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RESEARCH ARTICLE

Optimization and modeling of biohydrogen production by mixed bacterial cultures from raw cassava starch

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Abstract The production of bio-hydrogen from raw cassava starch via a mixed-culture dark fermentation process was investigated. The production yield of H_2 was optimized by adjusting the substrate concentration and the microorganism mixture ratio. A maximum H_2 yield of 1.72 mol H_2/mol glucose was obtained with a cassava starch concentration of 10 g/L to give a 90% utilization rate. The kinetics of the substrate utilization and of the generation of both hydrogen and volatile fatty acids were also investigated. The substrate utilization follows pseudo first order reaction kinetics, whereas the production of both H_2 and the VFAs correlate with the Gompertz equation. These results show that cassava is a good candidate for the production of biohydrogen.

Keywords cassava, biohydrogen, mixed cultures, kinetics

1 Introduction

Energy shortages and environmental pollution caused by the excessive use of fossil fuels are two of the most serious problems faced by humans in the 21st century, and therefore the development of renewable green energy is critical [1,2]. Hydrogen is considered to be one of the most promising alternatives to fossil fuels because of its high conversion capabilities and its non-polluting nature [3–5]. Moreover, it has a high energy content of 122 kJ/g, which is at least 2.75 times greater than that of any of the hydrocarbon fuels [6,7].

Currently, hydrogen is most commonly produced by chemical methods such as the steam reforming of hydrocarbons, the partial oxidation of fossil fuels and the gasification of biomass. However, these methods require high temperatures making the methods energy intensive and expensive [8]. Biological fermentation methods offer distinct advantages for hydrogen production such as mild operating conditions and specific conversions. However cost and low hydrogen yields and rates are major obstacles for bio-hydrogen production [8–10]. To overcome these problems, low cost substrates, more effective organisms, mixed cultures and optimizing environmental conditions have all been investigated [8].

Many studies have been carried out using single cultures; however, when complex substrates are used, mixed cultures are advantageous because of their robustness and metabolic flexibility. In addition, the presence of different microorganisms generally improves substrate utilization and consequently hydrogen production [11]. Many studies have shown that mixed cultures enhance hydrogen yields [12–14]. Argun and Kargi investigated bio-hydrogen production from waste ground wheat starch, and the highest hydrogen yield of 0.6 mol H_2/mol glucose was obtained using a mixture of *Clostridium beijerinkii* (DSMZ-791) and *Rhodobacter sphaeroides*-RV [15]. Bao et al. used a mixed culture to ferment hydrogen from corn starch [16]. This mixed culture significantly enhanced the biohydrogen production yield giving a H_2 yield of 1.04 mol H_2/mol glucose. This was twice the yield obtained with a single culture.

Several studies have investigated the feasibility of hydrogen production from simple sugars such as glucose [17], sucrose [18], xylose [19] or starches [20]. However, these substrates are food-sources and expensive. Thus they are not viable for the large-scale economic production of hydrogen.

Cassava, as a starch-containing, non-food crop in China [21], accounts for around 35% of China's total bioethanol production capacity [22]. In China, around 440000 ha of cassava are cultivated annually which yields about 9110000 tons of roots, most of which are used for the production of starch [23,24]. There are many reports in the literature on the bio-conversion of cassava to bioethanol

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[25,26][1)]. However, few papers have addressed the fermentation of raw cassava for the production of biohydrogen.

Thus, the present study investigates the feasibility of producing biohydrogen by the mixed culture fermentation of raw cassava. The substrate concentration and microorganism mixture ratio were optimized. In addition, the reaction kinetics were analyzed.

2 Materials and methods

2.1 Microorganisms, mediums and experimental set-up

Two bacteria strains, A1 and B1, were first isolated from an anaerobic digestion sludge. Both strains A1 (*Bacillus cereus*) and B1 (*Brevumdimonas naejangsanensis*) are facultative anaerobic bacteria and are efficient hydrogen producers [27,28].

The seed medium consisted of 3 g/L of beef extract, 10 g/L peptone, and 5 g/L NaCl. The fermentation medium contained 2 g/L peptone, 5 g/L NaCl, 1 g/L KH_2PO_4 and 1 g/L K_2HPO_4, with different concentrations of raw cassava starch. All the chemical and biochemical reagents were purchased from Beijing Chemical Works (China). The bacteria were first grown in the seed medium for 4–5 d at 37 °C. The cultures were then transferred to the fermentation medium to produce the hydrogen. The initial biomass concentration was 0.05±0.01 g/L.

