

# Soft Nitrogen and Sulfur Incorporated into Enzymatic Hydrolysis Lignin as an Environmentally Friendly Antioxidant and Mercury Adsorbent

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Soft donors of nitrogen and sulfur were incorporated into enzymatic hydrolysis lignin (EHL-NS) to make it suitable for multiple applications. Characterizations of the environmentally friendly material by Fourier transform infrared spectroscopy, scanning electron microscopy, energy dispersive spectroscopy, and thermogravimetric analysis confirmed the layered porous structure with nitrogen and sulfur groups. It exhibited high antioxidant activity due to the strong electron-donating capability of the soft donors. Moreover, the soft donors also contributed to the chemical complexation of Hg(II) with EHL-NS, which distinctly enhanced the adsorption of Hg(II) in water ( $Q_e=180$  mg/g, 25 °C). Given that the free radicals were highly effective at scavenging and adsorption, the functionalized enzymatic hydrolysis lignin is expected to serve a useful role.

*Keywords:* Lignin; Natural polymer; Antioxidant; Adsorption; Mercury

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## INTRODUCTION

The design of green materials using biomass has attracted increased attention due to the depletion of fossil resources and environmental concerns (Thakur *et al.* 2014). Lignin, the second most abundant biomass in the world, has many potential applications in polymers (Thakur *et al.* 2014), but it must be separated from the plant before its utilization. Compared with the conventional alkaline pulping process, enzymatic hydrolysis of lignocellulose uses mild conditions and is considered a clean and green method. It has been predicted that the US bio-refinery industry will generate an estimated 60 Mt of enzymatic hydrolysis lignin (EHL) annually by 2022 (Bugg and Rahmanpour 2015). Hence, the exploitation of this material is crucial for improving the economics of the bio-refinery industry.

Increasing interest in antioxidants from lignins to prevent radical attack, as well as for use as adsorbents for water purification has been observed (Salanti *et al.* 2010; Li *et al.* 2015a; Li *et al.* 2017). For example: Li *et al.* (2015b) prepared a new lignin xanthate resin (LXR) from alkaline lignin. The incorporation of xanthate functional groups (-CSS<sup>-</sup>) onto lignin matrix enhanced its interaction with Pb(II) and adsorption capacity, which is 64.9 mg/g at 30 °C, 4.8 times higher than the original lignin. Ge *et al.* (2014) developed a lignin-based dithiocarbamate (LDTC) by anchoring -NCSS<sup>-</sup> to the alkaline lignin matrix. Due to the cross-linked polymer matrix, high content of -NCSS<sup>-</sup> groups, the LDTC showed a high adsorption amount of 175.9 mg/g for Cu(II) at 25 °C. Moreover, the resultant metal-loaded

solid bio-sorbents were firstly estimated as free radical scavengers due to the inherent merits of naturally occurring polyphenols, which therefore could provide a potential way for value-added usage of the material. However, due to lack of functionality, there has been little research into advanced materials generated from EHL. The combination of soft sulfur and nitrogen represents a potentially powerful strategy for multi-functionality that utilizes their strong electron-donating abilities (Kundu and Bhaumik 2016). In this study, functionalized EHL with soft nitrogen and sulfur (EHL-NS) was designed to enhance its antioxidant activity and to improve its adsorption of metal ions.

## EXPERIMENTAL

### Materials

EHL was provided by Longli Biotech Co., China. It was purified before usage with the method by Li *et al.* (2011). Diethylenetriamine (DETA), CS<sub>2</sub>, formaldehyde (37%), methanol, and Hg(NO<sub>3</sub>)<sub>2</sub> were purchased from Guanghua Chem. Co. (Guangzhou, China). The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was purchased from Sigma-Aldrich (Shanghai, China).

### EHL-NS Synthesis

A total of 8.0 g EHL and 7.0 mL DETA were added to 120 mL of water (pH 12) by stirring at 120 rpm. Next, 9.4 mL of formaldehyde was added dropwise, and the mixture was subjected to microwave heating (XH-MC-1, 300 W, 25 min). After cooling to 40 °C, 7.8 mL of CS<sub>2</sub> was added for 2 h of esterification. The product was obtained by filtration, washing with deionized water, and drying under a vacuum at 50 °C for 24 h, which was named as EHL-NS (purity 95.2%).

