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Article

Optimizing Xylose Yield from Coffee Cherry Processing Waste (CCPW) via PEG-Assisted Enzymatic Hydrolysis

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Abstract: Coffee cherry processing waste is a lignocellulosic residue with considerable potential as a renewable feedstock for sugar production. This study investigated the effect of depectination and polyethylene glycol (PEG) 6000 supplementation on enzymatic sugar release from Robusta CCPW. CCPW was depectinated and subsequently pretreated with 3% NaOH at 80°C for 2.5 h to reduce lignin and pectin contents and improve hemicellulose accessibility. Enzymatic hydrolysis was performed using xylanase with PEG 6000 concentrations of 3, 5, 7, and 9 g/L under fed-batch conditions for 96 h at 40°C. We determined xylose- and glucose-equivalent reducing sugar concentrations using the DNS method with separate calibration curves and spectrophotometric measurements at 550 and 540 nm, respectively. Depectination reduced pectin content from 11.5% to approximately 1.4%; PEG 6000 supplementation significantly increased sugar release compared with the 0g/L control. The highest xylose-equivalent concentration and yield were obtained at 9 g/L, reaching 1.96 g/L and 78.76%, respectively, compared with 0.602 g/L and 24.18% in the control. The highest glucose-equivalent concentration and yield were obtained at 7 g/L PEGG 6000, reaching 2.77 g/L and 71.02%, respectively. The enhanced hydrolysis observed in PEG-Supplemented treatment may be associated with improved enzyme accessibility and reduced non-productive enzyme adsorption onto lignin, as proposed in previous studies. Unlike the authors' previous work focusing on downstream crystalline xylose production from CCPW, the present study specifically evaluates depectination and PEG 6000 supplementation as strategies to enhance enzymatic sugar release prior to purification and crystalization.

Keywords: coffee cherry processing waste; depectinization; PEG 6000; xylose; lignocellulosic biomass

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1. Introduction

The rapid growth of the coffee processing industry has resulted in increased generation of agricultural residues, especially coffee cherry processing waste (CCPW)(1). CCPW consists of coffee pulp, parchment, and silver skin (2). It has significant lignocellulosic components, including cellulose, hemicellulose, lignin, protein, tannin, and pectin, making it a promising raw material for bioconversion into fermentable sugar and other value-added products (3–6). This composition makes CCPW a promising renewable feedstock for producing fermentable sugars and other value-added bioproducts. However, efficient bioconversion remains challenging because lignin and

pectin contribute to biomass recalcitrance by limiting hydrolytic enzyme access to structural polysaccharides. Pectin is particularly relevant in coffee-processing residues because coffee pulp contains a considerable pectic fraction that may form a physical barrier around cellulose and hemicellulose and restrict mass transfer during enzymatic hydrolysis.

Hemicellulose is one of the most important components in CCPW because it contains xylan, which can be hydrolyzed into xylose. Xylose is an industrially valuable pentose sugar that can be converted into xylitol, bioethanol, and functional sweeteners (7–10). Nevertheless, xylose recovery from lignocellulosic biomass is limited by the heterogeneous association among lignin, cellulose, hemicellulose, and pectin (11). Pectin can decrease enzyme accessibility by restricting diffusion through the plant cell-wall matrix, while lignin can physically limit polysaccharide accessibility and interact non-productively with hydrolytic enzymes (12). Previous studies have demonstrated that enzymatic or chemical removal of pectin improves the accessibility of lignocellulosic carbohydrates and subsequently enhances enzymatic sugar release (13) . Therefore, reducing both pectin- and lignin-associated barriers is expected to provide a more accessible xylan fraction for subsequent xylanase hydrolysis.