Bottles were filled with 0.8 L fermentation medium, and then different ratios of A1 and B1 were inoculated so that the total amount of inoculums was 0.2 L. The air in the head space of each bottle was flushed with nitrogen gas to provide anaerobic conditions. The reactor temperature was maintained at 35 °C, using a thermostatic bath (HHeS6, MAI KENUO, China). The partial pressure of hydrogen gas was continuously reduced by connecting vacuum collection bags with a maximum volume of 500 mL to the outlet of the reactor. Once the bag was full, it was replaced with a new collection bag.

2.2 Analytical methods

The total sugar concentration were determined by 3,5-dinitrosalicylic acid (DNS) method and the pH was monitored by using a pH meter with appropriate probe (MP511, SANXIN, China). Volatile fatty acids and alcohols were determined by using a gas chromatograph (GC-2014C, SHIMADZU, Japan).

Gas samples were periodically taken from the head space of the reactor. The volume of gas produced was determined using a water displacement method and he composition of that gas was determined using a gas chromatograph (GC-2014C, SHIMADZU, Japan), detailed in a previous work [29]. All reported data are the average of triplicate experiments. To simplify the graphical presentation of the results, error bars are not included in the figures, however all experimental errors are < 5%.

3 Results and discussion

3.1 Effect of substrate concentration on hydrogen production

Substrate concentration is one of the most important factors in bio-hydrogen production processes [30]. For an appropriate concentration range, the activity of hydrogen-producing bacteria usually increases with increasing substrate concentration. Concentrations that are not in the optimum range negatively impact activities [31–33]. Therefore, the effect of the initial cassava starch concentration on the hydrogen production with an A1/B1 ratio of 1:1 was tested. Cassava starch concentrations of 5, 10, 15 and 20 g/L were tested and Fig. 1 shows the cumulative H_2 production and the change in pH with time. The highest cumulative hydrogen production was obtained for substrate concentrations of 10 and 15 g/L, with the final pH of 3.7–3.9. However, when the substrate concentration increased to 20 g/L, the pH dropped continuously to ~3.0, which would inhibited the biohydrogen production.

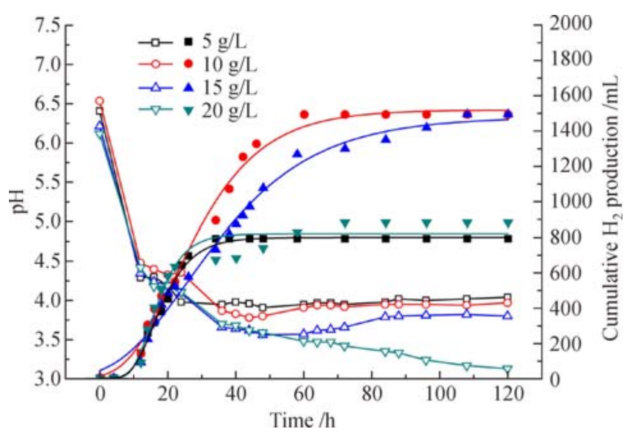

Fig. 1 Cumulative H_2 production and pH with time for different substrate concentrations for A1:B1 = 1:1. Filled symbols are cumulative H_2 production and hollow symbols are pH

To determine the optimal concentration of substrate for biohydrogen production, the hydrogen yields and compositions of different groups were further analyzed and summarized in Table 1. As the substrate concentration increased from 5 to 20 g/L, the hydrogen yield decreased

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Table 1 Hydrogen yields and concentration for different substrate concentrations

Substrate concentration /(g·L^{-1})	H_2 yield/(mol H_2·mol^{-1} glucose)	H_2 composition in biogas /%
5	1.44	61
10	1.39	56
15	0.91	53
20	0.41	53

from 1.44 to 0.41 mol H_2/mol glucose (A1:B1 = 1:1), and the H_2 composition decreased from 61% to 53%, which might be attributed to the inhibition of low pH as well as high concentration of substrate (Fig. 1). Although starch concentration 5 g/L showed the highest yield and hydrogen composition, the total amount of hydrogen was much lower than that of 10 g/L. All this considered, 10 g/L was chosen as the optimum substrate concentration.

