

Article

Sulfite Pretreatment Enhances Tobacco Stalk Deconstruction for Cellulose Saccharification and Lignin Pyrolysis

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Abstract: Sulfite-catalyzed acid pretreatment to overcome the inherent recalcitrance of biomass offers a significant advantage in terms of obtaining high glucose conversion. However, the residual lignin after enzymatic hydrolysis has not been fully exploited. Herein, this study introduced a joint approach using sulfite-catalyzed acid pretreatment (SPROL) and pyrolysis to upgrade tobacco stalk to produce fermentable sugar, and the resulting lignin is used to produce bio-oil and bio-char. The results suggest that SPROL pretreated tobacco stalk yields a high cellulose-based glucose selectivity of 75.9% with 15 FPU/g substrate enzyme dosage at 50 °C after 72 h of enzymolysis. Lignin characterization reveals that sulfonation occurred during SPROL pretreatment, and as the dosage of sulfonating agent increased, the thermal stability of the residue lignin decreased. After sample pyrolysis at 600 °C for 30 min, approximately 22%, 33%, and 45% of the lignin undergoes conversion into bio-oil, bio-char, and gas products, respectively. The bio-oil analysis results demonstrated that acetic acid is the most abundant identified GC-MS component at around 69.91% at the optimal condition, which implied that it could be of high value when utilized for pyrolygneous acid. This research provides a synthetic approach using the SPORL technique to process tobacco stalk into fermentable sugar, bio-oil, and bio-char, which is significant for the commercial utilization of agricultural waste into value-added products.

Keywords: tobacco stalk; sulfite-catalyzed pretreatment; lignin; pyrolysis



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1. Introduction

The global challenges of climate change and the depletion of petroleum resources have intensified the focus on sustainable alternatives, particularly the conversion of agricultural biomass waste into biofuels [1]. Agricultural biomass predominantly comprises lignin, cellulose, and polysaccharides, which are interconnected to form the lignin-carbohydrate complex (LCC) [2,3]. This complex plays a pivotal role in biomass recalcitrance, a phenomenon that significantly impedes the efficient utilization of biomass. Addressing the inherent recalcitrance of biomass is a pivotal step towards the effective conversion of these renewable resources into a myriad of valuable products [4,5].

Chemical pretreatment methods, such as acid [6,7] alkali [8,9], deep eutectic solvent (DES) [10,11], organosolv [12,13], and sulfite pretreatment (SPORL) [14,15], are commonly employed to disrupt the recalcitrance of lignocelluloses, thereby facilitating the release of fermentable sugars. These sugars are then utilized in the sugar platform through saccharification and fermentation processes [16]. However, a significant challenge in this process

is the fate of lignin, which either remains unutilized as an inhibitor of enzymatic saccharification or is isolated directly. Moreover, during chemical pretreatment, hemicelluloses are often removed, resulting in an increased lignin content in the pretreated substrates. This alteration in the lignin-to-cellulose ratio within the biomass feedstock exacerbates the non-production adsorption, where cellulase enzymes, instead of depolymerizing cellulose, bind to lignin components. As a result, it is necessary to increase the dosage of cellulase enzymes to achieve effective saccharification [17,18]. This requirement poses economic constraints on the commercial viability of current biomass conversion technologies.

Lignin, however, presents an untapped potential for conversion into valuable products. Through thermochemical processes, such as pyrolysis, lignin can be transformed into liquid, gas, and solid products [19]. Among these, bio-char, the solid residue from pyrolysis, is particularly noteworthy due to its role as a carrier of electron acceptors and donors, along with its pH buffering capacity and cation exchange capabilities [20]. These properties, coupled with the influence of raw material composition and production methods, render bio-char highly reactive and suitable for various applications. On the other hand, bio-oil, the liquid product of pyrolysis, is characterized by a complex mixture of molecules derived from the pyrolysis of cellulose, hemicellulose, and lignin [21]. Unlike conventional petroleum fuels, bio-oil is distinguished by its dark brown, its viscous nature, and the absence or low content of harmful substances, such as sulfur, alongside a high concentration of organic acids [22]. These characteristics make bio-oil a promising candidate for high-value applications. Previous studies have highlighted that hydrophobic interaction is the primary driver of protein adsorption onto lignin, whereas a hydrophilic lignin surface, modified with acid groups, such as $-SO_3H$ or $-COOH$, exhibits reduced affinity for cellulase, thereby enhancing enzymatic hydrolysis [23]. This insight into lignin's surface chemistry has significant implications for improving the efficiency of lignocellulosic biomass conversion processes.

In this study, tobacco stalks, an abundant agricultural waste [24], were subjected to a biomass-conversion system encompassing carbohydrate saccharification and lignin pyrolysis (Figure 1). The stalks were pretreated with a sulfuric acid/sodium bisulfite mixture at 160 °C, followed by enzymatic hydrolysis to assess the saccharification efficiency of the pretreated biomass. Subsequently, the structure, thermal stability, and pyrolysis properties of the isolated lignin were analyzed, with the major components of the resulting pyrolytic oil identified using gas chromatography–mass spectrometry (GC–MS). This study aims to contribute to the development of more efficient and economically viable methods for biomass conversion, with a particular focus on the integrated utilization of lignin.

