

## Article

# Effects of Different Hydrolysis Methods on the Hydrolysate Characteristics and Photo-Fermentative Hydrogen Production Performance of Corn and Sorghum Straw

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**Abstract:** The effects of hydrolysis methods (hydrothermal, acid, alkali, hydrothermal-enzyme, acid-enzyme, and alkali-enzyme) on hydrolysate characteristics and photo fermentative hydrogen production (PFHP) of corn straw (CS) and sorghum straw (SS) were investigated. The optimum production of reducing the sugar of straw in different solvent environments was studied by one-step hydrolysis and co-enzymatic hydrolysis pretreatment through a 3,5-dinitrosalicylic acid method. The hydrogen production process by photolytic fermentation of hydrolysates of *Rhodobacter sphaeroides* HY01 was further analyzed through a gas chromatograph, including the differences in accumulated PFHP yield, chemical oxygen consumption (COD), and volatile fatty acid (VFA) composition. The results showed that the highest reducing sugar yield was obtained by the acid method among one-step hydrolysis. In contrast, acid-enzyme hydrolysis can further increase the reducing sugar yield, which reached  $0.42 \text{ g}\cdot\text{g}^{-1}$ -straw of both straws. Both CS and SS had the highest hydrogen yield from acid-enzyme hydrolysate,  $122.72 \pm 3.34 \text{ mL}\cdot\text{g}^{-1}$ -total solid of straw (TS) and  $170.04 \pm 4.12 \text{ mL}\cdot\text{g}^{-1}$ -TS, respectively, compared with their acid hydrolysates with 40.46% and 10.53% higher hydrogen yields, respectively. The use of enzymatic hydrolysis showed a significantly higher hydrogen yield for CS compared to SS, indicating that acid hydrolysis was more suitable for SS and acid-enzyme hydrolysis was more suitable for CS.



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**Keywords:** agricultural straw; hydrolysis; reducing sugar; hydrogen; photo-fermentation

## 1. Introduction

The development of human society leads to increasing demand for energy. However, at the moment, energy consumption is still dominated by fossil fuel energy, which has limited reserves and causes environmental problems. As a result, the utilization of renewable energy is critical for long-term sustainable human development [1]. Because of its high energy density and environmental friendliness, hydrogen is regarded as the most promising alternative fuel for the future [2]. However, the majority of hydrogen is still produced using fossil fuels, which is not environmentally friendly [3]. Biological hydrogen production is regarded as one of the most promising renewable methods due to its mild reaction conditions, cheap and abundant substrates, and positive environmental impact [4,5].

Many research efforts have focused on photo-fermentative hydrogen production (PFHP), a suitable and attractive method that uses photosynthetic bacteria to convert solar energy and organic substrates into hydrogen and various hydrocarbons [6]. Agricultural straw is a high-yielding renewable organic biomass, and billions of tons of straw are annually produced in the world [7]. Agricultural straw is composed of large amounts of cellulose and hemicellulose, which can be utilized as a substrate for PFHP after hydrolysis [8]. However, the tightly bound cellulose, hemicellulose, and lignin in agricultural straws make it challenging to be destroyed, so the pretreatment of straw is essential for efficient utilization. It is estimated that the cost of pretreatment alone accounts for about

50% of the total cost of straw bioconversion processes [9]. Therefore, agricultural straw pretreatment research has a high economic value [10,11].

Pretreatment methods for straw currently include physical, chemical, biological pretreatment technologies, and so on. Hydrothermal treatment of straw refers to the application of water at a high temperature and pressure to the straw [12]. Its advantages are cleanness and less pollution [13], but its disadvantage is high energy consumption [14]. Acid can directly hydrolyze hemicellulose in lignocellulose and degrade it into reducing sugar, disaccharide, and other substances, while lignin is hardly degraded in the dilute acid pretreatment process [15]. Acid can destroy the connection among hemicellulose, lignin, and cellulose, improve the hydrolysis efficiency of hemicellulose and the removal rate of lignin, and further accelerate saccharification [12]. Acid hydrolysis has the disadvantage of producing inhibitory products, such as furfural, phenolic acids, and aldehydes [16]. Alkali hydrolysis can dissolve lignin in lignocellulose and remove hemicellulose, resulting in increased porosity of straw biomass and a greater impact of subsequent straw hydrolysis [17,18]. Alkali hydrolysis is a cheap method for straw treatment. It has the advantages of low cost, less corrosion, and fewer inhibitors [19]. The disadvantages of alkaline hydrolysis are the production of salts in the hydrolysate, which are difficult to recover [20], and the production of a black liquor that is very dark in color. Biological pretreatment usually refers to the use of fungi, bacteria, and other microorganisms, as well as enzyme degradation of straw [21]. The advantages of the biological method are less pollution and lower energy consumption. The disadvantage of microbial degradation is a very low degradation rate, and the disadvantage of enzymatic degradation is extremely high cost.

