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# Evaluating Bio-Hydrogen Production Potential and Energy Conversion Efficiency from Glucose and Xylose under Diverse Concentrations

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**Abstract:** Lignocellulose bioconversion to hydrogen has been proposed as a promising solution to augment the fossil fuel dominated energy market. However, little is known about the effects of the substrate concentration supplied on hydrogen production. Herein, the hydrogen producing bacteria *Thermoanaerobacter thermosaccharolyticum* W16 feeding with respective glucose, xylose, and glucose and xylose mixture (glucose–xylose) at different concentrations was evaluated, to study whether substrate concentration could impact the lignocellulose bioconversion to hydrogen and the associated kinetics. An average bio-hydrogen yield of 1.40 ± 0.23 mol $H_2$·mol$^{-1}$ substrate was obtained at an average substrate concentration of 60.89 mM. The maximum bio-hydrogen production rate of 0.25 and 0.24 mol $H_2$·mol$^{-1}$ substrate h$^{-1}$ was achieved at a substrate concentration of 27.75 mM glucose and 30.82 mM glucose–xylose, respectively, while the value reached the high point of 0.08 mol $H_2$·mol$^{-1}$ xylose·h$^{-1}$ at 66.61 mM xylose. Upon further energy conversion efficiency (ESE) analysis, a substrate of 10 g·L$^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose) provided the maximum ESE of 15.3 ± 0.3%, which was 15.3% higher than that obtained at a substrate concentration of 5 g·L$^{-1}$ (amounting to 27.75 mM glucose, 33.30 mM xylose or 30.28 mM glucose–xylose). The findings could be helpful to provide effective support for the future development of efficient and sustainable lignocellulosic bio-hydrogen production.

**Keywords:** bio-hydrogen production; glucose; xylose; lignocellulose; energy conversion efficiency



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## 1. Introduction

Hydrogen ($H_2$) is considered as a sustainable alternative to fossil fuels due to its carbon neutral characteristic during combustion [1,2]. To date, the majority of $H_2$ is produced from fossil fuels via physical or chemical processes, which require significant energy consumption and environmental management input [3,4]. By contrast, biological hydrogen (bio-hydrogen) production presents obvious benefits, such as having no dependence on fossil fuels, being renewable in nature, and being ecofriendly [5,6]. Among all the bio-hydrogen production processes, dark fermentative hydrogen production is considered to be one of the most promising methods for hydrogen production due to its dual effect on waste treatment and clean energy recovery [7,8]. A wide range of feedstocks have been investigated for bio-hydrogen production [9,10]. Lignocellulose is one of the most desirable substrates for bio-hydrogen production, due to its huge yield and difficulty in being processed [11].

Lignocellulose consists of cellulose, hemicellulose, and lignin. Current research has focused on obtaining pentoses and hexoses contained in cellulose and hemicellulose using appropriate pretreatment methods [12,13]. The results showed that the reducing sugars obtained from different straws and different pretreatment methods were generally glucose, xylose, or glucose and xylose mixture (glucose–xylose) in concentrations ranging from

5 to 30 g·L$^{-1}$ [14–16]. Examples regarding the hydrogen yield from different sugar concentrations and glucose/xylose ratios are shown in Table 1. Thus, the hydrogen yield is determined by the glucose and xylose consumption capacity of the hydrogen producing biocatalysts. For example, a hydrogen yield of 3.1 mol $H_2$·mol$^{-1}$ substrate was obtained by *Clostridium butyricum* at 55.51 mM glucose [17]. Microorganisms of this genus have also been found to use xylose for hydrogen production; a hydrogen production rate of 0.76 mol $H_2$·mol$^{-1}$ substrate was obtained by *Clostridium butyricum CGS5* from the rice straw hydrolysate with a xylose concentration of 61.28 mM [18]. Reference [19] found a coculture of *C. thermocellum* could achieve 0.85 mol $H_2$·mol$^{-1}$ substrate from sugarcane bagasse hydrolysate (69.38 mM glucose and 25.31 mM xylose). When the cornstalk hydrolysate concentration was increased to 20 g·L$^{-1}$ (84.37 mM glucose and 31.97 mM xylose), the strain *Clostridium* sp. T2 could accomplished a hydrogen production rate of 1.70 mol $H_2$·mol$^{-1}$ substrate [20]. Additionally, [21] used corn stover hydrolysate (11.66 mM glucose and 6.66 mM xylose) as substrate and obtained the maximum cumulative hydrogen yield of 0.27 mol $H_2$·mol$^{-1}$ substrate by dark fermentative bacteria *Enterobacter aerogenes*. In view of the above studies, the gap to be addressed is evaluating the bio-hydrogen production potential from different reducing sugar concentrations, which is crucial for both efficient lignocellulosic biomass conversion to bio-hydrogen, and minimizing of the consideration of sugar concentration during lignocellulose pretreatment [10,22,23].

