

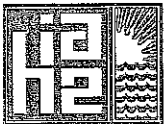
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APEC Advanced Biohydrogen Technology
Conference with Short-term Training Course

Asian Biohydrogen Symposium

Asia Bio-HyLinks Meeting



IAHE-Biohydrogen Division Business Meeting

■ **Date**

November 15th-20th, 2010

■ **Venue**

Feng Chia University, Taichung

■ **Organizers**

Green Energy Development Center, Feng Chia University
APEC Advanced Biohydrogen Technology Center
Asia Bio-HyLinks
IAHE

■ **Sponsors**

National Science Council
Bureau of Energy, Ministry of Economic Affairs

■ **Website**

<http://www.greenenergy.fcu.edu.tw>



Preface

The challenges and solutions to the development of technology for sustainable energy is a major issue that bridges the academic endeavors of the scientific community to the real problems of the global community. In this light, bio-hydrogen stands as a remarkably useful energy source for alleviating the pressures of limited resources as well as promoting the use of environmental friendly energy. Being produced from renewable resources such as water and organic wastes by biological means, biohydrogen represents a hopeful solution to some of the everyday problems that people face in terms of energy needs all around the world.

The first Asian Biohydrogen Symposium was organized and held in 2006 in Feng Chia University (FCU), Taiwan. Whereas the 2nd to 4th symposia were organized by Korea Institute of Energy Research (Korea), Harbin Institute of Technology (China) and Khon Kaen University (Thailand), respectively. Coming together a year the 5th symposium returns to Taiwan and organized by FCU and Asia Bio-HyLinks (an Asia-based bio-hydrogen association) from November 15th to 20th, 2010. The 2010 APEC Advanced Bio-Hydrogen Technology Conference with Short-term Training Course, Asia Bio-HyLinks (ABHL) and IAHE-Biohydrogen Division Business Meetings are also organized by Feng Chia University. These events were financially supported by the department of International Cooperation, National Science Council and Bureau of Energy, Ministry of Economic Affairs.

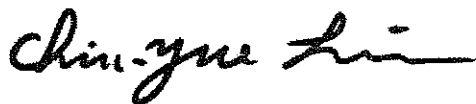
FCU will offer facilities such as laboratories to the students from the APEC member economies for short-term study and visit. The aims of this symposium were to facilitate the communication and exchange of ideas, evaluate current progress on early-stage findings and inform to identify promising research and development directions in the field of bio-hydrogen in Asian countries as well as all around the world.

These conferences focused on four major topics:

- Biohydrogen Application
- Biohydrogen Process Design
- Biohydrogen Economic Analysis
- Molecular Biology on Biohydrogen Production

In these conferences, a total of 100 papers from 20 countries were presented. In addition, we have invited 14 keynote and 17 invited lectures in the conference by some very well-respected scholars. High quality papers will be selected and published in special issues of the International Journal of Hydrogen Energy after a regular peer-reviewed process. We believe that through these conferences activities and the publication of this special issue would not only promote the advances on biohydrogen technology, but also strengthen the friendship and partnership of biohydrogen researchers in Asia and in the rest of the world.

On behalf of the organizing committee, I am grateful to all my colleagues and conference committee members who have helped to make these conferences a worthwhile and memorable event. I am sure APEC and ABHL Biohydrogen conferences will be an excellent opportunity for having constructive discussions and for participants to develop a fruitful cooperation amongst themselves.



Chiu-Yue Lin

Chairman,

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- Green Energy Development Center, Feng Chia University

Co-Organizers:

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- APEC Advanced Biohydrogen Technology Center
- International Association for Hydrogen Energy Taiwan Chapter

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Asia Bio-HyLinks



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Simultaneous Sacharification and Fermentation for Hydrogen Production from Cellulosic Fraction of Sugarcane Bagasse by Anaerobic Mixed Cultures

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Abstract

This research attempted to produce hydrogen from cellulosic fraction of sugarcane bagasse by anaerobic mixed cultures obtained from hydrogen fermenter. The cellulosic fraction was prepared by soaking sugarcane bagasse in 1% (w/v) NaOH aqueous solution for 30 min and then heated at 500 watt for 30 min in domestic microwave or direct heated by boiling for 60 min. The dilute-basic hydrolysate was discarded leaving the solid cellulosic fraction, which was further used to produce hydrogen by simultaneous saccharification and fermentation (SSF). The cellulase enzyme derived from *Trichoderma reesei* was added along with the inoculum from hydrogen fermenter to start the SSF process. The optimum condition for SSF of cellulosic fraction from microwave/alkali pretreated bagasse and direct heated pretreated bagasse was 20 g/l substrate, 0.05 g-cellulase/g-substrate, 35°C, initial pH 7.2 and 60 g/l substrate, 0.08 g-cellulase/g-substrate, 55°C, initial pH 6, respectively. Maximum hydrogen production of 1.932 l/l-substrate and 0.9980 l/l-substrate was obtained from SSF of cellulosic fraction from microwave/alkali pretreated bagasse and direct heated pretreated bagasse, respectively. Since hydrogen production obtained from SSF of cellulosic fraction from microwave/alkali pretreated bagasse was higher than direct heated pretreated bagasse but used a lower substrate concentration and less enzyme loading, therefore microwave/alkali pre-treatment was considered as the efficient method for preparing the cellulosic fraction from sugarcane bagasse.

