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ORIGINAL PAPER

Simultaneous organosolv pretreatment and detoxification of agro-biomass for efficient lignin extraction and characterization

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Abstract

A sequential acid extraction approach using organic acid followed by peroxy acid to enhance the recovery of lignin from *Bambusa bambos* was investigated. The conventional organic acid process was utilized in the pulping step to recover lignin. The residual organic acid-treated pulp was subjected to additional delignification step with peroxy acid to recover additional lignin. A five-level central composite design (CCD) was used to optimize this sequential organic acid followed by peroxy acid extraction process. Three factors, organic acid and peroxy acid concentration, biomass concentration, and duration of extraction, were optimized to maximize OAT–lignin (organic acid-treated lignin) and PAT–lignin (peroxy acid-treated lignin) recovery. Analysis of variance (ANOVA) was used to statistically analyze the significance of these variables on lignin recovery. The optimization process (83.45% v/v organic acid, 8.65% w/v biomass, and 3.35 h duration of extraction) resulted in the maximization of OAT–lignin recovery (123.6 mg/g of biomass) in the organic acid treatment step. The peroxy acid treatment was optimized (83.81% v/v peroxy acid, 6.41% w/v OAT pulp, and 3.23 h duration of extraction) to show that additional 47.2 mg/g of PAT–lignin can be recovered in the delignification step. The physicochemical characteristics of the lignin recovered with the sequential strategy were determined by fourier transform infrared spectroscopy (FTIR), thermo gravimetric analyzer (TGA), and ^{1}H Nuclear magnetic resonance spectroscopy (^{1}H NMR). The results show that the sequential treatment strategy significantly enhances the lignin extracted from *Bambusa bambos*.

Keywords Lignin recovery · Sequential extraction · Delignification · Central composite design

Introduction

One of the major problems facing the world today is the depletion of crude oil source and fast-growing demand for energy, agricultural biomass has been put forward as a route to solve this problem. Agriculture-based renewable biomass is of low cost and abundantly available; it is composed of three major constituents: cellulose, hemicelluloses, and lignin. In this context, lignin is an abundantly available aromatic biopolymer to replace petroleum-based resource (Lange et al. 2013; Lee et al. 2009; Brosse et al. 2011). In a recent study, large amounts of lignocellulosic wastes are produced at cost-effectively rates from many industries including those of paper and pulp and agriculture waste residues. This process effluent contains huge amounts of lignin and lignin-derived substance remaining in the waste material. In this interest, lignin extracted from the lignocellulosic feedstock and transform into valuable compounds (Monteil-Rivera et al. 2013).

Lignin is an amorphous and aromatic polymer, which acts as binding material between cell walls and cells. Lignin is a three-dimensional, highly cross-linked coniferyl, sinapyl, and p-coumaryl alcohol-bearing polymer containing various functional groups such as hydroxyl, methoxy groups, and ethers' linkages (Monteil-Rivera et al. 2013). Lignin-derived chemicals already find applications in phenolic resins (Santos et al. 2014), phenol–formaldehyde polymers (Park et al. 2008), emulsifiers (Boeriu et al. 2004), adhesives, composites, and carbon fibers (Schorr et al. 2014; Lourencon et al. 2015; Sales et al. 2007; Marshall and Alaimo 2010; Ben et al. 2013; Gao et al. 2014; Kumar et al. 2018).

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The pretreatment methods utilized for lignocellulosic biomass include alkali treatment, acid treatment (Scordia et al. 2013; Uppugundla et al. 2014), organic solvents treatment (Hage et al. 2010), ionic liquid treatment (Uppugundla et al. 2014), steam explosion treatment (Pinto et al. 2015), and ammonia fiber explosion (Liang et al. 2016). Among these processes, organosolv pretreatment method utilizing organic acids such as acetic acid and formic acid gives a sulfur-free (high-quality) product and hence is considered as an effective alternative to traditional pulping techniques. Conventional biomass treatment involves two stages, a preliminary pretreatment step (pulping) to hydrolyze the biomass and release lignin. The secondary delignification step further hydrolyzes the biomass to recover residual lignin. Hence, the structure, properties, and yield of lignin depend on the type of biomass and treatment process (Watkins et al. 2015). Acetic and formic acid pretreatment methods are reported to recover high-quality organosolv lignin from both woody and non-woody materials (Pablo et al. 2008; Alvira et al. 2010; Nitzsche et al. 2016; Wen et al. 2013). Organosolv lignin produced at moderate conditions is also reported to contain the least amount of contaminants (carbohydrates and modifiers from the digestion medium) and is relatively low in molecular weight (Cybulska et al. 2012). The residual pulp maybe subjected to secondary treatment with peroxy acids to recover residual lignin.

Statistical optimization is an essential step to maximize the yield with minimum cost of operation. Statistical optimization techniques may be used as an effective tool to for process improvement utilizing minimum number of experimental trials (Padmanaban et al. 2015; Manisha et al. 2015). Central composite design is used as widely used statistical optimization tool due to its ability to predict the mutual interactions between variables, thereby saving time, money, and effort (Srikanth et al. 2014). The objective of this work is to evaluate optimal condition for lignin recovery from bamboo as raw material by sequential organic acid and peroxy acid extraction. The recovered OAT–lignin and PAT–lignin were characterized by UV–Vis spectrophotometer (UV), FTIR, TGA, and ^{1}H NMR spectra analysis.

Experimental

Materials and methods

Materials

Air-dried bamboo wood (*Bambusa bambos*) was obtained from Tirupathur, Vellore District, Tamilnadu, India. It was grinded into powder and passed through a 40–50 mesh screen. The collected raw wood powder was dried in oven for 10 h at 100 °C. Formic acid, acetic acid, and hydrogen peroxide were obtained from SRL Pvt. Ltd, Mumbai, India.