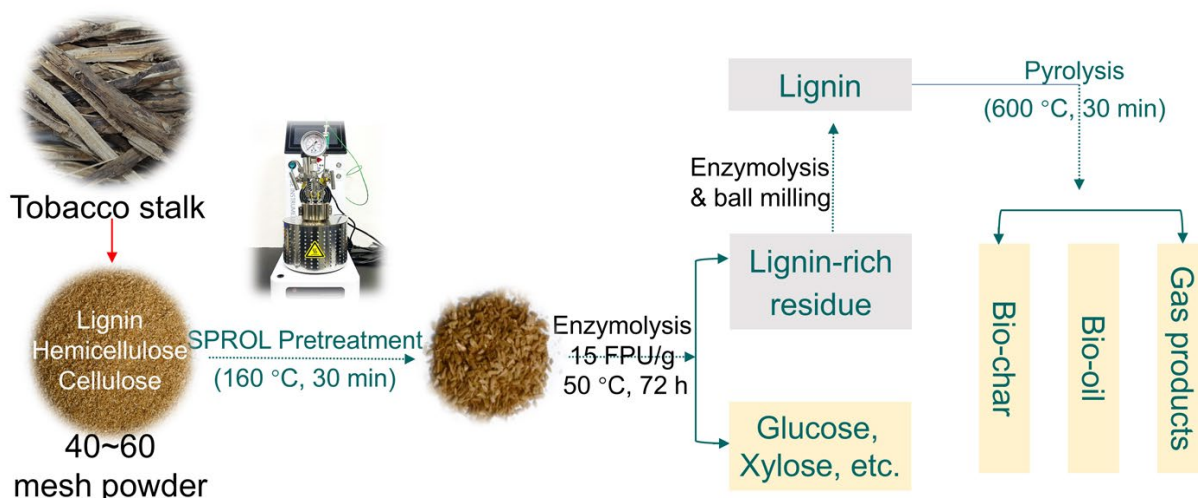


Figure 1. Schematic representation of sulfite pretreatment of tobacco stalk for bioenergy.

2. Results and Discussion

2.1. Composition Analysis

The compositional analysis results of the tobacco stalk powder before and after pretreatment using the conventional NREL method are shown in Table 1. The untreated tobacco stalk powder contains 19.4% lignin, 21.8% hemicellulose, and 34.1% cellulose. After 2.5% H₂SO₄ treatment, the hemicellulose content decreased to 16.4%, and around 60.8% of the hemicellulose was removed. Due to the reduction of the relative content of hemicellulose, the cellulose and lignin contents increased to 51.4% and 31.5%, respectively. Increasing the sulfite also increased the treated stalk yield and decreased the lignin content, which was attributed to the dissolution of lignin to enhance the pretreated tobacco stalk enzymolysis [25]. The lignin removal rate, compared with the raw material, was increased from 15.5% (DA pretreatment) to 27.7% (SP-B6 pretreatment) in general at a high sulfite dosage, but both the cellulose and the hemicellulose retention rates and the content in the pretreated substrates were enriched because of the reduced carbohydrate degradation, and it promoted delignification through sulfonation. When sulfite is introduced into the pretreatment liquor, it serves a pivotal role in adjusting the pH environment. By elevating the pH value, sulfite is a weak base, mitigating the acidity that could otherwise lead to undesirable consequences. This buffered environment prevents the cellulose and hemicellulose from undergoing excessive acid-catalyzed hydrolysis [26,27].

Table 1. Chemical compositions of the untreated and pretreated tobacco stalk.

Sample Label	^a Yield (%)	^b Cellulose (%)	^b Hemicellulose (%)	^b Lignin (%)
Raw material	-	34.1	21.8	19.4
DA	52.2 (67.4)	51.4 (78.5)	16.4 (39.2)	31.5 (84.5)
SP-B2	53.2 (67.7)	52.7 (82.1)	14.8 (36.2)	31.0 (84.8)
SP-B4	55.2 (70.4)	53.4 (86.3)	17.8 (45.0)	28.3 (80.2)
SP-B6	56.1 (71.0)	54.8 (90.0)	19.7 (50.6)	25.1 (72.4)

Note: ^a The yield of the tobacco stalk after pretreatment = the weight of the tobacco stalk after pretreatment/the weight of raw feedstock. The yield in parenthesis = the weight of (cellulose + hemicellulose + lignin) in the tobacco stalk after pretreatment/the weight of (cellulose + hemicellulose + lignin) in the raw feedstock. ^b The yield in parenthesis = the weight of cellulose or hemicellulose or lignin in the tobacco stalk after pretreatment/the weight of cellulose or hemicellulose or lignin in the raw feedstock. The margin of yields and composition error is within $\pm 5\%$.

