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An integrated green fractionation of coconut husk: Hydrothermal and deep eutectic solvent pretreatment for enhanced sugar and lignin production

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ABSTRACT

Coconut husk was explored for sugar and lignin production using an integrated approach combining hydrothermal treatment (HT) and deep eutectic solvent (DES) pretreatments, followed by enzymatic saccharification, to enhance lignocellulose fraction recovery. Optimized HT conditions yielded 4.802 g/L of total reducing sugars, including 0.223 g/L glucose and 0.998 g/L xylose, with minimal degradation products. Subsequent DES pretreatment with choline chloride and lactic acid effectively removed lignin, recovering high-purity lignin with a native-like structure. The combined pretreatment resulted in a cellulose-rich solid, enhancing saccharification yield by 3.24-fold compared to single pretreatments. This improvement stemmed from the extensive disruption and removal of non-cellulose components, confirmed by solid characterizations. The integrated process demonstrated a synergistic effect, achieving 31.3 % total sugar recovery and 48 % lignin recovery, highlighting the potential of coconut husk as a promising biorefinery feedstock.

1. Introduction

The depletion of fossil fuel resources and the high greenhouse gas emissions (GHG) from petroleum consumption have created an urgent need to find eco-friendly alternatives (Kumar et al., 2023). Lignocellulose, with its significant potential as a renewable carbon source, can be converted into bioenergy, biochemicals, and high-value biomaterials (Gundupalli et al., 2022; Yaashikaa et al., 2022; Zhang et al., 2023). Additionally, the abundance of lignocellulose, particularly from agricultural waste, presents a nearly limitless source that could be utilized without a net carbon footprint (Jennita Jacqueline et al., 2022). Lignocellulose is primarily composed of 38–50 % hemicellulose, 23–32 % cellulose, and 15–25 % lignin (Hoang et al., 2022; Sun et al., 2023). However, for lignocellulosic biomass with lignin-rich content, such as coconut husk, the composition is reported to be 15–17 % hemicellulose, 26–28 % cellulose, and 32–41 % lignin (Anuchi et al., 2022; Mankar et al., 2022; Muharja et al., 2018). These values contribute further to the structure recalcitrant nature in lignin-rich biomass. Consequently, an extensive pretreatment is required to obtain the three major fractions separately (Banu Jamaldheen et al., 2022; Han et al., 2022).

Coconut husk (CCH) is a potential agricultural waste in Indonesia with an abundant availability of coconut fruit around 2.86 million tons produced annually (Zikria, 2022). Compared to other agricultural wastes, CCH has a high lignin content, which can be valorized into valuable biochemicals through biorefinery processes. However, this rich lignin content also makes CCH more resistant to pretreatment compared to other biomasses. The lignin in coconut husk acts as a physical barrier, protecting the biomass from chemical degradation and microbial attack. Furthermore, during enzymatic hydrolysis, lignin prevents the productive binding of cellulase and xylanase to the biomass (Jia et al., 2022; Liu et al., 2023). Therefore, an efficient delignification process must be conducted prior to the biorefinery of coconut husk to achieve excellent performance.

Various pretreatment methods, including hydrothermal, alkali, microwave, ultrasound, ionic liquid, and deep eutectic solvent, have been investigated for overcoming the recalcitrant nature of lignocellulosic biomass especially CCH (Anuchi et al., 2022; Jose and Sajeena Beevi, 2023; Mankar et al., 2022; Muharja et al., 2020). Each process has its own advantages and disadvantages. Observed advantages include increased reducing sugars yield from pretreatment hydrolysates or enzymatic hydrolysis, decreased lignin content, and higher cellulose purity. However, some drawbacks have also been reported, such as the

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need for further downstream processing, high energy and chemical costs, and low biodegradability.

Deep eutectic solvent pretreatment, a green solvent with high biodegradability and low toxicity, has shown promise in extracting hemicellulose and lignin from lignocellulose (Rodríguez-Rebelo et al., 2024; Sun et al., 2023). Consequently, additional steps are required after the pretreatment in order to separate the hemicellulose and lignin from the mixture. On the other hand, hydrothermal pretreatment or hydrothermal treatment (HT) has demonstrated an appreciable yield of reducing sugars and only significantly removes the hemicellulose component. Moreover, the nature of HT is considered a green and relatively inexpensive process, as it only uses water and heat (Yang et al., 2023; Yue et al., 2022a). The high-temperature shift in water properties creates an acidic environment that facilitates the hydrolysis of more amorphous structure polysaccharide, hemicellulose into their monomeric components by cleavage of glycosidic bond, while preserving the more resistant constituents, cellulose and lignin in the solids (Saini et al., 2024; Singh et al., 2023; Sun et al., 2021; Yue et al., 2022a). Both pretreatment methods have shown promising results in enhancing the enzymatic saccharification process and fractionation of the lignocellulose component. However, a stand-alone pretreatment is insufficient to yield the hemicellulose, cellulose, and lignin fractions. Therefore, the combination of pretreatments is necessary for effective lignocellulose valorization.

The development of a consecutive HT and deep eutectic solvent (DES) pretreatment process would yield selective results. In HT, besides hydrolyzing hemicellulose, the cleavage of lignin-hemicellulose bonds occurs, which enhances the subsequent delignification step (Brenelli et al., 2022). To improve the benefits, cost-effectiveness, and energy requirements of the pretreatment, and minimize downstream processing, optimization to maximize sugar concentration and minimize sugar degradation products is crucial to achieve an economically feasible process (Ruiz et al., 2023). In the subsequent DES pretreatment, solvents consisting of hydrogen-bonding donors and acceptors form eutectic mixtures with very low melting points (Provost et al., 2022; Yue et al., 2022b). DES with acid-based hydrogen-bonding donors, such as lactic acid and acetic acid, have demonstrated desirable delignification. For instance, ChCl:LA DES has been reported to delignify 80.1 % of lignin from bamboo biomass (Wang et al., 2021). The proposed proton-catalyzed mechanism of bond cleavage suggests that stronger acidity would result in higher delignification performance, making ChCl:LA a promising candidate (Hong et al., 2020). However, the strategy for utilizing both the sugar and lignin fractions from the high-lignin coconut husk (CCH) biomass has not been thoroughly investigated.

From aforementioned background, this study aimed to optimize the HT process to maximize the yields of total reducing sugars, xylose, and glucose, while minimizing the formation of degradation products (furfural and 5-hydroxymethylfurfural). Additionally, the pretreated solids obtained from the optimized conditions were further subjected to deep eutectic solvent (DES) pretreatment to facilitate the delignification process. The regeneration of the extracted lignin and enzymatic hydrolysis were investigated. Finally, the synergistic effects to produce lignocellulose fractions between the two pretreatment methods were discussed.

2. Materials and methods

2.1. Materials

The CCH waste biomass was obtained from a local traditional market in Surabaya City, Indonesia. It was prepared adapting to NREL method (Hames et al., 2008), which the CCH was washed, sun-dried, and mechanically ground to a 40–60 mesh size. L-lactic acid and choline chloride, were used as a constituent of the deep eutectic solvents and purchased from Merck, Germany and Sigma-Aldrich, USA, respectively. Cellulase enzyme from *Trichoderma reesei* was purchased from Sigma-Aldrich, USA. Other chemicals of analytical grade purity were acquired from Sigma Aldrich, USA.

2.2. HT optimization by using RSM

For the purpose of multi-response optimization, certain constraints were set. The optimization process considered the presence of sugar degradation products, as these can inhibit fermentation and microbial growth, thereby affecting the overall efficiency of biofuel production processes (Yang et al., 2022). Hence, the goal of this RSM study was to maximize the reducing sugars yield while minimizing the degradation products, within acceptable limits for the fermentation process. Furthermore, the CCH pretreated under the optimal conditions was subsequently subjected to DES pretreatment.

The HT was conducted in a stainless-steel reactor, which was adapted from the work of Muharja et al., 2018. For the HT, 6 g of coconut husk were mixed with 120 mL of distilled water and loaded into the stainless-steel reactor. The reactor pressure was maintained at 6 MPa using CO_2 gas. The reactor was then heated to the selected HT conditions. After the pretreatment was complete, the reactor was cooled in an ice bath, and the mixture of pretreated solids and HT hydrolysate was separated by vacuum filtration. The filtrate and pretreated solids were subsequently subjected to analytical procedures.

The independent variables of HT, temperatures and times, were chosen to be optimized by RSM with a central composite design (CCD). This design of experiment offers fewer experimental runs to fit a quadratic surface rather than full factorial design, making it more efficient and cost-effective since multiple response and factors were explored (Koraqi et al., 2024). It also allows for the estimation of curvature and interaction effects between factors, providing a more comprehensive understanding of the system under study while keeping the efficiency of experimental cost (Gundupalli and Bhattacharyya, 2021). Statistical and design of experiment evaluation were conducted on Minitab 16 statistical software and MATLAB R2023b. The selected values for the RSM designs are shown in Table 1.

From Table 1, the CCD used in this study consisted of 4 factorial runs, 4 axial points, and 5 center point runs, for a total of 13 runs. Meanwhile, the responses for the optimization of this process were total reducing sugars (TRS), glucose, xylose, furfural, 5-HMF, recovered solids, and pH of HT hydrolysate as to be modeled by following the nonlinear regression equation below (1).

$$Y_n = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j \tag{1}$$

where Y_n is the response, β_0, β_i, β_{ii}, and β_{ij} are constant, linear, quadratic, and interaction coefficients respectively. The independent factors, temperature and time, are denoted as X_1 and X_2 respectively. The significance of the regression models was evaluated using analysis of variance (ANOVA), the coefficient of determination (R^2), and lack-of-fit analysis (Jose and Sajeena Beevi, 2023; Mohan et al., 2014). Lastly, the multi-response optimization was conducted, and the optimal conditions were repeated in triplicate to validate the RSM optimization.

2.3. DES pretreatment, DES recycle, and lignin recovery

DES was prepared by mixing lactic acids and choline chloride by a molar ratio equal to 10:1 and heated in a closed round bottom flask at

Table 1
RSM designs of hydrothermal pretreatment.

Independent factors	Coded values & natural values				
	−α	−1	0	1	α
Time (min)	17.6	60	80	100	108.3
Temperature (°C)	167.6	180	210	240	252.4

70 °C with stirring until a clear and homogeneous liquid was formed. Then the mixture was cooled down to room temperature before use (Alvarez-Vasco et al., 2016).

