Comparison of Calcium Oxide and Calcium Peroxide Pretreatments of Wheat Straw for Improving Biohydrogen Production

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**ABSTRACT:** Wheat straw was pretreated with either CaO2 or CaO to improve biohydrogen production. Both CaO and CaO2 pretreatments improved the biodegradability of the wheat straw. CaO pretreatment raised the H2 yield by between 48.8 and 163.9% at CaO contents ranging from 2 to 4%. The highest H2 yield (144 mL/g total solid [TS]) was obtained at 121 °C and 6% CaO. In addition, the highest H2 yield from wheat straw pretreated at the same temperature and dosage of CaO2 was 71.8 mL/g TS, which was higher than that of the control group (43.2 mL/g TS), with hot water (121 °C) treatment. Considering pretreatment costs and H2 production potential, CaO was a better pretreatment agent than CaO2.

1. INTRODUCTION

With the increasing shortages of fossil energy and the concomitant aggravation of environmental problems, the use of biomass waste products, as potential biomass resources, has been considered worldwide in recent decades.1 Such wastes have advantages, such as high abundance, low costs, and carbon neutrality.2 These biowastes are generally treated by direct incineration, being buried in soil, or subjected to anaerobic digestion.3 Direct incineration is the main technique used for treating biomass wastes.4 However, this technique harms the environment and likely generates considerable amounts of harmful and toxic dust and gases, causing both air pollution and fire risks. Lignocellulose, as an agricultural biomass, is a unique source of energy for the sustainable production of biofuels.4 Among lignocellulosic biomasses, wheat straw is an attractive raw material for large-scale biorefineries as it is inexpensive, has a high sugar content, and is widely available.5 Large quantities of wheat straw are produced around the world every year. In 2018, world wheat production was 763 million tons.6 One kilogram of wheat grain generates an average of 1.3 kg of wheat straw.6 In particular, China produces approximately 80 million tons of wheat straw annually.7 Wheat straw is considered one of the most promising renewable feedstocks for sustainable biohydrogen and methane production.8,9

Hydrogen (H2) is a highly combustible energy carrier (141.9 kJ/g H2) that is considered a promising alternative to fossil fuels, as it offers many social, economical, and environmental benefits. Using H2 as a transportation fuel can help meet global energy demands. H2 is referred to as a green fuel because it is entirely carbon-free. To be economically feasible and realistically viable, H2 production must be sustainable. This can be achieved by electrolyzing water, pyrolyzing biomass, or gasifying suitable materials. In particular, H2 can be produced by biological processes, particularly by anaerobic microorganisms that convert carbohydrates into H2.10 Anaerobic fermentation is a cost-effective and reliable technique for producing H2 directly from renewable biomass or organic waste.11 Monomeric sugars are consumed by fermentative microbes during the dark fermentation processes, usually involving the acetate and butyrate pathways.12 The advantages of this approach are significant: inexpensive biomass and byproduct of agricultural food production can be used as the fermentation feedstocks. Lignocellulosic waste is a potential feedstock for the production of biofuels such as H2, CH4, and ethanol, which can replace fossil fuels and reduce greenhouse gas emissions. However, the high cost of the hydrolysis of lignocellulosic wastes remains a matter of considerable concern.

Two sugars, sucrose and glucose, are readily degradable and thus are the preferred model substrates for H2 production. The major fermentable sugars in lignocellulosic waste are glucose and xylose in the form of cellulose and hemicellulose polymers,
respective. Although the conversion of wheat straw biomass to biofuels has significant economic and commercial potential, the cell wall of wheat straw can naturally protect cellulose from attack through many complex physicochemical factors, such as the dense cell wall structure, the limited accessible surface, the cellulose crystallinity, and the existence of both hemicelluloses and lignin. In addition, the high silica content and extremely complex lignin–carbohydrate structure greatly hinder the efficient conversion of wheat straw into biofuels through biotechnology. Cellulose and hemicelluloses can be degraded under the same conditions, but costs are increased because of the complex structure. Therefore, to more efficiently utilize wheat straw, an effective pretreatment technique must be carried out to overcome the recalcitrance of the wheat straw cell wall and increase the accessibility of the pretreated matrix to cellulase.

To date, several pretreatment methods, such as those based on hydrothermal, dilute acid, dilute alkaline, steam explosion, organic solvent, and autohydrolysis pretreatment strategies, have been developed. Among these methods, dilute acid hydrolysis has a significant effect, but it causes the decomposition of lignocellulose into inhibitors that negatively impact subsequent enzymatic hydrolysis and fermentation. In addition, this method significantly corrodes equipment and requires a neutralization step to treat the wastewater, increasing the cost of pretreatment. As an effective pretreatment technique, alkaline pretreatment has been widely used in the biogas production from lignocellulosic wastes such as corn stover and wheat and rice straws. These alkaline processes have some advantages, including changing the chemical composition and structure, lowering cellulose crystallinity, neutralizing acidic reagents used during production, and preventing decreases in pH during subsequent acidogenic processes.