2.2. Enzymolysis of the Pretreated Tobacco Stalk

To understand the chemical conversion capacity of the pretreated stalks, their enzymatic hydrolysis properties were characterized. As expected, Figure 2 shows that cellulose-based glucose selectivity of untreated tobacco stalks (the control) was the lowest, with a value of only around 26.8%. This is attributed to the inherent resistance of the lignocellulose. After pretreatment, there is a noticeable trend of increased glucose production. Notably, when enzymatic hydrolysis is carried out for 60 h, the saccharification approaches saturation, suggesting that the sample has undergone nearly complete conversion of its glucose content. Comparing samples treated with different amounts of sodium sulfite, when the amount of sodium sulfite increased from 0% to 6%, the glucose selectivity increased from 54.2% to 75.9% after 72 h of reaction. With the increase in the dosage of the sulfonating agent during pretreatment, the enzymatic hydrolysis activity of the treated tobacco stalks was significantly enhanced, which could be attributed to the destruction of the intact cell wall structure and the delignification. Compared to previous studies based on pretreatment with SPROL, the cellulose-based glucose selectivity in this study is higher than that of hardwood (e.g., aspen, 2% NaHSO₃) and softwood (e.g., pine, 8% NaHSO₃), with glucose selectivities of around 50~60% [28,29]. This is also comparable to corn stalks, which have glucose selectivities of about 80% [30]. In addition, the result is also similar to those from NaOH-treated tobacco stalks (69.0%) [31].

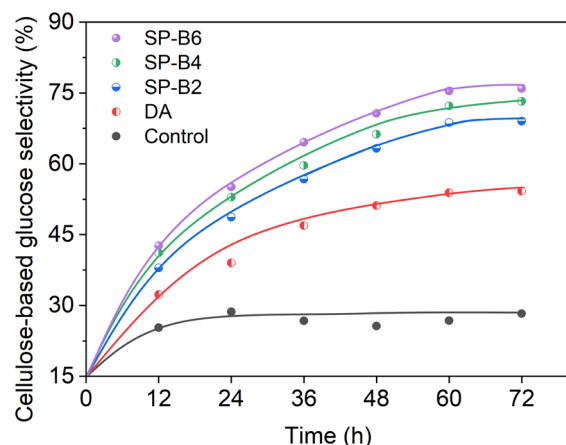


Figure 2. Results for cellulose based glucose selectivity arising from enzyme hydrolysis of raw material and pretreated tobacco stalks. (3 g pretreated tobacco stalk, 500 mL acetate buffer (200 mM, pH = 4.8), CTec3 enzyme 15 FPU/g substrate, 1 L Erlenmeyer flask, in a water bath shaker at 50 °C, 160 rpm, and atmospheric pressure; The margin of glucose selectivity error is within $\pm 5\%$).

2.3. Lignin Structure Analysis

2.3.1. Molecular Analysis

The molecular weight and distribution of lignin are important structural characteristics that affect its properties and reactivity. Figure 3 and Supplementary File Table S1 displays the Mw, Mn, and polydispersity index (where Mw is the mass-weighted molecular weight, Mn is the polymer chain number weighed molecular weight, and $PDI = Mw/Mn$, which is a measure of the broadness of the molecular weight distribution; the larger the PDI, the broader the molecular weight) of the lignin analyzed through GPC. For example, the Mw and Mn of the lignin resulting from the enzymatic hydrolysis of sample SP-B6 are 686 and 595 g/mol, respectively, with a PDI of 1.15. Figure 3 shows a slight decrease in Mw and Mn values with an increase in the content of sodium bisulfite during pretreatment, which is mainly due to the increase in the dosage of sulfonation agent ($NaHSO_3$) during the SPROL pretreatment process, leading to the breakage of molecular chains. The smaller the PDI, the narrower the molecular weight distribution, which means the obtained lignin is more uniform and favorable for utilization.

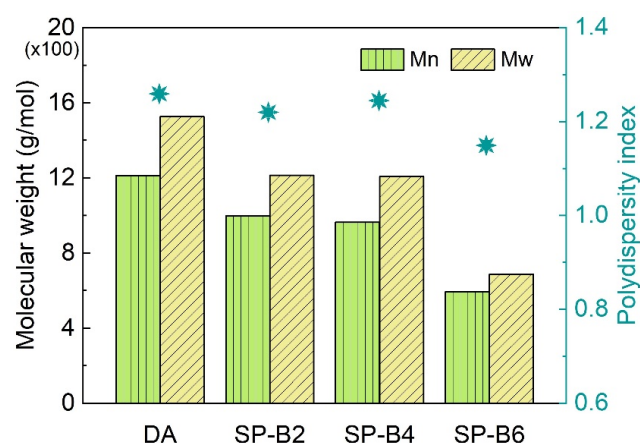


Figure 3. Mass-average (Mw) and number-average (Mn) molecular weight, and polydispersity index (PDI, Mw/Mn) of the isolated lignin from the enzymolysis treatment of tobacco stalks.

2.3.2. FT-IR and UV-Vis Spectra Analysis

The fingerprint region of the FT-IR spectra of different isolated tobacco stalk lignin after the enzymolysis step are shown in Figure 4a, where typical lignin bands are observed. Similarly to the original lignin from tobacco stalks (the control), the lignin extracted after

the enzymolysis of the sulfite treated stalks exhibits typical characteristics of grass lignin including the benzene ring vibrations of the phenylpropane skeleton at 1601, 1509, and 1423 cm^{-1} , as the intensity increased after the pretreatment [32]. Compared with the control lignin, the lignin treated through the SPROL process also exhibit new absorption peaks at 1050 and 1267 cm^{-1} assigned to sulfonic acid groups and whose intensity increased with the dosage of NaHSO_3 , indicating that the lignin was sulfonated [33]. Another significant difference is observed at 1652 cm^{-1} , which is attributed to the conjugated carbonyl groups ($\text{C}=\text{O}$ stretching vibration) during the sulfonation process [34].

