Enhancement of Biohydrogen Production via pH Variation using Molasses as Feedstock in an Attached Growth System

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Abstract. In this study, mesophilic biohydrogen production by a mixed culture, obtained from a continuous anaerobic reactor treating molasses effluent from sugarcane bagasse, was improved by using granular activated carbon (GAC) as the carrier material. A series of batch fermentation were performed at 37°C by feeding the anaerobic sludge bacteria with molasses to determine the effect of initial pH in the range of 5.5 to 7.5, and the effect of repeated batch cultivation on biohydrogen production. The enrichment of granular activated carbon (GAC) immobilised cells from the repeated batch cultivation were used as immobilised seed culture to obtain the optimal initial pH. The cumulative hydrogen production results from the optimal pH were fitted into modified Gompertz equation in order to obtained the batch profile of biohydrogen production. The optimal hydrogen production was obtained at an initial pH of 5.5 with the maximum hydrogen production (H_m) was found to be 84.14 ml, and maximum hydrogen production rate (R_m) was 3.63 mL/h with hydrogen concentration of 759 ppm. The results showed that the granular activated carbon was successfully enhanced the biohydrogen production by stabilizing the pH and therefore could be used as a carrier material for fermentative hydrogen production using industrial effluent.

1 Introduction

Today, energy demand and resources has become a crucial aspect in sustainable development due to the growing concerns over the environmental impact and the inevitable depletion of fossil fuels. Due to that reason, the development of alternative energy sources has become matter of urgency. According to Ni et al. [1], two important issues which are energy crisis and environmental sustainability were the major concern in order to achieve global sustainable development. Since mostly the focus on fossil fuels, it leads to climatological changes and rapid exhaustion of natural energy resources.

Hydrogen is well known as clean energy carrier since it is renewable energy source with carbon free [2]. Hydrogen also contributes substantially to the reduction of greenhouse gas (GHG) emissions as well as its might be considered as ultimate clean and climate neutral energy system [3]. If compared to fossil fuels, hydrogen yield obtained higher energy. Hydrogen gas guaranteeing other possibility to fossil fuels, otherwise it suggests the possibility of generating a valuable energy carrier that is renewable and carbon unbiased.

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Besides, hydrogen need an helter - skelter vitality yield about 122 kJ/g, which will be 2.75 times more terrific over hydrocarbon fills [4]. Various processes are being used in order to produce hydrogen gas, including photo fermentation, dark fermentation, photolysis and electrolysis of water. Among all, the most suitable processes are to be dark fermentation [5].

Dark fermentation is an anaerobic digestion of organic substances and it is considered the simplest processes of obtaining biohydrogen. Different factors have been investigated to improved biohydrogen production either as a suspended cells system or immobilised cells system using dark fermentation processes in this study. Many factors that affecting dark hydrogen fermentation such as inhibits of undissociated acid and changes of pH, hydrogen partial pressure and metal ions.

In terms of feedstock, real wastewater have demonstrated strong evidence of hydrogen production. Sugarcane, Saccharum officinarum L sp. is carbon - rich as well as the biomass residue [6]. There are various products that can be obtained from sugarcane, and one of it is molasses that are widely used and known to be practical feedstock in terms of availability, cost, and biodegradability for fermentative hydrogen production.

Therefore, this study will focus on the enhancement of batch biohydrogen production using immobilised cells system by feeding molasses from sugarcane bagasse as feedstock. This study will emphasis on the effect of initial pH on fermentative hydrogen production by varying the pH, which is the key environmental parameter in biohydrogen process.

2 Material and methods

2.1 Sample collection

The origin of seed sludge and molasses used in the study were from sugarcane bagasse that was obtained from Fempro, Chuping, Perlis, an industry producing ethanol. The bacterial sludge used in this study was the continuity from previous study that had operated for two months in a 14 L anaerobic reactor by feeding with molasses. The operation was done in continuous mode at 37ºC. The effluent samples of the reactor were collected every week and stored at 4ºC as stock culture for further use as inoculum.

2.2 Mixed culture and carrier material

The anaerobic sludge underwent heat shock by heating at 80°C for 60 minutes prior to be used for experiment. This is to inactivate methanogenic bacteria prior to batch fermentation. Granular activated carbon (GAC) in the range of 2 – 3 mm size was used as carrier material to attach the hydrogen - producing bacteria in this study.