Keywords: hydrogen production; sacchrification; fermentation; cellulosic fraction; sugarcane bagasse

1. Introduction

Current energy sources mainly come from fossil fuels. However, the reserves of fossil fuels have been decreasing resulting rise in oil and natural gas prices. In addition, the combustion of fossil fuels shows negative impacts on environment of emission of greenhouse gases (CO, CO₂ and CH₄), warrant the necessity to find clean, sustainable energy source that could substitute fossil fuels. Hydrogen is considered to be an ideal energy carrier for the future due to its clean and high energy yield (122kJ.g⁻¹) and it can be efficiently converted to electricity via fuel cell technology. Hydrogen can be divided into physical/chemical methods and biological methods. The physical/chemical methods cannot be considered as an alternative, nonpolluting energy source since traditional nonrenewable fossil fuels are used to produce the hydrogen gas. In contrast, complex substrates have been used in anaerobic fermentation to produce hydrogen gas routes which is more environmentally friendly [1].

Biological production of hydrogen using glucose is too expensive, the most promising method to produce hydrogen is the production of hydrogen from low cost substrates to support economic hydrogen production. Biohydrogen from renewable sources such as biomass appear to be economically feasible [2]. Biomass, which is widely available, is a renewable resource for alternative fuel product. An interesting option is the conversion of biomass to hydrogen. The major constitute of plant biomass is cellulose and highly available in agricultural wastes and industrial effluents such as food and pulp industry [3,4]. Biological hydrogen production from cellulose as a high value-added product, since cellulosic biomass is a valuable and most abundant renewable resource on earth. In addition, it might use non food crops of inedible waste products and does not divert food away from the animal or human food chain. However, pretreatment is an important tool for practical cellulosic bioconversion processes, because it had great potential for improving the efficiency and lowering of cost.

Thailand is an agricultural country and sugarcane is one of the important industrial crops. It can be cultivated in all parts of Thailand, except in Southern of Thailand, with the cultivation area of more than 960,000 ha; approximately 48 million ton of sugarcane is produced per year [5] Sugarcane bagasse (SCB) is a waste left after sugarcane extraction process. Since the bagasse accounted for approximately 25% of sugarcane

mass, about 12 million tons of SCB is produced annually. The most common use for SCB is the energy production by combustion [6] which can cause environmental problem from the emissions of CO₂. In addition, SCB can be used also to produce chemical compounds such as furfural or hydroxymethylfurfural [7], paper paste [8] or ethanol [9]. In addition, SCB is a very attractive raw material for the production of bio-hydrogen. The conversion of SCB to biohydrogen includes two processes: saccharification and fermentation. Due to its composition, three main fractions of SCB i.e., cellulose, hemicelluloses and lignin. Pretreatment technology is the most important to be considered in biohydrogen production, cellulose fraction in SCB containing mainly glucose and xylose [10] with small amount of arabinose [11]. Several processes have been developed for pretreatment of SCB including steam explosion, wet oxidation, liquid hot water process, acid hydrolysis and alkali pretreatment. Among many pretreatment methods of SCB, the alkali pretreatment is one of the most widely employed because alkali pretreatment removes lignin and various uronic acid substitutions on hemicelluloses that lower the accessibility of enzyme to the hemicelluloses and cellulose [12]. Although the alkali pretreatment of biomass has been extensively investigated, future improvements are strongly needed to develop a cheap, efficient and environmental friendly pretreatment technique for the biohydrogen production from SCB.