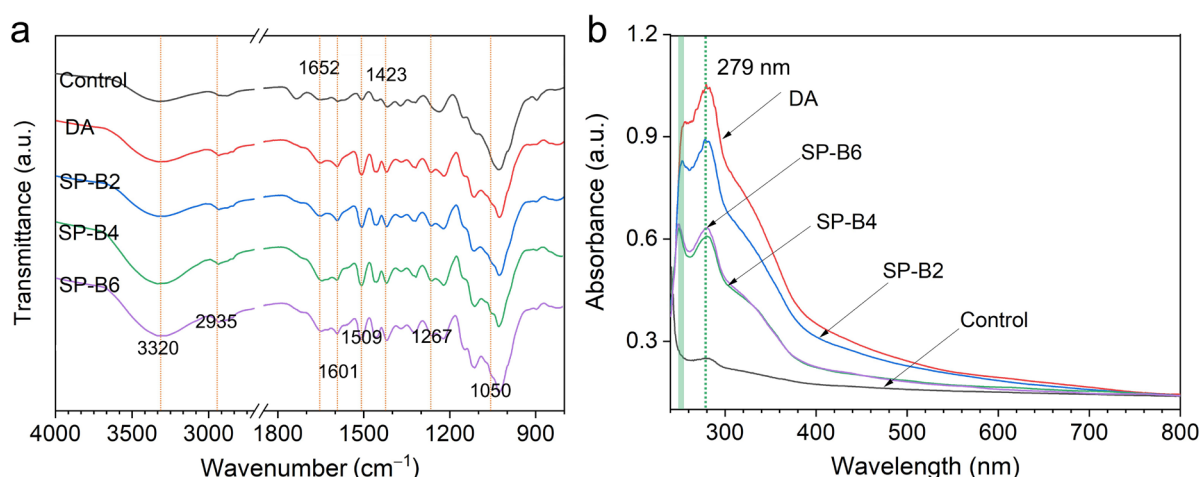


Figure 4. (a) FT-IR and (b) UV-Vis spectra of various isolated lignins from the enzymolysis treatment of tobacco stalks.

The UV-Vis absorption spectra of the original lignin and the pretreated lignin are presented in Figure 4b. It can be observed that the original lignin exhibits only a minor absorption at 279 nm, which corresponds to the electronic transition in aromatic-ring-conjugated molecular structures [32]. In contrast, the pretreated lignin exhibits a strong absorption peak at 279 nm, accompanied by a small shoulder peak at around 315 nm. Furthermore, as the dosage of sulfonation reagent increases, the absorption peak at 280 nm in UV-Vis decreases significantly, indicating that the lignin was esterified during the pretreatment process, which is consistent with the FT-IR results. Furthermore, the wavenumber of the phenolic hydroxyl group absorption peak also increased from 249.0 to 255.5 nm [35], which should be induced by the introduction of the sulfonic acid group. The induced functional group can affect the distribution of electron clouds, thereby changing the energy required for electron transition and resulting in the shift of absorption peaks to the long-wave direction.

2.4. Lignin Thermostability Analysis

The thermal stability of lignin is crucial for probing into its subsequent pyrolysis conversion [36]. Therefore, thermogravimetric analysis (TGA), which can reflect the thermal information of lignin in a straightforward manner, was applied to analyze all of the lignin samples [37,38]. Figure 5 shows the TG/DTG curves of all the studied lignin. The thermal decomposition process of the isolated lignin can be segmented into three stages. Firstly, some lower peaks at the temperature between 100 $^{\circ}\text{C}$ and 250 $^{\circ}\text{C}$ were observed, owing to the degradation of the weak or labile linkages in the residual polysaccharides and small fragments of lignin molecules. When the temperature increased up to 250–400 $^{\circ}\text{C}$, severe mass loss occurred, which could be attributed to the cleavage of β -O-4 and α -O-4 and the side chain oxidation of lignin, and simple substituted aryl compounds released [39]. The third stage occurs when the temperature is further elevated to above 400 $^{\circ}\text{C}$, at which the rate of weight change slows down. This process involves the cleavage of the C-C bond (5-5) in the lignin interior, and aromatic rings which collapsed and sedimented into residual char.

Furthermore, the shedding of methoxy (OCH_3) can also occur, and methane is released at this stage [40]. It was found that the DA lignin showed better thermal stability compared with other lignin samples. The maximum weight loss rate of DA lignin was at 304 °C, while the maximum weight loss rate of SPROL lignin samples was at 296~301 °C, indicating that as the dosage of sulfonating agent increased, the maximum weight loss temperature of lignin decreased. Moreover, compared with the control lignin, the lignin after pretreatment shows higher thermal stability as well as more pyrolytic residue, which could be due to its high purity.

