

Research Article

Steam Explosion Pretreatment with Different Concentrations of Hydrogen Peroxide along with Citric Acid: A Former Step towards Bioethanol Production

Akanksha Shukla ¹, Deepak Kumar ², Madhuri Girdhar ¹, Ajit Sharma ²,
and Anand Mohan ¹

¹School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, India

²School of Chemical Engineering and Physical Sciences, Lovely Professional University, Phagwara 144411, India

Correspondence should be addressed to Anand Mohan; anandmohan77@gmail.com

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The present paper focuses on the utilization of the steam explosion method for efficient pretreatment of rice straw, used as a prominent substrate for bioethanol production. Native rice straw contains abundant chemical compounds in the form of cellulose, hemicellulose, and lignin 32.4%, 57%, and 12.5%, respectively. Pretreatment with different concentrations of H₂O₂ (0.05%, 0.1%, 0.25%, 0.5%, 0.75%, and 1%) was used as efficient impregnating agents at an optimum temperature of 121°C at 130 kPa pressure. After pretreatment, cellulosic content increases up to 54.5% while there was a reduction in the hemicellulose and lignin content up to 46.5% and 6.7%, respectively. The morphological changes were analyzed both before and after pretreatment using FTIR, TGA, and XRD. The reducing sugar estimation was carried out using DNS reagent and the absorbance was measured using a UV spectrophotometer at 540 cm⁻¹ wavelength. Estimated results show a reducing sugar yield of 220.05 g/l from 0.05% (v/v) H₂O₂ pretreated sample and 273.21 g/l from sample, pretreated with H₂O₂, citric acid in the ratio of 1:1 with the same previous concentration. The XRD data shows enhancement of cellulose accessibility upon pretreatment to 13.3% and thereafter reduction with an increase in the concentration of H₂O₂. While pretreatment with H₂O₂ combined with citric acid in 1:1 ratio shows enhanced accessibility of 19.6% than untreated rice straw. This work mainly focuses on the core objective of an efficient pretreatment method for sustainable bioethanol production through a novel approach to the production of fermentable sugar.

1. Introduction

With an increase in population, global consumption has led countries towards a path of renewable energy sources, which can reduce global warming and the dependency on conventional fossil fuels. Biofuel production has become a sustainable option that will reduce dependency on the import of crude oil [1, 2]. Major countries are dependent on the export of crude oils which was considered a wasteful expenditure for their economies. Countries such as Brazil and the United States have started an active program on the usage and technological innovation in the production of biofuels over the

past 30 years [3] which has led to a reduction in greenhouse gas emissions. Various countries including India are heading towards clean-tech energy sources that will reduce carbon emissions. As a consequence of attempts being taken in this direction, the national biofuel policy of India has mandated the blending of 10% green biofuels with petrol [4]. Still, India barely manages to achieve 3% of blending with petrol. To meet the requirements, there was a need for 2G biofuels to achieve the mark of 10% blending and aim to go beyond the minimum mark in near future [5]. However, this path was far from simple and can take years of effort and capital to be spent by the government and industries worldwide [6].

As of now, various bioresources are available that have the potential for the production of bioethanol, these include industrial waste, agricultural biomass (cotton stalk, rice husk, rice straw, sorghum stalk, corn cob, wheat straw, sugarcane bagasse, and jatropha pruning), energy crops, and woody biomass as it was plentiful and cheap, and it did not overlap with the usage of food and feed naturally available [7, 8].

Lignocellulosic biomass (LCB) is the recalcitrant structure containing cellulose $(C_6H_{10}O_5)_n$, hemicellulose $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3(OCH_3)_{0.9-1.7}]_x$. It is considered that rice straw (RS) contains 35-50% cellulose, 20-25% hemicellulose, 8-10% lignin, and little traces of extractive and ash content [9]. However, the presence of high ash and silica concentration might sometime hinder bioethanol synthesis. As a result, treating RS in the first step with sodium carbonate is necessary to successfully remove 90% of the silica content from the biomass, progressively increasing the glucose output [10–13].

The cellulosic material is more resistant to bioconversion due to its recalcitrant structure. Pretreatment is considered an expensive process and the least technological step toward converting biomass to fermentable sugar. It makes structural polysaccharides accessible for hydrolysis of reducing sugar [14]. Among these processes, steam explosion (SE) is a distinctly efficient and favourable method with a maximum solubility of carbohydrate and lignin expulsion. SE partly melts lignin, depolymerizes hemicellulosic content, and disrupts the complex carbohydrate structure making complex biomass structure accessible for enzymatic hydrolysis [15]. It is estimated that SE-induced reduction in the acid groups present in the biomass due to enhancement in the severity of autohydrolysis causes higher losses of functional groups present in the lignin structure [16, 17].

Hydrogen peroxide along with citric acid is the green and safe route for a cost-effective and sustainable approach to bioethanol production. The H_2O_2 is used for delignification due to the presence of highly reactive radicals namely hydroxyl radical (OH^\cdot) and superoxide anion radical (O_2^\cdot). These forms of active radical delignify lignocellulosic biomass structure through the process, namely, oxidation and degradation [18]. By performing single-stage hydrogen peroxide pretreatment, H_2O_2 levels are relatively high, raising the processing cost. As a result, a combination of pretreatment that has the potential to reduce chemical inputs while increasing sugar yields should be identified to improve the process economically [17]. Liao et al. [19] performed hydrogen peroxide-acetic acid (HPAC) pretreatment with H_2SO_4 used as a catalyst for effective removal of 94.1% lignin from the biomass but it has risk of explosion due to the formation of strong oxidant of peracetic acid. To overcome the recalcitrance of poplar, a milder HPAC pretreatment was carried out by Liao et al. and Huang et al. [19, 20]. Furthermore, HPAC pretreatment has the potential to increase the sugar yield as it was stated that it would enhance hydrolytic enzyme adsorption onto the lignocellulosic structure. Based on our findings, HPAC pretreatment could be used for biomass with varied cell wall types to produce cellulosic ethanol effectively through the available green solvent. Overall, these

outcomes suggest that HPAC treatment is a beneficial approach for lowering the industry's costs of the 2G bioethanol processing technique [21]. The findings suggested that HPAC pretreatment eliminates interlinked glucan linkage by remoulding the composition of hemicellulose [22].

In the present study, physiochemical pretreatment impregnated with hydrogen peroxide combined with citric acid (HPCA) was performed for the enhancement of fermentable sugar production. After pretreatment, the structural composition of rice straw changed and was analyzed using XRD, FTIR, and TGA analysis. It was estimated that the crystallinity of cellulose, thermal decomposition of biomass, and the changes in the functional group of biomasses in the rice straw altered the structural component of the biomass after pretreatment. Estimation of reducing sugar was determined by using 3,5-dinitrosalicylic acid reagent and the absorption was measured at 540 cm^{-1} wavelengths. This research focuses on the establishment of an exact relationship between optimal pressure conditions along with hydrogen peroxide treatment conditions to shatter the recalcitrant structure of rice straw and make it affordable for converting rice straw into bioethanol.

