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# BIOHYDROGEN PRODUCTION FROM FERMENTATION OF SWEET SORHGUM (*SORGHUM BIOCULAR L*) BY *INTEROBACTER AEROGENES* ADH-43 IN THE PACKED-BED REACTOR

M. A. Rachman [a,b*], L.D. Eniya [b] , and E.T. Widyastuti [b]

[a]Centre of Hydrogen Energy (IHE-ERA), Malaysia Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia (UTM), Jl Semarak 54100, Kuala Lumpur, Malaysia
[b]Agency for The Assesment and Application of Technology (BPPT), Puspiptek, Serpong, Tanggerang, Banten, Indonesia

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*Corresponding author
yudinr@ic.utm.my

## Graphical abstract

## Abstract

Hydrogen gas ($H_2$) is one of a clean energy because its combustion produces only water vapor and heat, and leaves no carbon emissions. $H_2$ gas is an energy future that promises both from the aspect of social, economic, or environmental. One of potential raw material for $H_2$ gas production is Sweet sorghum (Sorghum bicolor). It is an annual plant native of tropical adaptive in hot and dry season. Moreover, it has a high biomass production , it also can adapt to extreme and sub-tropical regions. The objective of this experimental work was to produce gas $H_2$ using sweet sorghum at packed-bed reactor by *Enterobacter aerogenes* ADH-43 and to get optimum dilution rate in order to increase gas $H_2$ production. The reactor used is a packed bed with a working volume of 450 mL and total volume of 900 mL, height 60 cm with a diameter of 4 cm. The reactor is equipped with a coat of water associated with water heating to the temperature maintained at 37 ° C ± 1 °C. It also linked to the flask containing the Ca (OH) $_2$ which serves to capture the $CO_2$ gas produced, so expect only the $H_2$ gas. Batch experiments were performed in the beginning, the fresh sorghum medium was fed into the reactor before two hours of the stationary phase in order to achieve continuous culture. The steady state condition showed that that optimum dilution rate was 0.15 $h^{-1}$ with $H_2$ gas production 81.50 mmol/L·h and yield 0.87 mol $H_2$/mol total sugar.

*Keyword*: $H_2$ gas, Sweet sorghum, packed bed reactor, and *Enterobacter aerogenes*

## Abstrak

Gas hidrogen ($H_2$) adalah salah satu daripada tenaga bersih kerana pembakarannya menghasilkan wap air sahaja dan haba, dan tidak meninggalkan pengeluaran karbon. Gas $H_2$ adalah masa depan tenaga yang menjanjikan kedua-dua dari segi sosial, ekonomi, atau alam sekitar. Salah satu bahan mentah yang berpotensi untuk pengeluaran gas $H_2$ adalah sorgum Sweet (Sorgum bicolor). Ia merupakan tumbuhan asli tahunan penyesuaian tropika di musim panas dan kering. Selain itu, ia mempunyai pengeluaran biojisim yang tinggi, ia juga boleh menyesuaikan diri dengan kawasan-kawasan yang melampau dan sub-tropika. Objektif ujikaji ini adalah untuk menghasilkan gas $H_2$ menggunakan sorgum manis di reaktor padat-katil oleh *Enterobacter aerogenes* ADH-43 dan untuk mendapatkan kadar pencairan optimum bagi meningkatkan pengeluaran gas H2. Reaktor digunakan adalah katil yang penuh sesak dengan jumlah kerja 450 mL dan jumlah dagangan sebanyak 900 mL, ketinggian 60 cm dengan diameter 4 cm. Reaktor dilengkapi dengan lapisan air yang berkaitan dengan pemanasan air kepada suhu dikekalkan pada 37 ° C ± 1 °C dan dikaitkan dengan kelalang yang mengandungi Ca (OH) $_2$ yang berfungsi untuk menangkap gas $CO_2$ yang dihasilkan, sehingga diharapkan hanya $H_2$ gas. Eksperimen kumpulan telah dijalankan pada permulaan,

sederhana sorgum yang segar telah dimasukkan ke dalam reaktor sebelum jam dua fasa pegun. Syarat yang telah dicapai dalam usaha untuk mempunyai budaya yang berterusan. Keadaan keadaan mantap menunjukkan bahawa kadar pencairan optimum adalah 0.15 $h^{-1}$ dengan pengeluaran gas $H_2$ 81.50 mmol / L·jam dan menghasilkan 0.87 mol $H_2$ / mol jumlah gula

Kata kunci: gas $H_2$, sorgum Sweet, reaktor lapisan terpadat, dan *Enterobacter aerogenes*.