### Analysis

The surface morphology was identified using a Hitachi S-340 scanning electron microscope (SEM) (Tokyo, Japan, 20 kV, and ×1.00k) with energy dispersive spectroscopy (EDS) by immobilizing the sample with conductive glue. Fourier transform infrared spectroscopy (FTIR) was conducted with a Nexus 470 (Nicolet Instruments, Offenbach, Germany) in the range of 500 to 4000 cm<sup>-1</sup> by the KBr pellet method. Thermogravimetric analysis (TGA) was conducted with a Netzsch STA449C (10 °C min<sup>-1</sup>, N<sub>2</sub> 10 mL min<sup>-1</sup>) (NETZSCH, Germany). The Brunauer-Emmett-Teller surface area (*S*<sub>BET</sub>) was measured with a Quadrasorb SI analyzer (Quantachrome, Boynton, USA), the total pore volume (*V*<sub>p</sub>, cm<sup>3</sup>/g) and average pore size (*d*<sub>p</sub>, nm) of the samples were also determined.

Radicals scavenging activity (RSA) was determined by the DPPH method using a Shimadzu UV-2101PC spectrophotometer at λ<sub>max</sub> = 520 nm (Kyoto, Japan) (Salanti *et al.* 2010). The inhibition of DPPH (%) was calculated as follows,

$$RSA (\%) = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where *A*<sub>0</sub>, *A*<sub>1</sub> is the absorbance of the DPPH solution with and without the antioxidants.

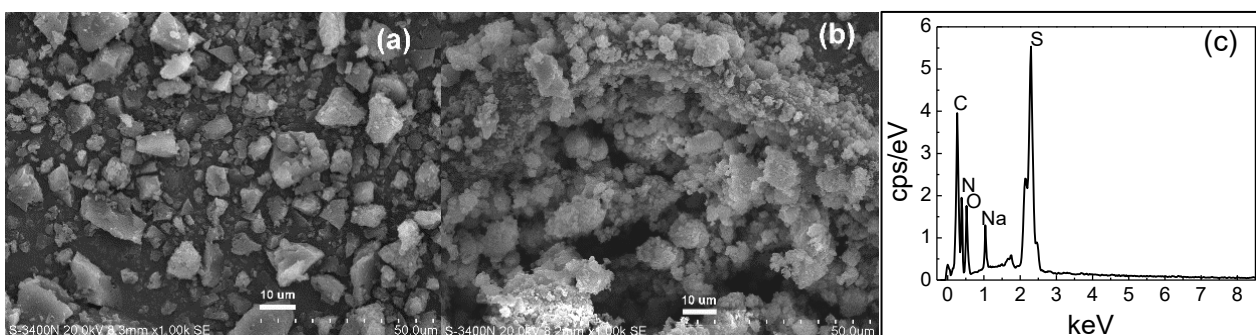
The adsorption of Hg(II), one of the most toxic heavy metals, was used as a model metal ion. EHL-NS was tested by a batch-wise mode in one set of 100-mL conical flasks. The adsorbent was added to each flask containing Hg(II) solution with different

concentrations. The mixtures were stirred at 10, 25, and 40 °C for a period, and then the supernatants were taken out and filtered (0.22 μm) and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 5300DV, Perkin-Elmer, Waltham, MA, USA). All experiments were carried out in triplicate with the relative deviations of <5%.

