Using Biomass of Starch-rich Transgenic Arabidopsis Vacuolar as Feedstock for Fermentative Hydrogen Production

Y.-C. Lo, C.-L. Cheng, C.-Y. Chen, L.-F. Huang, J.-S. Chang

This document appeared in

Detlef Stolten, Thomas Grube (Eds.):
18th World Hydrogen Energy Conference 2010 - WHEC 2010
Parallel Sessions Book 2: Hydrogen Production Technologies – Part 1
Proceedings of the WHEC, May 16.-21. 2010, Essen
Schriften des Forschungszentrums Jülich / Energy & Environment, Vol. 78-2
Institute of Energy Research - Fuel Cells (IEF-3)
Forschungszentrum Jülich GmbH, Zentralbibliothek, Verlag, 2010
ISBN: 978-3-89336-652-1

Using Biomass of Starch-rich Transgenic *Arabidopsis Vacuolar* as Feedstock for Fermentative Hydrogen Production

Yung-Chung Lo, **Chieh-Lun Cheng**, **Chun-Yen Chen**, Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
Li-Fen Huang, **Jo-Shu Chang**, Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Tao-yuan, Taiwan

Abstract

Cellulose is the major constitute of plant biomass and highly available in agricultural wastes and industrial effluents, thereby being a cost-effective feedstock for bioenergy production. However, most hydrogen producing bacteria (HPB) could not directly convert cellulosic materials (such as rice husk and rice straw) into hydrogen whereas most HPB could utilize sugar and starch for hydrogen production. In this work, we used an indigenous bacterial isolate *Clostridium butyricum* CGS2 as HPB, which could directly convert soluble starch into H_2 with a maximum H_2 production rate and a H_2 yield of 205.07 ml H_2/h/l and 6.46 mmol H_2/g starch, respectively. However, *C. butyricum* CGS2 could not ferment pure cellulosic materials such as carboxymethyl cellulose and xylan. Moreover, we found that *C. butyricum* CGS2 could utilize rich husk to produce H_2 at a rate of 13.19 ml H_2/h/l due to the starch content in rice husk (H_2 yield = 1.49 mmol H_2/g rice husk). In contrast, since lacking starch content, rice straw cannot be converted to H_2 by *C. butyricum* CGS2. The foregoing results suggest that increasing the starch content in the natural agricultural wastes may make them better feedstock for fermentative H_2 production. Hence, a genetically modified plant (*Arabidopsis vacuolar*) was constructed to enhance its starch concentration. The starch concentration of mutant plant S1 increased to 10.67 mg/fresh weight, which is four times higher than that of wild type plant. Using mutant plant S1 as carbon source, *C. butyricum* CGS2 was able to give a high cumulative H_2 production and H_2 production rate of 285.4 ml H_2/l and 43.6 ml/h/l, respectively. The cumulative H_2 production and H_2 production rate both increased when the concentration of the transgenic plant was increased. Therefore, this study successful demonstrated the feasibility of expressing starch on genetically-modified plants to create a more effective feedstock for dark H_2 fermentation.

1 Introduction

The increasing demand of petroleum and the decreasing global petroleum reserve triggered the crude oil price rise from 30 to 54–70 US$ per barrel since 1990 (OPEC., 2007 [1]). Combustion of fossil fuels has also caused global warming due to excessive emission of greenhouse gases. As a result, developing a new and more environmentally compatible alternative energy has become a hot issue in recent years. Among the potential energy alternatives being developed, hydrogen is recognized as the most promising energy carrier since it is clean, pollution-free, sustainable, and efficient (Das and Veziroglu, 2001 [2]).