Each hydrolysis method has advantages and disadvantages; the key is to determine which hydrolysis method is optimal for a chosen straw biomass. Numerous studies on agricultural straw hydrolysis, including various methods and straw types, have been reported [22–24]. Banerji et al. [25] investigated the effects of dilute sulfuric acid concentration, reaction temperature, and reaction time on sugar release from sweet SS. It was found that increasing the reaction time and acid concentration could increase the reducing sugar yield. According to this study, acid hydrolysis improved the sugar release of straw. This type of work is specific to the optimization of the conditions of a particular hydrolysis method. Zhang et al. [8] used enzymes to hydrolyze different agricultural straws under hydrolysis conditions: 2.5 g of straw was hydrolyzed at 50 °C for 36 h using 100 mg of cellulase (enzymatic activity of 30 units·mg<sup>-1</sup>). The PFHP for each straw was 145.8 mmol·L<sup>-1</sup> for CS, 150.4 mmol·L<sup>-1</sup> for SS, 140.3 mmol·L<sup>-1</sup> for rice straw, 130.7 mmol·L<sup>-1</sup> for soybean straw, 119.3 mmol·L<sup>-1</sup> for cotton straw, and 589.2 mmol·L<sup>-1</sup> for corn cob. This type of work was about pretreating different straws using the same hydrolysis method. In addition, in the case of alkali heat treatment, the hydrogen production effect is also superior to other single hydrolysis and acid heat conditions. Mirza et al. [26] treated wheat straw with different hydrolysis methods for PFHP. The hydrogen production was 254 mL·L<sup>-1</sup> for straw treated with 4% H<sub>2</sub>SO<sub>4</sub>, 372 mL·L<sup>-1</sup> for combined 4% H<sub>2</sub>SO<sub>4</sub>-Ca(OH)<sub>2</sub>, and 712 mL·L<sup>-1</sup> for combined 30% NH<sub>3</sub>-enzyme. The same straw was studied using different hydrolysis methods.

Although a lot of research has been conducted on straw hydrolysis, the comparison of photo-fermentation under different hydrolysis methods, particularly the influence of straw and hydrolysate properties on photo-fermentation, is rarely discussed. The effects of straw composition and hydrolysate properties on PFHP were investigated in this paper by studying the PFHP of different straws under different hydrolysis methods. Hydrothermal, acid, alkali, hydrothermal-enzyme, acid-enzyme, and alkali-enzyme methods were used to hydrolyze CS and SS to obtain a suitable hydrolysis method for PFHP from agricultural straw. CS and SS were used as substrates, and the photosynthetic bacterium *Rhodobacter sphaeroides* HY01 was used as a hydrogen-producing bacterium for PFHP. The effects of various methods on hydrolysis extent, reducing sugar yield, and photo-fermentation performance was thoroughly investigated.

## 2. Materials and Methods

### 2.1. Microorganism and Media

*Rhodobacter sphaeroides* HY01 [27] was used as a hydrogen producer for photo-fermentation. The bacteria were inoculated on a MedA [28] plate medium for 48 h at 35 °C, then grown for 48 h in a MedA liquid medium at 35 °C, and the bacterium was cultivated in a dark environment at 150 rpm. The culture media of MedA contained (per L) 20 mL of Solution C [29], 5 mL of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (10%), 20 mL of sodium succinate (10%), 2 mL of L-glutamic acid (5%), 2 mL of L-aspartic acid (2%), 1 g of NaCl, 1 mL of vitamin solution, 20 mL of phosphate buffer (pH 7.2, 1 M). The vitamin solution contained (per 1 L) 0.5 g of thiamine hydrochloride, 1 g of nicotinic acid, and 10 mg of biotin. The solid medium required adding 1.5–2.0% agar powder based on the above formula. The reagents used in this experiment are commercially available and analytical grade.

### 2.2. Hydrolysis Methods

CS and SS were provided by China Agricultural University. A fiber analyzer (FIWE 3, VELP Scientifica, Milan, Italy) was used to determine the composition of straw with Van Soest's method [29]. The calorific value of the straw was determined using an automatic calorimeter (5E-AC/PL, Kaiyuan Instruments Co., Ltd., Changsha, China). A certain sample mass was fully combusted with oxygen, and the released heat heated a quantitative amount of water. Its calorific value was calculated according to the change in water temperature.

The dried straw was shattered into 60 meshes before hydrolysis. The hydrolysis parameters are listed in Table 1, and 25 g straw was employed in each method. All parameter settings were based on the laboratory's previous investigation [30,31]. In the single-step hydrolysis process, CS and SS were hydrolyzed at 108 °C for 30 min supplemented with the proper amount of chemicals, keeping the solid-liquid ratio at 1:10 (*w/v*). In the two-step hydrolysis process, the hydrolysate mixture obtained through the previous step needed to adjust the pH to 4.8 with 1 M HCl/NaOH and then add 2.25 g cellulase keeping the temperature at 50 °C for 10 h.

**Table 1.** Experimental parameters of different hydrolysis methods.

Method <sup>1</sup>	Material	Reaction Conditions
WH	250 mL deionized water	108 °C for 30 min
AcH	250 mL HCl (1.5%)	108 °C for 30 min
AlH	250 mL NaOH (0.75%)	108 °C for 30 min
WEH	hydrolysate by WH, 2.25 g cellulase <sup>2</sup>	pH 4.8, 50 °C for 10 h
AcEH	hydrolysate by AcH, 2.25 g cellulase	pH 4.8, 50 °C for 10 h
AlEH	hydrolysate by AlH, 2.25 g cellulase	pH 4.8, 50 °C for 10 h

<sup>1</sup>: WH: hydrothermal hydrolysis; AcH: acid hydrolysis; AlH: alkaline hydrolysis; WEH: hydrothermal -enzymatic hydrolysis; AcEH: acid-enzymatic hydrolysis; AlEH: alkaline-enzymatic hydrolysis. <sup>2</sup>: Commercial cellulose from Chengdu, China Tian Feng Co., 100 U·g<sup>-1</sup>, U: μmol glucose·min<sup>-1</sup>·g<sup>-1</sup>.

