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The photosynthetic hydrogen production performance of a newly isolated *Rhodobacter capsulatus* JL1 with various carbon sources

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ABSTRACT

Rhodobacter capsulatus is a photosynthetic bacterium with the ability to produce H_2 under photosynthetic condition. In this study, a new strain JL1 isolated from lake water was identified as *Rhodobacter capsulatus* by phylogenetic analysis of 16S ribosomal DNA (rDNA) sequence. Initial medium pH and L-glutamate (nitrogen source) concentration were optimized. At optimum pH 7.0 and 7 mmol/L L-glutamate, *R. capsulatus* JL1 could grow and produce hydrogen on the carbon sources of acetate, butyrate, glucose, xylose and fructose with the maximum substrate to H_2 conversion efficiencies of 67.5%, 26.6%, 46.1%, 46.2% and 46.6%, respectively. The maximum H_2 production rate, 124 ± 0.6 mL/(L·h), was obtained using 20 mmol-glucose/L as the carbon source. The addition of appropriate acetic acid to the tests with low concentration of glucose was able to improve the H_2 yield. Under the optimum operation parameters, the maximum H_2 yield and H_2 production rate of *R. capsulatus* JL1 from 16.4 g-corn straw/L-culture were 2966.5 ± 43.2 mL/L and 71.1 ± 4.5 mL/(L·h), while the chemical oxygen demand (COD) removal rate was up to 49.6%. This study indicates that *R. capsulatus* JL1 can serve as good candidate strain for H_2 production with organic waste water as well as effluent of dark-fermentation.

Introduction

In the past century, the consumption of traditional fossil fuels leads to global warming issue, and the reserves of the non-renewable energy are limited. For these reasons, developing renewable effective cleaning energy attracts more and more concerns, such as wind energy, solar power, and biofuels. Among these, sustainable hydrogen (H_2) is an ideal fuel for its high combustion value (122 kJ/g) and clean characteristic [1,2]. Among H_2 production processes, biological H_2 production ($BioH_2$) process is attractive due to its ambient pressure and temperature, low energy requirement, environmentally friendly and broad range of substrates. $BioH_2$ can be produced by dark-fermentative or photo-fermentative process. Dark-fermentation is characterized by its relatively high H_2

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production rate (HPR), but low substrate to H_2 conversion efficiency (CE) with amount of volatile fatty acids (VFAs) as the by-products. Meanwhile, photosynthetic bacteria (PSB) has been reported to degrade these VFAs into hydrogen, and therefore implementing simultaneous energy recovery and environmental treatment under light anaerobic condition [3,4].

However, the industrialization of photo-fermentative H_2 production appears to be hindered by its low HPR [5]. To solve this problem, researchers have done a lot of work on strain breeding [6–8], fermentative process [9–11], reactor designs [12] and so on to increase the H_2 production performance. The current strain selection and improvement methods commonly used are: isolating new PSB strain from nature and constructing mutants by genetic engineering. PSB grow in a wide variety of environmental conditions, such as *Rhodobacter sphaeroides* HY01 isolated from farmland [6], *Rhodovulum sulfidophilum* P5 isolated from marine habitats [13], *Rubriviuax gelatinosus* L31 isolated from sediments [14], and *R. faecalis* RLD-53 screened from indigenous pond sludge [15]. These PSB species utilize VFAs and carbohydrates as carbon sources for H_2 production, and many studies on photo-fermentative H_2 production were reported to optimize different parameters including pH value, carbon source, nitrogen carbon and light intensity to enhance the H_2 yield (HY) from various agricultural or industrial effluents. The maximum HY of *R. capsulatus* PK from wheat straw was 1.32 mol/mol-substrate [16]. *R. capsulatus* DSM155 produced 1.18 mol/mol-substrate from thermophilic fermentation effluent [17], 4.87 mol/mol-DS and 90 mL/(L·h) were obtained by *R. sphaeroides* HY01 from corn stalk hydrolysate [18]. HY of 6 mol-H_2/mol-glycerol was obtained by *Rhodopseudomonas palustris* from crude glycerol [19]. Thus, the performances vary among different carbon sources with different PSB species, only few strains are reported to directly utilize reducing sugars (RS) with relatively high H_2 yield up to now.

