Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

Optimization of hydrogen production by *Rhodobacter sphaeroides* NMBL-01

*Anjana Pandey**, *Neha Srivastava*, *Pallavi Sinha*

Nanotechnology and Molecular Biology Lab, Centre of Biotechnology, University of Allahabad, Allahabad 211002, U.P., India

ARTICLE INFO

Article history:
Received 5 September 2011
Received in revised form
5 December 2011
Accepted 7 December 2011
Available online 30 December 2011

Keywords:
Purple non-sulfur bacteria
H_2 production
C/N ratio
Substrate conversion
R. sphaeroides NMBL-01

ABSTRACT

Water samples collected from the Northern region of India were used for isolation of anoxygenic photosynthetic (purple non-sulfur) bacterial isolate. The isolate was grown in modified Sistrom's media at pH 7.0 and characterized as new strain of *Rhodobacter sphaeroides* NMBL-01 by 16S rDNA sequencing analysis and used for current study. Effect of pH on growth kinetics of the bacteria showed maximum growth rate at pH 8.0 using malic acid as carbon source. The effect of C/N ratio (molar ratio of carbon to nitrogen) at 1.5, 5, 10, 13, 15, 20, 25, 30, 40, 50 and 80 using malate as carbon and glutamate/ammonium sulfate as nitrogen source on hydrogen production was investigated. The maximum hydrogen potential and hydrogen production rate were $2000 \pm 45\ cm^3\ m^{-3}$ and $11.8\ cm^3\ m^{-3}\ h^{-1}$, respectively, at C/N 13 using glutamate ($1.7\ mmol\ m^{-3}$) as nitrogen source and malate ($3\ g\ m^{-3}$) as carbon source with 66.5% malate conversion efficiency at initial medium pH 8.0. Further optimization of hydrogen production was performed keeping nitrogen source, glutamate ($1.7\ mmol\ m^{-3}$) constant with variable concentration of different carbon sources (succinate, butyrate, malate and acetate). The bacteria produced maximum hydrogen using malic acid at a concentration of $4\ g\ m^{-3}$ as source of carbon, i.e. $2755 \pm 32\ cm^3\ m^{-3}$ with 68.3% conversion efficiency followed by succinate ($1980 \pm 25\ cm^3\ m^{-3}$ with 58% conversion), butyrate ($1400 \pm 12\ cm^3\ m^{-3}$ with 14.1% conversion) and acetate ($650 \pm 12\ cm^3\ m^{-3}$ with 23.2% conversion).

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Biological routes for the hydrogen production are direct photolysis by blue green algae, indirect biophotolysis by cyanobacteria, dark fermentation, photofermentation and hybrid system using both dark fermentation and photofermentation [1–3]. Photosynthetic bacteria are one of the most favorable microorganisms for biological hydrogen production processes, in accordance with their high substrate conversion efficiencies and capabilities of the utilization of variable substrate sources [4]. Hydrogen production by photosynthetic bacteria (i.e. *Rhodobacter sphaeroides*) occurs under illumination in the presence of anaerobic atmosphere through the breakdown of organic substrates [5], by the process known as photofermentation. A range of organic substrates such as carbohydrates [6], lactate, malate, etc. are utilized by different species of phototrophic bacteria as electron donors during hydrogen production. It has been observed that the substrate specificity for hydrogen production varied with the species of the bacteria and the performance of photofermentation are highly dependent on the composition and the concentration of the substrate used for photofermentation [7,8]. The efficacy of *Rhodospirillum rubrum* and *Rhodobacter capsulatus* KU002 to utilize different organic substrates for hydrogen production was investigated by Melnicki et al. [9]. Purple non-sulfur bacteria such as *Rhodospirillum* sp. or *Rhodobacter* sp. can use

* *Corresponding author*. Tel.: +91 0532 2271977; fax: +91 0532 2461157.
E-mail address: apandey70@rediffmail.com (A. Pandey).
0961-9534/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biombioe.2011.12.005

a range of small organic acids and carbohydrates as substrates for H_2 [10,11].

In photosynthetic hydrogen production, H_2 production is driven by nitrogenase activity, which simultaneously converts molecular nitrogen (N_2) to ammonia (NH_3), thus, H_2-producing activity of photofermentative bacteria is strongly inhibited by an excessive amount of nitrogen [5]. Both the activity and gene expression of nitrogenase are inhibited by NH_4^+ [12–14]. Therefore, the hydrogen potential is greatly affected by nitrogen sources and their concentration [15,16]. The C/N ratio (molar ratio of carbon to nitrogen) in the culture medium is very important factor affecting hydrogen production. Hydrogen production decreased as the concentration of $(NH_4)_2SO_4$ was increased from 2 mmol m^{-3} to 7 mmol m^{-3} by *R. sphaeroides* [17]. Hydrogen yield was increased 2.5 folds by decreasing NH_4Cl concentration from 2 mmol m^{-3} to 1 mmol m^{-3}. Maximum hydrogen production rate at C/N 60 was 4.6 $cm^3\ m^{-3}\ h^{-1}$ by *R. sphaeroides* O.U. 001 [18].

In the present study, the optimization of hydrogen production by newly isolated *R. sphaeroides* NMBL-01 was investigated and the results are discussed.

