

Isolation and Irradiation-Modification of Lignin Specimens from Black Liquor and Evaluation of Their Effects on Wastewater Purification

Ke-qin Wang,^{a,1} Jing-ping Chen,^a Liang Chen,^a Xiao-fen Wu,^a Xiao-jun Su,^{b,1,*} Samuel Amartei,^c and Wensheng Qin^{d,1,*}

In this study, crude lignin extracted from the black liquor generated by a pulp and paper mill was modified by different doses of irradiation. The crude and irradiation-modified lignins were used to treat wastewater that was generated during the production of starch glucoamylase. Changes to the physical and chemical properties and structure of the irradiation-modified lignins were determined using scanning electron microscopy, solubility analysis, elemental analysis, analysis of phenolic hydroxyl group, ultraviolet-visible spectroscopy, and Fourier transform infrared spectroscopy. Irradiation reduced the phenolic hydroxyl content in the lignin but increased its solubility by about 40%; analysis revealed that irradiation also destroyed the skeletal structure of the benzene ring in the lignin. After four minutes of settling, the total nitrogen (TN) and chemical oxygen demand (COD) in the wastewater reached 7.0 mg/L and 1573.1 mg/L, respectively. The settled solids content and protein recovery were 1.12 g/L and 98%, respectively. This study suggested that irradiation-modified lignin extracted from black liquor generated in the pulp and paper industry can be used to treat wastewater from the production of starch glucoamylase.

Keywords: Black liquor; Irradiation modification; Lignin; Wastewater treatment

Contact information: a: Hunan Institute of Nuclear Agricultural Science and Space Breeding, Hunan Academy of Agricultural Sciences, Changsha 410125, China; b: Hunan Provincial Key Laboratory of Crop Germplasm Innovation and Utilization, Hunan Agricultural University, Changsha 410128, China; c: Division of Biology, Imperial College of Science, Technology and Medicine, London, SW72AZ, UK; d: Department of Biology, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, P7B 5E1, Canada.

¹These authors contributed equally to this work;

* Corresponding authors: suxiaojun5606@163.com; wqin@lakeheadu.ca

INTRODUCTION

Lignin, the second most abundant naturally renewable resource following cellulose, is present in the cell walls of plants. It is a highly reticulated polymer that consists of three monomers: guaiacyl, syringyl, and p-hydroxyphenylpropane. The lignin molecule also contains a large number of phenolic hydroxyl, carboxyl, and carbonyl groups. Lignin plays a number of important roles for the plant in providing structural support, ensuring appropriate impermeability, and resisting microbial attack. Approximately 1.5 to 1.8 million tons of industrial lignin is produced globally every year, but less than 2% is utilized. Most of the lignin produced is either used as fuel or indiscriminately discharged (Yao *et al.* 2013). For example, the black liquor generated from the pulp and paper industry contains a large amount of lignin and its degradation products, in addition to other products such as wood extractives. The indiscriminate discharge of this black liquor is not only a

waste of resources but also causes serious environmental pollution. Extracting and reusing lignin from the black liquor could render much of the waste usable, while also preventing environmental pollution (Guo *et al.* 2009).

Due to the relatively low solubility and the presence of oxygen-containing groups, lignins are widely used as adsorbents (Lin and Dence 1992; Wang *et al.* 2009). Currently, hydrolyzed lignins are used in the purification of wastewater. However, in order to improve the adsorption sites and capacity, the lignin needs to be modified using different methods such as hydroxymethylation, phenolation, phenolic aldehyde formation, oxidation, epoxidation, urea-aldehyde preparation, and polyesterification (Shu-nan *et al.* 2004). Radiation treatment is the use of ionizing radiation (X-ray, Y-ray, high-energy charged particles, and fission neutrons) to induce the material's physical and chemical changes (cross-linking, polymerization, grafting, and degradation, *etc.*) for processing or modifying the materials. The final resulted products do not contain radioactivity. Comparing with other treatment methods, radiation treatment can be advantageous in terms of energy saving, avoidance of pollutants, and so on (Ha and Wu 2002). Hu *et al.* (2006) used amphoteric lignin-based flocculants to purify wastewater from the dyeing industry and from montmorillonite suspensions, and found that both decolorization and turbidity removal reached up to 85% when the flocculants concentrations were 300 mg/L and 25 mg/L, respectively. Dizhbite *et al.* (1999) also found that the modification of lignin with quaternary ammonium compounds significantly increased their adsorption capacity for heavy metal ions.