Lignocellulosic resources, such as agricultural residues, paper wastes, wood chips, etc., are the most abundant organic substance in nature and considered to be promising and economically feasible feedstock for producing the new generation of biofuels (Kim et al., 2004 [3]). However, cellulosic materials are usually not readily fermentable by microorganisms for the yield of energy products, such as ethanol and hydrogen, thereby pretreatment or hydrolysis steps are often required (Xia et al., 2004 [4]). However, most hydrogen producing bacteria (HPB) could not directly convert cellulosic materials (such as rice husk and rice straw) into hydrogen whereas most HPB could utilize sugar and starch for hydrogen production. Dark fermentative hydrogen production from direct starch utilization was achieved using starch-fermenting bacteria, such as *Clostridium butyricum* (Yokoi et al., 1998 [5], Yokoi et al., 2001 [6], Yokoi et al., 2002 [7]). By using a mixed culture of *C. butyricum* and *Enterobacter aerogenes*, Yokoi, et al. reported a H_2 yield of 2.0–2.7 molH_2/mol glucose from single-stage H_2 production with sweet potato starch (Yokoi et al., 1998 [5], Yokoi et al., 2001 [6], Yokoi et al., 2002 [7]). Our recent work has identified several powerful H_2-producing bacterial isolates (mainly *Clostridial* species) from municipal sewage capable of producing H_2 from sugar very efficiently (Lo et al., 2008 [8]).

2 Materials and Methods

2.1 Microorganism and medium

Hydrogen-producing bacterial strain *Clostridium butyricum* CGS2 was isolated from effluent sludge of a continuous dark fermentation bioreactor able to produce H_2 from synthetic wastewater containing sucrose (20 g COD l^{-1}) or xylose (20-40 g COD l^{-1}) as the sole carbon source (Lo et al. 2008). The detailed procedures for strain isolation and identification were described in our recent work (Lo et al., 2008). The 16S rRNA gene sequence of *C. butyricum* CGS2 used in this study has been deposited in the NCBI nucleotide sequence database with an accession number of AY540106. The pure strain was pre-cultured under anaerobic conditions (Lo et al., 2008) on the medium consisted of (g l^{-1}): sucrose, 17.8; NH_4HCO_3, 6.72; $NaHCO_3$, 5.24; K_2HPO_4, 0.125; $MgCl_2.6H_2O$, 0.1; $MnSO_4.6H_2O$, 0.015; $FeSO_4.7H_2O$, 0.025; $CuSO_4.5H_2O$, 0.005; $CoCl_2.5H_2O$, 1.25×10^{-4}.

2.2 Fermentation medium and condition for bioH2 production

The medium for dark H_2 fermentation with the pure cultures was (g/l): starch or *Arabidopsis vacuolar*, (adjustable); NH_4HCO_3, 6.72; $NaHCO_3$, 5.24; K_2HPO_4, 0.125; $MgCl_2.6H_2O$, 0.1; $MnSO_4.6H_2O$, 0.015; $FeSO_4.7H_2O$, 0.025; $CuSO_4.5H_2O$, 0.005; $CoCl_2.5H_2O$, 1.25×10^{-4}. The culture temperature and pH was 37 °C and 7.5, respectively. Batch fermentation was carried out by static incubation. During the course of fermentation, cell concentration, pH, residual carbon substrate concentration, and production of biogas and soluble metabolites were monitored with respect to culture time.

3 Results and Discussion

3.1 Effect of soluble starch, rice husk and rice straw on the H_2 production performance

Using soluble starch (include starch), rice husk (include lignocellulose and starch) and rice straw (include lignocellulose) as carbon source to investigate dark-H_2 production performance of *Clostridium butyricum* CGS2. Fig. 1 shows the dark-H_2 production performance of *C. butyricum* CGS2 from soluble starch, rice husk and rice straw. Soluble starch and rice husk could be convert into hydrogen, the maximum H_2 production rate was 205.07 ml/h/l and 13.19 ml/h/l, respectively. Rice straw was not utilize. Model simulation analysis by modified Gompertz equation (Eqn. 1) shows that using soluble starch as carbon source resulted in maximum H_2 production rate (R_{max}) of 30.8 ml/h (Table 1). The lag time was similar (λ=9.7 h) for all carbon sources examined (Table 1).