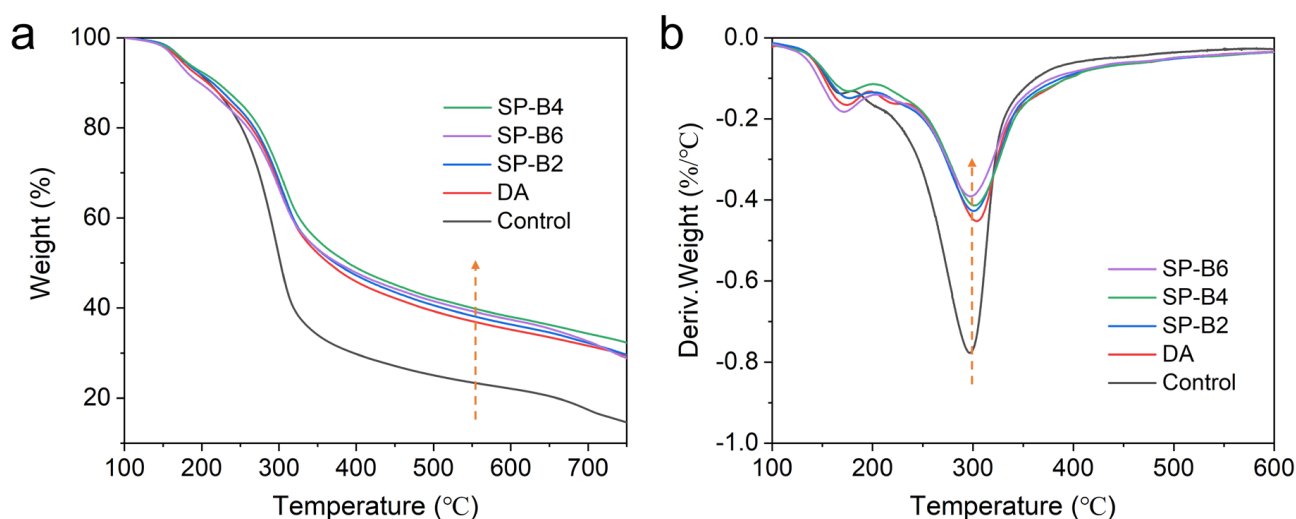


Figure 5. (a) TGA and (b) DTG curves of the studied lignin: control and isolated from the enzymolysis treatment of tobacco stalks.

2.5. Lignin Pyrolysis Properties and Pyrolysis Oil Analysis

Pyrolysis is one of the most promising technologies for converting lignin into biochemicals or bio-char. Typically, the optimal yield of bio-oil derived from biomass pyrolysis is obtained within the temperature range of 500 to 650 °C [41,42]. Therefore, the pyrolysis experiments in the present study were conducted at 600 °C, with simultaneous collection of pyrolysis oil. From Table 2, we can see that approximately 33~34% of the lignin undergoes conversion into bio-char as a result of the swift removal of hydrogen and oxygen elements within its molecular structure during the pyrolysis process [43]. Compared with the yield of DA lignin bio-char, the yield of bio-char for SPROL-pretreated lignin decreased slightly. In addition, the result of the bio-char yield is similar to the thermogravimetric analysis, in which the residue rate at 600 °C was around 35~38%. Lignin is a hydrogen and oxygen-deficient material, and the decomposition of hydrogen and oxygen atoms prompts the formation of aromatic-ring-positive ions that subsequently engage in intramolecular ring restructuring and intermolecular aromatization reactions, ultimately culminating in the condensation of these components into bio-char. The experimental results also display that the yield of bio-oil for SPROL lignin decreased and the bio-gas yield increased a bit compared with the DA lignin pyrolysis result. However, there were no obvious differences between different SPROL lignin in the bio-oil, bio-char, and bio-gas yield, which could be due to their similar structure.

Table 2. The yield of pyrolytic char, oil, and, gas for the lignin samples at 600 °C.

Sample ID	Bio-Char (%)	Bio-Oil (%)	Gas Products (%)
DA	34.26	23.60	42.14
SP-B2	33.08	22.07	44.85
SP-B4	33.00	22.38	44.62
SP-B6	33.32	21.95	44.73

As is shown in Figure 6 and Supplementary File Table S2, the main detectable components of bio-oil obtained from lignin pyrolysis are acid (e.g., acetic acid), aromatic derivatives (e.g., phenol, 3-methyl-phenol, etc.), and aliphatic hydrocarbon (1,5-Heptadien-3-yne, 2,4-Hexadiyne, etc.). Acetic acid is the most abundant identified GC-MS component and the proportions of acetic acid content are 45.27 wt.%, 57.93 wt.%, 69.91 wt.%, and 67.64 wt.% for DA, SP-B2, SP-B4, and SP-B6 lignin pyrolysis identifiable oil, respectively. The main source of acetic acid could be from the breakdown of the acetyl group in the hemicellulose, the further degradation of unhydrolyzed carbohydrates, or the cleavage of the C α -C β bond in the alkyl side chain of lignin units with the -CH₂OH or -COOH structure at the γ position [44,45]. The most abundant monomeric aromatic are 2-(2-Hydroxy-2-phenylethoxy)phenol and 3-methyl-phenol, which could be attributed to the stabilization process of the cracked radicals of C β -O and C α -O [46]. The generation of aliphatic hydrocarbons that contain two double bonds, such as 2,4-Hexadiyne and 1,5-Heptadien-3-yne, is attributable to the aromatic ring's propensity for undergoing ring-opening reactions and the secondary reaction of furans, propargyl, and monocyclic aromatic hydrocarbons at high temperatures [47]. The high acetic acid content enabled the bio-oil to further separate into bio tar and pyroligneous acid (a dark liquid produced through the destructive distillation of wood and other plant materials; it mainly contains acetic acid, and acetone), which have been widely applied in the food industry, agriculture, and the horticultural field [48,49]. Taken together, these results show that tobacco stock pretreated at SP-B4 or SP-B6 would be the ideal conditions for its comprehensive utilization, which ensures that the pretreated tobacco stock has high enzymatic hydrolysis efficiency while realizing high value utilization of lignin for pyroligneous acid and wood tar.