Then, the supernatant obtained by vacuum filtration of the hydrolyzed straw was used in the following experiment. The hydrolysis extent is calculated as follows:

$$\eta = \frac{m_0 - m_1}{m_0} \times 100\% \quad (1)$$

where  $\eta$  is hydrolysis extent/%,  $m_0$  is straw mass before hydrolysis/g, and  $m_1$  is the quantity of straw residue after hydrolysis/g.

### 2.3. Photo-Fermentative Hydrogen Production Processes

The liquid medium for PFHP was a slightly modified MedA, the hydrolysate was used as a carbon source, and the initial pH was adjusted to 7.2. The culture contained (per

L) 20 mL of Solution C, 1 g of L-sodium glutamate, 2 mL of 2% L-aspartic acid, 1 g of NaCl, 10 g-TVS of hydrolysate, 20 mL of phosphate buffer (pH 7.2, 1 M), 1 mL of vitamin solution.

Bacteria were centrifuged and collected, then diluted to  $OD_{660} = 1$  before use. A sterile syringe with a capacity of 30 mL was used as a reactor with a 10 mL working volume, including 1 mL inoculum. The reactors were placed in an incubator, and the light intensity was kept at around 4000 lux. The temperature was maintained at  $30 \pm 0.5$  °C.

The  $H_2$  production was equal to the gas production under standard atmospheric conditions multiplied by the hydrogen concentration. Cumulative  $H_2$  production ( $mL \cdot L^{-1}$ ) was the total amount of  $H_2$  produced in each experiment, and the  $H_2$  yield ( $mL \cdot g^{-1}$ ) was calculated as the ratio of the cumulative  $H_2$  production to the hydrolyzed straw. All the  $H_2$  production experiments were conducted independently in triplicates.

#### 2.4. Analysis Methods

The reducing sugar concentration of the hydrolysate was determined by a DNS (3,5-dinitrosalicylic acid) method [32]. The COD values were determined by a multifunction water quality determinator (ET99732, Lovibond, Dortmund, Germany). The concentration of photosynthetic bacteria was determined with the absorbance at the wavelength of 660 nm ( $OD_{660}$ ) with a spectrophotometer (U4100, Hitachi, Tokyo, Japan). The pH was determined with a pH meter. The light intensity was determined with a photometer.

$H_2$  concentration was determined by a gas chromatograph (GC, CP-3800, Varian Co., Palo Alto, CA, USA) equipped with thermal conductivity detectors (TCD) and a  $4\text{ m} \times 3\text{ mm}$  stainless column filled with Hayesep padding. The volatile fatty acids were determined by the same GC equipped with a flame ionization detector (FID) and a  $30\text{ m} \times 0.32\text{ mm}$  glass column [33,34].

Origin 8.0 software was used to simulate the kinetic parameters of  $H_2$  production by the modified Gompertz equation [35]:

$$H = H_{\max} \exp \left\{ - \exp \left[ \frac{R_e e}{H_{\max}} (L - t) + 1 \right] \right\} \quad (2)$$

where  $H$  is the cumulative  $H_2$  yield/ $mL \cdot L^{-1}$ ,  $H_{\max}$  is the  $H_2$  yield potential/ $mL \cdot L^{-1}$ ,  $R_e$  is the maximum  $H_2$  production rate/ $mL \cdot L^{-1} \cdot h^{-1}$ ,  $L$  is the lag time/h,  $t$  is the time/h, and  $e$  is 2.718281828.

### 3. Results and Discussions

#### 3.1. Basic Characteristics of CS and SS

Table 2 shows CS and SS's calorific value and wood fiber content. The calorific values of CS and SS are  $15.80\text{ MJ} \cdot \text{kg}^{-1}$  and  $17.78\text{ MJ} \cdot \text{kg}^{-1}$ , respectively. The calorific value of agricultural straw is highly dependent on its composition. Similar to the previous description, the content of cellulose and lignin in straw may be correlated with calorific value [36,37]. Hemicellulose showed a positive but non-significant correlation with the calorific value of straw [38–40], owing to its low thermal stability and activation energy [41]. The difference in calorific value between the two straws corresponds to the difference in wood fiber content. As shown in Table 2, there were some differences in the wood fiber composition of the two straws, with SS having a slightly higher total fiber content of 66.80% than the 64.29% of CS. The lignin content of CS was 17.50%, while SS had a relatively higher lignin content of 23.82%. Lignin was the most stubborn and difficult component of wood fiber to decompose, so SS might be more difficult to hydrolyze than CS [42–44].

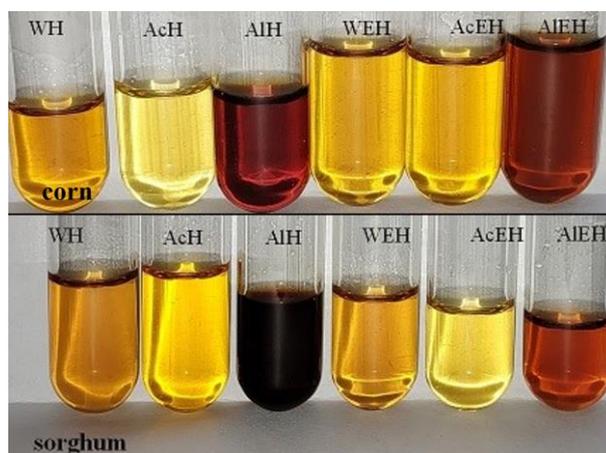
**Table 2.** Calorific value and wood fiber content of CS and SS.

