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Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials

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ABSTRACT

The effectiveness of alkali, acid and chlorite pretreatment of lignocellulosic feedstocks for improving the enzymatic saccharification of cellulose has been evaluated. The feedstocks such as Corncob, *Prosopis juliflora* and *Lantana camara* were pretreated with varied concentration of sulfuric acid, sodium hydroxide and sodium chlorite at 121 °C for 15–60 min. Among different methods used, chlorite pretreatment removed maximum lignin with ~90% (w/w) residual holocellulose content in all the substrates tested. Moreover, irrespective of the substrates used, the chlorite treated substrates were enzymatically saccharified from 86.4% to 92.5% (w/w). While, the alkali treated substrates containing 66.0–76.0% (w/w) holocellulose could be enzymatically saccharified up to 55% (w/w). The acid pretreated substrates were found to contain almost 54–62% (w/w) holocellulose, which on enzymatic hydrolysis could result in 39.5–48% (w/w) saccharification.

1. Introduction

Lignocellulosics, the most abundant biomass available on earth, have attracted considerable attention as an alternative feedstock for the production of various value added products due to their renewable nature and low cost availability (Kuhad & Singh, 2007). Various technological developments have improved the bioconversion of these substrates into bioethanol (Kapoor, Chandel, Kuhar, Gupta, & Kuhad, 2007). Enzymatic saccharification is one of the promising strategy to convert cellulosic biomass into sugars because of low energy requirement and less pollution. However, the primary challenge in enzymatic hydrolysis of cellulose is its low accessibility due to association with lignin. Therefore, efficient pretreatment of lignocellulosic substrates has become a pre-requisite to improve enzymatic saccharification (Zhao, Zhang, & Liu, 2008). The main focus of different pretreatment methods is to remove the lignin content and to decrease the cellulose crystallinity (Mosier et al., 2005). Although various physical (comminution, hydrothermolysis), chemical (acid, alkali, solvents, ozone), and biological pretreatment methods have been investigated over the years (Gupta, Mehta, Khasa, & Kuhad, 2010; Kuhar, Nair, & Kuhad, 2008; Kumar, Barrett, Delwiche, & Stroeve, 2009), thermo-chemical pretreatment of biomass has been a pretreatment of choice to enhance substrate accessibility for efficient enzymatic hydrolysis (Himmel et al., 2007).

The thermo-chemical pretreatment strategies such as acid, alkali and oxidation are commonly used for lignocellulosic biomass. The dilute mineral acids have been reported to remove the hemicellulosic fraction from substrates to improve enzymatic saccharification of cellulose (Gupta, Sharma, & Kuhad, 2009; Kuhad, Gupta, Khasa, & Singh, 2010; Schell, Farmer, Newman, & McMillan, 2003). It has dual advantage of solubilizing hemicellulose and subsequently converting it into fermentable sugars. Whereas, the alkali pretreatment remove lignin and various uronic acid substitutions responsible for inhibiting the cellulose accessibility for enzymatic saccharification (Chang & Holtzapple, 2000). Moreover, alkali treatment is also reported to increase the biodegradability of the cell walls due to cleavage of the lignin bonds with hemicellulose and cellulose (Spencer & Akin, 1980). In contrast to acid and alkali treatments, sodium chlorite, a powerful oxidizing agent has been used frequently to delignify wood for cellulose isolation (Sun, Sun, Zhao, & Sin, 2004). The chlorine dioxide produced in this pretreatment method oxidizes lignin to the phenolic compounds and in turn makes cellulose accessible.

Since there is no universal and economically viable pretreatment method available, which could be used to pretreat varied cellulosic biomass, in the present study, it has been attempted to evaluate the suitably used three pretreatment methods (acid, alkali and chlorite treatment) for lignocellulosic feedstocks viz., *Prosopis juliflora* (PJ, a woody biomass), *Lantana camara* (LC, a shrub and

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weed) and Corncob (CC, agricultural residue). The pretreatments of the substrates were attempted at varied chemical dosage and pretreatment time. The pretreated plant materials were enzymatically hydrolysed and the cellulosic saccharification efficiency was determined to evaluate the efficacy of these methods. Irrespective of the substrates used, the chlorite treatment was found to be an efficient method for delignification and producing cellulose rich plant material, which was almost 90% hydrolysable by cellulases into glucose.

2. Materials and methods

2.1. Raw materials

The lignocellulosic substrates: Corncob (CC), *P. juliflora* (PJ) and *L. camara* (LC) were collected locally, dried in sunlight and then cut into small pieces. The dried material was ground and passed through a 40–60 mesh size screen using a laboratory knife mill (Metrex Scientific Instrumentation, Delhi, India). The processed substrate was thoroughly washed, dried at 60 °C and stored in sealed plastic bags at room temperature.

2.2. Pretreatments

2.2.1. Acid pretreatment

The dilute sulfuric acid pretreatment of lignocellulosic substrate (100.0 g) was carried out using varied acid concentration (1–5% (w/v)) and incubation time (15–60 min) at 121 °C. The hydrolysates after treatment were separated by filtering the contents through double layered muslin cloth. The residual biomass was washed with tap water till neutral pH and dried in a hot air oven at 60 °C.