**Table 1.** The hydrogen yield from different sugar concentrations and glucose/xylose ratios.

| Microorganism | Substrate Concentration (mM) | | Hydrogen Yield (mol $H_2$·mol$^{-1}$ Substrate) | References |
|---|---|---|---|---|
| | Glucose | Xylose | | |
| *Clostridium butyricum* | 27.75–111.01 | 0 | 2.4–3.1 | [17] |
| *Clostridium butyricum CGS5* | 0 | 87.26/61.28 | 0.70–0.76 | [18] |
| *C. thermocellum* | 69.38 | 25.31 | 0.85 | [19] |
| *Clostridium* sp. T2 | 84.37 | 31.97 | 1.70 | [20] |
| *Enterobacter aerogenes* | 11.66 | 6.66 | 0.27 | [21] |
| *Clostridium beijerinckii* | 15.26 | 7.99 | 1.051 | [24] |
| *Clostridium roseum ATCC* 17,797 | 145.32 | 125.89 | 0.014 | [14] |
| *Escherichia coli* WDHL | 2.78 | 86.59 | 0.95 | [16] |

Our previous work on bio-hydrogen production from lignocellulose has resulted in the isolation of a glucose and xylose co-fermentation bacterium *Thermoanaerobacter thermosaccharolyticum* W16, making it a suitable candidate for bio-hydrogen production from lignocellulose hydrolysate [25]. Therefore, this study determined the hydrogen production potential of *Thermoanaerobacter thermosaccharolyticum* W16 at different concentrations of glucose, xylose, and glucose–xylose, respectively. Moreover, the kinetic analysis of hydrogen production under different conditions was also provided. The results obtained in this study will help to predict the bio-hydrogen production potential from lignocellulose hydrolysates of different origins, and will also give feedback on the choice of lignocellulose pretreatment methods.

## 2. Materials and Methods

### *2.1. Microorganism and Medium*

The thermophilic hydrogen producing bacteria *T. thermosaccharolyticum* W16 used in this study was isolated by Ren et al. [25]. *T. thermosaccharolyticum* W16 was routinely grown at 60 °C in the medium containing: 1.0 g·L$^{-1}$ $K_2HPO_4$, 1.0 g·L$^{-1}$ $KH_2PO_4$, 1.0 g·L$^{-1}$ NaCl, 0.2 g·L$^{-1}$ KCl, 0.5 g·L$^{-1}$ $MgCl_2·6H_2O$, 1.0 g·L$^{-1}$ $NH_4Cl$, 2.0 g·L$^{-1}$ yeast extract, 2.0 g·L$^{-1}$ peptone, 0.5 g·L$^{-1}$ cysteine, 10 g·L$^{-1}$ glucose, 1.0 mL·L$^{-1}$ vitamin solution, 1.0 mL·L$^{-1}$ trace element solution [25]. The strain at its exponential growth phase was prepared as the inoculum.

### 2.2. Bio-Hydrogen Potential Tests

A suite of 100 mL serum bottles with 48 mL aforementioned culture medium containing inorganic salts in addition to the carbon source was prepared. Glucose, xylose, and glucose–xylose (50% glucose and 50% xylose) at concentrations of 5 $g \cdot L^{-1}$, 10 $g \cdot L^{-1}$, 15 $g \cdot L^{-1}$, 20 $g \cdot L^{-1}$, and 25 $g \cdot L^{-1}$ were dispensed into serum bottles, respectively, to conduct bio-hydrogen potential tests. The initial pH for each bottle was adjusted to 6.5 using 2 mM NaOH. After degassing with nitrogen for 30 min, the serum bottles were sealed by the caps and autoclaved at 121 °C for 20 min. Then, the cooled serum bottles were inoculated with 2 mL inoculum solution and incubated in an orbital incubator shaker at 60 °C and a rotation speed of 150 rpm for 36 h until a negligible amount of bio-hydrogen was observed. The substrate consumption, hydrogen production, and cell growth profile were monitored with time, and all batch tests were performed in triplicates.

### 2.3. Analytical Methods

All liquid samples taken every 4–8 h during the batch tests were filtered through disposable sterile millipore filter units (pore size: 0.22 μm) for chemical analysis. The glucose and xylose concentrations were detected by high-performance liquid chromatography (HPLC) equipped with a refraction index detector (LC-10A, Shimadzu Corporation, Kyoto, Japan) [22,26]. A gas chromatograph (7890A, Agilent, Santa Clara, CA, USA) with a flame ionization detector (FID) and DB-FFAP (30 m × 50 μm, 0.25 μm) capillary column was employed to qualify and quantify the metabolic end products such as acetate and butyrate [27]. The volume of gas produced ($H_2$ and $CO_2$) was collected and measured by gas bag; meanwhile, the hydrogen fraction in the gas bag was determined by a gas chromatograph (7890A, Agilent, USA) with a thermal conductivity detector (TCD) [27]. The hydrogen amount was then calculated as accumulated gas production quantity * hydrogen content in the gas.