For DES pretreatment, 4 g of CCH was mixed with 120 g of DES in a 250 mL 3-neck round bottom flask. The pretreatment was conducted at 120 °C and 6 h (Wang et al., 2021). After the end of pretreatment, 100 mL of ethanol was added to the slurry and subsequently vacuum-filtrated. Then, the residual solids were washed with 300 mL of ethanol and followed by 300 mL of water. Afterward, the residual solids were dried in an oven at 60 °C before further analysis. The lignin rich DES was further processed to regenerate the lignin (Alvarez-Vasco et al., 2016). Deionized water was added with ratio filtrate to water 1:5 and let the lignin precipitated. Precipitated lignin separated by using centrifuge (HERMLE Z306 universal centrifuge) with 10,000 rpm for 10 min. Lignin was dried at oven with temperature 60 °C before characterization. The DES pretreatment experiment were repeated 3 times to maintain the reproducibility.

Following the DES pretreatment and lignin recovery, the recycling process of the deep eutectic solvent (DES) was carried out to ensure sustainable reuse in subsequent pretreatment cycles which adapted from elsewhere (Shen et al., 2019). After separating the lignin-rich DES filtrate through centrifugation, the recovered DES was subjected to ethanol and water removal by rotary evaporation at approximately 50 °C to eliminate residual solvents. The recovered DES was directly reused for subsequent pretreatment cycles without additional purification to assess the performance of pretreatment cycle with compositions and removals of CCH.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated CCH samples was conducted using cellulase enzyme, following a method adapted from the work reported by Huo et al., 2023. The experiments were carried out in triplicate. In this process, 0.5 g of the solid residues (2.5 % *w/v*) of raw or pretreated CCH was combined with a buffer solution containing citric acid and sodium citric (pH 4.8). Subsequently, 20 FPU/g of cellulase, was added to the mixture. The resulting mixture was then placed in a shaker at 50 °C for 72 h, with a rotational speed of 150 rpm. After the end of the process, the hydrolysates were separated with residual solids and subsequently proceed to sugar analysis. The yield of saccharification was calculated by Eq. (2) (Tang et al., 2023).

$$\%Saccharification\ Yield = \frac{released\ glucose \times 0.9_{(gram)}}{cellulose\ in\ substrate_{(gram)}} \times 100\% \tag{2}$$

2.5. Analytical method

2.5.1. HT liquid fraction and solid fraction compositional analysis

Solid and liquid fraction from HT were separated by vacuum filtration. TRS, glucose, xylose, furfural and 5-HMF were determined from liquid fraction. TRS was measured by dinitrosalicyclic acid (DNS) method (Miller, 1959). Monomeric sugars, xylose and glucose were determined by high performance liquid chromatography (HTLC), Waters 2414 with refractive index detector (RID), Rezex ROA-Organic Acid H^+ Column (Phenomenex, California, USA). The mobile phase is H_2SO_4 0.005 M with flowrate 0.6 mL/min. Temperature of column and detector were maintained at 60 °C and 40 °C respectively (Muharja et al., 2019). Besides, Furfural and 5-HMF were analyzed by gas chromatography-flame ionization detection (GC-FID) method (GC-2010 Plus Shimadzu, Kyoto, Japan), based on the approach described by (Gaspar and Lopes, 2009; Muharja et al., 2019). The separation was performed on an RTX-Wax column, 30 m × 0.25 mm internal diameter, with a film thickness of 0.25 μm. The injection and detector temperatures were set to 250 °C and 275 °C, respectively, with the injector operating in split mode. The initial column temperature was maintained at 80 °C for 5 min, then increased to 110 °C at a rate of 10 °C per minute and held for 3 min. Subsequently, the oven temperature was raised to 120 °C at 2 °C per minute, further increased to 150 °C at 15 °C per minute, and held for 3 min. Finally, the temperature was set to 165 °C and held for 8 min. Nitrogen and hydrogen were used as carrier and ignition gases, with flow rates of 522.6 and 40 mL per minute, respectively.

The solid fractions compositional analysis of optimal HT, DES, Hydrothermal-DES pretreatment and raw CCH were analyzed by Chesson-Datta method (Datta, 1981) and NREL protocol for ash content (Sluiter et al., 2008). These methods determined lignocellulose composition (hemicellulose, cellulose, and lignin) with step-by-step fractionation and gravimetric procedure.

Meanwhile, the recovered solids of CCH, removal of CCH components, and lignin yield were also quantified as the following Eqs. (3)–(5).

$$Recovered\ Solids\ (\%) = \left(\frac{Pretreated\ CCH\ mass_{(g)}}{Initial\ CCH\ mass_{(g)}}\right) \times 100 \tag{3}$$

$$R_i(\%) = \left(1 - \frac{pretreated\ CCH\ component\ mass_{(g)}}{initial\ CCH\ component\ mass_{(g)}}\right) \times 100 \tag{4}$$

$$Lignin\ Yield\ (\%) = \left(\frac{recovered\ lignin_{(g)}}{initial\ lignin\ in\ raw\ CCH_{(g)}}\right) \times 100 \tag{5}$$

where R_i is the removal of CCH component i (hemicellulose, cellulose, or lignin).

2.5.2. Crystallinity, morphology, and functional group analyses of raw and pretreated CCH

Solid fractions of CCH from optimal HT, DES, hydrothermal-DES, and raw CCH were analyzed to understand the effect of pretreatments with crystallinity, morphology, and functional group of pretreated CCH. Crystallinity was investigated according to Segal's method as it compares crystallinity as a relative value, crystallinity index (CrI), (Fatmawati et al., 2023; Segal et al., 1959) by using X-ray diffraction (XRD) (X'Pert PRO, PAN-analytical BV, Netherland). The XRD was operated using Cu Kα radiation, 40 kV, 30 mA. Index crystallinity was used as parameter and calculated as follows:

$$CrI\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \tag{6}$$

where I_{002} is the intensity of 002 cellulose diffraction plane at (2θ) 22° and I_{am} is the intensity of amorphous region for cellulose which identified at (2θ) 18°. The morphological changes of the CCH after pretreatments were observed using a scanning electron microscope (SEM) (Hitachi FlexSEM 1000, Japan), with a beam accelerating voltage of 5000 V, magnification of 2000 times, and a working distance of 7.7 mm. Lastly, the functional group change of the CCH was analyzed by Fourier transform infrared (FT-IR) spectroscopy (FT-IR MODEL 4200 JASCO, Japan) over a spectral range of 400 to 4000 cm^{-1}, with a resolution of 1 cm^{-1} and 64 scans.

2.6. Lignin characterization

Purity of lignin was determined by acid hydrolysis as Klason's lignin according to NREL protocol (Yue et al., 2022b). NMR spectroscopy analysis was conducted to examine the DES Lignin samples from sequential hydrothermal-DES pretreatment (Mankar et al., 2022; Sunar et al., 2024). Specifically, 30 mg of samples were dissolved in 0.6 mL of DMSO-d_6 in Teflon tape-sealed vials and sonicated below 50 °C. Subsequently, the ^1H spectra of DES lignin were measured using a NMR, Bruker AMX-400 spectrometer at 298 K. Meanwhile, 2D-HSQC NMR of lignin was measured by using Bruker Avance Neo-Ascend 500 with frequency 500 MHz. Lastly, the functional group analysis of regenerated lignin was determined by FT-IR spectroscopy (FT-IR MODEL JASCO, Tokyo, Japan).

2.7. Significance test

One-way ANOVA and post-hoc Tukey's analysis by using MATLAB R2023b were conducted to assess the significance of each pretreatment impact to the saccharification yield, composition and removal of CCH components.

3. Results and discussions

3.1. Hydrothermal pretreatment optimization

The surface plots in Fig. 1, illustrate the responses of reducing sugars, degradation products, recovered solids, and pH of the hydrothermal pretreatment hydrolysates of pretreated coconut husk. Fig. 1a–c demonstrate that the responses for total reducing sugars, glucose, and xylose follow a similar trend, initially increasing with temperature and time until they reach stationary values, after which they begin to decline. The glucose, xylose, and TRS concentrations range from 0.089 g/L to 0.225 g/L, from 0.105 g/L to 0.917 g/L, and from 2.455 g/L to 5.125 g/L, respectively. This initial increase was caused by the intensifying hydrolysis of the polysaccharides within the lignocellulose, which released the sugars through the autohydrolysis action of the hydrothermal pretreatment as temperature and time were increased. Further increases, however, led to the degradation of these sugars into furfural and 5-HMF. This behavior is also similar to previous research on HT of sunflower straw (Yang et al., 2023), which showed that glucose and xylose concentrations increased from 0.3 to 1.7 g/L and 0.6 to 5.1 g/L, respectively, as the temperature was raised from 140 to 220 °C. However, prolonged pretreatment time led to a decrease in glucose and xylose values, down to 0.7 and 2.5 g/L, respectively. Hence, based on the aforementioned result, there are optimum temperature and time condition to acquire the maximum amount of sugar.

In contrast, Fig. 1d–e indicate that the concentrations of furfural and 5-HMF show a linear increase with rising temperature and time, with no stationary point. Furfural and 5-HMF concentrations range from 0.105 g/L to 0.917 g/L and from 0.004 to 1.575 g/L, respectively. This finding aligns with previous research on hydrothermal pretreatment of lignocellulosic biomass, where at temperatures above 160 °C and times exceeding 60 min, furfural and 5-HMF start to accumulate due to the decomposition of C5 and C6 sugars (Ma et al., 2021; Wang et al., 2016). The increasing trend of degradation products under harsher conditions explains the declining responses of reducing sugars, as xylose is extensively converted into furfural and glucose is converted into 5-HMF. This competing effect between sugar production by hydrolysis and sugar loss due to degradation reactions highlights the importance of optimizing the hydrothermal pretreatment to maximize the yield of reducing sugars while minimizing the undesirable degradation products, a critical aspect of the overall biorefinery process (Ruiz et al., 2023).