2.3 Repeated batch cultivation

Repeated batch cultivation was conducted in 100 mL serum bottle with 50 mL working volume filled with 10 % of anaerobic sludge, and 90 % of molasses. This repeated batch of fermentation were conducted into different range of initial pH from 5.5 to 7.5 with 0.5 of increments (pH 5.5, 6.0, 6.5, 7.0, and 7.5). The immobilization of the anaerobic sludge was carried out by adding GAC in a 1 : 1 ratio of anaerobic sludge volume (mL) to GAC weight (g) in the serum bottle, while fermentation without GAC was conducted in parallel as control. Each of the serum bottle was then purged with nitrogen for a few minutes to provide an anaerobic conditions. Finally, the serum bottle was incubated at mesophilic temperature, 37 °C for 24 hours in incubator. This method was repeated for several successive batch until the performance of hydrogen gas is stable in order to compare the

hydrogen production at different initial pH values.

2.3.1 Biogas collection

Sampling of biogas from the serum bottles were done after 24 hours for each cycle of fermentation. The collected biogas were kept in 30 mL serum bottle containing acid at pH 2.0 before the biogas were analysed. The production of hydrogen was measured by using Gas Analyser (biogas 5000 standard) Model GA 5000. The samples were also analysed for other biogas composition such as carbon dioxide, oxygen and methane (if presence).

2.3.2 Batch profile fermentation

Generally, the experimental procedure on batch profile was same as in the Section 2.3. However, this batch profile was only employed for the initial pH with optimal hydrogen production that was obtained from Section 2.3. The data were recorded and the sampling were done every 3 hours of interval for 24 hours of incubation time.

2.3.3 Calculation of hydrogen production

The cumulative hydrogen production in the batch experiments was determined according to a modified Gompertz equation using Sigma Plot 10.0 (Systat Software Inc., USA). Theoretically, the modified Gompertz equation is as follows.

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

where H_t is the cumulative hydrogen production (mL), H_m is the maximum hydrogen production (mL), R_m is the maximum hydrogen production rate (mL h^{-1}), e is Euler's number (e =2.73), λ is the lag phase time (h), and t is the incubation time (h).

2.4 Analysis procedure

2.4.1 Total carbohydrate

The Phenol - Sulphuric acid reaction is feasible method used to determine the amounts of sugar for carbohydrate analysis of the fermentation broth and effluent. This method was widely used to determine sugar compositions due to its simplicity and sensitivity. Simple sugar such as glucose are stained orange - yellow when treated with phenol and concentrated sulphuric acid. This quality can be used to perform quantitative analyses of sugars using colometric methods such as spectrophotometer. The absorption of each sample is then measured by using spectrophotometer at 490 nm.

2.4.2 Alkalinity

The titration was used to determine the alkalinity of the effluent sample after fermentation to obtained the total value of volatile fatty acids. 50 mL of the sample was taken and then titrated with 0.1 N of sulphuric acid solutions. The electrode of pH meter was rinsed and the placed into the sample. The sample was mixed thoroughly until constant reading is obtained. The sample was titrated to pH 4.5 to an end point value for alkalinity. Then, the

value of alkalinity of the sample were calculated by using the equation 2:

$$Alkalinity,\ mg\ CaCo_3/\ l = \frac{S\ x\ N\ x\ 50000}{Volume\ of\ sample,\ ml} \quad (2)$$

where S = ml of titrant to achieve pH 4.5 as end point and N = equivalents H_2SO_4 per liter titrant.

2.4.3 Chemical Oxygen Demand (COD)

In this study, 2.5mL of the mixed culture samples were added in vial tubes. Then, the vial tubes were added with 1.5mL potassium dichromate solutions and 3.5mL of sulphuric acid solutions. Once it was done, the vials were placed in preheated DRB200 Reactor for two hours. After that, the vials were placed at room temperature for 30 minutes. The COD value were measured by using spectrophotometer.

3 Results and discussions

3.1 Characterization of seed sludge and molasses

Before conducting the batch fermentation, the sample of seed sludge and molasses were characterised. The results were presented in Table 1.

