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Determination of Lignin Concentration in Bleaching Effluent *via* Coomassie Brilliant Blue G-250 Method

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Kraft pulp and alkali peroxide mechanical pulp were prepared with aspen and wheat straw and bleached with chlorine dioxide, an alkali extraction strengthened by hydrogen peroxide, hydrogen peroxide, or peroxyacetic acid. Eight different bleaching effluents were obtained, and a Coomassie brilliant blue G-250 method was applied to determine the lignin content in each of the bleaching effluents. The visible light spectra indicated that the alkali-soluble lignin and the alkali bleaching effluent could increase the absorbance of Coomassie brilliant blue G-250 at 640 nm, but the acidic soluble lignin and the acidic bleaching effluent did not increase absorbance. Thus, the Coomassie brilliant blue G-250 method applied to alkali bleaching effluents but did not apply to acidic bleaching effluents. An analysis of the pH, particle size, zeta potential, and visible light spectra determination showed the Coomassie brilliant blue G-250 absorbed on lignin particles, which caused the absorbance increase at 640 nm. With the actual measured value of the lignin content obtained *via* the Klason lignin method, the relative error of the results obtained *via* the Coomassie brilliant blue G-250 method was less than or equal to 3.8%.

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INTRODUCTION

The lignin content in the bleaching effluent was dependent on the bleaching method (Kaur *et al.* 2018, 2019). Whether the lignins in bleaching effluent were recovered, adsorbed (Andersson *et al.* 2012), removed (Azadi Aghdam *et al.* 2016), or degraded (Singhal and Thakur 2009), the lignin content of the bleaching effluents needed to be determined. Methods to determination the lignin content in the bleaching effluent included the ultraviolet light absorption method (Andersson *et al.* 2012; Gao and Fatehi 2018), the Folin phenol method (Azadi Aghdam *et al.* 2016; Kaur *et al.* 2018, 2019), the Pearl-Benson method (Singhal and Thakur 2009), and the Klason lignin method. Carbohydrate degradation products were found to interfere with the ultraviolet light absorption method (Andersson *et al.* 2012). The Folin phenol method was not lignin-specific and was affected by compounds containing aromatic hydroxyl groups (Clescerl *et al*. 1998). Phenolic compounds interfered with the Pearl-Benson method, which caused errors. The Klason lignin method required a relatively long time for achieving determination.

The Coomassie Brilliant Blue G-250 (CBBG) method is suitable for determining the lignin content in aspen kraft pulping black liquor and it is rapid, cheap, and free from interference of carbohydrate degradation products and phenolic compounds (Wang *et al.* 2016). However, the applicability of the method to determine the lignin content in bleaching effluent was unclear. Because of high temperatures, high-pressure levels, high

dosages of chemical reagents, and strong mechanical action, the pulping effluent has higher lignin concentrations than the bleaching effluent. In addition, there are many different bleaching methods. Thus, the authors described the applicability of the CBBG method in determining lignin content in bleaching effluent.

EXPERIMENTAL

Reagents and Materials

CBBG, ethanol (95% v/v), H_3PO_4 (85% w/w), H_2O_2, peroxyacetic acid, ClO_2, NaOH, $MgSO_4$, Na_2SiO_3, and diethylenetriaminepentaacetic acid (DTPA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and the NaOH, $MgSO_4$, Na_2SiO_3, and DTPA were analytically pure. The exact concentrations of the H_2O_2, peroxyacetic acid, and ClO_2 purchased were determined before use. The concentrations of peroxyacetic acid and H_2O_2 were determined according to Karst's method (Pinkernell *et al.* 1997). The concentration of ClO_2 was determined according to the malonic acid-iodine method (Wang *et al.* 2003).

Kraft pulp (KP) and alkaline peroxide mechanical pulp (APMP) from aspen and wheat straw were prepared beforehand. Aspen KP (25% w/w), wheat straw KP (20 % w/w), aspen APMP (23.5% w/w), and wheat straw APMP (15% w/w) samples were put into a sealing bag, respectively, and stored in 4 °C.

Bleaching

The amount of oven dried pulp (ODP) used for all bleaching methods was 200 g. The charge of the chemicals added during the bleaching process was calculated *via* the mass percentage of the ODP. The KP underwent a single stage bleaching treatment with ClO_2 (D) as well as an alkaline extraction strengthened by hydrogen peroxide (Ep). The APMP underwent a single delignification process, which utilized either H_2O_2 (P) or peroxyacetic acid (Pa). The conditions for the D, Ep, P, and Pa bleaching methods were as shown in Table 1.

Table 1. Conditions of D, Ep, P, and Pa Bleaching Methods

Bleaching Method	D	Ep	P	Pa
Pulp Consistency Charge (% w/w)	10	10	10	10
ClO_2 Charge (% w/w)	1	-	-	-
NaOH Charge (% w/w)	-	2.5	5	-
H_2O_2 Charge (% w/w)	-	1	8	-
Peroxyacetic acid Charge (% w/w)	-	-	-	1
$MgSO_4$ Charge (% w/w)	-	0.5	-	-
DTPA Charge (% w/w)	-	0.3	0.2	-
Na_2SiO_3 Charge (% w/w)	-	-	2	0.1
Initial pH	4	≥ 9	≥ 9	5
Temperature (°C)	70	70	70	70
Time (min)	120	90	90	60

The pulp, all other chemical reagents besides the bleaching reagent, and deionized water were added to a clean sealing bag and were mixed evenly. The sealing bag was placed in water bath of 70 °C, and after 30 min, the pH was adjusted to the desired pH (as shown

in Table 1). The bleaching reagent was added, and the sealing bag was rubbed repeatedly in order to blend, and then placed back in the (70 °C) water bath. The sealing bag was rubbed repeatedly every 15 min during the bleaching process. After the bleaching process, the slurry was transferred into a clean cloth bag. The bleaching effluent was extruded from the slurry into a sample bottle, filtered, and then stored at 4 °C.