Alkaline pretreatment and depectinization are complementary approaches for reducing these structural barriers (14,15) Sodium hydroxide (NaOH) pretreatment disrupts lignin–carbohydrate associations, promotes lignin solubilization, and can simultaneously facilitate the removal of part of the pectic fraction (16–18). In the present study, 3% NaOH at 80°C for 2.5 h was applied as a fixed pretreatment condition to reduce the lignin and pectin barriers prior to enzymatic conversion. The NaOH concentration was not treated as an optimization variable in the present experimental design; rather, the study focused on the effect of PEG 6000 concentration following alkaline pretreatment and depectinization. Integrating depectinization with the hydrolysis sequence was intended to reduce the pectic barrier before and during enzymatic conversion, thereby increasing the exposure of hemicellulose-rich regions to xylanase. This rationale is supported by previous evidence showing that removal of pectin increases biomass porosity, cell-wall separation, and enzyme accessibility (13) .

In addition to structural pretreatment, surfactant supplementation has been investigated to increase enzymatic hydrolysis efficiency. Non-ionic surfactants and hydrophilic polymers, including polyethylene glycol (PEG), Tween 20, Tween 80, and Triton X-100, have been reported to improve enzymatic hydrolysis of several lignocellulosic substrates, although their effectiveness depends strongly on biomass composition, pretreatment severity, enzyme system, and surfactant concentration (19,20) (21–23). PEG 6000 is of particular interest because its hydrophilic polymer chains may interact with residual lignin and thereby decrease the tendency of hydrolytic enzymes to undergo non-productive adsorption. However, this mechanism is substrate-dependent and should be considered a proposed explanation rather than a directly demonstrated mechanism in the present study. Comparisons among PEG 6000, Tween 80, and Triton X-100 have also shown that no single surfactant is universally superior across different lignocellulosic substrates (19). Therefore, PEG 6000 was selected in this study as a non-ionic polymeric additive whose effect on xylanase-mediated hydrolysis of depectinated CCPW remains insufficiently characterized.

The PEG 6000 concentrations of 3, 5, 7, and 9 g/L were selected to evaluate the response of enzymatic hydrolysis across a moderate concentration range, while a treatment without PEG 6000 (0 g/L) was included as an experimental control. Previous studies have demonstrated concentration-dependent effects of PEG 6000 during lignocellulose hydrolysis; for example, PEG 6000 concentrations ranging from 0 to 2.5% have been evaluated in pretreated wheat straw, with hydrolysis performance varying according to PEG concentration and process configuration (24). Other lignocellulosic systems have reported beneficial effects at approximately 0.5–1% PEG 6000, indicating that the effective dosage is strongly dependent on substrate and processing conditions

(20,24). The range of 3–9 g/L used in the present study, corresponding to approximately 0.3–0.9% (w/v), was therefore chosen to investigate whether increasing PEG 6000 concentration within this moderate range enhances xylose release from CCPW and whether the response approaches a maximum or plateau. Including the 0 g/L treatment allows the contribution of PEG supplementation to be directly distinguished from enzymatic hydrolysis without PEG.

Despite previous studies on alkaline pretreatment, depectinization, and surfactant-assisted enzymatic hydrolysis of lignocellulosic materials, their combined application for improving xylose release from robusta CCPW has received limited attention. More importantly, the specific contribution of PEG 6000 to xylanase-mediated hydrolysis of depectinated and alkali-pretreated CCPW has not been clearly established using a PEG-free control. The authors' previous study on CCPW focused on optimizing crystalline xylose production by varying substrate concentration, xylanase concentration, and commercial xylose crystal concentration (2). That study addressed downstream crystalline xylose production but did not specifically investigate pectin removal as a structural barrier or quantify the contribution of PEG 6000 supplementation to enzymatic hydrolysis. The present study therefore extends the previous work by focusing on upstream biomass deconstruction and enzymatic sugar release through the integration of alkaline pretreatment, depectinization, and PEG 6000-assisted xylanase hydrolysis.