### 2.4. Data Analysis

In order to compare the bio-hydrogen production potential of *T. thermosaccharolyticum* W16 from different substrates under different concentrations, the hydrogen production capacity was fitted to a modified Gompertz model [26], as given in the formula (1) shown below:

$$H = P \times \exp\{-\exp[\frac{Rme}{P}(\lambda - t) + 1]\} \quad (1)$$

where $H$ (mol $H_2 \cdot mol^{-1}$ substrate) represents the total amount of $H_2$ at culture time $t$ (h), $P$ (mol $H_2 \cdot mol^{-1}$ substrate) is the maximum cumulative amount of $H_2$ produced, $Rm$ (mol $H_2 \cdot mol^{-1}$ substrate $h^{-1}$) is the maximum $H_2$ production rate, $\lambda$ (h) is the lag time before exponential $H_2$ production, $t$ (h) is the bio-hydrogen production tests time, and $e$ = 2.71828.

The energy conversion efficiency (ECE) of the overall bio-hydrogen production from different substrates under different concentrations was computed by using the following formula:

$$\text{ECE\%} = \frac{\text{Heat value of } H_2 \left(\text{kJ} \cdot \text{mol}^{-1}\right)}{\text{Heat value of glucose} \left(\text{kJ} \cdot \text{mol}^{-1}\right) + \text{Heat value of xylose} \left(\text{kJ} \cdot \text{mol}^{-1}\right)} \times 100\% \quad (2)$$

where the heating values (HV) of $H_2$, glucose, and xylose were 284 $kJ \cdot mol^{-1}$, 2810.5 $kJ \cdot mol^{-1}$, and 2432.1 $kJ \cdot mol^{-1}$, respectively [28].

One-way factor analysis of variance (ANOVA) was utilized to analyze the significance of experimental data.

## 3. Results and Discussion

### *3.1. Bio-Hydrogen Production from Glucose, Xylose, and Glucose–Xylose*

The bio-hydrogen production profile of *T. thermosaccharolyticum* W16 feeding with glucose, xylose, and glucose–xylose at different concentrations was shown in Figures 1–3, and the corresponding kinetic parameters were given in Table 2. The hydrogen yield increased with glucose concentration rising from 27.75 to 55.51 mM, and the maximum hydrogen yield obtained was 1.32 mol $H_2$·mol$^{-1}$ glucose (Figure 1). Further increasing the glucose concentration to 83.26 mM reduced the hydrogen yield to 61.8% of the maximum value. When the glucose concentration was improved to 138.77 mM, the highest hydrogen yield was only 0.60 mol $H_2$·mol$^{-1}$ glucose. The cumulative bio-hydrogen yield was simulated with the modified Gompertz equation (Figure 1); the determination coefficient ($R^2$) of over 0.99 confirmed that the experimental data fitted the modified Gompertz equation well. In contrast to the bio-hydrogen production performance, the highest predicted bio-hydrogen production rate (*Rm*) occurred at 27.75 mM glucose, which was 0.25 mol $H_2$·mol$^{-1}$ glucose h$^{-1}$, although the lag time ($\lambda$) was 7.5 h. With the rising of glucose concentration from 27.75 to 138.77 mM, the *Rm* and the lag time decreased accordingly. The lowest *Rm* was 0.04 mol $H_2$·mol$^{-1}$ glucose h$^{-1}$ at 138.77 mM glucose, while the lag time ($\lambda$) was shortened to 6.3 h (Table 2).

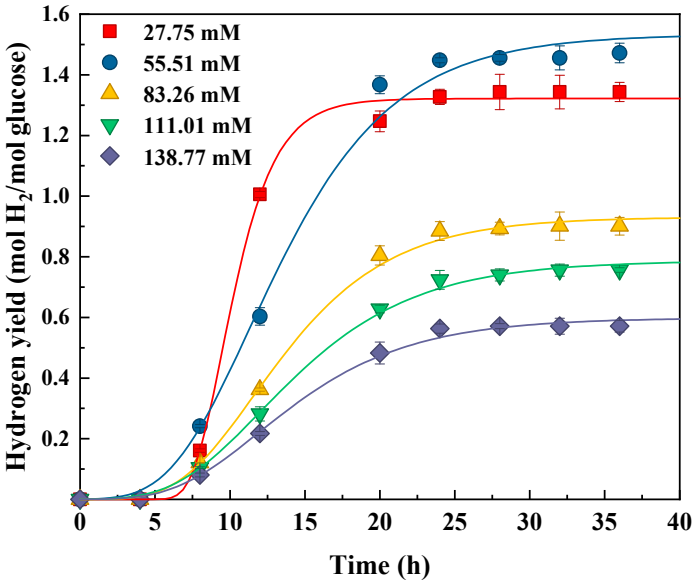


**Figure 1.** Cumulative bio-hydrogen yield from glucose at varying concentrations with simulation curves based on the modified Gompertz equation.