Fig. 1f shows that the recovered solids of coconut husk decrease as temperature and time increase. The plot of the recovered solids response suggests that under milder conditions, the decomposition of coconut husk is more rapid compared to harsher conditions, due to most of hemicellulose is hydrolysed at milder conditions, which is more susceptible than cellulose and lignin. At harsh conditions, high temperature and time, the decomposition is focused on more resistant components such as cellulose and lignin. Consequently, the rate of solid decomposition is decreasing as the pretreatment getting harsh (Shen et al., 2021a; Trajano et al., 2013). Meanwhile, main products as depicted in Fig. 1a–c, can be correlated with the recovered solid in Fig. 1f. As the recovered solids was decreasing, the conversion or depolymerization of hemicellulose and cellulose are getting intense as well, which leads to higher sugars product (xylose and glucose) content until the certain stationary point of sugar response and depleting as harsher conditions. This information is needed to understand how much the impact of pretreatment with pretreated CCH solids that can be correlated with cellulose and lignin content. These two components, were processed further into combined pretreatment such as subsequently continued to DES pretreatment then fed to the enzymatic hydrolysis or directly from HT to enzymatic hydrolysis.

Last surface plot, Fig. 1g demonstrates that the pH of the hydrothermal pretreatment hydrolysates decreases with increasing temperature and time, which can be attributed to the greater solubilization of the acetyl component of hemicellulose and the degradation of furfural and 5-HMF into organic acids as the conditions become more severe (Ma et al., 2021; Wang et al., 2016). This trend was also observed in a previous study (Wang et al., 2016), where the pH was initially 4.9 at a temperature of 145 °C and a time of 15 min, but it decreased as the temperature and time increased, reaching a value of 3.42 at a temperature of 205 °C and a time of 120 min.

The findings of this study indicate that the hydrothermal pretreatment hydrolysates derived from CCH have the potential to serve as a source of reducing sugars for various applications, such as biofuel fermentation. However, the presence of degradation products like furfural and 5-HMF can inhibit some biofuel-producing microbes (Vanmarcke et al., 2021; Yang et al., 2022). Consequently, a multi-response optimization was conducted to maximize the reducing sugars concentration while minimizing the levels of these inhibitory compounds. The optimization process set an upper limit of 0.8 g/L for furfural and 5-HMF, as concentrations beyond this level have been reported to hamper biohydrogen production (Yang et al., 2022). The results of this optimization and validation are presented in Table 2. Validation was performed by conducting the optimum condition three times, and the results were comparable to the predicted values.

The optimal values of hydrothermal pretreatment demonstrate the potential for utilizing hydrolysates directly in fermentation processes without the need for an additional furan compound detoxification step. This advantage significantly minimizes downstream processing costs, enhancing the overall economic feasibility of the process (Gundupalli et al., 2022; Guo et al., 2022; Ruiz et al., 2023). From a scalability perspective, the mild operational conditions employed in the hydrothermal pretreatment process, including moderate temperatures and pressures, offer compatibility with existing industrial reactor designs, such as batch or continuous flow systems (Peng et al., 2021; Yukesh Kannah et al., 2020). These factors make scaling up the process more feasible compared to more severe pretreatments that require specialized or costly equipment.

Furthermore, the selective removal of hemicellulose during hydrothermal treatment improves the accessibility of cellulose for subsequent enzymatic hydrolysis while producing fermentable sugars. This capability has practical implications for biorefineries aiming to convert lignocellulosic biomass into biofuels and chemicals efficiently. However, the scalability of hydrothermal pretreatment must also consider challenges such as the energy requirements for heating large volumes and potential fouling issues within reactors (Ruiz et al., 2023, 2020). Addressing these challenges may involve optimizing heat recovery systems, exploring continuous pretreatment processes, and integrating hydrothermal treatment with other pretreatment or recovery systems to enhance energy efficiency and operational stability at an industrial scale.

The study investigated the optimization of hydrothermal pretreatment with multiple responses. Multiple regression analyses were performed, yielding the following regression model equations:

$$Y_{TRS}\left(\frac{g}{L}\right) = -64.8135 + 0.5651X_1 + 0.2488X_2 - 0.001242X_1^2 - 0.0009884X_2^2 - 0.0004633X_1X_2 \tag{7}$$

$$Y_{Glucose}\left(\frac{g}{L}\right) = -3.8267 + 0.031X_1 + 0.02014X_2 - 0.0000672X_1^2 - 0.00007939X_2^2 - 0.0000361X_1X_2 \tag{8}$$

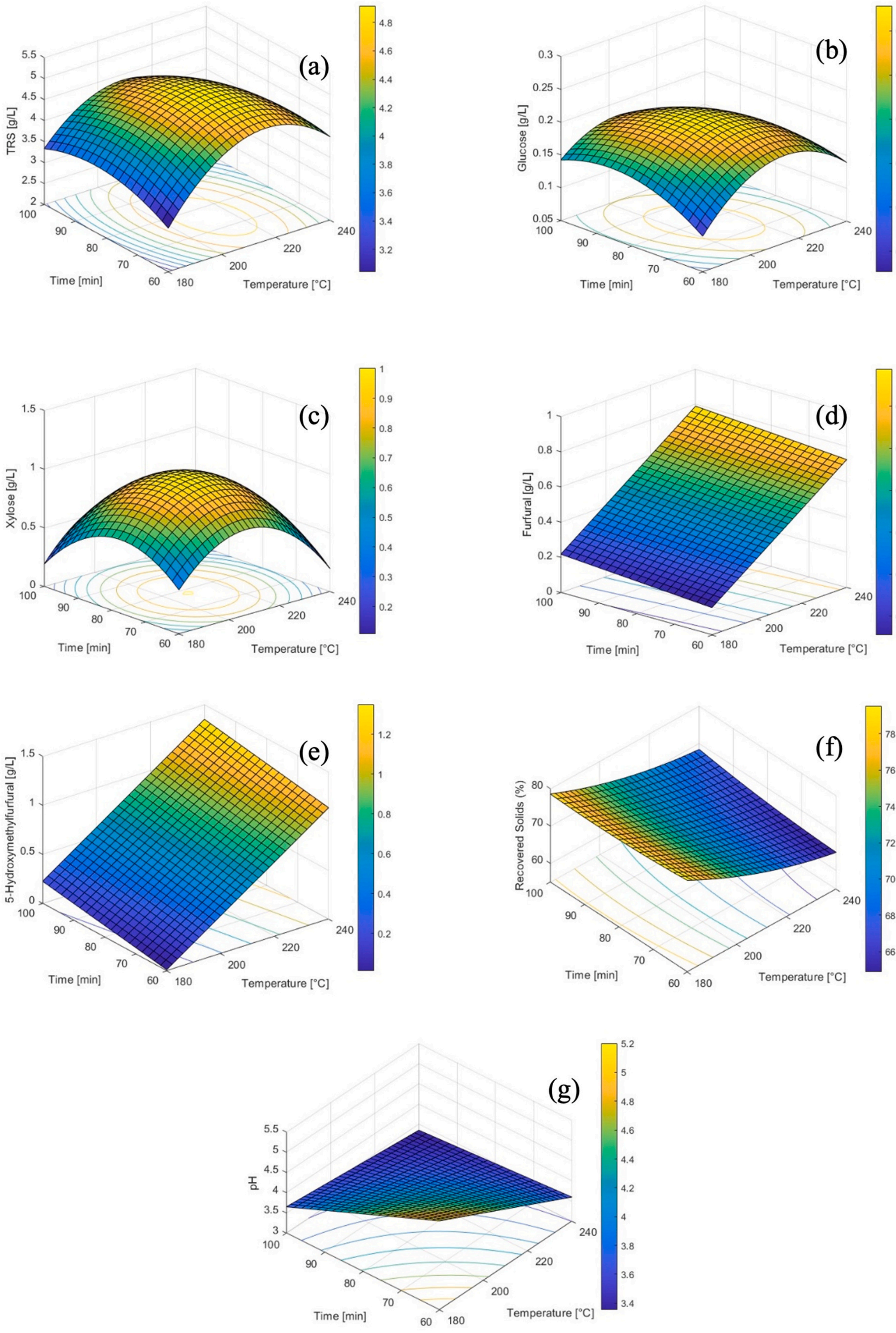


Fig. 1. Surface plots of different responses with temperature and time of pretreatment as factors. (a) TRS (g/L), (b) Glucose (g/L), (c) Xylose (g/L), (d) Furfural (g/L), (e) 5-Hydroxymethylfurfural (g/L), (f) Solid Residue (%), and (g) pH.

Table 2
HT hydrolysate responses optimization.

Factors	Remarks	$Y_{TRS}\left(\frac{g}{L}\right)$	$Y_{Glucose}\left(\frac{g}{L}\right)$	$Y_{Xylose}\left(\frac{g}{L}\right)$	$Y_{Furfural}\left(\frac{g}{L}\right)$	$Y_{5-HMF}\left(\frac{g}{L}\right)$	$Y_{Rec.Solids}\%$	Y_{pH}
X_1=210.7 °C	Predicted	4.908	0.211	0.997	0.476	0.683	65.2	3.92
X_2=77.9 min	Observed	4.802 ± 0.16	0.223 ± 0.012	0.998 ± 0.045	0.464 ± 0.053	0.634 ± 0.17	66.4 ± 2.05	4.04 ± 0.1

X_1- temperature; X_2- time; $Y_{Rec.Solids}$- recovered solids, and Y_{pH}- pH.
Concentrations: $Y_{Glucose}\left(\frac{g}{L}\right)$, $Y_{Xylose}\left(\frac{g}{L}\right)$, $Y_{Furfural}\left(\frac{g}{L}\right)$, $Y_{5-HMF}\left(\frac{g}{L}\right)$.

$$Y_{Xylose}\left(\frac{g}{L}\right) = -24.5795 + 0.2X_1 + 0.1235X_2 - 0.0004893X_1^2 - 0.000847X_2^2 + 0.000041X_1X_2 \quad (9)$$

$$Y_{Furfural}\left(\frac{g}{L}\right) = -1.6501 + 0.009512X_1 + 0.001559X_2 \quad (10)$$

$$Y_{5-HMF}\left(\frac{g}{L}\right) = -3.6768 + 0.01872X_1 + 0.005333X_2 \quad (11)$$

$$Y_{Rec.Solids}\% = 235.1045 - 1.1365X_1 - 0.6196X_2 + 0.001859X_1^2 + 0.00163X_2^2 + 0.001859X_1X_2 \quad (12)$$

$$Y_{pH} = 18.2147 - 0.05938X_1 - 0.1367X_2 + 0.0005444X_1X_2 \quad (13)$$

$$Y_{5-HMF}\left(\frac{g}{L}\right) = -3.6768 + 0.01872X_1 + 0.005333X_2 \quad (14)$$

where $Y_{TRS}\left(\frac{g}{L}\right)$, $Y_{Glucose}\left(\frac{g}{L}\right)$, $Y_{Xylose}\left(\frac{g}{L}\right)$, $Y_{Furfural}\left(\frac{g}{L}\right)$, $Y_{5-HMF}\left(\frac{g}{L}\right)$, $Y_{Rec.Solids}\%$, and Y_{pH} are responses or dependent variables: TRS, glucose, xylose, furfural, 5-HMF, recovered solids, and pH respectively. Model terms included into these models, X_1 and X_2 are linear model terms, X_1^2 and X_2^2 are quadratic model terms, and X_1X_2 are two-factor interaction model terms.