In this study, crude lignin present in black liquor generated from the pulp and papermaking industry was extracted and modified using different doses of irradiation. The irradiation-modified lignin was then characterized using a number of physical and chemical methods. The effects of the irradiation-modified lignin on the treatment of wastewater generated from the production of starch glucoamylase were studied.

EXPERIMENTAL

Materials

Black liquor and other wastewater

Mixtures of 90% dry mass of hardwood (aspen) and 10% dry mass of softwood (spruce and pine) were used as raw materials for the production of the black liquor. Wastewater generated in the production process of starch glucoamylase was provided by Hunan Hong Ying Xiang Biological Engineering Co., Ltd (Changsha, Hunan, China).

Methods

Extraction and irradiation of crude lignin from black liquor

Table 1 shows the composition of the black liquor from which the lignins were extracted (Shi and He 2009).

Table 1. Composition of the Black Liquor Used for the Extraction of the Lignin

Solids Content (%)	Total Alkali (Based on Na ₂ O) (g/L)	Inorganics (g/L)	Organics (g/L)	pH
7.84	18.67	17.54	48.29	11.5

The procedures for the extraction and irradiation modification of lignins are shown in Fig. 1. In brief, the pH of the black liquor (1 L) was adjusted to 2.0 by adding H₂SO₄ (4 M) and then stirring. The solution was then centrifuged at 3000 rpm for 3 min. The precipitate was washed once using H₂SO₄ (0.005 M) and dissolved in NaOH (2 M) until the solution was dark; the pH was then adjusted to 2.0 with H₂SO₄ (0.005 M). The solution was centrifuged again at 3000 rpm for 3 min, and the precipitate was washed for five times using H₂SO₄ (0.005 M) and three times using distilled water. Finally, the precipitate was dried at 110 to 120 °C for 24 h, which yielded 21.5 g of crude lignin (L1).

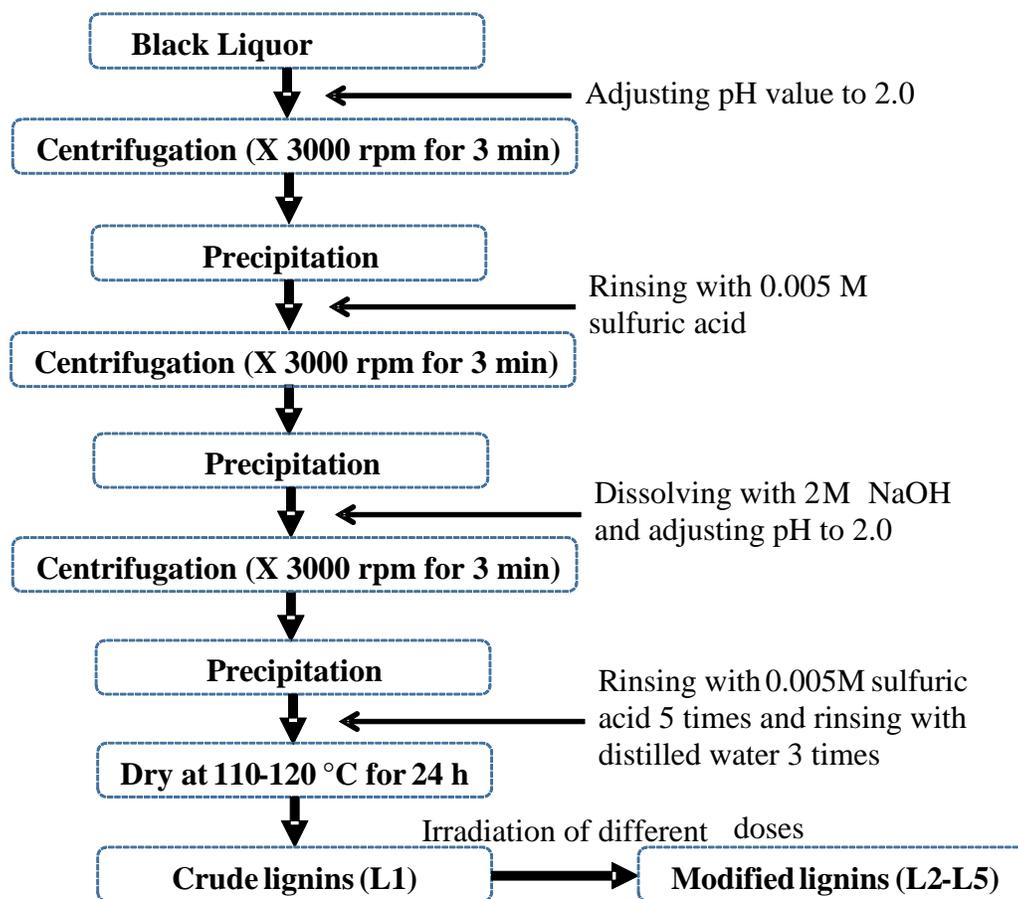


Fig. 1. Flow chart showing the extraction and irradiation modification of the lignin