## 1.0 INTRODUCTION

The world's dependency on fossil fuels, besides resulting in the depletion of fuel reserve, the use of fossil fuels also causes global warming as the impact of greenhouse gas emissions resulting from combustion. Hydrogen ($H_2$) gas is one of clean energy for combustion produces only water vapor and heat, and leaves no carbon emissions. $H_2$ gas is an energy future that promises both from the aspect of social, economic, and environmental. Approximately 90% of $H_2$ gas production resulting from the thermochemical process of non-renewable sources such as high temperature breakdown (thermal cracking) of natural gas and coal gasification. Fermentation is an anaerobic biological process is advantageous for the production of $H_2$ gas which can be produced continuously for a feed of carbon and C / N ratio sufficient [2]. Excellences $H_2$ gas production via fermentation is not require solar energy, various waste or energy crops can be used, and a simple reactor technology [3].

Various organic farming has identified potential fermentation substrate on $H_2$ gas. Sweet sorghum (Sorghum bicolor L) fermentation of biomass that is a potential raw material for fermentation in the production of $H_2$ gas. Sweet sorghum is an annual plant native to tropical regions of adaptive in hot and dry and can adapt in extreme regions and sub-tropical and has a high biomass production. In addition, sweet sorghum has a much higher production than sugarcane and sweet sorghum harvest faster just 4 months, compared with sugar cane harvested at 7 months [4] sweet sorghum stalk of sugar, which is 55 % sucrose and 3.2 % glucose (dry weight) and also contains cellulose and hemicellulose 12.4 % and 10.2 % [5]. The study of $H_2$ gas production using sweet sorghum substrates indicated for results

**Figure 1** The tree and seed of Sweet sorghum

$H_2$ gas producing microbes in dark fermentation is divided into two, facultative anaerobes such as Escherichia coli, *Enterobacter aerogenes* and Citrobacter as well as obligate anaerobes such as Clostridium, methylotrophic methanogens, and rumen bacteria. Facultative anaerobic bacterial culture producing $H_2$ gas which has been widely studied is *Enterobacter aerogenes* [8]. According Tanisho *et al*. [9]. these bacteria have no resistance when the partial pressure of $H_2$ gas produced $H_2$. *Enterobacter aerogenes* ADH-43 is able to produce 1.59 moles $H_2$/mol sugar by using Stirred Batch Tank Reactor (BSTR) 10 and 1.84 mol $H_2$/mol sugar in Continuous-flow Stirred-Tank Reactor (CSTR) [11]. Increase in the rate and yield of $H_2$ gas production to an economical process is a challenge in the production. Utilization of carbon from biomass sources that do not compete with food is expected to be an alternative in addressing energy issues.

The general research outlined in this paper aims to produce $H_2$ gas through fermentation with substrate Sweet sorghum using cells of *Enterobacter aerogenes*-ADH43 in continuous packed bed bioreactor. Furthermore learned how to do with changes in dilution rate to the rate of $H_2$ gas production, as it also studied the changes that occur in the formation of acids and alcohols metabolism and ends by comparing with previous paper by previous researchers to see the progress of research in this field.

## 2.0 MATERIALS AND METHODS

### 2.1 Microorganism and Culture Condition

$H_2$ gas producing bacteria of *Enterobacter aerogenes* ADH-43 was obtained by classical mutagenesis working and was maintained at -80 °C with 15 % glycerol. A synthetic medium used in this contained (per liter) 7.0 g $K_2HPO_4$, 1.0 g$(NH_4)_2SO_4$, 0.25 g $MgSO_4{\cdot}7H_2O$, 0.021 g $CaCl_2{\cdot}\ 2H_2O$, 0.029 g $Co(NO_3)_2{\cdot}6H_2O$, 0.039 g $Fe(NH_4)_2SO_4{\cdot}6H_2O$, 0.172 mg $Na_2Se0_3$, 0.02 mg $NiCl_2$, 0.5 g $MnCl_2{\cdot}4H_2O$, 0.1 g $H_3BO_3$, 0.01 g $AlK(SO_4)_2{\cdot}12\ H_2O$, 0.001 g $CuCl_2{\cdot}2H_2O$, 0.5 g $Na_2EDTA{\cdot}2H_2O$, and 2.0 mg nikotenic acid [12].