Accordingly, this study aimed to evaluate the effect of PEG 6000 supplementation on enzymatic sugar release from depectinated and alkali-pretreated robusta CCPW. Specifically, the study evaluated xylose- and glucose-equivalent reducing sugar concentrations, and their corresponding hydrolysis yields at PEG 6000 concentrations of 0, 3, 5, 7, and 9 g/L. We hypothesized that removing pectin and partially disrupting lignin with alkaline pretreatment would improve hemicellulose accessibility to xylanase, and that PEG 6000 supplementation would further increase sugar release relative to the 0 g/L PEG control. It was further hypothesized that the response to PEG 6000 would be concentration-dependent rather than necessarily linear, because the beneficial effect of surfactant supplementation may reach a saturation level at higher concentrations.

2. Materials and Methods

2.1. Materials

Robusta coffee cherry processing waste (CCPW) was obtained from a local farmer group in Bondowoso, East Java, Indonesia. The CCPW originated from Coffea canephora (Robusta) cherries harvested predominantly at the mature-to-ripe stage and processed using the routine postharvest practices applied by the farmer group. The collected residue consisted mainly of coffee pulp, parchment, and residual fruit tissues generated during coffee cherry processing. To minimize raw-material variability, CCPW was collected from the same production area and processing batch and was manually screened to remove soil, stones, leaves, and visibly deteriorated materials before further processing.

The chemicals and reagents used in this study included sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), polyethylene glycol 6000 (PEG 6000), acetate buffer, 3,5-dinitrosalicylic acid (DNS) reagent, analytical-grade xylose, and analytical-grade glucose standards. The xylanase used was a technical-grade industrial enzyme obtained from Natural Micron Chem Tech Co. Ltd., Jinan City, China. All laboratory chemicals were Merck analytical grade.

2.2. Biomass Preparation, Alkaline Pretreatment, and Delignification [2]

CCPW was washed with distilled water to remove adhering impurities and subsequently oven-dried at 80°C for 18 h. The dried material was ground and sieved through a 60-mesh sieve to obtain a relatively uniform particle size.

Alkaline pretreatment was performed by mixing CCPW powder with 3% (w/v) NaOH solution at a solid-to-liquid ratio of 1:4 (w/v). The suspension was incubated in a water

bath at 80°C for 2.5 h. After pretreatment, the solid fraction was separated by filtration and washed repeatedly with distilled water to remove residual alkali. Washing was continued until the filtrate reached approximately neutral pH. The final pH of the wash water was measured using a calibrated pH meter and recorded as 7. The neutralized biomass was then oven-dried at 80°C for 12 h and stored in a sealed container until subsequent treatment. The 3% NaOH concentration was used as a fixed pretreatment condition and was not investigated as an independent optimization variable in the present study.

2.3. Enzymatic Hydrolysis (2,25) and Depectinization (26)

Depectinization of the alkali-pretreated CCPW was conducted before or in conjunction with enzymatic hydrolysis, following the procedure described in reference (20), with modifications. Briefly, 12.5g of pretreated CCPW was suspended in 100mL *of* acetic buffer. The suspension was sterilized using an autoclave. The suspension was then treated with 4.01 g of xylanase and PEG 6000 at the specified concentration and hydrolyzed under semi-aerobic conditions. Hydrolysis was conducted in a fed-batch system at 40 °C and 125 rpm for 96 h. The initial substrate concentration was 60%, and the rest was fed at 12, 24, 36, and 48 h in 5% increments of the total substrate. The hydrolysate was centrifuged to separate the liquid fraction for the analysis of xylose, glucose, and pectin.

2.4. Analysis of Pectin, Xylose, and Glucose Content (2,27)

We determined pectin content gravimetrically after acid extraction with H_2SO_4 and ethanol precipitation. Xylose- and glucose-equivalent reducing sugar concentrations in the hydrolysate were determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method. We prepared separate calibration curves using xylose and glucose standards. Absorbance was measured spectrophotometrically at 550 nm for the xylose-calibrated assay and at 540 nm for the glucose-calibrated assay. We calculated sugar concentrations from the corresponding standard calibration curves. Because the DNS method responds to reducing sugars rather than selectively distinguishing individual monosaccharides, we expressed the measured concentrations as xylose- and glucose-equivalent reducing sugars. We calculated hydrolysis yields based on the proportion of sugar released relative to the corresponding carbohydrate fraction available in the pretreated CCPW substrate (9).

