INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2013) 1–8

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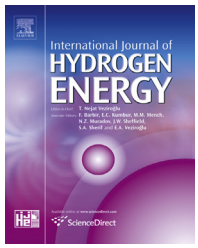

Dark fermentative hydrogen production with crude glycerol from biodiesel industry using indigenous hydrogen-producing bacteria

Yung-Chung Lo [a,d], Xue-Jiao Chen [a,b], Chi-Yu Huang [c], Ying-Jin Yuan [b], Jo-Shu Chang [a,d,e,*]

[a] Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[b] School of Chemical Engineering and Technology, Tianjin University, Tianjin, China
[c] Department of Environmental Science and Engineering, Tunghai University, Taichung, Taiwan
[d] Center for Biosciences and Biotechnology, National Cheng Kung University, Tainan, Taiwan
[e] Energy Technology and Strategy Center, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 31 March 2013
Received in revised form
13 May 2013
Accepted 15 May 2013
Available online xxx

Keywords:
Biohydrogen
Glycerol
Biodiesel
Clostridium butyricum
Clostridium pasteurianum
Klebsiella sp.

ABSTRACT

Glycerol is an inevitable by-product from biodiesel synthesis process and could be a promising feedstock for fermentative hydrogen production. In this study, the feasibility of using crude glycerol from biodiesel industry for biohydrogen production was evaluated using seven isolated hydrogen-producing bacterial strains (*Clostridium butyricum*, *Clostridium pasteurianum*, and *Klebsiella* sp.). Among the strains examined, *C. pasteurianum* CH4 exhibited the best biohydrogen-producing performance under the optimal conditions of: temperature, 35 °C; initial pH, 7.0; agitation rate, 200 rpm; glycerol concentration, 10 g/l. When using pure glycerol as carbon source for continuous hydrogen fermentation, the average H_2 production rate and H_2 yield were 103.1 ± 8.1 ml/h/l and 0.50 ± 0.02 mol H_2/mol glycerol, respectively. In contrast, when using crude glycerol as the carbon source, the H_2 production rate and H_2 yield was improved to 166.0 ± 8.7 ml/h/l and 0.77 ± 0.05 mol H_2/mol glycerol, respectively. This work demonstrated the high potential of using biodiesel by-product, glycerol, for cost-effective biohydrogen production.

1. Introduction

The combustion of fossil fuels gives serious negative effects on the environment mainly because of the emissions of greenhouse gases (e.g., CO, CO_2) and air pollutants (e.g., NOx, SOx and fly ash). For these reasons, many researchers have been searching new sustainable energy sources that could replace fossil fuels [1]. Biomass energy is a promising alternative to fossil fuels. It is produced by microbial conversion of biomass, such as agricultural, forestry, husbandry wastes or lignocellulosic materials to methane, hydrogen, ethanol, gasoline or biodiesel. In the past decades, hydrogen, the intermediate of anaerobic digestion process, has gained increasing attention since it is clean, efficient, and sustainable [2–4].

Biological hydrogen production is considered the most environmentally friendly way of producing energy [5–13].

* *Corresponding author*. Department of Chemical Engineering, National Cheng Kung University, No. 1 University Road, Tainan 701, Taiwan. Fax: +886 6 2357146, +886 6 2344496.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

0360-3199/$ – see front matter
http://dx.doi.org/10.1016/j.ijhydene.2013.05.083

Please cite this article in press as: Lo Y-C, et al., Dark fermentative hydrogen production with crude glycerol from biodiesel industry using indigenous hydrogen-producing bacteria, International Journal of Hydrogen Energy (2013), http://dx.doi.org/10.1016/j.ijhydene.2013.05.083

Biological H_2 production can be carried out by either photosynthetic microorganisms or fermentative anaerobes. Hydrogen fermentation process exhibits preference over phototrophic hydrogen production process in three aspects: (1) Simpler process on technical ground, (2) faster H_2 production rates, (3) easier conversion of refuse or waste products into hydrogen, thereby facilitating waste recycling [3,4,14]. Therefore, dark fermentative hydrogen production seems to be the most commercially viable biological hydrogen production process [9–13].

Biodiesel, currently used as fuel in conventional diesel engines, is composed of ethyl esters produced from vegetable oils or animal fats [15]. Biodiesel has drawn much attention recently due to several advantages over petroleum-based diesel, such as favorable energy balance, lower harmful emissions and nontoxic [16]. Biodiesel can be produced through transesterification catalyzed by chemical catalysts (e.g., NaOH or metal oxides) or by enzymes (e.g., lipase). Regardless of chemical or enzymatic biodiesel synthesis process, significant amount of glycerol will be generated as a main by-product [17,18], resulting in a significant decrease in market price of glycerol. Therefore, finding a feasible way for the reutilization of the massive amount of glycerol generated from biodiesel industry has become a hot issue [19–22]. Glycerol serves as an excellent substrate for fermentation industry, as it is often fermentatively converted to 1,3-propanediol for making polyesters [23,24]. Using this wasted glycerol for clean energy (e.g., H_2) production is also very attractive since this waste-to-energy concept is able to significantly enhance the overall economic benefits of bioenergy processes and human sustainability. However, conversion of glycerol into biohydrogen has been much less studied when compared with biohydrogen production from carbohydrate-based feedstocks [19–22,25]. The metabolisms for dark fermentative H_2 production from carbohydrate-based and glycerol-based feedstock with *Clostridium pasteurianum* were a bit different, as the major metabolites of *C. pasteurianum* when using sugars as substrate are butyrate, acetate, lactate and ethanol, whereas 1,3-propandiol, butanol, butyrate, acetate, lactate and ethanol become dominant metabolites when using glycerol as carbon source [26,27].

