

Calcium peroxide pretreatment to facilitate the delignification and enzymatic hydrolysis of wheat straw

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Research Article

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Abstract

Calcium peroxide (CaO_2) pretreatment was employed to remove lignin and subsequently facilitate enzymatic digestibility of wheat straw. An optimal condition was obtained at 130°C for 10 min with 0.35 g CaO_2 /g dried material of wheat straw and a 1:8 solid-liquid ratio. Under this condition, 57.8% of initial lignin, 7.2% of initial glucan, and 30.6% of initial xylan were removed from CaO_2 pretreatment, respectively, meanwhile, a glucose recovery of 90.6 % and a xylose recovery of 65.9 % were obtained from the subsequent enzymatic hydrolysis of treated wheat straw, respectively. CaO_2 pretreatment was proved to be a very effective method in delignification and improving enzymatic digestibility. Compared to raw material, the complex structure of lignocellulose was drastically disrupted with a wide emergence of scaly bulges and fully exposed microfibrils, which still retained in the solid.

Introduction

At present, the severe challenges posed by the scarcity of energy and resources and the increasingly serious environmental problems have aroused widespread concern among researchers in related fields. Bioethanol is regarded as one of the effective ways to achieve sustainable use of clean energy because of its renewable, low cost and abundance ^[1]. Wheat straw represents about 110–120% dry weight of the harvested wheat, which is a potential feedstock for bioethanol production in China and other wheat-producing countries. Wheat is one of the most widely cultivated food crops throughout the world, with about 40% of the world's populations depending on it as their staple food. China is the leading producer and consumer of wheat in the globally. In 2020, wheat yield was about 127 million tons (Mt), and the total available wheat straw was about 146 Mt in China. Wheat straw can be easily collected from the process of harvesting wheat and consists ~ 26% cellulose and ~ 26% hemicellulose, but a recalcitrant structure in which lignin is intertwined with cellulose and hemicellulose, hinders the polymer to produce monosaccharides in the step of enzymatic hydrolysis ^[2]. Therefore, pretreatment is a key process to remove lignin and disrupt the resistance barrier of the wheat straw to improve their enzymatic digestibility for enhancing fermentable sugar production ^[3, 4].

Presently, a variety of pretreatments to improve enzymatic digestibility by enhancing delignification, increasing porosity of the lignocellulosic biomass and disrupting its recalcitrant structure have been investigated ^[5]. Previous research showed that lignin is the main reason for depressing enzymes efficiency during enzymatic saccharification ^[6, 7]. Alkali and oxidant agents are widely used to remove lignin from biomass, which have many favorable advantages such as less sugar loss, few inhibitors production and effective delignification ^[8, 9]. Among these pretreatments, alkaline hydrogen peroxide (AHP) has attracted much attention due to its high efficiency, low secondary pollution and effective lignin removal at mild temperatures ^[10]. Additionally, AHP pretreatments still have a fast reaction rate of lignin removal at mild conditions ^[6]. It was noted that the structure of AHP treated biomass was destroyed, which enhanced the substrate accessibility to enzymes and reduced the non-productive adsorption of

Loading [MathJax]/jax/output/CommonHTML/jax.js enzymatic digestibility ^[9, 11, 12]. It was reported that AHP

pretreatment resulted in 91.53% lignin removal of corn stover at 30°C for 24 h with 0.5 g H₂O₂/g substrate (pH = 11.5), and increased the cellulose accessible pore volume and the area of exposed cellulose, which effectively enhanced the enzymatic hydrolysis efficiency [10]. However, as the principal reagent of AHP pretreatment (e.g. NaOH/H₂O₂), H₂O₂ is not only expensive but also difficult to store. Calcium peroxide (CaO₂) reacts with water to form calcium hydroxide and hydrogen peroxide, and the latter further produces highly oxidizing hydroxyl radical under alkaline conditions [8]. The mechanism is as follows [10, 13].



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Based on the described mechanism, CaO₂ possesses the function of alkaline hydrogen peroxide. Moreover, CaO₂ is more cost-effective and easier to transport and store due to its better thermal stability in comparison with hydrogen peroxide. CaO₂ pretreatment removed major part of lignin and preserved most of carbohydrates in the kenaf core powder [5]. Therefore, CaO₂ treatment is a promising method to remove lignin and improve enzymatic saccharification of lignocellulose.