2.2.2. Alkali pretreatment

The substrate (100.0 g) was presoaked in different concentrations of alkali (NaOH) ranging from 1% to 5% (w/v) for 2 h and thereafter thermally pretreated at 121 °C for 15, 30, 45 and 60 min. The pretreated material was filtered through double layered muslin cloth, washed extensively with tap water until neutral pH and dried at 60 °C.

2.2.3. Chlorite pretreatment

The lignocellulosic substrate (100.0 g) was treated with different concentrations of sodium chlorite (1–5% (w/v)) at 121 °C for 15, 30, 45 and 60 min. The pretreated material was filtered through double-layered muslin cloth, washed thoroughly till neutral pH and dried in a hot air oven at 60 °C.

3. Enzymatic saccharification of pretreated substrates

Cellulase from *Trichoderma reesei* (ATCC 26921) with an activity of 6.5 FPU/g, supplemented with β-glucosidase from *Aspergillus niger* (Novozyme 188) having 250 U/g was used for saccharifying the cellulosic material obtained after each pretreatment type.

Enzymatic hydrolysis of each type of pretreated plant materials (10.0 g each) was carried out at 5% (w/v) substrate consistency in 50 mM citrate phosphate buffer (pH 5.0). The substrate with buffer was pre-incubated at 50 °C on a rotatory shaker (Innova-40, New Brunswick Scientific, Germany) at 150 rpm for 2 h and thereafter the slurry was added with cellulase (3 FPU/ml) and β-glucosidase (9 U/ml). Tween 80 (1% (v/v)) was also added to the reaction mixture and the reaction continued up to 36 h. Samples of enzymatic hydrolysate were withdrawn at regular intervals and analysed for amount of glucose released.

Table 1
Proximate chemical composition analysis of Corncob, *P. juliflora* and *L. camara* using TAPPI (1992) protocols.

Components (% (w/w))	Corncob	*P. juliflora*	*L. camara*
Cellulose	37.4 ± 4.18	47.5 ± 3.27	44.1 ± 1.72
Pentosans	34.2 ± 1.02	18.7 ± 1.09	17.0 ± 0.81
Holocellulose	71.6 ± 3.21	66.2 ± 4.54	61.1 ± 2.53
Klason lignin	19.2 ± 0.83	29.1 ± 2.05	32.3 ± 1.57
Moisture	7.4 ± 0.42	2.7 ± 0.28	4.4 ± 0.13
Ash	1.8 ± 0.17	2.0 ± 0.12	2.3 ± 0.13

4. Analytical methods

The chemical composition (α-cellulose, klason lignin, pentosans, moisture and ash) of all the three substrates and their residual solid fraction post pretreatment were determined following standard TAPPI (1992), protocols. The reducing sugars released were estimated using the DNS method (Miller, 1959) and the yield of reducing sugars in enzymatic hydrolysate (YRSEH) was calculated as follows:

$$\text{YRSEH}(\%) = \frac{\text{Sugars released in enzymatic hydrolysate}}{\text{Total carbohydrate content in pretreated substrates}} \times 100$$

The percent loss and gain of different components in pretreated substrates were calculated using following equations:

$$\text{Loss}(\%) = \frac{M_i - M_f}{M_i} \times 100 \quad (1)$$

$$\text{Gain}(\%) = \frac{M_f - M_i}{M_i} \times 100 \quad (2)$$

where M_i is the amount of component in the untreated substrate and M_f is the amount of the component in the substrate after pretreatment.

5. Statistical analysis

All the experiments were performed in triplicate and the results are presented as mean ± standard deviation.

6. Results and discussion

6.1. Compositional analysis of different lignocellulosic substrates

The chemical composition analysis of different lignocellulosic biomass revealed that the holocellulose content was in the range of 61.1–71.6% (w/w), where *P. juliflora* (PJ) contained maximum cellulose content (47.5 ± 3.27%) followed by *L. camara* (LC; 44.1 ± 1.72%) and Corncob (CC; 37.4 ± 4.18%). The lignin contents observed in CC, PJ and LC were 19.2 ± 0.83%, 29.1 ± 2.05% and 32.3 ± 1.57% (w/w), respectively. However, the hemicellulose content was maximum in CC (34.2 ± 1.02%) compared to PJ (18.7 ± 1.09%) and LC (17.0 ± 0.81%). Moreover, all the substrates had almost equal amount of ash content ranging from 1.8% to 2.3%, and the moisture content in CC, PJ and LC were 7.4 ± 0.42%, 2.7 ± 0.28% and 4.4 ± 0.13%, respectively (Table 1). The considerably high carbohydrate content in all the three lignocellulosic substrates qualified them as potential feedstocks for bioethanol production.

6.2. Effect of chemical pretreatment

6.2.1. Acid treatment

The dilute acid treatment is the most commonly used method to pretreat the lignocellulosic biomass to hydrolyse hemicellulosic

Table 2
Effect of acid treatment on the chemical composition of lignocellulosic substrates.