Several factors affect hydrogen production as well as the nature of the microbial flora [13]. Therefore, the optimization of fermentation conditions, particularly nutritional and environmental parameters are of primary importance for biohydrogen production process. In conventional multifactor experiments, optimization is usually carried out by varying a single factor while keeping all other factors fixed at a specific set of conditions. It is not only time-consuming, but also usually incapable of reaching the true optimum due to ignoring the interactions among variables. Taguchi design, which is a fractional factorial design using orthogonal array, allows the effects of many factors with two or more levels on a response, to be studied in a relatively small number of runs. In addition, the orthogonal array facilitates the analysis of the design. When used properly, Taguchi design may provide a powerful and efficient method to find an optimal combination of factor levels that may achieve optimum. Usually, with the aid of range analysis, analysis of variance or analysis of signal to noise ratio, the key factors having significant effects on a response can be identified and the best factor levels for a given process can be determined from the pre-determined factor level [14,15].

The present study investigated the SSF of two different pretreatment sugarcane bagasse to biohydrogen were optimized and the comparison was made between microwave/alkali pre-treatment and direct heated pretreatment to find out the suitable pre-treatment method for biohydrogen production from sugarcane bagasse.

2. Material and methods

2.1 Materials

Sugarcane bagasse was obtained from a local sugar industry (Thai Roong Ruang sugar industry, Phitsanulok, Thailand). SCB were air dried, milled and sieved through a 0.5 mm screen before stored at room temperature prior to use. The composition (w/v) of the SCB was 38.13% cellulose; 21.18% hemicelluloses; 8.29% lignin; 2.54% ash and 40.14% of other components.

The seed microorganism was taken from the effluent of a hydrogen reactor, while its volatile suspended solid (VSS) was 3.23 g/L. Prior to use, the seed sludge was first washed with 0.75% w/v of NaCl₂ and heated at 94 °C for 30 min to inhibit the bioactivity of hydrogen consumers and to harvest spore-forming anaerobic bacteria.

The cellulase enzyme was a commercial *Trichoderma reesei* cellulase (Sigma Chemical Co., Japan). Its carboxymethyl cellulose (CMC) saccharification activity was 15 IU/mg, measured as the initial rate of reducing sugars formation during hydrolysis of 0.5% carboxymethyl cellulose at pH 5.0 and 50 °C [16]. Its filter-paper activity was 0.54 FPU/mg, determined following the standard procedure recommended by the Commission on Biotechnology, IUPAC [17]. Its cellobiose activity was 0.18 CBU/mg, measured as the initial rate of hydrolysis of 2 mM cellobiose to glucose at pH 5.0 and 50 °C [18].

2.2 direct heated pretreatment

50 grams samples of mill bagasse were suspended in 200 ml of 1% NaOH aqueous solution and boiled in a 250 ml beaker for 60 min. The residues were collected and washed extensively with tap water until neutral pH. Then they were used as the substrate of the saccharification and fermentation to biohydrogen.

2.3 Microwave/alkali pre-treatment

50 grams samples of mill bagasse were suspended in 200 ml of 1% NaOH aqueous solution in a 250 ml beaker for 30 min and then heated in microwave. The beaker was positioned at the centre of a rotating circular glass plate in a domestic microwave oven (SANYO model EM-2450 MHZ) for microwave treatment. The

applied microwave power was 500 W for 30 min. The residues were collected and then washed extensively with tap water until neutral pH and used as the substrate of the saccharification and fermentation to biohydrogen.

2.4 Experimental design

A series of nine batch experiments with different combinations of selected factors viz., (i) substrate concentration, (ii) initial medium pH (iii) temperature and (iv) cellulase enzyme loading were investigated based on biohydrogen production. Two types of SCB pretreatment viz., direct heat pretreated or microwave/alkali pretreated were used as substrate in order to optimize the simultaneous saccharification and fermentation condition. The L9(3⁴) orthogonal table was chosen in this work. Factors and their levels of the orthogonal experiment are designed in Table 1.

2.5 Hydrogen production

Hydrogen production experiments were performed in 100 mL serum bottle with a working volume of 70 mL containing concentration of substrate ranged from 20 to 60 g/L, enzyme loading ranged from 0.02 to 0.08 mg/g [substrate]. Each bottle was supplemented with nutrients (Endo formulation; mg/dm³) following; 5240 NH₄HCO₃, 125 K₂HPO₄, 100 MgCl₂ · 6H₂O, 15 MnSO₄ · 6H₂O, 25 FeSO₄ · 7H₂O, 5 CuSO₄ · 5H₂O, 0.125 CoCl₂ · 5H₂O which was modified from Lin and Lay (2005) [13]. Each bottle was then filled to 70 mL with distilled water. Initial pH was adjusted ranged from 4.8 to 7.2 using either 1 N HCl or 1 N NaOH. Subsequently, the bottles were tightly sealed using rubber septa and aluminum cap. After replacement of the gas phase with argon to create anaerobic condition, the serum bottle was incubated at 35, 45 or 55°C and 150 rpm in the water bath shaker. All treatments were carried out in triplicate.

