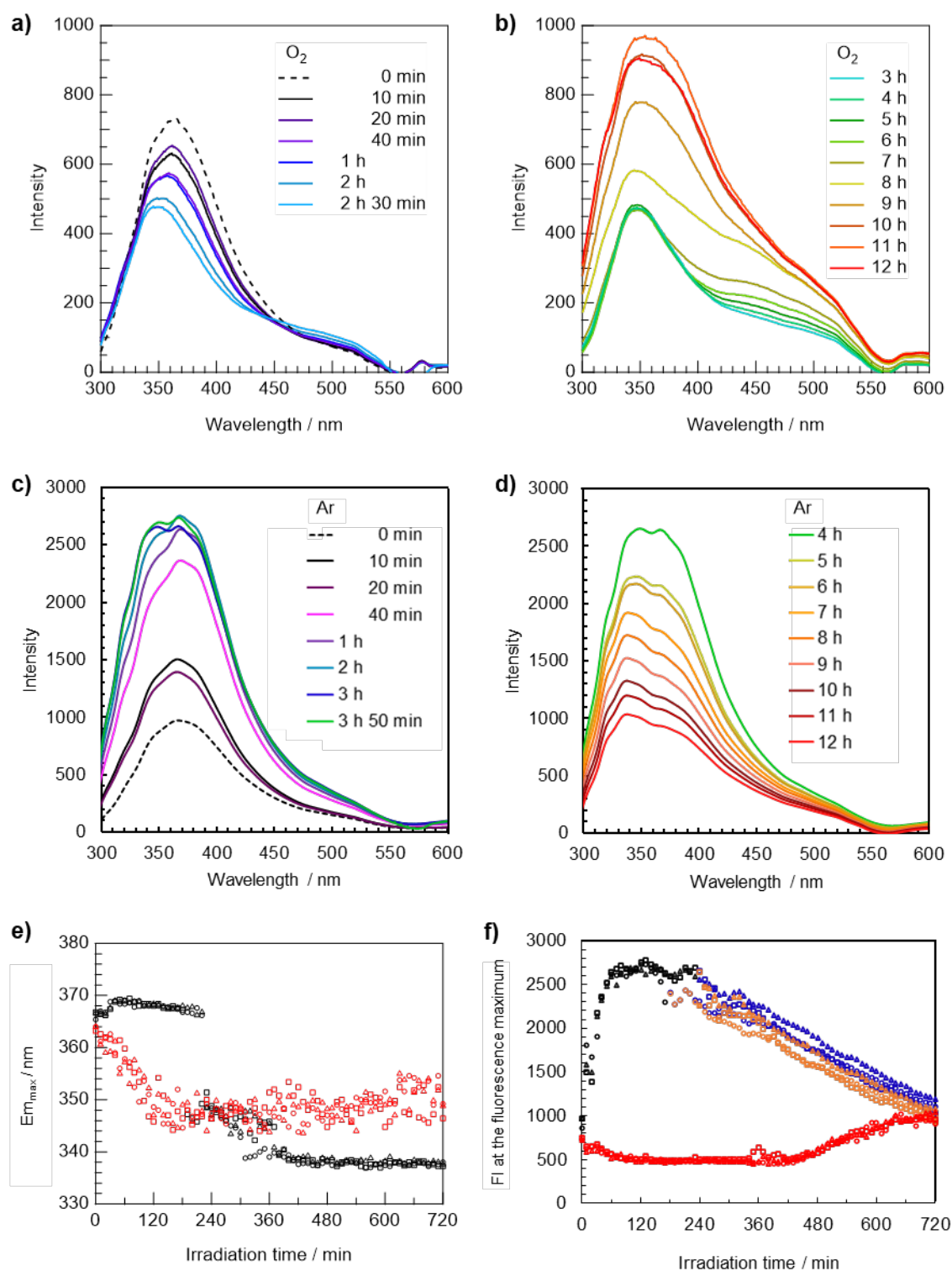


Figure 2.