Isolation of the Lignins in the Bleaching Effluent

The four kinds of 400 mL filtered bleaching effluent respectively obtained by D, Ep, P, and Pa bleaching methods was concentrated to 10 mL under a vacuum. Next, 2 M H_2SO_4 and 2 M NaOH were added into the concentrates of the alkaline bleaching effluent and the acidic bleaching effluent, respectively, until completely precipitated. After each bleaching effluent was centrifuged (5000 rpm, 30 min), the precipitate was obtained, and it was washed with deionized water until the pH of the supernatant liquid became neutral (pH 7). The precipitant (*i.e.* the bleaching effluent lignins) was stored at 35 °C under a vacuum for drying.

Determination of Cellulose and Hemicellulose Content in the Bleaching Effluent Lignins

The dried bleaching effluent lignin (25 mg) was transferred to a pressure bottle and hydrolyzed *via* 72% sulfuric acid and 4% sulfuric acid, successively, according to the methodology of Sluiter *et al.* (2011). The final hydrolysis liquid was used to determine the cellulose and hemicellulose content *via* the phenol-sulfuric acid method (Funaoka *et al.* 1995). Glucose (0, 2, 4, 6, 8, and 10 µg/mL) and xylose ((0, 2, 4, 6, 8, and 10 µg/mL) gradient solution was prepared. One mL of each gradient solution mentioned above was taken. Then, 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 were added. Those mixtures were thoroughly mixing and allowed to stand for 10 min. After being incubated in 30°C water bath for 20 min, the absorbance at 490 nm and 480 nm of the mixture were determined and recorded using a UV−visible spectrophotometer (Agilent 8453, Agilent Technologies, Inc., Santa Clara, CA, USA). The standard curve of glucose was established by the absorbance at 490 nm as the ordinate, and the concentration of glucose as the abscissa. The standard curve of xylose was established by the absorbance at 480 nm as the ordinate and the concentration of xylose as the abscissa. Cellulose and hemicellulose content was calculated by an anhydro correction of 0.88 for C-5 sugars and a correction of 0.90 for C-6 sugars. The amounts of hexose and pentose sugars in hydrolysis liquid were determined at 490 nm and 480 nm, respectively, by the same method of standard curve except the hydrolysis liquid substitute for the gradient solution. Every sample had each measurement repeated three times.

Assay of Lignin Content in Bleaching Effluent *via* the Klason Lignin Method

For each sample, 50 mL of the bleaching effluent was transferred to a pressure bottle and was evaporated at 105 °C until dry. After cooling to room temperature, the assay of the acid insoluble lignin and acid soluble lignin in each dried bleaching effluent sample was performed according to Sluiter *et al*. (2011). Every sample assay had three replicates.

Preparation of Coomassie Brilliant Blue G-250 Reagent

The Coomassie Brilliant Blue G-250 reagent (CBBG reagent) was prepared as previously reported by the author (Wang *et al.* 2016).

Visible Light Spectroscopy

The lignins isolated from the P and Ep bleaching effluent were dissolved using 0.1 M NaOH. The lignins isolated from the D and Pa bleaching effluent were dissolved using 0.1 M H_2SO_4. A volume of 0.2 mL of lignin solution or bleaching effluent and 8 mL of CBBG reagent were pipetted into a centrifuge tube and mixed. Pipetting 3 mL from 8.2 mL total solution was added into a cuvette with a path length of 1 cm. Its visible light spectrum (400 nm to 700 nm) was monitored with the Agilent 8453 UV−visible spectrophotometer.

The pH, Solution Stability, Zeta Potential, Particle Size Determination

The pH was determined with a Leici PHS-3E (Shanghai, China) (pH meter at 21 ± 2 °C). The solution stability of the mixture of CBBG and lignin solution was monitored with a multiple light scattering instrument (Turbiscan Lab, Formulaction Co., Paule Raymondis, Toulouse, France). Dynamic light scattering to measure the particle size and the zeta potential was carried out using a Zetasizer Nano ZS 90 (Malvern Instrument Ltd., Malvern, Worcestershire, UK).

Determination of the Lignin Content in the Bleaching Effluent *via* the CBBG Method

The preparation of the standard curves for each lignin solution and the determination of the lignin content in the corresponding bleaching effluents were performed according to the previous report of the authors (Wang *et al.* 2016).