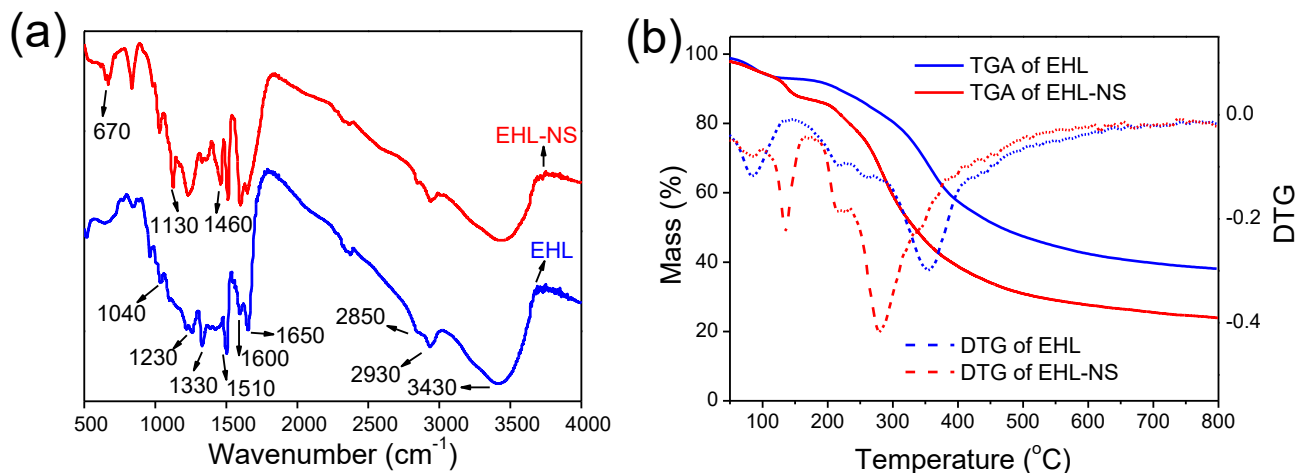
## RESULTS AND DISCUSSION

### Characterization

The SEM images show that EHL displayed a compact surface with irregular blocks (Fig. 1a), while EHL-NS contained various micro-scale interstitial spaces, indicating a layered porous structure (Fig. 1b). The layered porous structure resulted in a higher  $S_{\text{BET}}$  of EHL-NS ( $3.9 \text{ m}^2 \text{ g}^{-1}$ ) than EHL ( $2.9 \text{ m}^2 \text{ g}^{-1}$ ), as determined by the BET method. The average pore size and total pore volume of EHL-NS ( $d_p=16.4 \text{ nm}$ ,  $V_p=0.016 \text{ cm}^3 \text{ g}^{-1}$ ) were also higher than EHL ( $d_p=13.8 \text{ nm}$ ,  $V_p=0.011 \text{ cm}^3 \text{ g}^{-1}$ ) that suggested the presence of mesopores in EHL-NS. The EDS results (Fig. 1c) showed obvious signals of N (0.392 keV) and S (2.307 keV) in EHL-NS.



**Fig. 1.** SEM images of EHL (a) and EHL-NS (b) at magnification of 1 k and EDS spectrum of EHL-NS (c).



**Fig. 2.** FTIR spectra (a); TGA (b) of EHL and EHL-NS

FTIR spectra (Fig. 2a) confirmed the incorporation of N and S in EHL-NS. For example, the bands at 1130 and 670  $\text{cm}^{-1}$  indicated the stretching vibrations of C=S and C-S (Qin *et al.* 2017), respectively; the band at 1460  $\text{cm}^{-1}$  indicated the stretching vibration of C-N (Ge *et al.* 2014). As shown by the TGA curves (Fig. 2b), the weight loss of EHL was attributed to the vaporized water (greater than 200  $^{\circ}\text{C}$ ) and lignin decomposition (355  $^{\circ}\text{C}$ ) (Li *et al.* 2015b). The new sign at 282  $^{\circ}\text{C}$  indicated the C-N and C-S bonds decomposing in EHL-NS.

### Antioxidant Activity

The antioxidant activities are given in Fig. 3. EHL-NS exhibited a higher antioxidant activity, which was indicated by a lower IC<sub>50</sub> (25  $\mu\text{g mL}^{-1}$ ) than EHL (32  $\mu\text{g mL}^{-1}$ ). The EHL-NS exhibited higher antioxidant activity than EHL due to the incorporation of soft N and S into the lignin matrix, which increased the electron-donating capabilities and therefore enhanced the antioxidant activity (Kundu and Bhaumik 2016).