The crude lignins (L1) were irradiated with different doses (500 kGy, 1000 kGy, 1500 kGy, and 2000 kGy) using the Co-60 γ Irradiation Facility (Nuclear Agriculture and Space Breeding Institute, Hunan Academy of Agricultural Sciences, Changsha, China). The irradiation source strength was 9.99×10^{15} Bq, and the dose rate was 2.0 kGy/h. The modified lignins were labeled L2, L3, L4, and L5.

Structural characterization of lignin

A JSM-6380LV (JEOL, Japan) scanning electron microscope (SEM) was used to investigate the surface structure of the lignin. The lignin samples were first dried at 105 °C for 12 h, attached to an aluminum stub using a conductive adhesive, and coated with gold.

The surface morphology of the samples was analyzed on the SEM at an accelerating voltage of 10 kV.

Solubility determination

One gram of lignin (m1) and 100 mL of 50% ethanol were mixed in an Erlenmeyer flask at 200 rpm and at room temperature for 5 h. The solution was then filtered, and the residual powder (m2) on the filter paper was weighed to calculate the solubility of the lignin, according to the following formula. This was carried out in duplicates.

$$\text{Lignin solubility} = m1 - m2 \quad (1)$$

Elemental analysis

Lignin samples (irradiated and non-irradiated) were dried at 105 °C for 12 h, transferred into sample boxes, and pressed. The mass fractions of the elements carbon, hydrogen, oxygen, and nitrogen (C, H, O, and N) in the lignins were determined using a CE440 Elemental Analyzer (Exeter Analytical Inc., USA). This was carried out in duplicates.

Determination of phenolic hydroxyl groups in the lignin molecule

The determination of phenolic hydroxyl groups was conducted using ion differential spectrophotometry (Goldschmid 1954). Lignin samples (0.10 g) were dissolved in dioxane, and the volume was set to 100 mL. The lignin-dioxane solution (2 mL) was then transferred into each of three 50-mL volumetric flasks. Phosphate buffer (pH 6.0), NaOH (0.20 M), and disodium hydrogen phosphate-sodium hydroxide buffer (pH = 12.0) were added to each of the three volumetric flasks to make up the volume. The absorbances at 300 nm and 360 nm were measured for each sample in a quartz cuvette. The phosphate buffer diluted sample was used as a control.

The molar extinction coefficient ($\Delta\epsilon$) was calculated using the following formula,

$$\Delta\epsilon = D/(cl) \quad (2)$$

where D is absorbance, c is lignin concentration (g/L), and l is the cuvette light path length (cm).

The different structures of the phenolic hydroxyl groups in lignin molecules are shown in Fig. 2.

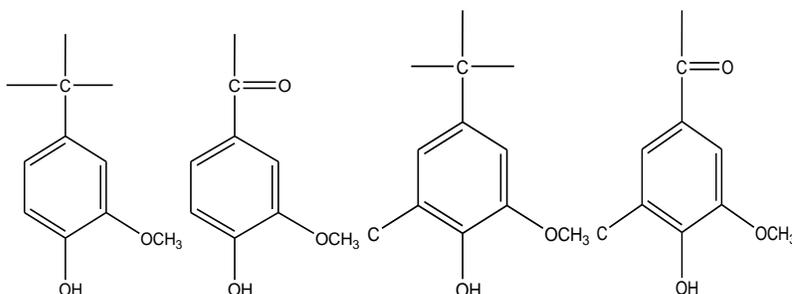


Fig. 2. Phenolic hydroxyl groups with different structural characteristics in lignin molecules

The formulas used to calculate [OH] are shown below.

$$[\text{OH I}] = 0.425\Delta\epsilon_2' \quad (3)$$

$$[\text{OH II}] = 0.081\Delta\epsilon_2'' \quad (4)$$

$$[\text{OH I} + \text{OH III}] = 0.425\Delta\epsilon_1' + 0.101\Delta\epsilon_1'' \quad (5)$$

$$[\text{OH II} + \text{OH IV}] = 0.081\Delta\epsilon_1'' \quad (6)$$

$$[\text{OH III}] = [\text{OH I} + \text{OH III}] - [\text{OH I}] \quad (7)$$

$$[\text{OH IV}] = [\text{OH II} + \text{OH IV}] - [\text{OH II}] \quad (8)$$

$$[\text{OH I} + \text{OH II} + \text{OH III} + \text{OH IV}] = 0.425\Delta\epsilon_1' + 0.182\Delta\epsilon_1'' \quad (9)$$

