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Kinetic Analysis of Biohydrogen Formation using Immobilized Hydrogen-producing Bacteria on Activated Carbon Sponge from Pineapple Residues

(Analisis Kinetik Penghasilan Biohidrogen dari Proses Fermentasi Menggunakan Bakteria Penghasilan Hidrogen ke atas Span Karbon Aktif daripada Sisa Nanas)

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ABSTRACT

Pineapple residues are one of potential biomass feedstock for biohydrogen production. The most convenient way to produce biohydrogen from pineapple residual is through fermentation proses. The process is environmentally friendly and consumes low energy, but generally the process has low yield production. Various strategies can be used to increase production, including the use of immobilized cells in fermentation. The performance of the process can be explained as realistically as possible by the appropriate kinetic model. In this work, a kinetic analysis on fermentative biohydrogen production using different hydrogen-producing bacteria immobilized onto activated carbon sponge has been performed. The performance of cumulative and biohydrogen production rate were assessed using modified Gompertz equation via Excel solver application. All fermentation processes were carried out at a condition of initial pH 7 and temperature of 32 ± 1°C, with 30% v/v inoculum of working volume in batch process. Three different hydrogen-producing bacteria were used, namely Escherichia coli, Enterobacter aerogenes and Clostridium sporogenes, were immobilized onto activated carbon sponge and in free cell form as comparison. Based on best fitting curve results on the cumulative biohydrogen production, it was found that modified Gompertz equation were fitted well with all the experimental results of all regression values, R^2 were greater than 0.9. This study also presented that E. aerogenes and C. sporogenes able to produce better result compared to E.coli in term of production of biohydrogen The modified Gompertz equation would be useful for further analysis of biohydrogen production performance of selected hydrogen-producing bacteria culture immobilized onto activated sponge from pineapple residues.

Keywords: Kinetic analysis, Gompertz model, bacteria culture, immobilization, biohydrogen production, pineapple residues

ABSTRAK

Sisa nanas adalah salah satu bahan biojisim mentah yang berpotensi untuk pengeluaran biohidrogen. Cara yang paling mudah untuk menghasilkan biohidrogen dari sisa nenas adalah melalui proses penapaian. Proses ini mesra alam dan menggunakan tenaga yang rendah, tetapi secara amnya proses ini mempunyai hasil pengeluaran yang rendah. Pelbagai strategi boleh dilakukan untuk meningkatkan pengeluaran, antaranya penggunaan sel tersekatgerak dalam penapaian. Prestasi proses dapat dijelaskan secara realistik oleh model kinetik yang sesuai. Dalam kerja ini, analisis kinetik mengenai pengeluaran biohidrogen fermentasi menggunakan bakteria penghasil hidrogen yang berbeza yang tidak aktif ke span karbon diaktifkan telah dilakukan. Prestasi kadar pengeluaran kumulatif dan biohidrogen dinilai dengan menggunakan persamaan Gompertz diubahsuai melalui aplikasi "Solver" didalam Excel. Semua proses penapaian dijalankan pada keadaan awal pH 7 dan suhu 32 ± 1° C, dengan inokulum 30% v / v dari jumlah isipadu dalam proses kelompok. Tiga bakteria penghasil hidrogen yang berbeza, iaitu Escherichia coli, aerobes Enterobacter dan Clostridium sporogenes, telah disekatgerakkan ke span karbon teraktif dan dalam bentuk sel bebas sebagai perbandingan. Berdasarkan hasil lengkungan terbaik pada pengeluaran biohydrogen kumulatif, didapati persamaan Gompertz diubahsuai disuaipadankan dengan baik dengan semua keputusan eksperimen dengan nilai regresi, R^2 lebih besar daripada 0.9. Kajian ini juga menunjukkan bahawa E. aerogenes dan C. sporogenes mampu menghasilkan hasil yang lebih baik berbanding dengan E.coli dari segi pengeluaran biohidrogen.

Persamaan Gompertz yang diubahsuai akan berguna untuk analisis lanjut mengenai prestasi pengeluaran biohidrogen bagi bakteria penghasil hidrogen yang terpilih yang tersekatgerak ke span teraktif dari sisa nanas

Kata kunci: Analisis kinetik, Model Gompertz, kultur bakteria, imobilisasi, penghasilan biohidrogen, sisa nanas

INTRODUCTION

Kinetic modeling is very important in production of biohydrogen as growth associated product (Singh et al. 2015). Different model has been tested in order to analyze the effect, relationship, role of the parameter and predict the performance of biohydrogen during fermentation. Table 1 displays the model and function in biohydrogen production (Singh et al. 2015).