Straw	Calorific Value/MJ·kg <sup>-1</sup>	Compose	Content/%
CS	15.80	Hemicellulose	22.48
		Cellulose	24.31
		Lignin	17.50
SS	17.78	Hemicellulose	21.51
		Cellulose	21.47
		Lignin	23.82

### 3.2. Characteristics of CS and SS Hydrolysates

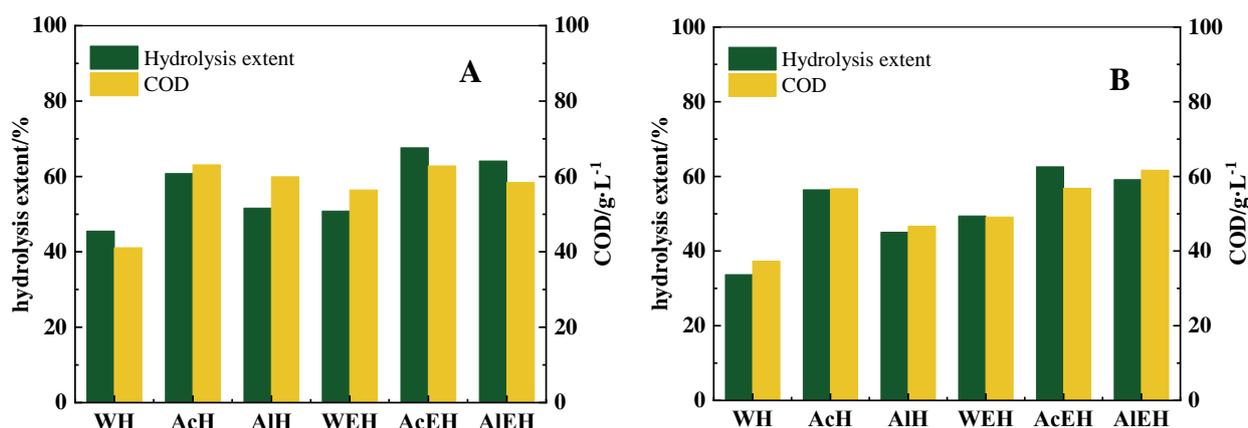
#### 3.2.1. Color Characteristics of CS and SS Hydrolysates

In this section, the properties of straw hydrolysates obtained from different hydrolysis methods were investigated. First, the photos of six hydrolysates are shown in Figure 1. The colors of the CS and SS hydrolysates with alkali participation (AIH and AIEH) were darker than other hydrolysates. Alkaline hydrolysis can dissolve and degrade lignin, an amorphous high-complex aromatic polymer. Alkaline degradation results in a darker-colored hydrolysate, similar to the paper industry's black liquor, and contains alkali lignin, aliphatic acids, acid grease, resins, and polysaccharides [45–47]. The chelate formed during the lignin degradation reaction imparts a brownish-black color to the solution. Alkali lignin is alkali-soluble, but water and acid are insoluble. As the pH drops, alkali lignin deposits, and the black liquor lightens in color. The acid hydrolysate, consisting primarily of soluble sugar, is much lighter in color.

**Figure 1.** Photos of hydrolysate samples of CS and SS.

#### 3.2.2. The Hydrolysis Extent and COD of the Hydrolysates

Figure 2 depicts the effect of the hydrolysis method on the hydrolysis extent and COD, which shows that the COD of hydrolysate is positively correlated with the hydrolysis extent. This is because the hydrolysis extent reflects the mass of straw converted into the hydrolysate, which is roughly reflected in the COD value. The hydrolysis extent of CS was slightly greater than that of SS with the same hydrolysis method. As CS contains less lignin than SS, which is a difficult component of wood fiber to hydrolyze, it is easier to hydrolyze CS than SS. In WH hydrolysis, both straws had the least hydrolysis extent. The hydrolysis extent of CS was 45.51%, while that of SS was 33.68%. This is because neutral hydrolysis at 108 °C only provides pyrolysis with little chemical degradation [48]. Because acid and alkali can reduce the crystallinity in the fiber, which is conducive to further hydrolysis, their hydrolysis extent is significantly greater than that of WH [49].



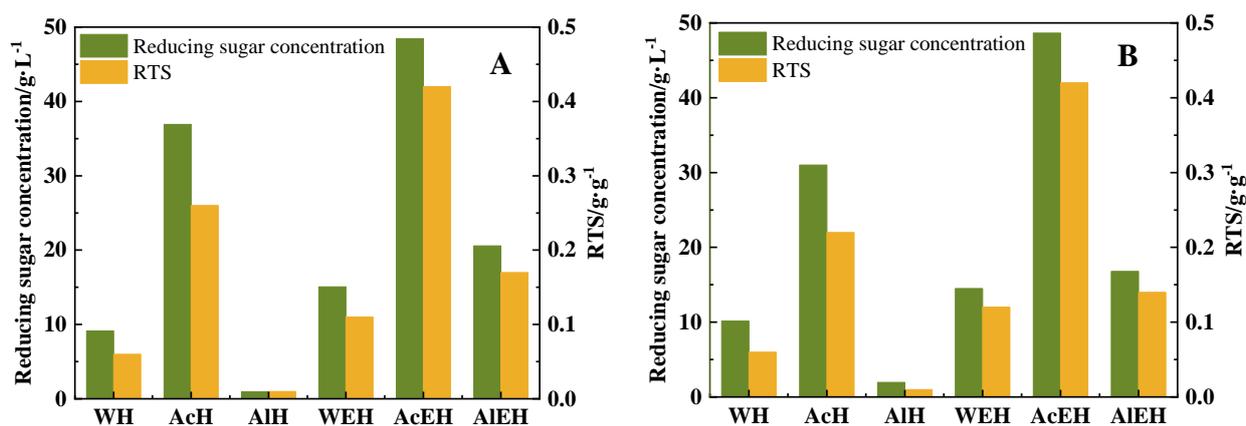
**Figure 2.** Hydrolysis extent and COD of different straw hydrolysates. (A) CS hydrolysates; (B) SS hydrolysates.