Organic acid and peroxy acid treatment process

Pulping process: the oven-dried bamboo biomass was subjected to an initial pulping step by presoaking in 75–95% organic acid [70:30 ratio of formic acid (FA): acetic acid (AA)] mixture with 5–15% solid loading and refluxing at 100 °C for 1–5 h (Watkins et al. 2015). The extract was filtered with a cloth and washed further with formic acid and hot distilled water to obtain the OAT–lignin-rich filtrate (Fig. 1, Section A).

Delignification process: the residual fibrous material retained on the filter cloth termed organic acid-treated pulp (OAT pulp) was further treated with 75–95% peroxy acid [70:30 ratio of peroxy formic acid (PFA): peroxy acetic acid (PAA)] solution at 100 °C for 2–4 h. The PFA/PAA solution was prepared by mixing 75–95% of formic acid/acetic acid (70:30 v/v) with 2.5% H_2O_2 as described elsewhere (Watkins et al. 2015). The mixture was filtered to separate PAT–lignin-rich filtrate (Fig. 1, Section B) from the delignified biomass.

Lignin precipitation process

The OAT–lignin and PAT–lignin black liquor obtained from pulping and delignification processes were diluted with 5X volume of distilled water. The diluted liquor at around pH 2 was subjected to centrifugation. The supernatant was discarded and the lignin precipitate was washed two-to-three times with distilled water to eliminate the residual acid in the precipitate. The lignin precipitate was transferred to a watch glass and dried in an oven at 50 °C for 12 h. The weight of the dried lignin was used to calculate the yield of the pulping and delignification processes.

Statistical optimization of sequential acid treatment of bamboo biomass

Central composite design is one of the frequently used statistical methods for fitting second-order models. The statistical software package, Design Expert 7.1.6 from State-Ease, Inc., Minneapolis, USA, was used to design the experiments and analyze the data. Two series of 20 experiments each for (1) pulping and (2) delignification steps were carried out with three independent variables. The three independent variables selected for the optimization of lignin yield in the pulping process were organic acid concentration (A_1), bamboo biomass concentration (A_2), and duration of extraction (A_3). The three independent variables selected for optimizing lignin yield in the delignification process were peroxy acid concentration (B_1), OAT-pulp concentration (B_2), and duration

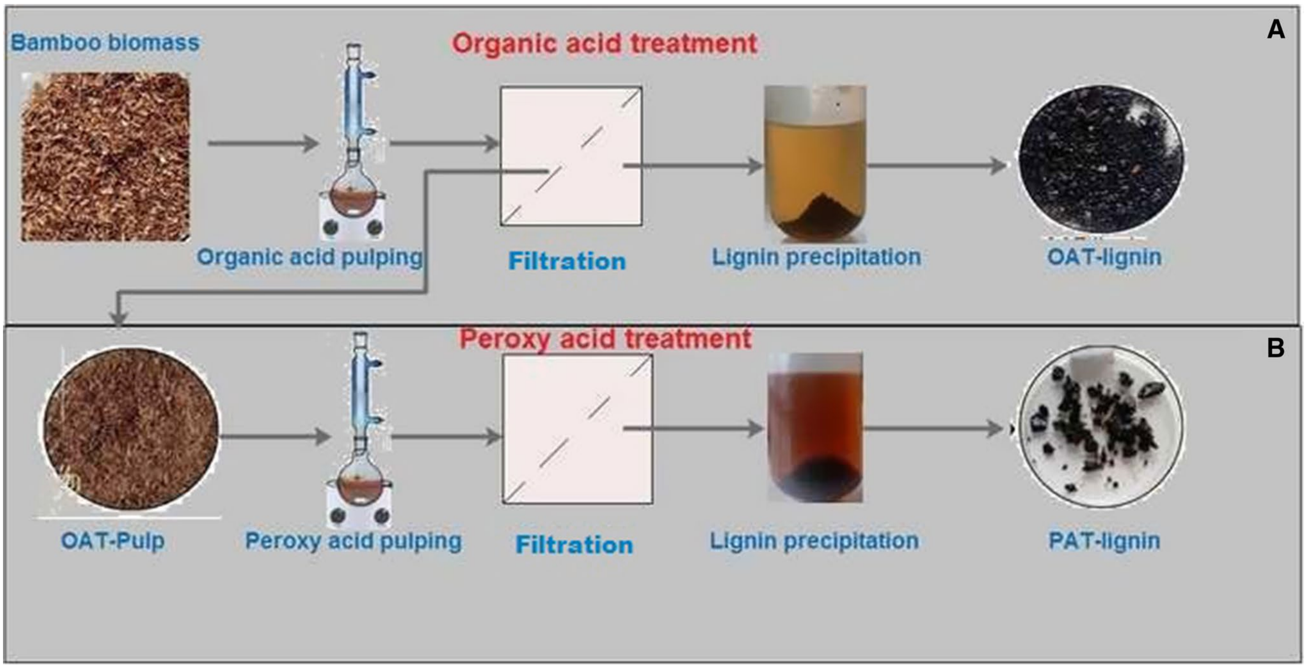

Fig. 1 Schematic diagram of the process of OAT–lignin and PAT–lignin extraction

of extraction (B_3). There variables were studied at five levels with six repetitions at the central point and two replicates at axial and factorial points. Table 1 lists the coded and actual values of these experimental variables with resultant lignin yield (Y_i). The Design-Expert statistical software was also utilized for analysis of variance (ANOVA) and multiple regressions tests. The ANOVA involved an (P) probability test and (F) Fisher's test to determine the regression coefficient and fitness of model.