The responses for TRS, glucose, xylose, and recovered solids exhibited quadratic or second-order model behavior. In contrast, the responses for furfural and 5-HMF fit a linear or first-order model. Furthermore, the pH response fit a linear or first-order model with a two-factor interaction model. The significance of each response model was evaluated using ANOVA as presented in detail in Table S1. The *p*-values obtained from each model were <0.05, indicating the statistical significance of the regression models. Furthermore, the fit of the experimental data to the regression models was assessed using the coefficient of determination (R^2), which showed strong correlations between the experimental and predicted values. The R^2 values for the responses were 0.978, 0.979, 0.980, 0.957, 0.942, 0.957, and 0.963, respectively. The models exhibited a strong correlation between the experimental and predicted values, indicating those models can effectively represent the system's performance (Asadi and Zilouei, 2017; Gundupalli et al., 2022). The lack-of-fit analysis further confirmed the significance of the established models, as the obtained p-values were >0.05 for all models, indicating that the models fit the experimental data well (Pihlajaniemi et al., 2021; Walls et al., 2023).

3.2. DES, HT-DES pretreatment, and DES recycle

To understand the effects of combined pretreatments and single pretreatment alone, an investigation was conducted to examine the impacts of pretreatment on the CCH components. These results are presented in Fig. 2.

From Fig. 2, the HT from optimized condition significantly reduced the hemicellulose content of the coconut husk, from 20.29 % to 3.76 %, leading to a higher proportion of cellulose and lignin in the pretreated CCH residue. This pretreatment achieved a substantial 87.7 % removal of hemicellulose, while the removal of cellulose and lignin was more moderate at 14.39 % and 21.68 %, respectively. Consequently, the pretreated CCH had a cellulose content 1.25 times higher than the raw CCH. The substantial and selective removal of hemicellulose indicates the potential to generate valuable hemicellulosic sugars from the process (Gong et al., 2022; Ma et al., 2021). Furthermore, the DES pretreatment alone also significantly reduced the hemicellulose content of coconut husk, from 20.29 % to 8.47 %. Furthermore, it removed a considerable amount of lignin, lowering it from 39.8 % to 29.7 %. The removal of hemicellulose and lignin was 79.96 % and 64.18 %, respectively. Consequently, the cellulose content increased from 32.14 % to 59.2 %, which is 1.84 times higher than the raw CCH, with a relatively low cellulose removal of 11.59 %. The significant removal of lignin demonstrates the effectiveness of DES in selectively delignifying coconut husk. The interaction of hydrogen bond donors and acceptors in the DES has been reported to disrupt the lignin-carbohydrate complex,

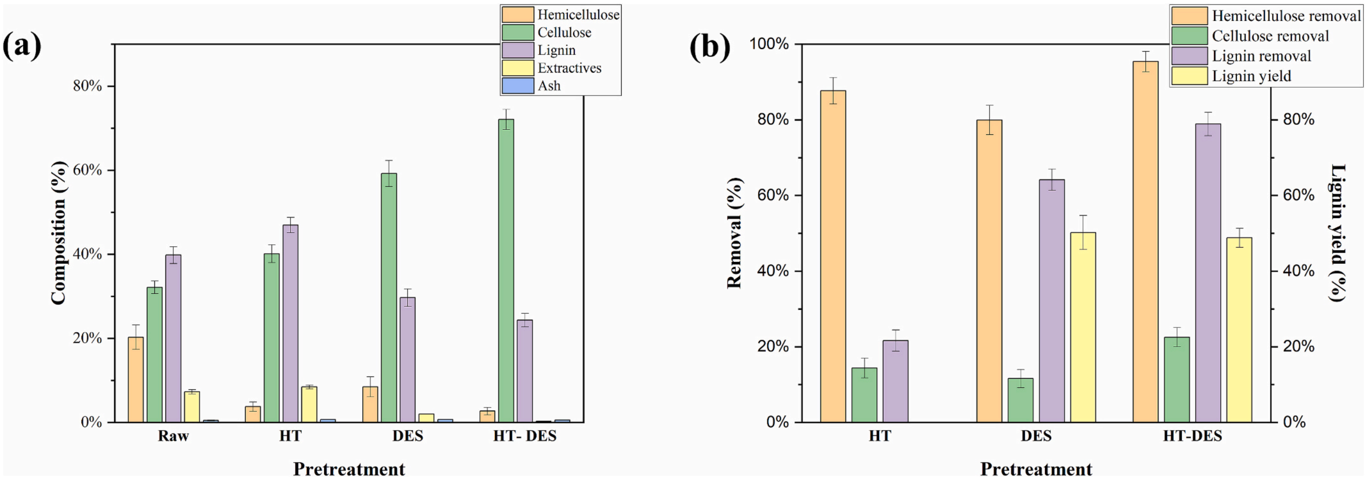


Fig. 2. Composition (a), component removal, and lignin yield (b) of raw and pretreated CCH.

depolymerize the ether linkages within the lignin, and the acidic nature of DES leads to the hydrolysis of glycosidic bonds in hemicellulose and catalyzes the ether bonds of lignin (Hong et al., 2020; Liu et al., 2019; Tan et al., 2019). This DES pretreatment approach showcases the potential to leverage the increased cellulose content of CCH, which can be utilized for valuable applications such as nanocellulose production, cellulosic sugar generation, and aerogel fabrication (Huo et al., 2023; Liu et al., 2019; Wu et al., 2024; Xie et al., 2023; Zhao et al., 2011).

Meanwhile, the combined hydrothermal-DES pretreatment, as shown in Fig. 2, achieved the highest delignification or lignin removal and hemicellulose removal. With these removals, the cellulose content was increased to 72.1 % from the initial 32.14 %, the hemicellulose content decreased to 2.7 % from 20.29 %, and the lignin content was reduced to 24.34 % from 39.8 %. The combined hydrothermal-DES pretreatment resulted in a cellulose content that was 2.24 times higher than the raw CCH. Besides, overall removal for each component is 95.41 % for hemicellulose, 22.54 % for cellulose, and 78.89 % for lignin. Notable removal for two step pretreatment shows a synergistic effect of enhanced lignin removal by the effect of prior HT. This confirmed that hydrothermal pretreatment affects the lignin structure inside the CCH and eases the lignin removal (Shen et al., 2021b; Sun et al., 2023; Wang et al., 2021). These results indicate that the combined hydrothermal-DES pretreatment significantly increases the purity of cellulose content, potentially enhancing enzymatic hydrolysis while producing hemicellulosic sugar and lignin fractions. Aforementioned benefits and mechanism of hydrothermal-DES pretreatment are illustrated in Fig. 3.

The ash content in biomass plays a critical role in evaluating pretreatment effectiveness on coconut husk (CCH) as it affects enzymatic digestibility and overall process efficiency. In Fig. 2a, raw CCH exhibited an ash content of 0.49 %. The hydrothermal (HT) pretreatment resulted in a slight increase to 0.68 %, possibly due to concentration effects as organic matter was removed. The DES pretreatment maintained a stable ash level at 0.65 %, while the combined hydrothermal-DES approach led to a modest reduction to 0.57 %. Excessive ash can hinder enzymatic reactions and reduce biomass reactivity. These results are consistent with previous research (Prempeh et al., 2021) which highlights the critical role of minimizing ash content to improve the efficiency of biomass conversion. Optimizing pretreatment conditions to balance component removal and ash content is key to achieving higher-quality biomass fractions and improving downstream process performance.

The statistical analysis was conducted using one-way ANOVA, followed by Tukey's HSD post hoc tests, to confirm the significant differences in the effects of single and combined pretreatments on CCH components. The results, summarized in Tables S2 and S3, provide robust statistical evidence for the observed compositional changes and removals of hemicellulose, cellulose, and lignin. Specifically, *p*-values for most group comparisons were found to be well below 0.05, indicating statistically significant differences among the pretreatment groups (Morán-Aguilar et al., 2022; Muharja et al., 2018).

The %Lignin yield in Fig. 2, shows that considerable amounts of lignin, 50.2 % and 48.8 %, were recovered from the DES pretreatment alone and the combined hydrothermal-DES pretreatment, respectively. Since the combined pretreatment had already removed some portion of the lignin during the prior hydrothermal pretreatment step, the extent of recovered lignin was not significantly different between the individual and combined pretreatment approaches. However, the hydrothermal-DES pretreatment scheme has an advantage, as it can preserve the hemicellulosic fractions in the initial hydrothermal pretreatment step, whereas the DES pretreatment alone would result in a mixture of hemicellulose and lignin fractions. Therefore, the combined pretreatment approach minimizes the need for downstream separation of the lignin and hemicellulose fractions.

