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Enhancement of Biohydrogen Yield by Co-digestion of Waste Glycerol and Glucose

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Abstract

Biological hydrogen production from glycerol waste with glucose as co-substrate was tested in batch reactor at the varying mass ratios of glycerol and glucose. All ratios have equivalent extent of total chemical oxygen demand of 9.13 g L⁻¹.COD. Fermentation was setup in 0.5 L glass bottle, 37° C, and pH 6. Liquid and gas samples were collected to analyze concentrations of volatile fatty acids and glycerol; and gas compositions. The results showed that glycerol/glucose ratio plays an important role on the H₂ yield. Maximum H₂ yield was obtained at the glycerol/glucose ratio of 75:1. Acetate and butyrate were the main fermentative end products. This work demonstrated glucose can be used as a co-substrate to promote the H₂ yield in the fermentation of waste glycerol.

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1. Introduction

The rise of energy demand results the rapid growth of biodiesel industry. In the biodiesel production process, fatty acids in vegetable/animal oils are converted to methylester of fatty acids in the tranesterification reaction. Crude glycerol is the major byproduct of the biodiesel industry. In general, about 10 kg crude glycerol is produced for every 100 kg of biodiesel. The productivity increased rapidly in 2007, with a productivity of biodiesel worldwide, 450 million gallons per year, an increase from 2005,

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with a productivity of less than 100 million gallons per year [1], and is expected to reach 37 billion gallons in 2016 with an average annual growth of 42%, which equals 4 billion gallons of waste glycerol will be produced [2, 3]. The crude glycerol with 50 to 60 percent purity is required a filtering process, chemical addition, and vacuum distillation to achieve up to 95 to 99 percent before using in the food industry, cosmetics and pharmaceuticals. The rapid growth of biodiesel industry causes the oversupply of purified glycerol and its price drop. Therefore, purification of the crude glycerol appeared not economically, and thus it tended to become waste required treatment before releasing to the environment. Many studies reported that many high-value chemicals including hydrogen [4], citric acid [5], lactic acid [6], docosahexaenoic acid (DHA) [7], 1,3-propanediol [8], and ethanol [9] could be biologically produced from glycerol as a feedstock. The current work aimed to improve the hydrogen yield in the fermentation of the crude glycerol by using glucose as a co-substrate. Optimisation of glucose addition in the batch fermentation was performed.

2. Materials and Methods

2.1 Microbial seed and feedstock

Microbial seed was obtained from a full-scale up-flow anaerobic sludge blanket (UASB) reactor (Eiamburapa Co., Ltd. Sakaew, Thailand). Granular seeds with diameter >0.5 mm were washed with tap water twice. The fine granules, used in the hydrogen fermentation were boiled at 100°C for 30 min to deactivate methanogens, before sampled for total solid (TS) and total volatile solid (TVS) analyses. Glycerol waste with brownish colour and 63.9% purity was obtained from Trang Plam Oil Co., Ltd. Trang, Thailand.

2.2 Batch fermentation

The batch fermentation was setup in 0.5 L Scott Duran bottles with varying the glycerol and glucose ratios. All ratios have equivalent extent of total chemical oxygen demand of $9.13 \text{ g L}^{-1} \cdot \text{COD}$. Fermentation was setup in 0.5 L glass bottle. Feedstock and microbial seed were mixed in the batch reactor (375 mL of feedstock and 125 mL of microbial seed, which is equivalent to 7.91 g TS). The reactor content was adjusted to pH 6, purged with nitrogen gas to create an anaerobic condition, and incubated in 37°C incubator. Air and liquid samples were collected every 12 hr for gas composition, pH, volatile fatty acids (VFA), chemical oxygen demand (COD), glycerol and solids (TS and TVS) analyses. Air samples were collected by gastight syringe (Hamilton, USA) 500 μL for injection volume of gas composition and the volume of biogas was collected with measured by water displacement method in a 500 mL graded cylinder.

2.3 Analytical methods

Analyses of COD and TS were conducted according to the standard methods for the examination of water and wastewater [10]. Gas composition (H_2 , CH_4 , and CO_2) in the headspace of batch reactor was measured on a gas chromatograph (Shimadzu GC-2014, Japan) equipped with thermal conductivity detectors (TCD) fitted with stainless steel column packed with Unibeads C (80/100 mesh). Helium was used as a carrier gas. The temperatures of the injection port, column and detector were 120, 70 and 150 °C, respectively. Volatile fatty acids (VFAs) and ethanol were analyzed by a gas chromatograph (Shimadzu GC-2010, Japan) equipped with a flame ionization detector (FID) fitted with Stabilwax DA capillary column (Restek, USA). Hydrogen, air, nitrogen and helium were used as a carrier gas. The temperature of the injection port, column and detector were set up at 230, 80 and 250°C, respectively.

2.4 Kinetics of batch hydrogen production

Kinetics of hydrogen production was calculated from the cumulative hydrogen production versus time data of each batch experiment fitted with the modified Gompertz equation [11].

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

where $H(t)$ is the cumulative volume of hydrogen production (mL), t is time of fermentation (hr), H_m is the hydrogen production potential (mL), R_m is the maximum hydrogen production rate ($mL \text{ hr}^{-1}$), λ is lag phase (hr) and e is a constant (2.71828). The methane yield (Y) is calculated by dividing the production potential (H_m) at 24 hr of by the amount of glucose COD added.

