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Effect of Substrate Concentration on Photo-Fermentation Bio-Hydrogen Production Process from Starch-Rich Agricultural Leftovers under Oscillation

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Abstract: China has plenty of starch-rich agricultural leftovers, which can be degraded and further utilized for biogas production. Potato, which has more and more cultivated areas, was taken as a substrate. The pH, OD_{540}, biogas yield, hydrogen yield, biogas production rate, and hydrogen production rate were determined to evaluate the effect of substrate concentration on the photo-fermentation bio-hydrogen production process under an oscillation condition. Results showed that the photo-fermentation period was extended to 264 h under oscillation, which was two times longer than the static condition. It was found that 8 g per 100 mL fermentation broth was the most suitable substrate concentration under oscillation, the cumulative hydrogen yield was 510 mL VS^{-1}, and the hydrogen content was 38.36%.

Keywords: photo-fermentation bio-hydrogen production; starch-rich agricultural leftover; substrate concentration; oscillation

1. Introduction

Currently, resources and environmental problems have attracted more and more attention due to the over-combustion of fossil fuels and the emission of greenhouse gases [1]. To solve these problems, the priority is to promote an energy revolution, encouraging innovation in new energy technologies and pursuing sustainable energy development [2]. Energy is the foundation of national development and the lifeline of the national economy. As a green and efficient renewable energy source, hydrogen has been extensively used by researchers worldwide, and the development of hydrogen is one of the attractive measures for solving environmental pollution and energy shortage [3]. Therefore, hydrogen has been considered the most promising and ideal alternative energy source for fossil fuels due to its high energy content (122 kJ/g) [4–7].

So far, the methods of hydrogen production mainly consist of coal gasification, pyrolysis of fossil fuels, biomass gasification, and biological hydrogen production. Previous studies on hydrogen production are mostly based on chemical methods [8]. Compared with traditional hydrogen production by chemical method, biological hydrogen production has obtained more attention because of its mild reaction conditions, low cost, and environmental friendliness [9].

Biological hydrogen production can be done by using photosynthetic bacteria through photosynthesis. There are lots of papers about dark fermentation using sugar/starch-rich substrates to produce hydrogen, but few papers using photo-fermentation. Photosynthetic bacteria (PSB) can produce hydrogen from organic wastes with a high substrate conversion rate [10]. The main organic

component of cassava is starch, which has great potential as a feedstock for hydrogen fermentation [11]. Potatoes are rich in starch, and if we use potatoes as effective energy plants, there will be a great number of biomass resources. China creates a vast amount of starch-rich agricultural leftovers such as potatoes, whose cultivation area is increasing. The world production of potatoes has reached 324.18 million tons, and China is the world's largest producer, with nearly 75 million tons [12]. As a potential substrate, the substrate concentration should be determined since it is a key factor in the photo-fermentation bio-hydrogen production process. Eker and Sarp studied the effect of initial concentration and biomass on hydrogen production from waste paper in a batch model and found the hydrogen yield decreased as initial sugar concentration increased [13]. The higher sugar concentration resulted in lower hydrogen yield due to the volatile fatty acids in the higher concentration products. Jiang et al. investigated the initial biomass concentration and initial pH of photo-fermentation hydrogen production from corn stalk pith; optimal biomass concentration and initial pH were found to be 0.18 g/L and 7, respectively [14].

Because starch-rich agricultural leftovers always have high substrate concentration, when agricultural leftovers are utilized as substrate, the solid phase substrate is divided into three layers: the upper layer floats on the surface, the bottom layer is sediment, and the middle layer is low suspended solid [15]. These situations have a negative influence on the light transmission and mass transfer inside the photo-bioreactor, especially with high substrate concentration. Oscillating is critical to improving the mass and heat transfer in the process of photo-fermentation bio-hydrogen production, as it can expose more processes of hydrogen production to light effectively. It can enhance the homogenization of fermentation liquid and prevent stratification, making the temperature of fermentation liquid more uniform, and finally increasing the hydrogen production [16–18]. Hence, it is better to adopt a mixing strategy to ensure uniform distribution of the substrate and enhance the mass transfer in the reaction.