Investigation of DES pretreatment impact under different cycle were investigated and shown in Fig. 4. In Fig. 4a The DES pretreatment recycling results illustrate notable changes in hemicellulose, cellulose, and lignin compositions across multiple cycles. Initially, the raw material displayed a composition of 20.29 % hemicellulose, 32.14 % cellulose, and 39.8 % lignin. In the first cycle of DES pretreatment, hemicellulose composition dropped sharply to 8.47 %, while cellulose content increased to 59.2 %, and lignin content decreased to 29.7 %. This demonstrates the high initial efficiency of DES in removing hemicellulose and lignin, which enhances the relative cellulose content. As the cycles progressed, a gradual decrease in hemicellulose content and a slight reduction in cellulose were observed, with cycle 4 showing hemicellulose at 10.8 %, cellulose at 48.8 %, and lignin at 33.3 %. The data suggest that while the DES solution remains effective in hemicellulose and lignin removal, there is a diminishing return in efficacy with successive cycles. This trend could be due to the accumulation of solubilized components in the DES solution or changes in substrate characteristics, making the remaining components more recalcitrant (Shen

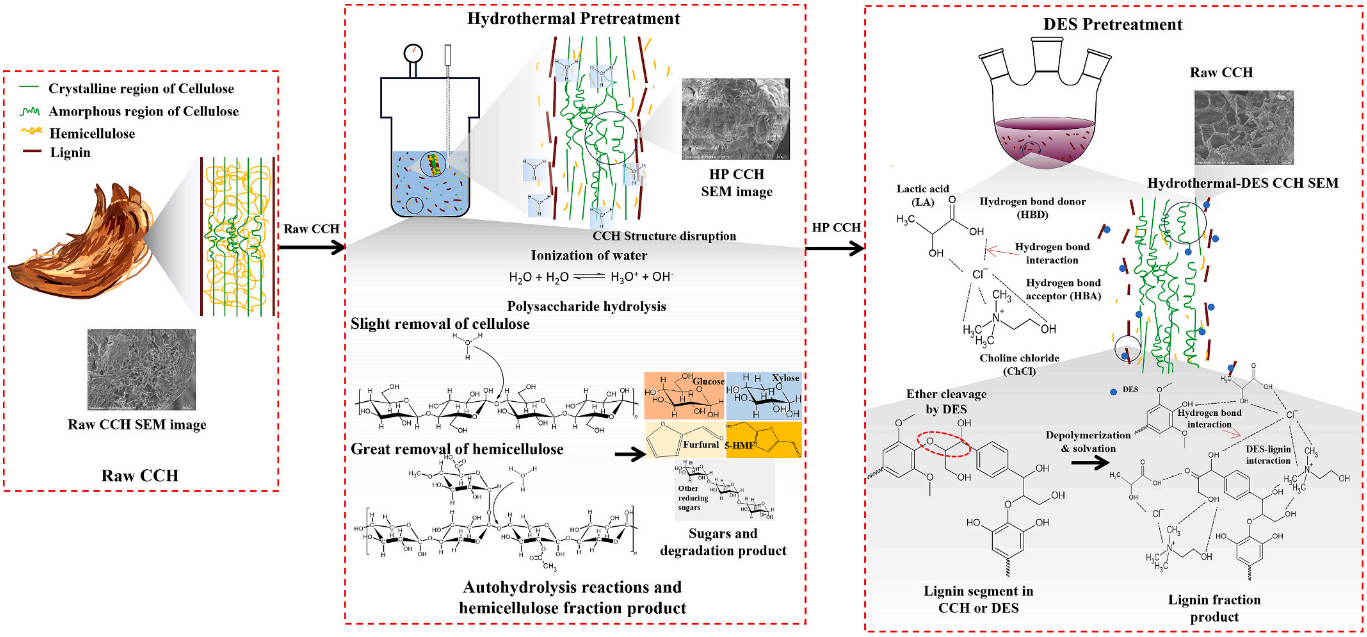


Fig. 3. Mechanism of hydrothermal-DES pretreatment.

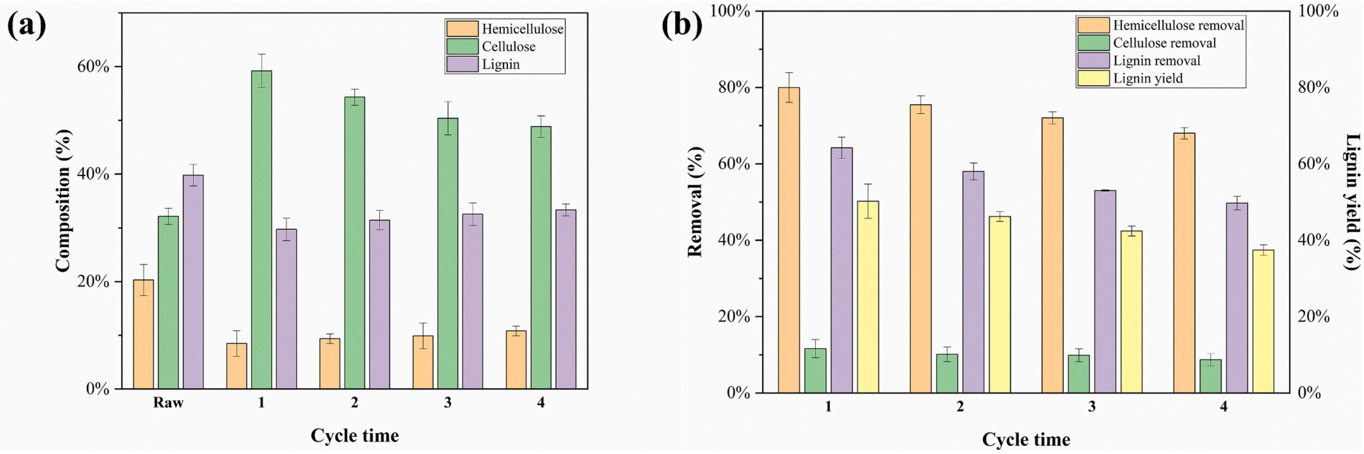
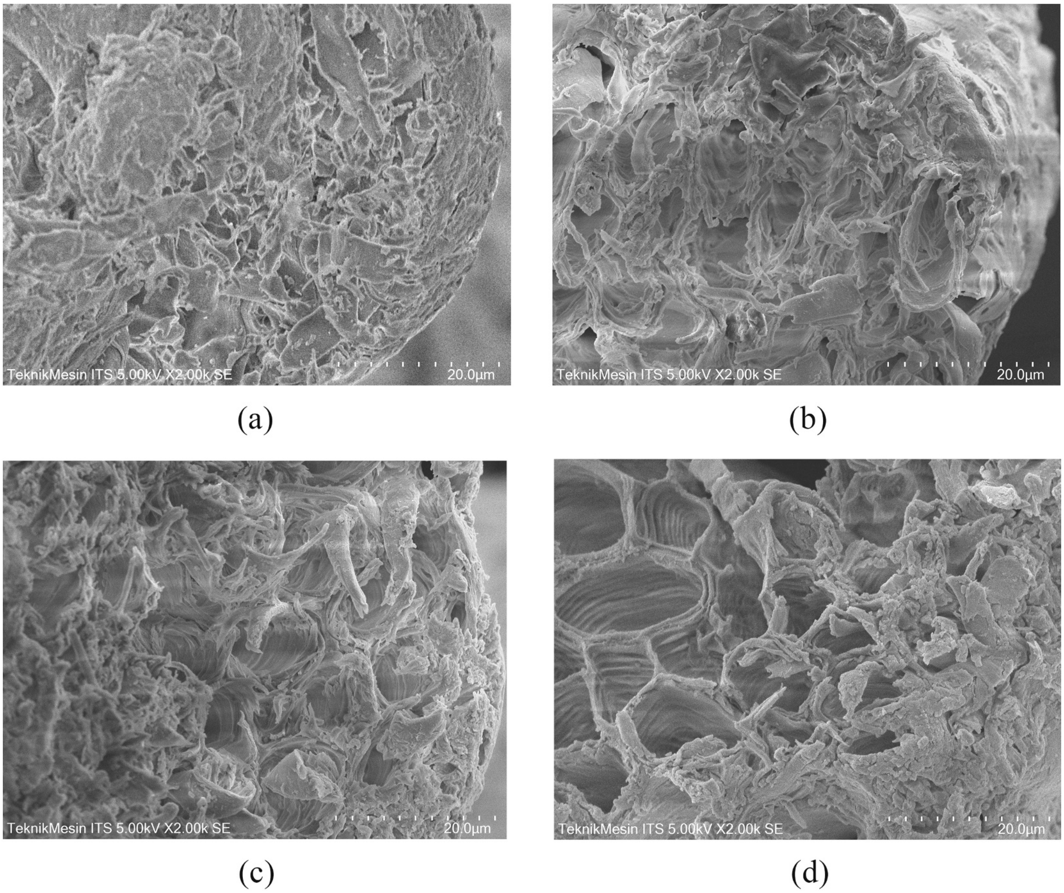


Fig. 4. Compositions (a), component removals (b), and lignin yield of each DES pretreatment cycle.

et al., 2019; Wang et al., 2020). The observed trend highlights the potential for DES recycling in achieving high cellulose purity across multiple cycles, though the reduced efficacy indicates a need for regeneration or optimization strategies for the DES solution to sustain its effectiveness over extended cycles (Peng et al., 2021). This balance between efficiency and sustainability in recycling cycles is crucial for practical and economical biomass pretreatment processes.

Fig. 4b, the removal efficiency of hemicellulose, cellulose, and lignin components during successive DES pretreatment cycles highlights a consistent decline in effectiveness over four cycles. In the first cycle, hemicellulose removal reached 79.96 %, significantly reducing its content in the substrate. Cellulose removal remained low at 11.59 %, indicating selective hemicellulose removal, which is beneficial for retaining cellulose. Lignin removal was also substantial at 64.18 %, with a lignin yield of 50.2 %, reflecting the efficacy of the DES pretreatment in breaking down lignin structures. In subsequent cycles, hemicellulose removal decreased progressively, from 75.46 % in the second cycle to 68 % in the fourth cycle. Similarly, lignin removal dropped from 58 % in cycle 2 to 49.7 % in cycle 4. This declining trend indicates that the DES solution's performance deteriorates over repeated use, possibly due to saturation with solubilized compounds or a reduced ability to disrupt the lignin-carbohydrate complex (Leal Silva et al., 2022; Sunar et al.,



(a) (b)

(c) (d)

Fig. 5. SEM Images of (a) Raw CCH, (b) Optimal HT CCH, (c) DES Pretreated CCH, and (d) Hydrothermal-DES Pretreated CCH.

2024). The cellulose removal remained low across all cycles, ranging from 10.1 % to 8.7 %, preserving cellulose content and further validating the selective removal capabilities of DES pretreatment.

The observed trend in lignin yield, with values decreasing from 50.2 % in the first cycle to 37.4 % in the fourth cycle, underscores the diminishing recovery efficiency of lignin over recycling cycles. This outcome suggests that while DES recycling is viable for multiple cycles, the gradual decline in component removal and yield necessitates periodic regeneration or replacement of the DES solution to maintain high pretreatment efficiency (Wang et al., 2024). These results emphasize the trade-offs between operational sustainability and pretreatment efficacy when recycling DES solutions, offering critical insights for future research in optimizing the economic and practical aspects of DES-based biomass pretreatment processes.