Furthermore, the distribution of VFAs of different groups were also measured to determine to fermentation type and date are shown in Fig. 2. The products were ethanol, acetate and butyrate, while butyrate was the dominant product in all groups and accounted for 60%–75% of the total VFAs, indicating that the fermentation is a butyric acid-type [34]. The concentration of acetate remained relatively stable in all groups. The production of acetic acid and butyric acid during the hydrogen production process were responsible for the lower pH values that occurred in these reactions over time (Fig. 1) [35]. It should be noticed that ethanol concentration increased from 0.06 to 0.81 g/L with an increasing substrate concentration. In comparison, butyrate concentration increased from 1.28 to 2.73 g/L when substrate concentration continuously increased to 15 g/L, but it significantly decreased to 1.45 g/L when substrate concentration reached to 20 g/L. This further suggested that a high substrate concentration could shift the metabolic flux from butyrate to ethanol, thus avoiding or decreasing the inhibition of low pH.

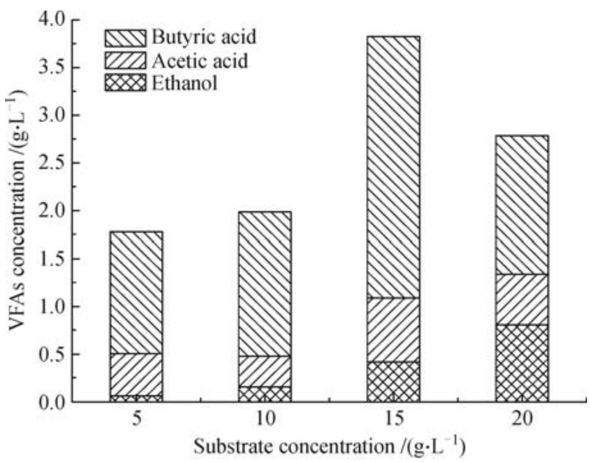

Fig. 2 Types of VFAs produced with different substrate concentrations (A1:B1 = 1:1)

3.2 Effect of the A1/B1 (V/V) ratio on hydrogen production

Both single and mixed cultures have been extensively studied for hydrogen production [36,37]. The mixed culture A1/B1 is more effective for producing hydrogen than single cultures because of the synergetic effect between the two microorganisms [16]. Argun and Kargi [38] have shown that the composition of the bacterial culture significantly affects the yield and rate of hydrogen production. So the cumulative hydrogen yields with different ratios of A1/B1 were tested and the results are shown in Fig. 3. The highest cumulative hydrogen production was obtained for A1/B1 = 1/0.5 and the lowest was with A1/B1 = 1/3. In the early phase of the gas production, the samples with a larger proportion of B1 (A1/B1 = 1/2 and 1/3) had higher hydrogen production rates than the other two samples. In the later phase of hydrogen production, the results are just the opposite. This is because strain A1 is responsible for starch hydrolysis [16]. After the initial soluble sugars are completely consumed, the availability of glucose is dependent on the efficiency of the hydrolysis of cassava starch. Therefore, the samples with a higher proportion of A1 exhibited higher hydrogen production rates. The maximum hydrogen yield of 1.72 mol H_2/mol glucose was obtained when the mixture ratio was A1/B1 = 1/0.5.

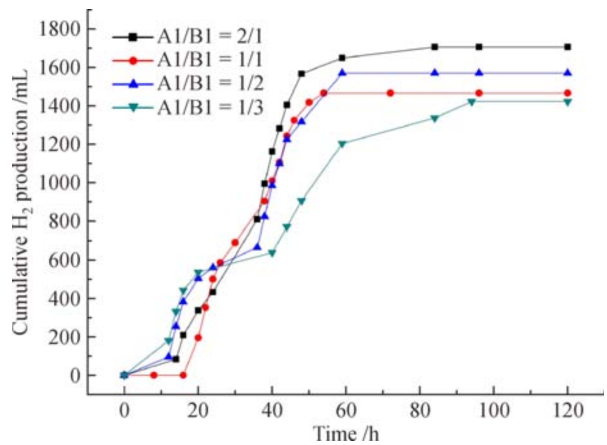

Fig. 3 Cumulative H_2 production with time with different mixture ratios

3.3 Substrate consumption and product formation kinetics

In order to determine the effect of the mixed bacteria ratio on hydrogen production from cassava starch and to obtain kinetic parameters for the fermentation process, the pseudo first order reaction equation and a Gompertz equation were used [7].

3.3.1 Substrate consumption kinetics

A first order kinetic dependence of the substrate utilization (C_0, C_t) can be described by the equation $\ln C_t - \ln C_0 = -kt$ where C_0 and C_t are substrate concentrations of time 0 and t, respecitvely, k is slope and t is time. The plots of time *versus* $\ln(C_t)$ are shown in Fig. 4 and the values for k and the correlation coefficients are listed in Table 2.