In this work, the feasibility of enhancing enzymatic digestibility of the wheat straw by application of CaO₂ pretreatment was discussed at different conditions. The influences of reaction time, temperature and CaO₂ loading on lignin reduction and carbohydrates degradation were determined. Treated samples were subjected to enzymatic hydrolysis with different enzymes loadings to evaluate the effects of CaO₂ pretreatment. Finally, macro-structural changes in the untreated and treated wheat straw was analyzed by scanning electron microscopy (SEM).

Material And Methods

Wheat straw

Wheat straw was collected from rural areas of Hebei Province, China. Wheat straw was air-dried and ground to pass a 20 meshes screen in preparation for the experiment. The principal compositions of the raw wheat straw were cellulose (36.92 ± 0.98%), hemicellulose (25.11 ± 0.77%), and lignin (17.77 ± 1.26%).

CaO₂ pretreatment

CaO₂ pretreatment was performed in a 200 mL reactor with a magnetic stirrer. The temperature and stirring speed of the reactor was adjusted by an automatic controller. The speed of the stirrer was set at 200 rpm during the pretreatment process. Raw wheat straw was loaded into the reactor at a solid-liquid ratio of 1:8 with CaO₂ loading of 0.15, 0.25, 0.35, 0.45 and 0.55 g/g dried material (DM). All the CaO₂ pretreatments were performed at varying pretreatment temperatures (90, 110, 130 and 150°C) and times (10, 30, 60, 90, 120, 150 min). After the reaction, the reactor was taken out of the heating jacket and immediately put into ice water to complete the reaction. The pretreated slurry was quantitatively transferred to a 500 mL glass beaker. The reactor was washed several times with distilled water after the transfer. Then, the mixture in the glass beaker was neutralized with acid (0.1 mol/L HCl) to pH 7.0, added distilled water to 300 mL, and filtered. The filtrate obtained was used to determine sugar and their degradation products. Wet solids were washed to remove the residual sugar. The way of washing the solids was centrifuged in two weight volumes, decanted the water and repeated six times. The washed solids were stored in a sealed plastic bag and placed in a 4°C freezer for chemical compositional analysis and subsequent enzymatic hydrolysis.

Enzymatic hydrolysis

Enzymic saccharification tests were performed in sodium citrate buffer (0.1 M, pH 4.8) with using of cellulase (Novozyme NS50013, 77.25 FPU/mL), β-glucosidase (Novozyme NS50010, 107.13 pNPGU/mL) and xylanase (UTC-X50, 25,000 IU/g) in a shaking incubator. Enzyme doses of cellulase 20 FPU/g-substrate, of β-glucosidase 30 pNPGU/g-substrate, and of xylanase 60 IU/g-substrate were used. Weigh out CaO₂-treated wheat straw to the equivalent of 0.2000 g on an oven dry weight basis. To each saccharification vial was pipetted prepared substrate, 5.0 mL (pH 4.8) sodium citrate buffer, 400 Fg tetracycline and 300 g cycloheximide. After addition of the enzymes, added distilled water to a total volume of 10 mL. Closed tightly vials were stirred thoroughly, and then returned to the shaking incubator (50°C, 150 rpm) for 48 h. After enzymatic saccharification test, the aliquot was withdrawn, and then diluted 10-fold. Diluted samples were boiled in a water bath for 10 min to put an end to the enzymatic digestion before being centrifuged and filtered for sugar analysis. All the saccharification tests were conducted in duplicate.

The recoveries of xylose and glucose were calculated as the percent conversion of gulcan and xylan in enzymatic saccharification process, as following equations (4) and (5), respectively.

$$\text{Glucoserecovery} = \frac{n_1 \times 0.9}{m_1} \times 100\%$$

4

$$\text{Xyloserecovery} = \frac{n_2 \times 0.88}{m_2} \times 100\%$$

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where n_1 is the amount of glucose released in enzymatic hydrolysis, m_1 is the amount of gulcan in untreated wheat straw, n_2 is the amount of xylose released in enzymatic hydrolysis, m_2 is the amount of xylan in untreated wheat straw, 0.9 and 0.88 are the conversion factors for polymer to monomer sugars by the water of hydrolysis ^[14].