Media complex solution was prepared by adding 2% Reducing sugar molasses or equal to 4% of the total sugar synthetic media. For anaerobic bacterial culture, modified Hungate technique for the

combination of serum bottle technique used for culture of anaerobic bacteria [13]. Medium without phosphate buffer sorghum boiled for 20 minutes, cooled on ice with continuous $N_2$ gas bubbles, dispered into serum bottles sealed with black butyl rubber stoppers and then sterilized (18 minutes, 121 ° C). Sweet sorghum and autoclaved separately phosphate buffer and injected into the serum bottle. After inoculation of 10 % of the seed culture into serum bottles and adjusting the pH to 6.8, bottles were incubated at 37 ° C with 50 rpm agitation [12]. Seed culture was obtained with 40 m*L* of pre-culture of *Enterobacter aerogenes* ADH - 43 (Optical Density/OD = ± 0.82 ), before the end of the logarithmic growth phase were inoculated into 400 m*L* of complex medium supplemented with 2 g / *L* of total sugar from molasses, and then incubated at 37 $^0$ C, 120 rpm, 8 hours of temperature, agitation time and fermentation time.

### 2.2 Analyses

The number of bacterial colonies was measured by the method of total plate count (TPC). Gas volume measurements made using respirometer connected with the holes on the top of the fermentor. $CO_2$ gas and $H_2$ gas are formed will flow through the hose into the erlenmeyer containing a solution of Ca (OH) $_2$. $CO_2$ gas will react with Ca (OH) $_2$ to form $CaCO_3$, while the $H_2$ gas will get into the respirometer containing a saturated NaCl as follow the reaction:

$CO_2$ (g) + Ca (OH) $_2$ (*l*) → $CaCO_3$ $_{(s)}$ + $H_2$ (g) ... eq 1).

The amount of $H_2$ gas produced is shown by the difference in volume between the small cylinders in a NaCl solution with the outer cylinder (large cylinder) on the respirometer. The measuring volume of $H_2$ gas is calculated based on the difference in volume that occurs due to gas pressure cylinder between two cylinders. Furthermore, the concentration of $CO_2$ gas and $H_2$ gas were determined by gas chromatography (GC 8A, Shimadzu Kyoto) with a thermal conductivity detector [14]. On the other hand Total sugar (TS) and reducing sugars (RS) were measured by phenol sulfuric method [15].

### 2.3 Packed Bed Reactor

In Figure 2, the reactor used was a packed bed with with working volume of 450 m*L* and total volume of 900 m*L*, height 60 cm reactor with a diameter of 4 cm. The reactor is equipped with a coat of water which is connected to the water heater so the temperature maintained at 37 ° C ± 1°C and connected with the flask containing the Ca (OH) $_2$ which serves to capture the $CO_2$ produced so expect only the $H_2$ is measured in the respirometer

**Figure 2** $H_2$ gas fermentation by using Sweet sorghum and *Enterobacter aerogenes* ADH-43 in a packed-bed reactor continuous system

## 3.0 RESULTS AND DISCUSSIONS

### 3.1 Raw Material Composition

Analysis of total sugar, reducing sugar and nitrogen content are shown in Table 1. Low values are influenced by the age of the plant when the main crop is harvested at low planting. Further variation of this material composition is influenced by soil conditions, climate, crop varieties and maturity. According to Saraphirom P *et al*, [16] in total sugars and other components were increased from 75 to 120 days age for Sweet sorghum. Sweet sorghum used in this study were harvested at the age of less than 75 days of planting. The content of carbon and nitrogen from sweet sorghum obtained at relatively low when compared with the reference. Conditions that cause the ratio of Carbon (C) / nitrogen (N) in the Sweet sorghum is 17.64. The C / N as sweet sorghum used in this study were harvested at vegetative stage (before flowering), where C is required for the formation of leaves, stems and roots. In the reproductive phase, C / N ratio will increase due to the accumulation of sugar and other food reserves to reproduce the content of the N source and extract tryptone added about 0.5 g in 100 ml of media or equal to 0.116% to achieve the optimum ratio of 13-15% [14].