Alkaline pretreatment has also been shown to be an efficient strategy for removing lignin from straw to enhance methane production from wheat straw. Ca(OH)2 pretreatment has been utilized to improve enzyme hydrolysis and biogas production from rice straw. After Ca(OH)2 treatment, the xylose and glucose conversion rates during enzymatic hydrolysis rose from 22.4 and 36.0 to 50.2 and 66.8%, respectively. The highest biogas production obtained with 10% Ca(OH)2-treated rice straw was 574.5 mL/g volatile solids (VS), which was 36.7% higher than the control group, without Ca(OH)2 treatment. This alkaline pretreatment technique utilizes rigorously controlled physicochemical conditions to obtain monomeric sugars from lignocellulosic wastes. The operational conditions including temperature, reaction time, alkaline concentration, and solid/liquid ratio of the biowaste and alkaline solution can significantly influence the efficiency of the alkaline pretreatment.

A NaOH/urea solution at −12 °C was used to treat rice straw to improve saccharification and bio-H2 generation. The NaOH/urea process showed excellent pretreatment performance and afforded 80.22% conversion to reducing sugars, which is 31.89% higher than that of the control group. Further fermentation of the sugars resulted in a higher H2 yield (45.98%), which was higher than the control yield.

On the other hand, calcium peroxide (CaO2) is commonly employed as an oxidant because of its ability to generate stable oxidation conditions. When CaO2 is dissolved in hydrous media, it reacts with water and slowly produces oxygen (O2), calcium hydroxide [Ca(OH)2], and hydrogen peroxide (H2O2), as described by eqs 1 and 2. H2O2 is considered as a classic and nontoxic oxidant. CaO2 is one of the safest and most versatile solid inorganic peroxy-compounds, and it can be considered a “solid form” of H2O2.

\[
\text{CaO}_2 + 2\text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 + \text{H}_2\text{O}_2 \tag{1}
\]

\[
2\text{CaO}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Ca(OH)}_2 + \text{O}_2 \uparrow \tag{2}
\]

Previous studies have demonstrated that CaO2 is an efficient oxidant for contaminant removal by the action of reactive oxygen species generated from CaO2. CaO2 treatment could also improve sludge solubilization. Moreover, CaO2 can also oxidize the most refractory organic compounds that are difficult for anaerobes to biodegrade. Fu et al. found that CaO2 was also an effective intermediary for improving biogas production from corn straw. They also observed that the digestate from the anaerobic digestion conducted with CaO2 had a high CaCO3 content, improving its potential as a soil conditioner for acidic soil.

Nonetheless, there are few reports on CaO2 pretreatment of biomass for enhancing bio-H2 generation. The main characteristics of wheat straw pretreated with CaO and CaO2 and the effects of these treatments on subsequent bio-H2 production processes are investigated and compared for the first time here. The aims of this work were: (1) to investigate whether wheat straw pretreated with CaO or CaO2 is a suitable feedstock for bio-H2 generation; (2) to compare the effects of CaO and CaO2 pretreatments on the hydrolysis of wheat straw; and (3) to evaluate the influences of CaO and CaO2 pretreatments and the biodegradability of wheat straw and further H2 production.

2. MATERIALS AND METHODS

2.1. Feedstock and Inoculum. The hydrogen-producing substrate used in this study was wheat straw, which was collected from rural areas in Shandong, China. The wheat straw had to be pretreated before H2 production. First, the wheat straw was dried at room temperature (30–35 °C) for 3–5 d and then chopped to a particle size of approximately 3.0 cm. Subsequently, the straw was further homogenized with a grinder (COSUAICS-700Y, China) to produce a 40 mesh (0.45 mm) straw powder that was packed in sample bags for further utilization.

The anaerobic sludge collected from the UASB system in the Shandong Engineering Lab of Light Waste Cleaner Energy Technology was stored at 4 °C until use. The sludge incubation process lasted until biogas production was accomplished (40 d). Subsequently, the incubated sludge was heated at 85 °C for 30 min, and then, glucose (2 g/L) was added. The material was cultured at 37 ± 1 °C for 36 h to enrich H2-producing bacteria (HPB), affording the H2-producing inoculum. The thermal treatment method such as this have been shown to be a simple and effective technique for enriching HBP. The main characteristics of both the wheat straw powder and inoculum are described in Table 1.