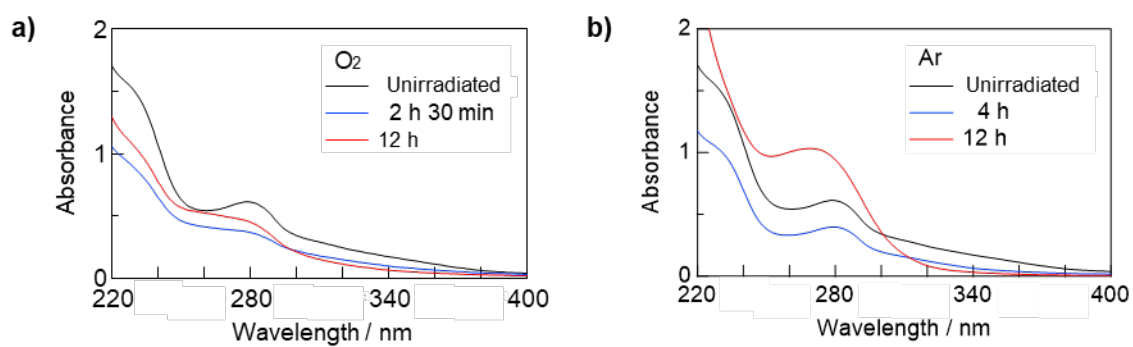


Figure 3.

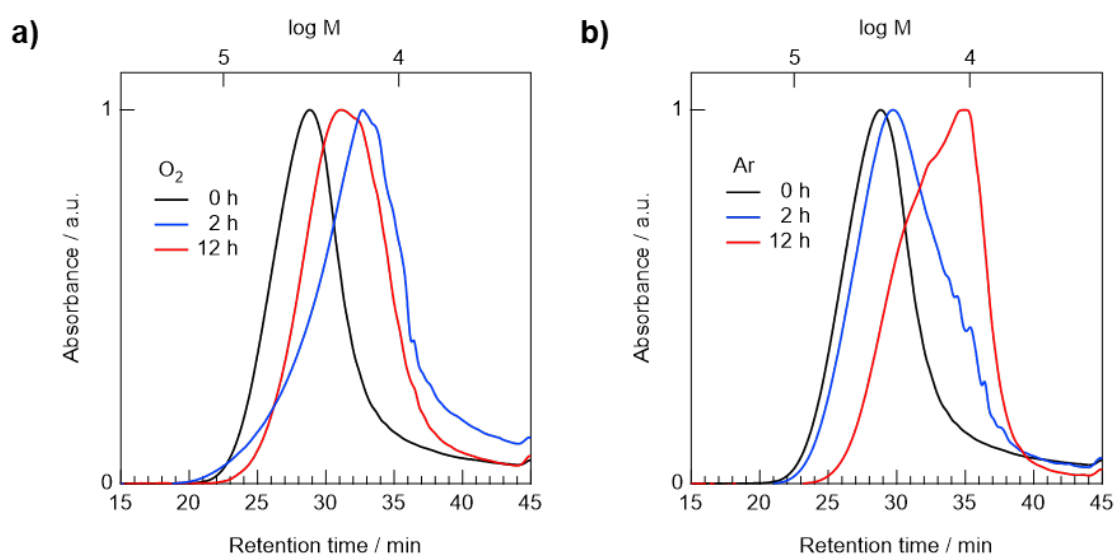


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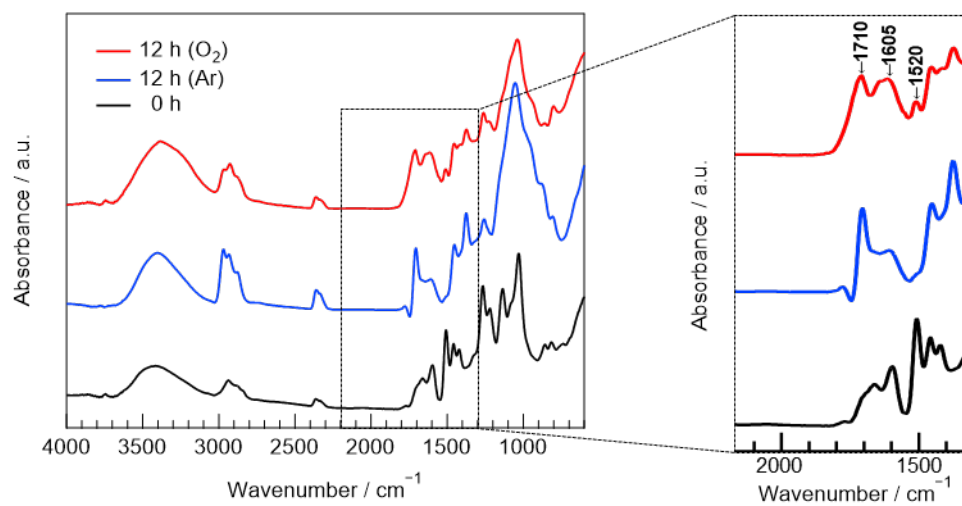