TABLE 1 Kinetic Modelling of Biohydrogen Production

No	Models	Functions
1	Arrhenius	Effect of temperature on H_2 production
2	Monod or Michaelis-Menten	Microbial growth on H_2 production
3	Logistic	Describe bacterial cell growth
4	Haldane-Andrew and Hans-Levenspiel	Substrate inhibition or substrate dependent on specific growth
5	Leudeking-Piret	Relation between cell growth and H_2 production rate
6	Andrew	Relation between pH and substrate consumption
7	Gompertz	Progress of cumulative H_2 production

The modified Gompertz equation has been widely used for the performance of biohydrogen production by fermentation which defined as follow:

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

Where H_t is the cumulative biohydrogen production (mL) at culture time t, P_m is the maximum amount of biohydrogen production (mL), R_m is the maximum biohydrogen production rate (ml_{H2}/hr), λ is the lag time (hr) and the value of is 2.71828. The correlation coefficient (R^2 value range over 0.99) indicates a strong correlation between the experimental data curve. The best fit curve (modified Gompertz equation) which describe the formation progress of biohydrogen.

The Gompertz model is beneficial for estimating the biohydrogen potential, specific rate of biohydrogen production and lag phase time in batch for various parameters setting based on the cumulative biohydrogen production. Besides the biohydrogen production, this equation also works to describe the progress of bacteria growth and substrate degradation (Boni et al. 2013). Other than that, models 1-6 stated in the Table 1 also been used by researchers to investigate the effect of different parameters that influence fermentative hydrogen production. It is based on the kinetic constant obtained from the models.

Commonly, fermentative biohydrogen production was derived from the biological process of organic or carbon-based feedstock or substrate by anaerobic bacteria. The plant biomass for the second-generation biofuels including agricultural waste, forest wastes, municipal solid waste (MSW) and industrial wastes. All these feedstocks categorized as second-generation feedstock which are non-food materials. Pineapple residues included as cellulosic resources in which about 70% of the whole pineapple will become residues after the processing (MPIB 2015). From the review, renewable feedstock like biomass, agricultural waste by-products, agricultural and livestock effluents and the most recent, lignocellulosic products are available and abundant for biohydrogen production.

Instead of free or suspended cell culture, immobilized cell has been used widely for hydrogen production either in lab or industrial scale as alternative to enhance microorganisms activity in fermentation system. This is because, most studies on biohydrogen production using suspended cells reported the washout problem in continuous process which affected the operational stability and the production yield (Sekoai et al. 2017). Immobilization has promote more advantages including of require less volume of growth medium, enhance the mass transfer, less space required (Basak et al. 2014), enhance microorganisms' activity in fermentation system so that can maintain high cell density (Argun & Kargi, 2011; Balachandar et al. 2013; Basak et al. 2014; Bru et al. 2012; Goers et al. 2014; Hu, 2013; Kao et al. 2014; Tuba Keskin et al. 2012; Tuğba Keskin et al. 2011; Koskinen, 2008; Rahma, 2013; Sekoai & Kana, 2013; Singh et al. 2013a, 2013b; Sivagurunathan et al. 2017; Tenca et al. 2011), reduce the risk of contamination (Sekoai et al. 2017; Baptista et al., 2007), enhance metabolic activity (Kao et al. 2014), increase substrate conversion efficiency (Sekoai et al. 2017) and reusable (Sekoai et al. 2017; Basak et al. 2014; Singh et al. 2013b).

The main objectives of the present study is to perform kinetic analysis of batch fermentation of pineapple residues using different H_2-producing bacteria of *Escherichia coli*, *Enterobacter aerogenes*, and *Clostridium sporogenes* in order to analyze the performance of biohydrogen production between free cell and immobilized cell and at the same time determine the most suitable H_2-producing bacteria for fermentation of pineapple residues.