The oscillating state is a significant abiotic factor. There is little research about the effect of oscillation on photo-fermentation bio-hydrogen production, especially when using starch-rich agricultural leftovers as substrate. Generally, with non-oscillation, the fermentation time of hydrogen production by photosynthetic organisms is 120 h. In this paper, the experiment shows that the fermentation time under the condition of oscillation can reach 264 h, two times longer than the non-oscillation condition. The oscillating table cultivation can increase the contact between the substrate and PSB. Meanwhile, oscillating is beneficial for the growth and metabolism of PSB. Finally, it can increase the hydrogen yield. Lindmark J et al. studied mixing intensity as an important abiotic factor, directly affecting the growth and metabolism of microorganisms on anaerobic digestion [19]. Oncel and Sabankay investigated the influence of mixing time on bio-hydrogen production, determining which mixing time provides the shortest transfer time [20]. Hence, in this study, with potato taken as a starch-rich agricultural leftover, diverse substrate concentrations were added to evaluate the influence on the photo-fermentation bio-hydrogen production process under an oscillating condition and to find the suitable substrate concentration.

2. Materials and Methods

2.1. Preparation of Substrate

As seen from Table 1, carbohydrate contents of several starchy crops are listed. Potato starch has an average carbohydrate content. Given that potatoes are being grown more and more widely, and its application is broadening, hence, in this paper, potato was selected as a representative of the starch-rich crops.

Table 1. Carbohydrate contents of several starchy crops.

Name	Corn	Soybean	Potato	Peanut
Carbohydrate content (g/100g)	22.8	18.7	17.2	13

Potatoes were collected from a local vegetable market (Zhengzhou, Henan, China). Potatoes were cleaned, skins removed, and cut into slices. Potato slices were air-dried and ground to pass 200-mesh sieve. The potato powder was kept in a sealed bag.

Diverse amounts of potato powder were added into each conical flask with settled concentration (2, 4, 6, 8, and 10 g per 100 mL), and 100 mL pH 6.0 citrate sodium citrate buffer was added into the conical flasks separately. Alpha-amylase was utilized to degrade the starch with the enzyme load of 0.1mL/g substrate at 80 °C for 15 min. Reducing sugars formed from starch enzymatic hydrolysis were adopted for the photo-fermentation bio-hydrogen production process. All tests were conducted in triplicate.

2.2. Microorganisms and Medium

Hydrogen-producing photosynthetic bacteria (PSB): PSB HAU-M1 was provided by Key Laboratory of New Materials and Facilities for Rural Renewable Energy, Ministry of Agriculture, China, which consists of *Rhodobacter sphaeroides*, *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, and *Rhodopseudomonas palustris* [21].

Growth medium and hydrogen production medium for the PSB HAU-M1 were listed in the previous study [18]. The pH of the cultivation medium was adjusted to 7 by using 50% (*w*/*w*) KOH solution. The total cultivation time was 48 h. The illumination intensity of 3000–4000 Lux was provided by tungsten lamps for the PSB's growth.

2.3. Procedures of Bio-Hydrogen Production Process

Bio-hydrogen production processes were conducted in the conical flasks containing amylase hydrolysate that mentioned above. The initial pH was adjusted to 7 by adding 50% (*w*/*w*) HCl and KOH (5 mol/L) solution. After neutralization titration, HAU-M1 in the logarithmic growth phase were added into the conical flasks with an inoculum size of 30% (*v*/*v*). The conical flasks were sealed with rubber stoppers, and airbags were connected to collect produced biogas. All experiments were settled in a constant-temperature incubator with a light intensity of 7000 Lux and a temperature of 30 °C. The oscillation frequency was set at 150 rpm. Samples were determined at a time interval of 12 h. All runs were conducted in triplicate to assure the data repeatability.

2.4. Analytical Methods

Volume of biogas was measured by the water-displacement method. The composition of the produced biogas was determined by using gas chromatography (6820GC-14B, Agilent, USA). Nitrogen at a flow rate of 45 mL/min was the carrier gas. The temperatures of the injector, detector, and column oven were 100, 80, and 150 °C, respectively. The solution pH was measured by a pH meter (PHS-3C, Shanghai, China) with a measurement range of 0.00~14.00 and a resolution of 0.01.