Chemical analysis

Total solid and constituents (structural carbohydrates, lignin and ash) of untreated and treated wheat straw were determined referring to LAP standard procedures established by NREL ^[15]. Sugar was measured by HPLC (Lab Alliance Series III) equipped with refractive-index detection and a Biorad Aminex HPX-87H column. The operating procedure in detail was similar to previous report ^[3]. All samples were measured in duplicate, with the averages of measured values.

SEM

Surface morphological feature changes caused by CaO₂ pretreatment were captured at a magnification of 1000 using a field emission SEM (Nova NanoSEM 50, USA). Prior to imaging, the sample was coated by spraying the palladium-gold mixture to it conductive. Coated sample was placed in the chamber of scanning electron microscope and then observed under vacuum.

Results And Discussion

Effects of CaO₂ loading on pretreatment and enzymatic hydrolysis

The CaO₂ loading is a critical factor in pretreatment as it has a great influence on delignification, cellulose and hemicellulose degradation of the biomass, as well as the cost of the reagent. The effects of CaO₂ loadings on lignin removal and carbohydrate degradation of wheat straw were measured in the range of 0.15, 0.25, 0.35, 0.45, 0.55 g/g DM at 130°C for 120 min (Fig. 1). The delignification rate increased significantly from 26.2–57.8%, as CaO₂ loading elevated from 0.15 to 0.35 g/g DM; nevertheless, with no significant decreasing trend on delignification with above 0.35 g/g DM. Due to the low solubility of CaO₂, it is difficult to increase the active ingredient by increasing the concentration of a certain value. Yang et al. ^[16] observed similar results in the pretreatment of sisal waste with alkaline H₂O₂, which reported that the lignin removal increased significantly as H₂O₂ loading increased from 0.1–0.6 g/g, and little above 0.6g/g. Increasing CaO₂ loading form 0.15 to 0.55 g/g DM, the degradation rates of glucan and xylan for pretreatment increased from 4.8% and 20.9–7.6% and 36.7%, respectively. The result indicated that lignin removal was significantly affected by the CaO₂ loading. The degradation of hemicellulose and some part of cellulose were also observed. However, both glucan and xylan were retained in wheat straw in large proportion even after CaO₂ pretreatment.

Pretreated wheat straw by CaO_2 with different loadings was subjected to enzymatic saccharification test. Enzymatic hydrolysis processes were performed in 48 h with complex enzyme of cellulase (20 FPU/g-substrate), β -glucosidase enzyme (30 pNPGU/g-substrate) and xylanase (60 IU/g-substrate). The recoveries of glucose and xylose for the CaO_2 loading studies were presented in Fig. 1. Glucose recovery increased from 52.1 to 93.2% with CaO_2 loading increasing from 0.15 to 0.55 g/g DM. CaO_2 loading showed a significant impact on enhancing glucose recovery. The results showed that increasing CaO_2 loading from 0.15 to 0.35 g/g DM glucose recovery increased by 73.9%. However, only 2.9% glucose recovery improvement was achieved by CaO_2 loading from 0.35 to 0.55 g/g DM. It was noted that xylose recovery increased with elevated CaO_2 loading within 0.35 g/g DM, and then decreased with continued CaO_2 loading. In totally, as CaO_2 loading increased from 0.35 to 0.55 g/g DM did not evidently improve both the lignin removal and cellulose digestibility significantly; instead, decreased the hemicellulose digestibility. The maximum recovery of xylose was 65.9%, which was obtained at 0.35 g/g DM. This trend may be due to higher loss of hemicellulose from CaO_2 pretreatment. The maximum recovery of xylose in this work was higher than that for alkaline H_2O_2 pretreated sisal waste (pH = 11.5, 6h, at room temperature, 0.6 g H_2O_2 /g), though slightly higher delignification rate^[16]. It could be attributed to the addition of xylanase improved the cellulose and hemicelluloses enzymatic hydrolysis effect. These results demonstrated that CaO_2 pretreatment was an efficient pretreatment wheat straw in enhancing the subsequent enzymatic digestibility. Lignin removal was positively correlated with enzymatic hydrolysis of cellulose. This was probably attributed to the removal of lignin and the destruction of the complex cross-linking structure of wheat straw during CaO_2 pretreatment, which effectively improved the accessible surface area of cellulose and hemicellulose straw, and thereby enhanced enzymes digestibility^[17, 18]. In terms of enzymatic saccharification test, 0.35 g/g DM as an optimum CaO_2 loading was used for subsequent experiments.