The fit of the regression models was evaluated by the determination coefficient (R^2). The suggested quadratic model for the independent variables is indicated in Eqs. (1) and (2):

$$Y_1 = \alpha_0 + \sum_{j=1}^{k} \alpha_j A_j + \sum_{j=1}^{k} \alpha_{jj} A_j^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \alpha_{ij} A_i A_j \tag{1}$$

$$Y_2 = \beta_0 + \sum_{j=1}^{k} \beta_j B_j + \sum_{j=1}^{k} \beta_{jj} B_j^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} B_i B_j, \tag{2}$$

where Y_1 and Y_2 are the predicted response, regression coefficients for intercepts (α_0, β_0), linear (α_j,β_j), quadratic (α_{jj},β_{jj}), and interaction effects (α_{ij},β_{ij}) of pulping and delignification process. The predicted polynomial equation was utilized to plot the 3D surface contour diagrams used to depict the interactions effects and validate the model (Manisha et al. 2016).

Analytical methods

Scanning electron microscopy (SEM) was used to compare the morphological changes in acid-treated biomass. Samples were imaged by SEM (FEI, USA) after coating with a gold layer by a cathodic sputtering process on voltage of 15 kV. Fourier transform infrared spectroscopy analysis was carried out in a Cary 600 series FTIR spectrometer, Agilent technologies in the direct transmittance mode in the range of 400–4000 cm^{-1} with a 4 cm^{-1} resolution. UV–visible spectra of lignin samples were analyzed on a Cary 60 UV–Vis spectrophotometer, Agilent technologies. Lignin samples (10 mg) were dissolved in 20 ml of 0.5% NaOH solution. A 1 ml lignin sample was diluted to 20 ml with deionized water and the absorbance between 200 and 400 nm was recorded (Bu et al. 2011). Thermal stability behaviors of the lignin sample were analysis by thermo gravimetric analyzer (TGA) of type Perkin Elemer STA-6000. Approximately 5 g of lignin sample was placed in thermo gravimetric analyzer and dynamic scans were recorded from 25 to 800 °C with 20 °C/min heating rate. The solution state ^{1}H NMR experiments were performed on a Bruker Avance-500 spectrometer operating in FT mode at a frequency of 125 MHz. The ^{1}H NMR spectra were analyzed using 100 mg of isolated lignin dissolved in 1 ml DMSO-d_6 with slight heating and stirring with a micro stir bar.

Table 1 Central composite design (conditions and responses) for pulping (A) and delignification (B)

Run	(A) Pulping process			
	Organic acid % (v/v)	Biomass % (w/v)	Extraction time (h)	OAT–lignin (mg/g of biomass)
	A_1	A_2	A_3	Y_1
1	75 (− 1)	5 (− 1)	1 (− 1)	56.0
2	95 (+ 1)	5 (− 1)	1 (− 1)	50.0
3	75 (− 1)	15 (+ 1)	1 (− 1)	26.7
4	95 (+ 1)	15 (+ 1)	1 (− 1)	36.7
5	75 (− 1)	5 (− 1)	5 (+ 1)	108.4
6	95 (+ 1)	5 (− 1)	5 (+ 1)	60.0
7	75 (− 1)	15 (+ 1)	5 (+ 1)	71.3
8	95 (+ 1)	15 (+ 1)	5 (+ 1)	48.4
9	68.1 (− 2)	10 (0)	3 (0)	85.0
10	101.8 (+ 2)	10 (0)	3 (0)	38.0
11	85 (0)	1.59 (− 2)	3 (0)	41.4
12	85 (0)	18.4 (+ 2)	3 (0)	47.7
13	85 (0)	10 (0)	− 0.36 (− 2)	0.0
14	85 (0)	10 (0)	6.36 (+ 2)	121.0
15	85 (0)	10 (0)	3 (0)	117.0
16	85 (0)	10 (0)	3 (0)	119.0
17	85 (0)	10 (0)	3 (0)	120.0
18	85 (0)	10 (0)	3 (0)	121.0
19	85 (0)	10 (0)	3 (0)	117.7
20	85 (0)	10 (0)	3 (0)	118.0

(B) Delignification process

Run	Peroxy acid % (v/v)	OAT pulp % (w/v)	Extraction time (h)	PAT− lignin (mg/g of OAT pulp)
	B_1	B_2	B_3	Y_2
1	75 (− 1)	3.0 (− 1)	2 (− 1)	21.4
2	95 (+ 1)	3.0 (− 1)	2 (− 1)	23.0
3	75 (− 1)	9.0 (+ 1	2 (− 1)	38.7
4	95 (+ 1)	9.0 (+ 1	2 (− 1)	34.8
5	75 (− 1)	3.0 (− 1)	4 (+ 1)	35.7
6	95 (+ 1)	3.0 (− 1)	4 (+ 1)	29.3
7	75 (− 1)	9.0 (+ 1	4 (+ 1)	31.7
8	95 (+ 1)	9.0 (+ 1	4 (+ 1)	28.1
9	68.1 (− 2)	6.0 (0)	3 (0)	21.6
10	101.8 (+ 2)	6.0 (0)	3 (0)	13.8
11	85 (0)	1.0 (− 2)	3 (0)	18.0
12	85 (0)	11.0 (+ 2)	3 (0)	27.2
13	85 (0)	6.0 (0)	1.3 (− 2)	29.6
14	85 (0)	6.0 (0)	4.6 (+ 2)	40.1
15	85 (0)	6.0 (0)	3 (0)	48.8
16	85 (0)	6.0 (0)	3 (0)	46.9
17	85 (0)	6.0 (0)	3 (0)	49.2
18	85 (0)	6.0 (0)	3 (0)	44.1
19	85 (0)	6.0 (0)	3 (0)	48.7

Table 1 (continued)

(B) Delignification process

Run	Peroxy acid % (v/v)	OAT pulp % (w/v)	Extraction time (h)	PAT− lignin (mg/g of OAT pulp)
	B_1	B_2	B_3	Y_2
20	85 (0)	6.0 (0)	3 (0)	43.8

Results and discussion

Statistical optimization of pulping and delignification process

Statistical methods are generally used to investigate the interaction effect of selected variables on any process. In the pulping step, optimization studies were conducted with different levels of the three variables: (1) organic acid concentration (A_1) from 75 to 95% (v/v); (2) biomass concentration (A_2) from 5 to 15% (w/v); and (3) duration of extraction (A_3) from 1 to 5 h. In the delignification step, optimization studies were conducted with different levels of the three variables: (1) peroxy acid concentration (B_1) from 75 to 95% (v/v); (2) OAT-pulp concentration (B_2) from 3 to 9% (w/v); and (3) duration of extraction (B_3) from 2 to 4 h.