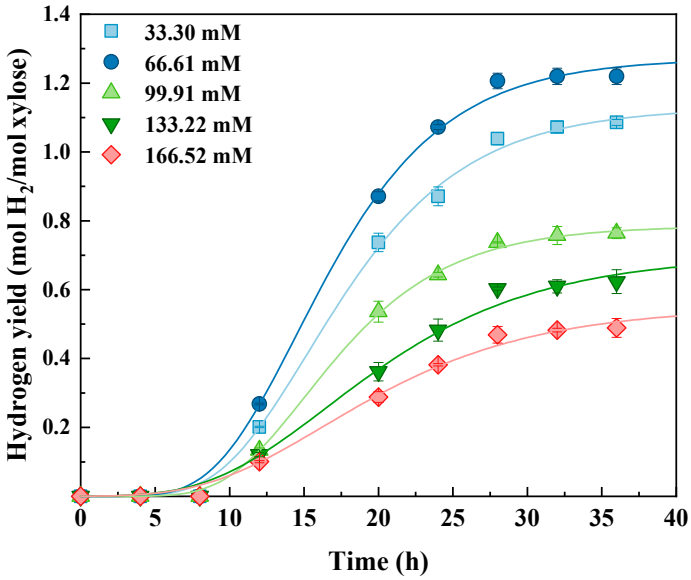


**Figure 2.** Cumulative bio-hydrogen yield from xylose at varying substrate concentrations with simulation curves based on the modified Gompertz equation.

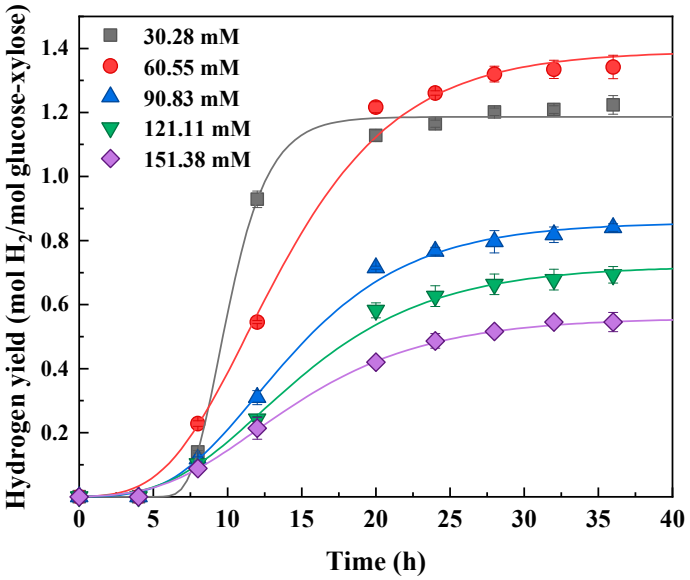


**Figure 3.** Cumulative bio-hydrogen yield from glucose–xylose at varying substrate concentrations with simulation curves based on the modified Gompertz equation.

**Table 2.** Estimated bio-hydrogen production potential (*P*) and maximum bio-hydrogen production rate (*Rm*) from glucose, xylose, and glucose–xylose at varying concentrations.

| Substrate Types | Substrate Concentration ($g \cdot L^{-1}$) | Substrate Concentration (mM) | *P* (mol $H_2$ $mol^{-1}$ Substrate) | *Rm* (mol $H_2$ $mol^{-1}$ Substrate $h^{-1}$) | $\lambda$ (h) | $R^2$ |
|---|---|---|---|---|---|---|
| Glucose | 5 | 27.75 | 1.32 ± 0.11 | 0.25 ± 0.02 | 7.4 ± 0.4 | 0.997 |
| | 10 | 55.51 | 1.53 ± 0.12 | 0.11 ± 0.00 | 6.0 ± 0.3 | 0.997 |
| | 15 | 83.26 | 0.93 ± 0.07 | 0.07 ± 0.00 | 6.6 ± 0.4 | 0.999 |
| | 20 | 111.01 | 0.79 ± 0.06 | 0.05 ± 0.00 | 6.3 ± 0.3 | 0.991 |
| | 25 | 138.77 | 0.60 ± 0.05 | 0.04 ± 0.00 | 6.3 ± 0.5 | 0.992 |
| Xylose | 5 | 33.30 | 1.13 ± 0.09 | 0.07 ± 0.01 | 9.3 ± 0.8 | 0.995 |
| | 10 | 66.61 | 1.27 ± 0.10 | 0.08 ± 0.01 | 8.9 ± 0.7 | 0.988 |
| | 15 | 99.91 | 0.79 ± 0.05 | 0.05 ± 0.00 | 9.7 ± 0.8 | 0.996 |
| | 20 | 133.22 | 0.70 ± 0.05 | 0.03 ± 0.00 | 8.6 ± 0.7 | 0.998 |
| | 25 | 166.52 | 0.55 ± 0.03 | 0.03 ± 0.00 | 8.3 ± 0.6 | 0.994 |
| Glucose–xylose | 5 | 30.28 | 1.19 ± 0.09 | 0.24 ± 0.02 | 7.5 ± 0.5 | 0.994 |
| | 10 | 60.55 | 1.39 ± 0.11 | 0.09 ± 0.01 | 5.7 ± 0.4 | 0.993 |
| | 15 | 90.83 | 0.86 ± 0.07 | 0.06 ± 0.00 | 6.2 ± 0.5 | 0.998 |
| | 20 | 121.11 | 0.72 ± 0.05 | 0.04 ± 0.00 | 5.9 ± 0.3 | 0.998 |
| | 25 | 151.38 | 0.56 ± 0.04 | 0.03 ± 0.00 | 5.5 ± 0.3 | 0.999 |