2. Materials and methods

2.1. Microorganisms and their isolation

R. sphaeroides NMBL-01, photosynthetic non-purple sulfur (PNS) bacteria was isolated from water sample collected from 15 to 20 inch beneath the surface of ponds from Northern region of India in modified Sistrom's media (120 cm^3) containing 3 g m^{-3} malate and 1.2 g m^{-3} ammonium sulfate. The isolation was done in air-tight serum bottles (120 cm^3). Samples were kept under tungsten bulb (1.8 klux) at 30 °C ± 2 °C. The bacteria were purified by clonal selection method (serial dilution) followed by plating procedure and characterized by 16S rDNA sequencing using universal primers 27 F and 1492 R (Eurofins, Bangalore, India). The PNS isolate was identified as *R. sphaeroides* NMBL-01 (ID: 1467404, Accession BANKIT: JN256029) and used for optimization studies of molecular hydrogen production.

2.2. Media composition

One liter of modified Sistrom's media contains Macro solution, Trace elements and Vitamin solutions. Macro solution constitutes D-L malic acid 3 g m^{-3}, $MgSO_4 \cdot 7H_2O$ 0.2 g m^{-3}, $(NH_4)_2SO_4$ 1.2 g m^{-3}, $CaCl_2 \cdot 2H_2O$ 0.07 g m^{-3}, KH_2PO_4 1.2 g m^{-3}, K_2HPO_4 1.8 g m^{-3} and NaCl 0.4 g m^{-3}. Trace elements contain Fe (III) citrate 500 mg m^{-3}, $MnCl_2 \cdot 4H_2O$ 20 mg m^{-3}, $ZnCl_2$ 5 mg m^{-3}, LiCl 5 mg m^{-3}, KI 2.5 mg m^{-3}, $NiCl_2 \cdot 6H_2O$ 20 mg m^{-3}, KBr 2.5 mg m^{-3}, $CuSO_4 \cdot 5H_2O$ 0.15 mg m^{-3}, $Na_2MoO_4 \cdot 2H_2O$ 1 mg m^{-3}, $CoCl_2 \cdot 6H_2O$ 5 mg m^{-3}, $SnCl_2 \cdot 2H_2O$ 0.5 mg m^{-3}, $BaCl_2 \cdot 2H_2O$ 0.5 mg m^{-3}, H_3BO_3 10 mg m^{-3} and EDTA 20 mg m^{-3}. Vitamin solution includes nicotinic acid 200 mg m^{-3}, thiamin HCl 400 mg m^{-3}, nicotinamide 200 mg m^{-3} and biotin 8 mg m^{-3} which was filter sterilized. The pH of the media was adjusted to 8.0. Vitamin solution was added after autoclaving the media at 103.5 kPa (15 psi) pressure and 121 °C ± 2 °C temperature for 15 min.

2.3. Experimental condition

Batch experiments were performed in air-tight stoppered 120 cm^3 serum bottles containing 100 cm^3 of inoculated medium under still condition with intermittent shaking. After capping the bottle with a gas-tight rubber stopper and an aluminium cover, solution was carefully deaerated with argon and 5 cm^3 CO_2 introduced prior to illumination. The experimental setup was maintained at 32 °C ± 2 °C. The illumination was provided by 200 W tungsten lamp adjusted to provide a uniform light intensity of 1.8 klux at the surface of batch reactors. The hydrogen produced was collected in the 20 cm^3 disposable syringes. Initial pH of media was adjusted ranging from 5.0 to 10.0 in step of 1 unit using 1 mol m^{-3} NaOH to study the effect of pH on growth kinetics. The experiments with different concentrations of nitrogen sources (maintaining C/N ratio at 1.5, 5, 10, 13, 15, 20, 25, 30, 40, 50 and 80) i.e. ammonium sulfate and glutamate were carried out in a modified Sistrom's medium containing 22.4 mmol m^{-3} malate. Initial cell concentration and pH were set to 0.40 g dcw m^{-3} (48 h grown cells) and 8.0, respectively.

The experiment with different concentrations of acetate (a two carbon compound), butyrate, succinate and malate (a four carbon compound) was carried out in a modified Sistrom's medium containing 1.7 mmol m^{-3} glutamate as nitrogen source. All carbon sources were evaluated at concentrations of 1 g m^{-3}, 2 g m^{-3}, 3 g m^{-3}, 4 g m^{-3}, 5 g m^{-3} and 6 g m^{-3} keeping all other conditions same as mentioned above.

2.4. Analytical method

The bacterial cell concentration was measured by UV–Vis spectrophotometer (Labomed, USA) using the standard curve where one unit of optical density at 660 nm corresponded to 0.50 g dcw m^{-3}-medium. Hydrogen gas in the collected gas was analyzed with a gas chromatograph (Agilent 7890) equipped with a thermoconductivity detector and a capillary column (HP-PLOT/Q). Nitrogen gas served as carrier and pure hydrogen gas served as standard. The oven temperature was 90 °C, and the temperature of detector and injector was 90 °C and 80 °C, respectively. The analysis of organic acids was done using HPLC (Agilent 1200) fitted with C-18 column using 0.05 mol m^{-3} KH_2PO_4 buffer at pH 2.5 using phosphoric acid with a flow rate of 0.15 $cm^3\ min^{-1}$ for 10 min.

3. Results and discussion

The ability of *R. sphaeroides* NMBL-01 to grow at different pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) was tested. The comparison of initial and final pH of the medium indicated that media at pH 4.0 did not support the growth; in turn no change in the final pH was observed. At pH 6.0 there was significant growth rate. However, at pH 7.0, 8.0 and 9.0 good growth was observed. Maximum growth was achieved at pH 8.0 (Fig. 1). An initial pH of 7.0 has been found as the best value for cell growth by *R. sphaeroides* and *R. capsulatus* [19,20]. This study indicates that *R. sphaeroides* NMBL-01 may have potential for the treatment of waste water and waste biomass utilization

Download English Version:

https://daneshyari.com/en/article/677617

Download Persian Version:

https://daneshyari.com/article/677617

Daneshyari.com