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Short Communication

Effect of substrate concentration on hydrogen production by photo-fermentation in the pilot-scale baffled bioreactor

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ABSTRACT

Effect of substrate concentration on photo-fermentative hydrogen production was studied with a self-designed 4 m^3 pilot-scale baffled photo-fermentative hydrogen production reactor (BPHR). The relationships between parameters, such as hydrogen production rate (HPR, mol $H_2/m^3/d$), hydrogen concentration, pH value, oxidation–reduction potential, biomass concentration (volatile suspended solids, VSS) and reducing sugar concentration, during the photo-fermentative hydrogen production process were investigated. The highest HPR of 202.64 ± 8.83 mol/m^3/d was achieved in chamber #3 at a substrate concentration of 20 g/L. Hydrogen contents were in the range of 42.19 ± 0.94%–49.71 ± 0.27%. HPR increased when organic loading rate was increased from 3.3 to 20 g/L/d, then decreased when organic loading rate was further increased to 25 g/L/d. A maximum HPR of 148.65 ± 4.19 mol/m^3/d was obtained when organic loading rate was maintained at 20 g/L/d during continuous bio-hydrogen production.

1. Introduction

Bio-hydrogen production has been regarded as a pollution-free bioenergy production process (Hosseini et al., 2015). One of the bio-hydrogen production methods is photo-fermentative hydrogen production which is conducted using photosynthetic bacteria under light-illuminated and anaerobic conditions. Compared to other bio-hydrogen production methods, photo-fermentative hydrogen production has many advantages, such as effective removal of organic pollutants with a wide range of substrates, high substrate conversion efficiency, high hydrogen production rate (HPR, mol $H_2/m^3/d$) and hydrogen content (Pattanamanee et al., 2015; Trchounian and Trchounian, 2015; Zhang et al., 2017b).

Among various factors that can significantly affect bio-hydrogen production, (Buitron and Carvajal, 2010; Guo et al., 2015; Hallenbeck and Liu, 2016; Krujatz et al., 2015; Li et al., 2017; Wang et al., 2015), substrate concentration and organic loading rate (OLR, g/L/d) are particularly crucial for continuous bio-hydrogen production (Mariakakis et al., 2011; Zhang et al., 2015). Substrate concentration below the optimal value always leads to a low HPR, hydrogen content, and biomass concentration (Guo et al., 2014; Mariakakis et al., 2011). When substrate concentration is higher than its optimal value, hydrogen producing microorganism could overproduce inhibitory substances, such as volatile fatty acids (VFAs) and ethanol, leading to decreased HPR. A negative correlation was found between HPR and concentration of volatile fatty acids. Akutsu et al. (2009) reported that maximum HPR of 197.77 ± 21.43 mol/m^3/d using starch was obtained at a substrate concentration of 30 g/L; however, HPR decreased drastically to 162.05 ± 33.48 mol/m^3/d when substrate concentration increased to 50 g/L (Akutsu et al., 2009). Antonopoulou et al. (2011) reported that maximum HPR of 130.80 ± 4.02 mol/m^3/d was observed at a substrate concentration of 17.50 ± 2.0 g carbohydrates/L using sweet sorghum extract as substrate. Whereas HPR decreased to 120.54 ± 8.93 mol/m^3/d when substrate concentration increased to 20.99 ± 2.0 g carbohydrates/L (Antonopoulou et al., 2011). Hydrogen yield, HPR and hydrogen content are also significantly affected by OLR. HPR increased with OLR, whereas it may decrease when substrate concentration reach a high level (Lee et al., 2014). Zahedi et al. (2013) reported that HPR fell drastically when OLR was higher than 110 g TVS/L/d. Tawfik and Salem (2012) reported that HPR went down when OLR exceed 21.4 g COD/L/d.