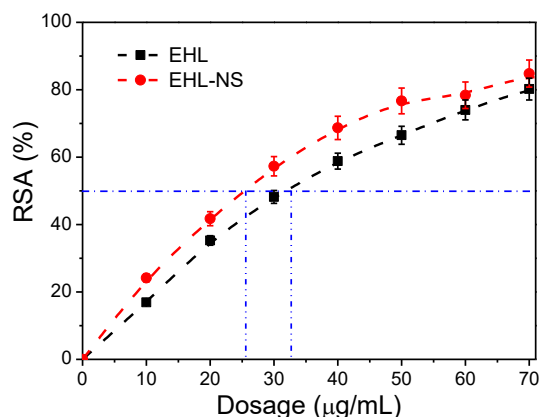


Fig. 3. Radicals scavenging activity of EHL and EHL-NS

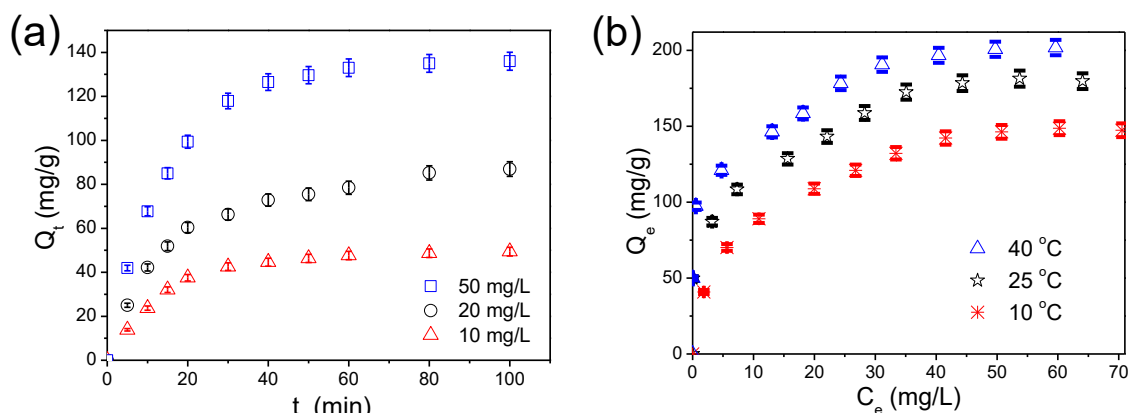
### Adsorption

The adsorption kinetics of Hg(II) on EHL-NS are shown in Fig. 4(a) (dosage 0.2 g/L, pH 6, 25  $^{\circ}\text{C}$ ). The  $Q_e$  increased sharply during the first 20 min and slowed gradually as equilibrium was approached after 50 min. The pseudo-first-order and pseudo-second-order kinetic models were used to fit the experimental data, as given by (Li *et al.* 2017),

$$\log(Q_e - Q_t) = \log Q_e - \frac{k_1 t}{2.303} \quad (2)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e} \quad (3)$$

where  $Q_t$  (mg/g) is the adsorption amount at  $t$  (min),  $Q_e$  (mg/g) is the equilibrium adsorption amount,  $k_1$  ( $\text{min}^{-1}$ ), and  $k_2$  ( $\text{g}/(\text{mg min})$ ) is a constant. As exhibited in Table 1, fitting using the pseudo-second-order model had a greater correlation coefficient ( $R^2 > 0.99$ ), and the calculated  $Q_e$  values were close to the experimental values. Therefore, the pseudo-second-order model fit well to the adsorption kinetics and accordingly indicated the adsorption was based upon chemical interactions.



**Fig. 4.** Adsorption kinetics (a) and isotherms (b) of Hg(II) on EHL-NS

**Table 1.** Parameters of the Pseudo-First-Order and Pseudo-Second-Order Models

$C_0$ (mg/L)	Pseudo-first-order			Pseudo-second-order		
	$Q_e$ (mg/g)	$k_1$ ( $\text{min}^{-1}$ )	$R^2$	$Q_e$ (mg/g)	$k_2$ ( $\text{g}/(\text{mg min})$ )	$R^2$
10	48	0.07	0.998	57	0.001	0.990
20	82	0.06	0.985	97	0.0007	0.998
50	135	0.06	0.989	159	0.0005	0.993

The adsorption isotherms are presented in Fig. 4 (b) (dosage 0.2 g/L, pH 6,  $t = 180$  min). The  $Q_e$  increased with an increasing  $C_e$ , and the saturated  $Q_e$  increased with increasing temperature, which indicated the adsorption was endothermic (Li *et al.* 2015b). The maximum  $Q_e$  (180 mg/g, 25 °C) was higher than other biomass-derived adsorbents listed in Table 2. This might be due to the porous structure and incorporated soft N and S in the EHL-NS.