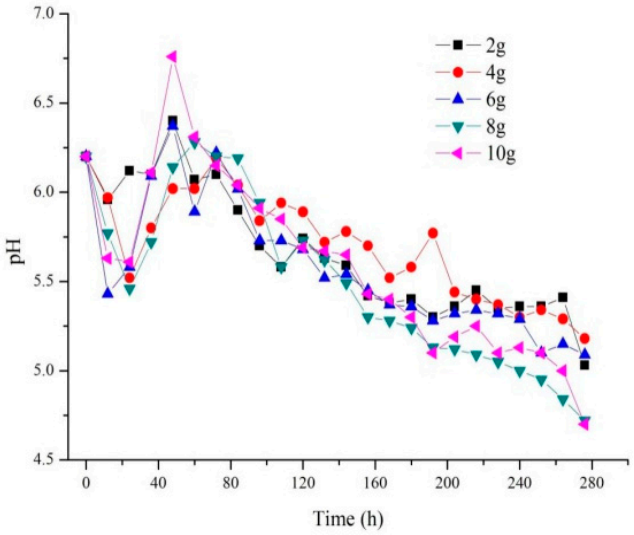
The oxidation-reduction potential (ORP) value of the fermented liquid was monitored by using a redox potentiometer (Az8851, Guangzhou, China). The reducing sugar concentration (RSC) was determined by the dinitrosalicylic acid (DNS) method [22,23] with a spectrophotometer at 540 nm (HP8453 Ultraviolet Spectrophotometer, Agilent, USA). Samples were centrifuged at 5000 rpm for 5 min, and the supernatant was used for RSC analysis. Volatile fatty acid (VFA) analyses were carried out by using gas chromatograph (6820GC-14B, Agilent, USA) equipped with a 30 × 0.320 mm × 1.0 μm capillary column (DB-FFAP) and a flame ionization detector (FID).

3. Results and Discussion

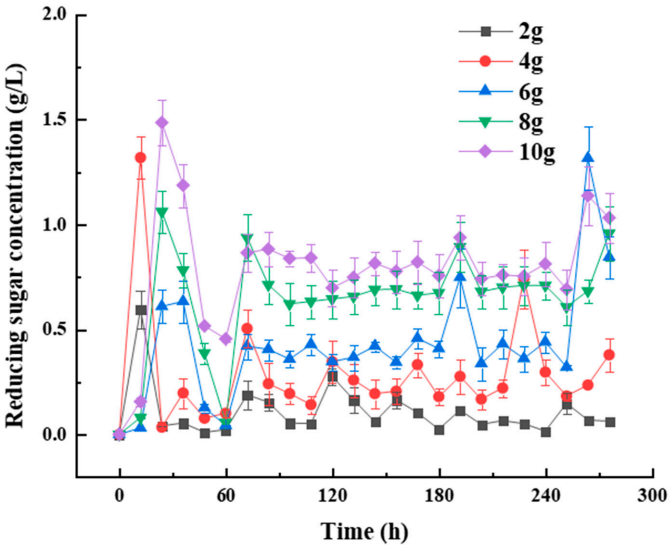
3.1. Evaluation of pH and RSC Changes under Diverse Substrate Concentrations

The effects of substrate concentration on RSC and pH are shown in Figure 1. From Figure 1a, the variation of pH showed similar trends. The pH in the fermented liquid remarkably decreased to a range of 5.4–5.9 during the period from 0 to 24 h. The reason might be that reducing sugars

were digested by PSB to support its growth. Hence, a lot of small-molecule acids were generated, which made the fermented liquid acidic. Moreover, the activity of PSB did not recover yet, so no acid was utilized to produce hydrogen. The high yield and low consumption of organic acids caused the accumulation of acids, leading to the decreasing of pH. After 24 h, the pH of fermented liquid began to increase quickly during the period from 24 to 48 h. The reasons for this phenomenon might be owed to two aspects: one reason was that the shaking table cultivation at a uniform speed increased the contact between the substrate and photosynthetic bacteria. PSB began to produce hydrogen because of the activity recovery and suitable acid environment [24], so organic acids were utilized to generate hydrogen. The other reason was that the acid environment resulted in further degradation of the unconverted substrate, which can be confirmed from the changes of RSC. Hence, the pH displayed a rising trend. After 48 h, the overall trend of pH was downward in fluctuation and maintained around 5 at the end of all tests. The reasons might be the results of the interaction between acid consumption for bio-hydrogen production and the accumulation from further substrate degradation.



(**a**)



(**b**)

Figure 1. Effect of substrate concentrations on pH (**a**) and reducing sugar concentration (**b**) during photo-fermentation using potato starch as a carbon source.