Acid could significantly improve the hydrolysis extent of both straws over alkali, but the advantage of acid-enzyme over alkaline-enzyme was insignificant. CS hydrolysis extent under AcH was 60.79% and significantly greater than AIH (51.60%). The AcH hydrolysis extent of SS was 56.43% and significantly greater than AIH (45.06%). The hydrolysis extent of AcEH of CS was 67.60%, which was only 3.50% greater than that of AIEH of 64.10%, and the hydrolysis extent of AcEH of SS was 62.60%, which was only 3.45% greater than that of AIEH of 59.15%. After acid treatment, the hemicellulose in the straw degraded to monosaccharides, increasing the cellulose's surface area with the hydrolysis solution. The lignin content in the solution was essentially constant after acid hydrolysis. Hemicellulose content was low; both the content and the degree of cellulose polymerization were reduced [50]. Alkali treatment primarily degrades lignin and reduces the crystallinity of cellulose. However, the effect on cellulose and hemicellulose degradation was negligible [51]. Although acid and alkali hydrolyze straw differently, the dense crystalline structure of lignocellulose decayed after the acid or alkali step, increasing the active area of subsequent cellulose enzymatic hydrolysis.

The hydrolysis extent of the same straw with enzyme-assisted hydrolysis was greater than that of the corresponding hydrolysis without enzymes. The hydrolysis extents of WEH, AcEH, and AIEH of CS increased by 5.30%, 6.81%, and 12.50%, respectively, compared with WH, AcH, and AIH, and by 15.71%, 6.17%, and 14.09% of SS compared with WH, AcH and AIH, respectively. This was due to the involvement of cellulase, which caused more hydrolysis of cellulose, increasing to hydrolysis extent.

### 3.2.3. Reducing Sugar Yield of CS and SS Hydrolysate

Figure 3 depicts the reducing sugar concentrations and RTS (reducing sugar produced by total straw, which is the ratio of reducing sugar to total straw/ $\text{g}\cdot\text{g}^{-1}$ ) of CS and SS hydrolysates obtained through various hydrolysis methods. The figure clearly shows that there was a strong correlation between reducing the sugar concentration and RTS for a specific hydrolysate. However, due to the different volumes of hydrolysates obtained by the different methods, the ratio of the reducing sugar concentration to RTS was not consistent across hydrolysates. This also implies that reducing the sugar concentration is not a substitute for RTS in the case of a specific hydrolysate. The difference among RTS of diverse hydrolysis methods was significant but insignificant between RTS of different straws, which was due to the slightly different cellulose and hemicellulose contents of the two straws. Hemicellulose and cellulose contents in CS were 22.48% and 24.31%, respectively, while in SS were 21.51% and 21.47%, respectively. Cellulose and hemicellulose are the primary sources of reducing sugar in straw. Different straw types represent different saccharifiable substrates with minimal differences, whereas different hydrolysis methods represent differences in the ability to saccharify substrates.



**Figure 3.** The concentration of reducing sugar and RTS of different straw hydrolysates. (A) CS hydrolysates. (B) SS hydrolysates.

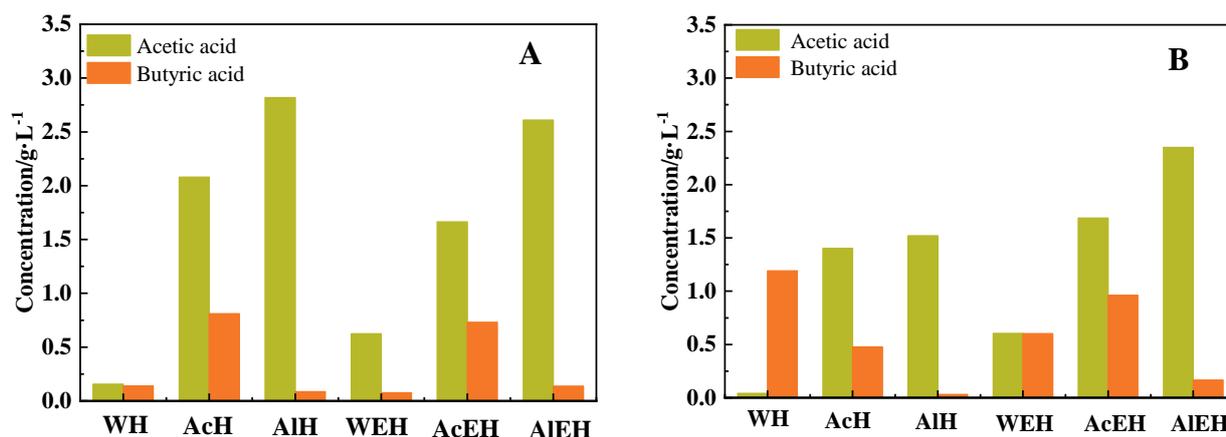
CS contains 22.48% hemicellulose, which is slightly higher than that of SS (21.51%). Because acid hydrolysis can directly hydrolyze hemicellulose into monosaccharides, the RTS ( $0.26 \text{ g}\cdot\text{g}^{-1}$ ) of CS acid hydrolysate is slightly higher than that of SS acid hydrolysate ( $0.22 \text{ g}\cdot\text{g}^{-1}$ ). Furthermore, the RTS of the hydrolysates hydrolyzed with AcH and AcEH was significantly higher than that of other hydrolysates.