Effect of temperature on pretreatment and enzymatic hydrolysis

Temperature is a vital factor in the chemical reaction, as well as in the pretreatment biomass process, which directly influenced the effect of pretreatment and energy consumption^[19]. Influences of pretreatment temperature (90, 110, 130, 150°C) in relation to CaO_2 pretreatment efficacy were investigated for 120 min with the CaO_2 loading of 0.35 g/g DM (Fig. 2). As the temperature increased from 90°C to 150°C, the lignin removal rate increased significantly from 18.7–60.8%. In addition, the temperature rising from 90°C to 130°C has a greater impact on delignification than the temperature rising from 130 to 150°C. As showed in Fig. 2, increasing temperature could accelerate the loss of hemicellulose and cellulose. It can be observed that the removal rate of the gulcan and xylan was quite different depending on the temperature. The maximum removal of gulcan and xylan reached 8.7 and 48.1%, respectively, which suggested that hemicellulose could be degraded more easily than cellulose at the same pretreatment condition, due to their different structures^[20].

The recoveries of glucose and xylose for enzymatic digestibility were plotted along pretreatment temperature in Fig. 2. An increase of the pretreatment temperature favored the enzymatic hydrolysis of cellulose, which was similar to that of lignin removal. As it was observed for glucose recovery increased from 40.9–91.4%, with the temperature rising from 90 to 130°C. As temperature increased to 150°C, it didn't significantly affect glucose recovery (91.4%). It was considered that CaO₂ pretreatment was effective in breaking apart lignin and hemicellulose while keeping cellulose intact at high temperature, thereby enhanced in its surface area, by which it becomes more accessible to enzymatic hydrolytic treatment [5]. Xylose recovery initially increased with temperature, while it gradually declined with further increasing temperature. The maximum recovery of xylose was 65.9%, which was obtained at 130°C. It was attributed that a partial hemicellulose degradation during CaO₂ pretreatment at 150°C resulted in the decrease of xylose recovery of enzymatic hydrolysis. In terms of enzymatic hydrolysis, 130°C was taken for the appropriate reaction time.

Effect of time on pretreatment and enzymatic hydrolysis

Reaction time affects the utilization of the reagent and the effect of pretreatment. The effects of reaction time varying from 10 to 150 min were investigated at 130°C with CaO₂ loading 0.35 g/g. The results of delignification and enzymatic hydrolysis were listed in Fig. 3. Lignin removal significantly enhanced from 29.3 to 57.3%, as the reaction time rising from 10 to 90 min. However, further enhance time at 120 and 150 min, no significant increase of delignification was exhibited. The removal of gulcan and xylan slowly increased with the reaction time increasing. However, it was observed that reaction time had little effect on the degradation of cellulose and hemicellulose at 130°C with CaO₂ loading 0.35 g/g. As the reaction time increasing from 10 to 120 min, the removal rates of glucan and xylan enhanced from 5.5 to 7.3%, and from 26.4 to 31.4%, respectively.

Both xylose and glucose recoveries for enzymatic hydrolysis gradually ascended first and then declined with increasing reaction time, as it was seen in Fig. 3. The maximum recovery of glucose and xylose of 90.6% and 65.9%, respectively, was available at the time of 120 min. Further increasing the reaction time to 150 min, both the recovery of glucose and xylose decreased for enzymatic hydrolysis. This was caused by the loss of cellulose and hemicellulose fraction during the pretreatment process. In general, the reaction time is not a variable leading to major changes in enzymatic hydrolysis. Hence, increased reaction time did not favorably enhance enzymatic hydrolysis efficiency. For sugar recovery of enzymatic hydrolysis, the reaction time of 120 min was selected as the optimization of CaO₂ pretreatment time for the further work. It is important to note that CaO₂ pretreatment of wheat straw only resulted in the loss of a small amount of carbohydrates, and the conversion rate of cellulose and hemicellulose increased significantly during the enzymatic digestion process.

