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Enhanced hydrogen production from corn starch wastewater as nitrogen source by mixed cultures

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ABSTRACT

The feasibility of hydrogen production from mixed corn starch wastewaters was investigated to recover energy from the treatment of corn starch wastewater. Effects on hydrogen production using peptone, NH_4Cl and a mixture of CSL and CGW as nitrogen sources were evaluated with supplement of GW. Experimental results showed that the optimum initial C/N ratio and total sugar concentration was 9.1 and 10.0 g/L respectively with the maximum hydrogen yield of 1.88 mol H_2/mol glucose consumed by mixed culture of *Bacillus* sp. A1 and *Brevumdimonas* sp. B1. A modified Gompertz equation is able to describe the batch production of hydrogen from wastewater. The maximum hydrogen production rate (Rm) of 165.89 mL/L/h calculated by modified Gompertz equation was obtained using CSL/CGW as nitrogen source, which was 20.8 times higher than that using peptone as nitrogen source. The results revealed an excellent potential for hydrogen production using a mixture of CSL/CGW as a nitrogen source.

1. Introduction

The energy consumption continues to rise with constantly increasing world economy and population. However, the ongoing energy crisis and environmental pollution have been given more attention. The development of renewable resource alternatives to fossil fuels is imminent. The bio-hydrogen, an alternative and eco-friendly fuel, has attracted an intensive interest because of its high energy content of 122 kJ/g, renewable, and non-polluting by-products generation during combustion (CO_2-neutral) [1–3].

In recent years, bio-hydrogen production, focusing upon bio-photolysis of water, photo and dark fermentation of organic matter, has gradually replaced hydrogen generated from fossil fuels by thermochemical processes, for it need less energy [4]. Especially, anaerobic fermentative hydrogen production is more practical than photo fermentation which requires expensive and large surface area photo bioreactors for photosynthetic bacteria to utilize solar energy [5]. Several issues have been investigated the feasibility for hydrogen production from food-competitive simple sugars such as glucose [6], sucrose [7], xylose [8] or starches [9] using anaerobic microorganism. However, it is not considered economical to commercially produce hydrogen due to high cost of these raw materials and the food crisis.

Many studies have been conducted using agricultural wastes (such as corn stalk, grass silage, wheat straw) [10–12] and wastewaters (such as food processing wastewater, chemical wastewater and domestic wastewater) [13–17] as substrates for hydrogen production. Nevertheless, a pretreatment of decomposing cellulose compounds into soluble sugar is required before agricultural residues converted biologically to hydrogen, leading to a high cost and energy consumption. High-carbohydrate wastewaters show a great potential for sustainable production of hydrogen owing to the advantages of high hydrogen production rate, less energy requirement, reduction in waste management costs and carbon dioxide emissions [18]. Besides, it provides dual environmental benefits in the direction of wastewater treatment along with sustainable bio-energy generation. A techno-economic evaluation of biohydrogen production from wastewater has proven its economic feasibility, and the results showed that the optimal size of the commercial hydrogen fermenter of wastewater was 52.51 m^3 according to the local price [19].

Corn starch wastewater (CSW) has a high chemical oxygen demand (COD) consisting of simple sugar, starch, protein and traces of vitamin and inorganic salts. Therefore, it is proposed as an optimum feedstock for offering necessary nutrients for microbial growth and metabolism. According to statistics, more than 20 million tons of corn starch wastewater (CSW) is produced by over 600 companies in China [20,21]. Using CSW as substrate for bio-

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hydrogen production is an effective and attractive way of wastewater treatment and sustainable bio-energy generation [22–26].

Several kinds of CSW are produced in different stages of corn starch processing, including corn steep liquor (CSL), corn gluten water (CGW) and glucose wastewater (GW). CSL is produced in the process of corn steeping and wet-milling, which contains about 2% proteins and traces of Fe^{2+}, Zn^{2+}, Mg^{2+} and biotin. CGW, rich in proteins, is obtained from the separation process of corn starch milk by centrifugation. Then, refined starch milk is partly processed into glucose and GW is generated during this process. Different from the two kinds of nitrogen-rich wastewater above, GW has a very high viscosity and high content of glucose, but is deficient in nitrogen source. Numerous studies have been reported to produce hydrogen from various kinds of wastewater as the carbon source. However, only a few papers address fermentative production using wastewater as nitrogen source. Besides, to the best of our knowledge, rare reports are available for hydrogen production using the mixture of three kinds of wastewater.

Thus, the present study investigates the feasibility of hydrogen production using a mixture of CSL and CGW as the main nitrogen source, supplemented with GW as the main carbon source. The bioconversion of wastewater to hydrogen by dark fermentation is a series of complex reactions [27]. Mixed culture could produce more various hydrolases which could utilize complex substrates present in wastewater than pure culture [28]. Therefore, two particular strains, *Bacillus* sp. A1 and *Brevumdimonas* sp. B1, were employed for hydrogen production in mixed culture system. Optimal C/N and substrate concentration to maximize H_2 production from wastewater was identified. Moreover, the effect on H_2 production using CSL/CGW as nitrogen source was evaluated.