3. Results and discussions

The cumulative H_2 fermentation profile data were fitted to the modified Gompertz equation ($R^2 > 0.99$). The hydrogen evolution had the S-shape trend and the kinetics data from the equation was statistically significant. Lag phase approximately 6 h prior to the evolution of H_2 was observed in the first stage fermentation. No CH_4 was detected in all cases. The H_2 content in the reactor headspace was in the range of 25-50%. The H_2 yield was increased after the increase of glycerol/glucose ratios (Fig 1). The highest H_2 yield of $1,990 \text{ mL g}^{-1} \text{ COD}$ glucose was achieved after 24 hr fermentation (glycerol/glucose of 75:1). The results showed that the glycerol/glucose ratios have strong effect on the H_2 production, and the optimum glycerol/glucose ratio was 75:1. pH dropped 0.5-1.0 pH unit after 12 hr incubation and became constant until the end of fermentation. Acetate and butyrate were main fermentative end products.

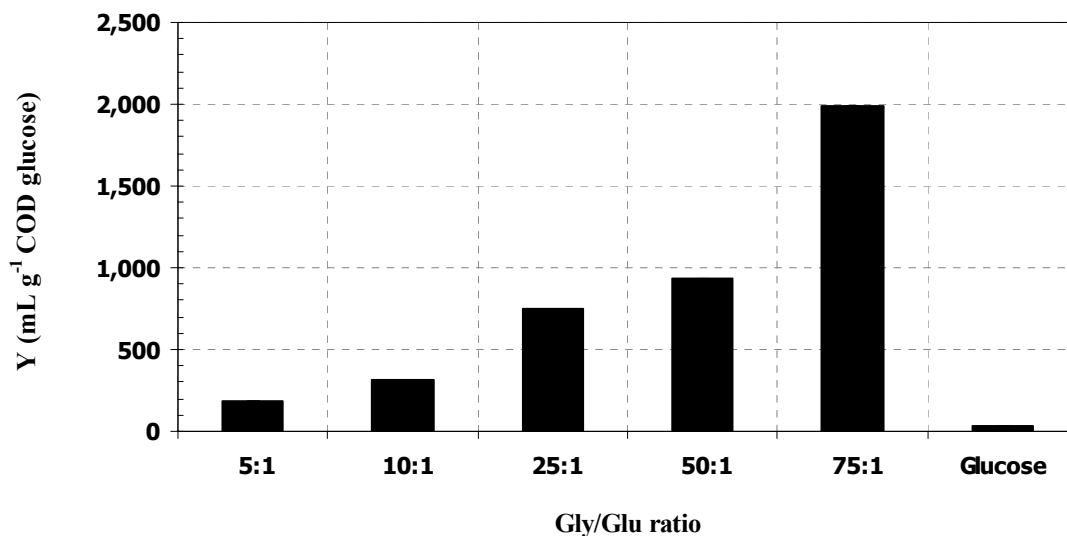


Fig. 1. Hydrogen yield at varying glycerol and glucose ratios. Histogram bars represents average value of duplicate experiment (n=2) and I-bar represents standard deviation.

4. Conclusion

The current work successful demonstrated that glucose can be used as a co-substrate to promote the H₂ yield in the fermentation of waste glycerol. The optimum H₂ yield was achieved at the glycerol/glucose ratio of 75:1.

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References

- [1] National Biodiesel Board. Biodiesel production; accessed on October, 2009. URL: <http://www.biodiesel.org/resources/reportsdatabase/default.aspx?AspxAutoDetectCookieSupport=1>.
- [2] Wang L, Du W, Liu DH, Li LL, Dai NM. Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system. *J. Mol. Catal. B-Enzyme* 2006;43:29-32.
- [3] Fan X, Burton R, Zhou Y. Glycerol (Byproduct of Biodiesel Production) as a Source for Fuels and Chemicals – Mini Review. *The Open Fuels & Energy Science Journal* 2010;3:17-22.
- [4] Dharmadi Y, Murarka A, Gonzalez R. Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering. *Biotechnol. Bioeng* 2006;94:821-9.
- [5] Rymowicz W, Rywińska A, Żarowska B, Juszczyk P. Citric acid production from raw glycerol by acetate mutants of *Yarrowia lipolytica*. *Chem. Pap* 2006;60:391-4.
- [6] Hong AA, Cheng KK, Peng F, Zhou S, Sun S, Liu CM, Liu DH. Strain isolation and optimization of process parameters for bioconversion of glycerol to lactic acid. *J. Chem. Technol. Biotechnol* 2009;84:1576-81.

- [7] Pyle DJ, Garcia RA, Wen Z. Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: effects of impurities on DHA production and algal biomass composition. *J. Agric. Food Chem* 2008;56:3933-9.
- [8] Mu Y, Teng H, Zhang DJ, Wang W, Xiu ZL. Microbial production of 1,3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel preparations. *Biotechnol. Lett* 2006;28:1755-9.
- [9] Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N. Hydrogen and ethanol production from glycerol-containing waste discharged after biodiesel manufacturing process. *J. Biosci. Bioeng* 2005;100:260-5.
- [10] American Public Health Association, American Water Works Association, Water Pollution Control Federation Standard methods for the examination of water and wastewater, 20th edn. Washington DC, USA, 1998
- [11] Nathao C, Sirisukpoca U, Pisutpaisal N. (2013) Production of hydrogen and methane by one and two stage fermentation of food waste, *International Journal of Hydrogen Energy* 2013; **38(35)**, 15764-15769..