Pilot-scale bio-hydrogen production test is necessary prior to a

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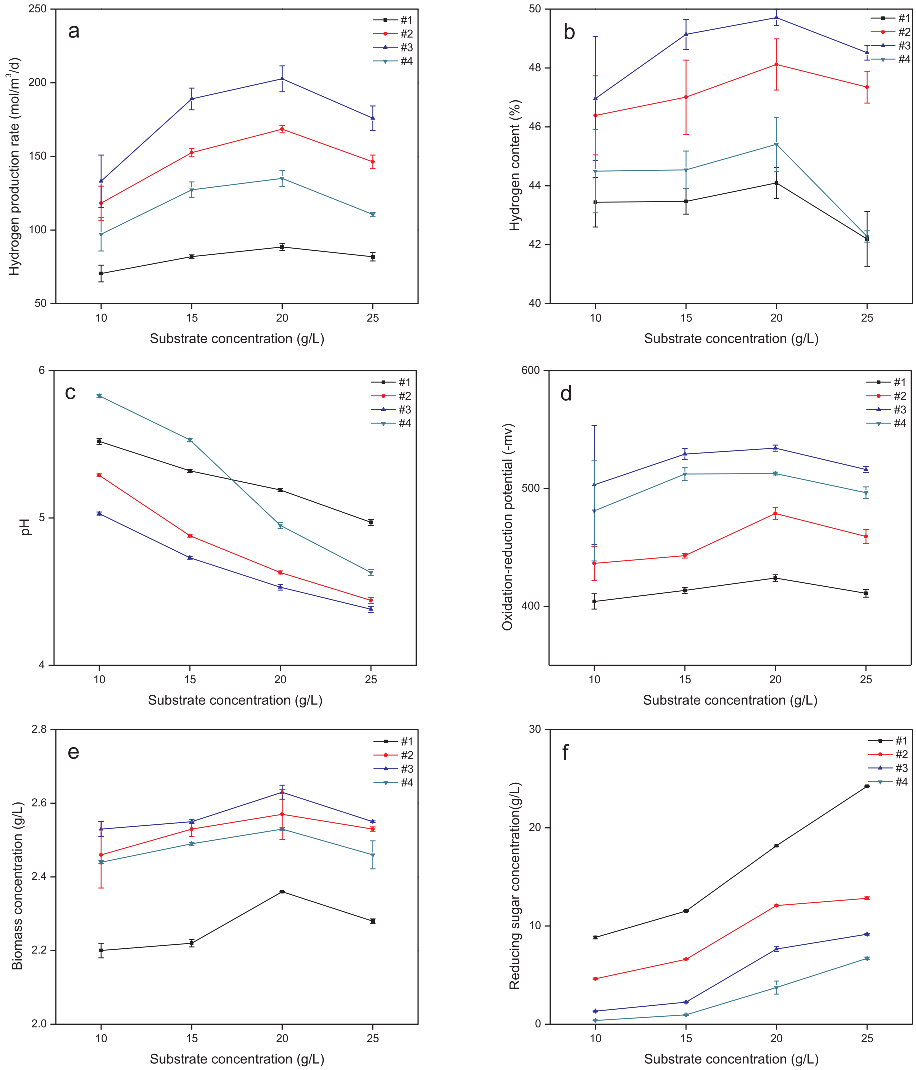

Fig. 1. Effect of substrate concentration on (a) hydrogen production rate, (b) hydrogen content, (c) broth pH, (d) broth oxidation-reduction potential, (e) broth biomass concentration, and (f) residual glucose concentration in broth.

commercial-scale application. At present, there are very few investigations on pilot-scale photo-fermentative bio-hydrogen production (Zhang et al., 2017a). In a previous study, a baffled photo-fermentative hydrogen production reactor (BPHR) was developed and an optimal HRT for bio-hydrogen production was determined (Zhang et al., 2017a). During the feeding process, reaction liquid was rolled up and down along with baffled structure, which improved mixing and consequently improved hydrogen production (Zhang et al., 2015). Up to date, there have been no reports on the effect of substrate concentration on pilot-scale photo-fermentative bio-hydrogen production.

In this study, pilot-scale continuous bio-hydrogen production with mixed microflora HAU-M1 was operated in BPHR system at different substrate concentrations. Effect of substrate concentration on HPR, hydrogen content, and broth characteristics was evaluated. Effect of OLR on HPR was also investigated. Kinetic analysis of hydrogen production process and analysis of variance were further conducted to quantitatively and statistically describe the effect of key factors on the bio-hydrogen production process.