UV spectroscopy

To determine the UV spectra using a UV-visible spectrophotometer (UV-2450, Shimadzu Corporation, Japan), 1 mg of lignin was dissolved in 25 mL of a dioxane/water (9:1, v/v) solution. A dioxane/water (9:1) solution without lignin was used as the control. The spectra were recorded in the wavelength range of 220 nm to 500 nm (Zhao and Liu 2010).

Infrared spectroscopy

Crude lignin and an appropriate amount of spectrally pure KBr were mixed by grinding under dry conditions and made into a tablet. Infrared spectroscopy was then carried out using an 8400S Fourier transform infrared spectrometer (FTIR-8400 S-type, Shimadzu Corporation, Japan). The spectral scanning range was about 4000 cm^{-1} to 400 cm^{-1} .

Purification of wastewater using irradiation-modified lignin

The wastewater generated during starch glucoamylase production was first passed through a polyethersulfone (PES 8040-10) external pressure tubular membrane filtration unit. The pH of the wastewater (100 mL, pH 3.0 to 4.0, 8% proteins) was adjusted to 9.0 using 5% NaOH. Appropriate amounts of lignin and a control adsorbent (chitosan) were added to the wastewater and allowed to settle for 1 h. The settled solution was then centrifuged at 3000 rpm for 5 min, and the supernatant was filtered through a filter paper using vacuum filtration. The filtered liquid was then treated with activated carbon (about 0.35 g/cm^2 to 0.5 g/cm^2). The sediments were collected and added to the animal feed as feeding protein for follow-up use.

Determination of settling time and amount of settled solids

Appropriate amounts of lignin adsorbents or chitosan (control) were added to the wastewater samples, and the free settling time of the supernatants (time that flocs began to form until stable flocs were formed) was recorded. The absorbance of the supernatant at 600 nm, the amount of suspended solids, and the amount of settled solids of each adsorbent were all measured and compared after 1 h of settling. The amount of settled solids was determined as follows: (i) filter paper (12.5 cm diameter) was weighed, (ii) the floc was filtered through the paper, and (iii) the filter paper containing floc was dried at 105 °C for 4 h and weighed again. The difference in the weight was calculated to determine the weight of settled solids.

Determination of total nitrogen (TN), chemical oxygen demand (COD), and protein recovery rate

After 1 h of settling, the wastewater with different adsorbents was centrifuged and filtered to obtain its solid contents. The proteins in the solid contents, as well as the TN and COD in the liquid filtrate, were quantified. The determination of total nitrogen was conducted using alkaline potassium persulfate digestion and UV spectrophotometry (Yuan *et al.* 2004). The protein content was determined using the Kjeldahl method (Wan 2012), and COD was determined using the potassium dichromate oxidation method (Yuan *et al.* 2004). The protein recovery rate was calculated with the following formula:

$$\text{Protein recovery rate (\%)} = \frac{\text{protein content of the sediment}}{\text{total protein content in wastewater samples}} \times 100 \quad (10)$$

RESULTS AND DISCUSSION

Effects of Irradiation on Lignin Structure

Untreated lignins (control) were found to have a smooth surface to which a small amount of irregular materials are attached (Fig. 3a). After irradiation with 500 kGy, the surfaces of the lignin were broken, and small, irregular protrusions appeared (Fig. 3b). With each increased radiation dose, the structure of the lignin became looser, the quantity of particulates increased, and more irregularly shaped debris appeared on the surface (Figs. 3c, 3d, and 3e), which significantly increased the total surface area. Holes appeared on the lignin surface when the radiation dose reached 2000 kGy (Fig. 3e), suggesting that irradiation had changed the morphology and increased the surface area of the lignin (Zhang *et al.* 2010).

Effects of Irradiation on Lignin Solubility

Previous studies have reported that irradiation increases the solubility of lignin by degrading the macromolecules into a variety of small aromatic compounds (Wang *et al.* 2012; Jiang *et al.* 2013). As shown in Table 2, with increased doses of irradiation, the solubility of lignin in 100 mL of 50% ethanol increased from 0.38 to 0.54g/L (40.32%), confirming that irradiation probably changed the molecular structure of lignin by degrading the large polymers into smaller molecules.