RESULTS AND DISCUSSION

Residual Cellulose and Hemicellulose in the Bleaching Effluent Lignins

Table 2 shows the residual carbohydrate content in the lignins isolated from the bleaching effluent. The residual carbohydrates contained residual cellulose and hemicellulose. The residual carbohydrate content in the aspen KP (Ep and D) lignins was lower than 1.4%. In the aspen APMP (P and Pa) lignins, the residual carbohydrate content was between 2% and 3.1%. The residual carbohydrate content in the wheat straw KP (Ep and D) lignins was 1.6% and 2.12%, respectively. The residual carbohydrate content in the wheat straw APMP (P and Pa) lignins was 4.66% and 3.59%, respectively. The residual carbohydrate content in the lignins separated from the bleaching effluent of wheat straw KP and APMP were higher than the lignins from the aspen KP and APMP bleaching effluents. The residual cellulose and hemicellulose content in lignins isolated from the bleaching effluents did not exceed 5%. This meant that lignins separated from the Ep, P, D, and Pa bleaching effluents (both aspen and wheat straw KP and APMP) were higher than 95%. Therefore, the lignins isolated from the bleaching effluents can be used in a standard curve preparation.

Lignin Content in the Bleaching Effluents Obtained *via* the Klason Lignin Method

The results in Table 3 show that the lignin content in the bleaching effluents determined *via* the Klason lignin method. The lignin content in the P bleaching effluents of aspen APMP and wheat straw APMP was 4.6343 mg/mL and 8.9172 mg/mL, respectively. The lignin content in the other bleaching effluents calculated *via* the Klason lignin method were all less than 1 mg/mL.

Table 2. Cellulose and Hemicellulose Content in Lignins from Bleaching Effluents

Lignin	Abs$_{490nm}$[1]	Abs$_{480nm}$[2]	Cellulose Content (w/w %)	Xylan Content (w/w %)	Carbohydr-ate Content (w/w %)
Aspen KP Ep Lignin	1.07 (0.0004)	1.07 (0.0009)	0.67	0.63	1.30 (0.0013)
Aspen APMP P Lignin	2.24 (0.0370)	2.24 (0.0390)	1.63	1.38	3.01 (0.076)
Wheat Straw KP Ep Lignin	1.24 (0.0057)	1.31 (0.0062)	0.82	0.78	1.60 (0.0119)
Wheat Straw APMP P Lignin	3.28 (0.0164)	3.47 (0.0057)	2.49	2.17	4.66 (0.0221)
Aspen KP D Lignin	1.11 (0.0050)	1.09 (0.0041)	0.71	0.64	1.35 (0.0091)
Aspen APMP Pa Lignin	1.61 (0.0301)	1.65 (0.0314)	1.12	1.00	2.12 (0.0614)
Wheat Straw KP D Lignin	2.18 (0.0061)	2.14 (0.0049)	1.59	1.31	2.90 (0.0100)
Wheat Straw APMP Pa Lignin	2.58 (0.0440)	2.70 (0.0574)	1.92	1.67	3.59 (0.1014)
[1,2] represented the absorbance at 490 nm and 480 nm, respectively.					

Table 3. Lignin Content in the Bleaching Effluents Determined *via* the Klason Lignin Method

Bleaching Effluent	Acid Insoluble Content (mg/mL)	Acid Soluble Content (mg/mL)	Gross Lignin Content (mg/mL)
Aspen KP Ep Effluent	0.2140 (0.0043)	0.5629 (0.0134)	0.7769
Aspen APMP P Effluent	1.9300 (0.0572)	2.7043 (0.0814)	4.6343
Wheat Straw KP Ep Effluent	0.2060 (0.0022)	0.4553 (0.0127)	0.6613
Wheat Straw APMP P Effluent	5.0200 (0.0992)	3.8972 (0.1032)	8.9172
Aspen KP D Effluent	0.0002 (1.213×10^6)	0.2377 (0.0069)	0.2379
Aspen APMP Pa Effluent	0.0015 (2.382×10^5)	0.0956 (0.0009)	0.0971
Wheat Straw KP D Effluent	0.0007 (1.039×10^5)	0.3192 (0.0083)	0.3199
Wheat Straw APMP Pa Effluent	0.0068 (1.938×10^4)	0.0936 (0.0179)	0.1004

Visible Light Spectra of Lignin Solution Mixed with CBBG Reagent

Lignins isolated from the alkali bleaching effluents were dissolved in 0.1 M NaOH and was prepared to a certain concentration solution (the aspen KP Ep lignin and APMP P lignin for 1.5 mg/mL, the wheat straw KP Ep lignin and APMP P lignin for 2.25 mg/mL). After water, 0.1 M NaOH, the aspen KP Ep lignin, the aspen APMP P lignin, the wheat straw APMP KP Ep lignin, and the wheat straw APMP P lignin solutions were mixed with the CBBG reagent, Fig. 1 shows the absorbance changes of those mixed solutions between 400 nm and 700 nm. The mixture of water and CBBG reagent was the blank. A mixture of just NaOH and CBBG was the control. All curves in Fig. 1 had two absorbance peaks, at approximately 460 nm and 640 nm, respectively. The CBBG mixture absorbance of the aspen KP Ep lignin, the aspen APMP P lignin, the wheat straw APMP KP Ep lignin, and the wheat straw APMP P lignin around 640 nm increased by 71%, 46%, 44%, and 26%, respectively, when compared to the control mixture absorbance at 640 nm. However, their CBBG mixture absorbance at 460 nm changed less than 5%, when compared to the control mixture absorbance at 460 nm. This indicated that the lignin concentrations in the four

kinds of bleaching effluents possibly can be determined *via* the CBBG method, similar to pulping black liquor (Wang *et al.* 2016).