2.5. Statistical Analysis

The experiment used a completely randomized design with five PEG 6000 treatments (0, 3, 5, 7, and 9 g/L). Each treatment was performed using three independent experimental replicates. Analytical measurements for each independent replicate were performed in duplicate. Data are presented as mean $\pm$ standard deviation. We evaluated the effect of PEG 6000 concentration using one-way ANOVA. When a significant treatment effect was detected, means were compared using Duncan's multiple range test at a significance level of $p<0.05$. We reported exact ANOVA p-values with the corresponding results rather than indicating significance only by a threshold value. Statistical analysis was performed using *Microsoft software Version 16.113.2 (26092012).*

3. Results and Discussion

3.1. Change in Lignocellulosic and Pectin Contents of CCPW.

Alkaline pretreatment followed by depectinization–hydrolysis markedly altered the lignocellulosic composition of CCPW (Table 1). Raw CCPW contained 15.10 ± 0.03% hemicellulose, 28.30 ± 0.05% cellulose, 19.00 ± 0.02% lignin, and 11.50 ± 0.01% pectin on a

dry-weight basis. After treatment with 3% NaOH at 80°C for 2.5 h, lignin decreased from 19.00 to 15.00%, corresponding to a 21.05% reduction, while pectin decreased from 11.50 to 3.00%, equivalent to a 73.91% reduction. Cellulose remained relatively stable, increasing slightly from 28.30 to 29.30%, while apparent hemicellulose increased from 15.10 to 28.00%.

Table 1. Changes in lignocellulosic and pectin contents of CCPW during alkaline pretreatment and depectinization-hydrolysis.

Conditions	Hemicellulose (% Dry Basis)	Cellulose (% Dry Basis)	Lignin (% Dry Basis)	Pectin (% Dry Basis)
Raw CCPW	15.1 ± 0.03	28.3 ± 0.05	19 ± 0.02	11.5 ± 0.01
Alkaline-pretreated CCPW	28 ± 0.05	29.3 ± 0.02	15 ± 0.07	3 ± 0.04
Depectinated-hydrolized CCPW	17.5 ± 0.09	28.1 ± 0.08	15 ± 0.04	1.4 ± 0.02

The decrease in lignin indicates that alkaline pretreatment partially disrupted the lignin-associated matrix while largely retaining the carbohydrate fractions. A previous study showed substantial modification of coffee-pulp lignocellulose after alkaline pretreatment and reported 4% NaOH for 25 min as the best condition in their experimental design, leaving 69.18% cellulose, 44.15% hemicellulose, and 25.19% lignin in the treated material (28) . The residual lignin concentration in the present study (15.00%) was numerically lower; however, the values cannot be used to conclude that the present treatment was superior because the initial biomass composition, treatment severity, analytical basis, and process objectives differed between studies (29) .

The apparent increase in hemicellulose from 15.10 to 28.00% after alkaline pretreatment represents relative enrichment on a dry-weight basis rather than formation of additional hemicellulose. Removal of pectin, lignin, and other alkali-soluble constituents decreases the total residual biomass mass, thereby increasing the proportional contribution of retained carbohydrates (30) . The minor change in cellulose, from 28.30 to 29.30%, further indicates that the 3% NaOH treatment predominantly altered non-cellulosic structural components rather than extensively degrading cellulose.