2. Material and Methods

2.1. Proximate Analysis of the Sample

2.1.1. Description of Lignocellulosic Biomass. Rice (*Oryza sativa*) is the staple food grown in most of northern India. The rice straw utilized for the process was collected from the local farm in February 2022 from Kanpur, Uttar Pradesh. After that, the rice straw is dried in the air in the presence of natural sunlight at 21°C temperature. After drying up in the air, it was further dried in the oven at 110°C for a duration of 6 hrs and mechanically grinded up to $<3\text{ mm}$ mesh size particle and stored at room temperature $25 \pm 2^\circ\text{C}$. According to the technique developed by Ayeni et al. [23], the compositional analysis of dried biomass is 32.5% cellulose, 57% hemicellulose, 12.5% lignin, 10.12% extractive, and 7.4% ash and was performed according to the National Renewable Energy Laboratory (NREL) protocol [24]. All the chemicals were of reagent grade and acquired from Hi-Media Lab.

2.2. Compositional Analysis of Biomass. Soxhlet's extraction was set up and the weight of the extractive-free sample was measured both before and after extraction to find out the constant weight of extractive present in the rice straw. After that, hemicellulose determination was carried out according to the protocol of Ayeni et al. to obtain the constant weight of hemicellulose [23, 25]. Similarly, for lignin determination, 1 gm of extractive-free dried sample was taken and dissolved in 50 ml of 96% H_2SO_4 and was undertaken for acidic hydrolysis with the method of Cornejo et al. [26]. The acid-soluble lignin was evaluated by measuring its absorbance at wavelength 205 cm^{-1} . After that, it was dried at ambient temperature to obtain the constant weight of lignin present in the rice straw [27, 28]. For the determination of cellulosic content, it was accounted with the difference

obtained from extractive, hemicellulose, lignin, and ash content. The residue was left with the dried constant weight (%w/w) of cellulosic biomass.

2.3. H_2O_2 -Impregnated Steam Explosion. Following the determination of moisture content, the dried 2 gm RS was subjected to acid impregnation with different concentrations of 0.05% (v/v), 0.1% (v/v), 0.25% (v/v), 0.5% (v/v), 0.75% (v/v), and 1% (v/v) H_2O_2 with a stock solution prepared in 100 ml of distilled water. Afterward, impregnations were carried out in other batches combining H_2O_2 (0.05%, v/v) and citric acid in 1:1, 1:2, and 2:1 ratio. The RS was dissolved in the solvent for 1 hr before being subjected to steam explosion and then autoclaved at 121°C for 45 min at 103 kPa pressure. After that, the slurry was vacuum filtered for the separation of liquid (water-soluble) and solid (water-insoluble) fractions. The water-insoluble fraction was rinsed with distilled water to obtain the desired pH using 6 mol/l of NaOH for further designated hydrolysis conditions and is then stored at 4°C for further analysis. The reducing sugar was estimated by the 3,5-dinitrosalicylic acid (DNS) method [29]. All experiments were performed at least in duplicate.

2.4. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. The dried RS sample weighed 0.3 mg and was agitated for 2 minutes with 50 mg of KBr using an FTIR grid. With a maximum resolution of 4 cm^{-1} , this reveals structural variations that happened between 4000 and 400 cm^{-1} wavelength, and an average of 20 scans were performed using an FTIR spectrophotometer (CIF Lab, LPU). Both before and after pretreatment was analyzed at different peaks on different wavelength range to detect the spectra of various functional group present in the biomass [16].

2.5. Thermogravimetry Analysis (TGA). TGA was performed using Perkin Elmer TGA 4000 and was used to measure the kinetics, proximate analysis, and composition of the biomass. It was used to measure the weight of the decomposed biomass on heating from room temperature to 600°C at 10°C/min. Throughout the procedure, the weight was recorded so that with the onset temperature, the reaction accelerates and the weight reduces significantly, which can be determined. TGA measured the mass changes as a function of temperature or time with a constant heating rate.

2.6. Powder X-Ray Diffraction. The cellulose crystallinity index of both native and pretreated samples was analyzed using the Bruker D8 Advance XRD system (CIF Lab, LPU). X-pert pro diffractometer with scanning range 2θ within 5-40° at 0.03°/s using Cu-K α radiation X-ray. The crystallinity index was calculated from PXRD analysis using a formula earlier developed by [30],

$$CrI(\%) = \left[\frac{(I_{002} - I_{am-})}{I_{002}} \right] \times 100, \quad (1)$$

where CrI shows the crystallinity index, I_{002} shows maximum intensity in the crystalline region of cellulose at the 002 planes while I_{am} shows the minimum intensity in the amorphous region of cellulose between its lattice plane [31].

TABLE 1: Comparison of compositional analysis of Rice straw used in previous studies.

Component	Present study	Syaftika et al. [36]	Semwal et al. [34]
Cellulose (glucan)	32.4	28	37.8
Hemicellulose (xylan and arabinan)	57	55	21.6
Lignin	12.5	11	13.6
Ash	7.4	6	13.2
Extractive	10.12	NA	16.1

2.7. Analytical Methods. The moisture content in the biomass was determined by drying to constant weight at 110°C for 2 hrs in the hot air oven and was estimated to be 13%. The compositional analysis of raw rice straw was obtained by Soxhlet's extraction and using NREL standard protocol [32]. In short, a two-step hydrolysis technique using sulfuric acid (H_2SO_4) was performed to digest 1 g of 1-3 mm sample size. After hydrolysis, the Klason lignin was separated by filtration through medium porosity filtering crucibles and quantified after drying at 105°C. At 205 cm^{-1} wavelengths, the acid-soluble lignin in the hydrolysate was determined using a UV-vis spectrophotometer [33]. The amount of lignin is calculated as the sum of acid-soluble lignin and the Klason lignin. While NaOH was used to digest 1 gm of the extractive-free biomass sample. Hemicellulose content was filtered via vacuum filtration and weighed after drying at 110°C for 1 hr. The amount of hemicellulose was calculated as the constant weight of dried biomass. The pretreated rice straw is subjected to a steam explosion along with the different concentrations of H_2O_2 and with varied ratios of citric acid and H_2O_2 . The solid residue obtained after pretreatment was analyzed for characterization using FTIR, XRD, and TGA. The quantification of reducing sugar was performed using DNS reagent test and absorbance was measured at 540 cm^{-1} wavelength [31].