3.3. *Pretreated solid characterization*

Various techniques, including SEM, FTIR, and XRD, were used to investigate the structural changes of the pretreated CCH samples, as shown in Fig. 5.

The SEM images in Fig. 5 show that the raw CCH has a compact, dense, and rigid fiber structure. The HT disrupted this compact structure, causing the fibers to become more irregular, porous, and fragmented. The DES pretreatment alone also disrupted the structure compared to the raw CCH, resulting in a more porous, irregular, and fragmented surface, which is similar as HT result but in more greater effect. Furthermore, the combined hydrothermal-DES pretreatment shows the most substantial effect of surface disruption with fiber highly separated and the most porous structure compared to raw, HT, and DES alone. More disrupted structure of pretreated CCH corresponds to the extent of lignocellulose component removals as already shown in Fig. 2. As the removal of components is getting higher the surface becomes more fragmented, rougher, and porous. Hence, the hydrothermal-DES pretreated CCH exhibited the most significant morphology changes compared to the other pretreatment conditions (Shen et al., 2019; Wang et al., 2021). This change potentially increases the accessibility of enzymes to the cellulose during the subsequent hydrolysis step (Zhou et al., 2023).

Table 3
FTIR peak spectrum and functional group assignment of pretreated and unpretreated CCH.

Peak (cm^{-1})	Functional group	References
3345	Intra- and inter-molecular hydrogen bonds in hemicellulose, cellulose, and lignin	(Jose and Sajeena Beevi, 2023)
2907	C—H aliphatic in lignin	(Jose and Sajeena Beevi, 2023)
1729	Acetyl group in hemicelluloses or the carbonyl ester of lignin	(Gundupalli et al., 2022).
1603	Aromatic ring stretch vibration breathing in lignin	(Wang et al., 2021)
1507	Aromatic ring stretch vibration breathing in lignin	(Wang et al., 2021)
1371	C—H, O—H, or C—O bonds in hemicelluloses	(Sunar et al., 2024; Wang et al., 2021)
1327	C—H, O—H, or C—O bonds in hemicelluloses	(Sunar et al., 2024; Wang et al., 2021)
1238	C—H, O—H, or C—O bonds in hemicelluloses	(Sunar et al., 2024; Wang et al., 2021)

FTIR spectral analysis of the raw and pretreated CCH, as shown in Fig. 6a, was performed to illustrate the changes in functional groups that occurred during the various processing pretreatments. The peaks and group assignment were summarized in Table 3. The most typical absorption band appearing at around 3345 cm^{-1} could be attributed to the presence of intra- and inter-molecular hydrogen bonds, mainly from the cellulose, meanwhile, the 2907 cm^{-1} band indicated the presence of aliphatic C—H (Jose and Sajeena Beevi, 2023). The absorption near 1729 cm^{-1} represented the typical acetyl group in hemicelluloses or the carbonyl ester of p-coumaric lignin units (Gundupalli et al., 2022). The presence of C—H, O—H, or C—O bonds in hemicelluloses was confirmed by the absorption bands at 1371 cm^{-1}, 1327 cm^{-1}, and 1238 cm^{-1} (Sunar et al., 2024; Wang et al., 2021). Additionally, the bands around 1603 cm^{-1} and 1507 cm^{-1} could be attributed to the aromatic ring stretch vibration breathing in lignin. The intensities of absorption bands near 1371 cm^{-1}, 1327 cm^{-1}, and 1238 cm^{-1} decreased or disappeared from the spectra of pretreated CCH, indicating the removal of hemicelluloses during the HT, DES, and hydrothermal-DES pretreatment. Additionally, the intensities of absorption bands around 1603 cm^{-1} and 1507 cm^{-1} decreased after DES pretreatment and a greater decrease was observed in hydrothermal-DES pretreatment suggesting the partial separation of lignin from the pretreated CCH. The absorption band at 1729 cm^{-1} also confirms the extensive removal of hemicellulose and lignin in hydrothermal-DES pretreatment.

The functional group changes observed reflect the targeted removal of hemicellulose and lignin, which can potentially improve the digestibility of CCH by enzymes (Rodríguez-Rebelo et al., 2024). The decreased intensity of specific functional groups corresponding to non-cellulose components indicates improved enzyme binding to the cellulose in CCH (Sunar et al., 2024). Hence, these findings confirmed the

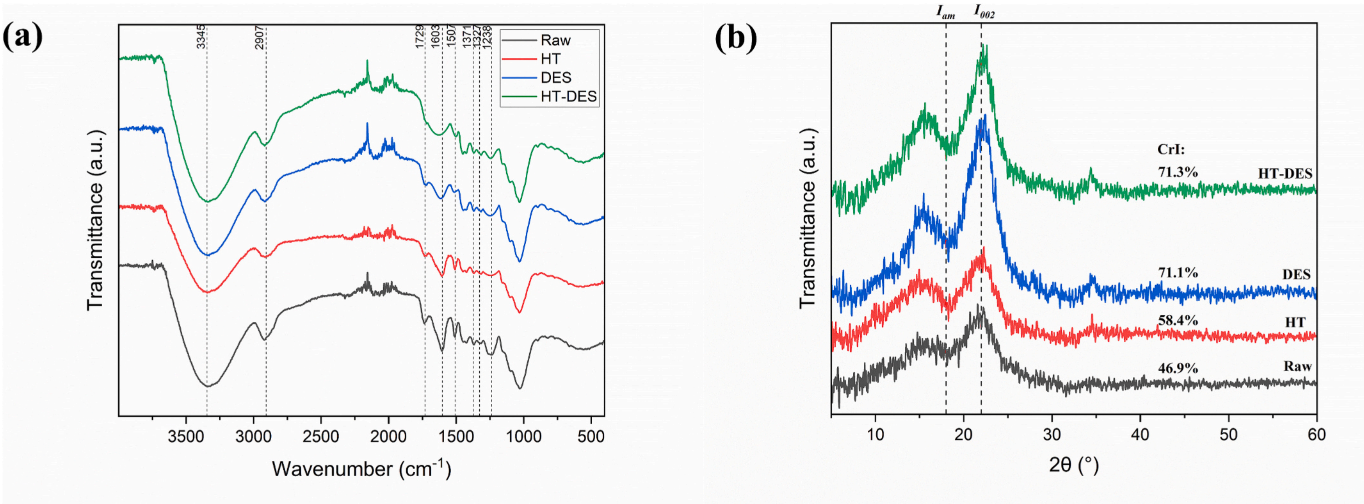


Fig. 6. (a) FT-IR spectra and (b) XRD pattern of raw and pretreated CCH.

effect of pretreatments on the CCH component removal as shown in Fig. 2.

The crystalline nature of the pretreated CCH after hydrothermal, DES, and combined hydrothermal-DES pretreatment was examined using XRD analysis, as shown in Fig. 6. The results indicate that the crystallinity index (CrI) increased for the pretreated CCH compared to the raw material. The CrI of the HT CCH was 58.4 %, higher than the raw CCH. Meanwhile, the CrI of the DES pretreated CCH was even higher at 71.1 %, a 27 % increase compared to the raw CCH. The significant increase in CrI for the DES pretreatment compared to HT was likely due to the more extensive removal of amorphous components like hemicellulose and lignin during the DES pretreatment (Zhou et al., 2023). However, the CrI of the combined hydrothermal-DES pretreatment was relatively similar to the DES pretreatment alone, with a CrI of 71.3 %, indicating that the crystallinity was not further increased despite the greater removals of hemicellulose and lignin achieved by the combined pretreatment. This suggests that the combined pretreatment led to a more amorphous cellulose structure in the CCH, which could potentially enhance enzymatic hydrolysis compared to the single pretreatment approaches (Xu et al., 2019).

The solid characterization of CCH using SEM, FTIR, and XRD techniques confirms the effects of pretreatments in removing non-cellulosic components. In conclusion, the removal of hemicellulose and lignin potentially could enhance the subsequent enzymatic hydrolysis, leading to improved cellulose to glucose conversion. Furthermore, the disrupted and porous structures of the pretreated CCH also contribute to enhanced digestibility by enzymes.

3.4. *Enzymatic hydrolysis*

The enzymatic hydrolysis performance of the pretreated coconut husk samples was evaluated, and the results are shown in Fig. 7.

The raw CCH had the lowest cellulose conversion, reaching only 7.6 % after 72 h of saccharification yield, as shown in Fig. 7. The hydrothermal pretreated CCH showed an improved saccharification yield of 14.3 % after 72 h, which was 1.88 times higher than the raw CCH. Meanwhile, the DES pretreated CCH had a 72 h saccharification yield of 16.7 %, which was 2.2 times higher than the raw CCH. These single pretreatments effectively improved the enzymatic digestibility of the CCH samples by removing some of the recalcitrant components such as hemicellulose and lignin (Shen et al., 2019), as evidenced by the compositional analysis and solid characterization results. The impact of HT to the removal of these components is strongly indicated by the changes in the FTIR spectrum shown in Fig. 6a, as mentioned in the previous subsection (Xu et al., 2019). Furthermore, the increase in the CrI in Fig. 6b due to the removal of amorphous non-cellulose components signifies an increase in the cellulose composition, which makes the cellulose more susceptible to cellulase attack (Yang et al., 2023). However, DES pretreatment alone was more rigorously removing the hemicellulose and lignin as the nature of DES that able to cleavage the glycosidic bond of hemicellulose and ether linkage of lignin (Wang et al., 2020). Consequently, leaving CCH with higher cellulose content, higher CrI as cellulose content increased, rougher morphology as confirmed by SEM image, losing more functional groups as detected in FTIR in wavenumber 1371 cm^{-1}, 1327 cm^{-1}, 1238 cm^{-1}, 1507, and 1729 cm^{-1} correspond to functional groups in lignin and hemicellulose. Consequently, these analysis confirms the superiority of DES pretreatment compared to HT to enhance saccharification yield (Gundupalli and Bhattacharyya, 2021; Shen et al., 2021a).