2.2. CaO2 and CaO Pretreatments. To compare the effects of CaO and CaO2 pretreatments on wheat straw hydrolysis and subsequent bio-H2 generation, wheat straw was hydrolyzed according to a previous report. Ten grams (dry weight) of wheat straw was mixed with different amounts (0.2–1.0 g) of CaO or CaO2 in a series of 200 mL glass bottles. The corresponding dosages of CaO or CaO2 were 2, 4, 6, 8, and 10% relative to dry wheat straw. Simultaneously, two groups were treated with hot water (121 °C) and served as the control groups. Deionized water was added into each bottle to...
keep the solid (dry wheat straw)/liquid (H2O) mass ratio at 1:8. To fully soak the straw, these bottles were tightly sealed and placed in an incubator at 35 °C for 120 min. Subsequently, the bottles were kept at 121 °C for 60 min and then cooled to room temperature to obtain a mixture of the hydrolysate and solid residue.

2.3. Batch H2 Fermentation. The batch H2 fermentation tests were conducted in an 800 mL bioreactor with a working volume of 500 mL. The mixtures containing different quantities (0.2–1.0 g) of CaO2 or CaO, which were labeled 2–10% (of wheat straw), were transferred to the corresponding bioreactors. The control mixtures (without CaO2 or CaO) were transferred to other bioreactors and used as the control groups. Then, 200 mL of deionized water was added to each bioreactor, and the contents were mixed to homogeneity. Subsequently, the initial pH of each bioreactor was adjusted to 7.0 using 1.0 mol/L HCl. Then, 150 mL of H2-producing inoculum were measured with a pH meter (PHS-3C, Leici, Brentford, UK). The pH values of the wheat straw and inoculum were measured, according to a previous report. In addition, ammonia nitrogen (NH4+-N) was determined by Nessler reagent spectrophotometry. The total organic carbon (TOC) was analyzed using a TOC analyzer (TOC-L CPH CN200, Shimadzu, Japan). Before the analysis, the samples were filtered through a 0.45 μm cellulose acetate filter (Whatman, Brentford, UK). The pH values of the wheat straw and inoculum were measured with a pH meter (PHS-3C, Leici, China). The initial pH of wheat straw was measured by mixing 5 g of sample with 25 mL of deionized water, soaking for 2 h, and measuring the pH of the supernatant. The total carbon (C) and nitrogen (N) contents of wheat straw were analyzed using an elemental analyzer (Elementar, Vario EL III, Germany). Volatile fatty acids (VFAs), such as acetate, propionate, and butyrate, were determined by using gas chromatography (GC-2014C/TCD, Shimadzu, Japan) with a thermal conductivity detector (TCD). The operational temperatures of the injection port and detector were 50 and 80 °C, respectively. The carrier gas was nitrogen (N2), and the flow rate was 20 mL/min. All gas volumes were measured under the standard temperature and pressure conditions (273 K, 101.325 Pa).

In addition, to investigate whether the effects of CaO and CaO2 pretreatments on the biogas production were statistically significant, the one-factor analysis of variance was carried out with the Microsoft Excel 2016 for these average values of SCOD, soluble total organic carbon (STOC), VFAs, and H2 yields. The p-value is less than 0.05, which is defined as the significance level.

2.5. Kinetic Model. The kinetics of the anaerobic digestion process can dramatically influence various physical, chemical, and biological factors. A cumulative hydrogen production (CHP) curve was employed to estimate the H2 production potential (HPP). The H2 fermentation process was divided into four phases: the lag, exponential, stationary, and decline periods. Both HPP and lag time were important parameters that indicated the overall performance of the H2 generation process. Moreover, these kinetic parameters were estimated according to the modified Gompertz model, as shown in eq 5

\[ \beta = \frac{k \lambda}{\tau \cos \theta} \]  



where \( \lambda \) represents the wavelength of the incident X-ray (0.15406 nm), \( \theta \) is related to the Bragg angles corresponding to the planes, \( \beta \) is the full-width at half maximum of the X-ray peak corresponding to the planes; \( \tau \) describes the X-ray crystallite size, and \( k \) is a constant of 0.89. In addition, the crystallinity index (CrI) was calculated according to eq 4

\[ \text{CrI} (%) = 100 \times \left[ \frac{I_{002} - I_{am}}{I_{002}} \right] \]



where \( I_{am} \) refers to the diffraction intensity of amorphous cellulose at 2θ = 18.4°, and \( I_{002} \) is related to the maximum diffraction intensity at 2θ = 22.5°.