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

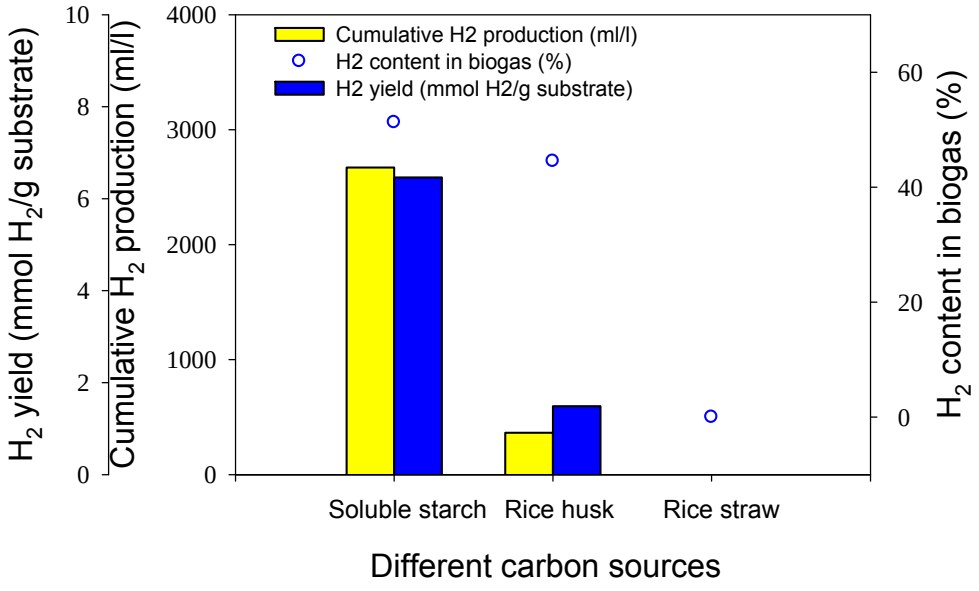

Figure 1: Dark-H_2 production performance of *C. butyricum* CGS2 at soluble starch, rice husk and rice straw.

3.2 Effect of *Arabidopsis vacuolar* on H_2 production performance

The foregoing results suggest that increasing the starch content in the natural agricultural wastes may make them better feedstock for fermentative H_2 production. Hence, a genetically modified plant (*Arabidopsis vacuolar*) was constructed to enhance its starch concentration. Figure 2 show the starch concentration was enhanced. The starch concentration of mutant

plant S1 increased to 10.67 mg/fresh weight, which is four times higher than that of wild type plant. Figure 3 show the H_2 performance of *C. butyricum* CGS2 from wild type plant and mutant S1 plant. Using mutant plant S1 as carbon source, *C. butyricum* CGS2 was able to give a high cumulative H_2 production and H_2 production rate of 285.4 ml H_2/l and 43.6 ml/h/l, respectively. The cumulative H_2 production and H_2 production rate both increased when the concentration of the transgenic plant was increased. Therefore, this study successful demonstrated the feasibility of expressing starch on genetically-modified plants to create a more effective feedstock for dark H_2 fermentation.

Table 1: Effect of soluble starch, rice husk and rice straw on H_2 production.

Carbon sources	H_2 content	Model simulation			
	(%)	Hmax(ml)	Rmax(ml/h)	λ(h)	R^2
Soluble starch	51	400.8	30.8	9.7	0.999
Rice husk	44	54.6	1.98	13	0.999
Rice straw	Cell non-growth				