When feeding with xylose, the bio-hydrogen yield tendency was similar to that of glucose. The maximum value was 1.27 mol $H_2 \cdot mol^{-1}$ xylose at 66.62 mM xylose (Figure 2). While the hydrogen production rate was slightly different from that of glucose, the highest value of 0.08 mol $H_2 \cdot mol^{-1}$ xylose $h^{-1}$ was also found at 66.62 mM xylose, which was only 34.3% that of 27.75 mM glucose (Figure 2). Additionally, the overall lag time was longer with xylose than that with glucose, indicating that the cell growth was directly related to the type of substrate. Moreover, it can be obtained from Figure 3 that the hydrogen production performance was similar to that of glucose alone when glucose–xylose was feeding as a substrate. The maximum bio-hydrogen yield and bio-hydrogen production rate was 1.39 mol $H_2 \cdot mol^{-1}$ glucose–xylose and 0.24 mol $H_2 \cdot mol^{-1}$ glucose–xylose $h^{-1}$, respectively. Additionally, it was noteworthy that the lag time at a high glucose–xylose concentration of 151.38 mM was shortened to only 5.5 h (Table 2).

From the above results, it can be concluded that, no matter what type of reducing sugar was used, the maximum bio-hydrogen yield emerged when the substrate concentration was 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose), indicating that different substrate types had little effect on the cumulative bio-hydrogen yield. The maximum hydrogen production rate appeared when the substrate concentration was 5 $g \cdot L^{-1}$ (amounting to 27.75 mM glucose, 33.30 mM xylose or 30.28 mM glucose–xylose) or 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose), and the value decreased significantly with the increase of substrate concentration. Using glucose alone or glucose–xylose as the substrate at a concentration of 5 $g \cdot L^{-1}$ (amounting to 27.75 mM glucose, 33.30 mM xylose or 30.28 mM glucose–xylose), the bio-hydrogen production rate was significantly higher than that of xylose. However, as the substrate concentration increased, the difference in bio-hydrogen production rates among glucose, xylose, and glucose–xylose continued to decrease. The results confirmed that the bio-hydrogen production performance decreased along with substrate concentration increasing, which was consistent with previous research [29,30]. Table 3 showed that the metabolic end products (mainly acetate and butyrate) increased with substrate concentration when using glucose or xylose as the sole substrate, while for the glucose–xylose one, ethanol and butanol have also appeared in metabolic end products. When the substrate concentration increased from 30.28 mM to 151.38 mM, the ethanol and butanol concentration decreased accompanied with the gradual increase of acetate and butyrate. In our previous studies [31,32], trace amounts of ethanol and butanol were

usually detected as by-products during $H_2$ production by *T. thermosaccharolyticum* W16, no matter what concentration of glucose or xylose was used as substrate. However, in this study, the ethanol and butanol appeared only in the glucose/xylose mixture; it is a very interesting phenomenon that can be further investigated by means of functional genomics in another study. Moreover, the metabolic end products' accumulation under high substrate concentration might also limit bio-hydrogen production [30,33]. It can also be found from the results that the substrate concentration had no significant effect on the lag time when the value was greater than or equal to 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose) (when the substrates were glucose, xylose and glucose–xylose, the $p$ values were all higher than 0.05). However, the lag time in hydrogen production from xylose was longer than that in the presence of glucose, which is consistent with previous studies [26]. The presence of xylose gives the strain W16 a greater preference for self-synthesis than for bio-hydrogen production.

**Table 3.** Metabolic end products of bio-hydrogen production from glucose, xylose, and glucose and xylose mixture at varying concentrations.

| Substrate Types | Substrate Concentration ($g \cdot L^{-1}$) | Substrate Concentration (mM) | Acetate (mM) | Butyrate (mM) | Ethanol (mM) | Butanol (mM) |
|---|---|---|---|---|---|---|
| Glucose | 5 | 27.75 | 13.4 ± 1.0 | 40.4 ± 3.3 | n.d. | n.d. |
| | 10 | 55.51 | 19.3 ± 1.4 | 39.5 ± 3.1 | n.d. | n.d. |
| | 15 | 83.26 | 20.3 ± 1.5 | 35.1 ± 2.8 | n.d. | n.d. |
| | 20 | 111.01 | 20.1 ± 1.4 | 37.3 ± 3.1 | n.d. | n.d. |
| | 25 | 138.77 | 28.3 ± 2.2 | 45.8 ± 3.7 | n.d. | n.d. |
| Xylose | 5 | 33.30 | 10.6 ± 0.8 | 38.1 ± 3.1 | n.d. | n.d. |
| | 10 | 66.61 | 15.9 ± 1.2 | 36.1 ± 2.9 | n.d. | n.d. |
| | 15 | 99.91 | 16.9 ± 1.3 | 32.6 ± 2.6 | n.d. | n.d. |
| | 20 | 133.22 | 17.8 ± 1.3 | 35.0 ± 2.8 | n.d. | n.d. |
| | 25 | 166.52 | 24.9 ± 1.9 | 41.2 ± 3.3 | n.d. | n.d. |
| Glucose and xylose | 5 | 30.28 | 4.1 ± 0.2 | 12.3 ± 0.8 | 34.0 ± 2.2 | 22.5 ± 1.4 |
| | 10 | 60.55 | 6.0 ± 0.4 | 19.4 ± 1.1 | 25.9 ± 1.9 | 21.6 ± 1.4 |
| | 15 | 90.83 | 10.8 ± 0.3 | 26.9 ± 1.6 | 20.7 ± 1.2 | 20.2 ± 2.3 |
| | 20 | 121.11 | 15.4 ± 0.4 | 29.8 ± 2.4 | 20.4 ± 0.9 | 20.0 ± 1.6 |
| | 25 | 151.38 | 25.8 ± 0.8 | 31.8 ± 2.1 | 17.5 ± 1.1 | 16.4 ± 1.2 |