These data were fed to the Design-Expert software to generate a design table with 20 suggested experimental runs including 6 replicates around the centre point (0,0,0), as shown in Table 1a, b. The 20 suggested CCD experiments were performed with the suggested value of variables, A_1, A_2, and A_3 for pulping and B_1, B_2 and B_3 for the delignification steps. The OAT–lignin and PAT–lignin yields from these experiments were used to conduct an ANOVA analysis (Tables 2, 3). The maximum OAT–lignin yield of 121 mg/g of biomass was obtained with the suggested process variables of run number 14 of (Table 1) 85% v/v organic acid (A_1), 10% w/v biomass (A_2), and 6.36 h duration of extraction (A_3). ANOVA was used to evaluate the interaction effects of the three process variables. The maximum PAT–lignin yield of 49.2 mg/g of OAT pulp was obtained with the suggested process variables of run number 17 of Table 1b: 85% (v/v) peroxy acid (B_1), 6% (w/v) OAT pulp (B_2) and 3 h duration of extraction (B_3). ANOVA was again used to estimate the interaction effects of the three process variables.

The coefficients of the full model were determine by regression analysis and tested for their significance as represented in Tables 2 and 3. Equation (3) describes the correlation between the significant variables for the OAT–lignin recovery during the pulping process:

Table 2 Analysis of variance (ANOVA) for lignin recovery by pulping process (R^2: 0.92)

Factors	Sum of squares	Degrees of freedom	Mean squares	*F* value	*p* value
Model	27162.78	9	3018.09	13.20	< 0.0001*
A_1: organic acid	1569.91	1	1569.91	6.87	< 0.0001*
A_2: biomass	478.02	1	478.02	2.09	0.1788
A_3: extraction time	7603.26	1	7603.26	33.26	<0.0001*
A_1A_2	214.45	1	214.45	0.94	0.3556
A_1A_3	710.27	1	710.27	3.11	0.1084
A_2A_3	4.59	1	4.59	0.02	0.8901
A_1A_1	5254.33	1	5254.33	22.98	<0.0001*
A_2A_2	9062.86	1	9062.86	39.64	< 0.0001*
A_3A_3	5450.71	1	5450.71	23.84	< 0.0001*
Residual error	2286.01	10	228.60		
Lack-of-fit	2274.60	5	454.92		
Pure error	11.41	5	2.28		
Total	29448.79	19			

*Significant model terms ($p < 0.05$)

Table 3 Analysis of variance (ANOVA) for lignin recovery by delignification process ($R^2=0.91$)

Factors	Sum of squares	Degrees of freedom	Mean squares	F value	p value
Model	2120.40	9	235.61	12.00	< 0.0001*
B_1: peroxy acid	47.11	1	47.11	2.40	0.1524
B_2: OAT pulp	114.35	1	114.35	5.83	< 0.0001*
B_3: extraction time	43.76	1	43.76	2.23	0.1663
B_1B_2	0.97	1	0.97	0.04	0.8290
B_1B_3	7.37	1	7.37	0.38	0.5536
B_2B_3	147.17	1	147.17	7.50	< 0.0001*
B_1B_1	1148.89	1	1148.89	58.54	< 0.0001*
B_2B_2	748.42	1	748.42	38.13	< 0.0001*
B_3B_3	119.19	1	119.19	6.03	< 0.0001*
Residual error	196.26	10	19.63		
Lack-of-fit	166.71	5	33.34		
Pure error	29.55	5	5.91		
Total	2316.72	19			

*Significant model terms ($p<0.05$)

$$Y_1 = 118.61 - 10.72A_1 - 5.92A_2 + 23.60A_3 + 5.18A1A_2 - 9.42A_1A_3 - 0.76\,A_2A_3 - 19.09\,A_1^2 - 25.08\,A_2^2 - 19.45\,A_3^2. \quad (3)$$

Table 2 also indicates that the model exhibits significant contribution to OAT–lignin yield from A_1, A_3, A_1A_1, A_2A_2, and A_3A_3 (p value <0.05). The fitness of the model was expressed by the coefficient of determination (R^2), which was reported to be 0.92. The proximity of R^2 value to 1 suggests significant correlation between the observed and predicted values. Table 3 shows the ANOVA results of delignification step. Results show that B_2, B_2B_3, B_1B_1, B_2B_2 and B_3B_3 had a significant effect on the PAT–lignin yield ($p<0.0001$):

$$Y_2 = 46.71 - 1.86\,B_1 + 2.89\,B_2 + 1.79\,B_3 - 0.35\,B_1B_2 - 0.96\,B_1B_3 - 4.29\,B_2B_3 - 8.93\,B_1^2 - 7.21\,B_2^2 - 2.88\,B_3^2. \quad (4)$$

The regression analysis of the data shows excellent fit into the proposed model with more than 91% variability. This indicates that Eq. (4) is a suitable model to describe the response of the experiment to PAT–lignin recovery. The optimal value of the variables for maximization of OAT–lignin and PAT–lignin recovery predicted by the response optimizer tool of Design-Expert software was 123.6 mg/g of biomass and 47.2 mg/g of OAT pulp in the pulping and delignification steps, respectively, as presented in Table 4. The optimized process variables predicted for the pulping step are 86.6% (v/v) organic acid, 9.4% (w/v) bamboo biomass, and 4 h duration of extraction. The optimized process variables for the delignification steps are 83.8% (v/v) peroxy acid, 6.4% (w/v) OAT pulp, and 3.2 h duration of extraction.

