Prosopis juliflora—A Potential Problematic Weed for Lignocellulosic Ethanol Production

Vijayakumar Palled, M. Anantachar, M. Veerangouda, K.V. Prakash, C.T. Ramachandra, Nagaraj M. Naik, R.V. Beladadhi, K. Manjunatha and Beerge Ramesh

Abstract Lignocellulose-to-ethanol conversion is a promising technology to supplement starch based ethanol production. *Prosopis juliflora*, a problematic weed has been recently suggested as one of the alternative lignocellulosic biomass materials for cellulosic ethanol production. Sodium hydroxide (NaOH) pretreatment performed at 100, 120 and 140 °C in an autoclave at 15 psi, with combination of residence times (15, 30, and 60 min) and NaOH concentrations (1, 2 and 3%) indicated that almost 51% of solids were dissolved at 140 °C after 60 min pretreatment with 3% NaOH concentration. The corresponding maximum lignin reductions of 48.39, 67.01 and 74.79% were obtained at 100, 120 and 140 °C respectively for 1 h, 3.0% NaOH concentrations. Hydrolysis was carried out with CTec2® Cellulase enzyme at different loading levels (0, 15 and 30%) and the results showed that the maximum rate of saccharification (26.07 mg/g/h) was attained at 12 h for sample pretreated at 120 °C, 60 min, 2% NaOH loaded with 30% enzyme with a total maximum sugar yield of 583.9 mg/g and the carbohydrate conversion of 90.86%. Batch fermentations of enzymatic hydrolyzates carried out with 5 g/l *Saccharomyces cerevisiae* at 30 °C indicated that fermentation of 46.71 g sugar/l sample resulted in maximum ethanol of 21.84 g/l with a productivity of 0.91 g/l/h and an ethanol yield of 0.27 g/g dry biomass.

V. Palled (✉)
AICRP on Energy in Agriculture, Department of Farm Machinery and Power Engineering, University of Agricultural Sciences, CAE, Raichur 584104, Karnataka, India
e-mail: vs.palled@gmail.com

M. Anantachar · M. Veerangouda · K.V. Prakash · K. Manjunatha · B. Ramesh
Department of Farm Machinery and Power Engineering, CAE, Raichur, India

C.T. Ramachandra
Department of Processing and Food Engineering, CAE, Raichur, India

N.M. Naik
AICRP on Post Harvest Technology, Department of Processing and Food Engineering, CAE, Raichur, India

R.V. Beladadhi
Department of Soil Science and Agricultural Chemistry, Agriculture College of Engineering, Raichur, India

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Introduction

One of the greatest challenges for the growing society in this century is to meet the energy demand for transportation, heating, lighting and industrial processes, which have significant impact on the environment. These challenges demanding an urgent need to carry out research work to find out the viable alternative fuels. One of the promising alternative fuels to gasoline in the transport sector is bioethanol. It is not only produced on a renewable basis from various biomass sources, including sugarcane, corn, trees, grasses, yard wastes, agricultural residues, and forestry wastes, but it also causes less net greenhouse gas (GHG) emissions during combustion. Given this reality, nations around the world are investing in alternative sources of energy, including bioethanol. Although currently most of the ethanol produced from renewable resources comes from sugarcane and starchy grains, significant efforts are being made to produce ethanol from lignocellulosic biomass (almost 50% of all biomass in the biosphere) such as agriculture residues (Bothast and Saha 1997).

For ethanol production; feedstock availability, its variability and sustainability are the main issues to be addressed. Though the generation of biomass residues in the country from agriculture is considerably large, the actual availability of a major share of these residues for bioethanol production is questionable. Thus the selection of feedstock for a future technology for lignocellulosic ethanol itself needs careful planning. With a huge population to feed and limited land availability, the nation needs to develop bioethanol technologies which use the biomass feedstock that does not have food or feed value. One of the fast growing trees which have the potential to substitute food crops for bioethanol production is *Prosopis juliflora*. It is a tree species native to Northern Mexico and the Southern U.S. that survives droughts and thrives in sunny arid regions. The plant fixes its own nitrogen, requires no seeding, fertilization or irrigation, and grows on dry, nutrient-poor soils. *P. juliflora*, a perennial deciduous thorny shrub, the common vegetation of semi-arid region of Indian subcontinent and considered to be as a problematic weed has been suggested recently to use as one of the alternative lignocellulosic biomass materials for long and sustainable production of cellulosic ethanol (Hopkins 2007). Its very nature to drought tolerance, grazing and more pellatable feed for animals like sheep and goats, could be grown in heavy sandy and saline soils of dryland tracts and not much competence to animal feed made, it could be selected as low value substrate for ethanol production. There is an urgent need for development of appropriate processing technologies to convert *P. juliflora* into a usable end product like bioethanol. Successful use of lignocellulosic biomass for bioethanol production depends on five important factors: composition of the biomass, pretreatment

methods, efficient microorganisms, process integration and optimization of processing conditions.