Table 2. Effects of Different Doses of Irradiation on the Solubility of Lignin

Irradiation-modified Lignin (Irradiation Dose)	Solubility (g/L)
L ₁ (without irradiation)	0.38±0.01
L ₂ (500kGy)	0.41±0.03
L ₃ (1000kGy)	0.40±0.01
L ₄ (1500kGy)	0.50±0.02
L ₅ (2000kGy)	0.54±0.01
Data reported are mean ± standard deviation	

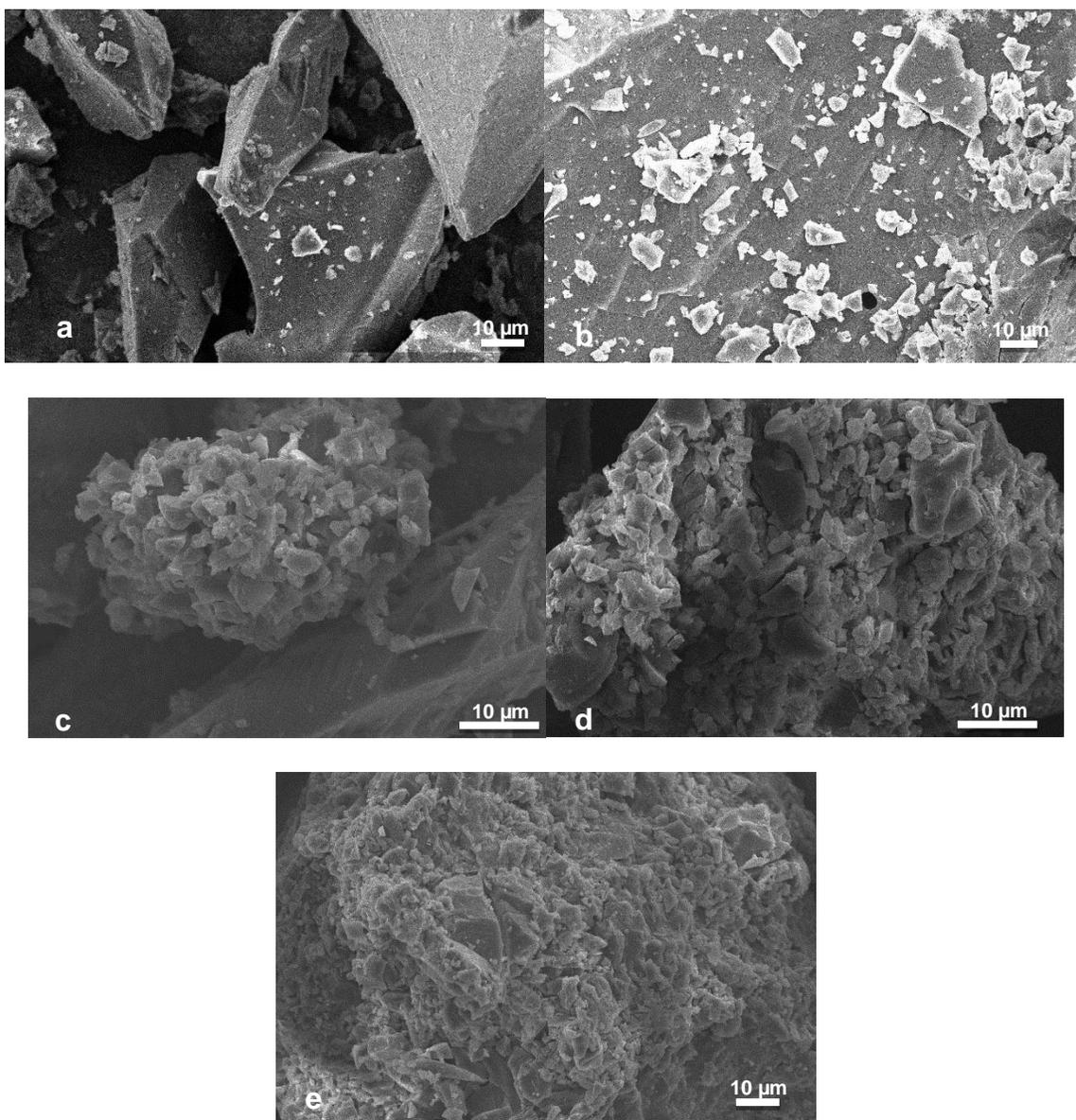


Fig. 3. SEM micrographs of the surface morphologies of the crude and irradiation-modified lignins. (a) 0 kGY, (b) 500 kGY, (c) 1000 kGY, (d) 1500 kGY, and (e) 2000 kGY

