Bioresource Technology 101 (2010) 5325–5329

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Enhanced bio-hydrogen production by the combination of dark- and photo-fermentation in batch culture

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ARTICLE INFO

Article history:
Received 7 December 2009
Received in revised form 3 February 2010
Accepted 4 February 2010
Available online 4 March 2010

Keywords:
Hydrogen production
Dark-fermentation
Photo-fermentation
Diluted ratio
Light/dark cycle

ABSTRACT

In this study, some key factors, for example, diluted ratio of effluents, the ratio of dark-photo bacteria, light intensity and light/dark cycle influencing hydrogen production by combining *Clostridium butyricum* and immobilized *Rhodopseudomonas faecalis* RLD-53 in batch culture, were investigated. Experimental results showed the photo-hydrogen yield decreased when increasing diluted ratio from 1:0.5 to 1:3, and it reached the maximum value of 4368 ml-H_2/l-effluents at the ratio of 1:0.5. When the ratio of dark-photo bacteria was at 1:2, the hydrogen yield reached highest value of 4.946 mol-H_2/mol-glucose and cumulative hydrogen volume was 5357 ml-H_2/l-culture during the combination process. When the light intensity was at 10.25 W/m^2, the hydrogen volume of photo-fermentation and the combination process reached maximum value of 4260 ml-H_2/l-effluents and 5892 ml-H_2/l-culture, respectively. During the combination process, maximum total hydrogen yield was 5.374 mol-H_2/mol-glucose. Meanwhile, hydrogen production under light/dark cycle was evaluated.

1. Introduction

It is well known that hydrogen is a clean energy carrier and it has been recognized as the most promising substitute for fossil fuels in future (Pan et al., 2008). Dark- and photo-fermentation were found to be important bio-hydrogen production technologies. However, the bottleneck of single dark-/photo-fermentation process is lower hydrogen yield due to the accumulation of short chain organic acids and expensive costs of pure organic acids, respectively. Photo-fermentation bacteria can utilize cheap substrate such as short chain organic acids from dark-fermentation as electron donors for hydrogen production depending on light energy. Therefore, the combination of dark- and photo-fermentation can resolve above problems, and it may increase hydrogen production and conversion efficiency of mono-substrate. Recently, several studies have already reported the overall hydrogen yield during the combination process was higher compared to a single dark- or photo-fermentation process (Tao et al., 2007; Chen et al., 2008; Liu et al., 2009) and the combination could be expected to reach as close to the theoretical maximum hydrogen yield of 12 mol-H_2/mol-glucose.

The combination system is mainly regulated by either dark- or photo-fermentation process. So, key factors influencing dark- or photo-fermentation can also affect hydrogen production of the combination system.

Some researches have studied the effect of certain parameters, for example, substrate concentration (Nath et al., 2005; Tao et al., 2007; Su et al., 2009; Argun et al., 2009; Liu et al., 2009), *C/N* ratio (Tao et al., 2007), initial pH (Nath et al., 2005; Tao et al., 2007; Nath and Das, 2009) and phosphate buffer concentration (Belokopytov et al., 2009; Liu et al., 2009) on hydrogen production by dark-fermentation in the combination system. A few studies reported about the control of photo-fermentation for hydrogen production during two-step process (Chen et al., 2008; Nath et al., 2005; Nath and Das, 2009). The combination system using dark- and photo-fermentative bacteria became an efficient bio-hydrogen production method. However, up to date, little information is known about the effect of dilution ratio of dark-fermentative effluent, the ratio of dark- and photo-fermentation bacteria and light/dark cycle on hydrogen production by combining dark- and photo-fermentation process.

Previous study demonstrated that *Rhodopseudomonas faecalis* RLD-53 can convert effluents from dark-fermentation by ethanol fermentation bacterium *Ethanoligenens harbinense* B49 with higher hydrogen yield (Liu et al., 2009).