H2 production was monitored by the drainage method, and saturated NaOH was used to absorb CO2 and H2S. The H2 concentration was determined by gas chromatography (GC-2014C, Shimadzu, Japan) with a thermal conductivity detector (TCD). The operational temperatures of the injection port and detector were 50 and 80 °C, respectively. The carrier gas was nitrogen (N2), and the flow rate was 20 mL/min. All gas volumes were measured under the standard temperature and pressure conditions (273 K, 101.325 Pa).

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Table 2. Lignin, Cellulose, and Hemicellulose Contents before and after Pretreatment

| addition (%) | cellulose (%) | hemicelluloses (%) | lignin (%) | ash (%) | others (%) |
|--------------|--------------|--------------------|------------|---------|------------|
| wheat straw  |              |                    |            |         |            |
| 0            | 36 ± 0.4     | 21 ± 0.1           | 17 ± 0.1   | 2.3 ± 0.1 | 23 ± 0.5   |
| 2            | 36 ± 0.6     | 20 ± 0.3           | 16 ± 0.5   | 2.4 ± 0.1 | 24 ± 0.5   |
| 4            | 36 ± 0.7     | 20 ± 0.3           | 15 ± 0.4   | 2.6 ± 0.1 | 27 ± 0.4   |
| CaO₂         |              |                    |            |         |            |
| 6            | 37 ± 0.5     | 19 ± 0.4           | 14 ± 0.3   | 2.7 ± 0.1 | 27 ± 0.4   |
| 8            | 40 ± 0.8     | 18 ± 0.2           | 13 ± 0.4   | 2.8 ± 0.2 | 26 ± 0.4   |
| 10           | 39 ± 0.5     | 18 ± 0.4           | 13 ± 0.2   | 2.9 ± 0.2 | 27 ± 0.5   |
| CaO          |              |                    |            |         |            |
| 6            | 36 ± 0.7     | 18 ± 0.2           | 16 ± 0.1   | 2.6 ± 0.1 | 27 ± 0.5   |
| 8            | 37 ± 0.6     | 17 ± 0.5           | 15 ± 0.6   | 2.7 ± 0.1 | 28 ± 0.4   |
| 10           | 38 ± 0.5     | 17 ± 0.3           | 15 ± 0.5   | 2.8 ± 0.2 | 28 ± 0.6   |

Table 3. Main Properties of the Wheat Straw Hydrolysates

| addition (%) | SCOD (mg/L) | NH₄⁺–N (mg/L) | STOC (mg/L) | pH     | solid residue yield (%) |
|--------------|-------------|---------------|-------------|--------|-------------------------|
| 0            | 7900 ± 100  | 90 ± 3.0      | 3200 ± 10   | 6.0 ± 0.1 | 90 ± 0.5               |
| 2            | 14600 ± 200 | 64 ± 2.0      | 4450 ± 12   | 7.5 ± 0.1 | 91 ± 0.2               |
| 4            | 16600 ± 200 | 52 ± 2.0      | 6000 ± 32   | 8.6 ± 0.1 | 91 ± 0.4               |
| CaO₂         |             |               |             |        |                         |
| 6            | 18200 ± 200 | 42 ± 2.0      | 7370 ± 30   | 8.9 ± 0.1 | 92 ± 0.1               |
| 8            | 19000 ± 200 | 42 ± 2.0      | 8740 ± 30   | 9.7 ± 0.1 | 93 ± 0.5               |
| 10           | 18200 ± 30  | 20 ± 1.0      | 8450 ± 30   | 10.2 ± 0.1 | 94 ± 0.6              |
| CaO          |             |               |             |        |                         |
| 6            | 17400 ± 200 | 34 ± 2.0      | 7735 ± 30   | 10.3 ± 0.1 | 92 ± 0.4              |
| 8            | 17600 ± 200 | 24 ± 2.0      | 7970 ± 30   | 11.6 ± 0.2 | 93 ± 0.2              |
| 10           | 20700 ± 250 | 20 ± 1.0      | 8860 ± 30   | 12.3 ± 0.2 | 95 ± 0.2              |

\[
P(t) = p_m \exp \left\{ -\exp \left( \frac{R_m \cdot e}{p_m}(\lambda - t) + 1 \right) \right\}
\]

where \( P(t) \) is the CHP (mL) at time \( t \) (d), \( P_m \) represents the HPP (mL), \( R_m \) is related to the maximum \( H_2 \) production rate (mL/d), \( e \) is 2.72, \( \lambda \) is the lag time (d), and \( t \) refers to the fermentation time (d). The values of \( R_m \), \( p_m \), and \( k \) for each experiment were determined using the software CurveExpert 1.4. In addition, these parameters were evaluated based on a correlation coefficient (\( R^2 \)).