SEM

Scanning electron microscope (SEM) was used to study the influence of calcium oxide pretreatment on Loading [MathJax]/jax/output/CommonHTML/jax.js wheat straw. Figure 4 showed that the untreated wheat straw

had a smooth, continuous, and dense structure, while the treated wheat straw presented a loose sheet structure due to the removal of more than half of the lignin. Although the complex structure of lignocellulose was drastically disrupted with a wide emergence of scaly bulges and fully exposed microfibrils, it still retained in the solid, which implied that there is no significant loss of cellulose. This was in agreement with the result of the degradation rate of cellulose in the pretreatment. Therefore, CaO_2 pretreatment effectively increased the accessibility of enzymes to cellulose and hemicellulose, which, in turn, enhanced enzymatic hydrolysis. Therefore, CaO_2 pretreatment was efficient in attacking the cellulose and hemicellulose fibers, which significantly improved the enzymatic hydrolysis.

Conclusion

CaO_2 pretreatment showed effective delignification of wheat straw and largely retained the cellulose and hemicellulose fraction. The pretreated wheat straw achieved a higher glucose recovery and xylose recovery in enzymatic hydrolysis. Meanwhile, the surface structure of cellulose bundle was disrupted by the CaO_2 pretreatment, which raised the accessibility of cellulose and hemicellulose to enzymes, and profitably enhanced the hydrolysis efficiency. Therefore, CaO_2 is a potential pretreatment method for improving enzymatic hydrolysis of lignocellulose.

Declarations

Conflict of interest

The authors declare no competing financial interest with the work submitted.

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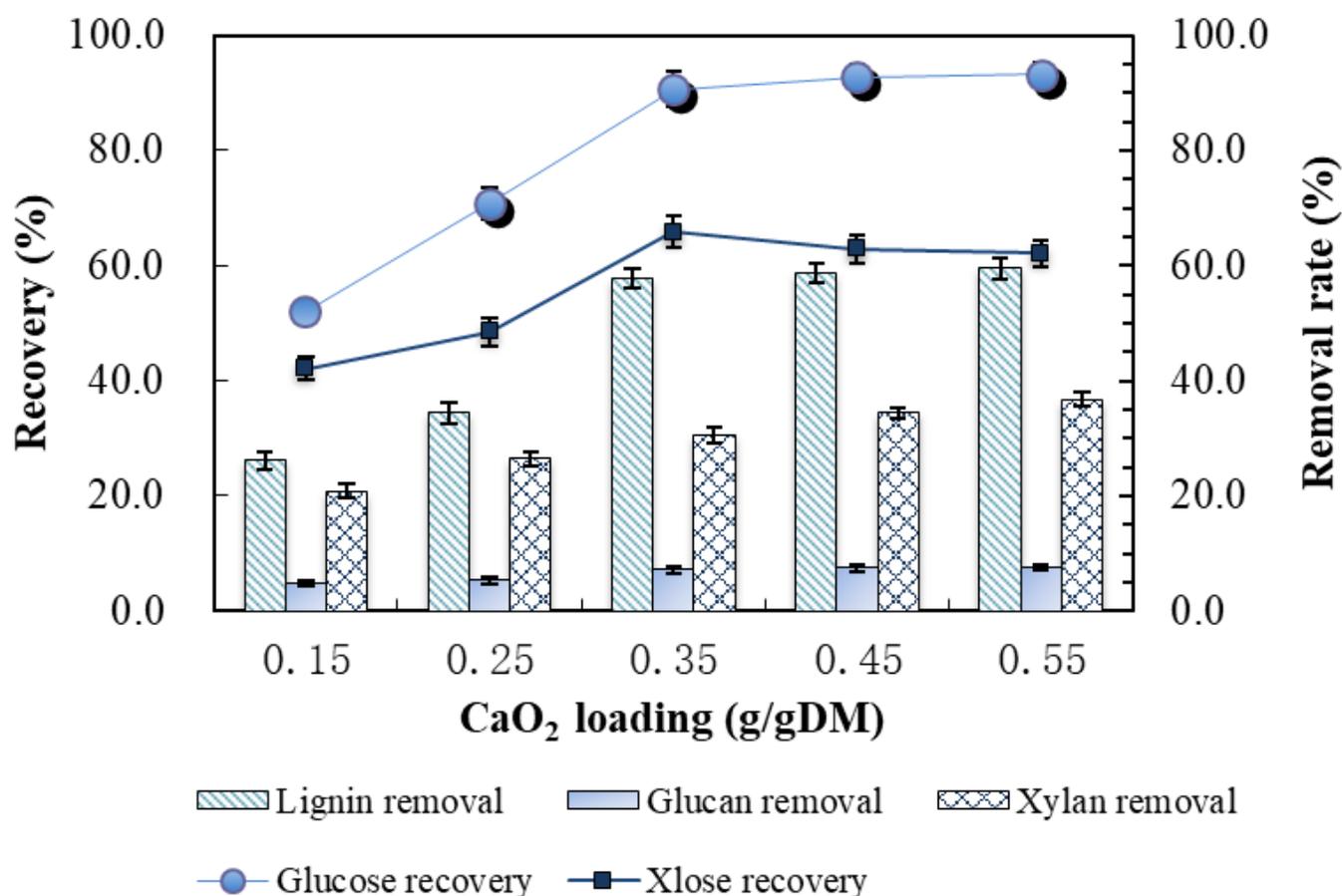
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21. A List of Figures.