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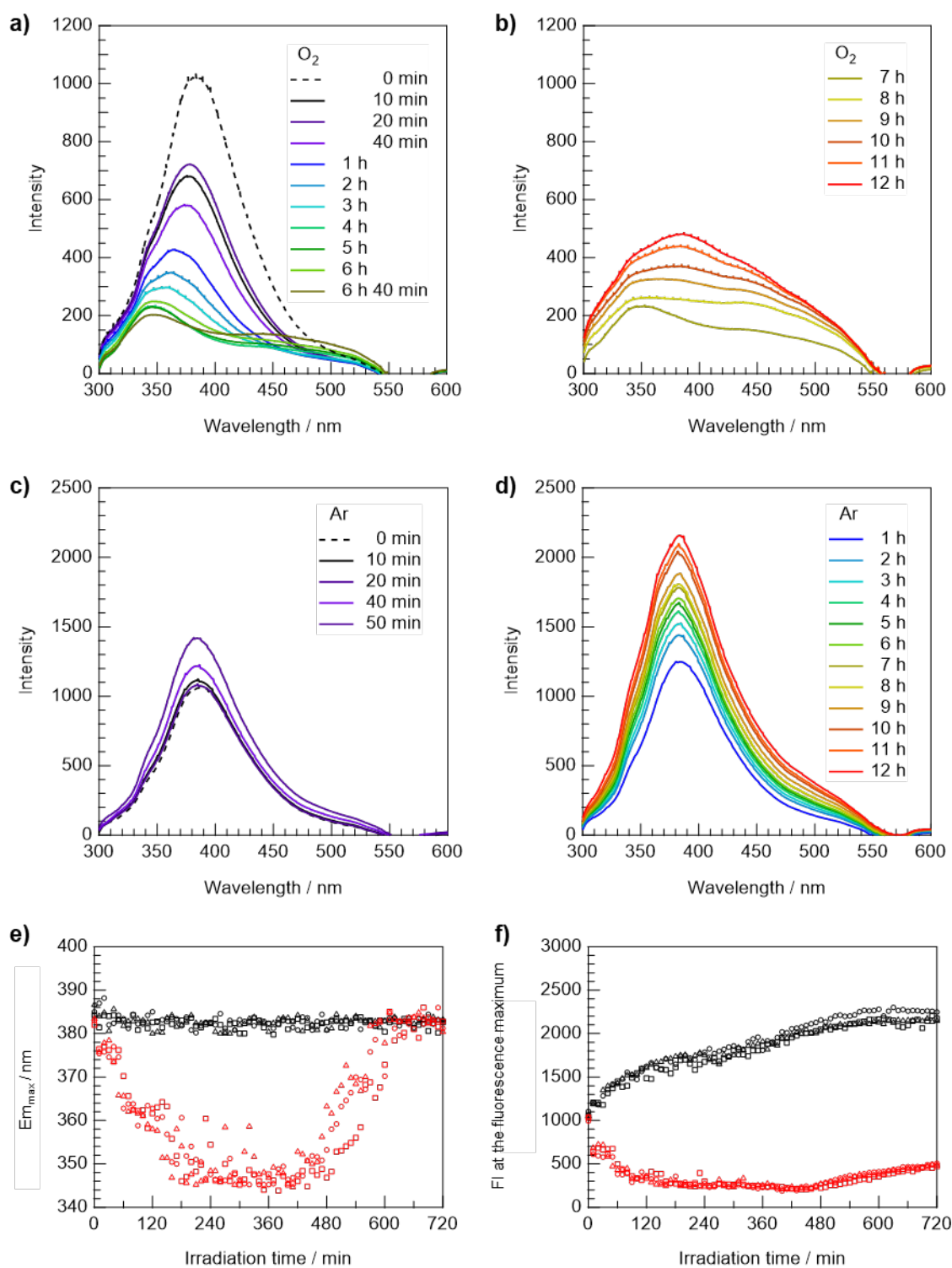