**Table 2.** Adsorption Capacity of Some Biomass-Derived Adsorbents for Hg(II)

Adsorbent	$Q_e$ (mg/g)	Reference
Rice straw	27.7	(Rocha <i>et al.</i> 2009)
Polyacrylamide-grafted cellulose	12.5	(Bicak <i>et al.</i> 1999)
Chitosan-thioglyceraldehyde base	98	(Monier 2012)
Malt rootlets	50	(Anagnostopoulos <i>et al.</i> 2012)
Chitosan spheres	31.3	(Vieira and Beppu 2006)
EHL-NS	180	this work

The isotherms were fitted by the Langmuir and Freundlich models as follows (Freundlich 1906; Langmuir 1918),

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{\max}} + \frac{1}{bQ_{\max}} \quad (4)$$

$$\log Q_e = \log k_F + \frac{1}{n} \log C_e \quad (5)$$

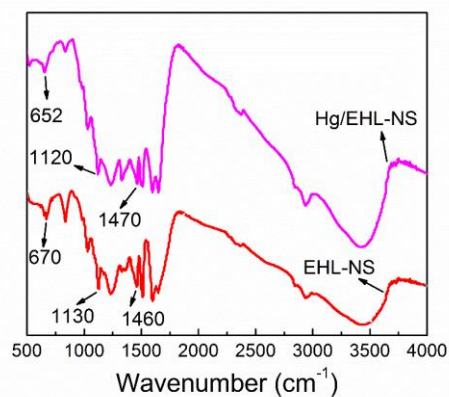
where  $C_e$  (mg/L) is the equilibrium concentration;  $Q_m$  is the saturated adsorption capacity

(mg/g); and  $b$  (L/mg),  $k_F$  (mg/g), and  $n$  are constants. As listed in Table 3, the Freundlich model provided better fitting for the greater correlation coefficients ( $R^2 > 0.98$ ), indicating the surface of EHL-NS was heterogeneous. The increasing  $n$  with temperature indicated the adsorption became more favorable at higher temperature (Li *et al.* 2015b).

**Table 3.** Parameters of the Langmuir and Freundlich Models

Temperature (°C)	Langmuir			Freundlich		
	$Q_m$ (mg/g)	$b$ (L/mg)	$R^2$	$k_F$ (mg/g)	$n$	$R^2$
10	165	0.12	0.986	41	3.1	0.983
25	192	0.18	0.905	73	4.4	0.983
40	223	0.21	0.864	97	5.5	0.989

The FTIR spectra of Hg(II) loaded EHL-NS (Hg/EHL-NS) was recorded to clarify the adsorption mechanism. As shown in Fig. 5, the bands of C=S and C-S shifted to lower frequencies while the N-CS<sub>2</sub> vibration shifted to higher frequencies. This result reflected the chemical bindings of Hg(II) to C=S. The C-S bond reduced the effects of electrostatic induction and enhanced the double bond characteristics of N-CS<sub>2</sub>. While -NH groups may form complexes with Hg(II) (Caner *et al.* 2015), no prominent changes in -NH were observed, but they may have been obscured by the absorption bands of -OH. In sum, a strong metal-ligand bond formed between Hg(II) and the soft nitrogen and sulfur.



**Fig. 5.** FTIR spectra of EHL-NS and Hg/EHL-NS

## CONCLUSIONS

1. An enzymatic hydrolysis lignin (EHL) derived polymer with soft nitrogen and sulfur (EHL-NS) was successfully synthesized.
2. The EHL-NS showed an enhanced antioxidant activity ( $IC_{50} = 25 \mu\text{g mL}^{-1}$ ) and a higher adsorption capability toward Hg(II) ( $Q_e = 180 \text{ mg/g}$ ,  $25 \text{ }^\circ\text{C}$ ) than other natural-based adsorbents.
3. The multi-functionality of EHL-NS was due to the incorporation of soft donors.
4. EHL is a low-cost and environmentally friendly material, and it has great potential as an antioxidant and for adsorption applications.

## ACKNOWLEDGMENTS

The authors are grateful for the support of the National Natural Science Foundation of China, Grant. No. 21464002, and Guangxi Natural Science Foundation (No. 2015GXNSFBA139215, 2016GXNSFAA380329, 2016GXNSFGA380003).

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Article submitted: May 21, 2017; Peer review completed: July 15, 2017; Revised version received and accepted: August 7, 2017; Published: August 21, 2017.

DOI: 10.15376/biores.12.4.7341-7348