The subsequent depectinization–hydrolysis stage produced a different compositional response. Hemicellulose decreased from 28.00 to 17.50%, corresponding to a 37.50% reduction, whereas cellulose decreased by only 4.10%, from 29.30 to 28.10%. The substantially larger decrease in hemicellulose is consistent with xylanase as the principal hydrolytic enzyme and indicates preferential conversion of the xylan-containing fraction (31). Pectin also decreased from 3.00 to 1.40%, representing a further 53.33% reduction and an overall decrease of 87.83% relative to raw CCPW. Removal of pectin has previously been shown to increase enzyme accessibility to structural carbohydrates (32); a previous study reported that chemical or enzymatic pectin removal increased hydrolysis yield by improving cell-wall separation and accessible surface area (33).

However, the reduction in pectin from 3.00 to 1.40% cannot be attributed specifically to PEG 6000 because pectin was determined at processing stages rather than separately for each PEG concentration. Thus, the value reflects the overall depectinization–hydrolysis process. Similarly, lignin remained at approximately 15.00% after hydrolysis, indicating that measurable lignin removal occurred primarily during alkaline pretreatment rather than during PEG-assisted enzymatic hydrolysis (34) .

The compositional changes are relevant to the subsequent sugar-release response. The alkaline pretreatment reduced two structural barriers—lignin by 21.05% and pectin by

73.91%—before hydrolysis, while hemicellulose subsequently decreased by 37.50%. This reduction in residual hemicellulose occurred concurrently with the release of xylose-equivalent reducing sugars. Nevertheless, because lignin removal, pectin removal, and enzymatic hydrolysis were sequential process stages rather than independently factorialized variables, their individual contributions to xylose yield cannot be quantitatively separated.

3.2. Effect of PEG 6000 Concentration on Reducing Sugar Release and Hydrolysis Yield.

PEG 6000 concentration significantly affected reducing-sugar release during enzymatic hydrolysis (Table 2). The inclusion of the 0 g/L PEG treatment provides a direct experimental baseline for evaluating the contribution of PEG supplementation. Without PEG, the hydrolysate contained only 0.602 ± 0.030 g/L xylose-equivalent reducing sugar, corresponding to a yield of 24.18 ± 0.03%. Addition of 3 g/L PEG increased these values to 1.750 ± 0.006 g/L and 70.31 ± 0.01%, respectively.

Table 2. Effect of PEG 6000 concentration on reducing-sugar concentration and hydrolysis yield of CCPW.

PEG 6000 Concentration (g/L)	Xylose Equivalent Reducing Sugar (g/L)	Glucose Equivalent Reducing Sugar (g/L)	Xylose Equivalent Yield (%)	Glucose Equivalent Yield (%)
0	0.602 ± 0.03^{e}	0.785 ± 0.05^{e}	24.18 ± 0.03^{e}	20.12 ± 0.05^{e}
3	1.75 ± 0.006^{d}	2.61 ± 0.004^{d}	70.31 ± 0.01^{d}	66.92 ± 0.03^{d}
5	1.89 ± 0.005^{c}	2.65 ± 0.004^{c}	75.94 ± 0.05^{c}	67.94 ± 0.01^{c}
7	1.93 ± 0.002^{b}	2.77 ± 0.061^{a}	77.94 ± 0.03^{b}	71.02 ± 0.01^{a}
9	1.96 ± 0.002^{a}	2.72 ± 0.003^{b}	78.76 ± 0.08^{a}	69.74 ± 0.04^{b}

The greatest PEG-associated improvement occurred between 0 and 3 g/L. Relative to the control, addition of 3 g/L PEG increased xylose-equivalent concentration by approximately 190.70% and xylose-equivalent yield by 190.78%. Increasing PEG from 3 to 5 g/L further increased concentration by 8.00% and yield by 8.01%. The incremental response became smaller at higher concentrations: increasing PEG from 5 to 7 g/L increased concentration by approximately 2.12% and yield by 2.63%, whereas increasing PEG from 7 to 9 g/L increased concentration by only 1.55% and yield by 1.05%.