3. RESULTS AND DISCUSSION

3.1. Characteristics of Wheat Straw. As described in Table 1, the wheat straw contained 91.5% (w/w) TS, and volatile solids (VS) accounted for 95% of the TS. The wheat straw was rich in total carbon (46.4% of TS) and had a low nitrogen content (0.45% of TS), indicating that wheat straw had high C/N ratio (100:1). Although the C content of wheat straw is high, it is present in the forms of lignin, hemicellulose, and cellulose. Proper pretreatments can decompose cellulose and hemicellulose into low molecular weight compounds, such as polysaccharides and monosaccharides, which can be converted into biofuels by anaerobes. The characteristics suggested that wheat straw is a suitable substrate for anaerobic digestion. The cellulose, hemicellulose, and lignin contents of the wheat straw used in this study were 36, 21, and 17%, respectively (Table 2). In addition, the pH of the wheat straw was approximately 6.9.

3.2. Effects of CaO₂ and CaO Pretreatments on Wheat Straw Hydrolysis. The hydrolysis of lignocellulosic biomass is the first step of straw utilization, and it is thought to be the rate-limiting step in batch H₂ fermentation. The hydrolysis of wheat straw in this study was mainly evaluated in terms of COD, and TOC, as shown in Table 3. Alkaline treatment is nearly always employed to remove lignin from lignocellulosic waste. Moreover, alkali pretreatment can enhance the biodegradability of wheat straw by releasing the organic-soluble fraction, which is represented by the STOC or SCOD. Therefore, SCOD and STOC are regarded as suitable indicators for estimating the performance of CaO and CaO₂ pretreatments. Different CaO₂ and CaO concentrations were used to hydrolyze the wheat straw, and the results are compared in Tables 2 and 3. Table 3 shows that the STOC values of the pretreated liquors are high, ranging from 3200 to 8860 mg/L, and carboxylic acids, phenols and furans, and other soluble materials from hemicellulose and lignin were the major components of the liquor. However, a decrease in the STOC was observed as the CaO₂ content of the pretreated liquor increased. This was likely because a portion of the soluble organic matter was oxidized to inorganic matter at a high concentration of CaO₂. The pH is another parameter of interest in the pretreatment process. The final pH values of the wheat straw samples were obviously increased because of pretreatment. During the pretreatment process, the CaO₂ or CaO reacted with water, and larger amounts of CaO₂ or CaO added led to higher pH values in the liquid samples. After pretreatment, the SCOD and STOC of the hydrolysate were notably increased. Considering the SCOD and STOC values, the treatment effects of CaO and CaO₂ at low dosages (≤6%) were different (Table 3). The statistical analyses of the control, 6% CaO, and 6% CaO₂ groups illustrated that the SCOD and STOC values of the three groups have a p-value of 0.00 (less than 0.05), indicating that different pretreatment methods have obvious influence on the SCOD and STOC in the substrate.
Figure 1. Comparisons of FT-IR curves of all the samples: (a) CaO$_2$-treated wheat straw; (b) CaO-treated wheat straw; (c) fermented residue of the CaO$_2$-treated wheat straw; and (d) fermented residue of the CaO-treated wheat straw.