n.d.: not detected.

### *3.2. Glucose and Xylose Consumption during Bio-Hydrogen Production*

Profiles of residual reducing sugars concentration with time were shown in Figures 4–6. When the initial glucose concentration was 27.75 mM (Figure 4), it could be fully utilized within 12 h of fermentation. The lack of carbon source might cause lower biomass and inactive metabolism. Therefore, this would explain why the highest hydrogen production rate was achieved at 27.75 mM glucose, but the final cumulative hydrogen yield was lower than that at 55.51 mM glucose. With the rising of glucose concentration from 27.75 mM to 55.51 mM, substrate consumption improved gradually, and the maximum substrate consumption of 39.47 mM was achieved. As the glucose concentration gradually increased to 138.77 mM, the substrate consumption decreased at the same time, and the lowest substrate consumption yield was only 30.88 mM. The results suggested that extremely high substrate concentration would restrict the metabolic growth activity of cells and cause harm to bio-hydrogen production.

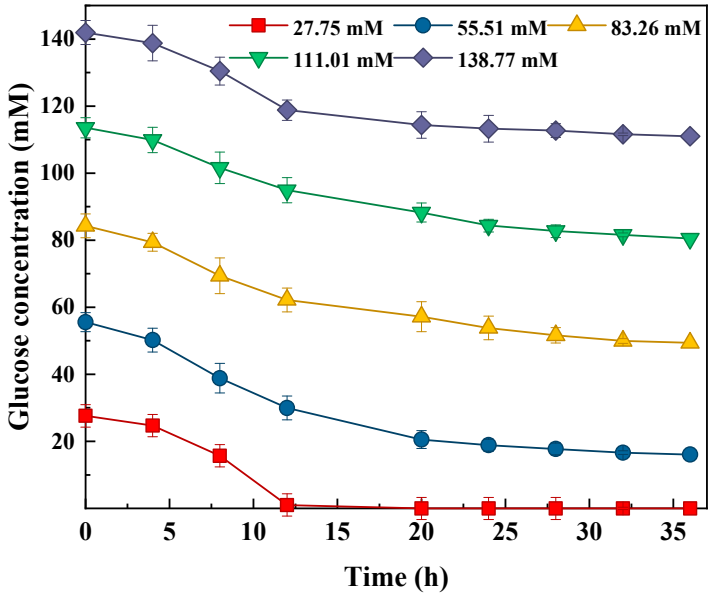


**Figure 4.** Residue glucose concentration during bio-hydrogen production at varying glucose concentrations.

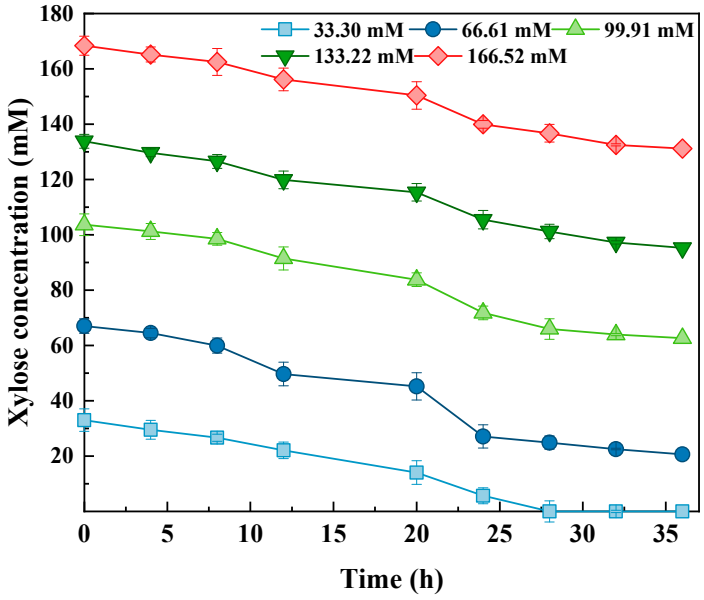


**Figure 5.** Residue xylose concentration during bio-hydrogen production at varying xylose concentrations.

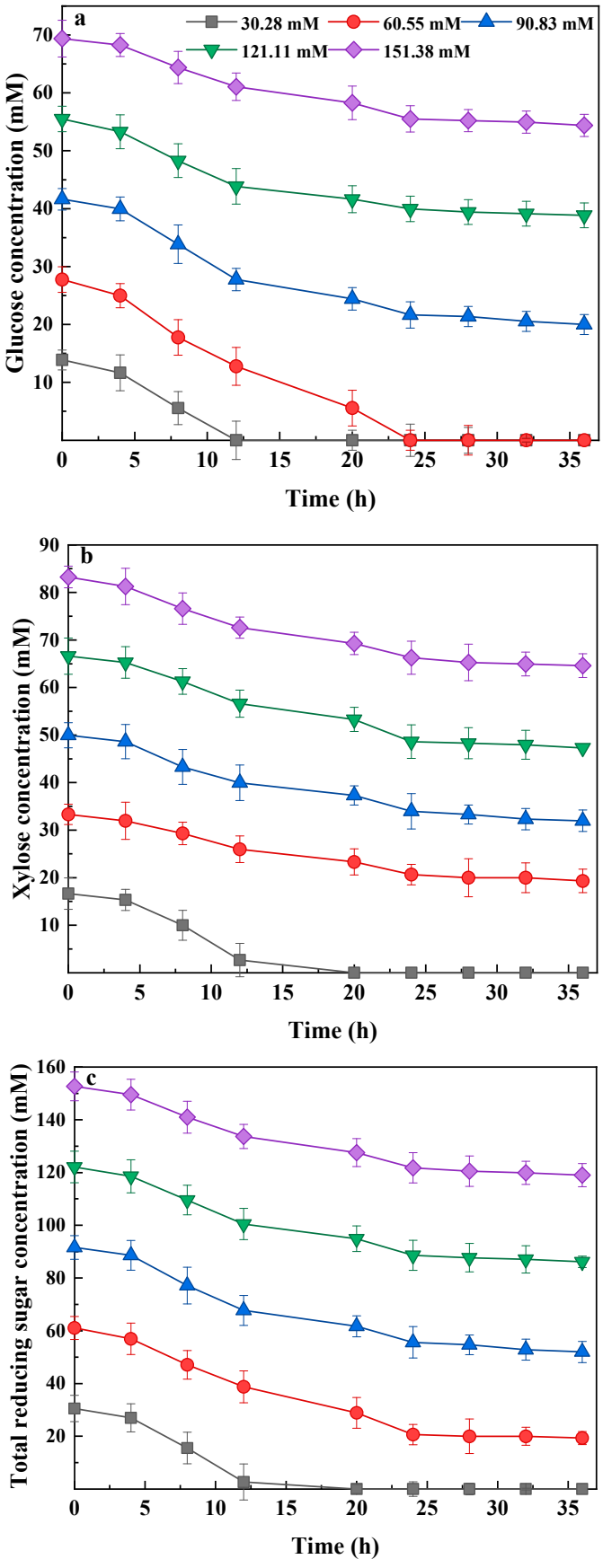