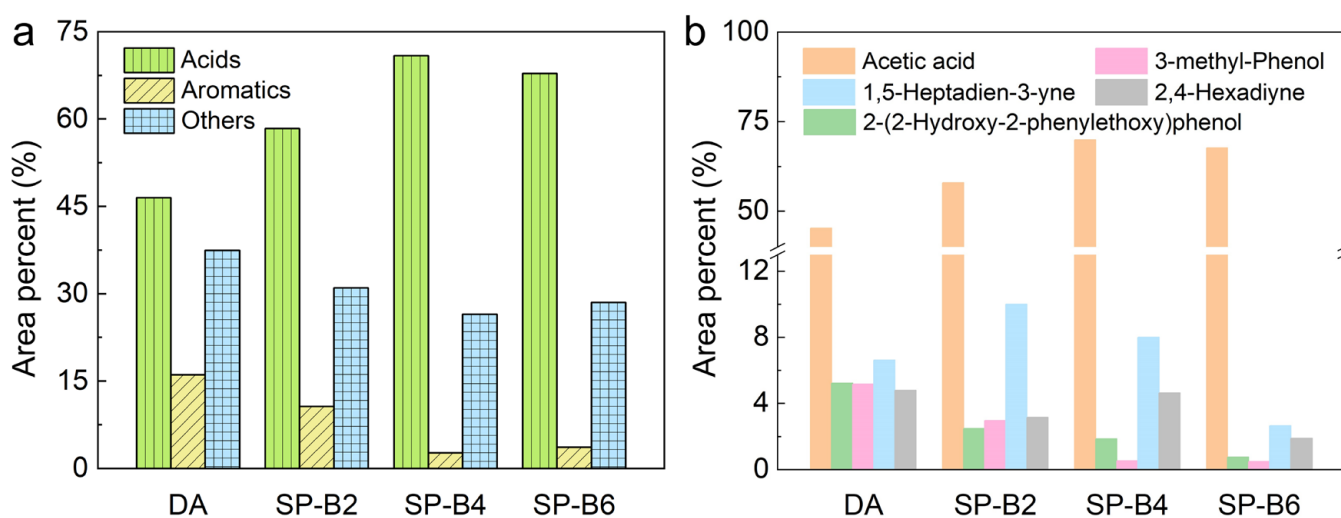


Figure 6. (a) Distribution of detectable chemical families: acids (e.g., acetic acid), aromatics (e.g., phenol, 3-methyl-phenol, etc.), and hydrocarbons (e.g., 1,5-heptadien-3-yne, 2,4-hexadiyne). (b) The main detectable components identified through GC-MS of bio-oil obtained from different pretreatments of tobacco stalk.

Overall, the SPORL pretreatment combined pyrolysis platform offered significant advantage. First, around 74% of the three major components was recovered following the SPORL pretreatment (Figure 7), which reduces the harm of tobacco stalk to the environment. Additionally, after pyrolysis, around 55% of the lignin components was converted to bio-char and bio-oil. Thus, this new model is green and sustainable for tobacco stalk utilization, with excellent environmental performance, and it will be conducive to a reasonable carbon balance due to less carbon waste is generated.

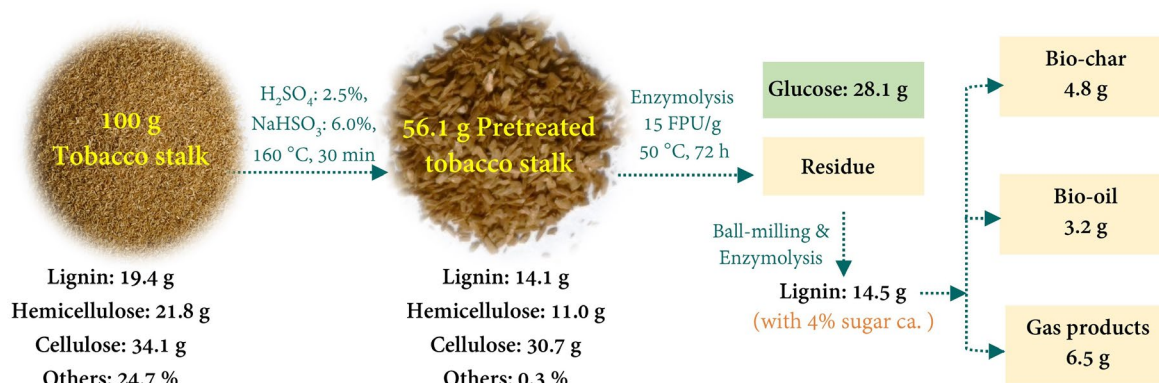


Figure 7. Mass balance around the whole fractionation process of tobacco stalk through SPORL pretreatment (H_2SO_4 2.5 wt.% and NaHSO_3 6.0 wt.%) and combined pyrolysis.