Table 1. Characteristics of anaerobic sludge and molasses.

Parameter	g/L (except pH)	
	Anaerobic sludge	Molasses
pH	7.583 ± 0.008	7.808 ± 0.004
TSS	0.0480	0.014
COD	28.053 ± 1.083	6.270 ± 0.673
Total Carbohydrate	3.46	2.60

3.2 Batch fermentation at various initial pHs

3.2.1 Effect of repeated batch cultivation at various initial pHs

pH can be the main control parameter for the bacteria activities in order to enhance the hydrogen performance. Therefore, batch fermentation were conducted in series to determine the effects of different initial pH during fermentative hydrogen production in this study. The fermentation broth consist of effluent sludge and molasses were conducted triplicate each for different initial pH of 5.5, 6.0, 6.5, 7.0 and 7.5. The result shown in Fig.1 was the data of hydrogen performance at different initial pH values for ten days of repeated batch cultivation. On the first day, the highest collected gas was at pH 6.0 with 44 ml. However, at pH 7.5, no gas was produced and this might be due to the bacteria still adjusting to their new environment. The performance of gas seems to be evolved for all pHs and start to stabilised starting from day 5.

At initial pH 5.5, the hydrogen produced only 37 ml on the first day, however starting the second day, the gas increased to 40 ml. Then it continue to increase up to day five which

reached to 70 ml. It can be stated that the hydrogen gas production was two folds. Start from day five, the gas from initial pH 5.5 reached its stability and it sustain until day ten. Meanwhile, for initial pH 6.0, the biogas produce almost 50 ml of hydrogen gas on the first day. Then, on the second day until third day the gas produced were increase continuously up to 70 ml. However, after day five, the hydrogen gas production at initial pH 6.0 had depicted its stability and could only produce a maximum of 60 ml of hydrogen gas. On the other hand, initial pH 7.5 did not show any production of gas on the first day. However, it start to produce gas on the second day until day ten. The maximum of gas that can be produced at initial pH 7.5 is only 30 ml of hydrogen.

From the graph, it shown that pH between 5.5 to 6.0 produce hydrogen gas the most while decrease when pH are set between 6.5 to 7.5. Our studies revealed that between those pH, the fermentation process do not favour the production of hydrogen. This result was found to be likely same as previous studies by Chen et al. (2009) [7]. Normally, pH values between 5.5 and 6.0 are reported to be appropriate for better microbial activity as well as produce better hydrogen. Meanwhile, pH between 6.5 to 7.5 that operates under natural condition reduced the hydrogen production. As previous reported, hydrogenase activity is suppressed at low pH during fermentation.

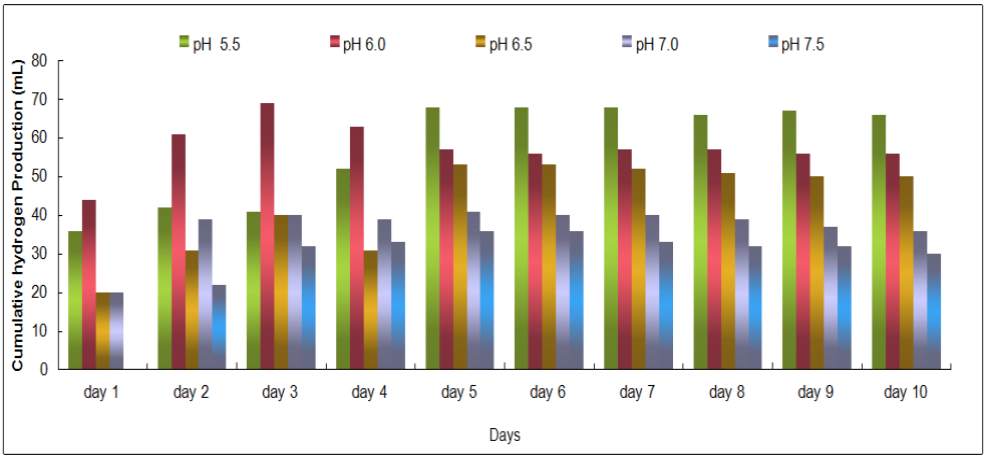

Fig. 1. Production of hydrogen with GAC immobilized cells at various initial pH.