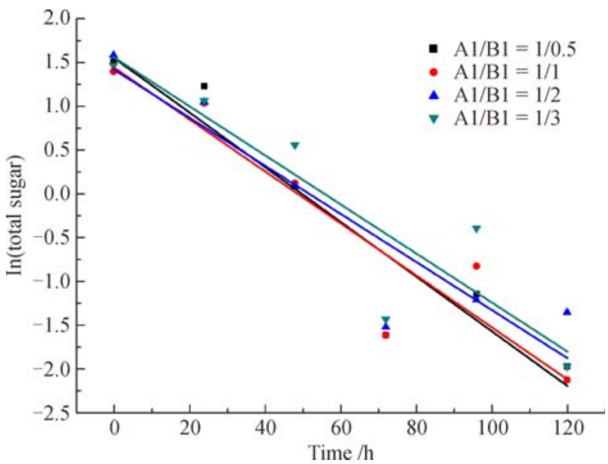

Fig. 4 First order kinetic fit of substrate utilization at different mixture ratios

Table 2 First order k values and correlation coefficients

Group	k	Correlation coefficient
A1/B1 = 1/0.5	0.0299	0.9809
A1/B1 = 1/1	0.0283	0.9597
A1/B1 = 1/2	0.0260	0.9658
A1/B1 = 1/3	0.0266	0.9089

From the k values in Table 2, the substrate consumption rate decreased as the A1 ratio decreased, indicating that the A1 strain does play a key-role in hydrolyzing the cassava starch and making it a more readily available for hydrogen production. As reported, hydrolysis is one of the key steps in biohydrogen production [39]. For A1/B1 = 1/3, the correlation coefficient was only 0.9089 which indicates that this reaction does not follow first order kinetics due to the limited substrate conversion rate.

3.3.2 Product formation kinetics

Figure 5 shows the Gompertz [40] fitted curves for the cumulative hydrogen productions with different mixture ratios. The correlation coefficient values are between 0.96 and 0.98. As the proportion of B1 increased, the correlation became lower. This may be because B1 cannot hydrolyze cassava starch and since the hydrolysis step is the rate-limiting step, as the reaction progressed there was not

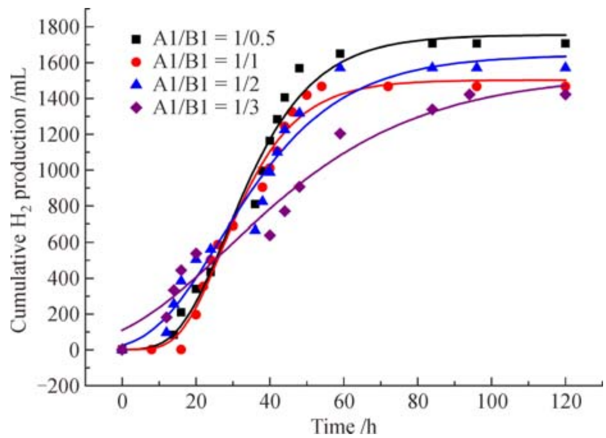

Fig. 5 Gompertz fitted curve for the cumulative H_2 production for different mixture ratios

enough glucose supply to maintain hydrogen production.

Figure 6 shows the Gompertz fitted curves for VFAs generation for the different mixture ratios. Clearly, the Gompertz equation can be well fitted to model the production of VFAs during the fermentation in Figs. 6(a–c). For A1/B1 = 1/3 in Fig. 6(d), the Gompertz equation can still be applied to model the ethanol and acetate production, while it cannot fitted well for butyrate production.

4 Conclusions

Cassava starch is a potential candidate for bio-hydrogen production. The maximum H_2 yield of 1.72 mol H_2/mol glucose was obtained when the substrate concentration was 10 g/L and the bacteria strain ratio was A1/B1 = 1/0.5. The substrate conversion follows pseudo first order reaction kinetics with a correlation coefficient of 0.98. The Gompertz equation provides a good fit for both the hydrogen production and VFA formation (such as butyrate and acetate) with (r^2 = 0.96–0.98). The A1 strain plays a key-role in hydrolyzing the cassava starch, making it a more readily available source for hydrogen production. Both bacteria strains contribute to the fermentation process in a symbiotic relationship.