2.5 Analytical method

Biogas volume was determined by a gas tight syringe at room temperature (20°C) and pressure (760 mm Hg). The cumulative hydrogen gas production was determined by using the following equation [19].

$$V_{H_{2,i}} = V_{H_{2,i-1}} + V_w CH_{2,i} + V_{G,i} CH_{2,i} + V_{G,i-1} CH_{2,i-1} \quad (3)$$

Where $V_{H_{2,i}}$ and $V_{H_{2,i-1}}$ are the volumes of cumulative hydrogen (ml) calculated after the *i*th and the previous measurement; V_w is the total gas volume measured by a gas tight syringe (ml); $CH_{2,i}$ is the concentration of hydrogen gas in the total gas measured by a gas tight syringe (%); $V_{G,i}$ and $V_{G,i-1}$ are the volumes of the gas in the head space of the bottle for the *i*th and the previous measurement (ml); $CH_{2,i}$ and $CH_{2,i-1}$ are the percent hydrogen in headspace of the serum bottle for the *i*th and the previous measurement. The amount of released hydrogen gas in the head space of the bottle was measured independently and added up to determine cumulative hydrogen formation for every period of sampling.

The components of biogas were analyzed using a gas chromatograph (GC) (Model 8APT, SHIMADZU, Japan) equipped with a thermal conductivity detector (TCD). A 3m x 3mm stainless-steel column packed with 60/80 mesh activated charcoal (Model 8APF, SHIMADZU, Japan) was used to analyze the percentage of hydrogen, nitrogen, methane and carbon dioxide in the biogas produced. Argon was used as the carrier gas at a flow rate of 70 mL/min. The temperatures of injector, detector and column were 50, 50 and 60°C, respectively.

The concentration of organic acids were determined by a gas chromatography (Model 8A, SHIMADZU, Japan) equipped with a flame ionization detector (FID) and a 3 m x 3.2 mm glass column packed with 30/60 mesh Unisole F-200 (GL Science Inc. Japan). Injector, detector and column temperatures were at 250, 140 and 140°C, respectively. Nitrogen, hydrogen and pressured air were used as carrier gases with flow rate of 50, 60 and 500 mL/min, respectively.

Total sugar, suspended solids (SS), VSS, and pH were determined according to Standard Methods [20].

2.6 Kinetic modeling

The cumulative volume of hydrogen production in the batch experiment followed the modified Gompertz equation:

$$H(t) = P \exp \left\{ - \left(\exp \frac{R_m e}{P} (\lambda - t) \right) + 1 \right\} \quad (4)$$

where H is the cumulative hydrogen production (ml); λ the lag time (h), P the hydrogen production potential (ml), R_m the maximum hydrogen production rate (ml/h), e is 2.718281828.

3. Result and Discussions

3.1 Optimization of the conventional direct heated pretreatment process

An orthogonal experiment was carried out according to the materials and method section. Tables 2 and 3 show the results and statistical analysis of the orthogonal experiment of optimization for the SSF of the conventional direct heated pretreatment bagasse in terms of biohydrogen cumulative. As indicated in Table 3, the average bio-hydrogen cumulative has the highest value for substrate concentration at levels 3. As for the initial medium pH and temperature the average biohydrogen production have the highest value at level 2 and 3, respectively. Which regard to enzyme loading, the average biohydrogen production has the highest values at level 3. Based on the above result, the conclusion that the optimal simultaneous SSF condition of the conventional cellulosic fraction of bagasse by direct heated in terms of biohydrogen production are substrate concentration of 60 g/l, initial medium pH of 6, temperature of 55 °C and enzyme loading of 0.8 mg/g [substrate] can be drawn. The optimal conditions, are not covered by the orthogonal experiments in Table 2, another experiment was conducted under the optimal condition. Under these optimum condition biohydrogen production was 0.9980 l/l-substrate, this still proves the correctness of the orthogonal optimization because the optimization objective is to achieve the highest biohydrogen production. In table 3, the biohydrogen production standard deviation display that the influential extent of the affecting factors to biohydrogen production during the simultaneous saccharification and fermentation is temperature > the initial medium pH > substrate concentration > enzyme loading.