Gupta et al. (2009) analyzed the proximate composition of *P. juliflora* wood and reported that it contained 66.20% total carbohydrate, 29.10% lignin, 2.68% moisture and 2.02% ash content. Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier et al. 2005). Alkali breaks the intercellular bonds cross-linking hemicellulose and other compounds (lignin and cellulose), resulting in increased porosity and internal surface of biomass, and decreased crystallinity and polymerization degree of carbohydrates (Sun and Cheng 2002). Xu et al. (2010) investigated sodium hydroxide pretreatment of switchgrass for ethanol production. At the best pretreatment conditions (50 °C, 12 h and 1.0% NaOH), the yield of total reducing sugars was 453.4 mg/g raw biomass which was 3.78 times that of untreated biomass. The maximum lignin reductions were 85.8% at 121 °C, 77.8% at 50 °C and 62.9% at 21 °C all of which obtained at the combinations of the longest residence times and the highest NaOH concentrations. Sharma et al. (2013) studied the potential of potassium hydroxide pretreatment of Switchgrass for fermentable sugar production. Performer Switchgrass was pretreated at KOH concentrations of 0.5–2% for varying treatment times of 6–48, 6–24, and 0.25–1 h at 21, 50, and 121 °C, respectively. They reported that the pretreatments resulted in the highest percent sugar retention of 99.26% at 0.5%, 21 °C, 12 h while delignification up to 55.4% was observed with 2% KOH, 121 °C, 1 h. The fermentation of both acid and enzymatic hydrolysates, containing 18.24 and 37.47 g/L sugars, with *Pichia stipitis* and *Saccharomyces cerevisiae* produced 7.13 and 18.52 g/L of ethanol with corresponding yield of 0.39 and 0.49 g/g, respectively (Gupta et al. 2009). Keeping the above points in view, the present paper investigated the potential of *P. juliflora*, a problematic weed for lignocellulosic ethanol production.

Materials and Methods

The stems of *P. juliflora* wood available in the University of Agricultural Sciences, Raichur campus, Karnataka, India, were harvested up to 6 in stubble and were dried in open sun for about a week. The wood were cut into small chips and oven dried at 70 °C in a forced air oven in cloth bags for 72 h. Then oven dried samples were ground to pass through a 2-mm sieve in a hammer mill (Crompton Greave Ltd., NDA 2 TOP) and stored at room temperature in zip-locked plastic bags for use in further studies.

The initial composition of *P. juliflora* was analyzed using Laboratory Analytical Procedures (LAP) adopted by National Renewable Energy Laboratory (NREL) for the measurement of total solids, acid-insoluble lignin (AIL), acid-soluble lignin (ASL) and ash content (Sluiter et al. 2005a, b; Sluiter et al. 2008). While the

structural carbohydrates (cellulose and hemicelluloses) represented by total reducing sugars of biomass was estimated by the 3,5-dinitrosalycylic acid (DNS) method (Ghose 1987; Miller 1959). In this experiment, sodium hydroxide (NaOH) pretreatment of *P. juliflora* woody substrate at different elevated temperatures ranging from 100 to 140 °C with various combinations of residence times and NaOH concentrations was explored. Pretreatment of *P. juliflora* woody substrate samples were performed at 100, 120 and 140 °C in an autoclave at 15 psi, with residence times of 15, 30, and 60 min each. All the temperature–time pretreatment combinations were performed with sodium hydroxide (NaOH) concentrations of 1, 2 and 3% (w/v) in a 3^3 factorial complete randomized block design.

Five grams of *P. juliflora* woody substrate samples was mixed with 50 ml of NaOH solution in 125 ml bottles using glass rods, and the bottles were sealed and kept in an autoclave. The pretreated samples were filtered through pre-weighed filter paper (*Whatman* filter paper No. 1) in vacuum flask using a vacuum pump. The bottles were rinsed with 50 ml DI water to recover the residual solids. All solids accumulated on the filter papers in the filtration set up were quantified by oven drying and considered in solid recovery calculations. Approximately 5 g of wet biomass was drawn from each pretreated sample and dried at 105 °C in conventional hot air oven for estimation of solid recovery. A similar amount was placed for vacuum drying at 40 °C in vacuum oven to obtain samples for estimation of acid-insoluble lignin to study the effect of pretreatment conditions on delignification of *P. juliflora*. Filtrate from the AIL acid hydrolysis was utilized to study the effect of sodium hydroxide (NaOH) pretreatment on reducing sugar content generated in each pretreated sample at various temperature–time combinations (100–140 °C and 15–60 min) using 1–3% NaOH concentrations.

Hydrolysis was carried out at 8% solid loading (of total volume 20 ml) to examine the effect of enzyme loading levels (0, 15 and 30%) on the untreated sample and selected pretreated samples for fermentable sugar production with a 3×4 factorial design. The CTec2® Cellulase enzyme complex sponsored by Novozymes, Beijing, China was used for conducting research on hydrolysis of samples for fermentable sugar production. The enzyme complex was reported to have an activity of 108.3–168.8 floating-point unit/ml (Eckard et al. 2012; Kodaganti 2011) and protein content 117–185.2 mg protein/ml (Eckard et al. 2012; Eylen et al. 2011).