2. Material and methods

2.1. Seed microflora, medium, and bio-hydrogen production process

The seed microflora HAU-M1 was provided by key laboratory of new materials and facilities for renewable energy ministry of agriculture, and cultured as described previously (Lu et al., 2016; Zhang et al., 2017a). The growth medium and photo-fermentative bio-hydrogen production medium were prepared using the same methods as in a previous study (Jiang et al., 2016). Continuous hydrogen production was conducted in a BPHR system as described by Zhang et al., (2017a,b), using a similar method except that the glucose concentration of influent was set at different concentrations (10, 15, 20, 25 g/L).

2.2. Analytical methods

Biogas composition was determined by a gas chromatograph (6820 GC–14B, Agilent) as described previously (Zhang et al., 2017a). Oxidation-reduction potential (ORP) was measured by an ORP metre (SX712, Shanghai San-Xin Instrumentation, Inc.). Biomass was measured by visible spectrophotometer (721, Shanghai Jinghua Technology Instruments Co., Ltd) at 660 nm. Reducing sugar concentration was determined by dinitrosalicylic acid (DNS) method with a visible spectrophotometer at 540 nm.

2.3. Kinetic analysis of hydrogen production

A modified Gompertz equation (Eq. (1)) was used to simulate the cumulative hydrogen production:

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

where, H is the cumulative hydrogen formation per cubic metre (mol/

Table 1
Effect of OLR on hydrogen production.

Trial	Hydraulic retention time (h)	Substrate concentrate (g/L)	Organic loading rate (g/L/d)	Hydrogen production rate ($mol/m^3/d$)	Hydrogen yield (mmol/g)
1	72	10	3.3	64.13 ± 4.58	19.43 ± 1.39
2	48	10	5	83.48 ± 5.84	16.70 ± 1.17
3	24	10	10	104.7 ± 4.72	10.47 ± 0.47
4	24	15	15	137.69 ± 2.89	9.18 ± 0.19
5	24	20	20	148.65 ± 4.19	7.43 ± 0.21
6	24	25	25	128.64 ± 4.17	5.15 ± 0.17

Table 2
Modified Gompertz Model parameters for different substrate concentrations.

Substrate concentration (g/L)	P (mol/m^3)	R_m ($mol/m^3/d$)	λ (d)	R^2
10	1097.32	117.91	2.04	0.9981
15	1447.95	154.50	1.99	0.9982
20	1616.38	166.13	1.90	0.9985
25	1413.07	143.76	1.89	0.9986

m^3) at time (d), P is the maximum potential hydrogen formation per cubic metre (mol/m^3), R_m is the maximum hydrogen formation rate ($mol/m^3/d$), λ is the lag-phase time (d), t is the time (d), and e is a numerical constant that is equal to 2.718. Values of P, R_m and λ for different substrate concentrations were determined by fitting the hydrogen production data using Origin 8.5.

The overall hydrogen formation rate ($R_{overall}$), shown in Eq. (2), is a performance indicator that takes account of hydrogen formation rate and time delay in hydrogen production (Su et al., 2010).

$$R_{overall} = \frac{P}{(P/R_m) + \lambda}\cdot\frac{1}{V} \tag{2}$$

where, V is the working volume of the reactor. For substrate concentration of 10, 15, 20, 25 g/L, the $R_{overall}$s were 86.50 $mol/m^3/d$, 114.29 $mol/m^3/d$, 125.09 $mol/m^3/d$ and 108.64 $mol/m^3/d$, respectively.

2.4. Statistical analysis

One-way ANOVA was used to analyze the significant differences. Both P-value and F-test were performed to further clarify the correlations among the different substation concentration and hydrogen production process (Kim, 2017).