Time (min)	Acid (% (v/v))	Corncob			*P. juliflora*			*L. camara*		
		RL (% (w/w))	RH (% (w/w))	Hemicellulose removal (% (w/w))	RL (% (w/w))	RH (% (w/w))	Hemicellulose removal (% (w/w))	RL (% (w/w))	RH (% (w/w))	Hemicellulose removal (% (w/w))
15	1	22.4 ± 0.5	70.1 ± 1.2	31.9 ± 1.5	32.4 ± 0.9	64.1 ± 2.7	41.8 ± 2.3	33.5 ± 3.1	60.2 ± 3.3	23.4 ± 3.1
	2	24.6 ± 0.6	66.7 ± 2.8	57.3 ± 2.7	33.7 ± 0.5	62.5 ± 3.4	61.1 ± 2.8	34.0 ± 3.1	59.5 ± 1.1	32.2 ± 2.8
	3	25.7 ± 1.1	65.1 ± 3.5	67.6 ± 2.9	34.5 ± 1.8	61.5 ± 4.0	72.3 ± 3.4	34.5 ± 2.3	58.8 ± 4.2	40.9 ± 5.1
	4	26.6 ± 0.4	63.7 ± 4.8	75.7 ± 3.1	34.7 ± 2.2	61.2 ± 4.3	64.9 ± 6.2	34.7 ± 3.8	58.6 ± 5.1	43.3 ± 6.3
	5	27.3 ± 1.8	62.5 ± 4.5	82.4 ± 2.7	34.8 ± 2.2	61.1 ± 1.8	62.6 ± 1.5	34.8 ± 2.7	58.4 ± 2.5	45.6 ± 2.9
30	1	26.6 ± 1.2	63.5 ± 1.7	76.5 ± 2.8	32.9 ± 1.7	63.5 ± 1.9	49.2 ± 4.2	34.1 ± 1.0	59.4 ± 3.0	33.7 ± 4.2
	2	27.3 ± 1.5	62.4 ± 2.6	82.2 ± 3.5	34.4 ± 2.2	61.6 ± 5.7	71.2 ± 2.9	34.8 ± 2.0	58.5 ± 2.8	44.6 ± 0.8
	3	27.4 ± 0.9	62.4 ± 1.2	82.8 ± 3.1	35.0 ± 1.0	60.8 ± 3.1	79.9 ± 3.4	35.5 ± 1.2	57.6 ± 3.2	55.5 ± .1
	4	27.4 ± 1.5	62.3 ± 3.8	83.1 ± 2.8	35.2 ± 0.6	60.5 ± 4.2	72.0 ± 1.8	35.6 ± 3.8	57.3 ± 2.4	58.5 ± 1.9
	5	27.4 ± 1.6	62.3 ± 4.2	83.0 ± 4.9	35.4 ± 0.9	60.1 ± 3.6	68.2 ± 1.0	35.8 ± 4.2	57.1 ± 2.9	61.5 ± 0.4
45	1	26.8 ± 1.4	63.2 ± 5.4	74.6 ± 2.3	33.8 ± 2.2	62.3 ± 4.6	63.4 ± 0.8	36.5 ± 1.3	56.2 ± 2.7	70.7 ± 5.3
	2	27.5 ± 0.9	62.3 ± 6.1	84.0 ± 4.1	35.3 ± 1.7	60.5 ± 3.1	83.3 ± 2.4	37.2 ± 0.8	55.2 ± 3.3	81.7 ± 3.1
	3	27.5 ± 0.8	62.2 ± 3.0	84.0 ± 6.0	35.9 ± 0.6	59.7 ± 4.1	91.2 ± 1.1	38.0 ± 2.6	54.1 ± 3.4	92.6 ± 2.9
	4	27.6 ± 1.1	62.1 ± 3.8	84.1 ± 3.8	35.9 ± 2.7	59.5 ± 3.0	86.0 ± 3.2	38.1 ± 3.1	54.1 ± 2.5	92.8 ± 5.1
	5	27.6 ± 0.6	62.1 ± 4.9	84.2 ± 2.8	36.1 ± 1.4	59.4 ± 4.7	85.7 ± 3.8	38.1 ± 3.4	54.0 ± 1.6	93.5 ± 2.7
60	1	27.8 ± 1.5	61.7 ± 1.1	87.6 ± 5.2	34.1 ± 1.3	62.0 ± 3.0	66.7 ± 2.9	36.8 ± 2.6	55.8 ± 2.6	75.2 ± 1.0
	2	28.0 ± 1.2	61.5 ± 6.5	86.4 ± 4.9	35.4 ± 0.9	60.3 ± 2.4	85.1 ± 3.5	37.6 ± 3.1	54.7 ± 2.3	86.6 ± 1.9
	3	28.1 ± 0.4	61.5 ± 3.6	87.5 ± 6.5	36.1 ± 1.2	59.5 ± 4.7	93.5 ± 3.8	38.0 ± 1.9	54.2 ± 4.2	92.6 ± 1.0
	4	28.3 ± 1.9	61.4 ± 5.9	86.1 ± 2.4	36.2 ± 2.3	59.2 ± 3.4	90.2 ± 4.9	38.0 ± 3.1	54.2 ± 1.9	92.1 ± 2.0
	5	28.3 ± 0.9	61.4 ± 4.8	87.9 ± 3.0	36.2 ± 0.6	59.1 ± 1.8	84.7 ± 2.1	37.9 ± 3.4	54.1 ± 3.5	90.9 ± 2.5

RL, residual lignin; RH, residual holocellulose.

