

Study on Molasses Concentration from Sugarcane Bagasse for Biohydrogen Production using Enriched Granular Activated Carbon (GAC) Immobilised Cells by Repeated Batch Cultivation

Norfatiha Idris¹, Nabilah Aminah Lutpi^{1*}, Che Mohd Ruhaizul Che Ridzuan¹, Wong Yee Shian¹, and Tengku Nuraiti Tengku Izhar¹

¹School of Environmental Engineering, Universiti Malaysia Perlis, Arau, Perlis, 02600, Malaysia

Abstract. Repeated batch cultivation is known as most attractive method in improving hydrogen productivity, due to the facts that this approach could minimize the reuse of the cell and the inoculum preparation. In addition, with the combination of attach growth system during the fermentation processes to produce biohydrogen, the density of cells will be increased and the cell washout could be avoided. Therefore, this study aimed to examine the effectiveness of repeated batch cultivation for enrichment of anaerobic mixed culture onto granular activated carbon (GAC) and investigate the effect of molasses concentration during immobilization of mixed culture onto the GAC. The molasses concentration using 50 %, 40 %, 30 %, 20 % and 10 % of diluted molasses were used as feedstock in the fermentation process. The maximum hydrogen production of 60 ml was obtained at 30 % of molasses concentration with 831 ppm of hydrogen concentration. Thus, the kinetic parameter obtained from the batch profiling based on modified Gompertz equation are, $H_m = 58$ ml for the maximum hydrogen production and $R_m = 2.02$ ml/h representing the hydrogen production rate.

1 Introduction

Nowadays, the utilization of fossil fuels was rapidly increase as energy supply especially in transportation and electricity. About 75% of the increasing CO₂ concentration in the atmosphere contributes from burning of fossil fuels. Burning fossil fuels resources could increase four times the present value of the CO₂ concentration in the atmosphere which of about 350 to 1500 ppmv (parts per million by volume) [1]. Momirlan et. al stated that in the next 30 years the consumption of petroleum based is predicted to increase 80% [2]. Therefore, there is a need to replace the fossil fuels with a new renewable energy sources such as hydrogen. Hydrogen has high energy yield which is 122 kJ/g compared with other fuels [3]. There is increased and developing interest in biological hydrogen production by

* Corresponding author: nabilahaminah@yahoo.com

utilizing the use of microorganism. In biological production, there are few types of processes, which are light-dependent processes (e.g. photo-fermentation) and light-independent processes (e.g. dark fermentation) [4]. However, dark fermentation is more attractive due to the ability of an anaerobic microorganism to produce hydrogen gas.

The use of simple sugars (e.g. glucose and sucrose) as substrate or feedstock for dark fermentation in biohydrogen production are widely researched but these are considered as expensive [5]. Therefore, reuse of waste such as molasses wastewater as substrate in fermentative biohydrogen production is the best choice because of its high composition of sugar, easy to obtain and cut off the overall project costing. Repeated batch cultivation is the most attractive method in improving the hydrogen production due to reuse of bacteria cells, minimizing fermentation preparation and cut the overall costing. Repeated batch cultivation is applied for process to attach the bacteria cell on the surface of support carrier [6].

In this study, mesophilic fermentative hydrogen production was carried out by immobilization of the anaerobic sludge through surface attachment on granular activated carbon (GAC). As a support carrier, GAC functioned to attach growth cells of molasses that operate under mesophilic temperature at suitable pH and nutrient needed [6]. This study will focus on to examine the effectiveness of repeated batch cultivation for enrichment of anaerobic mixed culture onto GAC and to optimize the concentration of molasses as the substrate use for the biohydrogen fermentation.

2 Material and methods

2.1 Sources of seed sludge, feedstock and support carrier for fermentative hydrogen production

The origin of seed sludge and molasses as feedstock in the study were from sugarcane bagasse that was obtained from Fempro, Chuping, Perlis. 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 effluent samples of the reactor were collected every week and stored at 4°C as stock culture for further use as inoculum. The anaerobic sludge will undergo heat shock by heating at 80°C for 60 minutes prior to be used for experiment. This is to inactivate methanogenic bacteria and non-hydrogen producing bacteria prior to batch fermentation. Granular activated carbon (GAC) in the range of 2 – 3 mm size was used as support carrier to attach the hydrogen producing bacteria in this study.