3. Results and discussion

3.1. Effect of substrate concentration on HPR and hydrogen content

HPR increased first and then decreased with substrate concentration or chamber number (Fig. 1a). HPRs of all the four chambers of BPHR showed increasing trends when substrate concentration increased from 10 g/L to 20 g/L. Further increasing substrate concentration to 25 g/L led to a significant drop in the HPR (Fig. 1a). Maximum HPR values of 88.52 ± 2.4, 168.4 ± 2.4, 202.6 ± 8.8, and 135.0 ± 5.5 $mol/m^3/d$ were obtained at a substrate concentration of 20 g/L for chambers #1, #2, #3, and #4, respectively (Fig. 1a). Hydrogen content showed similar trends to those of HPR, i.e. increased with substrate concentration (10–20 g/L) or chamber number (#1–3), then decreased when substrate concentration was further increased to 25 g/L, or at chamber #4 (Fig. 1b). Hydrogen contents with different substrate concentrations were in the range of 42.19 ± 0.94%–49.71 ± 0.27% (Fig. 1b). This result is similar to that (maximum hydrogen content of 49.5 ± 1.4%) reported in a previous study (Akutsu et al., 2009). In summary, the optimal substrate concentration for hydrogen production was 20 g/L in terms of HRP and hydrogen content.

3.2. Effect of substrate concentration on broth characteristics

pH values in all four chambers showed a decreasing trend when substrate concentration was increased from 10 g/L to 25 g/L (Fig. 1c). pH values decreased with chamber number for the first three chambers, then increased in chamber #4 (Fig. 1c). Similar result was also found in literature (Zhang et al., 2015). During photo-fermentative hydrogen production process, glucose is first converted into volatile fatty acids (VFAs) which are then utilized by photo synthetic hydrogen producing microorganisms for hydrogen production (Zhang et al., 2017b). Higher substrate concentration could lead to more VFAs which accumulate at an early stage of hydrogen production, and consequently decrease the pH in the medium. The accumulated VFAs could be consumed at later stages of hydrogen production (e.g. in chamber #4), which caused the increase of pH.

ORP decreased with substrate concentration that was at 10–20 g/L, but increased when substrate concentration was further increased to 25 g/L (Fig. 1d). The lowest ORP of −534.29 ± 2.56 mV was obtained in chamber #3 at a substrate concentration of 20 g/L, while the highest ORP of −404.14 ± 6.52 mV was obtained in chamber #1 at a substrate concentration of 10 g/L (Fig. 1d). The results showed that BPHR was in a favorable condition of bio-hydrogen production by photo-fermentation.

Similar to HPR, biomass concentration increased and then decreased with substrate concentration or chamber number (Fig. 1e). The highest biomass concentration of 2.63 ± 0.19 g VSS/L was obtained at a substrate concentration of 20 g/L in chamber #3, and the lowest biomass concentration of 2.20 ± 0.02 g VSS/L was obtained at a substrate concentration of 10 g/L in chamber #1 (Fig. 1e). It has been known that biomass has a positive correlation with HPR, which is the reason that why the effect of substrate concentration on biomass is similar to that on HPR (Fig. 1a and e).

Reducing sugar concentrations increased with substrate concentration (10–25 g/L), and decreased with chamber number (Fig. 1f). The highest reducing sugar concentration of 24.23 ± 0.03 g/L was obtained in chamber #1 at a substrate concentration of 25 g/L, and the lowest reducing sugar concentration of 0.37 ± 0.02 g/L in chamber #4 at a substrate concentration of 10 g/L (Fig. 1f). Low substrate concentration (e.g. 10 g/L) obtained lower residual sugar concentration

Table 3
Summary of one-way ANOVA results.

Parameters	Hydrogen production rate		Hydrogen content		pH		Oxidation-reduction potential		Biomass		Reducing sugar concentration	
Source of variation	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
#1	32.69	1.21E-08	8.82	4.05E-04	1243.78	1.87E-26	28.61	4.26E-08	301.33	3.56E-19	50062.58	1.12E-45
#2	72.62	3.50E-12	3.26	0.039	5315.113	5.35E-34	36.66	4.02E-09	4.15	0.017	15945.66	1.02E-39
#3	48.98	2.21E-10	8.06	6.92E-04	2423.808	6.46E-30	2.11	0.125	64.24	1.3E-11	6693.55	3.37E-35
#4	42.93	8.40E-10	15.04	1.02E-05	6805.942	2.76E-35	3.43	0.033	28.41	4.56E-08	525.42	5.18E-22

which is desirable in terms of feedstock utilization. However, the low substrate concentration is not optimal for hydrogen production. When substrate concentration was at 20 g/L, the reducing sugar concentration in the last chamber (#4) was 3.73 ± 0.66 g/L, i.e. most (> 81%) of the substrate had been utilized (Fig. 1f). Therefore, the optimal substrate concentration of 20 g/L is still acceptable when considering both hydrogen production and feedstock utilization.