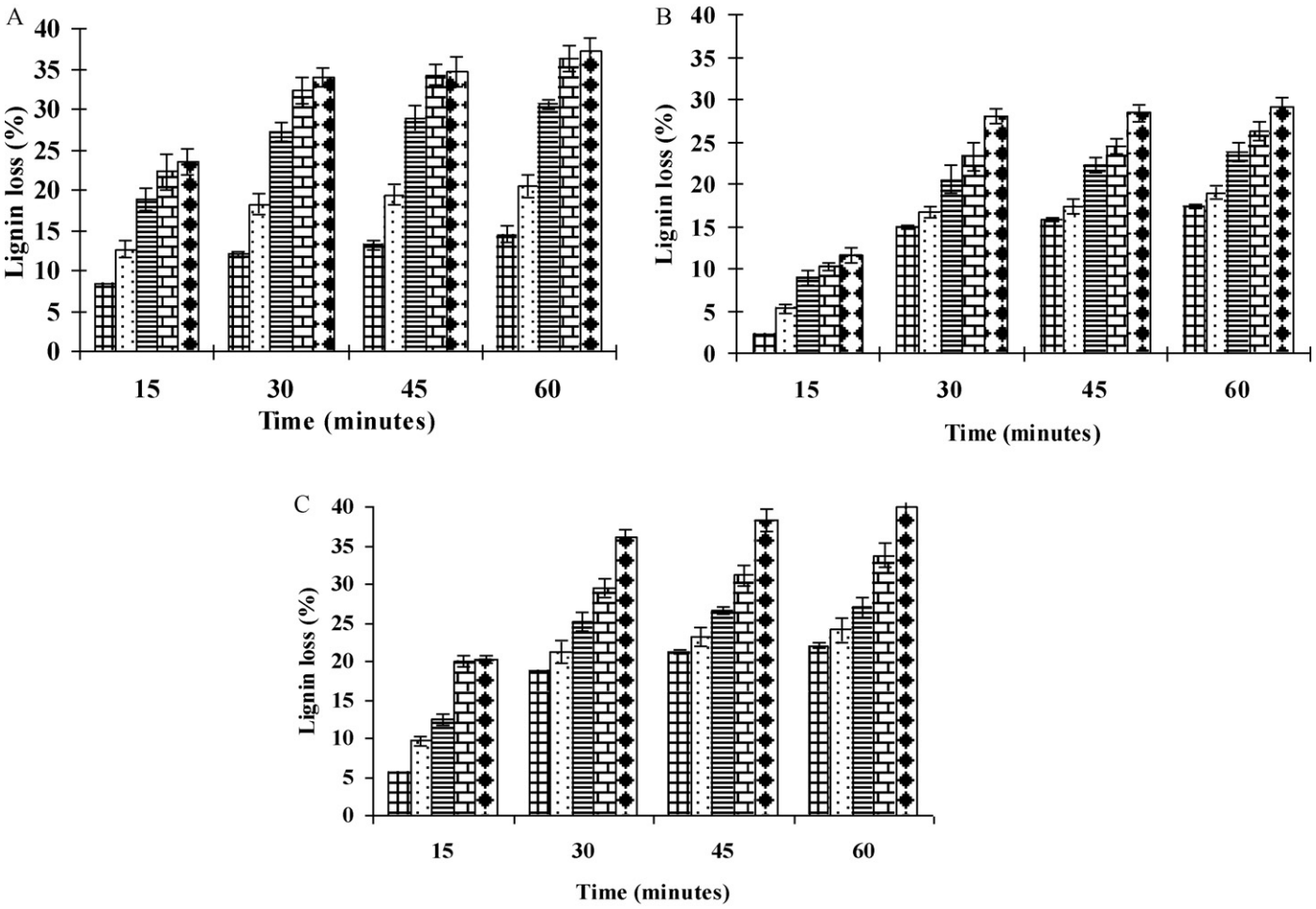

Fig. 1. Lignin removal from Corncob (A), *P. juliflora* (B) and *L. camara* (C) at different concentrations of alkali (▦ 1% NaOH, □ 2% NaOH, ▤ 3% NaOH, ▩ 4% NaOH and ⊞ 5% NaOH).