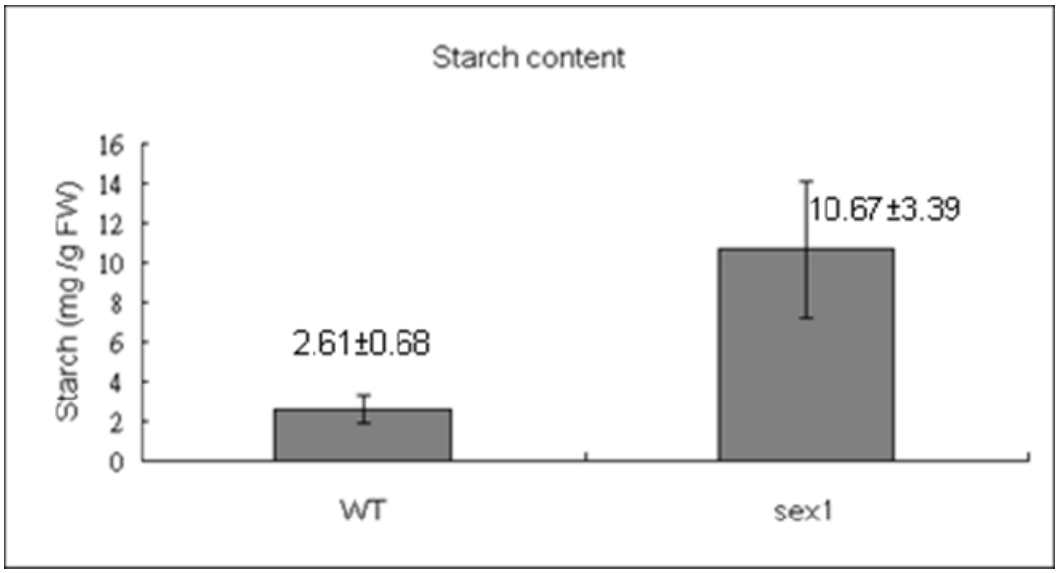

Figure 2: The starch concentration of wild-type plant and mutant plant S1.

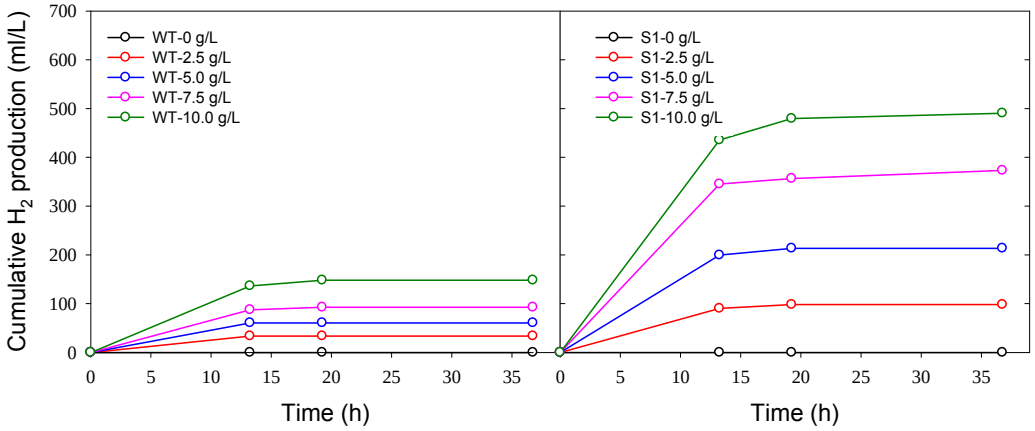

Figure 3: **H_2 performance of *C. butyricum* CGS2 from different wild type plant and mutant S1 plant concentration.**

References

[1] OPEC. Organization of the petroleum exporting countries,http://www.opec.orgi, 2007 [Retrieved March 22, 2007].

[2] Das D, Veziroglu TN. Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 2001;26(1):13–28.

[3] Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residules. Biomass Bioenergy, 26, 361-375.

[4] Xia, L.M., Sheng, X.L., 2004. High-yield cellulase production by *Trichoderma reesei* ZU-02 on corncob residues. Bioresour. Technol., 91, 259-262.

[5] Yokoi H, Tokushige T, Hirose J, Hayashi S, Takasaki Y. H_2 production from starch by a mixed culture of *Clostridium butyricum* and *Enterobacter aerogenes*. Biotechnol Lett 1998;20(2):143–7.

[6] Yokoi H, Saitsu A, Uchida H, Hirose J, Hayashi S, Takasaki Y. Microbial hydrogen production from sweet potato starch residue. J Biosci Bioeng 2001;91(1):58–63.

[7] Yokoi H, Maki R, Hirose J, Hayashi S. Microbial production of hydrogen from starch manufacturing wastes. Biomass Bioenerg 2002;22:389–95.

[8] Lo, Y.C., Chen, W.M., Hung, C.H., Chen, S.D., Chang, J.S., 2008. Dark H_2 fermentation from sucrose and xylose using H_2-producing indigenous bacteria: Feasibility and kinetic studies. Water Res. 42, 827-842.