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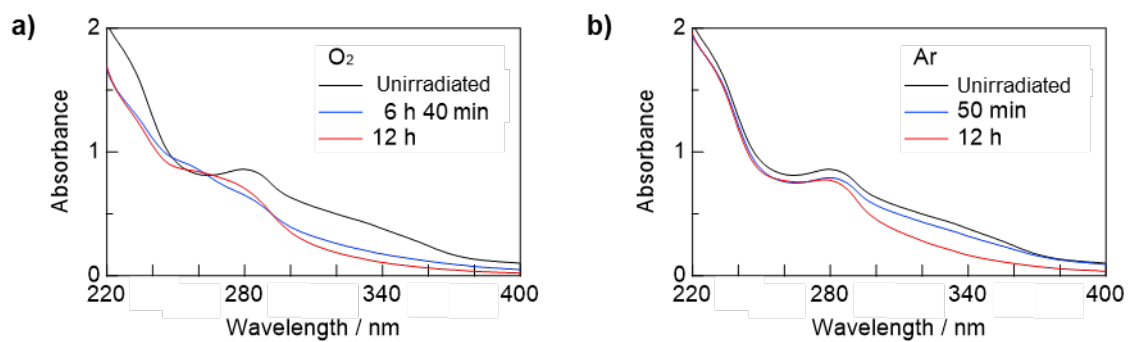


Figure 7.

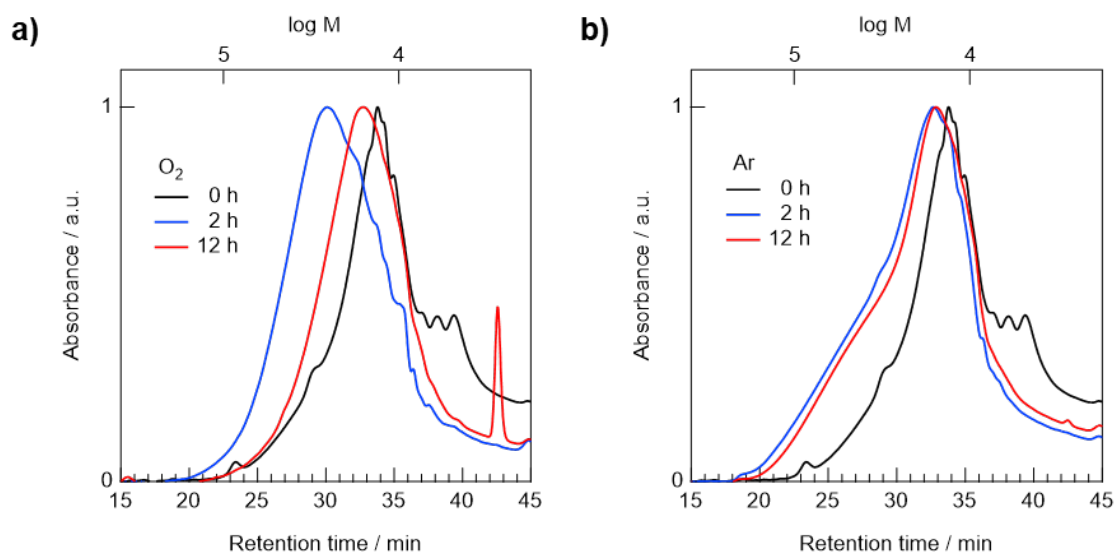
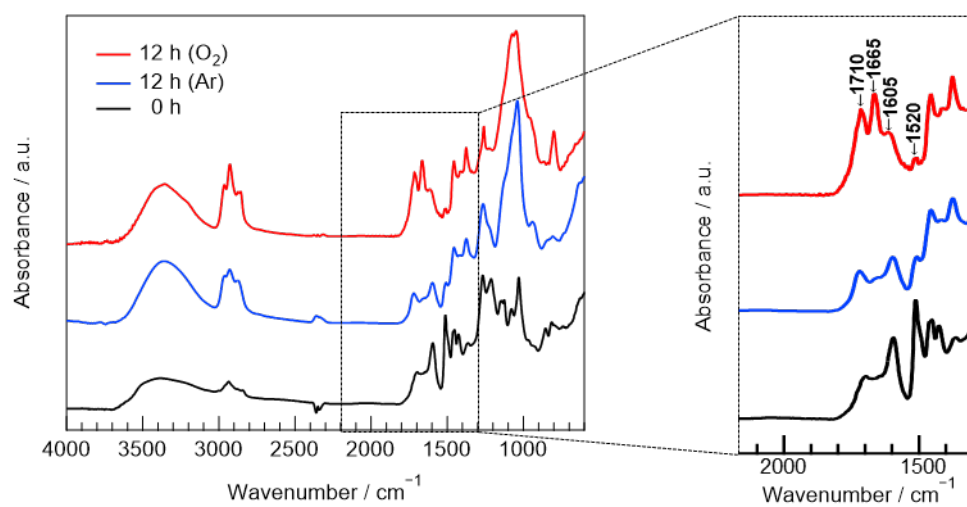