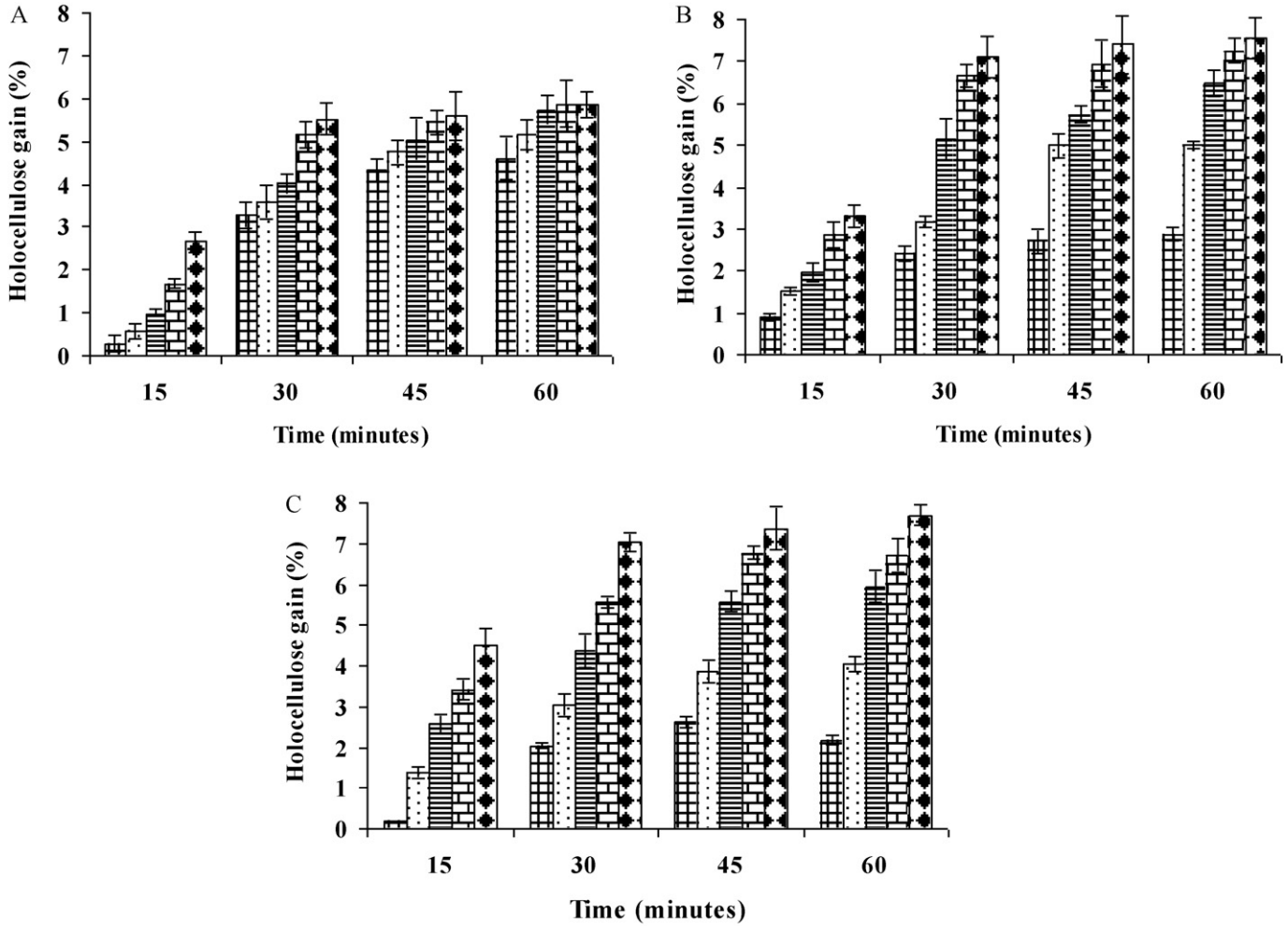

Fig. 2. Holocellulose enrichment in Corncob (A), *P. juliflora* (B) and *L. camara* (C), after treatment with different concentrations of alkali (▦ 1% NaOH, ▨ 2% NaOH, ▤ 3% NaOH, ▩ 4% NaOH and ▦ 5% NaOH).

fraction of lignocellulosic substrates, which is a critical parameter for process efficacy (Ishizawa, Davis, Schell, & Johnson, 2007). The hemicellulose fraction in CC was optimally hydrolysed to 82.2 ± 3.5% (w/w) with 2.0% (v/v) H_2SO_4 for 30 min. While, the optimum acid saccharification in PJ (91.2 ± 1.1% (w/w)) and LC (92.6 ± 2.9% (w/w)) was achieved with 3.0% (v/v) sulphuric acid for 45 min (Table 2). The higher acid concentration and pretreatment time requirement for optimal hydrolysis of PJ and LC may be attributed to the woody nature of these substrates. Irrespective of the substrates, increase in the acid dosage or pretreatment time beyond optimal conditions resulted in decrease of sugar yield, which may be because of the formation of sugar degradation products such as furfurals and hydroxyl–methyl furfurals (Chandel, Kapoor, Sigh, & Kuhad, 2007; Gupta et al., 2009). However, the acid hydrolysed CC contained 62.4 ± 2.6% (w/w) holocellulose and 27.3 ± 1.5% (w/w) lignin, while the acid treated PJ and LC were found to have 59.7 ± 4.1% (w/w) and 54.1 ± 3.4% (w/w) holocellulose with 35.9 ± 0.6% (w/w) and 38.0 ± 2.6% (w/w) lignin, respectively (Table 2). As compared to untreated substrates (control), the higher residual lignin and lower residual holocellulose in the pretreated substrates may be due to the removal of acid soluble carbohydrate fraction (hemicellulose). Our results are well in accordance with the previous reports. Chen, Zhao, and Xia (2009) reported an increase in lignin content from 19.3% to 28.4% (w/w) in acid hydrolysed corn stover. While, an increase in lignin content from 21.8% to 28.5% (w/w) was observed when switch grass was hydrolysed with 1.2% sulphuric acid at 160 °C for 20 min (Li et al., 2010).