Lignins isolated from the acidic bleaching effluents were dissolved in 0.1 M H_2SO_4 and was prepared to a certain concentration (the aspen KP D lignin and APMP Pa lignin for 2 mg/mL, the wheat straw KP D lignin and APMP Pa lignin for 2.5 mg/mL). Figure 2a demonstrates that after the four acidic lignin solutions (aspen KP D lignin, aspen APMPM Pa lignin, wheat straw KP D lignin, and wheat straw APMP Pa lignin) were mixed with CBBG reagent, their absorbance changed from 400 nm to 700 nm. Figure 2b shows the absorbance changes in the four separate acid bleaching effluent and CBBG reagent solutions from 400 nm to 700 nm. Compared to the absorbance of the blank or control (the mixture of water or the 0.1 M H_2SO_4 with CBBG reagent), the four kinds of acidic lignin solutions and bleaching effluents did not cause an obvious increase or decrease in absorbance like the alkali lignin solution. Thus, the lignin content in the acidic bleaching effluent was not suitable to be determined *via* the CBBG method.
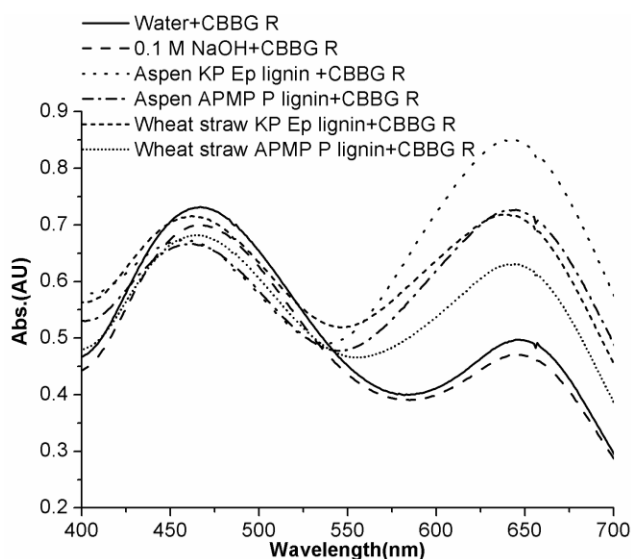

Fig. 1. Visible light spectra of alkali bleaching lignin solutions mixed with CBBG reagent (CBBG R)
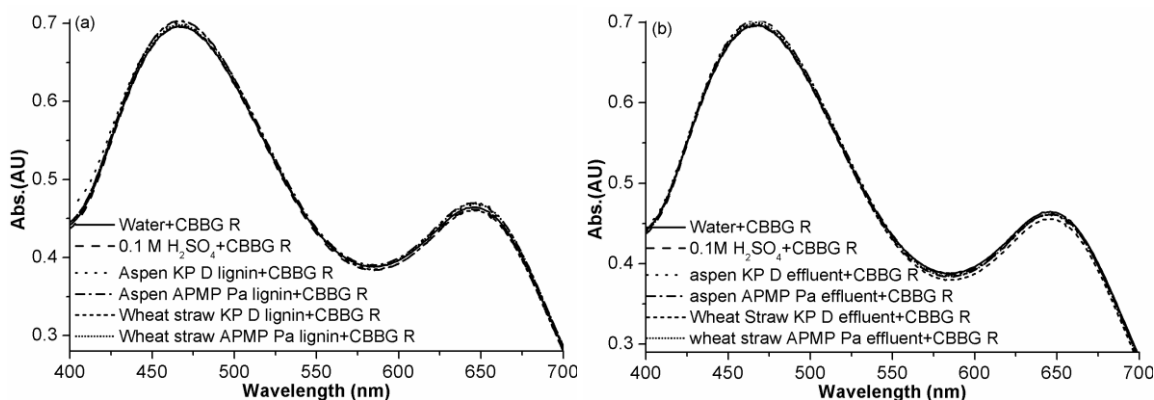

Fig. 2. (a) Visible light spectra of acidic bleaching lignin solutions mixed with CBBG reagent and (b) visible light spectra of acidic bleaching effluents mixed with CBBG reagent

The pH, Particle Size, Zeta Potential, and Solution Stability of the Mixtures Composed of CBBG Reagent and Bleaching Effluent Lignin Solutions

Table 4. The pH Changes of the Mixture

Name	Initial pH	pH After 24 h
CBBG Reagent	0.68	0.71
0.1M NaOH	9.01	9.01
CBBG Reagent +0.1M NaOH	0.70	0.70
CBBG Reagent +1 mg/mL Aspen KP Ep Lignin Solution	0.71	0.71
CBBG Reagent + 1 mg/mL Aspen APMP P Lignin Solution	0.71	0.71
CBBG Reagent +1 mg/mL Wheat Straw KP Ep Lignin Solution	0.70	0.71
CBBG Reagent +1 mg/mL Wheat Straw PMP P Lignin Solution	0.71	0.71
0.1M H_2SO_4	0.98	0.98
CBBG reagent +0.1M H_2SO_4	0.69	0.69
CBBG Reagent+1 mg/mL Aspen KP D Lignin Solution	0.69	0.71
CBBG Reagent +1 mg/mL Aspen APMP Pa Lignin Solution	0.69	0.71
CBBG Reagent +1 mg/mL Wheat Straw KP D Lignin Solution	0.69	0.71

After the CBBG reagent was mixed with the 0.1 M NaOH or the lignin solutions prepared with 0.1 M NaOH, the absorbance curve of those mixed solutions (at 640 nm) changed from down to up (as shown in Fig. 1), which suited the curve change of the CBBG reagent from a pH 0 to a pH 1.3 (Georgiou *et al.* 2008). But the pH after the CBBG reagent mixed with the bleaching effluent lignin solutions kept a pH of 0.69 to 0.71. Therefore, the increase in absorbance at 640 nm after the CBBG reagent mixed with the alkali bleaching lignin solutions was not due to pH change.