2. Material and methods

2.1. Microorganism

The strains A1 and B1 were screened and isolated from sludge, obtained from an anaerobic digestion reactor. The 16S rDNA gene sequence analysis and biophysical and biochemical tests were carried out by China Center for Type Culture Collection (CCTCC). The results showed A1 and B1 were *Bacillus cereus* and *Brevundimonas naejangsanensis*, respectively [29,30]. Both strains A1 and B1 were facultative anaerobic, acidogenic H_2 producers. Strain A1 was capable of hydrolyzing starch by secreting amylase and producing hydrogen; while strain B1 was only able to ferment glucose into hydrogen with high efficiency. The mixed culture of A1 and B1 makes them be able to utilize complex substrate in wastewater directly and produce H_2 efficiently.

2.2. Experiment set up

Batch experiments were conducted in 1.25 L bottles with 1 L reaction volume. Each reactor was filed with 0.8 L fermentation medium, and then inoculated with 100 mL A1 and 100 mL B1 seed solution. The air in the head space of each bottle was flushed with nitrogen gas to provide anaerobic condition. The reactor temperature was maintained at 35 °C, using a thermostatic bath (HH–S6, MAI KENUO, China).

2.3. Seed medium and corn starch wastewater

The seed medium contains beef extract (3 g/L), peptone (10 g/L), and NaCl (5 g/L). The cultivated bacteria were used for inoculation after 72 h of incubation at 37 °C.

The Corn starch wastewater (CSW) was obtained from Shan Dong Rui Xing Biological and Chemical Company, China. CSW contains three kinds of wastewater: corn steep liquor (CSL), corn gluten water (CGW) and glucose wastewater (GW).The characteristics of each wastewater were examined and shown in Table 1. CSL and CGW were acid and nitrogen-rich wastewaters, 6.3 and 6.0 of C/N ratio respectively, only slightly different leading to the use of a mixed CSL/CGW ratio of 1/1, applied in this work. However, GW has a high content of glucose (800.8 g/L) with little nitrogen source. CSL and CGW were diluted with deionized water to reach the set of C/N ratio and initial substrate loading rate with supplemented GW. The fermentative medium was sterilized for 25 min at 116 °C to remove the mixed population of microorganism in the wastewater, which could either compete with biohydrogen producers or inhibit their growth. The initial pH was adjusted to 6.5 prior to inoculation.

2.4. Analysis methods

2.4.1. Analysis of gas and broth

Total biogas was sampled from the head space of the reactors, and the volume of total biogas produced was determined by water displacement method. The data were averages of triplicate experiments. Due to the very limited experimental error, error bars were not included in the figures to simplify the graphical presentation of the results. The proportion of hydrogen in the biogas was determined by gas chromatograph (GC-2014C,SHIMADZU, Japan), equipped with a thermal conductivity detector (TCD) and a TDX-01 packed (3 mm inside diameter) stainless steel column (2 m × 3 mm). Argon was used as the carrier gas with a flow rate of 30 mL/min. Temperatures of the column oven, injection, and detector were 160, 160, and 180 °C, respectively.

The cumulative hydrogen gas production was determined by using the following equation.

$$V_{H2,i} = V_{H2,i-1} + V_w C_{H2,i} + V_{R,i} C_{H2,i} - V_{R,i} - 1 C H_{2,i-1} \quad (1)$$

where $V_{H2,i}$ and $V_{H2,i-1}$ are the volumes of cumulative hydrogen gas production (mL) calculated after the *i*th and the previous measurement; V_w is the total gas volume measured by the water displacement method (mL); $C_{H2,i}$ is the concentration of H_2 in the total gas measured by the water displacement method (%); $V_{R,i}$ and $V_{R,i-1}$ are the volumes of the gas in the head space of the reactor for the ith and previous measurement (mL); $C_{H2,i}$ and $C_{H2,i-1}$ are the concentration of H_2 in the head space of the reactor for the ith and the previous measurement (%).

The total sugar and soluble sugar concentrations were determined by 3,5-Dinitrosalicylic Acid (DNS) Method, detailed in previous literature [31]. COD and NH_4^+ were measured using standard methods [32]. TOC and TN were measured using an Organic Element Analyzer (TOC-V, SHIMADZU, Japan). Metal ion concentrations were detected by an Atomic Absorption Spectroscopy (SpectrAA55-B, Varian, USA). Volatile fatty acids and alcohols were determined by using a gas chromatograph (GC-2014C, SHIMADZU, Japan), equipped with a FID using a second-order temperature-programmed method. The pH of the fermentation medium was monitored by using a pH meter with appropriate probe (MP511, SANXIN, China).

2.4.2. Date analysis

Based on the results obtained in the batch texts, the hydrogen production potential, maximum hydrogen production rates and lag-phase time were determined using modified Gompertz Eq. (2).

$$H(t) = P \cdot \exp\left\{ - \exp\left[\frac{R_m \cdot e}{P} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where *H(t)* represents the cumulative volume of hydrogen