3. Result and Discussion

3.1. Compositional Analysis of Rice Straw. The majority of lignocellulosic biomass is composed of the polysaccharide cellulose and hemicellulose, as well as the aromatic polymer lignin. All of these components are meticulously woven together to offer unwavering support to the plant cell wall. This complicated ambiguity of LCB components impeded enzyme degradation into a low-molecular building block. The composition of the RS used in this study was initially compared to previous studies (Table 1) and was found as hemicellulose (57% w/w), cellulose (32.4% w/w), lignin (12.5% w/w), extractive (10.12% w/w), and ash (7.4% w/w). Semwal et al. [34] reported a higher cellulosic content (37.8 wt%) for the RS biomass along with hemicellulose (21.6 wt%), lignin (13.6 wt%), and ash (13.2 wt%) content. Ayeni et al. [23] reported higher cellulosic contents for the rice straw they used. Indeed, such varied compositional analyzes of lignocellulosic biomass due to soil type,

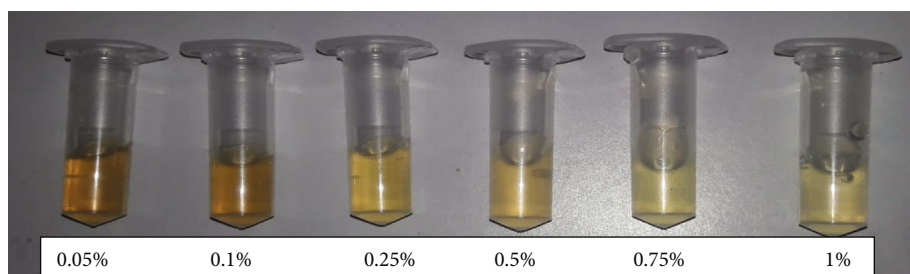


FIGURE 1: Liquid hydrolysate obtained after pretreatment of rice straw.

nitrogen fertilization, and harvest time, all had a strong influence on biochemical composition [35]. The presence of both lignin and hemicellulose had the potential to reduce overall efficacy in conventional bioethanol synthesis from cellulose alone, as these additional components could impair both the sample pretreatment and the enzymatic hydrolysis phases [36].

3.2. Biomass Digestibility under Various Concentrations of H_2O_2 along with a Steam Explosion. Rice straw was pretreated with different concentrations of H_2O_2 along with citric acid (HP-CA) at different ratios. The colour of the hydrolysate solution darkened during the pretreatment process on decreasing concentration of H_2O_2 , demonstrating the breakdown of the lignocellulosic materials from the RS, as illustrated in Figure 1. Lignin was considered a phenolic polymer that was hydrophobic in nature and nearly insoluble in water. The hydrophobic interactions between the substituent of phenolic lignin and the aromatic ring of the hydrotrope resulted in the preferential solubilization of lignin over cellulose and hemicellulose in the hydrotropic solution. For lignin separation, the hydrolysate solution containing the lignin extract was diluted with less concentration of H_2O_2 . Mejica et al. [37] stated that during the alkaline pretreatment process, the substrate primarily undergoes the crumbling of lignocellulosic structure, cellulose and hemicellulose dissolution, and saponification of ester intermolecular bonds, destroying the chemical bond of hemicellulose and other chemical compounds, resulting in lignocellulosic substrate delignification. Furthermore, the intensity of cross-linking polymers was altered, resulting in variations in the porosity, surface range, and crystallinity of the treated biomass. To prevent lignin precipitation on the surface of the biomass, the solid fraction extracted after pretreatment must be rinsed thoroughly with distilled water, followed by washing with hot water. The pretreatment method using SE and HP was considered to bring about hydrolysate with elevated glucose concentration from RS. The compositional analysis of RS was slightly influenced by SE treatment alone while a reduction in lignin content of up to 6.7% and hemicellulosic content of up to 46.5% while cellulosic content enhanced by up to 54.5% that was affected by HP treatment and the process was enhanced with HP-CA treatment. Gao et al. [38] performed wet disk milling with HP pretreatment at 70°C with 84.7% of enhanced glucan content in the sugarcane bagasse. The maximum delignification has a considerable impact on xylan hydrolysis

as the majority of xylan elimination was affected by WDM-HP pretreatment. A similar finding was confirmed with the low concentration of H_2O_2 along with weak acid, i.e., citric acid as it was one of the efficient methods towards bioethanol production as it efficiently removed lignin under mild temperature as well as utilization of weak acid was less hazardous as compared to stronger one. It was earlier revealed that HPAC pretreatment would enhance the capacity of enzyme adsorption up to many folds approximately 2.6 to 7.1 and also contributes efficiently toward the fermentation of bioethanol as it eliminated the formation of inhibitors in the form of furfurals from the hydrolytic medium [39]. Similarly, work was confirmed by using acetic acid pretreatment prior to HPAC that was mixed in a 1:1 ratio. Thus, 10 gm poplar was pretreated with 100 ml HPAC (80%, v/v) along with 100 mM H_2SO_4 as a catalyst at 60°C for 2 hrs retention time that was efficient in the delignification of biomass with 95.4% of lignin removal [40]. The findings suggested that coupling SE and H_2O_2 would not only improve saccharification efficiency but also lower enzyme loading during enzymatic hydrolysis. As a result, optimizing saccharification to decrease enzyme loading for low-cost ethanol production should be a priority in the future. Further recent studies on hydrogen peroxide pretreated samples on various lignocellulosic biomass are illustrated in Table 2.

3.3. Cellulose Crystallinity Index Analysis of the Sample. The cellulose crystalline index (CrI) was a key factor that influences lignocellulosic biomass enzymatic digestibility. The crystalline nature of cellulose varies depending on the biomass, and PXRD was used to analyze the variations in the crystallinity index of cellulose both for native and pretreated RS. There are crystalline and amorphous forms present in the cellulosic part of lignocellulosic biomass. To prevent cellulose degradation, the crystalline structure features a large intramolecular hydrogen bonding that was earlier confirmed by Liu et al. [42]. Zhang et al. [16] studied the sharp high-intensity peaks that indicated the crystalline nature of all the samples while a broad array of peaks in all biomass samples indicated that they were amorphous in nature. The strong diffraction peak at various 2θ values corresponds to the (110), (200), and (004) crystal phases of the biomass are illustrated in Figure 2, a similar finding was earlier observed by Malgas et al. [43]. The CrI was calculated using the intensity range of both amorphous and crystalline cellulose at the strong diffraction peak range of (200) and (110), respectively. It was estimated that the pretreated sample

TABLE 2: Recent studies on hydrogen peroxide pretreatment on various lignocellulosic biomass.

Pretreatment methods	Biomass	Required pretreatment condition	Changes after pretreatment	Ref.
Alkaline hydrogen peroxide	Wheat straw	0.2 Mol/l NaOH at 30°C for 5 hr 20 mg H ₂ O ₂ /g biomass 50°C for 7 hrs	42.7% of lignin removal	[17]
Hydrogen peroxide-acetic acid (75% HPAC)	Poplar	30%, w/w H ₂ O ₂ & 99%, w/w acetic acid in 1:1 ratio 100 mM H ₂ SO ₄ as catalyst (1:10, w/v) 80°C, 2 hr	Decrease in lignin concentration from 28.2% to 3.1% Glucan content increased from 40% to 67.2%, with 11.8% glucan removal	[19]
Alkaline hydrogen peroxide treatment	Bamboo	3%, v/v H ₂ O ₂ , 100°C, 2.2% w/v NaOH	76.5% of glucan, 56% of xylan recovered, & 79.25% of lignin removal	[20]
HPAC	Bamboo	30% H ₂ O ₂ : CH ₃ COOH (1:1, v/v)	Reducing sugar yield increases by more than 1.3-fold while cellulosic content increases by 1.7-fold	[22]
H ₂ O ₂ + H ₃ PO ₄ (PHP)	Wheat straw	74.92 gm H ₃ PO ₄ (85%, w/w) & 5.08 gm H ₂ O ₂ (30%, w/w)	54.1% of lignin removal with 92.4% of sugar conversion	[27]
HPAC	Maize straw, sugarcane bagasse, <i>Eucalyptus</i> bark	10 ml HPAC (1:1, v/v), incubated at 80°C for 2 hr	Cellulose crystallinity increases from 34% to 53%	[39]
Combined process of acetic acid and H ₂ O ₂ (AC-HPAC)	Poplar	5%, v/v acetic acid with solid to liquid ratio of 1:10 100 ml HPAC (80%, v/v) & 100 mM H ₂ SO ₄ at 60°C for 2 hr.	Glucan content increased from 42.1% to 54.5% with 85.8% glucan content	[40]
Alkaline hydrogen peroxide+ supercritical CO ₂ + ultrasound	Sugarcane bagasse	Pressured CO ₂ -20.6 Mpa, 453 K temperature for 1 hr, 0.6% H ₂ O ₂ treatment for 9 hr and 4 hr ultrasound retention time	97.8%, w/w of glucose recovery	[41]