Biohydrogen production has many advantages over petrochemical energy technologies such as less carbon dioxide emission and replacement of fossil fuels. However, cost and low hydrogen yields are still major challenges in

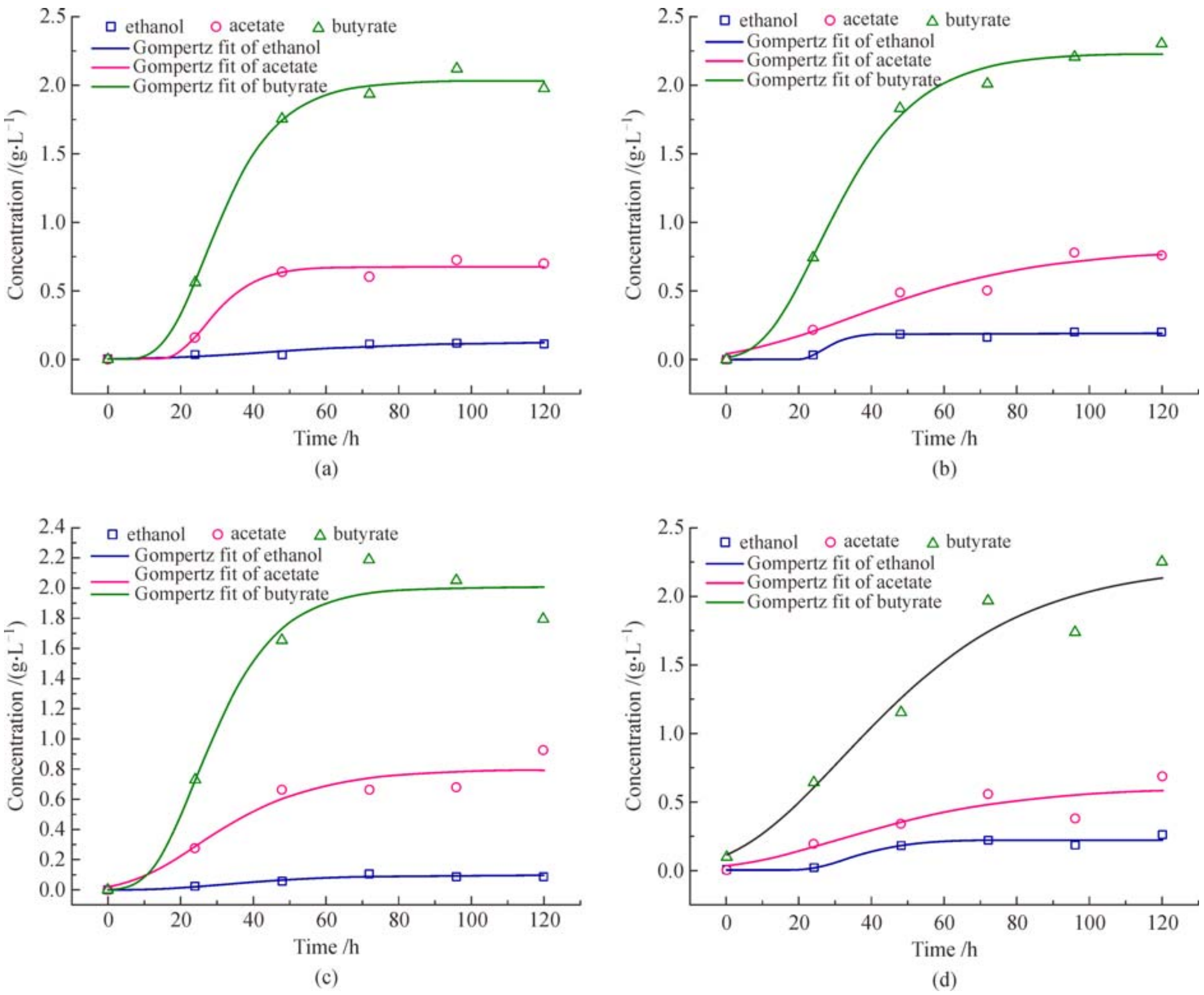

Fig. 6 Gompertz fit curve of VFAs generation at different mixture ratios. (a) A1/B1 = 1/0.5; (b) A1/B1 = 1/1; (c) A1/B1 = 1/2; (d) A1/B1 = 1/3

the development of biohydrogen production. Although cassava starch, a non-food product, has shown potential for biohydrogen production, further advancements are still needed before it will be economically feasible.

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