3. Materials and Methods

3.1. Materials

Tobacco stalk (Supplementary File, Figure S1) was kindly supplied by Hubei Xinye Reconstituted Tobacco Development Co. Ltd. (Wuhan, China). The chemical composition of the stalk includes cellulose (34.1 wt%), hemicelluloses (21.8 wt%), and lignin (19.4 wt%). The tobacco stalk was pulverized to a 40–60 mesh using the IKA MF-10 pulverizer and extracted using an ethanol/benzene (1:2, *v/v*) mixture to remove the extractives. H_2SO_4 , NaHSO_3 , and 1,4-Dioxane were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), CTec3 enzyme was kindly provided by Novozymes Biotechnology Co., Ltd. (Beijing, China, 160 FPU/mL, determined according to NREL method [50]).

3.2. Tobacco Stalk Pretreatment

In order to disrupt the lignin structure and break the linkages between lignin and the other carbohydrate fractions in the lignocellulosic biomass, thus making the carbohydrates in the hetero-matrix more accessible, dilute acid (DA) and SPORL treatment methods served to treat the tobacco stalk according to the previous literature, but with a slight modification [14]. The pretreatment conditions consist of a solid-to-liquid ratio of 1:5 (weight ratio), heating from room temperature to 160 °C (around 0.6 MPa) in 40 min, with a hold for 30 min and with a chemical dosage of H_2SO_4 and NaHSO_3 as listed in Table 3. For SP-B2, for example, 10 g of oven-dried stalks was treated with 0.25 g of H_2SO_4 , 0.20 g of NaHSO_3 , and 49.75 g of H_2O . The experiment was conducted in a lab-scale high-pressure reactor with a glass liner under magnetic stirring (600 rpm) (BE100, Labe Instruments, Beijing, China); after the reaction, the pretreated tobacco stalk was separated from the pretreatment hydrolysate and washed with deionized water (solid-to-water ratio around 1:100, washed 4–6 times until the pH test paper showed the washed water was neutral (Supplementary File, Figure S1)).

Table 3. Tobacco stalk pretreatment conditions.

Sample Label	Chemical Dosage on Tobacco Stalk [wt.%]
DA	H_2SO_4 : 2.5
SP-B2	H_2SO_4 : 2.5, NaHSO_3 : 2.0
SP-B4	H_2SO_4 : 2.5, NaHSO_3 : 4.0
SP-B6	H_2SO_4 : 2.5, NaHSO_3 : 6.0

The pretreatment conditions: amount of stalk: 10 g; solid-to-liquid ratio: 1:5 (weight ratio); time to heat up: 40 min; pretreatment temperature: 160 °C (around 0.6 MPa); holding time: 30 min; magnetic stirring: 600 rpm/min; reactor size: 100 mL.

3.3. Enzymatic Hydrolysis

The cellulose and hemicellulose in the tobacco stalk can be enzymatically hydrolyzed into fermentable sugars, and the lignin-rich residue that results from the saccharification

process can also be used for bioenergy. In order to reach that goal, 3 g of pretreated tobacco stalk was added to 500 mL of acetate buffer (200 mM, pH = 4.8) along with CTec3 enzyme at 15 FPU/g substrate in a flask. The enzymolysis was conducted in a water bath shaker at 50 °C and 160 rpm over a 72 h period. Once the enzymatic hydrolysis was complete, the hydrolysate was collected into tubes and subjected to a 5 min denaturation process in a boiling water bath, aimed at inactivating any residual enzymes. Subsequently, the mixture underwent filtration through a 0.22 µm syringe filter, yielding a clarified supernatant that was then injected into an autosampler vial for sugar analysis. Samples were analyzed by HPLC using a Biorad Aminex HPX-87P column equipped with a Biorad 1250119 Carbo-prefill 30 × 4.6 mm guard column. The HPLC conditions for sugar analysis are set as follows: mobile phase: deionized water (filtered through a 0.2 µm film and degassed twice); flow rate: 0.6 mL/min; column temperature: 85 °C; detector: refractive index; run time: 30 min.

The amount of glucose formed divided by the amount of initial cellulose in feed × 100 was used to express the “Cellulose-based glucose selectivity (%)”. The definition is based on the assumption that glucose primarily forms from cellulose, and the experimental data of the cellulose-based glucose selectivity are the average values based on triplicate runs.

3.4. Lignin Isolation

The abovementioned enzymatically hydrolyzed lignin-rich residue was washed extensively with acetate buffer (pH 4.8) and deionized water and then freeze-dried. The dried residual solid was further subjected to zirconia vibratory ball milling (Chuo-kakohki, Toyoake, Japan, MB-I type) with 50 Hz for 48 h. We repeated the abovementioned enzymatic hydrolysis and ball milling procedure was repeated, and the lignin present in this residue (Figure 1) was collected via centrifugal separation followed by deionized water washing; it was used for further analysis and a pyrolysis study.