Effects of Irradiation on the Composition of the Main Elements in Lignin Molecules

The results of the elemental analysis are shown in Table 3. The crude lignin (L1) had high percentages of C and H, indicating a relatively high content of benzene rings. However, with increasing irradiation doses, the C/H ratio decreased, suggesting that irradiation had destroyed the benzene rings. In addition, the O content increased, suggesting that the lignin molecule contained a large number of methoxy, hydroxy, and ether bonds, which are important for the adsorption capacity of the lignin. Moreover, the small percentage of N present in the lignin molecules suggested the presence of small amounts of nitrogen-containing compounds.

Table 3. Effects of Different Doses of Irradiation on the Elemental Composition of the Lignin Molecules

Irradiation Dose (kGy)	C (%)	H (%)	O (%)	N (%)	C/H ratio
0	62.93±0.13	5.51±0.03	31.40±0.05	0.17±0.01	11.42
500	63.26±0.07	5.74±0.02	30.84±0.06	0.16±0.02	11.02
1000	63.12±0.06	5.71±0.01	31.01±0.13	0.15±0.01	11.05
1500	63.22±0.04	5.71±0.04	30.93±0.07	0.14±0.01	11.07
2000	63.63±0.11	5.76±0.02	30.45±0.12	0.16±0.00	11.04
Data reported are mean ± standard deviation					

Effects of Irradiation on the Phenolic Hydroxyl Groups in Lignin

A number of functional groups, such as methoxy, hydroxyl, and carboxyl groups, connect the structural units in lignin. The phenolic hydroxyl group and the aliphatic chain hydroxyl group are the major hydroxyl groups that can exist either in a free form or coupled with other alkyl groups and aromatic groups via ether bonds. Large numbers of hydroxyl groups contribute to the strong hydrogen bonds that exist between lignin molecules (Schmidt *et al.* 1995). As shown in Table 4, the concentrations of the four phenolic hydroxyl groups, OH I, OH II, OH III, and OH IV, in lignin decreased greatly at the irradiation dose of 500 kGy. When the irradiation dose was increased to 1000 kGy, the concentrations of these groups increased. However, when the irradiation dose was further increasing, there was no great change in the concentrations of these four phenolic hydroxyl groups.

Table 4. Effects of Different Doses of Irradiation on the Content of Phenolic Hydroxyls in the Lignin Molecules

Irradiation Dose (kGy)	OH _I (%)	OH _{II} (%)	OH _{III} (%)	OH _{IV} (%)	OH _{I+II+III+IV} (%)
0	1.29±0.07	0.29±0.00	3.18±0.19	0.21±0.11	4.99±0.31
500	0.82±0.02	0.24±0.05	2.47±0.24	0.13±0.05	3.66±0.22
1000	1.10±0.12	0.28±0.01	3.02±0.06	0.21±0.00	4.61±0.06
1500	1.05±0.14	0.28±0.01	2.86±0.08	0.19±0.00	4.38±0.07
2000	1.08±0.23	0.28±0.02	2.95±0.04	0.20±0.00	4.51±0.20
Data reported are mean ± standard deviation					

UV Spectrum

The UV spectra of lignin molecules in the dioxane solvent are shown in Fig.4. A strong adsorption peak at 276 nm, primarily attributed to the phenyl-ring-B, can be observed. A large slope peak that can be observed at >300 nm may have been caused by a component of the conjugated system in the molecule, such as the adsorption of the aromatic ring (Chen *et al.* 2006; Ren *et al.* 2012). However, as the irradiation dose increased, the adsorption peak at 276 nm gradually decreased, suggesting that irradiation had destroyed the structure of the benzene ring. These observations were consistent with the results of the elemental analysis (Table 3).

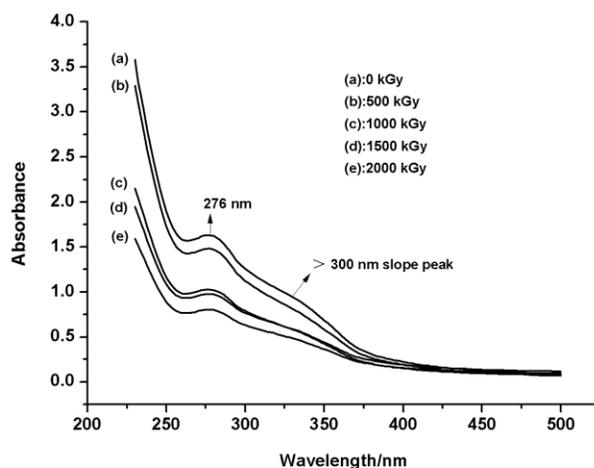


Fig. 4. UV spectra of the lignin modified with different doses of irradiation

Infrared Spectroscopy

The dynamic changes in the infrared spectroscopy of the irradiation-modified lignins are shown in Fig.5. The adsorption peaks and their origins are listed in Table 5.