showed slightly lower CrI than the native RS. It was reported that the CrI value of native and 0.05% H₂O₂ samples was 49.4% and 57%, respectively, and thereby started decreasing with an increase in the concentration of H₂O₂. Similarly, HP-CA pretreated sample in the ratio 1:1 shows a maximum CrI value of 61.5%, this shows 19.6% rise in cellulose crystallinity while 13.3% rise in crystallinity on treating with 0.05% H₂O₂ due to delignification of biomass. The CrI calculation based on intensity values at 18.5 (I_{am}) and 22.5 (I₀₀₂) is presented in Table 3. This reduction in CrI of the pretreated sample suggests that it was extremely amorphous, indicating that the lower concentration of HP has broken down intra- and interchain H-bonding in the crystalline structure of cellulose. The XRD pattern's large diffraction peak signifies that the crystalline form of processed biomass has undergone significant modifications. The rise in CrI was attributed mostly to the ablation of lignin and hemicellulose from the amorphous area. Thus, the similar findings from Paramasivan et al. [44] revealed that more amorphous cellulose was generated in the presence of greater surface accessibility, implying that more cellulolytic activity was potentially possible.

3.4. Functional Group Analysis of a Pretreated Sample. FTIR spectroscopy was used to evaluate potential changes in the polymer interlinkage in pretreated feedstock residues at distinct peaks. On comparing both steam explosion and native rice straw accountable for linkage between hemicellulose and cellulose microfibrils peak whereas untreated sample had not shown

those peaks clearly. The broader peak at 3306 cm⁻¹ showed the stretching of the O-H bond while with an increase in the concentration of H₂O₂ the peak got flattened. Similarly, stretching of the C-H bond occur at the 2916 and 2924 peak interpreting loosening of lignin microstructure for the further enzymatic activity that was earlier confirmed by Paramasivan et al. [44]. The absorption spectra pertaining to usual intermolecular H-bond and β-glycosidic linkages in polysaccharides, on the other hand, spectra were greatly elevated by the combined pretreatment used in this work. This indicates that the consolidated pretreatment used in this analysis should be a cellulose-friendly technology that sustains cellulose in the pretreated rice straw. As a result, the combined pretreatment effectively extracted hemicellulose-lignin complexes from the pretreated biomass. Namely, 2 peaks corresponding to 1056 cm⁻¹ and 1037 cm⁻¹ are associated with the stretching of C-O bond of acetamide group while the peak at 1625 cm⁻¹ and 1617 cm⁻¹ indicate the stretching of the amide group in acetamide, similar stretching was earlier studied by Ma et al. [45]. Figure 3 indicates the infrared spectrum of raw and pretreated rice straw (RS). The absorption peaks of cellulose were stronger for the sequential steam explosion and H₂O₂ pretreated RS as compared to the native sample. Thus, the change in functional group absorbance indicates that lignin structures have been destroyed, exposing more cellulose surface area for enzymatic hydrolysis.

3.5. Thermal Stability of the Sample. It was critical to investigate the thermal properties of the pretreated sample, which

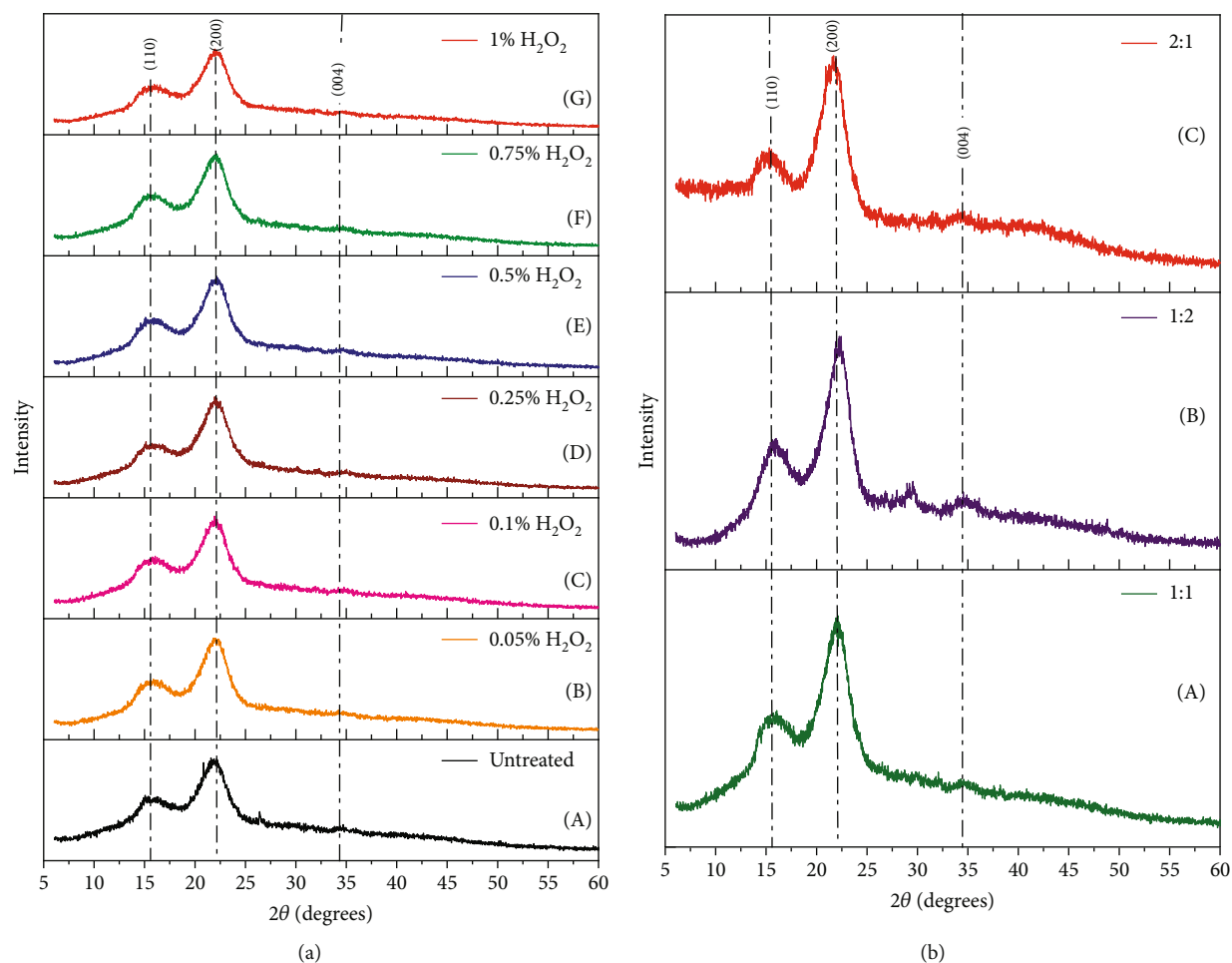


FIGURE 2: X-ray diffraction pattern. (a) Steam explosion impregnated with different concentration of H₂O₂ (A) untreated sample (B) 0.05% H₂O₂ pretreated sample, (C) 0.1% H₂O₂ pretreated sample, (D) 0.25% H₂O₂ pretreated sample, (E) 0.5% H₂O₂ pretreated sample, (F) 0.75% H₂O₂ pretreated sample, and (G) 1% pretreated sample. (b) At different ratio of HP-CA, (A) 1:1 ratio, (B) 1:2 ratio, and (C) 2:1 ratio.

