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Integrated Approach for Bioethanol and Paper Production using *Populus deltoides* Wood Biomass: An Experimental Study

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Abstract

Lignocellulosic materials contain two major sugar macromolecules, cellulose and hemicellulose, and polyphenolic lignin. During pulping, lignin and hemicellulose are broken down into smaller molecules such as organic acids, and removed in the black liquor, leaving cellulose fibers for papermaking. Lignocellulose consists of approximately 28-35% hemicelluloses, which are lost during the pulping process in black liquor and are an important source of sugars that can be used to produce bioethanol as a liquid fuel. The hemicellulosic sugars from the Populus deltoides (poplar) lignocellulosic biomass were partially extracted keeping in mind that it does not affect the properties of paper beyond acceptable limits, further converting these extracted sugars by fermentation to bioethanol, followed by pulping the residual biomass and papermaking and determining pulping and papermaking properties. With the increasing demand for lignocellulosic biomass by various industries, an integrated biorefinery approach for maximum utilization of its chemical components with minimum degradation is necessary in the future. The maximum bioethanol yield was found to be 3.58 g/L. On manufactured paper sheets, the mechanical properties tensile index and tear index of pre-extracted biomass were observed as 19.23 Nm/g and 3.5 mNm²/g and slightly lower against the control 21.34 Nm/g and 4.0 mNm²/g. The main objective of the present study is to recover reducing sugars before the pulping process for bioethanol production and to further utilize the remaining residue for papermaking without disturbing its fiber integrity.



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Statement of Sustainability: This work emphasized an integrated approach for bioethanol and paper production using *P. deltoides* wood biomass that aligns with sustainable practices of bioenergy generation and spent resource utilization. The proposed integrated approach strives to achieve a circular economy model where waste from the bioethanol production process can be repurposed for paper production, ensuring optimal resource utilization, and minimizing waste generation. This research endeavors to contribute to a more sustainable and environmentally conscious future by promoting the efficient use of resources and reducing the carbon footprint of the bioethanol and paper industries.

1. Introduction

Lignocellulosic materials contain three main components: cellulose fibers (desired for papermaking and other applications), lignin (a three-dimensional polymer that binds the cellulose fibers together), and hemicellulose (shorterbranched carbohydrate polymers). In pulping and papermaking, the goal is to break down the bulk structure of the fiber biomass, whether chips, stalks, or other plant parts, into its constituent fibers (Tarasov et al., 2018; Malik et al., 2020). During pulping, lignin and hemicellulose are broken down into smaller molecules as organic acids and removed in the black liquor, leaving cellulose fibers for papermaking (Woiciechowski et al., 2020). Hemicellulose is an important source of sugar and can be used to produce bioethanol as a liquid fuel. Lignocellulose consists of approximately 28–35% hemicelluloses, which are lost in the black liquor during the pulping process (Oriez et al., 2020). The hemicellulosic sugars from the lignocellulosic biomass of *P. deltoides* (poplar) have been partially extracted, considering that it does not affect the properties of paper beyond acceptable limits, further converting these extracted sugars through fermentation to bioethanol, followed by pulping the residual biomass and papermaking and determining the pulping and papermaking properties (Sannigrahi et al., 2010; Saeed et al., 2012; Yuan et al., 2017; Woiciechowski et al., 2020).

India is a fiber deficit country, and a significant amount of fiber material is imported by the paper industry to meet its domestic demand. On the other hand, the demand for lignocellulosic biomass by the paper industry and by other emerging bioethanol and briquetting industries is increasing (Purohit and Dhar 2018). Its availability is likely to continue to decline. An integrated biorefinery approach for maximum utilization of chemical components of lignocellulosic biomass with minimum degradation and generation of waste byproducts causing environmental problems is a requirement for the future (Fatma et al., 2018). İnan and Özçimen (2019) have also investigated the production of biodiesel from algal biomass. They reported the highest yield under the TOE conditions of 2N H₂SO₄ and 0.15N KOH solutions, with a pretreatment temperature of 100 °C and a pretreatment time of 60 min. Similarly, Antczak et al. (2019), evaluated the delignification process by the Kraft method, which was carried out with 19% and 26% of active alkali (NaOH and Na₂S). The sugars obtained (xylose and glucose) were analyzed by high-performance liquid chromatography. The pulp obtained from the wood of fast-growing poplar species (P. deltoides x maximowiczii and P. trichocarpa Torr. & A. Gray ex Hook) was used as feedstock, but the enzymatic saccharification rate was low, so bioethanol production from lignocellulosic biomass was found to be very important for the selection of the suitable inorganic salt (Sim et al., 2020). Environmentally friendly bioenergy requires renewable energy sources to reduce dependence on fossil fuels. As a renewable energy source, first-generation bioethanol has been produced from corn. However, the production of such a biofuel increases the price of corn-based food, leading to serious debates about food versus fuel. Financial incentives would motivate first-generation bioethanol producers to switch to second-generation bioethanol production.