For this study, mesophilic bacteria of *Escherichia coli*, *Enterobacter aerogenes* and *Clostridium sporogenes* were used as inoculum to avoid necessity of external heating in providing optimum condition for bacteria to grow well and have better performance (Ren et al. 2009). These bacteria

were then immobilized onto activated carbon sponge as the sponges has a proper pore size and large surface area for the cells to adhere (Kirli & Kapdan, 2016). The fermentation was performed at condition parameter of 33 -, 30 % v/v inoculum and pH of 7 using pineapple peel sample as substrate.

METHODOLOGY

PINEAPPLE SUBSTRATE PREPARATION

Pineapple waste was obtained from local market in Johor Bahru, Malaysia. The pineapple peel was selected and processed as hydrolysate or substrate in the fermentation. The substrate undergoes steam heat pre-treatment (autoclaved, T at 121 – for 20 mins) before being chopped into small pieces. Afterwards, an amount of 1 kg chopped pineapple waste was crushed using the steel blender (Waring Commercial Blender) with 2 L distilled water. Next, the mixture was filtered to obtain the hydrolysate or extract for the characterization analysis. The hydrolysate was then stored in refrigerator at 4°C and restored at ambient temperature, 25°C before used. The hydrolysate was neutralized to pH 7 before mixed with inoculum and used as substrate for the fermentation.

IMMOBILIZED BACTERIA AND INOCULUM PREPARATION

Facultative anaerobes (*Enterobacter aerogenes* – ATCC 13048 and *Escherichia coli* – ATTC 10799) and so known strict anaerobes bacteria (*Clostridium sporogenes* – ATCC 19404) purchased from Microbiologics were utilized as H_2-producing bacteria to perform the fermentation process. For inoculum preparation, each culture of bacteria was activated onto agar medium from agar-agar powder (QREC) and nutrient agar (Merck). The culture was then cultivated individually in nutrient broth (Merck) carefully and aseptically for 24 hours (overnight) in incubator (at 37°C).

Commercial activated carbon (AC) sponge was used as support materials to retain the bacteria culture. The AC sponge were cut into pieces (1 ± 0.2 cm x 1 ± 0.2 cm) and soaked in boiling water for 30 minutes. Then, the sponges were washed under tap water before left in distilled water for 24 h (changed 3-4 times). This is essential to remove all fine suspended particles (Rahma, 2013). Next, the sponges were dried in oven at 70°C overnight before uniformly dried in desiccator.

BATCH-KINETIC EXPERIMENTS

There are three different H_2-producing bacteria types used in this work, *E. coli*, *E. aerogenes* and *C. sporogenes*. The cultures were mixed together with immersion of AC sponges inside 90 ml of respective culture cultivation (30% v/v of working volume) to be incubated for another 24 hours at 130 r.p.m at 37°C before used for immobilization. The batch fermentation of pineapple substrate was carried out in 500 ml Dreschel bottle with the working volume of 300 ml. The 210 ml pineapple waste was first added to a 500 ml Dreschel bottle and another 90 ml was the inoculum with immobilized co-culture on AC sponges or the free cell. The initial pH of the substrate was adjusted using 0.5 M sodium hydroxide (NaOH) to achieve initial pH of 7. Nitrogen sparging was applied to provide anaerobic condition for the fermentation process and the bottles were sealed and put in a water bath to keep the culture medium at temperature 33°C ±1°C. Mixing was provided by a stirring magnetic bar in the bottle. The volume of biogas produced was measured using water displacement method whereas the gas captured in polyvinylidene fluoride (PVDF) gas bag was analyzed using gas chromatography (Agilent Technologies, 6890N, USA) equipped with a thermal conductivity detector (TCD) and Network GC System to obtain the composition and amount of biohydrogen produced.

RESULTS AND DISCUSSION

The result of cumulative biohydrogen production is shown in Figure 1. The values were used to fit the modified Gompertz equation as following where the maximum potential hydrogen formation (P_m), the maximum rate of hydrogen formation in ml_{H2}/hr (R_m) and the lag phase (λ) in hr of each culture type for free cell and immobilized cell on AC sponge are shown in Table 2.