In this study, a photosynthetic bacteria *R. capsulatus* JL1 was enriched, identified, and characterized. Parameters, which influenced photo-fermentative H_2 production significantly, including pH, concentration of nitrogen and carbon sources, were optimized to enhance the H_2 production performance. The ability of *R. capsulatus* JL1 in directly using corn straw hydrolysate (CS) as carbon source to produce H_2 in batch tests was also studied.

Materials and methods

Enrichment and identification of photo-fermentative H_2 production strain

The PSB were isolated from Xingqing Park lake water in Xi'an, China. Enrichment medium MPYE [20] contains the following components (g/L): yeast extract 3.0, $MgSO_4 \cdot 7H_2O$ 0.394, $CaCl_2 \cdot 2H_2O$ 0.147 and peptone 3.0. The initial pH value was tuned to 7.0 using 1 mol/L HCl or 1 mol/L NaOH. 20 mL lake water was mixed with 100 mL medium in sterile serum bottle and incubated under photosynthetic anaerobic condition (light intensity around 4000 Lux at 30 ± 0.5 °C) for several days until the culture turned red. Streaking the diluted culture on MedA media plate [21] to obtain colonies. Repeated plate streaking was carried out until purified isolates were obtained. The purified colonies were selected for further studies.

The genomic DNA of a purified strain JL1 was extracted and the amplification of its 16S rDNA gene conducted with the universal primers (27F-AGA GTT TGA TCM TGG CTC AG; 1492R-GGT TAC CTT GTT ACG ACT T) as described in the literature [6]. The polymerase chain reaction (PCR) was performed with PCR mix kit (2 × pfu Master mix, GK8008, Shanghai Generay Biotech Co., Ltd). PCR product were examined by electrophoresis with agarose gel (1% (w/v)) including 0.5 mg/mL ethidium bromide, then purified and sent to sequence (Sangon Biotech Co., Ltd). The obtained partial 16S rDNA sequence data were submitted to GenBank database for blasting. Several sequences similar with the submission data were obtained, and these data were submitted to multiple sequence alignments using ClustalX 2.0.3 [22]. The aligned data were used to construct a phylogenetic tree using the neighbor-joining method (N-J) by MEGA 5.0 [23]. The 16S rDNA sequence reported in the present study was submitted to the nucleotide sequence database of NCBI (No. KX853135).

Corn straw with acidification pretreatment

The main ingredients of corn straw were determined as described by Zhang Y et al. [24]. Corn straw was smashed and passed through 60 meshes, then 25.0 g powder sample was added to glass flask filled with 250 mL 1.5% diluted HCl (solid: liquid = 1:10). The mixture reacted at 108 °C for 30 min before vacuum filtration to obtain the corn straw hydrolysate used for the subsequent study. The concentration of RS in corn straw hydrolysate was determined according to the di-nitro salicylic (DNS) method [25]. The COD values were measured using a multifunctional water quality detector (ET99732, Germany lovibond) according to the instructions [26]. Pretreatments such as pH adjustment and filtration sterilization were processed before feeding to the H_2 production process by JL1.

Batch hydrogen production process

Single colonies of JL1 from fresh agar plate were incubated aerobically in 50 mL screwed tube with 10 mL MedA liquid medium at 35 °C, shaking darkly at 150 rpm for two days in the incubator. Cell harvesting was carried out by Beckman Coulter X22R centrifuge (9000 rpm for 9 min) and washed twice with MedA medium to remove the residue and metabolites. The pellets were resuspended in MedA medium and diluted to OD_{660} = 1.0. The photo-fermentative H_2 production was carried out using 30-mL syringes as the reactors [27]. 10 mL culture was filled into each syringe containing 1 mL PSB inoculum. The incubator was controlled at 30 ± 0.5 °C and illumination intensity at around 4000 Lux provided by 10 W filament lamps. All of the tests were conducted in triplets.

The effect of initial pH values of 5.5, 6.0, 6.75, 7.0, 8.0 and 9.0 on the H_2 production of JL1 was studied, using 20 mmol/L acetate and 30 mmol/L butyrate as carbon sources, 7 mmol/L L-glutamate as nitrogen source. All processes were operated as mentioned above, and the cultures were adjusted to different pH values before photosynthetic incubation.

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