Consequently, the highest xylose-equivalent response within the evaluated range was obtained at 9 g/L PEG, reaching 1.960 g/L and 78.76% yield. These values represent approximately 225.58% and 225.72% increases, respectively, compared with the PEG-free control. However, the progressively smaller incremental increases between 5 and 9 g/L indicate that the response was approaching a plateau. Therefore, 9 g/L should be described as the highest-performing concentration among those evaluated, rather than as an optimum concentration, because PEG concentrations above 9 g/L were not tested.

The response observed in this study is consistent with previous evidence that PEG can improve enzymatic conversion of lignocellulosic substrates containing residual lignin. Börjesson et al. reported that PEG addition increased enzymatic conversion of lignocellulose from approximately 42 to 78% while reducing cellulase adsorption from 81 to 59%, demonstrating the importance of PEG–lignin interactions in reducing non-productive enzyme binding [43]. Although that study used cellulase and softwood rather than xylanase and CCPW, the magnitude of improvement supports PEG's general role as an additive in lignin-containing enzymatic systems.

More directly relevant to the present study, the study investigated the interaction of xylanase with acid-insoluble lignin and showed that additives including PEG 6000 affected xylanase adsorption and desorption on lignin (35). Their findings support the hypothesis that PEG can alter xylanase–lignin interactions and thereby maintain a larger fraction of enzyme available for productive hydrolysis (36) . PEG 6000 should therefore not be described using a classical "hydrophilic head–hydrophobic tail" model.

PEG is a linear polyether containing repeating ethylene oxide units. A previous study demonstrated that PEG adsorption onto lignin is strongly associated with hydrophobic interactions and that increasing PEG surface coverage can reduce enzyme interaction with lignin (37). Importantly, the present experiment did not directly measure PEG adsorption, xylanase adsorption/desorption, or lignin surface characteristics.

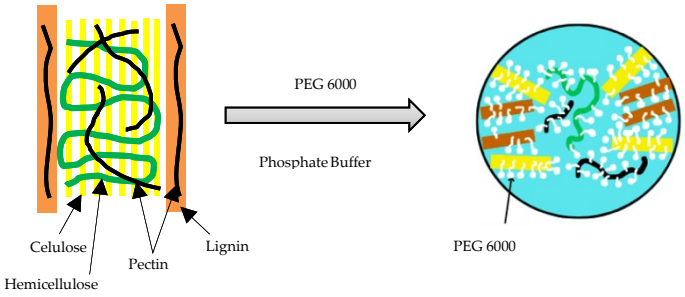


Figure 1. Proposed role of PEG 6000 in reducing non-productive xylanase adsorption onto residual lignin during enzymatic hydrolysis of CCPW.

The glucose-equivalent reducing-sugar response differed from that of xylose-equivalent reducing sugars. Glucose-equivalent concentration increased from 0.785 g/L in the control to 2.610 g/L at 3 g/L PEG, equivalent to an increase of approximately 232.48%. It subsequently increased to 2.650 g/L at 5 g/L and reached the highest value of 2.770 g/L at 7 g/L PEG, with a corresponding yield of 71.02%. Increasing PEG to 9 g/L slightly reduced concentration to 2.720 g/L and yield to 69.74%, corresponding to decreases of approximately 1.81% and 1.80%, respectively, relative to the 7 g/L treatment.

The different response patterns indicate that the PEG concentration associated with the highest measured response depended on the analytical parameter: 9 g/L for xylose-equivalent reducing sugars and 7 g/L for glucose-equivalent reducing sugars. However, because the DNS method detects reducing sugars rather than chromatographically separating individual monosaccharides, the higher glucose-equivalent values should not be interpreted as direct evidence of extensive cellulose conversion. The technical-grade xylanase may contain secondary enzyme activities, but we did not characterize them in this study.

The two units presented in Table 2 also describe different aspects of hydrolysis. Concentration (g/L) represents the amount of reducing-sugar equivalent present per unit volume of hydrolysate, whereas yield (%) represents the proportion of the corresponding carbohydrate substrate converted into reducing-sugar equivalents. Thus, increasing concentration indicates greater accumulation of soluble reducing sugars, while increasing yield reflects improved conversion efficiency relative to the available carbohydrate fraction.