Similar reports in the literature show that alfalfa fibers, pine straw, wheat straw, and flax fibers treated with FA/AA and PFA/PAA acid yielded lignin recovery of 34%, 22.6%, 20.4%, and 14.8%, respectively (Watkins et al. 2015). Wen et al. have reported that formic acid treatment at atmospheric pressure dissolved 62% of the lignin present in bamboo culms to produce a lignin yield of around 168 mg/g of biomass. Similar work with organic acid pulping followed by peroxy acid delignification steps has reported significant enhancement in lignin recovery from bamboo (Wen et al. 2013). Another report shows that 74% of the lignin in untreated wood poplar chip was recovered as organosolv lignin (Pan et al. 2006).

Interaction between the extraction condition and lignin yield

The 3D response surface plots provide a graphical depiction of the polynomial model and the nature of interaction between the variables. Graph showing the interactions between organic acid, biomass concentration, and duration

Table 4 Optimal values of the test variables for lignin recovery in decoded units

Pulping process		Delignification process	
A_1: organic acid %	83.45	B_1: peroxy acid %	83.81
A_2: biomass % (w/v)	8.65	B_2: OAT pulp % (w/v)	6.41
A_3: extraction time (h)	3.35	B_3: extraction time (h)	3.23
Y_1: OAT–lignin (mg/g of biomass)	123.6	Y_2: PAT–lignin (mg/g of OAT pulp)	47.2

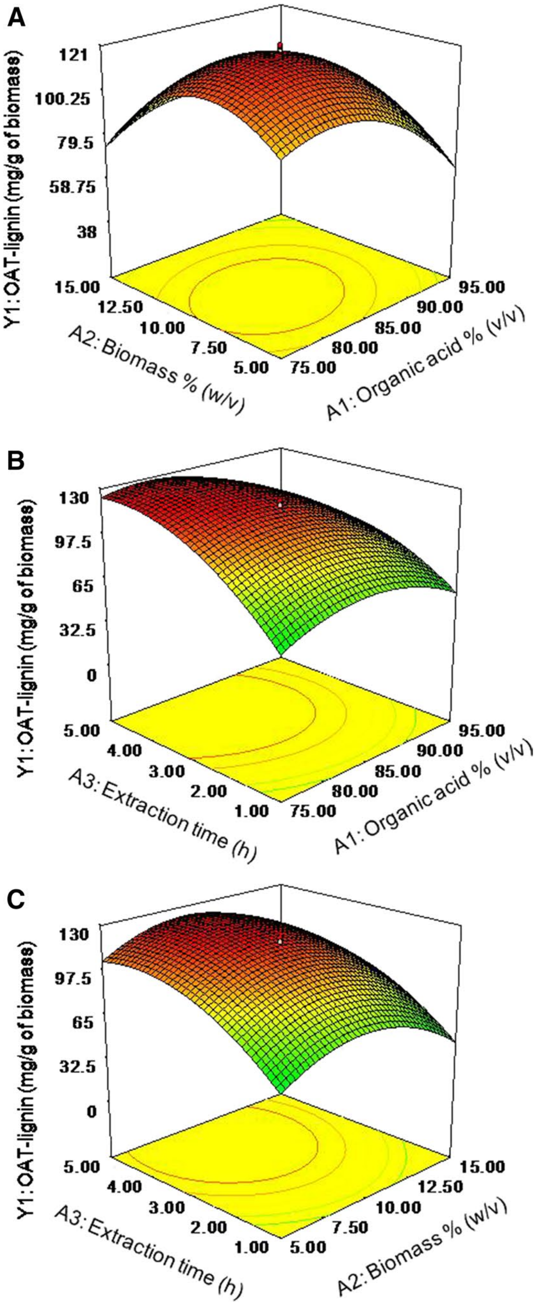

Fig. 2 Response surface plots showing the interactions between organic acid concentration, biomass concentration, and duration of extraction for the pulping process (**a**–**c**)

of extraction for the pulping process is depicted in Fig. 2a–c. The interactive effect between organic acid and biomass concentrations on OAT lignin yield is shown in Fig. 2a. The figure shows that increasing organic acid concentrations from 75 to ~ 85% at constant biomass concentrations led to an initial increase in OAT–lignin yield. However, further increase in organic acid concentrations led to a decline in the yield. A similar trend was observed for increasing biomass concentrations from 5 to 15% at constant organic acid concentrations with optimum yields realized at around 10% biomass concentrations. The highest OAT–lignin recovery (109 mg/g of biomass) was observed with ~ 85% organic acid, ~ 10% biomass concentrations, and 3 h duration of extraction. Figure 2b represents the interactive effect between organic acid concentration and duration of extraction on OAT–lignin recovery. The figure shows that with constant organic acid concentration, OAT–lignin recovery initially increases with duration of reaction until ~ 3 h. Further increase in duration of extraction does not lead to any appreciable increase in yield, suggesting that the optimum OAT–lignin recovery (124.9 mg/g of biomass) occurs with around ~ 3 h incubation, ~ 85% organic acid concentration, and 10% biomass concentration. The interaction effect of biomass concentration and duration of extraction on OAT–lignin recovery (Fig. 2c) show that the highest recovery was obtained with ~ 3 h duration, ~ 10% biomass concentration, and 85% organic acid concentration.