Figure 2. Comparisons of the XRD patterns of all the samples: (a) CaO$_2$-treated wheat straw; (b) CaO-treated wheat straw; (c) fermented residue of the CaO$_2$-treated wheat straw; and (d) the fermented residue of the CaO-treated wheat straw.
On the other hand, the wheat straw pretreated with CaO2 showed almost the same weight loss as that pretreated with CaO (Table 3). The weight loss of wheat straw was a result of the solubilization of components, such as cellulose, hemicellulose, and lignin by the solutions. The higher weight loss from the wheat straw indicated that more lignocellulosic components were converted into soluble substances.40 The weight loss from the wheat straw was predominant because of hemicellulose hydrolysis by both CaO and CaO2, and more hemicellulose in the wheat straw was converted to soluble sugars by CaO than by CaO2. In addition, soluble sugars are mainly generated from the hemicellulose fraction.41 Alkaline pretreatment could effectively degrade hemicellulose into soluble oligomers and monomeric sugars.42 Mustafa et al.43 observed the structural variations of the wheat straw (36% cellulose), the lignin contents in the solid fraction pretreatment decreased from 17 to 15% when the CaO2 content was increased from 2 to 10%. In addition, the lowest lignin content (13%) was present near 8% of CaO2, suggesting that such pretreatment methods were also effective in dissolving hemicellulose and removing lignin. These phenomena indicated that the two pretreatment techniques degraded portions of the hemicelluloses and lignin, increasing the solubilization of components, such as cellulose, hemicellulose in the wheat straw was converted to soluble oligomers and monomeric sugars. 23 Hemicellulose degradation of hemicellulose and lignin, increasing the CaO or CaO2 contents increased the degradation of hemicellulose and lignin; when the CaO2 content was increased from 2 to 10%. In addition, the intensity of the band at 1430 cm\(^{-1}\) (cellulose I) increased while the peaks at 898 cm\(^{-1}\) (cellulose II) decreased, which indicated that CrI changed (Figure 1). Moreover, this was confirmed by the obtained XRD pattern (Figure 2 and Table 4); the higher CrI (55%) was observed at 8% CaO/CaO2 than in the material (53.14%) because of the removal of the lignin and hemicellulose (Table 4). The peaks at 2920 cm\(^{-1}\) (C–H stretching vibration) and 3400 cm\(^{-1}\) (–OH stretching) were weaker in the spectra of all the pretreated samples, indicating that the alkali treatment destroyed the intermolecular and intramolecular hydrogen bonds, as well as the methylene and methyl groups of cellulose. The decrease in the intensity of the peaks at 1228 and 1720 cm\(^{-1}\), corresponding to the C–O and ester/carboxylic acid bonds of the acetyl units of hemicellulose, revealed the dissolution/degradation of the hemicellulose.42 The FT-IR curves were evaluated, according to previous works.42,43,45 The bands at 1260 cm\(^{-1}\) (related to the guaiacyl C–O bond) and 1635 cm\(^{-1}\) (assigned to the C==O vibration of lignin) decreased in intensity because of the removal/degradation of lignin.46 In addition, the peak at approximately 898 cm\(^{-1}\), related to the stretching of cyclic β-1,4-glycosidic linkages, decreased in intensity, confirming the degradation of amorphous cellulose.45 Similarly, the change in the absorbance of the C==O stretching vibration at 1736 cm\(^{-1}\) reflected the change in the hemicellulose content. After pretreatment, these peaks, such as those at 2920, 1720, 1228, and 1032 cm\(^{-1}\), were weakened, indicating a reduction in the hemicellulose content. Moreover, decreases in the intensities of the peaks at 1603 and 1511 cm\(^{-1}\) were indicative of the degradation of lignin.43

In addition, the pretreated and untreated wheat straw samples were characterized by XRD, and the results are shown in Figure 2. In a fiber structure, diffraction peaks indicate a relatively high degree of crystallinity. After alkali treatment, the cellulose chain segments were folded to form new crystal structures.46,47 This result was consistent with those of a previous study.47 Natural cellulose, composed of type-I cellulose crystals, underwent a structural change upon treatment with base above a certain concentration, resulting in a transition from cellulose I to II.48 As described in Figure 2, all the XRD patterns of untreated and CaO2/CaO-treated wheat straw samples showed a sharp and intense band at 2θ = 22.6° and two weaker diffraction bands at 2θ = 14.7 and 16.4°, which are typical of cellulose I.49 The pattern of wheat straw before pretreatment showed a strong diffraction band at 2θ = 16 and 22°. However, after pretreatment, the patterns of the wheat straw showed that the intensities of the diffraction peaks were lower (Figure 2). Figure 2 shows that the wheat straw samples treated with CaO/CaO2 had XRD patterns similar to those of the untreated straw. However, the 2θ = 29° band was shifted to a slightly higher angle in the pattern of 6% CaO2-treated wheat straw. When the CaO2 content was increased to

Table 4. Comparisons of Crystallinity Indices and Crystallite Size of All the Samples

| addition (%) | CrI (%) | crystallite size (nm) | strain |
|-------------|---------|-----------------------|-------|
| wheat straw | 53.14 ± 1.20 | 33.8 ± 2.5 | 0.624 ± 0.020 |
| 0           | 45.27 ± 1.00 | 27.6 ± 1.2 | 0.315 ± 0.015 |
| 2           | 50.98 ± 1.20 | 26.4 ± 1.1 | 0.124 ± 0.010 |
| 4           | 47.63 ± 1.50 | 18.1 ± 1.5 | 0.245 ± 0.013 |
| CaO2        | 53.41 ± 1.60 | 15.9 ± 0.9 | 0.284 ± 0.015 |
| 8           | 55.16 ± 1.50 | 5.5 ± 1.2 | 0.327 ± 0.020 |
| 10          | 53.33 ± 1.20 | 6.6 ± 0.8 | 0.715 ± 0.025 |
| 2           | 51.02 ± 1.20 | 17.5 ± 1.2 | 0.240 ± 0.013 |
| CaO         | 52.91 ± 1.10 | 16.5 ± 1.2 | 0.265 ± 0.013 |
| 6           | 54.35 ± 2.20 | 8.8 ± 1.0 | 0.593 ± 0.024 |
| 8           | 55.40 ± 1.20 | 7.0 ± 0.9 | 0.785 ± 0.028 |
| 10          | 53.52 ± 2.50 | 9.4 ± 1.2 | 0.636 ± 0.108 |
10%, the additional diffraction peak that appeared at $2\theta = 29^\circ$ was strong and sharp.