6.2.2. Alkali treatment

During the alkali pretreatment, an increase in alkali concentration up to 5.0% (w/v) caused a regular increase in removal of lignin and in turn holocellulose gain in PJ and LC, while a 4.0% (w/v) alkali concentration was found to be optimum for CC (Figs. 1 and 2). In all three substrates, the lignin removal increased with increase in pretreatment time till 30 min and remained almost constant thereafter. Irrespective of the substrates, lignin content was observed to be reduced in a range of 28–36% (w/w) (Fig. 1) with a concomitant enrichment in holocellulose content (5.2–7.1% (w/w)) (Fig. 2). The residual lignin in alkali treated CC, PJ and LC were 12.4%, 20.6% and 19.4% (w/w), while the holocellulose content were 75.8%, 71.3% and 65.8% (w/w), respectively. The increased lignin removal and enrichment of holocellulose in alkali treated substrates may be due to the cleavage of the ester bonds between hydroxycinnamic acids, and the α-benzyl ether linkages of the cell wall of plant materials by alkali (Mosier et al., 2005; Silverstein, Chen, Sharma-Shivappa, Boyette, & Osborne, 2007; Zhao et al., 2008). Similarly, other studies on alkali pretreatment have also reported approximately 40–60% delignification in crofton weed stem (Zhao et al., 2008), rice straw (Jeya, Zhang, Kim, & Lee, 2009) and switch grass (Nlewem & Thrash, 2010).

Table 3
Enzymatic hydrolysis of pretreated (under optimized conditions) lignocellulosic substrates.

Substrate	Pretreatment type	Reagent concentration	Pretreatment time (min)	RL (% (w/w))	RH (% (w/w))	YRSEH (% (w/w))
Corncob	Untreated	–	–	–	–	38.5 ± 2.5
	Alkali treatment	4% (w/v)	30	12.4 ± 1.0	75.8 ± 4.8	55.4 ± 3.7
	Chlorite treatment	4% (w/v)	30	5.4 ± 0.5	90.3 ± 6.7	91.5 ± 3.1
	Acid treatment	2% (v/v)	30	27.3 ± 1.5	62.4 ± 2.6	39.5 ± 4.2
P. juliflora	Untreated	–	–	–	–	33.2 ± 2.1
	Alkali treatment	5% (w/v)	30	20.6 ± 2.7	71.3 ± 3.5	52.2 ± 1.8
	Chlorite treatment	4% (w/v)	30	3.1 ± 0.2	90.7 ± 5.8	92.5 ± 4.3
	Acid treatment	3% (v/v)	45	35.9 ± 0.6	59.7 ± 4.1	47.7 ± 3.7
L. camara	Untreated	–	–	–	–	31.2 ± 0.9
	Alkali treatment	5% (w/v)	30	19.4 ± 1.1	65.8 ± 2.6	50.8 ± 2.4
	Chlorite treatment	4% (w/v)	30	6.5 ± 0.2	90.0 ± 7.2	86.4 ± 1.6
	Acid treatment	3% (v/v)	45	38.0 ± 2.6	54.1 ± 3.4	48.0 ± 3.4

RL, residual lignin; RH, residual holocellulose; YRSEH, yield of reducing sugars in enzymatic hydrolysate.

6.2.3. Chlorite treatment

All the three substrates when pretreated with 4% (w/v) sodium chlorite for 30 min, maximum lignin removal as well as gain in holocellulose content were observed. Thereafter, any further increase, either in the sodium chlorite concentration or pretreatment time did not cause any significant improvement in delignification (Fig. 3). Moreover, irrespective of the substrates tested, approximately 80–90% (w/w) delignification was obtained when pretreated under optimal conditions (Fig. 3). The pretreated substrates were found to have an increase in their holocellulose content by 26.2 ± 0.8%, 37.2 ± 5.2% and 47.3 ± 2.6% (w/w) in CC, PJ and LC, respectively, as compared to control, with almost 90% (w/w) holocellulose recovery (Fig. 4). Earlier reports with sodium chlorite treatment for the preparation of cellulose rich residue also showed similar trends (Sevenson, Cheng, Jameel, & Kadla, 2005; Sun et al., 2004; Wi, Kim, Mahadevan, Yang, & Bae, 2009). The extensive delignification of lignocellulosic substrates with chlorite treatment was due to the generation of chlorine dioxide (ClO_2),