**Figure 6.** Residue substrate concentration during bio-hydrogen production at varying glucose–xylose concentrations. (**a**) Residue glucose concentration; (**b**) Residue xylose concentration; (**c**) Residue glucose and xylose concentration.

When feeding with xylose or glucose–xylose, respectively, the trend in substrate consumption at different concentrations is the same as that in glucose. The only difference is that the substrate was completely consumed within 28 h and 20 h, respectively, at a concentration of 33.30 and 30.28 mM (Figures 5 and 6). Regardless of the substrate type, the highest substrate consumption (42.53 ± 3.86 mM) occurred at the substrate concentration of 55.51 mM glucose, 66.61 mM xylose and 60.55 mM glucose–xylose, which also corresponded to the maximum bio-hydrogen yield. It was found that the presence of glucose in the mixed substrate restricted the xylose utilization. For example, 27.75 mM glucose could be fully utilized in 60.55 mM glucose–xylose, while only 13.99 mM xylose was utilized for bio-hydrogen production and accounted for only 33.51% of the total sugar consumed. Increasing the glucose–xylose concentration from 60.55 mM to 151.38 mM, the xylose consumption ratio in the total substrates raised accordingly. The xylose consumption occupied 55.45% of the total substrate consumed when substrate concentration was 151.38 mM (Figure 6). In conclusion, the substrate consumption rule of three types of substrates with different concentration presented a positive correlation with total bio-hydrogen yield. That is, the bio-hydrogen production performance from lignocellulose hydrolysate was regulated by the substrate concentration rather than the substrate type.