Table 5. Particle Size and Zeta Potential of Lignin Solutions and the Mixture

Name	Z-Average Diameter (nm)	Zeta Potential (mV)
CBBG Reagent	1532.00 (24.05)	-16.10 (1.41)
CBBG Reagent + 0.1M NaOH	1199.00 (33.23)	-18.10 (0.14)
2 mg/mL Aspen KP Ep Lignin Solution	201.45 (4.17)	-21.37 (2.84)
CBBG Reagent +2 mg/mL Aspen KP Ep Lignin Solution	2853.5 (184.55)	-13.15 (0.35)
1 mg/mL Aspen APMP P Lignin Solution	109.73 (4.38)	-22.73 (2.85)
CBBG Reagent + 1 mg/mL A APMP P Lignin Solution	1571 (108.68)	-12.2 (0.85)
1 mg/mL Wheat Straw KP Ep Lignin Solution	162.07 (1.81)	-19.17 (1.85)
CBBG Reagent +1 mg/mL Wheat Straw KP Ep Lignin Solution	1489 (70.71)	-11.25 (1.06)
1 mg/mL Wheat Straw APMP P Lignin Solution	265.2 (20.22)	-25.43 (1.96)
CBBG Reagent +1 mg/mL Wheat Straw APMP P Lignin Solution	1684.5 (84.56)	-10.07 (1.32)
CBBG Reagent +0.1M H_2SO_4	1060.97 (89.08)	-17.4 (0.20)
1 mg/mL Aspen KP D Lignin Solution	230.53 (2.91)	-2.68 (0.23)
CBBG Reagent +1 mg/mL Aspen KP D Lignin Solution	1292.5 (6.36)	-20.5 (1.35)
1 mg/mL Aspen APMP Pa Lignin Solution	124 (6.40)	-1.45 (0.29)
CBBG Reagent +1 mg/mL Aspen APMP Pa Lignin Solution	1354.5 (26.16)	-15.35 (0.21)
1 mg/mL Wheat Straw KP D Lignin Solution	156.4 (7.35)	-2.62 (0.24)
CBBG Reagent +1 mg/mL Wheat Straw KP D Lignin Solution	1155 (7.85)	-21.3 (1.68)
1 mg/mL Wheat Straw APMP Pa Lignin Solution	120.43 (0.71)	-1.94 (0.45)
CBBG Reagent +1 mg/mL Wheat Straw APMP Pa Lignin Solution	1222 (16.97)	-16.53 (1.14)

Table 5 showed that the Z-average diameter for CBBG in the CBBG reagent, the mixture of CBBG and 0.1 M NaOH, and the mixture of CBBG and 0.1M H_2SO_4 was 1532 nm, 1199 nm, and 1060 nm, respectively. The size of the alkali bleaching lignins and the acidic bleaching lignins was between 100 nm and 300 nm. The particle size after mixing the CBBG reagent and the alkali bleaching effluent lignin solution ranged from 1489 nm to 2853.5 nm (as shown in Table 5). The particle size after mixing the CBBG reagent and the acidic bleaching effluent lignin solution ranged from 1155 nm to 1354.5 nm (as shown in Table 5). This indicated that the relatively large CBBG particles may absorb the relatively small lignin particles to aggregate into larger particles in dimensions, and these larger particles precipitated at different speeds in the mixtures. The zeta potential of the mixture of CBBG reagent and alkali bleaching effluent lignin solution ranged from -10.07 mV to -13.15 mV, and the zeta potential of the mixture of CBBG reagent and acidic lignin solution ranged from -15.35 mV to -21.3 mV (as shown in Table 5). The sedimentation velocity of the large particles depended on the type of lignin. The turbiscan stability index (TSI) of the mixture composed of CBBG reagent and lignin solutions (Table 6) demonstrated that the stability of the mixture of acidic bleaching effluent lignin solution and CBBG reagent was higher than the mixture of alkali bleaching effluent lignin solution and CBBG reagent. The strong and quick absorption between alkali bleaching effluent lignin and CBBG may increase the absorbance at 640 nm. In contrast, the weak and slow absorption between acidic bleaching effluent lignin and CBBG may have no the action. And the mixture of CBBG reagent and lignin solution was relative stable in one hour. The measurement of absorbance value can be done with acceptable accuracy during this period.