To generate enough biomass for hydrolysis at the various conditions, pretreatments were performed in six replicates and two replicates each were combined randomly and mixed thoroughly to generate three larger replicates. This was done to avoid the impact of any scale changes during pretreatment of larger amounts. Untreated samples with equivalent enzyme loading were also hydrolyzed as control. Pretreated and untreated samples with no enzyme were prepared to determine the effect of soaking. Hydrolysis was performed for 72 h at 50 °C in a shaking water bath (KEMI Make) at 150 rpm. The Laboratory Analytical Procedure (LAP) adopted by National Renewable Energy Laboratory (NREL) for enzymatic saccharification of lignocellulosic biomass (Selig et al. 2008) was followed for conducting enzymatic hydrolysis.

The samples were withdrawn at regular intervals of 12 h and centrifuged at 4,000 rpm for 10 min in a high speed refrigerated centrifuge (KEMI Make), and the filtrate was collected for sugar analysis. Upon termination of hydrolysis, the samples tubes were kept in high speed refrigerated centrifuge for centrifugation. The filtrate was collected and stored separately at 4 °C in a freezer for estimation of total sugar generated. The fermentable sugars generated during the hydrolysis were estimated by 3,5 dinitrosalycylic (DNS) acid method (Ghose 1987; Miller 1959).

The saccharification rate at regular intervals was calculated using the formula;

$$\text{Saccharification rate}\,(\text{mg/g/h}) = \frac{\text{Sugar yield}\,(\text{mg/g dry biomass})}{\text{Saccharification time}\,(\text{h})} \quad (1)$$

Further, carbohydrate conversion was calculated using the following formula (Gupta et al. 2009);

$$\text{Carbohydrate conversion}(\%) = \frac{\text{Reducing sugar concentration obtained}}{\text{Potential sugar concentration in the substrate}} \times 100 \quad (2)$$

Based on maximum total sugar yield obtained and highest carbohydrate conversion, the sample pretreated at optimal conditions (120 °C, 60 min, 2% NaOH) and hydrolyzed with 30% enzyme loading was selected for subsequent fermentation for bioethanol production. Batch fermentations of enzymatic hydrolyzates were carried out in 500 ml Erlenmeyer flask incubated with 5 g/l *S. cerevisiae* at 30 °C (Thuesombat et al. 2007). The yeast culture was obtained from the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur. The samples were withdrawn at regular intervals of 6 h and centrifuged at 10,000 rpm for 15 min at 4 °C in a high speed refrigerated centrifuge, and the filtrate was collected and saved for subsequent ethanol estimation. Upon termination of fermentation for 36 h, the samples tubes were centrifuged at 10,000 rpm for 15 min and the filtrate was collected for estimation of total ethanol yield. Ethanol concentration present in the fermented sample was estimated by titration method. Simultaneously, the samples were withdrawn at regular intervals of 6 h and analyzed for sugars (Gupta et al. 2009). All treatments in this study were conducted in triplicate. Design expert-7 Software was used for data analysis at 99% confidence level.

Results and Discussion

The results pertaining to the composition of biomass, effect of pretreatment, enzymatic hydrolysis of selected samples and fermentation of hydrolyzate are presented and discussed in the following sections.

Composition of Biomass

The initial composition of *P. juliflora* was analyzed and the results are presented in Table 1. The total solids content present in *P. juliflora* woody substrate selected for the study was 98.80%. This is in agreement with the moisture content (2.68%) reported by Gupta et al. (2009) which represents total solids. The average acid-insoluble lignin (AIL) of *P. juliflora* was observed to be 30.18% which is in close agreement with the lignin present in *P. juliflora* (31%) reported by Rajput and Tewari (1986). Similar results (29.10%) in *P. juliflora* were reported by Gupta et al. (2009) and 20–32% in dry wood (Alriksson 2006). The total carbohydrate portion (cellulose and hemicelluloses) represented by total reducing sugar content of *P. juliflora* was reported to be 64.26%. This is in close agreement with the findings of Gupta et al. (2009) who reported that *P. juliflora* wood contained total carbohydrate of 66.20%. Also, the total carbohydrate of *P. juliflora* pod flour (69.20%) reported by Choge et al. (2007) was in agreement with present findings. Ash content present in *P. juliflora* sample was 2.01% which is fairly close in agreement with the ash content of *P. juliflora* (2.02%) reported by Gupta et al. (2009).

Effect of Alkaline Pretreatment

The results pertaining to the effect of sodium hydroxide (NaOH) pretreatment at various temperature–time combinations (100–140 °C and 15–60 min) using different concentrations ranging from 1 to 3% NaOH on solids recovered after each pretreatment are presented in Table 2.

Solid recoveries after each pretreatment ranged between 49.77 and 91.43%. It was observed that the solids recovered were maximum (91.43%) in the sample pretreated at 100 °C, 15 min combination with 1% NaOH, whereas, a minimum of 49.77% was observed in the sample pretreated with 3% NaOH at 140 °C, 60 min. As the severity of pretreatment increased in terms of higher temperature, treatment time and NaOH concentration; the solids recovered in the sample decreased. The main effect of both treatment time and NaOH concentration had significant ($p < 0.01$) impact on solid recovery. The interaction effect between temperature and concentration had a significant ($p < 0.01$) impact on loss of solids and also, the combined effect of all the three factors had significant effect on solids recovery.