Two main processes are involved in bioethanol production from lignocellulosic biomass: hydrolysis of the total carbohydrate fraction of lignocellulosic biomass to produce reducing sugars (mainly glucose and xylose) and subsequent fermentation of the sugars to ethanol (Dubey et al., 2009; Dubey et al., 2015). *Saccharomyces cerevisiae* is the most used microorganism for ethanol production from sugars. Although it has a high ethanol tolerance, high yields, and fermentation rates, it is unable to ferment xylose sugars, the second most abundant sugar in nature (Kotter and Ciriacy, 1993). The xylose fraction represents 10–40% of the total carbohydrate of lignocellulosic biomass (Ladisch et al., 1983). A recent study shows that the yeast *Pichia stipitis* has the potential to ferment glucose and xylose with high fermentation efficiency and produce good ethanol yield (Agbogbo et al., 2008; Esmaeili et al., 2020).

Therefore, the present study focused on the pre-extraction of hemicellulosic sugars from lignocellulosic biomass *P*. *deltoides* and its end use in paper production.

2. Materials and Methods

2.1. Raw Materials

For the present study, the raw material under study i.e., poplar (*P. deltoides*) was procured from Forest Range office, FRI, Dehradun (30.3165° N and 78.0322° E) India. Yeast extract powder, peptone, and agar powder were procured from Himedia. Besides this, all standard chemicals were also procured from SRL Biochemical of high purity and analytical grade. All glassware used was borosilicate class glass material. Similarly, all standard plastic ware was also procured from Tarsons, India.

2.2. Proximate and Chemical Analyses of Raw Materials

For the experiment, the mature poplar logs were cut and converted into chips by a chipper from a woodchipper. Particle size optimization was carried out by converting the poplar chips into a 40–60 mesh size powdery mass in a Willey Mill (Christy and Norris LTD, Chelmsford England). The sieved mass was subjected to proximate chemical analysis and all experiments were carried out in triplicate. Samples were analyzed for moisture content (T 264 cm-9), ash content (T 211 om-02), hot and cold-water solubility (T 207 cm-99), N/10 NaOH solubility (T 212 om-02), alcohol-benzene solubility (T 204 cm-97), holocellulose (Useful method-249-75), and acid-insoluble lignin (T 222 om-02). All procedures were performed in triplicate according to the Technical Association of the Pulp and Paper Industry (TAPPI) standard method T 257 cm-02.

2.3. Pre-treatment of Poplar Chips

The poplar chips were pretreated in different media with acid, distilled water, and alkali for the extraction of reducing sugars. Poplar chips were soaked in a sulfuric acid solution of variable pH 4, 5, and 6; distilled water at pH 7; alkali solution with sodium hydroxide concentrations of 2% and 4%. A bath ratio of 1:6 on a biomass dry weight basis was maintained at room temperature for 24 h during the pretreatment. This was followed by autoclaving the soaked biomass with solutions in an oil bath digester at varying temperatures of 100, 125, and 150 °C for 1 h and at 150 °C for 2 h. After autoclaving, pretreated samples were filtered through nylon mesh, and the volume of extracted liquor was determined. The total reducing sugar concentration in the pre-extracted liquor was determined in mg/mL and the total extracted sugar content w/w % was estimated in terms of biomass on a dry weight basis.

2.4. Depolymerization of Pre-extracted Liquor

The pre-extracted liquor was depolymerized under reflux with sulfuric acid to a final solution of 1.2N at 100 °C for 150 min. The total reducing sugar concentration in the pre-extracted depolymerized liquor was determined in mg/mL, and the total extracted sugar content w/w % was estimated to the biomass on a dry weight basis. The phenolic content in the pre-extracted depolymerized liquor was also determined using a UV/Vis spectrophotometer (Shimadzu, Japan).