Table 6. Turbiscan Stability Index (TSI) of the Mixture of CBBG Reagent and Lignin Solution

Name	TSI at 1 h	TSI at 24 h
CBBG Reagent	0	0.2
CBBG + 0.1 M NaOH	0.1	0.7
CBBG Reagent + 2 mg/mL Aspen KP Ep Lignin solution	0.5	9
CBBG Reagent + 1 mg/mL Aspen APMP P lignin solution	0.1	3.2
CBBG Reagent + 1 mg/mL Wheat Straw KP Ep lignin solution	0.1	3.3
CBBG Reagent + 1 mg/mL Wheat Straw APMP P lignin solution	0.1	3.8
CBBG Reagent + 0.1 M H_2SO_4	0	0.4
CBBG Reagent + 1 mg/mL Aspen KP D lignin solution	0	0.7
CBBG reagent +1 mg/mL Aspen APMP Pa lignin solution	0	0.5
CBBG reagent +1 mg/mL Wheat Straw KP D lignin solution	0	0.7
CBBG reagent +1 mg/mL Wheat Straw APMP Pa lignin solution	0.1	1.6

After the mixtures of lignin solution and CBBG reagent had rested for 2 weeks, the visible light spectra of the supernatants were recorded (as shown in Fig. 3). Compared to the visible light spectrum of CBBG + NaOH (H_2SO_4) and the new prepared mixtures of lignin solution and CBBG reagent, the absorbance of all supernatants in the region of 550 nm to 700 nm were lower than the previous solutions (as shown in Fig. 3). This indicated that the increase in absorbance at 640 nm in the newly prepared mixtures of alkali lignin solution and CBBG was from a combination of CBBG and lignin; which was consistent with non-covalent combination between the lignins and the CBBG to increase the absorption at 640 nm as shown in previous research (Wang *et al*. 2016).
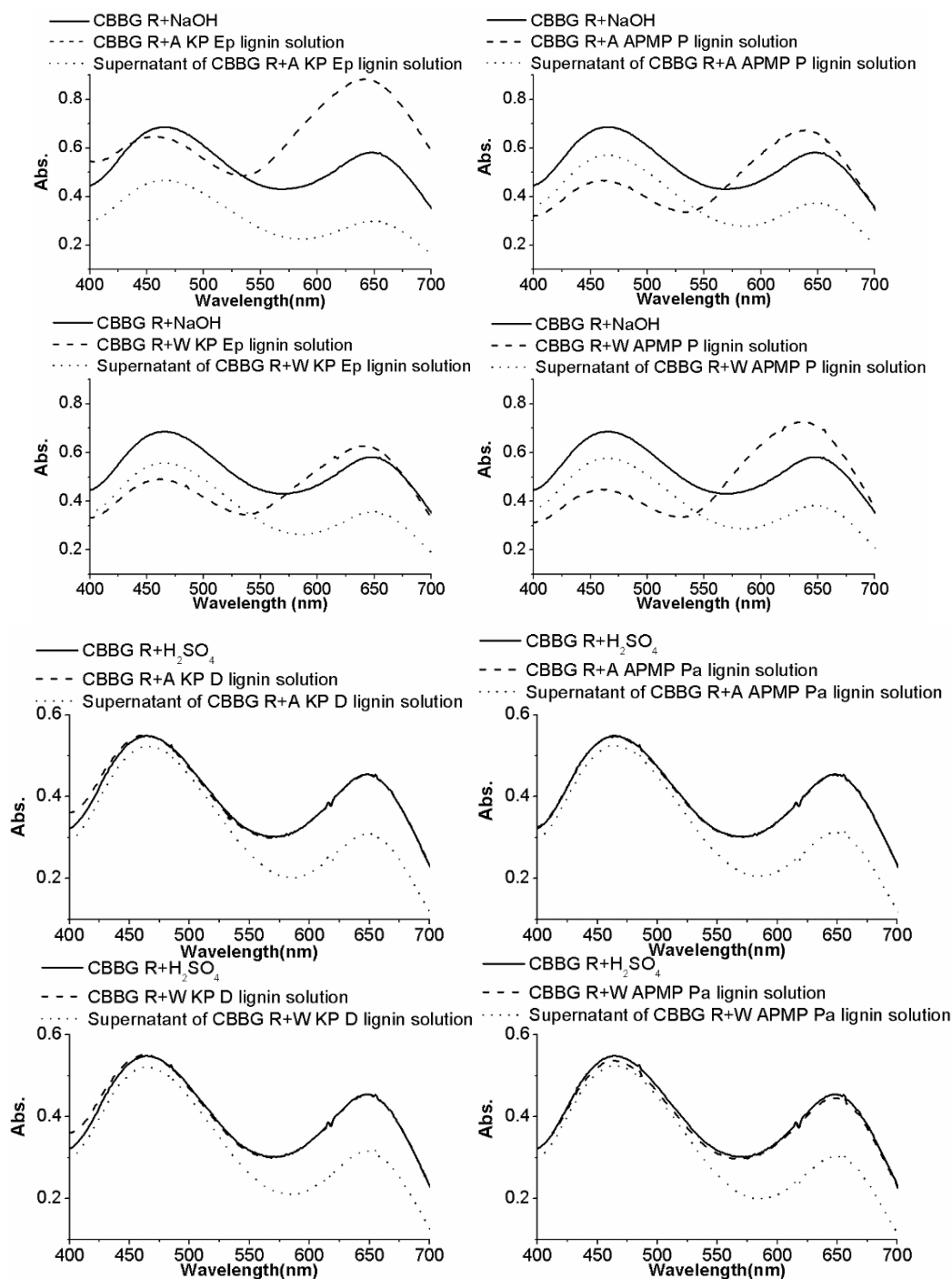
Fig. 3. Visible light spectra of the supernatant of the mixture of CBBG reagent and lignin solution after resting for 2 weeks. A and W in the legend represent aspen and wheat straw, respectively.