Table 1 Composition of *Prosopis juliflora*

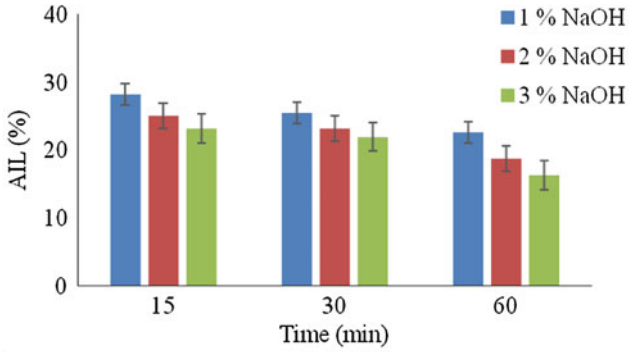
S. No.	Component	Dry weight (%)
1	Total solids	98.80 ± 0.71
2	Acid-insoluble lignin	30.18 ± 0.33
3	Acid-soluble lignin	1.67 ± 0.14
4	Carbohydrates (total sugars)	64.26 ± 0.53
5	Ash	2.01 ± 0.23

Table 2 Effect of sodium hydroxide (NaOH) pretreatment on solids recovery of *Prosopis juliflora*

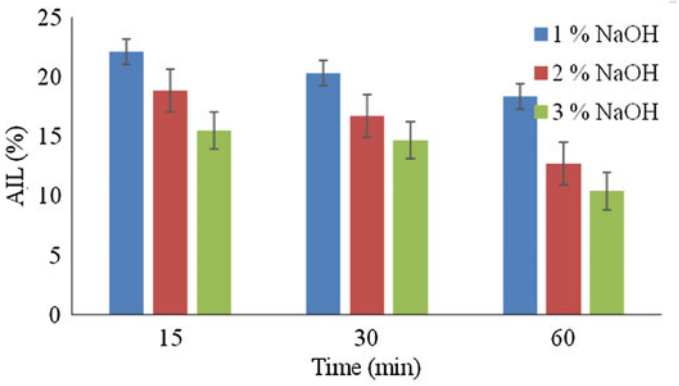
		Solids recovery (%)		
Temperature (°C)	Time (T-min)	NaOH concentration (%)		
		1	2	3
	15	91.43 ± 0.36	87.78 ± 0.59	79.88 ± 0.62
100	30	87.41 ± 0.51	83.23 ± 0.70	75.42 ± 0.38
	60	74.34 ± 1.21	68.85 ± 0.35	64.31 ± 1.84
	15	78.50 ± 0.72	73.90 ± 0.69	66.98 ± 0.24
120	30	74.54 ± 0.69	70.59 ± 0.86	64.52 ± 0.70
	60	72.21 ± 0.49	66.65 ± 1.06	62.94 ± 0.32
	15	74.40 ± 1.18	70.08 ± 0.28	63.88 ± 0.81
140	30	70.47 ± 0.42	64.76 ± 0.45	57.59 ± 0.17
	60	63.16 ± 0.53	58.99 ± 0.85	49.77 ± 0.56

KOH pretreatment of switchgrass followed a similar trend of increased solid loss with increasing intensity of treatments in terms of temperature and high concentration (Xu et al. 2010). Also, the results are in agreement with the findings of Sharma et al. (2013) for KOH pretreatment of switchgrass.

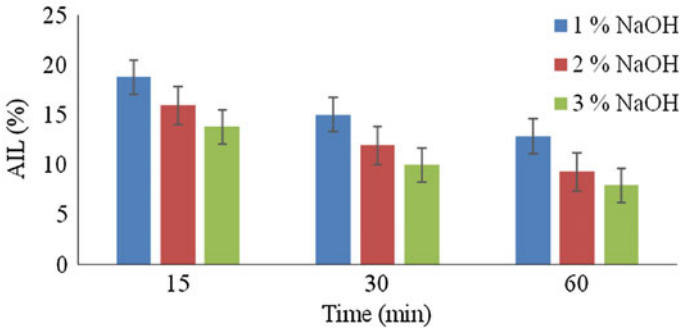
The results pertaining to the effect of sodium hydroxide (NaOH) pretreatment on acid-insoluble lignin (AIL) reduction using different concentrations ranging from 1 to 3% NaOH at various temperature–time combinations (100–140 °C and 15–60 min) are shown in Fig. 1a–c. At 100 °C, a maximum AIL of 28.25% was observed in the sample pretreated with 1% NaOH concentration for 15 min, while it was minimum (16.30%) in the sample pretreated for 60 min with 3% NaOH concentration. As the NaOH concentration and pretreatment time increased, the acid-insoluble lignin decreased (Fig. 1a). Acid-insoluble lignin in the samples pretreated at 120 °C ranged between 10.42 and 22.10% (Fig. 1b). As the severity of pretreatment time and NaOH concentration was more, the acid-insoluble lignin present in the sample was less. A maximum of 22.10% was observed in the sample pretreated with 1% NaOH concentration for 15 min, while it was minimum (10.42%) in the sample pretreated for 60 min with 3% NaOH concentration. Pretreatment with 2% NaOH concentration for 60 min resulted in 59.72% delignification at 120 °C which is in close agreement with the results of Silverstein et al. (2007) that NaOH pretreatment resulted in the highest level of delignification (65.63% at 2% NaOH, 90 min, 121 °C). At 140 °C, AIL after pretreatment ranged between 7.96 and 18.78% (Fig. 1c). It was observed that the maximum (18.78%) AIL was observed in the sample pretreated with 1% NaOH concentration for 15 min, while a minimum of 7.96% was recorded in the sample pretreated for 60 min with 3% NaOH concentration. About 10.54–74.79% of the original acid-insoluble lignin was lost during various combinations of pretreatments depending on severity. The corresponding maximum lignin reductions of 48.39, 67.01 and 74.79% were obtained respectively at 100, 120 and 140 °C, for 1 h,