2.2 Preparation of molasses at different dilution percentage for batch fermentation

First step prior to prepare the molasses concentration at different dilution percentage is to determine the total carbohydrates content of the raw molasses. After that, the suitable weight of glucose will be added to the sample according to equation 1 and 2 so that each of the molasses from different percentage of dilution will have the same concentration of 5g/l of total carbohydrate.

$$\text{Total carbohydrate still needed (g/L)} = 5\text{g/l} - \text{Total carbohydrate obtained (g/l)} \quad (1)$$

$$\text{Amount addition glucose (g)} = \text{Total carbohydrate still needed (g/l)} \times \text{Volume of sample (L)} \quad (2)$$

Experiment was conducted in 100 ml serum bottle with different percentage (50%, 40%, 30%, 20% and 10%) of molasses. Different glucose weight in (g) were added to the different percentage of molasses to equalised all of the sample to 5g/L of total carbohydrate (shown in Table 1). The batch experiments were carried out under mesophilic condition (37°C) in the incubator.

Table 1. Fermentation set-up parameter for batch experiment.

Serum bottle	Molasses concentration				
	50%	40%	30%	20%	10%
Total volume of serum bottle (ml)	100	100	100	100	100
Working volume (ml)	55	55	55	55	55
Ratio of molasses:distilled water (for 50 ml of working volume)	1: 1 (25:25)	1: 1.5 (20:30)	1: 2.33 (15:35)	1: 4 (10:40)	1: 9 (5:45)
Inoculum/sludge (mm)	5	5	5	5	5
Support carrier, GAC (g)	5	5	5	5	5
Glucose added (g)	0.82	0.86	0.92	0.94	0.97

2.3 Repeated batch cultivation

Repeated batch cultivation was conducted according to the set-up parameter in Table 1. Fermentation with GAC was conducted at various molasses concentration (50 %, 40 %, 30 %, 20 %, and 10 %) and similar experiments were conducted in parallel without GAC 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 molasses concentration.

2.3.1 Biogas production

The total gases collected were measured using Gas Analyser (biogas 5000 standard) Model GA 5000. The sulphuric acid with pH 2-3 was prepared in serum bottle to store the biogas prior to analysis. The biogas was analysed for the composition of hydrogen, carbon dioxide, oxygen and methane (if presence).

2.3.2 Kinetic analysis using Modified Gompertz equation for batch profiling

Kinetic analysis using Modified Gompertz equation for the optimal molasses concentration was applied whereby batch profiling for 24 hours with interval of 3 hours sample collection were observed. Theoretically, the modified Gompertz equation is as in equation 3:

$$H_t = H_m \exp \left\{ - \exp \left[\frac{R_m e}{H_m} (\lambda - t) + 1 \right] \right\} \tag{3}$$

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 fermentation time (h).

2.4 Analysis procedure

2.4.1 Total carbohydrate

The total carbohydrate present in the seed sludge, molasses, and fermentation effluent were tested by using phenol sulphuric acid method. Each of samples was prepared with volume of 0.5 ml in a vial. Then, 2.5 ml of sulphuric acid and 0.5 ml of phenol will be added to the vial. The samples were mixed evenly and leave for 30 minutes in dark place before being tested using spectrophotometer. The equation to determine the percentage of sugar consumption was as in equation 4.

$$\frac{\text{Total carbohydrate before} - \text{Total carbohydrate after}}{\text{Total carbohydrate before}} \times 100\% \quad (4)$$

2.4.2 Alkalinity

The dilution of 2.7 ml of sulphuric acid, (0.1N) in 1000 ml of volumetric flask and 50 ml of sample was prepared in a beaker. The diluted sulphuric acid was filled in 50 ml of burette and titration process is started. The titration stops when the samples reach pH 4.5. To determine the total alkalinity, the equation 5 is used.

$$\text{Total alkalinity, mgCaCO}_3 / \text{l} = \frac{S \times N \times 50000}{\text{volume of sample, ml}} \quad (5)$$

3 Results and discussions

3.1 Characterization of seed sludge and molasses

The characterization of seed sludge as inoculum to immobilized on GAC, and molasses as feedstock were listed as follow:

Table 2. Characteristics of seed sludge and molasses.

Parameter	Value	
	Molasses	Seed sludge
Chemical oxygen demand (COD), (g/L)	28.00	6.27
Total suspended solid (TSS), (g/L)	0.05	0.01
Initial pH	7.538	7.808
Initial temperature, ($^{\circ}\text{C}$)	26	26

3.2 Batch fermentation at various molasses concentration with the presence of GAC

The hydrogen production from the repeated batch fermentation for 5 different molasses concentration (50%, 40%, 30%, 20% and 10%) were collected for 10 repeated batch fermentation cycles (days) with the presence of GAC as support carrier.