Lignin Content in Alkali Bleaching Effluent Obtained *via* the CBBG Method

The lignin concentration in alkali bleaching effluent can possibly be determined *via* the CBBG method. Therefore, according the CBBG method, four different types of alkali bleaching effluent lignin standard curves were established (as shown in Fig. 4a, 4b, 4c, and 4d).
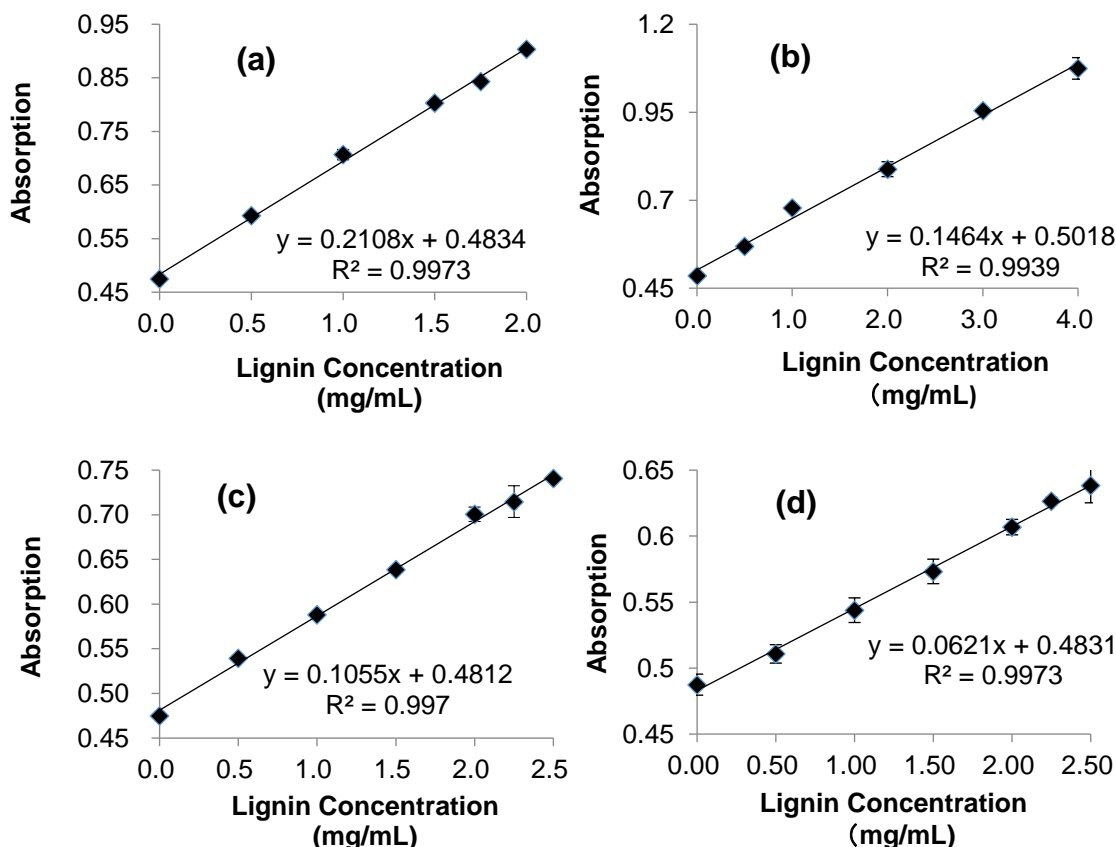
Fig. 4. Standard curves of aspen KP Ep lignins (a), aspen APMP P lignins (b), wheat straw KP Ep lignins (c), and wheat straw APMP P lignins (d) *via* the CBBG method

Table 7. Lignin Content in Alkali Bleaching Effluent Determined *via* the CBBG Method

Bleaching effluent	Abs. (640 nm)	Dilution Factor	Lignin Content (mg/mL)
Aspen KP Bleaching Effluent	0.65085 (0.008)	1	0.7943(0.0399)
Aspen APMP P Bleaching Effluent	0.6323 (0.007)	5	4.4568(0.2582)
Wheat Straw KP Ep Bleaching Effluent	0.5533 (0.004)	1	0.6837(0.0389)
Wheat Straw APMP P Bleaching Effluent	0.5743 (0.005)	6	8.8143(0.4657)