**Table 1** Chemical analysis of the ss used for biohydrogen fermentation

| Analyses | Sweet Sorghum (%) |
|---|---|
| Total Sugar (TS) | 7.233 ±0.673 |
| Reduction Sugar (RS) | 1.206±0.071 |
| Nitrogen Sugar | 0.410±0.047 |

### 3.2 Performance Fermentation in Batch Culture

The use of sweet sorghum as a source of carbon that contain complex sugars, based on the growth of cells

and other curves are shown in Figure 3. There diauxic pattern of cell growth (two exponential phase). The first phase lag is not visible, it may be very fast so it was not observed. Then the cells adapt during the lag phase-2 to break down the complex sugars into simple sugars. To the exponential phase 2 starts at and ends at the 10th hour. If microorganisms grown on a medium containing two types of sugar as a carbon source such as sweet sorghum, it will show a pattern diauxic growth. In diauxic growth, simple carbon sources such as glucose which is metabolized sugar will be consumed first by the cells of *Enterobacter aerogenes* ADH-43 After the first carbon source depleted, the cells will increase in number and enter to the stationery phase. Then in the second phase of growth, microorganisms will use a more complex carbon sources such as sugars saccharose [17].

Specific growth rate (μ) are not constant that depends on the physical and chemical environment in which the maximum value of the specific growth ($\mu_m$) was achieved in the supply of nutrients and substrate conditions are still excessive and inhibitor concentration is still low [18], This is shown by the curve coefficients worth Ln x = f (t). The curve is based on the values obtained from 0.198 $h^{-1}$ of $\mu_m$ at the exponential phase in which the substrate in the form of simple sugars (glucose) and the second exponential phase 0.119 $h^{-1}$ of $\mu_m$ wherein the substrate is a complex sugars (complex sugars occur decomposition into simple sugars). Specific growth in exponential phase 2 is smaller than the exponential phase 1, it is due to the exponential phase 2 cells must break down the complex sugars into simple sugars.

Based on Figure 3, indicated that the decrease in pH occurred during cultivation, there is an accumulation of metabolites in the form of organic acids (lactic acid and acetic acid), so that the pH becomes acidic media. $H_2$ production began in logarithmic phase-2 (6 h). However, its formation has decreased every hour and after hour 18 th (current cell death) fermentation was stopped. Total sugars (TS) consumption decreased during the period of cultivation. TS looks sharper decline than Reducing Sugars (RS). RS has been consumed not exhausted, but increases as the complex sugars are broken down into simple sugars during cultivation. The highest flow rate of $H_2$ gas production was obtained on 6 th when early detection of $H_2$ gas. The longer the flow rate begins to decline because of other metabolites produced and will reduce the amount of substrate. The highest flow rate was 645 m*L* /*L*· h and an average flow rate of 531 m*L* / *L*·h

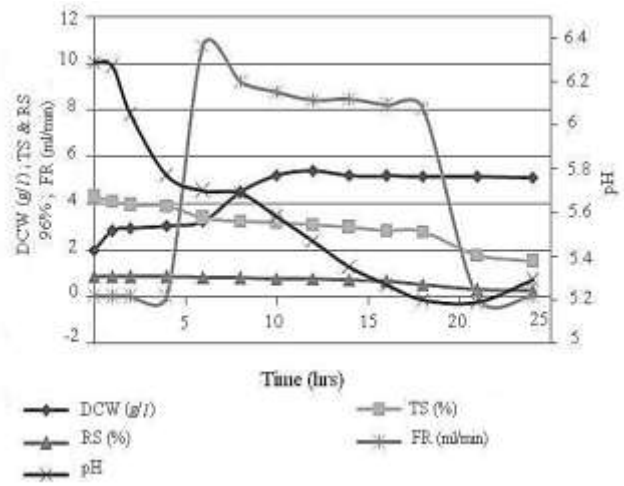


**Figure 3.** Fermentation performance of Sweet sorghum by *Enterobacter aerogenes* ADH-43 in packed bed reactor.

Based on Figure 4, $H_2$ gas is formed on the hour to 5 and 6 (early logarithmic phase 2), and then continued to increase until the 16 $^{th}$ hour in which the stationary phase started achieved. Furthermore, the growth of cells, which has a logarithmic phase 2 begins at the 6 $^{th}$ to the 12 $^{th}$, the next phase of the death or the stationary phase continues until the 14 $^{th}$ hour. When the rate of product formation is proportional to the rate of cell growth, it can be stated that the rate of growth of products such as $H_2$ gas proportional to the growth rate microorganisms.