These results may be explained with an example of two situations: at low organic acid and high biomass concentrations, the ratio of organic acid to biomass concentration is low enough to limit the yield of the lignin. Conversely, at high organic acid and low biomass concentrations, the high organic acid to biomass concentrations is likely to hydrolyze some of the extracted lignin and lower its overall yield in the OAT–lignin product (Pan et al. 2006). The interaction effects of duration of extraction, peroxy acid, and OAT-pulp concentrations on PAT–lignin recovery in the delignification process are depicted in Fig. 3. Figure 3d–f shows the interactive effects between OAT-pulp and peroxy acid concentrations; peroxy acid and duration of extraction; OAT-pulp concentration; and duration of extraction, respectively. The interactive effects between these variables were found to be similar to one found in Fig. 3 and maximum PAT–lignin recovery (47.2 mg/g of OAT pulp) was realized with peroxy acid and OAT-pulp concentrations of ~ 85% and ~ 6%, respectively, and 3 h of extraction.

Characterization of lignin

SEM images of raw biomass and sequential organic acid-treated biomass are shown in Fig. 4. A visual observation of the image from raw biomass (Fig. 4a), shows compact and rigid fibril structures. The organic and peroxy acid treatment leads to a disordered surface morphology of the biomass, as shown in Fig. 4b, c, respectively. Hence, acid treatment leads to the degradation of linkages in biomass fibers and this might be attributed to removal of lignin, furfural, and 5-hydroxymethylfurfural.

UV–Vis spectrophotometer in the 200-400 nm range was used to study the lignin isolated from OAT–lignin and PAT–lignin isolate with pulping delignification

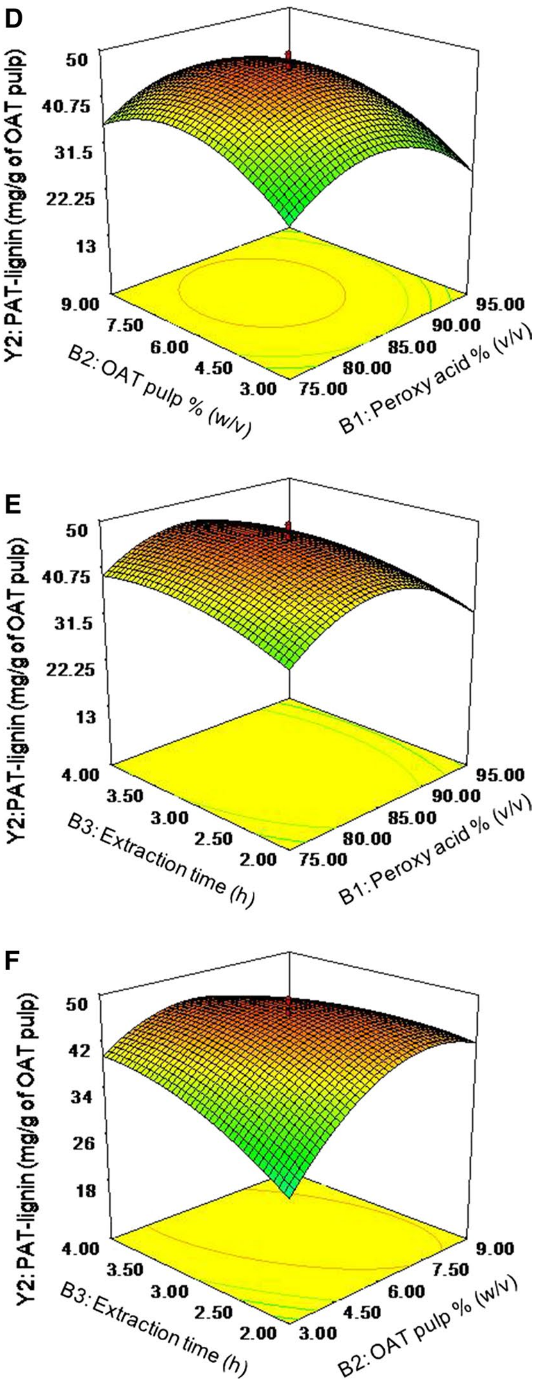

Fig. 3 Response surface plots showing the interactions between peroxy acid concentration, OAT-pulp concentration, and duration of extraction for the delignification process (**d**–**f**)

process, respectively (Fig. 5). The spectra exhibited discernable absorption maxima at around 270–280 nm and 320–350 nm ranges. The spectra peak at around 280 nm may be attributed to non-conjugated aromatic phenolic rings in lignin (Bu et al. 2011). Similar absorption peak maxima is observed around 320–350 nm range and maybe attributed to the conjugated phenolic groups in p-coumaric and ferulic acids (Pan et al. 2006; Nadji et al. 2009).

Structural analysis of isolated lignin

The FTIR spectra were recorded region between 600 and 4000 cm^1 for OAT–lignin and PAT–lignin samples and used to identify the functional groups present in the samples (Fig. 6). The spectra show strong absorption peaks observed at 1635 and 1606 cm^{-1} 1510 and 1459 cm^{-1} attributed to aromatic skeletal vibrations in both the lignin samples (Wang and Chen 2013). The spectra confirm that the treatment and isolation procedure did not alter the lignin structure. The spectra of both the samples showed dominant wide bands in the 3500–3400 cm^{-1} wave number range due to the presence of alcoholic groups in phenolic and aliphatic structures. The band in the 3000–2930 cm^{-1} range is likely to arise from the C–H stretching in aliphatic methyl and methylene groups of side chains in aromatic compounds. The bands at 1328–1336 cm−1 and 1267-1274 cm^{-1} are likely to be associated with the syringyl, guaiacyl units, respectively, in lignin molecules (Lourencon et al. 2015). The above absorption bands indicated the simultaneous presence of both guaiacyl and syringyl unit in the lignin samples (Goncalves et al. 2016). The sulfur-free organosolv lignin samples are considered beneficial, as it is known to interfere with further degradation of the lignin (Tan et al. 2009).