The linear correlation coefficient of the aspen KP Ep lignins, aspen APMP P lignins, wheat straw KP Ep lignins, and wheat straw APMP P lignins was 0.9973, 0.9939, 0.9970, and 0.9973, respectively (Fig. 4). This illustrated that the absorbance at 640 nm for the four different alkali bleaching effluent lignin solutions and the CBBG reagent had a strong linear correlation with the lignin concentration. The corresponding linear correlation equation for the four different alkali bleaching effluent lignin solutions was shown in Fig. 4a, 4b, 4c, and 4d. After the aspen and wheat straw APMP P bleaching effluent was directly mixed with the CBBG reagent, the absorbance at 640 nm exceeded the absorbance range in the standard curve. In order to gain accurate results, the aspen and wheat straw APMP P bleaching effluent was diluted, then mixed with the CBBG reagent; in order to determine the absorbance at 640 nm. The lignin content in the aspen KP Ep, the aspen APMP P, the wheat straw KP Ep, and the wheat straw APMP P bleaching effluent was calculated

utilizing the absorbance at 640 nm of the mixtures composed of four kinds of bleaching effluent and CBBG reagent, the dilution factor, and the linear regression equation, which equaled 0.7943 mg/mL, 4.4568 mg/mL, 0.6837 mg/mL, and 8.8143 mg/mL, respectively (as shown in Table 7). Compared to the result of Klason lignin method (as shown in Table 3), the relative error of the results of the aspen KP Ep, the aspen APMP P, the wheat straw KP Ep, and the wheat straw APMP P bleaching effluent obtained *via* the CBBG method was 2.2%, 3.8%, 3.3%, and 1.1%, respectively. This indicated that the lignin content calculated *via* the CBBG method was reliable.

CONCLUSIONS

1. Lignins isolated from the alkali bleaching effluent can cause an increase in the absorbance of the CBBG reagent at 640 nm. Lignins from the acidic bleaching effluent did not cause an increase in the absorbance of the CBBG reagent at 640 nm.

2. The rapid adsorption of CBBG in the mixtures of lignin solution and CBBG reagent into the lignin particles increased the absorbance of the CBBG reagent at 640 nm.

3. The lignin content in the alkali bleaching effluent can be determined *via* the CBBG method and the absorbance of the mixture of the lignin solution and the CBBG reagent needs to be determined in an hour in order to obtain a reliable result.

4. Because of the slow combination of CBBG and acid bleaching effluent lignin, the lignin content in the acidic bleaching effluent cannot be determined *via* the CBBG method.

5. Compared with the results obtained *via* the Klason lignin method, the relative error of the lignin content in four different alkali bleaching effluents calculated *via* the CBBG method was less than or equal to 3.8%.

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REFERENCES CITED

Aghdam, M. A., Kariminia, H., and Safari, S. (2016). "Removal of lignin, COD, and color from pulp and paper wastewater using electrocoagulation," *Desalination and Water Treatment* 57(21), 9698-9704. DOI: 10.1080/19443994.2015.1040461

Andersson, K. I., Eriksson, M., and Norgren, M. (2012). "Lignin removal by adsorption to fly ash in wastewater generated by mechanical pulping," *Industrial & Engineering Chemistry Research* 51(8), 3444-3451. DOI: 10.1021/ie202462z

Clescerl, L. S., Greenburg, A. E., and Eaton, A. D. (1998). "Tannin and lignin," in: *Standard Methods for the Examination of Water and Wastewater,* American Public Health Association, Washington DC, 5-51-5-52

Funaoka, M., Matsubara, M., Seki, N., and Fukatsu, S. (1995). "Conversion of native lignin to a highly phenolic functional polymer and its separation from lignocellulosics," *Biotechnology and Bioengineering* 46(6), 545-552. DOI: 10.1002/bit.260460607

Gao, W., and Fatehi, P. (2018). "Fly ash based adsorbent for treating bleaching effluent of kraft pulping process," *Separation and Purification Technology* 195, 60-69. DOI: 10.1016/j.seppur.2017.12.002

Georgiou, C. D., Grintzalis, K., Zervoudakis, G., and Papapostolou, I. (2008). "Mechanism of Coomassie brilliant blue G-250 binding to proteins: A hydrophobic assay for nanogram quantities of proteins," *Analytical and Bioanalytical Chemistry* 391(1), 391-403. DOI: 0.1007/s00216-008-1996-x

Kaur, D., Bhardwaj, N. K., and Lohchab, R. K. (2018). "A study on pulping of rice straw and impact of incorporation of chlorine dioxide during bleaching on pulp properties and effluents characteristics," *Journal of Cleaner Production* 170, 174-182. DOI: 10.1016/j.jclepro.2017.09.111

Kaur, D., Bhardwaj, N. K., and Lohchab, R. K. (2019). "Effect of incorporation of ozone prior to ECF bleaching on pulp, paper and effluent quality," *Journal of Environmental Management* 236, 134-145. DOI: 10.1016/j.jenvman.2019.01.089

Pinkernell, U., Effkemann, S., and Karst, U. (1997). "Simultaneous HPLC determination of peroxyacetic acid and hydrogen peroxide," *Analytical Chemistry* 69(17), 3623-3627. DOI: 10.1021/ac9701750

Singhal, A., and Thakur, I. S. (2009). "Decolourization and detoxification of pulp and paper mill effluent by *Cryptococcus* sp.," *Biochemical Engineering Journal* 46(1), 21-27. DOI: 10.1016/j.bej.2009.04.007

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO.

Wang, F., Ai, M., and Huang, F. (2016). "Development of method to determine the concentration of alkali-soluble lignin using Coomassie brilliant blue G-250," *BioResources* 11(3), 6137-6150. DOI: 10.15376/biores.11.3.6137-6150

Wang, S., Chen, J., and Yang, G. (2003). "Determination of ClO_2 concentration by malonic acid- Iodin method," *China Pulp & Paper Industry* (09), 43. DOI: 10.3969/j.issn.1007-9211.2003.09.015