Thus, the objective of this study was to determine the effect of diluted ratio of effluents from dark-fermentation, the ratio of dark- and photo-fermentation bacteria, light intensity and light/dark cycle on bio-hydrogen production by the combination of *Clostridium butyricum* and *R. faecalis* RLD-53. The results will shed light on large-scale studies for further bio-hydrogen production by combination system.

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doi:10.1016/j.biortech.2010.02.024

Table 1
The chemical composition of the mediums of dark- and photo-fermentation.

Dark-fermentation (1 l)	Photo-fermentation (1 l)	
Glucose 9 g		
Beef extract 2 g	CH_3COONa 1.0 g	$MgCl_2$ 0.2 g
K_2HPO_4 3.4 g	$C_4H_4Na_2O_4$ 1.0 g	$CaCl_2$ 0.08 g
KH_2PO_4 1.3 g	NH_4Cl 1.0 g	$NaHCO_3$ 1.0 g
$MgCl_2{\cdot}6H_2O$ 0.2 g	Beef extract 1 g	$FeSO_4{\cdot}7H_2O$ 12 mg
$CaCl_2$ 0.1 g	Peptone 0.5 g	EDTA–Na 0.1 g
NaCl 0.1 g	KH_2PO_4 0.5 g	L-Cysteine 0.5 g
L-Cysteine·HCl·H_2O 0.5 g	K_2HPO_4 0.5 g	Trace element 1 ml
Trace element 1 ml	NaCl 0.1 g	Vitamin 1 ml
Vitamin 1 ml		

2. Method

2.1. Bacteria strain and media

The dark-fermentative hydrogen producing bacterium, *C. butyricum*, was purchased from China General Microbiological Culture Collection Center, AS 1.209.

The previously isolated photo-fermentative bacterium *R. faecalis* RLD-53 used in this study (Ren et al., 2009).

The chemical composition of the medium for pre-culture of dark- and photo-fermentative bacterium is shown in Table 1, and pH of the medium adjusted to 7.0 by using 1 M HCl or NaOH solution. The media was sterilized at 121 °C for 15 min. The strain RLD-53 was pre-cultured at 35 °C for 24 h under light intensity of 2.93 W/m^2 with incandescent lamps (60 W) and argon was used to maintain anaerobic condition. Trace element consists of $FeCl_2{\cdot}4H_2O$ 1.8 g, $CoCl_2{\cdot}6H_2O$ 0.01 g, $CuCl_2{\cdot}2H_2O$ 0.01 g, $MnCl_2{\cdot}4H_2O$ 0.7 g, $ZnCl_2$ 0.1 g, H_3BO_3 0.5 g, $NaSeO_3{\cdot}5H_2O$ 0.01 g, ddH_2O 1.0 l. Vitamin consists of biotin 0.1 g, niacin 0.35 g, thiamine dichioride 0.3 g, p-aminobenzo acid 0.2 g, Ca-panthothenate 0.1 g, vitamin B_{12} 0.05 g, pyridoxolium hydrochloride 0.1 g, ddH_2O 1.0 l.

2.2. H_2 production experiments in batch culture reactors

The dark-fermentation hydrogen production experiment was carried out in triplicate using 100 ml serum bottles with a working volume of 80 ml, bottles were sealed by rubber plugs and top area of bottles filled with argon to maintain anaerobic conditions. The incubation temperature was controlled at 37 °C and the initial pH of the mediums was adjusted to 7.0, the bottles were shaken at 120 rpm. When the cells entered mid-exponential growth phase, the inoculants were added into serum bottles. The dark-fermentative effluents were centrifuged at 5000 rpm. The collected supernatant was properly diluted with pure water to desired initial fatty acids concentrations according to the proportion of 1:0.5, 1:1, 1:1.5, 1:2 and 1:3, and then the pH was adjusted to 7.0. The bottles with supernatant were autoclaved for 15 min. The pretreated supernatant was used for photo-hydrogen production. The bottles also were shaken at 120 rpm at constant temperature of 35 °C; the light intensity on the outside surface of the bottles was maintained at 5.86 W/m^2 by incandescent lamps (60 W). To determine the effect of light intensity on hydrogen production by the combination of dark- and photo-fermentation, light intensity of 1.46–13.2 W/m^2 was performed and adjusted by distance of the outside surface of the bottles from incandescent lamps.