Thermal degradation of the recovered lignin was analyzed by the weight loss percentage of materials with respect to the temperature and was shown in TGA curve (Fig. 7). The initial loss of lignin mass was observed at ~ 68 °C due to the moisture and volatile content present in the sample (Sun et al. 2001). The second stage significant mass loss occurs at ~ 176 °C, due to the degradation of hemicelluloses content in the lignin samples. The previous studies show similar behaviors of hemicelluloses degradation obtained in the lignin sample (Abbas et al. 2017). The maximum mass loss occurred over a wide range of temperatures from 292 to 361 °C; this region causes the fragmentation of inter linkages in the lignin molecule to releasing monomeric units. Watkins et al. studied the similar behavior of degradation observed by organic acid-treated lignin and peroxy acid-treated lignin samples (Watkins et al. 2015; El-Saied and Nada, 1993).

The chemical structure of extracted lignin molecule was analysis using ^1H NMR spectroscopy. The ^1H NMR spectra of the OAT and PAT–lignin fractions are shown in Fig. 8. The signals between 6.0 and 8.0 ppm range are attributed to the aromatic protons in the coumaryl, coniferyl, and syringyl units in lignin. The panels of Fig. 8 show a broad peak for the OAT–lignin and PAT–lignin due to the presence of these protons in varied chemical environment in the lignin structure. The lignin samples' NMR spectra absorbed at 7.5–7.3 ppm

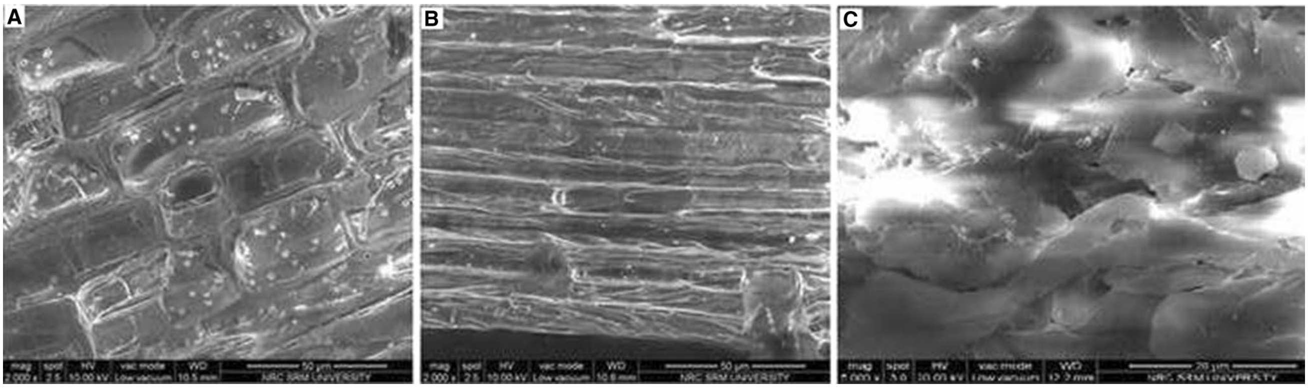

Fig. 4 SEM images of raw bamboo biomass (**a**), first organic acid-pretreated pulp (**b**), and the second sequential peroxy acid-treated pulp (**c**)

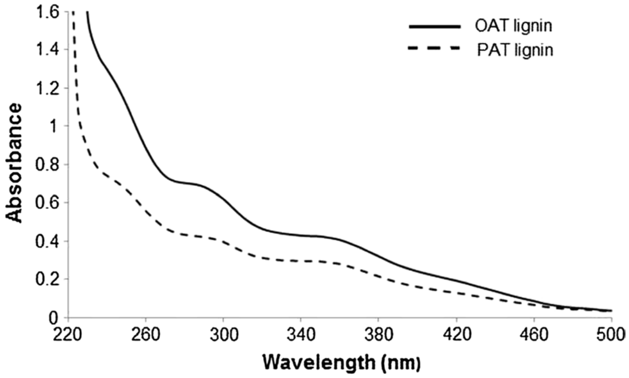

Fig. 5 UV spectra of OAT–lignin and PAT–lignin

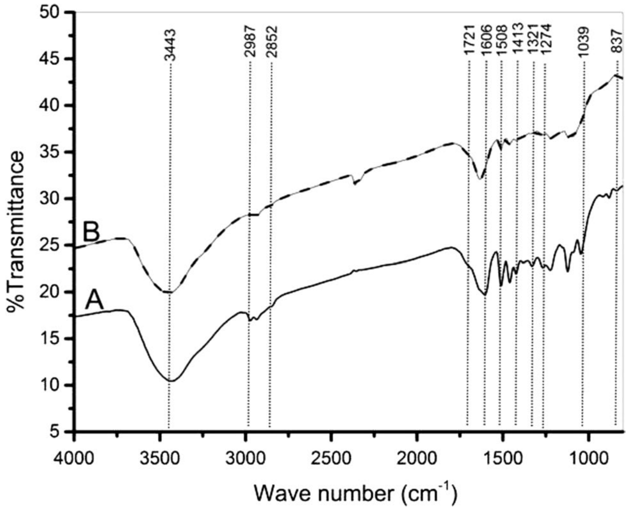

Fig. 6 FTIR spectra of OAT–lignin (**a**) and PAT–lignin (**b**)