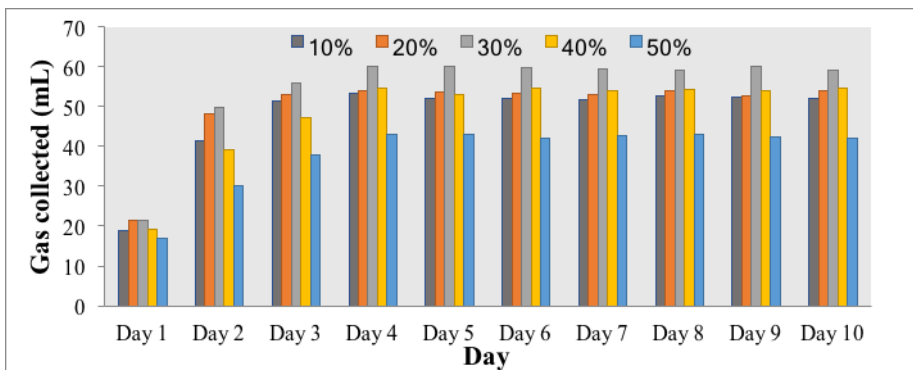


Fig. 1. Graph of hydrogen gas collection for different molasses concentration in repeated batch fermentation of 10 days.

Based on Fig. 1, the hydrogen produce by the mixed culture at different molasses concentration on the first cycle (day) was the lowest compared to other cycles. The hydrogen collected was increased from 19 ml to 21.3 ml when the molasses concentration is increase from 10% to 20% and 30%. However when further increased in substrate concentration to 40%, the hydrogen collected is decrease to 19 ml. The decreased trend was continued when the molasses concentration is increased to 50 % with 17.7 ml. The usage of 50% of molasses concentration was the lowest hydrogen production in all cycles (days) of repeated batch fermentation. It seem that the mixed culture bacteria were difficult to adjust to the environment with higher concentration of molasses. This might be due to the existence of fermentation inhibitor in the sugarcane bagasse molasses such as furfural. According to [7], pentose sugar such as in molasses could degrade to furfural. The inhibitory effects of furfural depend on its concentration and yet, no clear effect either positive or negative on the cell growth for molasses from sugarcane bagasse was reported. Further study and experimental need to be conduct for further justification on this matter. When the mixed culture bacteria growing in a rich medium, however were inoculated together with the presence of the inhibitor in the medium, the bacteria will take time to synthesising the necessary proteins, co-enzymes and vitamins needed for their growth.

Overall, the hydrogen production in all molasses concentration were rapidly evolved from day 1 to day 4 and start to stabilised until the day 10 of fermentation. The hydrogen producing bacteria might have increased their metabolic activity and also strengthen their adhesion onto GAC during the repeated batch cycle of fermentation. The repeated batch fermentation seems to caused the exploitation of growth medium at the maximal rate and growth of culture reaches the maximum rate. The used of repeated batch cultivation was successfull due to increasing in hydrogen performance. To sum up, the molasses concentration of 30 % depicted the highest hydrogen performance with 59 ml from day 4 of fermentation and the performance was sustained until day 10 of repeated batch fermentation.

3.3 Hydrogen production with GAC and without GAC (control) at various initial substrate concentration

The hydrogen production for 50%, 40%, 30%, 20% and 10% of molasses concentration with the presence of GAC were compared with the performance without GAC. The result obtained was shown in Fig. 2.

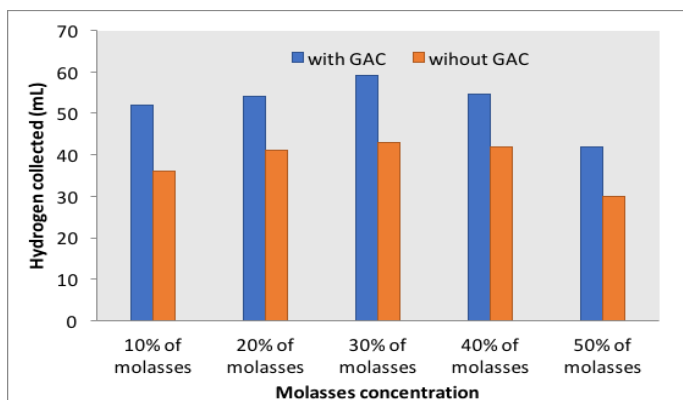


Fig. 2. Hydrogen production with GAC and without GAC at various molasses concentration.