Interestingly, CS has a cellulose content of 24.31%, which is slightly higher than SS (21.47%). Furthermore, the lignin content of CS (17.50%) is lower than that of SS (23.82%), while the RTS of CS acid-enzyme hydrolysate ( $0.42 \text{ g}\cdot\text{g}^{-1}$ ) did not show an advantage over that of SS acid-enzyme hydrolysate ( $0.42 \text{ g}\cdot\text{g}^{-1}$ ), indicating that the spatial structure of straw lignin also had a great influence on the degradation and saccharification of straw cellulose. The cellulose, hemicellulose, and lignin of straw are tightly woven into a network structure. Lignin is difficult to dissolve and degrade, and it will impede cellulose and hemicellulose degradation [52].

At the experimental temperature ( $108 \text{ }^{\circ}\text{C}$ ), acid hydrolyzes hemicellulose in straw to a monosaccharide, and hydrothermal also decomposes hemicellulose, but less effectively than dilute acid. At this temperature, alkali converts reducing sugar decomposed by hydrothermal action into short-chain acids and aldehydes [53]. It can be concluded that the presence of acid favors straw hydrolysis to produce reducing sugar. This is because cellulase hydrolyzes cellulose completely into glucose and other oligosaccharide compounds [54,55]. As shown in Figure 3, the reducing sugar concentrations and RTS of the two-step hydrolysates with enzyme participation were higher than the corresponding one-step hydrolysates without enzyme supplementation.

#### 3.2.4. Volatile Fatty Acids of CS and SS Hydrolysate

Figure 4 depicts the acetic acid and butyric acid concentrations in the hydrolysates. The concentrations of acetic acid and butyric acid in the hydrolysate did not differ significantly between the two types of straw, as shown in the figure. At the same time, the differences caused by the various hydrolysis methods were more pronounced. Since the composition of CS and SS differs little, the hydrolysis conditions are more important in determining the composition of the hydrolysate. Small molecule acids are more concentrated in acids or alkalis hydrolysates (AcH, AcEH, AIH, and AIEH) than in WH and WEH hydrolysates. Under the action of acid or alkali, the sugar in the hydrolysate will be degraded into short-chain acids and aldehydes in the hydrothermal environment. This means that the prolonged hydrolysis of straw with acids or bases at high temperatures is not suitable, as this would result in sugar degradation and inhibition of compound formation [56]. Short-chain acids can be used as PFHP substrates, but aldehydes can inhibit hydrogen production.

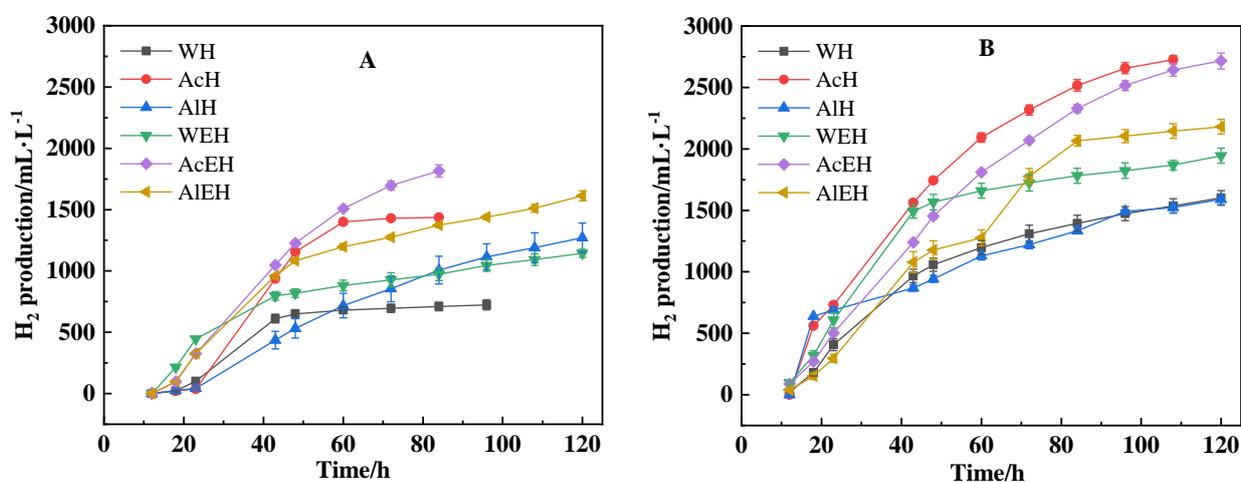


**Figure 4.** The concentration of acetic acid, butyric acid, and reducing sugar in the hydrolysates. (A) CS hydrolysates and (B) SS hydrolysates.

### 3.3. Photo-Fermentative H<sub>2</sub> Production of Straw Hydrolysate

#### 3.3.1. Hydrogen Production of Straw Hydrolysate by Photo-Fermentation

CS and SS hydrolysates were used as substrates for PFHP. Figure 5 depicts the cumulative PFHP time curves with hydrolysates. The cumulative hydrogen production from CS was highest for AcEH at  $1815.45 \pm 49.41$  mL·L<sup>-1</sup> and lowest for WH at  $723.15 \pm 41.34$  mL·L<sup>-1</sup>. For SS, the cumulative hydrogen production of AcH was the highest at  $2726.10 \pm 33.49$  mL·L<sup>-1</sup>, followed by AcEH at  $2716.25 \pm 65.77$  mL·L<sup>-1</sup>, and AIH was the lowest at  $1589.46 \pm 41.86$  mL·L<sup>-1</sup>.