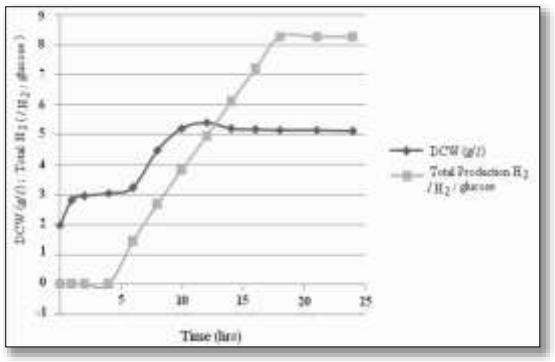


**Figure 4.** $H_2$ gas production curve of Sweet sorghum by *Enterobacter aerogenes* ADH-43 in packed bed reactor.

In Table 2 it is stated that the results of $H_2$ gas production in this study gives good results compared with the results of other researchers, but lower than that of Kumar *et al* [18] . both of the amount of $H_2$ gas and yield. The most important target of this research results showed that sweet sorghum potesial enough as a raw material for the production of $H_2$ gas. Before $H_2$ gas industry established then this approach needs to be deepened further biotechnological both involving the use of microorganisms, biomass and the selection of appropriate reactor.

**Table 2** Summary of $H_2$ gas production research

| Microbe and Carbon source | Flow rate of $H_2$ gas | Yield of $H_2$ gas |
|---|---|---|
| *Enterobacter cloacae* IIT-BT 08 and sucrose 1 % | 660 mL/L ·h | 6.0 mol/mol sucrose |
| *Enterobacter aerogenes and sucrose 2 %* | 138 mL/L· h | 1.5 mol/mol sucrose |
| *Klebsiella oxytoca* HP1 and Glucose 1 % | 87.5 mL/L·h | 1.0 mol/mol glucose |
| *The anaerobic mixed culture* | 6.9 L/L·h | 2.2 mol $H_2$/mol TS |
| *Enterobacter aerogenes* ADH- 43 and sweet sorghum 2.5 % | 8.3 L /L·h | 3.8 mol/mol TS |

### 3.3 $H_2$ gas Production in Continuous Culture in Packed-Bed Reactor

In three different dilution rate (D) treatment, the ethanol yield ($Y_{e/s}$) and lactic acid ($Y_{lac/s}$) is more dominant than the result of $H_2$ gas ($Y_{H2/s}$) and the results of acetic acid ($Y_{ac/s}$). This can be seen in Table 3. $Y_{H2/s}$ highest in D = 0.15 $h^{-1}$. The small $Y_{H2/s}$ in running reactors at D = 0.15 $h^{-1}$. this may be affected due to the dominance of the production of lactic acid and ethanol production that uses NADH which will eventually inhibit the formation of $H_2$ gas. This gas is produced by *Enterobacter aerogenes* ADH-43 through the release of electrons in the metabolic oxidation by the enzyme dehydrogenase that this enzyme interacts with NADH to produce $H_2$ gas. Compounds NADH generated in the catabolism of glucose to pyruvate via the glycolytic pathway. Conversion of pyruvate to alcohols and organic acids involves the oxidation of NADH, resulting in the formation of $H_2$ gas will be hampered if the oxidation of NADH is widely used for alcohols and organic acids.

**Table 3** Summary of yield of $H_2$ gas, ethanol, acetate and lactate production with different dilution rate in continuous culture of Sweet sorghum fermentation by *Enterobacter aerogenes* ADH-43 in packed bed reactor.

| Dilution rate ($h^{-1}$) | $Y_{H2\ gas/s}$ | $Y_{Lactate/s}$ | $Y_{acetate/s}$ | $Y_{ethanol/s}$ |
|---|---|---|---|---|
| 0.10 | 0.86 | 0.52 | 0.52 | 2.38 |
| 0.15 | 1.90 | 0.06 | 0.06 | 1.63 |
| 0.20 | 0.53 | 0.53 | 0.10 | 1.38 |

## 4.0 CONCLUSION

From this study it can be concluded that: Sweet sorghum can be used as a substrate in the production of $H_2$ gas;, D = 0.15 $h^{-1}$ is the optimum dilution rate to achieve a production rate of 81.51 mmol / L· h and a yield of 0.87 mol $H_2$/ mol total sugar.

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