3.2 Optimization of the microwave/alkali pretreatment process

As the pretreatment procedures change, the optimal SSF conditions may also change. In order to obtain the optimal SSF conditions and determine the extent of factors influencing the SSF, another orthogonal experiment was carried out. The results and statistical analysis are shown in Table 4 and 5. As shown in Table 5, the average biohydrogen production has the highest value of substrate concentration at levels 1 with regards to the highest initial pH. As for temperature and enzyme loading, the average biohydrogen production are the highest at level 1 and 2, respectively. From the above result, it can be concluded that the optimal SSF condition of bagasse by microwave/alkali pre-treatment are substrate concentration of 20 g/l, initial medium pH 7.2, temperature of 35 °C and enzyme loading of 0.05 mg/g [substrate]. The optimal conditions are not contained in Table 4, so another experiment was conducted under the optimal condition. The biohydrogen cumulative reached 1.932 l/l-substrate. In table 5, the biohydrogen cumulative standard deviation reveals that the influential extent of the affecting factors to bio-hydrogen production during the SSF is temperature > the initial medium pH > enzyme loading > substrate concentration

7. Conclusion

The SSF of the conventional direct heated and microwave/alkali pretreatment sugarcane bagasse to biohydrogen were investigated using cellulase enzyme from *Trichoderma reesei*. The SSF optima for conventional direct heat pretreated bagasse to biohydrogen was 60 g/l substrate, 0.08 g-cellulase/g-substrate, 55 °C and initial pH 6. The biohydrogen production was only 0.9980 l/l-substrate. The optimum condition for SSF of cellulosic fraction from microwave/alkali pretreated bagasse was 20 g/l substrate, 0.05 g-cellulase/g-substrate, 35 °C, initial pH 7.2. Under the optimum condition, maximum hydrogen production reached 1.932 l/l-substrate. Since hydrogen production obtained from SSF of cellulosic fraction from microwave/alkali pretreated bagasse was higher than direct heated pretreated bagasse but used a lower substrate concentration and less enzyme loading, therefore microwave/alkali pre-treatment was considered as the efficient method for preparing the cellulosic fraction from sugarcane bagasse.

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Table 1 Factors and levels of the orthogonal experiment

Level	Substrate concentration (g/l)	Initial medium pH	Temperature, (°C)	Enzyme loading mg/g [substrate]
1	20	4.8	35	0.02
2	40	6.0	45	0.05
3	60	7.2	55	0.08

Table 2 Condition and result of the orthogonal experiment of optimization for the SSF of the direct heated pretreatment bagasse to biohydrogen

Experiment No.	Substrate concentration, g/l	Initial medium pH	Temperature, °C	Enzyme loading mg/g [substrate]	Biohydrogen production (l/l-substrate)
1	20	4.8	35	0.02	0.0011
2	20	6	45	0.05	0.0023
3	20	7.2	55	0.08	0.0145
4	40	7.2	35	0.05	0.5896
5	40	4.8	45	0.08	0.0008
6	40	6	55	0.02	0.0410
7	60	6	35	0.08	0.9390
8	60	7.2	45	0.02	0.0097
9	60	4.8	55	0.05	0.0005

Table 3 Statistical analysis of the orthogonal experiment of optimization for the SSF of the conventional direct heated pretreatment bagasse to biohydrogen

Process variable	Biohydrogen production (l/l-substrate)			Standard deviation
	Average at level i			
	C ₁	C ₂	C ₃	
Substrate concentration	0.0059	0.2105	0.3164	0.1578
Initial medium pH	0.0008	0.3274	0.2046	0.1650
Temperature	0.5099	0.0043	0.0186	0.2879
Enzyme loading	0.0173	0.1975	0.3181	0.1514

Table 4 Condition and result of the orthogonal experiment of optimization for the SSF of the microwave/alkali pretreatment bagasse to biohydrogen

Experiment No.	Substrate concentration, (g/l)	Initial medium pH	Temperature, (C)	Enzyme loading mg/g [substrate]	Hydrogen production (l/l-substrate)
1	20	4.8	35	0.02	0.0020
2	20	6	45	0.05	0.0026
3	20	7.2	55	0.08	0.1300
4	40	7.2	35	0.05	1.1521
5	40	4.8	45	0.08	0.0006
6	40	6	55	0.02	0.0666
7	60	6	35	0.08	0.8625
8	60	7.2	45	0.02	0.0011
9	60	4.8	55	0.05	0.0009

Table 5 Statistical analysis of the orthogonal experiment of optimization for the SSF of the microwave/alkali pretreatment bagasse to biohydrogen

Process variable	Bio-hydrogen cumulative (ml)			Standard deviation
	Average at level i			
	C ₁	C ₂	C ₃	
Substrate concentration	0.0447	0.4064	0.2882	0.1845
Initial medium pH	0.0010	0.3106	0.4277	0.2205
Temperature	0.6720	0.0014	0.0658	0.3700
Enzyme loading	0.0231	0.3852	0.3310	0.1953