Figure 8.

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449 **Figure 9.**

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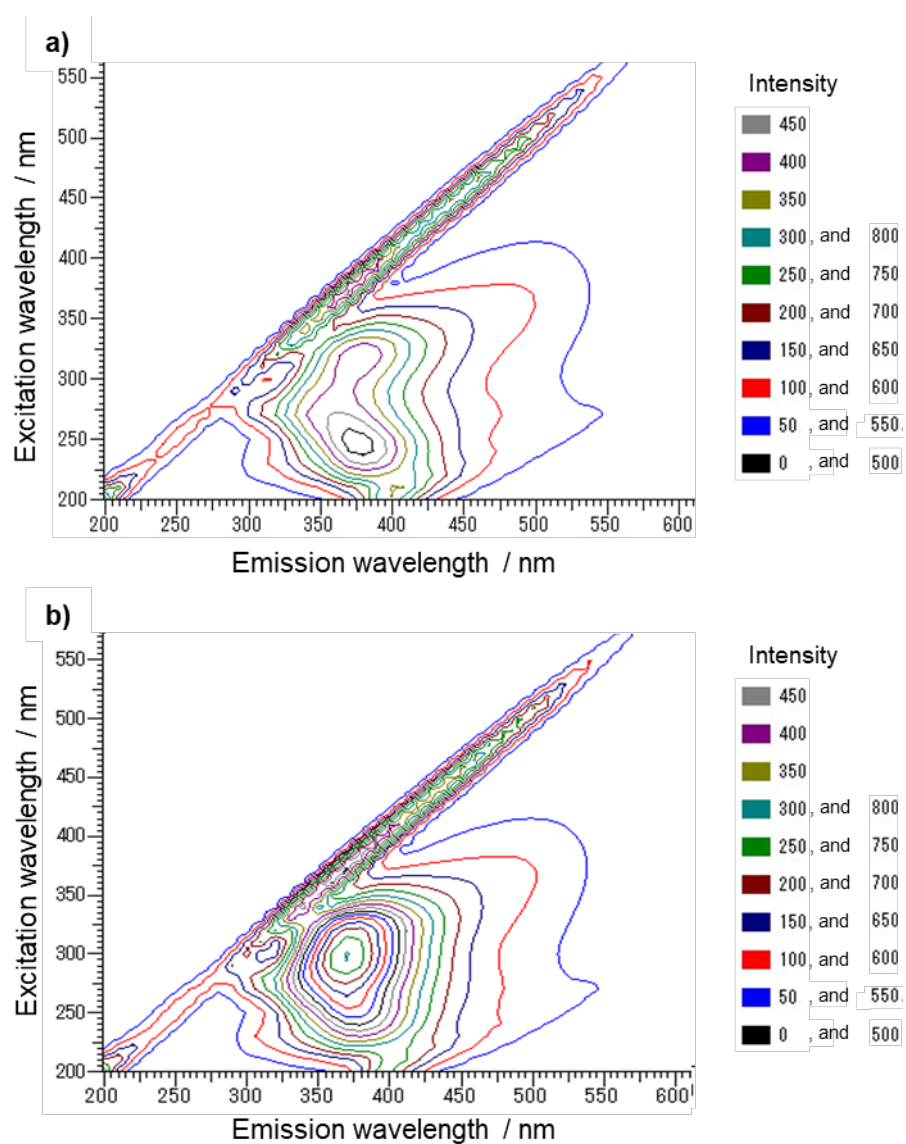