3.2.2 Production of Hydrogen with GAC and without GAC (control) at various initial pH

Fig.2 show the comparison of hydrogen production between different initial pH values with immobilised cells and control (without the presence of GAC). For all pH values, the samples which contains GAC were more efficient in producing biohydrogen compared to the sample without GAC. The study shows that pH 5.5 produce the highest hydrogen if compare to others pH values. This finding is similar to previous study by Wang & Wan (2009) [8] which state that the fermentative anaerobic bacteria were mostly favour to produced hydrogen at optimal pH of 5.5 and 6.0.

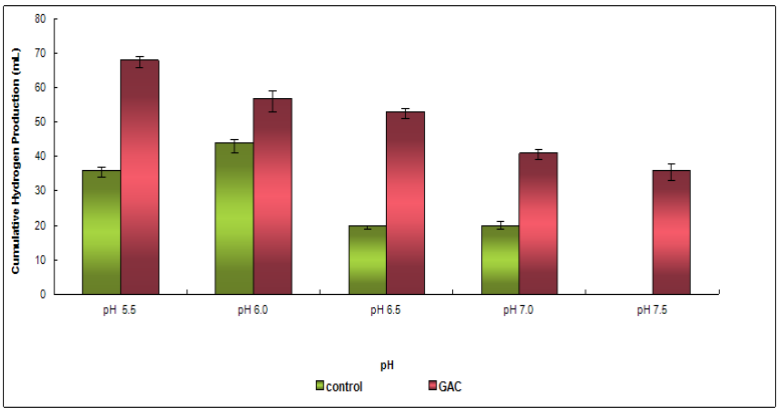

Fig. 2. Hydrogen production with GAC and without GAC (control) at 37ºC.

3.2.3 Kinetic analysis of batch fermentation using GAC immobilised cells

The kinetic analysis of batch fermentation using GAC immobilised cells were performed for only pH 5.5 due to the highest hydrogen performance in section 3.2.2. Fig.3 depicts the experimental data and the modified Gompertz model as in equation (1) of the cumulative hydrogen production of the GAC attached biofilm at pH 5.5. Table 2 presented the kinetic parameters obtained for hydrogen production based on data fitted by the modified Gompertz model, whereby H_m = 84.14 ml, R_m = 3.63 ml/h and λ = 2.95 h. The pH of 5.5 is considered weakly acidic, which urges microorganisms to release express protons from cytoplasm for hydrogen production evolution and for the recommencement of cell growth [9]. This study had revealed the greater effects of pH on biohydrogen production that influence the hydrogenase activity.

Table 2. Modified Gompertz equation parameter values for batch profile at optimal performance of biohydrogen.

pH		Modified Gompertz Equation Parameter values for hydrogen production			Hydrogen concentration GAC – cells (ppm)
Initial	Final	H_m (ml)	R_m (ml/h)	λ (h)	
5.5	4.1 ± 0.03	84.14	3.63	2.95	759

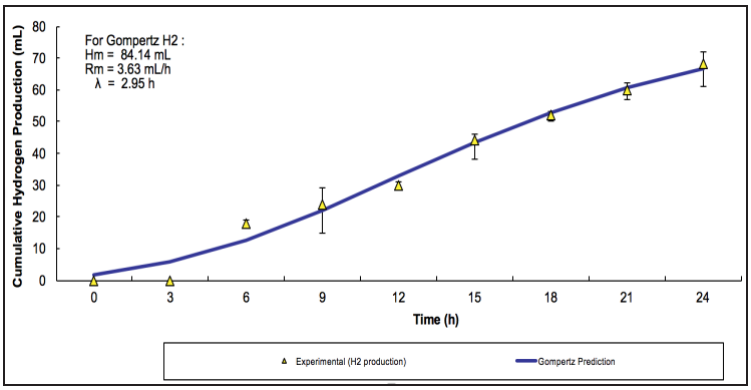

Fig. 3. Gompertz curve- fitting graph of cumulative gas production for pH 5.5.