Figures



Effects of CaO₂ loading on lignin removal, glucan removal, xylan removal and recovery of glucose and xylose after enzymatic hydrolysis. The pretreatment conditions: 130 °C, 120 min and a 1:8 solid-liquid ratio. Enzymatic hydrolysis conditions: 48 h, 20 FPU/g-sbustrate of cellucase, 30 pNPGU/g-sbustrate of β-glucosidase and 60 IU/g-xylan of xylanase, pH 4.8 at 50 °C.

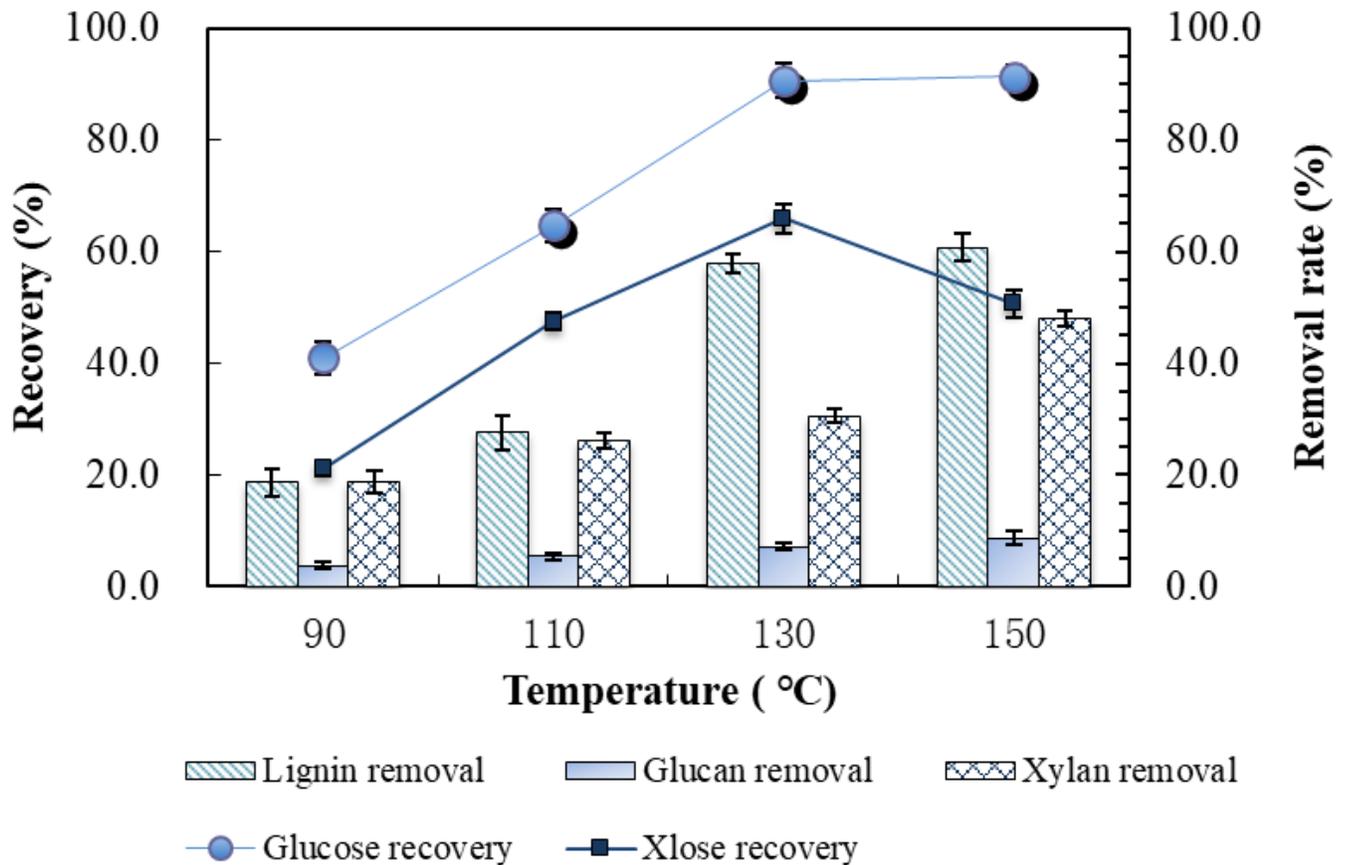


Figure 2

Effects of temperature on lignin removal, glucan removal, xylan removal and recovery of glucose and xylose after enzymatic hydrolysis. The pretreatment conditions: CaO₂ loading 0.35 g/g DM, 120 min and a 1:8 solid-liquid ratio. Enzymatic hydrolysis conditions: 48 h, 20 FPU/g-sbustrate of cellucase, 30 pNPGU/g-sbustrate of β-glucosidase and 60 IU/g-xylan of xylanase, pH 4.8 at 50 °C.