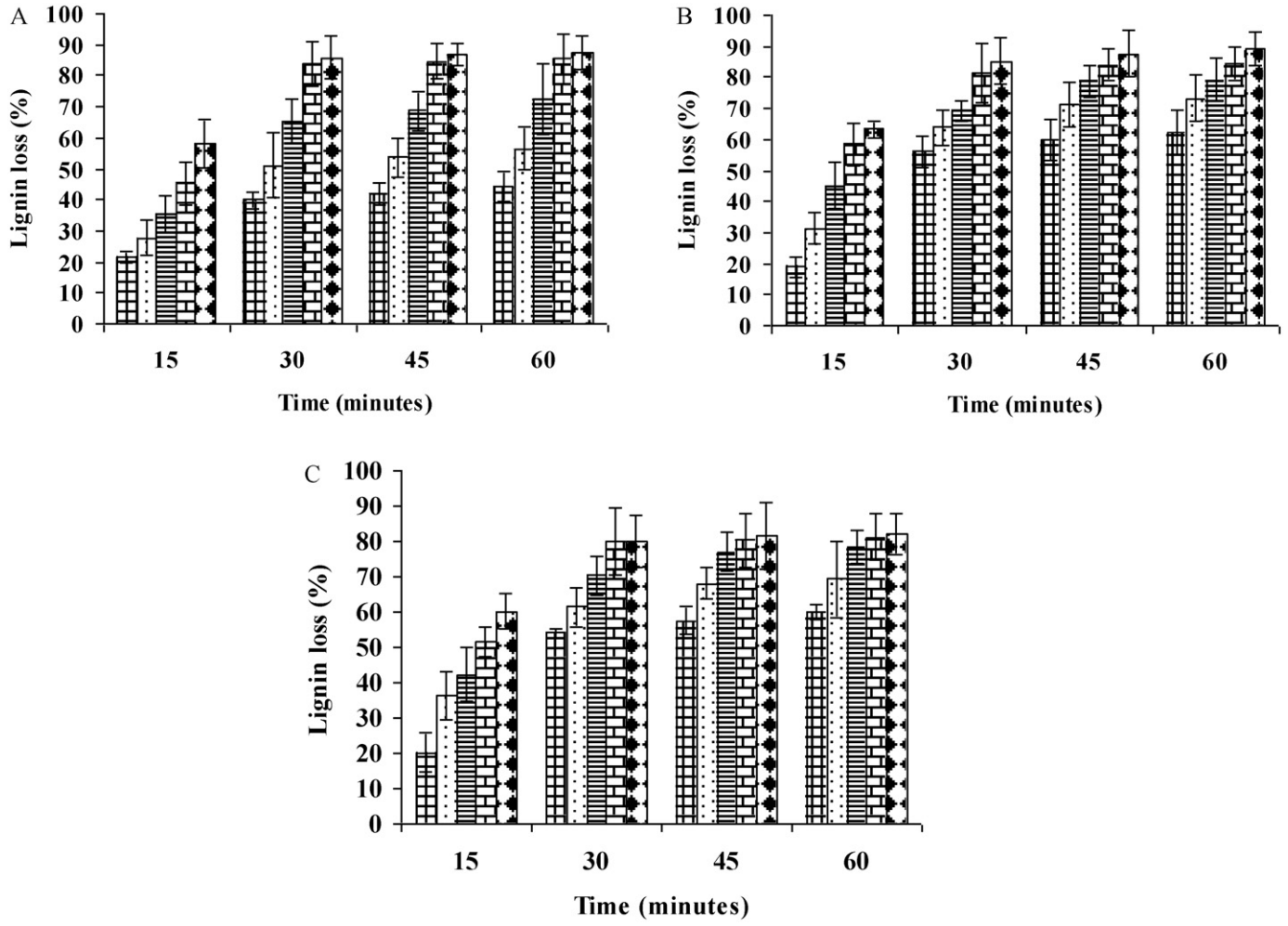

Fig. 3. Lignin removal from Corncob (A), *P. juliflora* (B) and *L. camara* (C) at different concentrations of Na-chlorite (▦ 1% Na-chlorite, ⬚ 2% Na-chlorite, ▤ 3% Na-chlorite, ▩ 4% Na-chlorite and ⊞ 5% Na-chlorite).