3.3. Effect of OLR on HPR

At a substrate concentration of 10 g/L, when HRT decreased from 72 h to 24 h (i.e. OLR increased from 3.3 g/L/d to 10 g/L/d), HPR increased from 64.13 ± 4.58 mol/m^3/d to 104.7 ± 4.72 mol/m^3/d, with a decrease of hydrogen yield from 19.43 ± 1.39 mmol/g to 10.47 ± 0.47 mmol/g (Table 1). At a HRT of 24 h, when substrate concentration was increased from 10 g/L to 20 g/L (i.e. OLR increased from 10 g/L/d to 20 g/L/d), HPR increased from 104.7 ± 4.72 mol/m^3/d to 148.65 ± 4.19 mol/m^3/d, and hydrogen yield decreased from 10.47 ± 0.47 mmol/g to 7.43 ± 0.21 mmol/g. A HPR of 128.64 ± 4.17 mol/m^3/d and a hydrogen yield of 5.15 ± 0.17 mmol/g were obtained when the reactor was fed at a substrate concentration of 25 g/L and a HRT of 24 h (i.e. an OLR of 25 g/L/d). Zhang et al. (2015) studied the effect of HRT on bio-hydrogen production with photosynthetic bacteria HAU-M1 in a baffled photo-fermentative reactor. Similarly, an optimal HRT of 24 h was reported with a maximum HPR of 185.71 mol/m^3/d (Zhang et al., 2015). Besides, the results showed that OLR had a great influence on the continuous hydrogen production, and should be controlled properly for maximizing the HPR (Lin et al., 2011).

3.4. Effect of substrate concentration on kinetics of hydrogen production

The parameters of Modified Gompertz Model varied substantially for different substrate concentrations (Table 2). The highest cumulative hydrogen formation P of 1616.38 mol/m^3 and hydrogen production rate R_m of 166.13 mol/m^3 were obtained at a substrate concentration of 20 g/L. The lowest P of 1097.32 mol/m^3 and R_m of 117.91 mol/m^3 were both obtained at a substrate concentration of 10 g/L. When substrate concentration was increased to 25 g/L, λ was reduced to 1.89 d, suggesting that high substrate concentration could result in shorter lag-phase time. The coefficients of determination (R^2) were very close to 1 for different substrate concentrations, which means that the Modified Gompertz Model fits the experiment data well.

3.5. Analysis of variance

Variations in the HPR, hydrogen content, pH, ORP, biomass and reducing sugar concentration were analyzed by one-way ANOVA in order to examine the effect of substrate concentration on hydrogen production (Table 3). In general, ANOVA analysis revealed that substrate concentration had a significant impact on most of parameters in chamber #1–#4. P-values for the effect of substrate concentration on all parameters in chamber #1 are less than 0.001. Similarly, very low P-values (< 0.001) were also observed for parameters in other chambers, expect for hydrogen content (P = 0.039) and biomass (P = 0.017) in chamber #2, and ORP in chamber #3 (P = 0.125) and #4 (P = 0.033).

4. Conclusion

Continuous bio-hydrogen production with photosynthetic bacteria HAU-M1 was studied in a pilot-scale baffled photo-fermentative hydrogen production reactor. Substrate concentration showed significant effects on HPR, pH, ORP and residual sugar. The optimal substrate concentration for hydrogen production was 20 g/L in terms of HRP and hydrogen content. A maximum HPR of 148.65 ± 4.19 mol/m^3/d was obtained at an OLR of 20 g/L/d during continuous bio-hydrogen production. Hydrogen yield decreased with OLR, while HPR increased with an increasing OLR (3.3–20 g/L/d) and decreased with a further increase of OLR to 25 g/L/d.

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