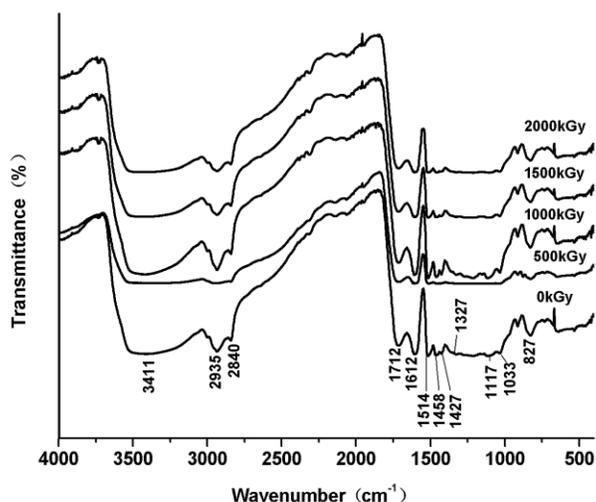


Fig. 5. FTIR spectra of the lignin modified with different doses of irradiation

The peak at 3411 cm^{-1} is the adsorption peak of a hydroxyl group, which was caused by the displacement and widening of the regular adsorption peaks of hydroxyl groups at 3650 cm^{-1} and 3600 cm^{-1} . The displacement and widening of these peaks resulted from the association of the hydroxyl groups with hydrogen bonds (Xu *et al.* 2012). The adsorption peaks at 1612 cm^{-1} , 1514 cm^{-1} , and 1427 cm^{-1} were caused by the skeleton vibrations of the benzene ring. The peaks at 1217 cm^{-1} and 1117 cm^{-1} are the characteristic peaks of lilac groups. The peaks at 1033 cm^{-1} and 827 cm^{-1} are attributed to the guaiacyl groups in lignin (Lei *et al.* 2013). Based on the infrared spectroscopy results, the adsorption peaks of benzene rings at 1612 cm^{-1} , 1514 cm^{-1} , and 1427 cm^{-1} decreased with increased irradiation modification of the lignin, indicating that the benzene ring structure had been degraded.

Table 5. Infrared Adsorption Bands of the Irradiation-modified Lignin

Wavenumber (cm ⁻¹)	Origin
3411	Stretching vibration of O-H in the -OH
2935, 2840	Stretching vibration of C-H in the -CH ₃ , -CH ₂ , and -CH
1712	Stretching vibration of C=O in the non-conjugated carbonyl group and ester group
1612, 1514, 1427	Aromatic ring skeleton vibration
1458	Bending vibration of C-H in the methyl group
1327	Stretching vibration of C=O in the lilac group
1117	Plane deformation vibrations of C-H in the lilac group
1033	Plane deformation vibrations of C-H in the guaiacyl group
827	Off-plane deformation vibrations of C-H in the guaiacyl group

Effects of the Irradiation-modified Lignin on Wastewater

The general effects of the irradiation-modified lignin on wastewater are shown in Table 6.

Table 6. Comparison of the Effects of Lignin Modified with Different Doses of Irradiation on Wastewater Purification

Lignin	Irradiation dose (kGy)	Settling time (min)	Supernatant absorbance (Abs)	Settled amount (g/L)
L ₁	0	4.00	0.242	0.79
L ₂	500	3.00	0.243	1.09
L ₃	1000	4.00	0.221	1.12
L ₄	1500	4.00	0.236	1.06
L ₅	2000	4.00	0.234	1.03

When 0.050 g/L irradiation-modified lignin was used, no great differences in the settling time were observed (Table 6). The adsorption value of the supernatant at 600 nm can be used to evaluate the purification capacity of lignin. Higher adsorption values at 600 nm correspond to lower supernatant cleanliness and therefore a lower adsorption capacity for the modified lignin. Therefore, considering both the adsorption values at 600 nm of the supernatant and the amount of settled solids, it could be said that the best purification was obtained when the irradiation dose was 1000 kGy. Under this condition, the adsorption value at 600 nm of supernatant was 0.221, and the amount of settled solids was 1.12 g/L.