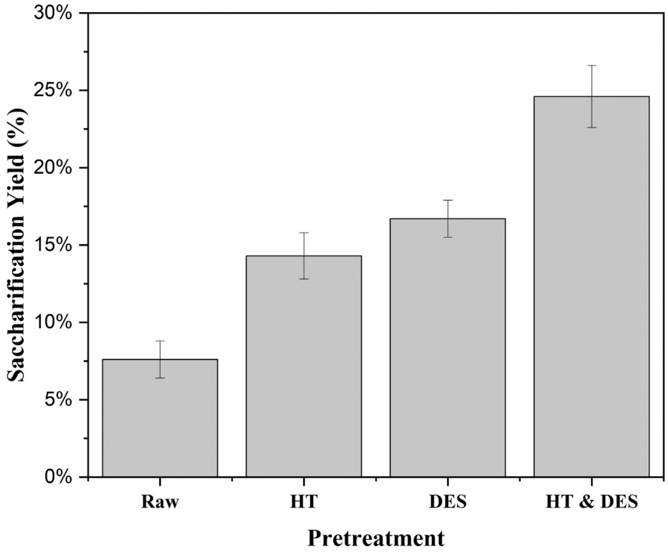


Fig. 7. Saccharification yield of each pretreated and raw CCH.

Remarkably, the integrated hydrothermal and DES pretreatment approach resulted in the highest cellulose conversion, achieving a 72 h saccharification yield of 24.6 %, which was 3.24 times higher than the raw CCH, 1.72 times higher than the hydrothermal pretreatment, and 1.47 times higher than the DES pretreatment. The significantly improved enzymatic digestibility of the integrated hydrothermal and DES pretreatment can be attributed to the synergistic effects of the two pretreatment methods, which were more effective in removing hemicellulose and lignin components than the individual pretreatments (Gundupalli and Bhattacharyya, 2021). This synergy substantially increased cellulose content while reducing lignin and hemicellulose, making the cellulose more susceptible to enzymatic hydrolysis (Wang et al., 2020; Xu et al., 2021). Additionally, the indication of a more amorphous cellulose structure from SEM image in Fig. 5d, CrI reduction of XRD data and the extensive removal of non-cellulose component by FTIR in Fig. 6 after the combined pretreatment compared to single pretreatment only may have further contributed to the enhanced saccharification performance (Gundupalli and Bhattacharyya, 2021; Shen et al., 2021a; Xu et al., 2019). The results align with findings from other studies on combined hydrothermal-DES pretreatment (Ma et al., 2021; Shen et al., 2021a).

The impact of the pretreatments on saccharification yield was further confirmed through one-way ANOVA and Tukey's HSD post hoc analysis, as summarized in Table S4. The statistical tests indicated significant differences ($p < 0.05$) among the pretreatment groups. The combined hydrothermal-DES pretreatment demonstrated a significantly higher saccharification yield compared to single pretreatments (HT and DES), reflecting its enhanced enzymatic digestibility (Ma et al., 2021; Shen et al., 2021a).

3.5. *Lignin characterization and 2D-HSQC NMR of lignin*

The lignin recovered from the integrated hydrothermal-DES pretreatment of coconut husk was further characterized to understand its structural features and compare them to previous reports on lignin. Additionally, the lignin purity was also checked by the acid hydrolysis method.

The FTIR and ^{1}H NMR spectral analyses, as shown in Fig. S1, provided insights into the lignin structure. The FTIR spectrum in Fig. S1a revealed the presence of characteristic lignin functional groups, including the O—H stretching vibration of aliphatic and phenolic hydroxyl groups at 3415 cm^{-1} (Mankar et al., 2022), alkane C—H stretching vibrations in methyl and methylene groups at 2925 cm^{-1} and 2860 cm^{-1} (Sunar et al., 2024), aromatic ring and aromatic skeletal vibrations with C═O stretching at 1600 cm^{-1} and 1515 cm^{-1} (Rodríguez-Rebelo et al., 2024), C—H bending in methyl groups at 1450 cm^{-1} (Wang et al., 2022), C—O stretching from guaiacol rings at 1264 cm^{-1}, and C—O stretching in syringyl units at 1220 cm^{-1} (Mankar et al., 2022). Additionally, the C—O deformation in primary and secondary alcohol groups, as well as aliphatic ether linkages, was observed at 1125

cm^{-1} and 1030 cm^{-1} (Sunar et al., 2024). These FTIR results confirm that the recovered lignin from the integrated pretreatment process exhibited the typical functional groups and structural characteristics commonly found in native lignin structures (Sharma et al., 2022; Sunar et al., 2024).

The ^{1}H NMR analysis presented in Fig. S1b reveals the characteristic signals associated with the lignin structure. The signals observed in the range of 6.5 to 8.5 ppm correspond to the aromatic protons (Sunar et al., 2024). Specifically, the peaks between 6.1 and 6.7 ppm indicate the presence of aromatic protons in the syringyl propane units, while the aromatic protons in the guaiacyl propane and *p*-hydroxyphenyl propane units appear in the ranges of 6.7 to 7.5 ppm and 7.4 to 8 ppm, respectively. The signals observed between 0.8 and 2 ppm are attributed to the aliphatic hydrocarbon region, primarily consisting of primary, secondary, and tertiary alkyl groups. The peaks within the range of 1.4 to 2.2 ppm correspond to the protons of aliphatic acetates, while the signals between 2.2 and 2.5 ppm represent the protons of aromatic acetates. A distinct peak at 2.5 to 2.7 ppm is attributed to the DMSO-d_6 solvent, and the peak between 3.5 and 3.7 ppm corresponds to the protons of water in DMSO-d_6. The methoxy protons in the lignin structure are represented by the signals observed in the range of 3.5 to 4.1 ppm (Mankar et al., 2022). This finding of ^{1}H NMR result also confirm the native lignin typical structure of CCH DES lignin.

The purity of the lignin obtained from the hydrothermal-DES pretreatment was verified through acid hydrolysis, which revealed that it contained 93.5 % lignin. This finding was consistent with the FTIR and ^{1}H NMR analysis results, confirming the high purity and typical structural characteristics of the recovered lignin. Additionally, indicating the successful extraction and recovery of lignin from the integrated hydrothermal and DES pretreatment of coconut husk.

The 2D-HSQC NMR spectra provide valuable insights into the structural transformations of lignin when treated with different pretreatment methods, specifically DES alone and the combination of HT followed by DES (HT-DES) which depicted in Fig. S2 and Table S5. For the DES-only treated lignin, key chemical shifts such as (3.84/55.50 ppm) correspond to C—H in methoxyl groups (Shen et al., 2019). The presence of these signals suggests that DES treatment preserves much of the methoxyl functionality, indicative of a relatively mild treatment that maintains the integrity of the aromatic structure while selectively cleaving interunit linkages such as β—O—4 bonds. This selective solubilization without extensive aromatic ring cleavage makes the lignin soluble while retaining core structural features (Sunar et al., 2024). Additionally, shifts at (6.69, 104.49/104.65 ppm) represent $C_{2,6}$—$H_{2,6}$ in syringyl units, further underscoring the preservation of key aromatic motifs in the DES-treated lignin (Wang et al., 2021).

Conversely, the HT-DES-treated lignin exhibits additional and more pronounced shifts that reflect extensive structural modification. For example, the shift around (4.49/58.40 ppm) and its presence in both DES and HT-DES indicate Cγ—Hγ in γ-acylated β—O—4 (A′). The HT step before DES pretreatment induces partial depolymerization, enhancing the reactivity and susceptibility of lignin to further chemical changes (Gong et al., 2022). This is particularly evident in the aromatic region, with signals like (6.71/104.65 ppm) for syringyl units showing modifications that signify more extensive cleavage and transformation of aromatic rings.

Shifts at (7.14/110.29 ppm) and (7.22/120.44 ppm) in guaiacyl units (C_2—H_2 and C_6—H_6, respectively) further illustrate the different degrees of modification between the two treatments. The HT-DES treatment, by breaking down rigid lignin bonds and opening up the structure, results in more reactive lignin, enhancing its potential for enzymatic or chemical transformations (Gong et al., 2022). The comparative analysis demonstrates that while DES-only treatment provides a moderately modified lignin structure with retained aromatic features, HT-DES yields a more fragmented, highly modified lignin, suitable for applications requiring high solubility and reactivity.

3.6. Process mass balance analysis

The mass balance of the overall integrated green fractionation process and the individual pretreatments, including HT, DES pretreatment, enzymatic saccharification, and lignin recovery, is demonstrated in Fig. 8. Additionally, the fractionation products of coconut husk from each scheme based on mass balance analysis are presented in Table 4. The integrated green fractionation process, combining HT and deep eutectic solvent (DES) pretreatment, demonstrates effective separation and recovery of valuable fractions from CCH. HT primarily yielded reducing sugars, recovering 26.4 % of total sugars from hemicellulose and cellulose, as depicted in Fig. 8a, while DES pretreatment excelled in lignin isolation, producing 50.2 % isolated lignin and recovering 10.1 % of the total sugars (Fig. 8b). The combined hydrothermal-DES process further enhanced the recovery of both sugars and lignin, yielding 48.8 % isolated lignin and recovering 31.3 % of total sugars from hemicellulose and cellulose, as shown in Fig. 8c. This combined pretreatment strategy highlights the potential for efficient fractionation of CCH into high-value products, such as reducing sugars and high-purity lignin, compared to single-step methods (Ma et al., 2021; Shen et al., 2021b; Wang et al., 2020).

Comparison against previous studies of lignocellulose fractionation is provided in Table 5. The integrated hydrothermal-DES pretreatment strategy demonstrates significant potential for enhancing the production of sugars and lignin fractions from coconut husk compared to other pretreatment methods. This combined approach achieved a total recovered sugar yield of 31.3 % and a lignin yield of 48.8 %, highlighting its effectiveness in fractionating biomass into valuable components. By comparison, previous studies using only hydrothermal pretreatment yielded a total recovered sugar of 34 % (Prado et al., 2014), while ionic liquid and enzymatic hydrolysis processes achieved sugar recoveries of 18.8–21.1 % and lignin yields ranging from 60 % to 64 % (Anuchi et al., 2022). Notably, a microwave-assisted DES pretreatment achieved an impressive 82 % lignin yield (Mankar et al., 2022) but did not report sugar recovery.