(a) at 100 °C



(b) at 120 °C



(c) at 140 °C

Fig. 1 Acid-insoluble lignin (AIL) of *P. juliflora* pretreated with 1.0–3.0% NaOH at different temperature–time combinations

Table 3 Effect of sodium hydroxide (NaOH) pretreatment on release of reducing sugar content of *Prosopis juliflora*

		Reducing sugar content (mg/g dry biomass)		
Temperature (°C)	Time (min)	NaOH concentration (%)		
		1	2	3
	15	339.40 ± 7.35	361.41 ± 7.46	349.99 ± 5.80
100	30	353.11 ± 1.75	392.73 ± 3.36	371.80 ± 4.48
	60	385.17 ± 7.20	394.19 ± 2.83	390.65 ± 2.94
	15	353.57 ± 8.37	378.72 ± 12.31	372.15 ± 12.62
120	30	361.93 ± 2.44	406.40 ± 4.21	392.38 ± 6.37
	60	398.60 ± 4.51	418.29 ± 3.22	389.38 ± 8.22
	15	356.33 ± 2.54	354.29 ± 4.35	348.03 ± 1.84
140	30	345.04 ± 3.82	337.03 ± 1.83	317.33 ± 1.46
	60	336.87 ± 9.14	324.77 ± 6.59	295.73 ± 3.35

3.0% NaOH concentrations. Maximum lignin reductions at different temperatures were all obtained at the combinations of highest NaOH concentrations and longest treatment times, which indicated a close relationship between pretreatment severity and lignin reduction. The similar results were reported by Xu et al. (2010), Sharma et al. (2013).

The results pertaining to the reduction in AIL were statistically analyzed and it was observed that the main effect of both treatment time and NaOH concentration had significant ($p < 0.01$) impact on lignin reduction at all the three temperatures. The interaction effect between temperature and concentration also had a significant ($p < 0.01$) impact on delignification. However, the combined effect of all the three factors on acid-insoluble lignin was not significant at 1% level of confidence. Statistical analysis indicated that at 120 and 140 °C, residence time had a significant impact ($p < 0.01$) on lignin reduction at all three NaOH concentrations. However, at 100 °C, residence time had significant impact ($p < 0.01$) on delignification of sample at higher concentrations.

On an average, reducing sugar (RS) content generated ranged between 295.73 and 418.29 mg/g dry biomass (Table 3) after pretreatment at different temperature–time combinations using various concentrations of NaOH.

It was observed that when the sample was pretreated with different concentrations of NaOH, at 100 and 120 °C, the release in sugar increased with increase in acid concentration up to 2.0% (v/v) NaOH and it declined thereafter. While at 140 °C, the acid concentration beyond 1.0% (v/v) resulted in continuous decrease in release of sugar as the pretreatment increased. The maximum sugars of 418.29 mg/g were released, when the sample was pretreated with 2.0% NaOH concentration at 120 °C for 60 min. Whereas, it was minimum (295.73%) in the sample pretreated at 140 °C, 60 min combination with 3% NaOH (Table 3). The highest carbohydrate retention of 65.09% was observed in the sample pretreated at 120 °C with 2% NaOH and 60 min. These results were more pronounced than the

sugars retained (60.6%) in KOH-pretreated samples of switchgrass (Sharma et al. 2013) and lesser than the sugars retained (74.8%) in NaOH pretreated switchgrass (Xu et al. 2010).

The effect of all the factors on the reducing sugar content was statistically analyzed. The main effect of temperature, time and concentration was significant ($p < 0.01$) on reducing sugar released. Also, their interaction effect and combined effect of all the factors were significant ($p < 0.01$) on carbohydrate availability. It was observed that 34.90–53.98% of the original untreated reducing sugar content was lost during various combinations of pretreatments depending on severity.