### *3.3. Energy Conversion Efficiency of Bio-Hydrogen Production from Glucose, Xylose, and Glucose–Xylose*

The energy conversion efficiency (ECE) of bio-hydrogen production from glucose, xylose, and glucose–xylose at varying concentrations was calculated and shown in Figure 7. The maximum ECE obtained by using glucose as a substrate was 15.6% at 55.51 mM glucose, 51.7% of the theoretical value. Increasing the glucose concentration to 83.26 mM, 111.01 mM, and 138.77 mM, the ECE went down to 9.5%, 8.0%, and 6.1%, respectively, which indicates that high substrate concentration was not conducive to energy conversion and bio-hydrogen production. Moreover, it can be clarified that ECE obtained from solely xylose with a concentration of 33.30 mM and 66.61 mM exhibits higher ECE, and 44.9% and 50.6% of the theoretical value were obtained, respectively. A similar value of the ECE (12.9% and 15.2%) has also been achieved from 30.28 mM and 60.55 mM glucose–xylose. In general, an energy conversion efficiency of less than 10% is not competitive compared to fossil-fuel-based bio-hydrogen production [28], and the maximal energy conversion efficiency of hydrogen production from solar is only 1.1% [34]. From the current results in this study, the ECE were all higher than 10% when the substrate concentration is less than 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose), which indicates that the reasonable control of the soluble reducing sugars concentration can make the lignocellulosic bio-hydrogen production more energy efficient.

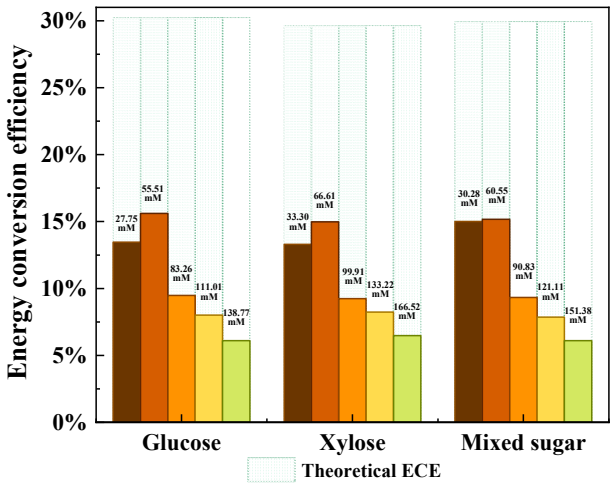


**Figure 7.** Energy conversion efficiencies of bio-hydrogen production from glucose, xylose, and glucose–xylose at varying concentrations.

### *3.4. Implications of the Study*

Bio-hydrogen production from lignocellulose has attracted serious concern from both environmental and economic points of view. However, lignocellulosic biomass presents a complex structure due to its recalcitrant nature, and appropriate pretreatment is necessary to release the reducing sugar for the subsequent bio-hydrogen production. Therefore, the rational use of lignocellulose hydrolysate is crucial to bio-hydrogen production. The results obtained in this study have confirmed that the total bio-hydrogen yield from lignocellulose hydrolysate is not related to the type of reducing sugar, but is mainly affected by the reducing sugar concentration. Taking *T. thermosaccharolyticum* W16 as an example, if continuous bio-hydrogen production from lignocellulose hydrolysate was operated, the hydrolysate containing glucose or glucose–xylose needs to be diluted to 5 $g \cdot L^{-1}$ (amounting to 27.75 mM glucose, 33.30 mM xylose or 30.28 mM glucose–xylose) to obtain the maximum bio-hydrogen production rate, while for the xylose one, it is better to be diluted to 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose) to ensure efficient lignocellulosic bio-hydrogen production. These collectively imply that if *T. thermosaccharolyticum* W16 is used as the candidate hydrogen producing bacteria, the lignocellulose pretreatment can be independent of the reducing sugar types. Instead, efficient substrate consumption and energy recovery can be achieved with reasonable hydrolysate dilution. On the other hand, excessively reducing sugar concentration could lead to more by-product (e.g., acetate and butyrate) generation, which is beneficial in integrating this dark fermentative hydrogen production with photo fermentative hydrogen production or bio-electrochemical hydrogen production, and therefore increasing the final hydrogen yield. Moreover, it should be noted that most lignocellulosic bio-hydrogen production was carried out at the laboratory scale, while pilot-scale experiments made slow progress. There are many obstacles that need to be solved to realize pilot scale, including low hydrogen yield due to incomplete utilization of lignocellulosic biomass, and reduced cost for lignocellulosic biomass pretreatment. This study provides a measuring tool in bio-hydrogen production, and through the reasonable control of the substrate loading rate, the maximum hydrogen production rate and yield could be obtained and avoid the excessive input caused by inappropriate pretreatment.

## 4. Conclusions

In this study, the bio-hydrogen production performance of *T. thermosaccharolyticum* W16 from glucose, xylose, and glucose–xylose with different concentrations was evaluated. Results showed that the bio-hydrogen yield and bio-hydrogen production rate were mainly related to the substrate concentration rather than substrate type. The maximum bio-hydrogen yields all appeared when the substrate concentration was 10 $g \cdot L^{-1}$ (amounting to 55.51 mM glucose, 66.61 mM xylose or 60.55 mM glucose–xylose), while the highest bio-hydrogen production rate of 0.25, 0.08, and 0.24 mol $H_2 \cdot mol^{-1}$ substrate $h^{-1}$ was obtained at 27.75 mM glucose, 66.61 mM xylose, and 30.28 mM glucose–xylose, respectively. These results suggest that diluting the substrate concentration is reasonably beneficial for bioenergy recovery. Moreover, more attention can be paid to the lignocellulose pretreatment efficiency than to the type of sugar obtained.

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