2.5. Detoxification of Hydrolyzate

The pre-extracted liquor after depolymerization with maximum TRS content was subjected to detoxification with calcium hydroxide slurry (20%) at 60 °C for 30 min to reach pH 7, centrifuged, and the supernatant was treated with 2% (w/v) activated charcoal under continuous stirring separately at ambient temperature for 1 h. The detoxified preextracted depolymerized liquor was analyzed for phenolic content using UV/Vis spectrophotometer (Dubey et al., 2009). Similarly, different types of detoxification viz. physical (membrane-mediated purification, evaporation), chemical (overliming, Ca(OH)₂, neutralization, ion exchange resins, activated carbon column and extraction with C₄H₈O₂), biological (microbial and enzymatic) and in situ microbial detoxification were studied to mitigate fermentation inhibitors (Chandel et al. 2012).

2.6. Reduction in Phenolics

The reduction in phenolic content of the pre-extracted depolymerized liquor obtained after detoxification was compared with the initial phenolic content of the hydrolysate and analyzed by the spectrophotometric method (Dubey et al., 2009). On the other hand, the phenolic compounds decreased sharply by direct observation of absorbance at 280 nm for hydrolysate and an accompanying decrease in concentrations of furans, phenolics, and weak acids (Chandel et al. 2012).

2.7. Fermentative Micro-organism and Maintenance of Culture

P. stipitis NCIM-3499 was obtained from the National Chemical Laboratory (NCL), Pune, India. *P. stipitis* was used for fermentation of detoxified pre-extracted depolymerized liquor obtained from hydrolysis experiments and was maintained on yeast extract peptone xylose (YEPX) medium containing xylose 20 g/L, yeast extract 10 g/L, peptone 20 g/L, and agar 20 g/L. The pH (5.0 ± 0.2) of the medium was adjusted with 1N H₂SO₄. After sterilization in an autoclave, the medium was cooled in laminar air flow and poured into Petri plates. After solidification, *P. stipitis* were plated on the agar plates and incubated in a BOD incubator (REMI) at 30 °C for 72 h. The prepared culture was maintained at 4 °C on an agar slant for future use.

2.8. Fermentation Conditions and Growth Curve

The sterile media (100 mL) were inoculated with a loop full of *P. stipitis* from the previously stored culture already incubated in the B.O.D. incubator. The composition of the media is yeast extract (5 g/L), peptone (5 g/L), KH₂PO₄ (5 g/L), (NH₄)₂SO₄ (0.2 g/L), MgSO₄ (0.4 g/L) pH of 4.5 and glucose (10 g/L). The culture was maintained at 30 °C for 24 h in an incubator shaker with continuous mixing at 150 rpm. After microbial growth, 5% (v/v) of the culture was used to inoculate sterile fermentation media. The growth curve of *P. stipitis* was developed for confirmation of different phases (lag, log, stationary, and death). The exponential growth of the cells was observed during the log phase. The growth curve was developed for the determination of the aerobic period during fermentation for the growth of inoculated cells. Fermentation was performed in a self-calibrated BIOSTAT® A-plus fermenter (Sartorius Germany, 5 L). The pH of the medium was controlled with 2N H₂SO₄ and 2N NaOH. Samples were taken every 8 h to monitor the production of

ethanol in the broth. Upon completion of the reaction, the fermented broth was further subjected to ethanol recovery. The ethanol produced was recovered by fractional distillation and further evaluated qualitatively and quantitatively.

2.9. Pulping of Poplar Chips

Kraft pulping of poplar chips was carried out in an electrically heated stainless steel oil bath (polyethylene glycol grade, 600) digester thermostatically controlled system. The digester (capacity 2.5–3.0 L) had six bombs, three bombs were loaded with pre-treated 200 g O.D. basis poplar chips and three bombs were used for control i.e., non-pre-treated sample. The digestion of control and pre-treated poplar chips was carried out at 20% sulfidity at 170 °C for 60 min and maintained a 1:4 bath ratio (Table 1). The maximum temperature was reached in 90 min. The samples were cooked to H-factor 1203.05, and the calculation of the H-factor of the cooking condition is given in Table 2. The pulp of poplar chips after digestion was defibrillated in a laboratory disintegrator and thoroughly washed with warm tap water at 40°C. The pulps were evaluated and screened. The unbleached pulp yielded rejects, Kappa number was determined in control and pretreated poplar pulp samples.