Selection of Optimal Pretreatment Conditions

The upper and lower limits of all the factors and also, the minimum and maximum values of solids recovered, acid-insoluble and soluble lignin and reducing sugars generated were given as input to the statistical software. Selections were based on maximum solids recovery, minimum AIL, i.e., maximum delignification and maximum carbohydrate (reducing sugar) retention.

The desirability index of solids recovery, acid-insoluble lignin, acid-soluble lignin and reducing sugar content retention were found to be 0.9935, 0.7348, 0.4009 and 0.9719, respectively for pretreatment combination of 2.0% NaOH, 60 min at 120 °C which has highest combined desirability of 0.7303 (Fig. 2). Hence, this pretreatment combination of 120 °C, 60 min, 2.0% NaOH was chosen as the optimum for further enzymatic hydrolysis for fermentable sugar production.

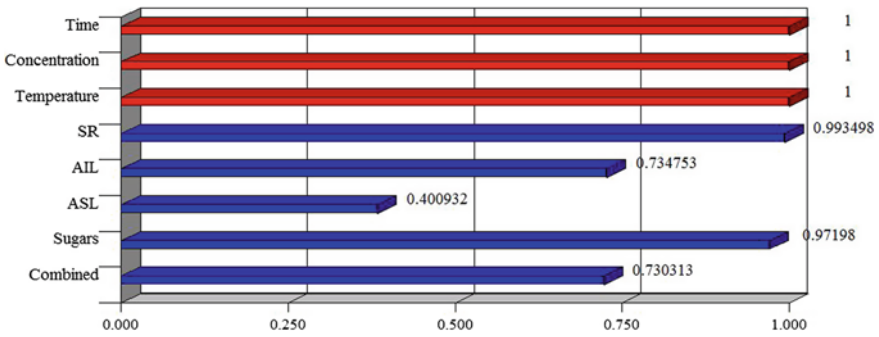


Fig. 2 Desirability index of solids recovery, lignin reduction and reducing sugar retention for optimal pretreatment condition (120 °C, 60 min, 2% NaOH)

Enzymatic Hydrolysis

The results pertaining to total sugar yield in the hydrolyzate of the untreated and selected pretreated samples with various enzyme loadings levels of 0, 15 and 30% are presented in Table 4.

The total sugar yield in the hydrolyzate of untreated and pretreated samples ranged from 26.4 to 583.9 mg/g biomass. A maximum total sugar yield of 583.9 mg/g biomass was recorded in the hydrolyzate obtained from the sample pretreated at optimal conditions (120 °C for 60 min with 2% NaOH) when loaded with 30% enzyme. Whereas, it was minimum (26.4 mg/g biomass) in the hydrolyzate of the sample pretreated at 140 °C for 30 min with 1% NaOH without enzyme loading (Table 4). The total sugar yield increased when the enzyme loading level increased for all the samples hydrolyzed. The maximum sugar yield obtained (583.9 mg/g biomass) at optimal pretreatment conditions of 120 °C for 60 min with 2% NaOH loaded with 30% enzyme in this study were in close agreement with maximum sugar yield of 582.4 mg/g biomass obtained from hydrolyzate of switchgrass pretreated at optimal conditions of 0.5% KOH, 12 h at 21 °C with same (CTec 2) enzyme loading level of 30% (Sharma et al. 2013). Similar findings were reported by Gupta et al. (2009) for *P. juliflora* with a total sugar yield of 586.6 mg/g biomass obtained from enzymatic saccharification of sulphuric acid pretreated delignified biomass at optimal conditions of 120 °C for 60 min with 3% H_2SO_4 and loaded with a mixture of 3.0 U of filter paper cellulase (FPase) and 9.0 U of β-glucosidase. The statistical analysis of total sugar yield obtained from hydrolysis of selected samples with different enzyme loadings indicated that there was a significant difference between the sugar yields obtained from the samples hydrolyzed. The effect of enzyme loading was significant ($p < 0.01$) on total sugar yield obtained from all the samples hydrolyzed at 1% level of significance.

The per cent carbohydrate conversion of different samples hydrolyzed with various enzyme loading levels ranged from 4.11 to 90.86. As the enzyme loading level increased, the per cent carbohydrate conversion also increased for both untreated and pretreated samples (Fig. 3).

The per cent carbohydrate conversion increased drastically with 15% enzyme loading and thereafter increased gradually with 30% enzyme loading for all the

Table 4 Total sugar yields of untreated and pretreated samples hydrolyzed with different enzyme loadings

Pretreatment	Total sugar yield (mg/g biomass)		
	Enzyme loading, % (g enzyme protein/g biomass)		
	0	15	30
Untreated	46.8 ± 2.4	233.2 ± 7.6	373.5 ± 8.7
120 °C, 60 min, 2% NaOH	33.2 ± 1.8	449.7 ± 8.2	583.9 ± 7.3
100 °C, 60 min, 2% NaOH	28.3 ± 2.1	378.3 ± 6.9	541.5 ± 4.3
140 °C, 30 min, 1% NaOH	26.4 ± 1.7	361.6 ± 5.4	514.4 ± 3.7