The combined hydrothermal-DES pretreatment provides a synergistic effect that enhances the breakdown of lignocellulosic structures, making cellulose more accessible for enzymatic hydrolysis and leading to higher sugar yields compared to many single pretreatment methods. The hydrothermal step disrupts the lignin structure, easing its subsequent removal by DES (Gong et al., 2022), which further enriches the cellulose fraction and enhances digestibility. This selective removal of lignin and hemicellulose increases the purity of cellulose, promoting efficient sugar production (Shen et al., 2021a). The high-purity lignin recovered through this method offers significant value for bioproduct development, including biopolymers, adhesives, coatings, and other specialty chemicals. Compared to lignin yields from other methods, such as the 60–82 % yields from ionic liquid and microwave-assisted DES treatments, the lignin produced through combined hydrothermal-DES pretreatment provides a balance between purity and functional properties, making it suitable for diverse applications (Gong et al., 2022). Furthermore, DES characteristics as biodegradable solvent, non-toxic, low cost, and recyclable are the main benefits compared to ionic liquid pretreatment (Li et al., 2022). In pretreatment modes, microwave heating process is still suffering in too energy intensive so that the conventional heating process still become the key of scalability of these pretreatments (Wang et al., 2024).

However, challenges remain, particularly in scaling up the process and optimizing DES recovery. Efficient recycling and reuse of DES solvents are critical for economic viability. Additionally, maintaining consistent DES performance over multiple cycles requires further process optimization to reduce costs and energy use (Leal Silva et al., 2022). Despite these challenges, this approach aligns with circular bioeconomy principles by enabling diverse bioproduct production from a single biomass source. With its potential for high-yield sugar and lignin production, the combined hydrothermal-DES pretreatment offers a

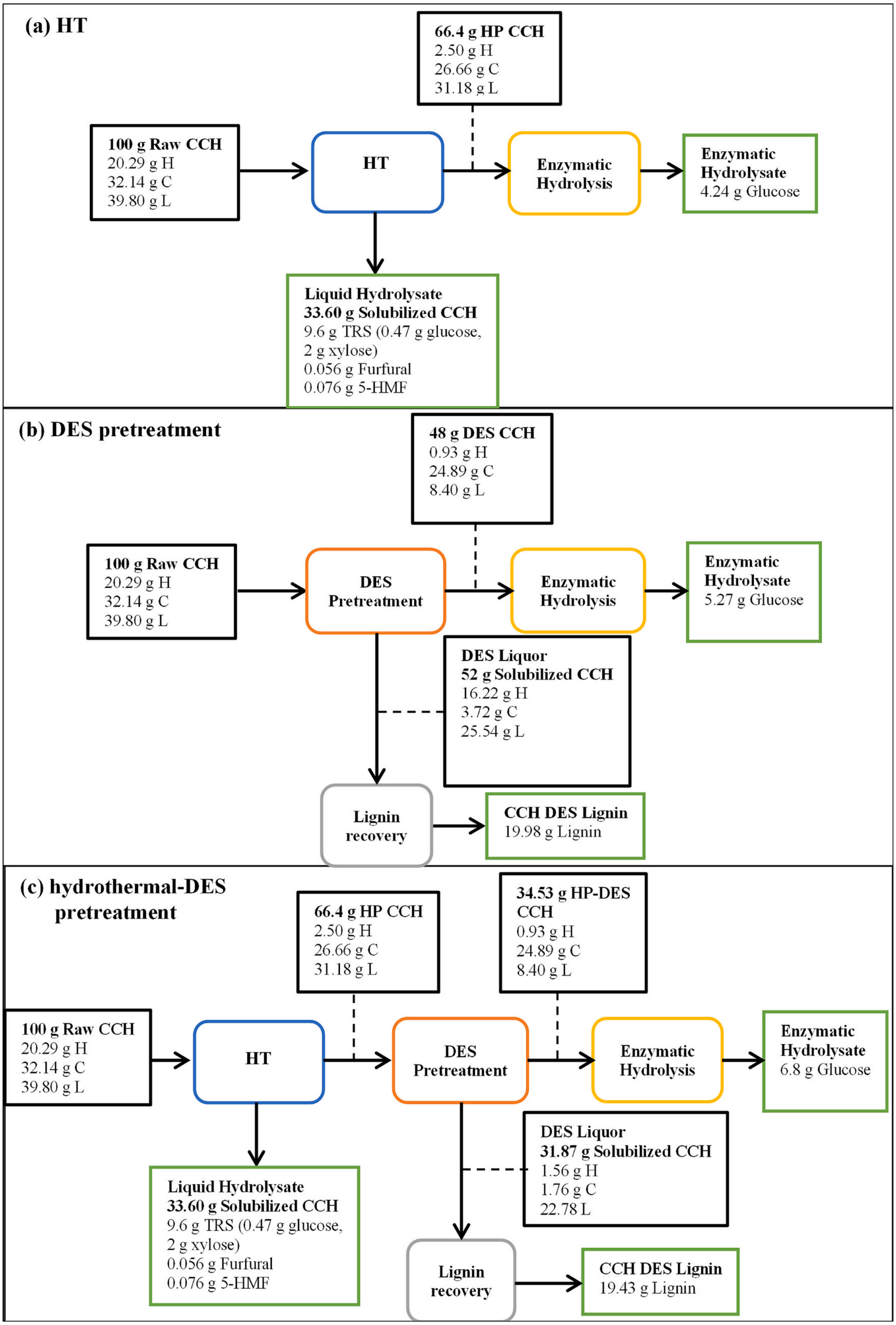


Fig. 8. Process mass balance of (a) HT, (b) DES, (c) hydrothermal-DES pretreatment with 100 g raw CCH basis.

Table 4
Fractionation products of CCH of each scheme from mass balance analysis.

Scheme	Fractions (per 100 raw CCH)			Total recovered sugar (%)
	Hemicellulose	Cellulose	Lignin	
HT	9.6 g TRS (0.47 g glucose, 2 g xylose), 0.056 g, Furfural 0.076 g, 5-HMF	4.4 g Glucose	–	26.40
DES pretreatment	–	5.27 g Glucose	19.98 CCH DES Lignin	10.1
Hydrothermal-DES pretreatment	9.6 g TRS (0.47 g glucose, 2 g xylose), 0.056 g, Furfural 0.076 g, 5-HMF	6.8 g Glucose	19.43 CCH DES Lignin	31.3

Table 5
Previous studies of lignocellulose fractionations.

Pretreatment/ biorefinery strategy	Biomass	Variables	Results	References
Hydrothermal pretreatment	Coconut husk	259 °C, 30 min	34 % total recovered sugar	(Prado et al., 2014)
Ionic liquid & Enzymatic hydrolysis	Coconut husk	[DMBA][HSO_4], 170 °C, 90 min	21.1 % total recovered sugar, 60 % lignin yield	(Anuchi et al., 2022)
Ionic liquid & Enzymatic hydrolysis	Coconut shell	[DMBA][HSO_4], 170 °C, 90 min	18.8 % total recovered sugar,64 % lignin yield	(Anuchi et al., 2022)
Microwave assisted DES	Coconut husk	ChCl:LA (1:4), 150 °C, 20 min	82 % lignin yield	(Mankar et al., 2022)
Hydrothermal, DES Pretreatment, & enzymatic hydrolysis	Coconut husk	HT: 210.7 °C, 77.9 min, DES: ChCl/LA (1:10) 120 °C, 6 h	31.3 % total recovered sugar, 48.8 % lignin yield	This study

versatile, sustainable pathway for lignocellulosic biomass valorization (Peng et al., 2021). By building upon these promising results and addressing scalability and process optimization, this strategy can contribute significantly to advancing bio-based industries.

Key economic considerations in scaling up this process include reducing capital expenditures (CAPEX) for pretreatment equipment, optimizing operating expenditures (OPEX) by minimizing energy and utility use, and improving the recycling efficiency of DES solvents. As noted in the referenced studies, achieving consistent DES performance across multiple reuse cycles remains a challenge but represents a critical step toward economic sustainability. (Peng et al., 2021) Additionally, while alternative methods such as microwave-assisted DES pretreatment offer high lignin yields, they are often more energy-intensive, limiting their scalability (source). In contrast, the conventional heating employed in our combined process offers a potentially more scalable and energy-efficient solution, aligning with broader industrial sustainability goals (Chen et al., 2015; Leal Silva et al., 2022).

In summary, the integrated hydrothermal and deep eutectic solvent pretreatment approach demonstrated effective fractionation of coconut husk into high-purity lignin and reducing sugars, outperforming the individual pretreatment schemes in terms of overall product yield and selectivity.

4. Conclusion

This study demonstrated that the combined hydrothermal-deep eutectic solvent pretreatment and enzymatic hydrolysis were more effective in producing various lignocellulosic fractions from coconut husk compared to single pretreatment methods. The hydrothermal pretreatment yielded a hemicellulose fraction with high sugar content and low sugar degradation products, as optimized through response surface methodology. The subsequent DES pretreatment after HT was able to effectively delignify CCH 78.89 %. and recover high-purity lignin with a similar structure to native lignin. The hydrothermal-DES pretreated CCH showed significantly higher glucose yields from enzymatic hydrolysis, outperforming the single pretreatment approaches by a factor of 3.24 compared to the unpretreated CCH. Solid characterization techniques, such as compositional analysis, scanning electron microscopy, X-ray diffraction, and Fourier-transform infrared spectroscopy, revealed the more disruptive effects and extensive removals of non-cellulose component of the combined pretreatment on the lignocellulosic structure compared to individual pretreatments, which was reflected in the improved enzymatic hydrolysis performance. Furthermore, the mass balance analysis indicated that the integrated pretreatment strategy yielded the highest total recovered sugars at 31.3 % while recovering 48 % of the original lignin content in the CCH, demonstrating its effectiveness in fractionating the lignocellulosic components. However, challenges remain in scaling up the combined process, including optimizing DES recovery and minimizing energy consumption to ensure economic viability. Future work should focus on improving the cost-effectiveness and consistency of DES performance across multiple cycles to enhance the overall sustainability of this approach.

CRediT authorship contribution statement

Candra Wijaya: Writing – original draft, Investigation, Conceptualization. **Ningsi Lick Sangadji:** Writing – review & editing. **Maktum Muharja:** Writing – review & editing, Conceptualization. **Tri Widjaja:** Writing – review & editing, Supervision. **Lieke Riadi:** Writing – review & editing, Supervision. **Arief Widjaja:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2025.102078.

Data availability

No data was used for the research described in the article.

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