TABLE 3: Cellulose crystallinity Index derived from XRD analysis.

Different pretreated sample	Intensity at I_{am}	Intensity at I_{002}	CrI
Untreated biomass	9.4	18.6	49.4
0.05% H ₂ O ₂	10.3	24	57
0.1% H ₂ O ₂	10.4	23.1	54.9
0.25% H ₂ O ₂	10.26	22.7	54.8
0.5% H ₂ O ₂	9.6	20.7	53.6
0.75% H ₂ O ₂	10.7	22.8	53.07
1% H ₂ O ₂	11.4	23.8	52.1
1:1	6.8	17.7	61.5
1:2	8.06	17.3	53.4
2:1	10.7	22.7	52.8

were linked to the chemical structure of LCB. TGA was carried out in order to effectively convey the thermal information of various samples as shown in Figure 4. The TGA result showed three major weight loss regions (A-zone, B-zone, and C-zone) of native and pretreated rice straw that

mainly correspond to moisture removal (dehydration), thermal decomposition (volatile material removal), and solid disintegration, respectively, as similar findings revealed by Monir et al. [46]. The initial degradation occurred at a temperature between 250°C and 380°C. It was noted from the graph that cellulose and hemicellulose started degrading at around 275°C and 376°C for all concentrations of H₂O₂ with an average weight loss of 0.08 (wt%) at 296°C and 0.06 (wt%) at 320°C temperature. The maximum weight loss percentage of raw rice straw was obtained at 365°C while the pretreated sample showed maximum weight loss at 376°C and 378°C temperatures and a similar finding was earlier observed by Huang et al. [20]. This was mostly due to the pretreatment elimination of a specific percentage of hemicellulose and lignin, which had a stochastic amorphous structure and was rendered obsolete with the rising temperature. After raising the temperature from 376°C leaving behind the minimum traces of ashes (14 wt%/°C) while the highest lignin breakdown occurred after 400°C with the highest weight loss of 0.96 (wt%/°C) which was attributed to the breakdown of rice straw fraction to gaseous compounds. It was also discovered that the lignin component of

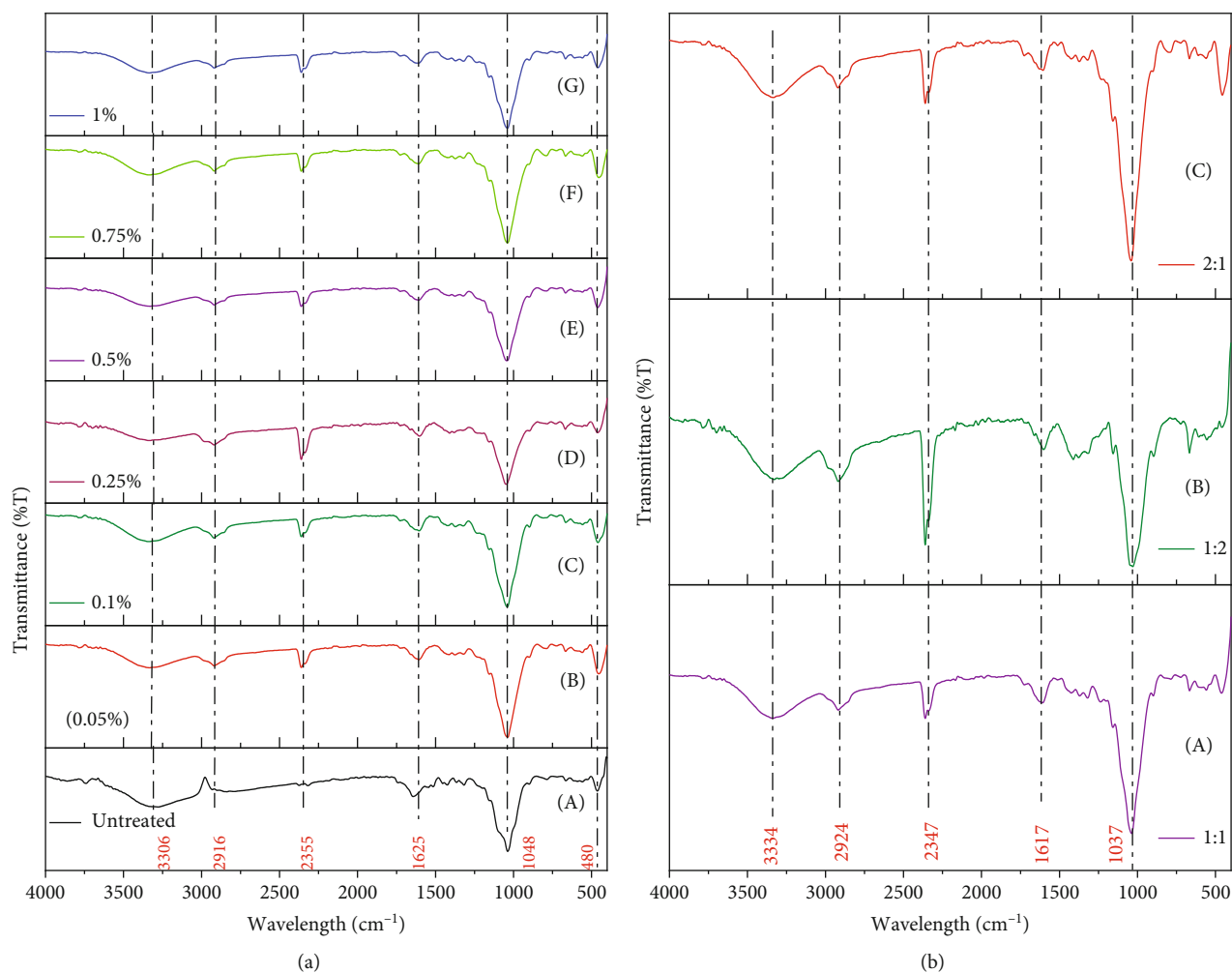


FIGURE 3: FTIR analysis. (a) Steam explosion pretreatment impregnated with H_2O_2 , (a) untreated sample, (b) 0.05% H_2O_2 pretreated sample, (c) 0.1% H_2O_2 pretreated sample, (d) 0.25% H_2O_2 pretreated sample, (e) 0.5% H_2O_2 pretreated sample, (f) 0.75% H_2O_2 pretreated sample, and (g) 1% H_2O_2 pretreated sample. (b) At different ratios of HP-CA, (a) 1:1 ratio, (b) 1:2 ratio, and (c) 2:1 ratio.

biomass was the most difficult counterpart to be degraded, and its breakdown occurred very gradually throughout the entire temperature profile (up to $600^\circ C$).