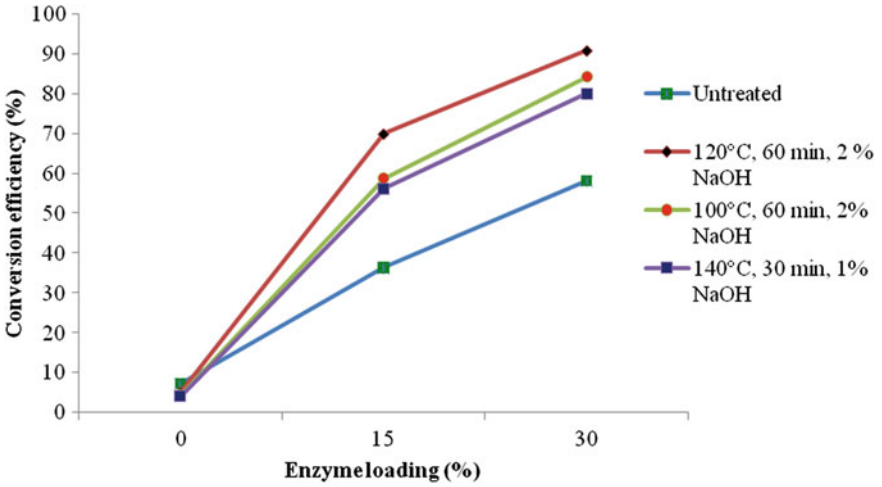


Fig. 3 Carbohydrate conversion of untreated and pretreated samples hydrolyzed with different enzyme loadings

samples. More than 70% of carbohydrates were converted with 15% enzyme loading. The maximum carbohydrate conversion of 90.86% was recorded for the sample pretreated at optimal conditions (120 °C, 60 min, 2% NaOH) which was loaded with 30% enzyme. Whereas the sample pretreated at 140 °C for 30 min with 1% NaOH recorded minimum carbohydrate conversion (4.11%) without enzyme loading. The maximum carbohydrate conversion (91.8%) was reported by Sharma et al. (2013) for KOH-pretreated switchgrass at 30% enzyme loading with Cellic$^{(R)}$ CTec2 cellulase enzyme (Novozymes, North America, Franklinton) at optimal pretreatment conditions were in close agreement with the maximum carbohydrate conversion (90.86%) obtained in this study for NaOH pretreated *P. juliflora* at 30% enzyme loading with CTec2 cellulase enzyme (Novozymes, Beizing, China). The statistical analysis of per cent carbohydrate conversion achieved from hydrolysis of selected samples with different enzyme loadings indicated that there was a significant difference between the per cent carbohydrate conversion obtained from the samples hydrolyzed. The effect of enzyme loading was significant ($p < 0.01$) on per cent carbohydrate conversion obtained from all the samples hydrolyzed at 1% level of significance.

However, the untreated sample without enzyme loading recorded higher carbohydrate conversion than all the pretreated samples with 0% enzyme loading. Similar trends were reported by Sharma et al. (2013). This may be due to the fact that only soaking effect could not convert all the carbohydrates of pretreated samples without enzyme loading which may be attributed to the loss of carbohydrates incurred during the pretreatment conditions. Based on maximum total sugar yield generated and highest carbohydrate conversion achieved, the sample pretreated at optimal conditions (120 °C, 60 min, 2% NaOH) and hydrolyzed with

30% enzyme loading was selected as optimum for subsequent fermentation for bioethanol production.

Fermentation Profile of Enzymatic Hydrolysate

The results pertaining to the batch fermentation of enzymatic hydrolyzates obtained from 30% enzyme loading on *P. juliflora* pretreated at 120 °C for 60 min with 2% NaOH concentration with *S. cerevisiae* are presented in Figs. 4 and 5.

As the fermentation time prolonged, ethanol yield increased regularly up to 24 h (21.84 g/l) and thereafter it declined. However, a regular decrease in sugars available in the sample was observed as the fermentation time increased. Further, it was observed that the ethanol productivity ranged from 0.39 to 1.14 g/l/h. As the fermentation time increased, drastic increase in ethanol productivity was observed up to 6 h, then it increased regularly up to 12 h and thereafter decrease in ethanol productivity was observed up to 36 h (Fig. 4).

The ethanol yield per g of fermentable sugars increased regularly up to 24 h (0.47 g/g) and thereafter it declined, as the fermentation time prolonged. Similar trend was observed for the ethanol yield per g of dry biomass (Fig. 5). Fermentation of enzymatic hydrolyzate containing 46.71 g sugar/l sample using *S. cerevisiae*, gave maximum ethanol of 21.84 g/l with yield (0.47 g/g sugar) and productivity (0.91 g/l/h) after 24 h of fermentation. The results are in close agreement with the