Table 1. Pulping conditions used in this study.

			(min)
01:04	170	0	60
	01:04	01:04 17	01:04 170

Table 2. Calculation of the H factor for the cooking condition.

Time from 100°C	Temp °C	Relative Rate	Average Rate, R	Time Internal	H factor R×T
0	100	1	-	-	-
15	110	3.1	2.05	0.25	0.51
30	120	9	6.05	0.25	1.51
45	130	24.9	16.95	0.25	4.24
60	140	65.6	45.25	0.25	11.31
75	150	165	115.3	0.25	28.83
90	160	397.8	281.4	0.25	70.35
105	170	921.4	659.6	0.25	164.9
165	170	921.4	921.4	1	921.4

Total= 1203.05, therefore, H factor = 1203.05.

2.10. Preparation of Laboratory Paper Hand Sheets and Physical Properties

The pulp obtained from the pulping of control and pre-treated poplar chips was converted into paper hand sheets (60 GSM) using a British Standard laboratory hand sheet machine, followed by pressing and drying. The paper sheets produced from the control and pre-treated poplar pulp were analyzed for their physical properties.

2.11. Analytical Methods

Bioethanol production was qualitatively tested using the Jones reagent ($K_2Cr_2O_7 + H_2SO_4$) (Jones, 1953). The reaction was carried out with 1 mL $K_2Cr_2O_7$ (2%), 5 mL H_2SO_4 (concentrated), and 3 mL of fermentation sample. Bioethanol was oxidized to acetic acid with potassium dichromate in the presence of sulfuric acid, giving a blue-green color. The green color indicates a positive test for ethanol (Caputi et al., 1968). Quantitative estimation of ethanol produced was performed at 585 nm in a UV-Vis spectrophotometer (Shimadzu, Japan).

The estimation of total reducing sugars, phenolics, and ethanol was carried out by UV-Vis spectrophotometer. The standard curve for total reducing sugar was prepared by the DNS method (Miller, 1959) using glucose as a standard, and the absorbance was measured at 510 nm by UV/Vis spectrophotometer (Shimadzu, Japan). Total reducing sugars of the hydrolysate were estimated after each treatment using the standard DNS method (Dubey et al. 2009). For the estimation of phenolic content, the different standard stock solutions of 0.002, 0.004, 0.006, 0.008, and 0.010% were prepared for the estimation of phenolic content (Lamuela-Raventós, 2018). The absorbance was recorded at 280 nm (Chandel et al., 2012).

3. Results and Discussion

3.1. Proximate and Chemical Properties of Raw Material

The proximate chemical analysis was determined according to the standards required for good product formation. The average moisture content in the samples was found to be 10.3%. Poplar chips powdered in Willy mill and sample passed through 40 mesh and retained on 60 mesh were taken for the study purpose. The cold and hot water solubilities indicate the presence of color pigments, phenols, starch, and water-soluble extractives and were 3.4 and 5.2% respectively. N/10-NaOH solubility and Klason lignin were found to be 18.4 and 24.6% respectively. Alcohol-benzene solubility is an indication of low molecular weight carbohydrates, salts, waxes, and resins in the sample and was found to be 15.2%. The holocellulose content in poplar chips was 74.10%. The content of holocellulose (total carbohydrates) in the raw material is important for the suitability of the raw material for bioethanol and paper. The ash content in the raw material was 0.52% (Table 3). The results of proximate analysis shown on biomass samples in terms of moisture content, volatile matter, ash, and fixed carbon, along with their respective dry values, were described by (Cavalaglio et al., 2020).