As seen from Figure 1b, substrate concentration shows a significant influence on RSC in the process of photo-fermentation bio-hydrogen production. The higher the substrate concentration is, the higher the RSC obtained. The RSC changed dramatically during the period of 0 to 84 h, no matter what the substrate concentration was. After 84 h, the RSC maintained at a certain range. The changes indicted the five main stages of RSC metabolism by PSB: adaptation and recovery phase of PSB (0–12 h), growth phase of PSB (12–36 h), acid inhibition of PSB (36–48 h), stable hydrogen production period of PSB (48–84 h), and the decay phase of PSB. The phenomenon might because that PSB cannot adapt to the new shaking environment immediately, which resulted in accumulation of RSC due to the lower metabolism of the PSB. After the PSB adapted to the new environment and the substrate was fully contacted with the PSB, the RSC was degraded fast because of the growth of PSB. Lower pH resulted in acid inhibition of PSB; hence, a slight RSC accumulation occurred around 36 h. Along with the utilization of organic acids, pH increased to a suitable level for the PSB. More RSC was utilized by PSB to produce hydrogen substrate for fermentation due to its activity recovery so the reducing sugar concentration decreased. When PSB turned into the decline phase, less reducing sugar was consumed. RSC remained stable in fluctuation because of the further degradation of the substrate and less consumption of RSC.

3.2. Variation of Bio-Hydrogen Yield under Diverse Substrate Concentrations

As shown in Figure 2, substrate concentration has a significant effect on the hydrogen yield. Cumulative hydrogen yield increased significantly along with the substrate concentration, increasing from 2 to 8 g, while lower cumulative hydrogen yield was obtained when the substrate concentration was higher than 8 g. The highest cumulative hydrogen yield of 510 mL H_2 VS was obtained at 8 g, followed by 433, 320, 303, and 180 mL H_2 VS at 10, 6, 4, and 2 g, respectively. Results demonstrated that the optimal substrate concentration was 8 g under the shaking table oscillation, which was higher than previous studies without shaking conditions [25]. The reason might be that the shaking condition improved the contact between the substrate and PSB, avoiding the deposition of the hydrogen-producing mixture. The disturbance provided by the shaking enhanced the mass transfer in the fermented liquid and promoted the escape of the biogas. Change trends of cumulative hydrogen yield with diverse substrate concentrations were roughly the same. Less hydrogen was generated within the first 48 h. The reason for this phenomenon might be that PSB needed time to get used to the new environment and to recover its activity. During the period from 48 to 132 h, the activity of the PSB was restored and began to metabolize vigorously. There were two peaks of hydrogen production that appeared in that period except for the group of 2-g substrate concentration. The first peak appeared at 36–60 h, and a second peak occurred at 90–120 h. The reason might be that the continuous degradation of the substrate under the acidic environment and supply of reducing sugars realized the longer growth and metabolism period of PSB. Then, PSB went through the process of rest and regeneration in the process of bio-hydrogen production under shaking conditions. After 132 h, the PSB cells entered the decay phase, and no more hydrogen was produced.

In summary, in a certain range of substrate concentration, the higher the substrate concentration is, the higher the hydrogen yield is. Substrate concentration of 8 g was found to be the optimal substrate concentration when taking potato starch as a carbon source for photo-fermentation bio-hydrogen production. The whole experiments last for 132 h until no further hydrogen was generated, which was two times longer than previous research, which took lignocellulosic agricultural waste as the carbon source and under static conditions [10].

3.3. Evaluation of the Byproducts of Photo-Fermentation Bio-Hydrogen Production from potato starch under Diverse Substrate Concentrations

As shown in Figure 3, several byproducts of the photo-fermentation bio-hydrogen production process from potato starch were obtained. The final pH of the fermented liquid under diverse substrate concentrations was inversely proportional to the byproducts. Butyric acid was the main soluble

byproduct followed by acetic acid, propionic acid, and pentanoic acid. Those volatile fatty acids (VFAs) led to the acid environment in the process of photo-fermentative bio-hydrogen production. Results showed that the concentration of VFAs increased along with the increase of substrate concentration, while VFA accumulated in different levels. The accumulation of acetic acid, propionic acid, and pentanoic acid were similar when the substrate concentration was lower than 8 g, which indicted that those organic matters can be effectively utilized by the PSB HAU-M1 to produce hydrogen. The vast majority of the byproduct was butyric acid, which meant butyric acid cannot be effectively adopted by PSB to produce hydrogen. Especially when the concentration of butyric acid was too high, the lower pH would result in the cease of the hydrogen-production process. Hence, the accumulation of butyric acid had a negative effect on the hydrogen yield. When the substrate concentration was higher than 8 g, all VFA concentrations were highest compared with the others. The phenomenon might illustrate that when substrate concentration is too high, the substrate cannot be effectively used, which will result in the waste of the substrate.