Taken together, the results establish a quantitative relationship between biomass modification and subsequent hydrolysis performance. Alkaline pretreatment reduced lignin by 21.05% and pectin by 73.91%, while the subsequent depectinization–hydrolysis stage reduced residual hemicellulose by 37.50%. Under these pretreated conditions, addition of PEG increased xylose-equivalent yield from 24.18% without PEG to 78.76% at

9 g/L PEG. The strong improvement between 0 and 3 g/L demonstrates a clear contribution of PEG supplementation relative to the control, whereas the progressively smaller improvement from 5 to 9 g/L suggests diminishing benefit at higher concentrations. Because structural changes and PEG–enzyme interactions were not independently manipulated or directly measured, these relationships should be interpreted as coordinated process responses rather than proof of a single causal mechanism.

4. Conclusions

Alkaline pretreatment followed by depectinization improved the accessibility of coffee cherry processing waste (CCPW) by reducing lignin from 19.00% to 15.00% and pectin from 11.50% to 3.00%. During the subsequent depectinization–hydrolysis stage, pectin decreased further to 1.40%, while hemicellulose decreased from 28.00% to 17.50%, indicating substantial conversion of the hemicellulosic fraction. PEG 6000 supplementation increased reducing-sugar release and hydrolysis yield compared with the PEG-free control. The highest xylose-equivalent reducing sugar concentration and xylose hydrolysis yield among the tested treatments were obtained at 9 g/L PEG 6000, reaching 1.96 g/L and 78.76%, respectively, compared with 0.602 g/L and 24.18% in the control. The highest glucose-equivalent reducing sugar concentration and glucose hydrolysis yield were observed at 7 g/L PEG 6000, reaching 2.77 g/L and 71.02%, respectively, compared with 0.785 g/L and 20.12% in the control. The improved hydrolysis observed in PEG-supplemented treatments may be associated with reduced non-productive enzyme–lignin interactions, as proposed in previous studies; however, this mechanism was not directly measured in the present study. Compared with the authors' previous work focusing on downstream crystalline xylose production from CCPW, the present study demonstrates the contribution of alkaline pretreatment, depectinization, and PEG 6000 supplementation to upstream enzymatic sugar release and hydrolysis efficiency. These findings support the potential use of CCPW as a lignocellulosic feedstock for producing xylose-containing hydrolysates for subsequent purification and downstream bioconversion.

5. Patents

Registered Patent Number S00202500187.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: Conceptualization, SON Yudiastuti, Wiwik Handayani, and Aulia Brilliantina; methodology, SON Yudiastuti and Remita Anggraini Permatasari; software, SON Yudiastuti and Remita Anggraini Permatasari; validation, SON Yudiastuti and Wiwik Handayani; formal analysis, SON Yudiastuti and Remita Anggraini Permatasari; investigation, SON Yudiastuti, EKN Sari, and Remita Anggraini Permatasari; resources, SON Yudiastuti, Rizza Wijaya, and Remita Anggraini Permatasari; data curation, SON Yudiastuti and AHH Slamet; writing—original draft preparation, SON Yudiastuti and Remita Anggraini Permatasari; writing—review and editing, SON Yudiastuti and Wiwik Handayani; visualization, SON Yudiastuti and Rizza Wijaya; supervision, SON Yudiastuti and Wiwik Handayani; project administration, SON Yudiastuti, Aulia Brilliantina, and Wiwik Handayani; funding acquisition, SON Yudiastuti, Wiwik Handayani, Aulia Brilliantina, Rizza Wijaya, and Ahmad Haris Hasanuddin Slamet. All authors have read and agreed to the published version of the manuscript.

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Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with "A"—e.g., Figure A1, Figure A2, etc.

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