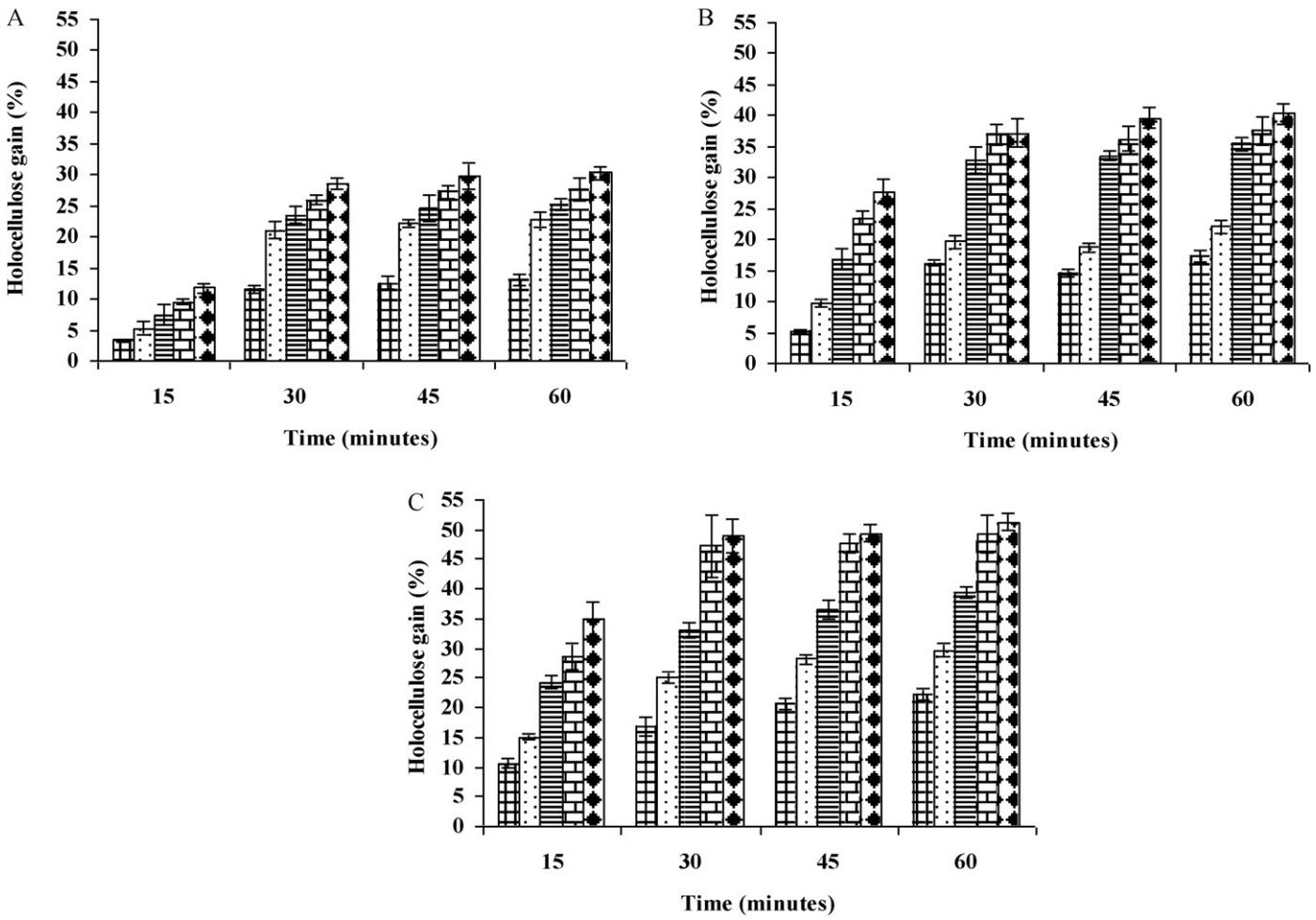

Fig. 4. Holocellulose enrichment in Corncob (A), *P. juliflora* (B) and *L. camara* (C), after treatment with different concentrations of Na-chlorite (▦ 1% Na-chlorite, ⬚ 2% Na-chlorite, ▤ 3% Na-chlorite, ▩ 4% Na-chlorite and ⊞ 5% Na-chlorite).

an oxidation product of chlorous acid ($HClO_2$) and hypochlorous acids (HOCl) produced during the thermal degradation of sodium chlorite, which degraded the lignin either by side-chain displacement or hydroxylation/de-alkylation reaction (Hamzeh, Mortha, & Lachenal, 2006).

6.3. Comparison of different pretreatments for improving enzymatic saccharification of cellulosic substrates

The enzymatic saccharification of all the three pretreated substrates showed an improved conversion of cellulose to glucose because of lignin and/or hemicellulose removal during pretreatments. The acid pretreated samples with minimum lignin removal showed lowest enzymatic hydrolysis (39.5–48.0% (w/w)), while, the alkali and sodium chlorite pretreated substrates caused higher enzymatic saccharification, which could be because of comparatively lower lignin content in the pretreated substrates (Table 3). Similar observations on pretreatment of lignocellulosic substrates followed by enzymatic hydrolysis have also been reported by Chen et al. (2009), Gupta et al. (2009) and Kuhad et al. (2010). The limited enzymatic saccharification in presence of higher lignin may be due to the high affinity of cellulases towards lignin, which resulted in unavailability of cellulase to cellulose moieties and led to poor enzymatic saccharification yields (Yang & Wyman, 2004).

Among different pretreated substrates evaluated here for the enzymatic hydrolysis, the chlorite pretreated substrates were observed to be more vulnerable to the enzymatic hydrolysis and resulted in maximum saccharification efficiency i.e., from 86.4% to 92.5% (w/w) (Table 3). The higher enzymatic saccharification in chlorite pretreated substrates may be attributed to the presence of higher holocellulose content (~90.0% (w/w)) with minimum amount of residual lignin (3.1–6.5% (w/w)). It has been shown by several workers that the delignification treatments not only remove the lignin but also act as a swelling agent, which in turn enhances the surface area of the substrate and make the substrate more amenable for enzymatic action (Gupta et al., 2009; Kuhad, Manchanda, & Singh, 1999; Kuhad et al., 2010). In contrast, the alkali treated substrates when subjected to enzymatic saccharification brought about approximately 51–55% (w/w) substrate hydrolysis (Table 3). As compared to chlorite pretreated substrates, the lower enzymatic saccharification efficiency in alkali treated substrates might be due to the lower delignification of the substrates by alkali treatment, which could be explained as the resistance of lignin removal due to strong lignin–cellulose attraction in the cell wall (Zhao et al., 2008). Our results of higher enzymatic saccharification with chlorite pretreated substrates strongly agree with the previous reports of achieving more than 65–67% saccharification in chlorite pretreated sugarcane bagasse (Adsul et al., 2005) and approximately 70% (w/w) hydrolysis from chlorite pretreated seaweed Ceylon moss (*Gelidium amansii*) (Wi et al., 2009).

7. Conclusion

Among different chemical pretreatments studied, the sodium chlorite pretreatment was found to be the most effective in lignin removal and led to the enrichment of the holocellulose content in treated substrates. This method offers the possibility of producing cellulosic material largely free from lignin, which eventually would be a good substrate for bioethanol production. However, there is a need to develop efficient biological delignification methods to make the process environmentally safe.

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