Effects of Different Amounts of Irradiation-modified Lignin on Wastewater

The effects of different amounts of irradiation-modified lignin on the purification of wastewater were investigated using only lignin irradiated with a 1000 kGy dose. Settling time (min), supernatant absorbance (Abs), and amount of settled solids (mg) were used to evaluate the wastewater purification capacity.

Table 7. Comparison of the Effects of Different Concentrations of Irradiation-modified Lignin on Wastewater Purification

Lignin amount (g/L)	Settling time (min)	Supernatant absorbance (Abs)	Settled amount (g/L)
0.000	12.00	0.411	0.26
0.025	4.00	0.223	1.02
0.050	4.00	0.221	1.12
0.100	6.00	0.291	1.06
0.200	6.00	0.290	0.82

As shown in Table 7, the settling time was significantly reduced when 1000 kGy irradiation-modified lignin was used. The highest purification capacity was observed when 0.050 g/L of 1000 kGy irradiation-modified lignin was used.

Amount of Settled Solids and Protein Recovery Rate

The wastewater generated in the production of starch glucoamylase is rich in proteins, and discharging this into the environment is not only a waste of resources, but also leads to environmental pollution. It is therefore very important to treat this wastewater, to protect both the environment and recycle the proteins.

Table 8. Comparison of the Effects of Different Adsorbents, including Chitosan and Lignin Modified with Different Doses of Irradiation, on Wastewater Purification

	Control	Chitosan	Lignin L ₁	Lignin L ₃
Amount of adsorbent (g/L)	0	0.05	0.05	0.05
Settled amount (g/L)	0.26	0.45	0.79	1.12
Protein recovery rate (%)	15.8	27	23	98
COD (mg/L)	8314.6	3895.4	7265.9	1573.1
Total nitrogen (mg/L)	325	237.3	250.2	7

As shown in Table 8, modifying the lignin by irradiation increased the protein recovery rate from the treated wastewater. The irradiation-modified L₃ lignins exhibited the highest protein recovery rate and the highest settled solids content.

COD Removal Efficiency and Total Nitrogen (TN)

The chemical oxygen demand (COD) is an indicator of reducing substances in water and is commonly used for assessing the extent of water pollution. In general, higher CODs represent more serious water pollution (You 1993). Nitrogen is one indicator of the nutrient level of water: high levels of nitrogen in water usually cause eutrophication (Sun *et al.* 2006). As shown in Table 9, the irradiation-modified lignin L₃ reduced the total nitrogen concentrations from 325.0 mg/L to 7.0 mg/L and reduced the COD concentration from 8314.6 mg/L to 1573.1 mg/L (Table 7). The purification rates of total nitrogen and the COD of the irradiation-modified lignin L₃ were 97.8% and 81.08%, respectively. These values are higher than those achieved with the other adsorbents, including chitosan and lignin without irradiation modification (L₁).

CONCLUSIONS

1. In this study, crude lignin was extracted from the black liquor produced from pulp and paper mills and was then modified by different doses of irradiation. The irradiation-modified lignins were characterized using scanning electron microscopy, solubility evaluation, elemental analysis, phenolic hydroxyl group analysis, UV spectroscopy, and infrared spectroscopy in order to investigate the effects of irradiation on the physical and chemical properties and the structure of the lignin. The results revealed that after irradiation, the lignin structure became loose, and holes appeared on the surface.
2. Additionally, irradiation increased the solubility of lignin by 40.3% and reduced the content of phenolic hydroxyl groups. The results of C/H elemental analysis, UV spectroscopy, and infrared spectroscopy suggest that irradiation also destroyed the skeletal structure of the benzene ring in lignin.
3. The irradiation-modified lignins were used to treat wastewater generated in the production of starch glucoamylase. The highest wastewater purification capacity was observed when 0.05 g/L of 1000 kGy irradiation-modified lignin was used. In this case, the settling time, the total nitrogen, and COD concentration in the supernatant were 4 min, 7.0 mg/L, and 1570 mg/L, respectively. The amount of solids that settled from the wastewater was 1.12 g/L, and the protein recovery rate was 98%. This study also showed that the irradiation-modified lignin L3 had a significantly higher purification capacity than chitosan, and that it can be used for the treatment of wastewater. Furthermore, it has been shown that the sorption properties of waste lignin extracted from black liquor can be improved by irradiation and used for the treatment of wastewater.

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