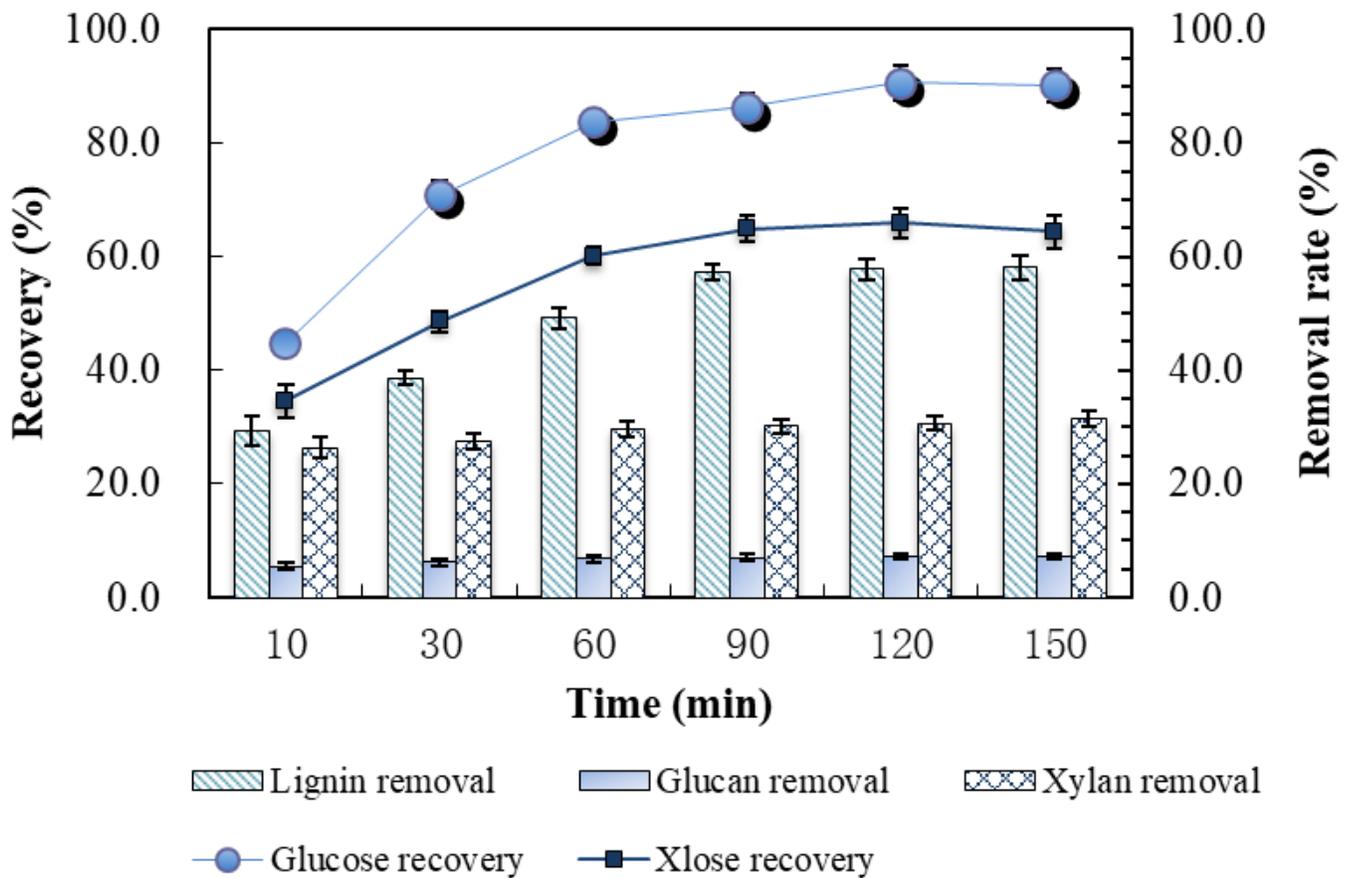


Figure 3

Effects of reaction time on lignin removal, glucan removal, xylan removal and recovery of glucose and xylose after enzymatic hydrolysis. The pretreatment conditions: CaO₂ loading 0.35 g/g DM, 130 °C and a 1:8 solid-liquid ratio. Enzymatic hydrolysis conditions: 48 h, 20 FPU/g-substrate