TABLE 2.Fermentation Results and Kinetic Parameter

Group	H2 production (mL)	Pm (mL)	Pm (mL/hr)	λ (hr)	R2	Difference (%)
Free cell E.coli	1857.39	2269.04	41.25	0.72	0.939	22.16
Free cell E.aerogenes	3520.2	4319.67	91.48	1.84	0.972	22.71
Free cell C.sporogenes	2807.1	2722.95	163.39	1.33	0.989	3.00
Immobilized E.coli	2707.9	2828.43	83.51	2.40	0.997	4.45
Immobilized E.aerogenes	3795.82	4519.22	109.33	2.35	0.977	19.06
Immobilized C. sporogenes	2885	2925.24	103.15	1.64	0.998	1.39

P_m: maximum potential hydrogen formation, R_m: the maximum rate of hydrogen formation in ml/hr and the λ: lag phase

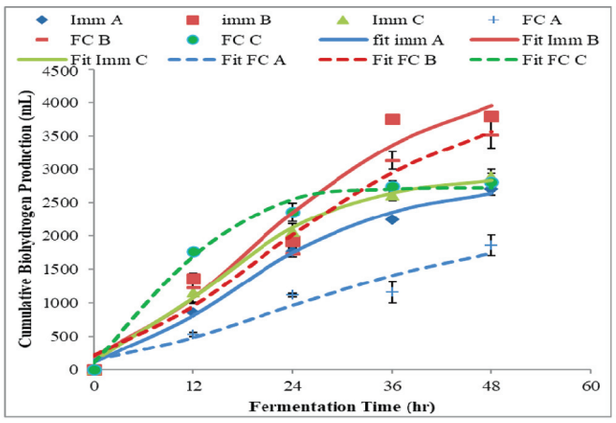

FIGURE 1. Cumulative biohydrogen production of free culture and immobilized of A,B,C

The results of fitting curve on Figure 1 and Table 2 showed that biohydrogen production fitted well with the modified Gompertz equation. The data presented in Figure 1 were correlated with the Gompertz equation and the constants were determined by regression analysis based on biohydrogen cumulative curves. The curve fitting showed that the equation was suitable to describe the progress of cumulative biohydrogen production from pineapple substrate.

It was observed that each of bacteria cultures displayed different duration of lag phase ranging from 0.72 to 2.40 hr. Rapid increase in biohydrogen production was observed after the lag phase until reach the stationary phase. Free cell *E. coli* had a shortest lag phase of 0.72 hr compared to other H_2-producing bacteria while immobilized *C. sporogenes* was observed to have the shortest lag phase of 1.64 hr compared to other immobilized H_2-producing bacteria used. However, both of these bacteria did not perform the maximum production of biohydrogen compared to *E. aerogenes* even the lag phase time was quite higher than others. Longer lag phase times could be attributed to lower degradability and require good environmental condition to release the products (Gupta 2014).

Examining the curves for cumulative biohydrogen production, the highest prediction was by immobilized *E. aerogenes* followed by free cell *E. aerogenes* with expected maximum production of 4519.22 mL and 4319.67 ml respectively. This indicates 19.06 % and 22.71 % differ from the experimental data accordingly. The fermentation by *C. sporogenes* for both free cell and immobilized cell noted the less difference where the measured biohydrogen production for free cell was 2807.1 ml. This result was 3 % higher than estimated biohydrogen production of 2722.95 ml confirming that *C. sporogenes* enhanced the production. Meanwhile, the immobilized *C. sporogenes* showed only 1.39 % difference of estimated production from the experimental data obtained. This could be attributed by the behavioral changes of the bacteria towards the surrounding. The porous structure on AC sponges may act as conductive platform for mass transfer of nutrients need by the bacteria (Hu 2013). It also provides a protective structure for bacteria in harsh environment. It is noted that from the kinetic reaction, the maximum production rate was observed by free cell *C. sporogenes* (163.39 ml/hr) followed by immobilized *E. aerogenes* (109.33 ml/hr) whereas the lowest production rate was by *E.coli* both for free cell or immobilized H_2-producing bacteria. Considering the overall performance of the three cultures, the immobilized cultures had a better performance compared to the free cell. Therefore, fermentation using immobilized H_2-producing bacteria has been identified as a potential process for biohydrogen production that favorable to reaction kinetics.

CONCLUSION

This study has concluded that higher final biohydrogen production was predicted than experimentally observed. *E. aerogenes* and *C. sporogenes* presented an outstanding result compared to *E.coli* by producing better production of biohydrogen. The model development and calibration provided useful information concerning the role of the kinetic constants in the analysis of a fermentative biohydrogen production process from organic waste. It may also represent a good foundation for the analysis of fermentative biohydrogen production from pineapple residues for pilot and full-scale applications.

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