**Figure 5.** Photo-fermentation of CS (A) and SS (B) hydrolysates.

The hydrogen production of AcH and AcEH hydrolysates of SS was very close. The hydrogen production of hydrolysates with enzyme participation was significantly higher than the corresponding hydrolysates without enzyme participation. WEH, AcEH, and AIEH of CS produced 58.25%, 26.32%, and 26.97% more hydrogen than WH, AcH, and AIH, respectively. WEH and AIEH of SS produced 21.42% and 37.19% more hydrogen than WH and AIH, respectively. The hydrolysates performed better due to more substances conducive to photo-fermentation in the hydrolysate with the participation of enzymes. Hydrogen production of AcH and AcEH hydrolysate was high due to the high content of reducing sugar. AIH hydrolysate has a lower reducing sugar content, a higher organic acid content, and a darker color with a higher shading effect, resulting in lower hydrogen production.

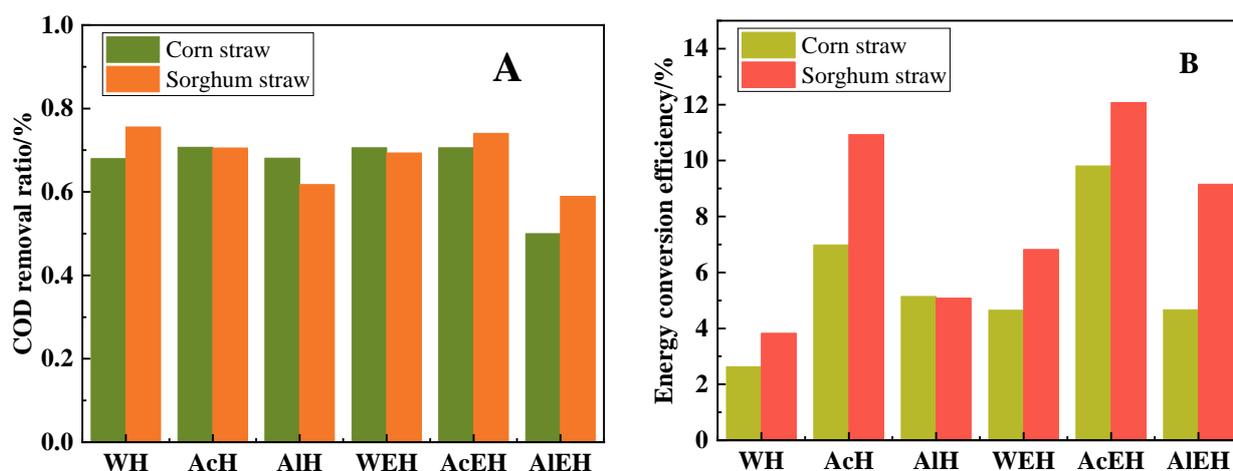
The hydrogen yields and parameter fitting of the photo-fermentation of CS and SS hydrolysates are shown in Table 3. AcEH had the highest hydrogen yield for CS at  $122.72 \pm 3.34 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$ . The hydrogen yield of AcH was the highest among the hydrolysates without enzyme supplementation at  $87.37 \pm 0.88 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$ . AcEH had the highest hydrogen yield for SS, which was  $170.04 \pm 4.12 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$ , followed by AcH at  $153.84 \pm 1.89 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$ . AcEH hydrolysate was a better photo-fermentation substrate for CS than the other hydrolysis methods because it produced significantly more hydrogen. However, in terms of hydrogen yield, the AcH hydrolysate was not significantly inferior to the AcEH hydrolysate for SS, and the ability to remove expensive enzymes made it more suitable as a substrate for photo-fermentative hydrogen production.

**Table 3.** Parameter fitting of photo-fermentation of SS hydrolysate.

Straw	Hydrolysates	Hmax/mL·L <sup>-1</sup>	Re/mL·L <sup>-1</sup> ·h <sup>-1</sup>	L/h	H <sub>2</sub> yield/mL·g <sup>-1</sup> -TS
CS	WH	707.52 ± 5.06	32.69 ± 1.24	20.23 ± 0.40	32.91 ± 1.88
	AcH	1449.78 ± 14.45	69.05 ± 7.31	28.81 ± 1.64	87.37 ± 0.88
	AIH	1359.93 ± 41.44	20.81 ± 1.36	21.69 ± 1.98	67.87 ± 4.02
	WEH	1053.80 ± 39.27	24.09 ± 3.53	8.76 ± 2.95	58.14 ± 1.70
	AcEH	1892.03 ± 43.79	41.47 ± 1.77	17.31 ± 0.96	122.72 ± 3.34
	AlEH	1495.70 ± 44.50	33.13 ± 3.40	14.78 ± 2.39	103.38 ± 2.58
SS	WH	1532.88 ± 37.70	30.00 ± 2.30	11.71 ± 1.92	53.94 ± 2.01
	AcH	2760.22 ± 95.25	47.11 ± 3.72	9.56 ± 2.14	153.84 ± 1.89
	AIH	1554.89 ± 54.77	26.71 ± 3.46	13.10 ± 4.46	71.62 ± 1.89
	WEH	1837.80 ± 26.25	53.16 ± 3.62	11.98 ± 1.05	96.03 ± 3.04
	AcEH	2797.95 ± 48.59	40.21 ± 1.44	12.83 ± 1.15	170.04 ± 4.12
	AlEH	2278.85 ± 86.80	35.77 ± 3.14	16.20 ± 2.68	128.96 ± 3.54