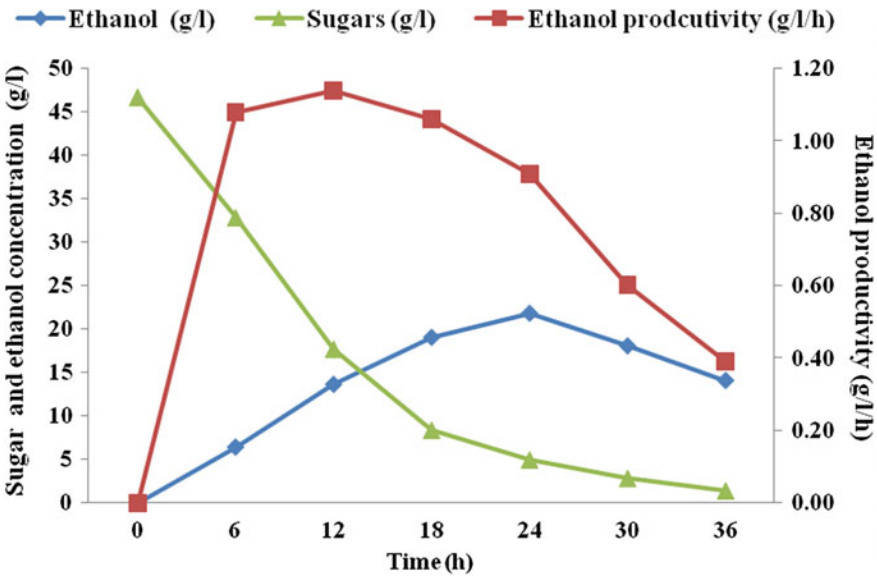


Fig. 4 Ethanol yield, sugar concentration and ethanol productivity during fermentation process

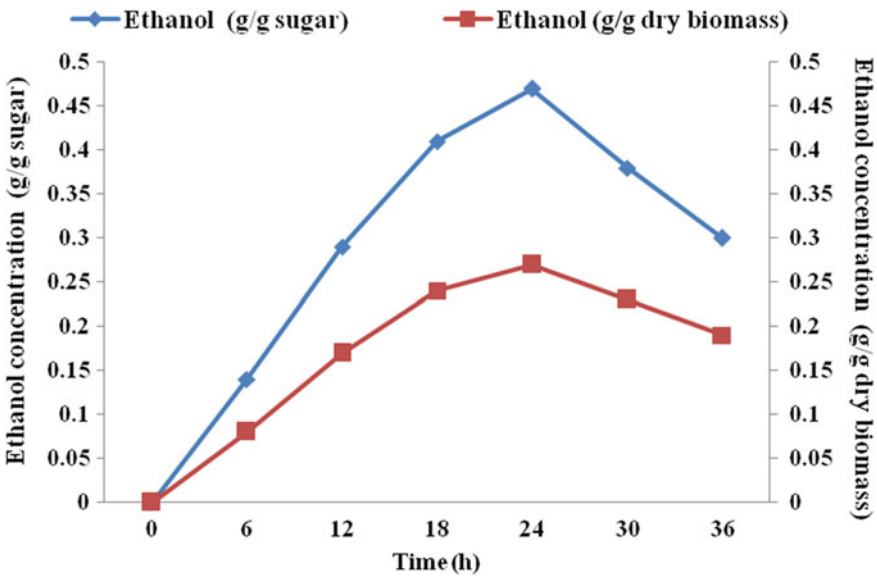


Fig. 5 Ethanol yield (g/g sugar) and ethanol yield (g/g dry biomass) during fermentation process

findings of experiment conducted by Gupta et al. (2009) on separate hydrolysis and fermentation (SHF) of *P. juliflora* for the production of cellulosic ethanol by *S. cerevisiae* with maximum ethanol (18.52 g/L) with yield (0.49 g/g sugar) and productivity (1.16 g/l/h) after 16 h.

Conclusions

Pretreatment of ground *P. juliflora* with NaOH resulted in promising lignin reduction of over 74% at 140 °C for 1 h with 3% NaOH concentration. Maximum lignin reductions at different temperatures were all obtained at the combinations of highest NaOH concentrations and longest treatment times, which indicated a close relationship between pretreatment severity and lignin reduction. Since, increasing pretreatment intensity does not necessarily lead to higher sugar recovery due to greater biomass solubilisation and lesser solids recovery, lignin reduction, though important, alone may not be an appropriate indicator for overall pretreatment effectiveness. The pretreatment combination of 120 °C, 60 min, 2.0% NaOH was chosen as the optimum for further enzymatic hydrolysis for fermentable sugar production as it had highest combined desirability. As the enzyme loading level increased, the per cent total sugar yield also increased for both untreated and pretreated samples. More than 70% of fermentable sugars were generated with 15%

enzyme loading. The sugar yield was maximum (583.9 mg/g biomass) with 30% enzyme loading at the end of 72 h of hydrolysis for the sample pretreated at optimal conditions (120 °C, 60 min, 2% NaOH) with a maximum carbohydrate conversion of 90.86%. A total ethanol of 270 kg per ton of dry *P. juliflora* could be produced by the fermentation of cellulosic hydrolyzates obtained from 30% enzyme loading on *P. juliflora* pretreated at 120 °C for 60 min with 2% NaOH concentration using *S. cerevisiae*.

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