of cellulase, 30 pNPGU/g-substrate of β-glucosidase and 60 IU/g-xylan of xylanase, pH 4.8 at 50 °C.

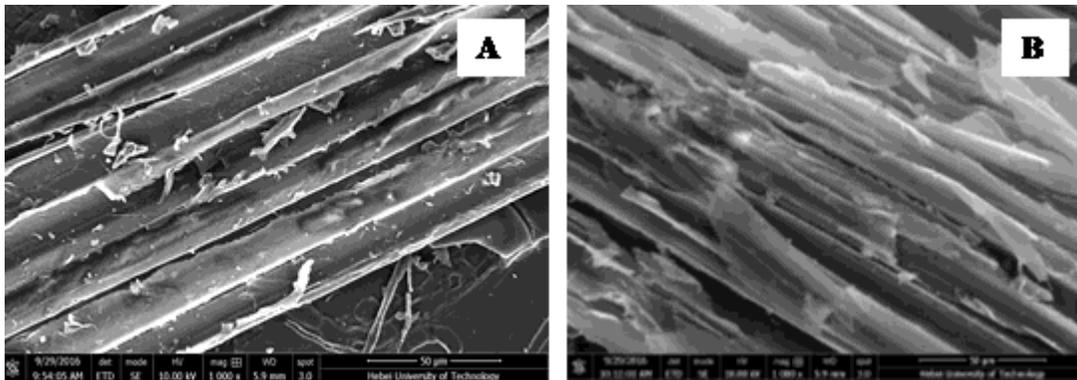


Figure 4

Scanning electron micrograph of untreated wheat straw (A) and pretreated (B) (CaO₂ loading 0.35 g/g DM, 120 min, 130 °C and a 1:8 solid-liquid ratio) at ×1,000 magnification.