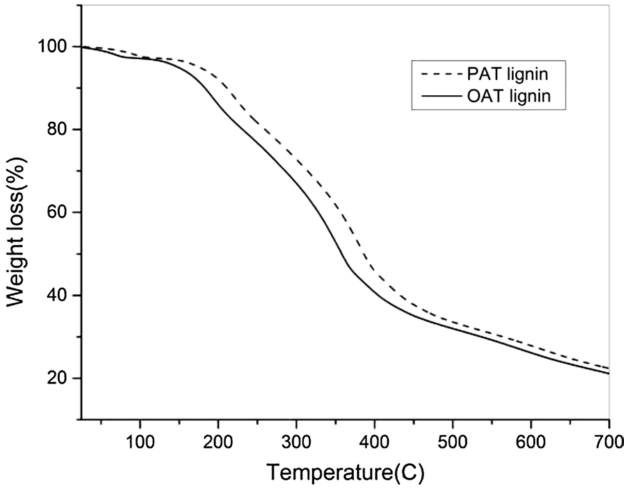

Fig. 7 TGA curves for OAT–lignin and PAT–lignin

is associated with the aromatic protons in p-hydroxyphenyl, while the peaks obtained at 7.3–6.7 and 6.7–6.3 ppm were corresponding to aromatic protons in guaiacyl and syringyl units, respectively (Bu et al. 2011; Wang and Chen, 2013; Xu et al. 2006, 2008). Signals between 4.3 and 6.3 ppm are due to the absorption of protons in the side chain of the aromatic rings. In this case, the signals are absent and this is in accordance with the literature, which cites that these signals are usually weak to be observed in spectra (Bu et al. 2011). The strong signal around 3.4 and 3.7 ppm have been reported to be due to the methoxyl protons (–OCH3) in the side chain of the aromatic ring. The peaks at around 2.5 and 2.1 ppm arise due to the absorption of acetyls protons attached to aromatic and the aliphatic groups in lignin, respectively (Garcia et al. 2009).The peaks between 0.8 and 1.5 ppm are attributed to aliphatic proton in the samples. This shows that the weak signal obtained in PAT–lignin suggests that peroxy acid treatment leads to more oxidation of lignin than mere organic acid treatment.

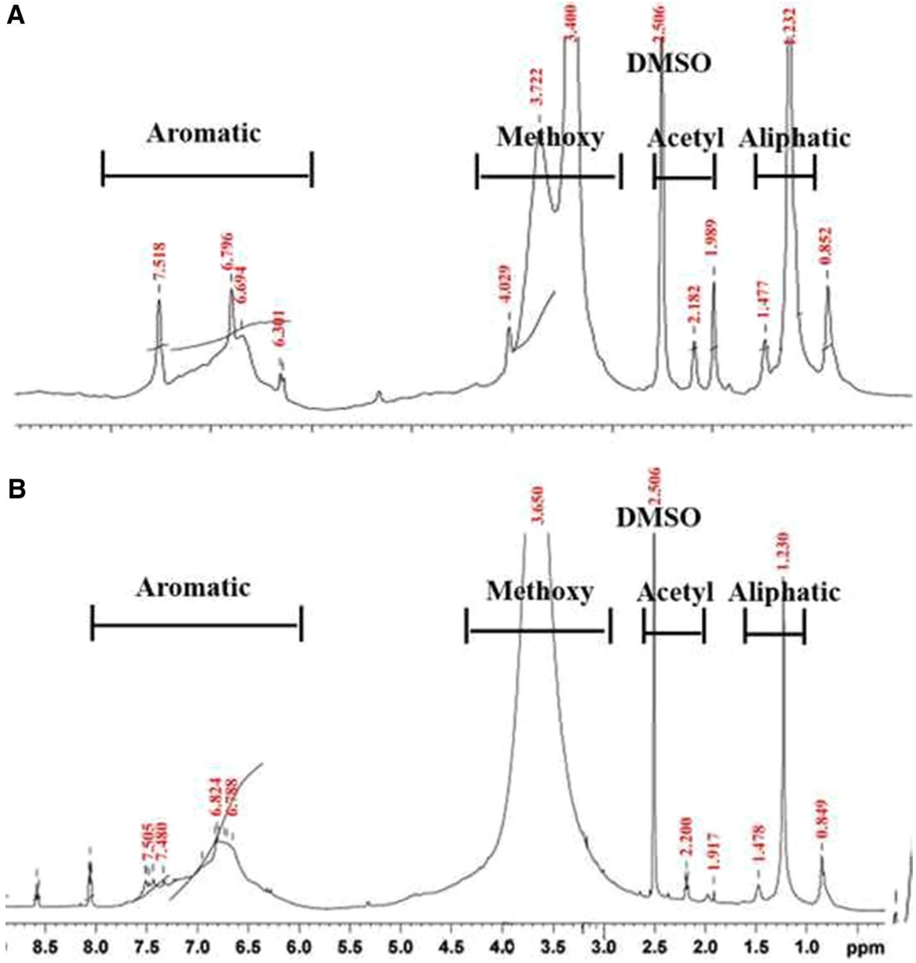

Fig. 8 ^{1}H NMR of OAT–lignin (**a**) and PAT–lignin (**b**)

Conclusion

A two-stage extraction employing pulping followed by delignification was employed to efficiently recover lignin from bamboo. The pulping process with aqueous organic acid was found to provide relatively high OAT–lignin yield 123.6 mg/g of biomass. The residual OAT pulp was further treated by delignification with peroxy acid to produce PAT–lignin yield 47 mg/g of OAT pulp. The sequential organic acid followed by peroxy acid treatment enhances the yield of lignin. FTIR and TGA analyses provided evidence that lignin extracted using both OAT and PAT processes showed similar structural characteristics. The ^{1}H NMR analyses show the presence of characteristic guaiacyl, coumaryl, and syringyl units in OAT–lignin as well as PAT–lignin samples. The results show that sequential acid treatments significantly enhance the lignin yield, which is possibility to use for large-scale industrial sectors.

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