The methods of cell immobilization adopted as described by Liu et al. (2009). After cultivation of *R. faecalis* RLD-53, the cells were collected by centrifugation at 5000 rpm/min. The immobilized bacterial cells were injected into the medium through a syringe. The average diameter of agar gel granule was about 3 mm and each agar gel granule contained approximately 0.211 mg cells dry weight. The collection of gases adopted drainage method. Gases were released continuously to outside of reactors. The hydrogen yield (mol-H_2/mol-glucose) during the whole combination process was calculated by dividing produced total hydrogen yield (mol) from dark- and photo-fermentation by the consumed glucose amount (mol).

2.3. Analytical method

Hydrogen gas analysis in evolved gas was performed using a gas chromatograph (GC) (Model SC-II, Shanghai Analysis Instrument Factory) equipped with a thermal conductivity detector and a 2-m stainless column packed with 5 Å molecular sieve. The operational temperatures at the injection port, the column oven and detector were 100, 60 and 105 °C, respectively. Argon was used as carrier gas at a flow rate of 70 ml/min. The volatile fatty acids in supernatant of culture broth were determined using a second GC (Model GC122, Shanghai Analysis Instrument Factory) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 mm fused-silica capillary column. The liquor samples were first centrifuged at 12,000 rpm for 5 min, and then acidified with hydrochloric acid and filtered through a 0.2-μm membrane before free acids were analyzed. Nitrogen was used as carrier gas. The light intensity (lux) was measured by using a digital luxmeter (TES1330A, Junkai Co.). Unit "lux" was converted into "W/m^2" according to the factors of 1 W/m^2 = 683 lux (Oh et al., 2004), wavelength was assumed at 555 nm in this study. Cell concentration was determined by an Amersham Pharmacia Biotech ultraspec 34300 UV/Vis spectrophotometer. Concentrations of glucose in the supernatants of culture broth were determined by the oxidase method.

3. Results and discussion

3.1. Effect of effluent's diluted ratio

To date, many researches have already indicated that the photo-fermentation bacteria could convert short chain organic acids into hydrogen gas with high conversion efficiency (Barbosa et al., 2001; Oh et al., 2004; Fang et al., 2005; Ren et al., 2009; Tao et al., 2008). In the present study, the main soluble metabolic products from dark-fermentation hydrogen production processed by *C. butyricum* were acetate and butyrate, which could be further converted into hydrogen gas by photo-fermentation bacteria *R. faecalis* RLD-53 (Ren et al., 2008). Glucose of 9 g/l and phosphate buffer of 50 mmol/l were used in this test; dark-fermentation produced more acetate, which may produce more hydrogen gas by further photo-fermentation (Ding et al., 2009; Ren et al., 2009). Acetate of 63.21 mmol/l and butyrate of 33.83 mmol/l were obtained in effluents from *C. butyricum*, and hydrogen yield was 1.63 mol-H_2/mol-glucose. Previous study showed the ratio of acetate to butyrate at 1:2 photo-hydrogen production can be achieved higher hydrogen yield (Ren et al., 2008). However, acetate of 40–50 mmol/l was the optimal concentration for hydrogen production by *R. faecalis* RLD-53 (Ren et al., 2009). So, to avoid the inhibitory effect of high substrate concentration on hydrogen production by photo-fermentation, the diluted ratio of dark-fermentative effluents was studied to determine suitable range of substrate concentration. The diluted ratio was 1:0.5, 1:1, 1:1.5, 1:2 and 1:3, respectively. The control was not diluted. The photo-hydrogen production by *R. faecalis* RLD-53 under different diluted ratio of dark-fermentative effluents is shown in Fig. 1.

When the diluted ratio was at 1:0.5, 1:1 and 1:1.5, hydrogen production capacity was higher than that of in the others ratio. Hydrogen yield decreased when increasing the diluted ratio from