3.6. Reducing Sugar Estimation Using Benedict's Reagent and DNS Reagent. One of the chemical methods used to determine the presence of reducing sugar in the obtained hydrolysate was Benedict's test which could be used to detect simple carbohydrates with a free aldehyde or ketone functional group. In this study, with a decrease in the concentration of H_2O_2 , the obtained hydrolysate got turbid which confirms the presence of reducing sugar in the 0.05% (v/v) H_2O_2 pretreated sample. Firstly, the reducing sugar was estimated using benedict reagent, and the observed colour is illustrated in Figure 5. The highly reactive radical in the form of hydroxide and superoxide anion would delignify the lignocellulosic biomass by oxidation and degradation of the rice straw. Further on, the reducing sugar presence was confirmed by using DNS reagent at 540 cm^{-1} wavelength in a UV spectrophotometer [39]. The graph obtained after UV spectrophotometry is illustrated in Figure 6. It was observed

that at a 0.05% H_2O_2 , 220.05 g/l of reducing sugar were quantitatively analyzed, and on increasing the concentration of H_2O_2 , the presence of reducing sugar gets reduced to 92.68 g/l from obtained hydrolysate. Similarly, HPCA pretreatment in the ratio 1:1 yielded 273.21 g/l of reducing sugar. This shows the increment in sugar yield of up to 19.4% with the utilization of citric acid along with H_2O_2 impregnation in a 1:1 ratio. Furthermore, the increased proportion of monomeric sugar in the pretreated analytes indicated that the hemicellulose was more easily dissolved at $121^\circ C$ temperature. It was worth mentioning that an increased concentration of xylose in the hydrolysate typically resulted in the maximum formation of inhibitors. While a maximum glucose concentration in the hydrolysate resulted in a lesser formation of hydroxymethyl furfural (HMF). This might be because xylan was more unstable and unpredictable for saccharification than glucan. Earlier studies had reported similar results for the pattern of degradation of product formed during the process. The illustration regarding the quantification of reducing sugar formed from hydrolysate is presented in Figure 7.

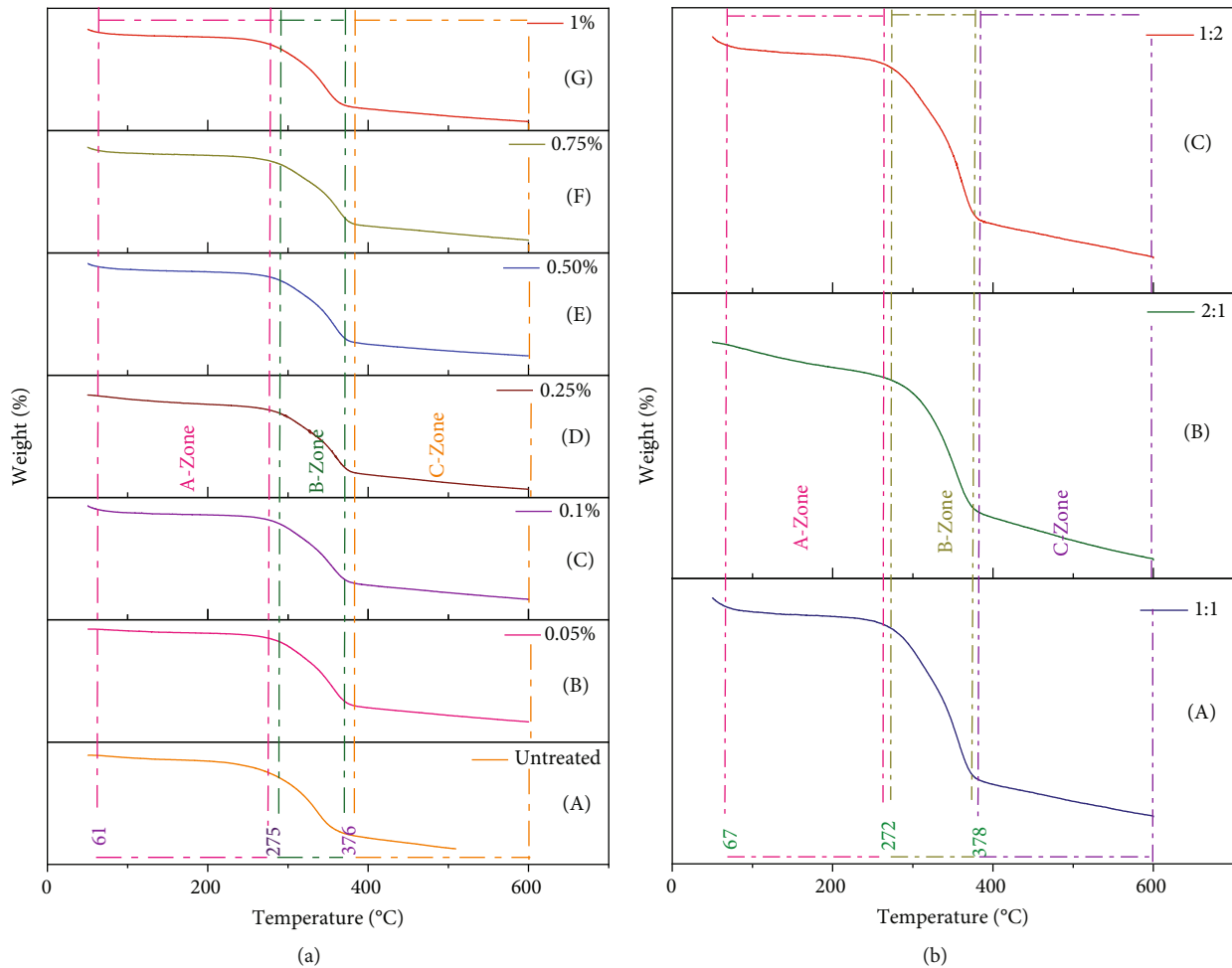


FIGURE 4: Thermogravimetric analysis. (a) Steam explosion impregnated with H₂O₂ pretreated sample, (a) untreated sample, (b) 0.05% H₂O₂ pretreated sample, (c) 0.1% H₂O₂ pretreated sample, (d) 0.25% H₂O₂ pretreated sample, (e) 0.50% H₂O₂ pretreated sample, (f) 0.75% H₂O₂ pretreated sample, and (g) 1% H₂O₂ pretreated sample. (b) Different ratio of HPCA pretreated sample, (a) 1:1 ratio, (b) 2:1 ratio, and (c) 1:2 ratio.