Lignin has been considered a cellulose adsorbent, limiting the cellulase accessibility in lignocellulosic materials during hydrolysis processes.\textsuperscript{50} All the removal efficiencies increased slightly when the contents of CaO/CaO\textsubscript{2} were high. Less lignin was present in the wheat straw samples treated with high contents of CaO, but the concentrations of potentially fermentable sugars (e.g., glucose and xylose) also decreased with increasing CaO/CaO\textsubscript{2} content. The fermentable sugars can react with CaO/CaO\textsubscript{2} or undergo the Maillard reaction, which could further reduce the concentrations of fermentable sugars and produce nondegradable substances. Moreover, excess Ca\textsuperscript{2+} was introduced into the subsequent bio-H\textsubscript{2} generation step by the CaO or CaO\textsubscript{2} pretreatment process. These factors are not favorable for microbial metabolism.

3.4. Effects of Ca\textsubscript{O\textsubscript{2}} and CaO Pretreatments on Bio-H\textsubscript{2} Production. Ca(OH)\textsubscript{2} was generated by the reactions of H\textsubscript{2}O and CaO or CaO\textsubscript{2}, which participated in hydrolysis—acidification, microbial metabolism, acid—base balance, and bio-H\textsubscript{2} production during wheat straw fermentation. Methane (CH\textsubscript{4}) was not detected in the bio-H\textsubscript{2} process, which indicated that the methanogenic activities of anaerobic sludge were sufficiently inhibited prior to the anaerobic fermentation. Figure 3 describes the effects of CaO/CaO\textsubscript{2} pretreatment of wheat straw for bio-H\textsubscript{2} production. As shown in Figure 3, the H\textsubscript{2} yield increased as the CaO and CaO\textsubscript{2} contents were increased from 0 to 6%, and the wheat straws treated with 6% CaO or CaO\textsubscript{2} achieved the highest H\textsubscript{2} yields of 114 and 71.8 mL/g TS, respectively. Further increasing the CaO or CaO\textsubscript{2} content reduced the H\textsubscript{2} yield. This was likely because of interactions between the biomass constituents and CaO/CaO\textsubscript{2} during the pretreatment processes. A moderate amount of Ca\textsuperscript{2+} (75–150 mg/L) facilitated the formation of granular sludge and bio-H\textsubscript{2} evolution while excess Ca\textsuperscript{2+} eroded H\textsubscript{2} productivity.\textsuperscript{51} Ca\textsuperscript{2+} could aggregate and precipitate lignin under alkaline conditions, leading to the formation of additional cross-links within the wheat straw structure. This further lowered the accessibility of carbohydrate polymers to microbes.\textsuperscript{52} Ca\textsuperscript{2+} also had high affinity for cellulose and pectin.\textsuperscript{52} Some toxic organic compounds, such as furfural, phenolic compounds, and levulinic compounds, could be generated during the pretreatment process, and they may have inhibited the activities of the anaerobes.\textsuperscript{53} In addition, CaO\textsubscript{2} could oxidize the dissolved organic matter, and a high CaO\textsubscript{2} content would decrease the substrate concentration. When excess CaO or CaO\textsubscript{2} was employed, cross-links formed within the biomass, which would likely reduce the accessibility of the carbohydrates to the anaerobes. The low observed H\textsubscript{2} yield of 43.2 mL/g TS from the control test without CaO or CaO\textsubscript{2} was because of the complex lignocellulosic structure and the low concentration of dissolved organic compounds. Pretreatment at 121 °C with CaO\textsubscript{2} resulted in some bio-H\textsubscript{2} production, but the H\textsubscript{2} yield remained low (Figure 3a) relative to that obtained from pretreatment at 121 °C with CaO (Figure 3b). The highest H\textsubscript{2} yields from wheat straw ranged from 59 to 168 mL H\textsubscript{2}/g VS, similar (71–114 mL H\textsubscript{2}/g TS) to those achieved in this work.\textsuperscript{54–56} Table 5 shows the results of other reports for comparison of the effects of alkaline or acid pretreatments of lignocellulosic waste on the production of H\textsubscript{2}. However, it was somewhat difficult to compare the H\textsubscript{2} yields even under comparable conditions. In addition, the statistical analysis results of the control, 6% CaO, and 6% CaO\textsubscript{2} groups show that all the H\textsubscript{2} yields have a p-value of 0.00, which are less than 0.05. The statistical analyses indicate that there are significant differences.
3.5. Effects of CaO2 and CaO Pretreatments on VFA Distribution. The data in Figures 4 and 5 show that CaO2 and CaO pretreatments could also affect the VFA distribution during bio-H2 production from the wheat straw samples. In the case of wheat straw, the wet oxidation process disrupts and oxidizes cellulose and lignin to CO2, H2O, and carboxylic acids.57 Excess CaO could also reduce the TOC in the hydrolysate of the wheat straw. Bio-H2 production is always accompanied by the formation of soluble metabolites. Therefore, the distribution of soluble metabolites under steady-state conditions must be monitored (Figures 4 and 5). VFAs are important intermediates generated during the bio-H2 evolution process, and they can also serve as substrates for H2 generation. A higher content of VFAs (approximately pH 4.5) can help provide a suitable microenvironment for HPB, thus increasing the generation of H2.58 The highest VFAs (1170 mg/L) were obtained from wheat straw pretreated with 4% CaO on the third day (Figure 4c). The VFAs from the wheat straw samples pretreated with 6% CaO and 6% CaO2 were similar, with values of 980 and 950 mg/L, respectively (Figures 4 and 5). The main VFAs in the fermentation residue were acetate and butyrate, and a small amount of propionic acid was observed (Figures 4 and 5). The percentage of VFAs (%)