Table 3. Proximate chemical ana	lysis of po	plar wood dust	used in this study.
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Chemical Analysis	Result (%)	Method References
Cold water solubility	3.4	T 207 cm-99
Hot water solubility	5.2	T 207 cm-99
N/10 NaOH solubility	18.4	T 212 om-02
Klason lignin	24.6	T 222 om-02
Alcohol/benzene solubility	15.2	T 204 cm-97
Holocellulose	74.1	method-249-75
Ash Content	0.52	T 211 om-02

3.2. Effect of Pre-treatment on Poplar Biomass

The poplar chips were pre-treated with acid, distilled water, and alkali media at a bath ratio of 1:6 for 24 h, followed by autoclaving the soaked biomass with solutions in an oil bath digester at varying times and temperatures. The total reducing sugar concentration in the pre-extracted liquor was also determined in mg/mL (Figure 1a), and the total extracted sugar content w/w % (after considering the amount of extracted liquor) was estimated for the biomass on a dry weight basis (Figure 1b). The results showed an increasing trend of sugar concentration in pre-extracted liquor and total sugar content extracted from biomass when treated with acid at lower pH than with water and alkali pretreatment, and the increase was also with increasing temperature and time of treatment. The maximum sugar concentration obtained in the pre-extracted liquor was 7.23 mg/mL and the maximum total sugar content extracted from the biomass on a dry weight basis was 2.8% w/w, at pH 4, time 2 h, and temperature 150 °C. Bajpai (2021) also reported the different methods of pretreatment in terms of bioethanol production.





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3.3. Depolymerisation of Pre-extracted Liquor and Reducing Sugars Extraction

The pre-extracted lye was depolymerized under reflux with sulfuric acid. There was an increase in TRS content in depolymerized pre-extracted liquor, which may be due to acid hydrolysis of oligomeric sugars present in the hydrolysate (Vena et al. 2015). The total reducing sugar concentration in the depolymerized pre-extracted liquor was determined in mg/mL (Figure 2a), and the total extracted sugar content was estimated in w/w % (after taking into account the amount of extracted liquor) in terms of biomass on a dry weight basis (Figure 2b). The results showed an increasing trend of sugar concentration in depolymerized pre-extracted liquor and total sugar content extracted from biomass when treated with acid at lower pH than with water and alkali pretreatment, and the increase was also with increasing temperature and time of treatment. The maximum sugar concentration obtained in the depolymerized pre-extracted liquor was 17.47 mg/mL and the maximum total sugar content extracted from the biomass on a dry weight basis was 6.9% w/w, at pH 4, time 2 h, and temperature 150 °C. It was considered an optimized condition for further detoxification and fermentation study. Malik et al. (2020) have also reported on the valorization of wheat straw for the paper industry: pre-extraction of reducing sugars and its effect on pulping and papermaking characterizations.



Figure 2. b) Concentration of sugar solution obtained in depolymerized pre-extracted liquor (mg/mL); b) amount of sugar extracted to biomass (OD basis) in depolymerized pre-extracted liquor (w/w %).

3.4. Detoxification of Depolymerized Pre-extracted Liquor

During the acid depolymerization process, phenolics are formed that interfere with the fermentation process (Dubey et al., 2009). Detoxification to remove phenolics was performed using calcium hydroxide slurry and activated carbon. The phenolic content was reduced from 1.15 to 0.008% in the depolymerized pre-extracted liquor.

3.5. Sugar Consumed and Growth of P. stipitis

The growth pattern of *P. stipitis* was monitored at regular time intervals for 120 h until the decay phase showed the characteristic sigmoidal pattern. Figure 3 shows the growth pattern of *P. stipitis* along with the corresponding sugar consumption during the fermentation of the hydrolysate. Germec et al. (2020) also evaluated ethanol from rice hull hydrolysates (RHHs) using *P. stipitis* strains and optimized the dilute acid hydrolysis and detoxification processes using response surface methodology (RSM).

3.6. Fermentation of Depolymerized Pre-extracted Liquor

The fermentation was performed with *P. stipitis* and the ethanol yield was monitored for up to a period of 120 h (Figure 4). An ethanol yield of 3.58 g/L was obtained after fermentation of detoxified depolymerized pre-extracted liquor with *P. stipitis* in 120 h. Similar results have been shown by using *P. stipitis* for the fermentation of lignocellulosic biomass extract (Dubey et al., 2015).