The effect of initial substrate concentration at temperature 37°C with GAC and without GAC were studied in the range of 10% to 50% of molasses concentration. Based on Fig. 2, with the presence of GAC attached biofilm, the hydrogen production is increased from 52 ml to 54 ml when the initial substrate concentration was increased from 10% of molasses to 20% of molasses. However, a further increase in the initial substrate concentration to 30% of molasses was found to be the highest hydrogen collected with 59 ml. However, the hydrogen production collected is decreased when the initial substrate increased from 40% of molasses to 50% of molasses. This observation is similar to that of [8], who also reported that the hydrogen production is increase from 2.82 mmol H₂L⁻¹h⁻¹ to 4.3 mmol H₂L⁻¹h⁻¹ in fermentation with GAC attached biofilm.

On other hand, the production of hydrogen trend without the presence of GAC were the same as with GAC attached biofilm when the concentration of molasses were increased. The biohydrogen collected is increase from 36 ml to 41 ml when the substrate concentration increased from 10 % to 20% of molasses. At 40 % to 50 % of molasses, the hydrogen collected was declined from 43 ml to 30 ml. The highest production of hydrogen without GAC is at 30% of molasses concentrtaion with 45 ml of hydrogen produced. However, the overall performance without the presences of GAC were lower than the performance of hydrogen with GAC immobilised cells.

3.4 Kinetic analysis of the optimal molasses concentration with GAC immobilised cells

The kinetic parameter of the optimal molasses concentration (30%) with GAC immobilised cells was obtained from modified Gompertz model as in equation 3. The batch profiling of cumulative hydrogen production between the data from experimental and predicted modified Gompertz model is shown in Fig. 3. Based on the predicted modified Gompertz model, the cumulative hydrogen data fitted with maximum hydrogen production, H_m = 58 ml, hydrogen production rate, R_m = 2.02 ml/h and lag phase time, λ = 6.13 h.

Based on Fig. 3, at the first 6 hours of fermentation process, the hydrogen production was starting to increase. This phase is known as the lag phase whereby the mixed culture bacteria have just started to adapt to new environment and medium (molasses). Mixed culture activity is accelerated due to its cellular metabolism and increasing in their cells size. The bacteria start to synthesize the necessary proteins, co-enzyme and vitamins needed for their growth rate hence it will take time to produce high hydrogen production.

At 9 hours to 24 hours of fermentation process, the hydrogen collected was increased rapidly from 7 ml to 35 ml and this is known as the exponential or logarithmic phase. At

this phase, bacteria are rapidly growing and dividing state occur due to their metabolic activity. The medium (molasses) is exploited at the higher rate and reaches their higher growth rate to produce higher hydrogen performance. The maximum of hydrogen concentration for this batch profiling is 831 ppm.

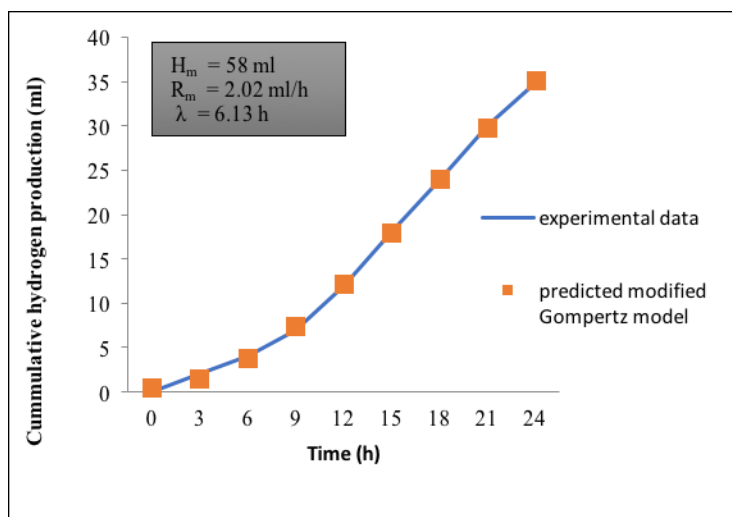


Fig. 3. Graph of experimental data and predicted Modified Gompertz Model for optimal molasses concentration (30 %).