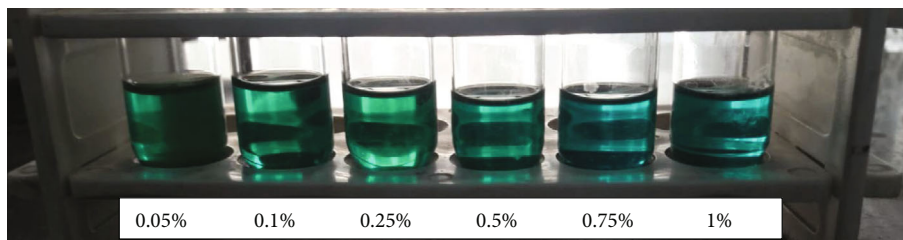


FIGURE 5: Benedict reagent test of different concentration of H₂O₂ pretreated sample.

3.7. Impact of Steam Explosion Pretreatment on HP and HPCA Impregnated Sample. The steam explosion pretreatment coupled with HP has the ability to break the bond amid the recalcitrant structure of lignin due to an increase in the degradation of lignin content along with oxidation of lignin. Thus, the greater the lignin degradation, the higher will be the accessibility of hydrolytic enzymes to access the cellulose and hemicellulose to loosen the recalcitrant structure. In previous literature, SE was considered to abrupt an initial explosion and fragmentation of the biomass cell wall structure into finer

constituents by improving shear strength, compression, bulk density, and mean particle size [47]. Higher hemicellulose degradation of up to 71% would occur at 1.5 MPa pressure during steam explosion methods due to partial hydrolysis of hemicellulose along with polymerization of lignin in the biomass was reported by Zhang et al. [16]. Thus, SE was an effective pretreatment as it enhances the water solubility of rice straw and also enhances the utilization of polysaccharides for the further process of hydrolysis. There were mainly two stages in the process, the biomass was firstly subjected to extremely

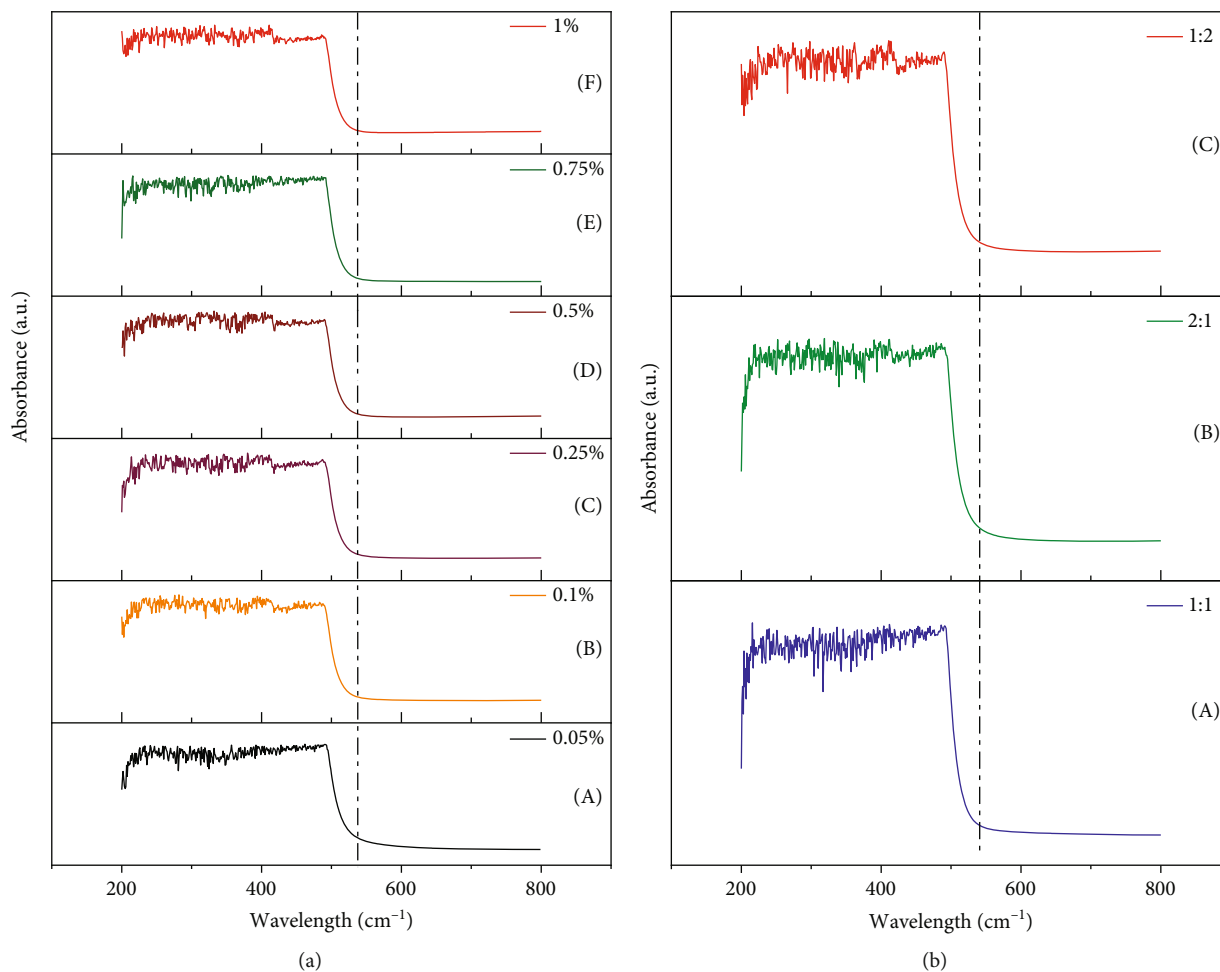


FIGURE 6: Absorbance graph at 540 cm⁻¹ wavelength. (a) Different concentrations of H₂O₂, (a) 0.05% H₂O₂ pretreated sample, (b) 0.1% H₂O₂ pretreated sample, (c) 0.25% H₂O₂ pretreated sample, (d) 0.5% H₂O₂ pretreated sample, (e) 0.75% H₂O₂ pretreated sample, and (f) 1% H₂O₂ pretreated sample. (b) At a different ratio of HP-CA, (a) 1 : 1 ratio, (b) 1 : 2 ratio, and (c) 2 : 1 ratio.