3.5. Lab-Scale Fixed-Bed Lignin Pyrolysis

The lignin obtained from Section 3.3 was pyrolyzed using a fixed-bed pyrolysis reactor (Carbolite Gero 30~3000 °C). First, the temperature of the tube (600 mm × Ø 26 mm) and the furnace was elevated to 600 °C, followed by introducing high-purity N₂ at an initial flow rate of 500 mL/min for 5 min to guarantee an oxygen-free environment (ambient pressure). Afterward, the N₂ flow was adjusted downwards to 200 mL/min. Around 0.5 g of the abovementioned lignin powder was carefully placed into a quartz wool-lined basket (r = 2 cm, h = 7 cm), which was promptly positioned in the heating zone of the furnace and pyrolyzed for 30 min (Figure S2). To collect the bio-oil, the tube's lower end was connected to a condenser, which was immersed in an ice-water bath. Subsequently, the bio-oil was collected by rinsing the condenser with acetone. The bio-char and bio-oil yields were calculated based on their weight ratio to the feedstock. The gas yields were calculated based on the mass balance, as follows:

$$\text{Gas yields} = (W_R - W_C - W_O) / W_R \times 100\%$$

where W_R is the weight of the raw stock, W_C is the weight of the bio-char, and W_O is the weight of the bio-oil.

3.6. Structural and Composition Characterization

The chemical compositions for cellulose, hemicellulose, and lignin of the tobacco stalk before and after treatment were determined according to the National Renewable Energy Laboratory (NREL) method [51], which is indicated in Table 1. In brief, about 0.3 g of (oven-dried) biomass was weighed into serum bottles. We then the bottles were kept under agitation with stirring rods in a water bath at 30 °C, followed by addition of 3 mL of 72% sulfuric acid into the bottles. Mixing with stirring rods was performed every 5 min. After 60 min, the content was diluted with 84 mL of water to stop the hydrolysis. The serum bottles were then sealed and autoclaved at 121 °C for 60 min. After that, the content was

passed through a crucible filter with known weights. Samples of the filtered liquor were collected for sugar analysis, after which more DI water was added to each bottle to recover any solid leftover in the bottles for acid-insoluble lignin analysis. For the lignin molecular weight, the lignin samples from Section 3.4 were dissolved in THF with a concentration of 2 mg/mL. The mass-average molecular weight (M_w), the number-average molecular weight (M_n), and the polydispersity (M_w/M_n) of lignin were determined through GPC (LC-20AD, Shimadzu, Osaka, Japan) with a column of 10- μ m PLgel MIXED-B. Fourier transform infrared (FT-IR) transmittance signals of the lignin powder were directly recorded using a Thermo Scientific Nicolet 6700 ATR-FTIR (Waltham, MA, USA, attenuated total reflection–Fourier transform infrared spectrometer) equipped with a Ge crystal, which scanned in the wavenumber range of 4000–800 cm^{-1} with 32 scans at a resolution of 4 cm^{-1} . The lignin sample was dissolved in a water/dioxane mixture ($v:v=1:1$) with a concentration of 0.15 mg/mL, the water/dioxane mixture was used as a reference, and the absorbance curve was detected using an ultraviolet spectrophotometer (Hitachi U-3900, Ibaraki, Japan) in the range of 200–800 nm. The thermostability of the lignin sample (around 5 mg) was characterized using a thermogravimetric analyzer (DTG-60, Shimadzu, Osaka, Japan) under N_2 condition with a heating rate of 10 $^{\circ}\text{C}/\text{min}$ from room temperature to 800 $^{\circ}\text{C}$. The pyrolysis liquid was detected using an Agilent 7890A-5975C gas chromatograph–mass spectrometer (Palo Alto, CA, USA, GCMS) with an Agilent HP-5MS column, following the previously described method. The peak area is used to semi-quantitate the proportion of each pyrolysis product [25].

4. Conclusions

In this study, a joint approach using the SPORL pretreatment and biomass pyrolysis to upgrade tobacco stalk into fermentable sugar, bio-oil, and bio-char was proposed. Tobacco stalk after pretreatment with H_2SO_4 2.5 wt.% and NaHSO_3 6.0 wt.% with a solid to liquid of 1:5 (weight ratio) at 160 $^{\circ}\text{C}$ for 30 min had a cellulose based glucose selectivity of 75.9% after 72 h enzymolysis. The lignin characterization reveals that sulfonation occurred during SPORL pretreatment, and as the dosage of the sulfonating agent increased, the maximum weight loss temperature of lignin decreased. After sample pyrolysis, approximately 22% and 33% of the lignin underwent conversion into bio-oil and bio-char, respectively. The bio-oil analysis results demonstrated that acetic acid is the most abundant identified GC-MS component at around 69.9 wt.% of the detectable chemicals in optimal conditions. This research provides a synthetic approach to the commercial utilization of tobacco stalk waste as value-added products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/catal14120889/s1>, Figure S1: Tobacco stalk with different forms: raw stalk, after smash, and after pretreatment.; Figure S2: Lab-scale fixed bed lignin pyrolysis schematic diagram and instrument. Table S1: Mass-average (M_w) and number-average (M_n) molecular weight, and polydispersity index (PDI, M_w/M_n) of the isolated lignin from the enzymolysis treatment of tobacco stalks; Table S2: Distribution of main GC-MS detectable chemicals.

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