Figure S1.

Figure Captions

Figure 1. Fingerprint fluorescence in the fluorescence spectra of MWL in propylene glycol (30 mg/L) under air: (a) first and (b) the second measurements. The different colored lines correspond to FI values listed on the right side of the figure. The second measurement was performed 2 h after the first. The diagonal region, where the excitation and emission wavelengths are the same, represents primary light and is not related to the intrinsic fluorescence of lignin.

Figure 2. Fluorescence spectra of MWL solutions continuously irradiated with UV light at 280 nm. The spectra were collected at an excitation wavelength (Ex) of 280 nm. Spectral changes over (a) 0–2 h 30 min and (b) 3–12 h under O₂ atmosphere; (c) 0–2 h and (d) 3–12 h under Ar atmosphere. Changes in the (e) $E_{m_{max}}$ and (f) FI of MWL over time at the fluorescence maximum under O₂ (red) and Ar (black) atmospheres. The FIs at the fluorescence maximum after 4 h and at 368 nm are shown using blue and orange markers, respectively. The measurements were carried out in triplicate, and the results were plotted using different symbols (circle, square, and triangle).

Figure 3. UV–vis absorption spectra of unirradiated MWL solution and MWL irradiated at 280 nm for (a) 2 h 30 min and 12 h under O₂ atmosphere and (b) 4 h and 12 h under Ar atmosphere.

Figure 4. Molar mass distribution of unirradiated MWL and UV-irradiated MWL for 2 h and 12 h under (a) O₂ and (b) Ar atmospheres, measured by SEC using a UV detector.

Figure 5. ATR-mode FT-IR spectra of untreated MWL (black) and UV-irradiated MWL for 12 h under O₂ (red) and Ar (blue) atmospheres.

Figure 6. Fluorescence spectra of SKL solutions continuously irradiated with UV light at 280 nm. These spectra were collected at an Ex of 280 nm. Spectral changes over time at (a) 0–6 h 40 min and (b) 7–12 h under O₂ atmosphere; (c) 0–50 min and (d) 1–12 h under Ar atmosphere. Changes in the (e) $E_{m_{max}}$ and (f) FI of SKL over time at the fluorescence maximum under O₂ (red) and Ar

(black) atmospheres. The measurements were carried out in triplicate, and the results were plotted using different symbols (circle, square, and triangle).

Figure 7. UV–vis absorption spectra of unirradiated SKL solution and SKL irradiated at 280 nm for (a) 3 h 40 min and 12 h under O₂ atmosphere and (b) 12 h and 50 min under Ar atmosphere.

Figure 8. Molar mass distribution of untreated SKL and UV-irradiated SKL for 2 h and 12 h under (c) O₂ and (d) Ar atmospheres measured by SEC using a UV detector.

Figure 9. ATR-mode FT-IR spectra of untreated SKL (black) and UV-irradiated SKL for 12 h under O₂ (red) and Ar (blue) atmospheres.

Figure S1. Fingerprint fluorescence in the fluorescence spectra of SKL in propylene glycol (30 mg/L) under air: (a) first and (b) the second measurements. The different colored lines correspond to FI values listed on the right side of the figure. The second measurement was performed 2 h after the first. The diagonal region, where the excitation and emission wavelengths are the same, represents primary light and is not related to the intrinsic fluorescence of lignin.