In this study, the H_2 fermentation performance from glycerol by using seven pure bacterial isolates was evaluated and compared. The H_2-producing strains examined (i.e., *Clostridium butyricum* CGS2 and CGS5, *C. pasteurianum* CH1, CH4, CH5, CH7 and *Klebsiella* sp. HE1) were isolated from high-rate H_2-producing bioreactors and were shown to possess high biohydrogen-producing efficiencies [9,28–30]. Optimal conditions for the bioH_2 yield and productivity of those H_2-fermentative bacteria were determined when using waste glycerol from biodiesel synthesis processes as the substrate.

2. Materials and methods

2.1. Bacterial strains and cultivation medium

The H_2-producing bacterial strains used in this study were isolated from effluent sludge of a continuous dark fermentation bioreactor capable of producing H_2 from synthetic wastewater containing sucrose (20 g COD/l) or xylose (20–40 g COD/l) as the sole carbon source as well as sufficient inorganic supplements [1,10,30]. The detailed procedures for the strain isolation and identification were described in our recent work [10]. The 16S rRNA gene sequence of *C. butyricum* CGS2, *C. butyricum* CGS5, *C. pasteurianum* CH1, *C. pasteurianum* CH4, *C. pasteurianum* CH5, *C. pasteurianum* CH7 and *Klebsiella* sp. HE1 used in this study has been deposited in the NCBI nucleotide database under the following accession number: AY540106 (strain CGS2), AY540109 (strain CGS5), EF140980 (strain CH1), EF140981 (strain CH4), EF140982 (strain CH5), EF140983 (strain CH7) and AY540111 (HE1). The pure strains were pre-cultured under anaerobic conditions [10] on the medium consisting of (g/l): glycerol, 15; $(NH_4)_2SO_4$, 3.0; Na_2HPO_4, 5.0; KH_2PO_4, 1.0; NaCl, 2.0; $MgSO_4$, 0.1; $Na_2S{\cdot}9H_2O$, 0.5. The H_2-producing medium used for assimilating glycerol (HMG medium) consisted of (g/l): glycerol, (adjustable); NH_4HCO_3, 6.72; $NaHCO_3$, 5.24; K_2HPO_4, 0.125; $MgCl_2{\cdot}6H_2O$, 0.1; $MnSO_4{\cdot}6H_2O$, 0.015; $FeSO_4{\cdot}7H_2O$, 0.025; $CuSO_4{\cdot}5H_2O$, 0.005; $CoCl_2{\cdot}5H_2O$, 1.25×10^{-4}; yeast extract, 1.0; casamino acid, 1.0; L-cystein·HCl, 0.5; sodium thioglycolate, 0.5.

2.2. Procedures of batch and continuous hydrogen fermentation

Batch fermentation was carried out by incubation in sealed serum bottles. The medium for dark H_2 fermentation was HMG medium as indicated in the preceding section. The initial glycerol concentration was adjusted to 1.0–40.0 g/l for the kinetic studies. The batch culture was conducted under different physical conditions: culture temperature, 30–50 °C; initial pH, 5.0–9.0; agitation rate, 0–200 rpm. During the course of fermentation, cell concentration, residual glycerol concentration, and production of biogas and soluble metabolites were monitored with respect to culture time.

Continuous bioH_2 production from glycerol feedstock was conducted with a continuous stirred tank bioreactor using *C. pasteurianum* CH4 as the bioH_2 producer. The operating conditions used to evaluate the feasibility of the continuous culture were: temperature, 35 °C; hydraulic retention time (HRT), 12 h; pH, uncontrolled; glycerol concentration (crude glycerol and pure glycerol), 10 g/l. During the course of fermentation, cell concentration, pH, residual glycerol concentration, and production of biogas and soluble metabolites were monitored with respect to culture time.

2.3. Data analysis

Time-course data of cumulative H_2 production were simulated by modified Gompertz equation (Eqn. (1)) [10,11,31–33] and the kinetic parameters were estimated via Sigma Plot 10.0 (SPSS Inc., Point Richmond, USA).

$$H = H_{max}\exp\left\{-\exp\left[\frac{R_{max,\,H_2} \times e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, H denotes cumulative H_2 production (ml), H_{max} denotes maximum cumulative H_2 production (ml), R_{max} denotes maximum H_2 production rate (ml/h), t denotes culture time (h), and λ denotes the lag time required for the onset of H_2 evolution (h).

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