3.7. Results of Pulping of Poplar Chips

Kraft pulping of poplar chips, control (untreated) and pretreated, was carried out in an electrically heated stainless steel oil bath digester at 20% sulfidity at 170 °C for 60 min, maintaining a 1:4 bath ratio. For pretreated chips, the residual biomass of the maximum sugar concentration obtained in the depolymerized pre-extracted liquor at pH 4, time 2 h, and temperature 150 °C was taken as the optimized condition. During digestion, the maximum temperature was reached

in 90 min. The samples were boiled to H-factor 1203.05. The untreated and pretreated poplar chips were converted into pulp. The pulp of poplar chips after digestion was disintegrated in a laboratory disintegrator and washed thoroughly. Evaluation and screening of the pulps were performed. The unbleached pulp yield rejects and Kappa numbers were determined in control and pretreated poplar pulp samples (Table 4). The pretreated poplar raw material yielded more pulp than the untreated raw material; after pulping, the pulp yield was 47.65% for the untreated raw material and 49.88% for the pretreated poplar chips. The possible explanation for this would be that during extraction for pretreatment of poplar chips there is a decrease in predominantly hemicellulose sugars making the biomass richer in cellulose content and leading to higher pulp yield, kappa number was also slightly higher in the pretreated 20.86% than untreated 19.93%. The rejects for untreated and pretreated chips were 0.92% and 0.96%, respectively. Garmaroody et al. (2016) also reported that the pre-treatment process for the poplar chips by Trametes versicolor for up to 21 days and successively used for kraft pulping to achieve a constant kappa number of about 20. The pretreated chips were categorized using SEM and FTIR analysis.



Figure 3. Sugar consumption and growth curve of P. stipitis at different times (h).



Figure 4. Bioethanol production and sugar consumption at different time intervals (h).

Table 4. Pulp yields, kappa number, and rejects of wood pulp after pulping process.

Sample Name	Pulp Yield (%)	Rejects	Pulp Yields after Screening (%)	Kappa No.
Non treated poplar	48.57	0.92	47.65	19.93
Treated poplar	50.84	0.96	49.88	20.86

Table 5. Physical properties of poplar sheets.

Sample Name	Tensile Index (Nm²/g)	Tear Index (mNm²/g)
Non-pre-treated	21.34	4
Pre-treated	19.23	3.5

3.8. Results of Physical Properties of Laboratory Paper Hand Sheets

The pulp obtained from the pulping of control and pre-treated poplar chips was converted into paper hand sheets of 60 g/m² (GSM) using a British Standard laboratory hand sheet-making machine and analyzed for physical properties. In making paper sheets, the physical properties of the tensile index and tear index of the pre-extracted biomass were 19.23 Nm/g and 3.5 mNm²/g and were slightly lower than the control 21.34 Nm/g and 4.0 mNm²/g, respectively (Table 5). The possible reason for lower strength properties in paper from pretreated chips may be due to the slight hydrolysis of cellulose during mild acid pretreatment of chips, but this decrease can be overcome by using strength additives. Jani et al. (2016) also showed significantly better apparent density and freeness than unbeaten pulp. The apparent density of beaten and unbeaten pulps was 0.74 and 0.69 g/cm³, respectively, while the freeness of beaten and unbeaten pulps was 250 and 415 mL, respectively. The paper from beaten pulp showed significantly better mechanical properties where the tensile index is 79.57 Nm²/g, burst index is 5.70 kPa.m², tear index is 4.17 mNm²/g and folding endurance is 508 whereas the unbeaten paper is 67.28 Nm/g, 4.98 kPa.m², 3.28 mNm²/g, and 195, respectively. The present studies agree with Obi Reddy et al. (2014), they have reported on the laboratory scale performance for the hand sheets made from Napier grass pulp. The abundance and fast growth of Napier grass is an added advantage as an alternative non-wood source for papermaking.

4. Conclusion

Based on the present study, it was concluded that the maximum reducing sugars extracted was 17.47 g/L from poplar biomass. Besides, the fermentation efficiency (60.13%) was achieved during bioethanol production at 3.58 g/L. The residue of pre-extracted hydrolysate was taken for pulping and paper production. The result showed that at H-factor 1203.05, a cooking temperature of 170 °C and a cooking time of 60 min for a pulp yield of 50.84% was obtained, which was better than the control performance of 48.57%. On production paper sheets the mechanical properties tensile index and tear index of pre-extracted biomass were observed as 19.23 Nm/g and 3.5 mNm²/g and slightly lower against the control 21.34 Nm/g and 4.0 mNm²/g. Overall, the present integrated studies were carried out significantly as an environmentally friendly fuel in terms of bioethanol and paper production from the *P. deltoides* wood biomass.

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