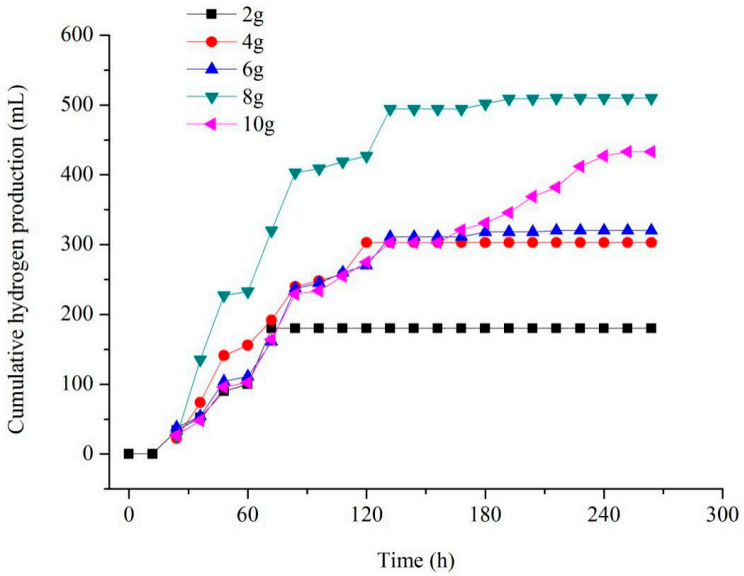


Figure 2. Effect of different concentrations of substrate on cumulative hydrogen production during photo-fermentation using potato as a carbon source.

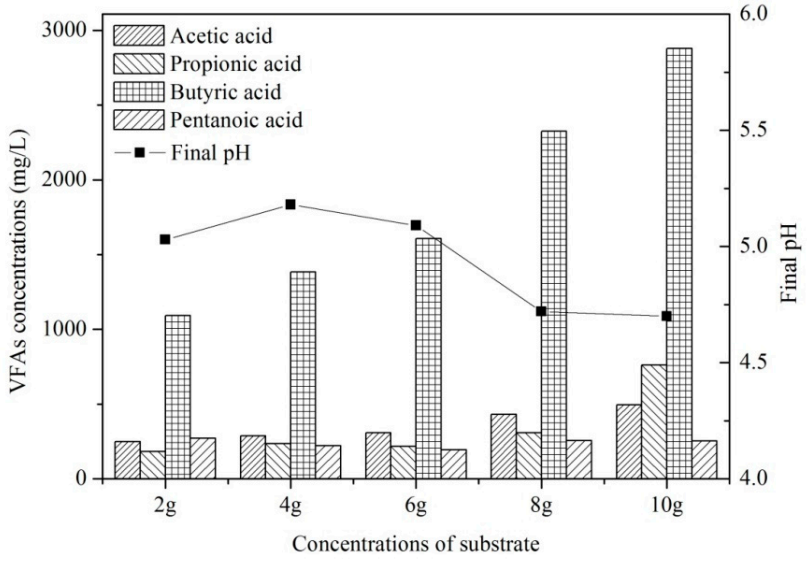


Figure 3. Effect of diverse concentrations of substrate on VFA production during photo-fermentation using potato as a carbon source.

4. Conclusions

Through the investigation of the effect of substrate concentration on the photo-fermentation bio-hydrogen production process, the feasibility of bio-hydrogen produced from starch-rich agricultural leftovers was confirmed. Results showed that the varied substrate concentration had a significant impact on hydrogen production performance under the oscillation condition. Too high or too low substrate concentration showed negative effects. Substrate concentration of 8 g per 100 mL fermentation broth was found to be optimal under oscillation. The highest cumulative hydrogen yield was 510 mL VS^{-1}, and the hydrogen content was 38.36%. The acid environment inhibited PSB activity, so that the fermentation process did not proceed efficiently. Oscillation enhanced the mass transfer inside the fermented liquid. Hence, the optimal substrate concentration and the whole bio-hydrogen generation period were 2 times higher and longer than the static condition, respectively. The results indicate that the oscillation condition is a beneficial attempt at high substrate concentrations.

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