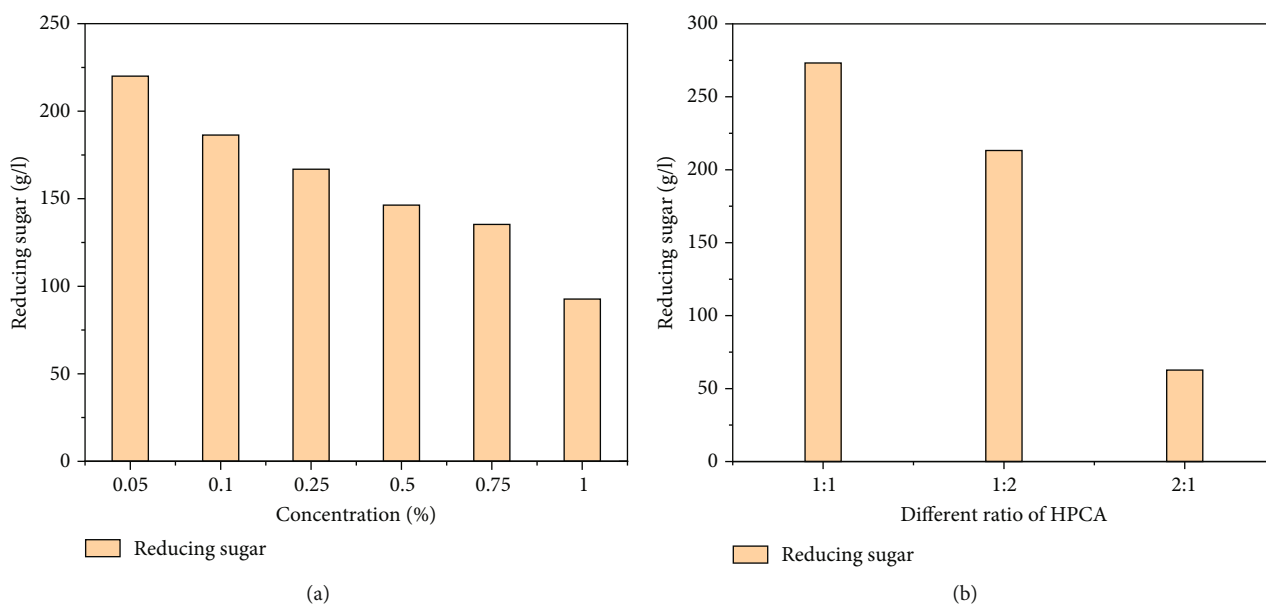


FIGURE 7: Quantification of reducing sugar elucidated taking $Y = 0.0019X - 0.0071$ with $R^2 = 0.9746$. (a) Quantification of produced reducing sugar from different concentrations of H₂O₂. (b) Quantification of produced reducing sugar at different ratio of HPCA.

TABLE 4: Recent analysis on steam explosion pretreatment of various lignocellulosic biomass.

Pretreatment techniques	Biomass	Required condition	Degradation in cellulose & hemicellulose	Major findings	Ref.
Steam explosion	Defatted soyabean meal	1.5 Mpa/193.1°C for 5 min	Cellulose-50.7% Hemicellulose-71.1%	At high pressure, biomass saturated with water vapour, the fiber network gets relaxed and lignin content migrates	[16]
Steam explosion +1%, <i>w/w</i> , dil. H ₂ SO ₄	Rice straw (10 mm particle size)	200°C for 10 min	Glucan conversion to 89.6%	Large particle size consumes less energy & low production cost	[34]
Steam explosion	<i>Miscanthus</i>	200°C, 15 bar, 10 min	Xylo-oligosaccharide yield 55%, <i>w/w</i>	Larger biomass (10-30 mm) particles led to higher XOS yield (55%, <i>w/w</i>)	[35]
Steam explosion +0.8% <i>w/v</i> NaOH +1.2% <i>w/v</i> CaO	Corn stalk	2.4 gm NaOH, 3.60 gm CaO in 5:1 ratio (<i>v/w</i>), 130 kPa pressure for 1 hr	54.7% reducing sugar yield	CrI has increased little due to an increase in the order of amorphous cellulose and degree of freedom	[42]
Steam explosion +0.5% H ₂ SO ₄	Rice straw	200°C/100 bar/10 min	Increase sugar concentration to 12.4 g/l	Max. Sugar yield 86%	[47]
Steam explosion & catalysed with H ₃ PO ₄ & H ₂ SO ₄	Sugarcane bagasse	195°C for 7.5 min	69.4% of free sugar released after pretreatment	Maximum mass recovery in presence of acid catalysts and higher xylan content up to 6.6 wt.%	[48]
Steam explosion + sulphonation (Na ₂ SO ₃ , 12%, <i>w/w</i> + NaHCO ₃ , 4%, <i>w/w</i>)	Woody <i>Eucalyptus</i>	210°C for 5 min	Glucan hydrolysis increased to 57.89% from 12.23%	Solubilization of hemicellulose Hydrolysis yield was higher at 210°C as compared to 200°C steam	[50]

saturated steam for a few minutes before being abruptly released, leading to a significant alteration in the composition and structure of the lignocellulosic material. The hemicelluloses are partially hydrolyzed to yield monomeric and oligomeric sugars due to the utilization of excessive pressure steam, owing to the emission of acetic as well as other organic compounds in the reaction environment [48]. The high-pressure steam explosion of 2.5 MPa for 1 min led to a higher degree of fragmentation of lignocellulosic biomass and also eliminated the intracellular structure of biomass [45]. The recent research on the impact of steam explosion on various LCBs is illustrated in Table 4. Thus, it was earlier noted that steam explosion impregnated in H₂O₂ resulted in an increase in 12% glucose and 34% xylose content while a 30% decrease in cellobiose yield during the pretreatment process, the presence of H₂O₂ reduces the accumulation of lignocellulosic by-products [49].

4. Conclusion

This research was performed as an integrated method of physiochemical pretreatment technique and has proven to be an efficient method for breaking down lignocellulosic biomass structure. The optimum SE pretreatment condition was 103 kPa pressure maintained for 45 min with different concentrations of H₂O₂ along with HPCA. It was concluded that less concentration (0.05%) of H₂O₂ showed a maximum reducing sugar formation of 220.05 g/l of hydrolysate. Thermal decomposition of native and pretreated RS was analyzed up to 600°C where lignin started its degradation. Similarly, different peaks were obtained at different wavelengths ranging from 4000 to 400 cm⁻¹. The reduction in the intensity peak resulted in the alteration in the C-O bond present in the recalcitrant structure of lignin. While the cellulose crystallinity was measured using XRD. Thus, steam explosion pretreatment of H₂O₂-impregnated RS showed better results with its ability to form radicals at higher concentrations of H₂O₂. The 0.05% (v/v) H₂O₂ loading along with citric acid in the ratio 1 : 1 was preferred due to its less toxicity and corrosivity than other pretreatment chemicals. The HPCA impregnation was performed using citric acid as one of the easily available weak acids to develop cost-effective physiochemical pretreatment for bioethanol production. Thus, it was concluded that due to the strong oxidizing ability of HP, it was efficient for hemicellulose and delignification of lignocellulosic biomass, causing detachment and solubilization of lignin along with loosening of lignocellulosic recalcitrant structure.

Abbreviations

FTIR:	Fourier transform infrared spectroscopy
TGA:	Thermogravimetric analysis
PXRD:	Powder X-ray diffractometer
DNS:	3,5-Dinitrosalicylic acid
H ₂ O ₂ /HP:	Hydrogen peroxide
HPCA:	Hydrogen peroxide-citric acid
2G:	Second generation
RS:	Rice straw

LCB:	Lignocellulosic biomass
SE:	Steam explosion
HPAC:	Hydrogen peroxide-acetic acid
HMF:	Hydroxymethyl furfural
KBr:	Potassium bromide
NREL:	National Renewable Energy Laboratory
ScCO ₂ :	Supercritical carbon dioxide.

Data Availability

The figures obtained from origin software used to support the findings of this study are included within the article.

Additional Points

Highlights. (1) Hydrogen peroxide is compatible with the bioconversion of rice straw. (2) The effect of acid impregnation on structural cross-linking of biomass. (3) Quantification of produced reducing sugar in prehydrolysate using DNS method. (4) The sequential pretreatment process formed lesser inhibitory by-products for further processing. (5) High retention of polysaccharide and lignin solubilization improve bioethanol yield

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this paper.

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