3.2.4 Total alkalinity and sugar consumption

The result in Fig.4 represented the total alkalinity and sugar consumption of the batch profile for initial pH 5.5, which also reflected the hydrogen production in Fig.3. The total alkalinity increased as the time increases in profile fermentation. The analysis of alkalinity as in equation (2) in this study represent the volatile fatty acids produced as by product besides the production of biohydrogen as the main product. Meanwhile, sugar consumption was decreased with an increase in fermentation time. At time 0 hour, the percentage of sugar is 100%, however after 24 hours, the sugar percentage start to reduce until 60 %. This reduction happened due to rapid growth and dividing state of bacteria resulting from the increasing of their metabolic activity. Thus, bacteria had consumed the sugar and start to adapt with the environment under mesophilic temperature.

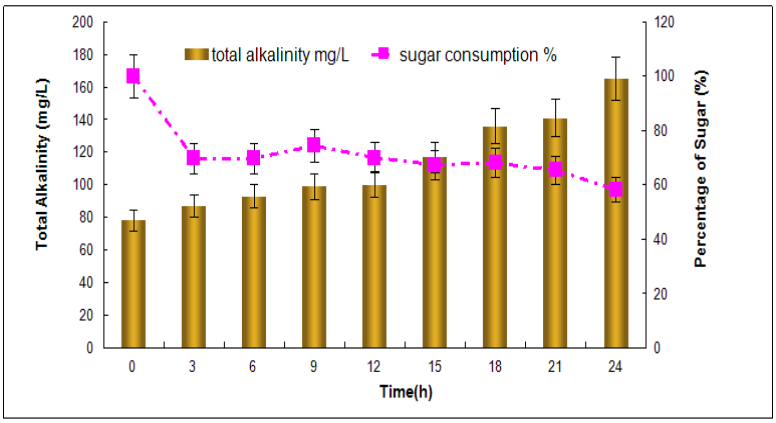

Fig. 4. Graph of total alkalinity and sugar consumption at pH 5.5.

3.2.5 Chemical oxygen demand (COD) analysis

Fig.5 illustrated the Chemical Oxygen Demand (COD) that were obtained after batch profile fermentation for pH 5.5 under mesophilic temperature. The result shows that there is reduction in COD, whereby this finding reveals that the process of fermentative biohydrogen production has simultaneous advantage by not only producing clean renewable energy (hydrogen), but also treating the wastewater used.

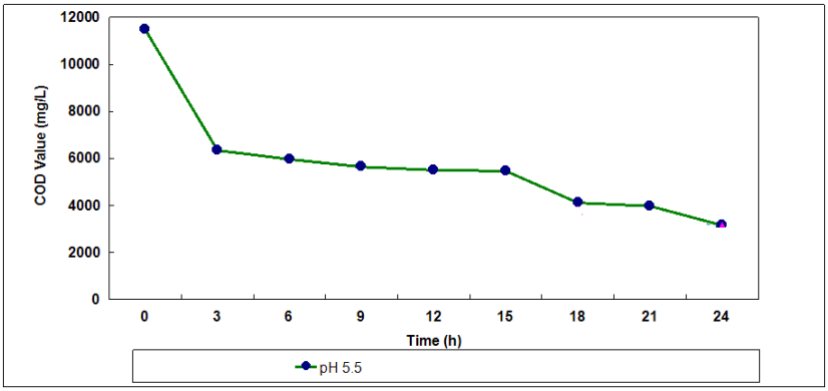

Fig. 5. COD results for pH 5.5 under mesophilic temperature.

4 Conclusions

The biohydrogen production performance of effluent sludge and molasses with GAC immobilized cells were investigated successfully. The optimal conditions for producing hydrogen using GAC immobilised cells at 37°C were achieved at initial pH of 5.5. Repeated batch cultivation have shown the enhancement of biohydrogen production and the durability of the enriched GAC immobilised cells were stable even for several cycles of repeated batch fermentation. The maximum cumulative hydrogen production from batch profiling of initial pH 5.5 was 84.14 ml and the maximum hydrogen concentration obtained was 759 ppm. Overall, the formation of total volatile fatty acid was increased parallel to the increased of the hydrogen production. Therefore, the hydrogen fermentation performed by mixed culture is very much dependent on the initial pH parameter to achieve better performance of hydrogen.

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