3.6. Bio-H2 Process Kinetics. Good curve fitting was obtained with the modified Gompertz model using the software CurveExpert 1.4, and the correlation coefficients (R2) between the measured CHP and the predicted CHP were more than 0.99 in all cases (Table 5). The values of the kinetic parameters were higher for the sample pretreated at 121 °C with 6% CaO (or CaO2) than they were for the control groups, which were treated with hot water (121 °C) (Table 5). When the CaO or CaO2 concentration was less than or equal to 6%, increasing the content of CaO or CaO2 led to greater H2 production rates. Indeed, the maximum H2 production rates (Rm) of 149.2 and 154.1 mL/d were achieved at 6% CaO2 and CaO, respectively. The HPP of Pm rose gradually from 463 to 1612 mL H2 with the increasing CaO content (Table 5). The magnitude of the increases in the H2 production rate was lower when the CaO content was increased beyond 6%. The results were consistent with the suitable concentration of carbohydrate obtained with 6% CaO relative to those obtained at other concentrations of CaO (Table 2). The primary effect of CaO or CaO2 pretreatment was the partial removal of lignin and hemicellulose, which enhanced the reactivity of the remaining polysaccharides. In addition, CaO or CaO2 pretreatment likely caused the solubilization, condensation, and redistribution of lignin, which could increase the crystallinity of the cellulose.60 These factors could partly counteract the positive effects of cellulose swelling and lignin removal because of alkaline pretreatment. In addition, the residual Ca57 from the CaO or CaO2 pretreatment process and the solubilized lignin components negatively impacted the anaerobes.

Figure 4. Effects of CaO pretreatments on VFA distribution: (a) control VFAs; (b) 2% CaO-treated VFAs; (c) 4% CaO-treated VFAs; (d) 6% CaO-treated VFAs; (e) 8% CaO-treated VFAs; and (f) 10% CaO-treated VFAs.

Differences among the three groups. These results reveal that different pretreatment methods can affect H2 yield. The effects of different pretreatments on total VFAs were significant during H2 production process.
4. CONCLUSIONS

CaO and CaO$_2$ pretreatments of wheat straw for bio-H$_2$ production were investigated and compared based on the main characteristics of wheat straw before and after pretreatment and subsequent H$_2$ generation. The highest H$_2$ yield obtained from wheat straw treated with CaO was 114 mL/g TS, which was higher than that (43.2 mL/g TS) obtained from the control group, which was not subjected to CaO or CaO$_2$ treatment. The hydrolysate determination showed the possible mechanisms by which the rigid structure of the wheat straw was disrupted by thermal/CaO (or CaO$_2$) pretreatment, and the cellulose and hemicellulose were degraded. Considering the cost and efficacy of the pretreatment methods, CaO was more effective than CaO$_2$ for improving the subsequent bio-H$_2$ generation.

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Notes

The authors declare no competing financial interest.

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