### 3.3.2. Energy Conversion Efficiency of Straw Hydrolysate by Photo-Fermentation

The COD removal ratio of each straw after photo-fermentation is shown in Figure 6A. The difference between the two straws is negligible, owing to the minor difference in the main fiber composition of the two straws. The WH has a high COD removal ratio; perhaps because the hydrolysate itself has a low COD content and limited organic matter available to microorganisms, it is more thoroughly utilized. The lowest COD removal ratio of AlEH is due to the increase of harmful substances to microorganisms in the hydrolysate after alkali treatment, and by adjusting the pH before and after enzymatic hydrolysis, the excessive addition of NaCl in the hydrolysate will aggravate the inhibition of microbial growth. Figure 6B depicts the energy conversion efficiency of photo-fermentation. CS hydrolysate had the lowest energy conversion efficiency of 2.63%. The highest was 9.81% for AcEH, and the next highest was 6.98% for AcH. The energy conversion efficiency of WH was approximately 3.83% for SS hydrolysate. AcEH was the highest at 12.07%, and AcH was the second highest at 10.92%. In terms of energy conversion efficiency, photo-fermentation of AcH hydrolysate and AcEH hydrolysate outperformed other hydrolysates. However, the data in the figure also shows that a significant amount of energy in the straw was wasted or unused for all hydrolysates, indicating that there is still much room for improvement.



**Figure 6.** The COD removal ratio (A) and energy conversion efficiency (B) of photo-fermentation of straw hydrolysate.

### 3.3.3. Comparison of Photo-Fermentative Hydrogen Yield Using Straws

The hydrogen yield obtained in this study is compared to other published literature in Table 4. As shown in the table, enzymes are used in the hydrolysis of straw in the majority of reports. Some of these studies used dual enzymes to hydrolyze straw, while others used mutant strains or coenobium in the photo-fermentation stage. In comparison, the hydrogen yields of CS and SS hydrolysates by acid-enzyme hydrolysis are  $122.72 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$  and  $170.04 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$ , respectively. In this study, acid hydrolysis without enzyme was used to hydrolyze SS with high hydrogen production of  $153.84 \text{ mL}\cdot\text{g}^{-1}\text{-TS}$  using wild strain. The results show that if the hydrolysis method is correct and the strain is selected appropriately, hydrogen production by photo-fermentation using straw as substrate can produce good hydrogen yields even without the use of enzymes. It also demonstrates the great potential of straw biomass for one-step PFHP.

**Table 4.** Comparison with other photo-fermentative hydrogen production using straws.

Straw	Hydrolysis	Inoculum	H <sub>2</sub> Yield	Reference
Wheat straw	Acid (4% H <sub>2</sub> SO <sub>4</sub> )	R. capsulatus PK	254 mL·L <sup>-1</sup>	[26]
CS	Acid (5% HCl)	R. sphaeroidesWH04 (hupSL-)	574.42 mL·g <sup>-1</sup> -reducing sugar	[57]
SS	Enzymatic	Photosynthetic consortium	150.4 mmol·L <sup>-1</sup>	[8]
CS	Enzymatic	Mixed culture HAU-M1	141.42 mL·g <sup>-1</sup> -TS	[58]
CS pith	Enzymatic	Photosynthetic consortium	136 mL·g <sup>-1</sup> -TVS	[59]
Medicago sativa L.	Enzymatic	Mixed culture HAU-M1	147.64 mL·g <sup>-1</sup> -TS	[60]
CS	Alkaline-combined double enzymatic	R. sphaeroides HY01	339.5 mL·g <sup>-1</sup> -TVS	[30]
Giant reed	Alkaline-enzyme	Mixed culture HAU-M1	98.3 mL·g <sup>-1</sup> -TS	[61]
CS	Alkaline-enzyme	Mixed culture HAU-M1	137.76 mL·g <sup>-1</sup> -TS	[52]
SS	Acid-enzyme	R. sphaeroides HY01	170.04 mL·g <sup>-1</sup> -TS	This study
CS	Acid-enzyme	R. sphaeroides HY01	122.72 mL·g <sup>-1</sup> -TS	This study
SS	Acid	R. sphaeroides HY01	153.84 mL·g <sup>-1</sup> -TS	This study
CS	Acid	R. sphaeroides HY01	87.37 mL·g <sup>-1</sup> -TS	This study

## 4. Conclusions

CS and SS were hydrolyzed using hydrothermal, acid, alkali, hydrothermal-enzyme, acid-enzyme, and alkali-enzyme methods, and the hydrolysates were used in PFHP. Because CS and SS contained similar amounts of cellulose and hemicellulose, comparable RTS would be obtained using the same hydrolysis method. Different hydrolysis methods

had a greater effect on straw saccharification; the use of acid or enzyme can significantly increase straw reducing sugar yield. The highest hydrogen yields were obtained by photo-fermentation with an acid-enzyme hydrolysate from CS and SS. The enzyme could significantly promote PFHP of CS. In contrast, the acid hydrolysis hydrolysate from SS had a high hydrogen yield, which could not be significantly improved by enzymatic hydrolysis. This implies that acid hydrolysis was more suitable for SS straw and acid-enzyme hydrolysis was more suitable for CS in PFHP.

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