3.5 Sugar consumption and total alkalinity of optimal molasses concentration with GAC immobilised cells

Fig. 4 shows the sugar consumption for the optimal molasses concentration (30 %) from the batch profiling. The results decreased over time of fermentation, which is from 100 % at first 3 hour to 36 % at 24 hour. This result was inline with the results obtained in Fig. 3, whereby the higher the sugar is consumed by the hydrogen producing bacteria, the higher the conversion of sugar into hydrogen is produced. Fig. 4 also depicted the total alkalinity, which represent the volatile fatty acid (VFAs) produced. VFAs and solvents, collectively known as soluble microbial products (SMPs) are considered as beneficial indicators to observe the hydrogen production performance. The result of total alkalinity was increased over time with 54 mg CaCO₃/l at first 3 hours and increased 3 fold to 94 mg CaCO₃/l at 24 hour of fermentation. The increment of total alkalinity was concurrent with the evolution of hydrogen production, which reflect the norm of anaerobic biohydrogen process.

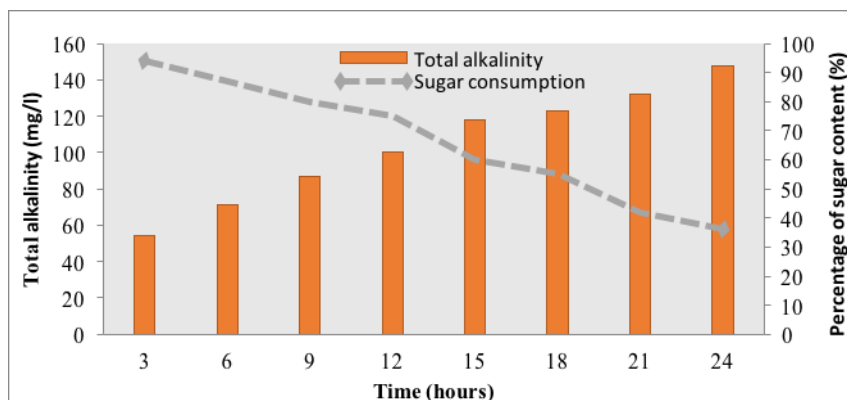


Fig. 3. Total alkalinity and sugar consumption of batch profiling fermentation process for optimal molasses concentration (30 %).

4 Conclusions

Ten cycles of repeated batch cultivation provide positive result in hydrogen production performance. The dark fermentation using GAC immobilised cell through repeated batch cultivation depict stable hydrogen production performance. The maximum biohydrogen obtained was optimal using 30% of molasses concentration. Batch profiling was done for the optimal molasses concentration, and the data was fitted with predicted modified Gompertz equation. The kinetic parameters were obtained with maximum hydrogen production, $H_m = 58$ ml and hydrogen production rate, $R_m = 2.02$ ml/h, with hydrogen concentration of 831 ppm. The performance of substrate concentration (molasses) during immobilisation of mixed culture bacteria onto granular activated carbon (GAC) has been proved by comparison with control sample (without GAC).

This research is fully supported by FRGS grant (FRGS No: 9003-00511). The authors fully acknowledged Ministry of Higher Education (MOHE) and Universiti Malaysia Perlis (UniMAP) for the approved fund, which makes this important research viable and effective.

References

1. R. R. Judkins, W. Fulkerson, M. K. Sanghvi, *Energy Fuels*. **7**, 14-22 (1993)
2. M. Momirlan, T. N. Veziroglu, *Int. J. Hydrogen Energy*. **30**, 795-802 (2005)
3. R. Chamousis, *Hydrogen: Fuel of the future*. (The Scientific Research Society, 2000)
4. J. J. Lay, Y. J. Lee, T. Noike, *Water Res.* **33**, 2579-2586 (1999)
5. W. Han, B. Wang, Y. Zhou, D. X. Wang, Y. Wang, L. R. Yue, N. Q. Ren, *Bioresour. Technol.* **110**, 219-223 (2012)
6. N. A. Lutpi, J. M. Jahim, T. Mumtaz, S. Harun, P. M. Abdul, *Process Biochem.* **51**, 297-307 (2016)
7. S. I. Mussatto, I. C. Roberto. *Bioresour Technol.* **93**, 1-10 (2004)
8. W. Q. Guo, N. Q. Ren, X. J. Wang, W. S. Xiang, Z. H. Meng, J. Ding, L. S. Zhang, *Int. J. Hydrogen Energy*. **33**, 4981-4988 (2008)